

MINIREVIEW—EXPLORING THE BIOLOGY OF GPCRS: FROM IN VITRO TO IN VIVO

Model Organisms in G Protein–Coupled Receptor Research

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ABSTRACT

The study of G protein–coupled receptors (GPCRs) has benefited greatly from experimental approaches that interrogate their functions in controlled, artificial environments. Working in vitro, GPCR receptorologists discovered the basic biologic mechanisms by which GPCRs operate, including their eponymous capacity to couple to G proteins; their molecular makeup, including the famed serpentine transmembrane unit; and ultimately, their three-dimensional structure. Although the insights gained from working outside the native environments of GPCRs have allowed for the collection of low-noise data, such approaches cannot directly address a receptor's native (in vivo)

functions. An in vivo approach can complement the rigor of in vitro approaches: as studied in model organisms, it imposes physiologic constraints on receptor action and thus allows investigators to deduce the most salient features of receptor function. Here, we briefly discuss specific examples in which model organisms have successfully contributed to the elucidation of signals controlled through GPCRs and other surface receptor systems. We list recent examples that have served either in the initial discovery of GPCR signaling concepts or in their fuller definition. Furthermore, we selectively highlight experimental advantages, shortcomings, and tools of each model organism.

Introduction

G protein–coupled receptor (GPCR) pharmacology began in earnest with Raymond Ahlquist's conjecture that there must be two types of adrenergic receptors to account for excitatory and inhibitory effects of the sympathetic adrenergic mediator, epinephrine. This conclusion was based on a set of experiments that characterized the effect of biogenic amines on a roster of vegetative functions in dogs, cats, rats, and rabbits (Ahlquist, 1948). Most interestingly, the proposal of adrenoceptor subtypes was achieved before the era of molecular biology, before receptors transformed from a physiologic concept into a molecular fact (De Lean et al., 1980; Dixon et al., 1986; Palczewski et al., 2000; Rasmussen et al., 2007).

Ahlquist's work illustrates one advantage of animal models in pharmacological research: the ability to learn about receptor functions on cellular, organ, and organismic states without

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ABBREVIATIONS: AC, adenylyl cyclase; aGPCR, adhesion G protein–coupled receptor; DREADD, designer receptors exclusively activated by designer drug; ECM, extracellular matrix; ETH, ecdysis triggering hormone; FRET, fluorescence resonance energy transfer; GPCR, G protein–coupled receptor; GPS, G protein–coupled receptor proteolysis site; LNV, ventrolateral clock neuron; MB, mushroom body; PDF, pigment dispersing factor; PDFR, pigment dispersing factor receptor; TM, transmembrane.

full knowledge of the molecular underpinnings of these effects. What may seem to be an experimental shortcoming at first sight in fact reveals its potential when considering the complex biology of signaling pathways involving GPCRs. Many GPCRs are “orphaned,” that is, they lack identified agonistic or antagonistic ligands, the elements that control receptor activity. This lack precludes classical pharmacological analyses that rely on the ability to challenge the receptors with a stimulus. In addition, downstream messaging cascades of many GPCRs are still unknown and can therefore not be readily assayed through available standard reporters. The advantage to in vivo model systems is that the signaling network used by a given receptor is completely present and set up in an optimal fashion, irrespective of whether all of its (main) components and their working conditions have been identified and characterized.

Clearly, the nature of questions about receptor signals that model organism research can answer differs from those addressed through canonical in vitro assays. Whereas the latter provides a means to quantitatively study individual receptor function, receptor research using animal models aids in defining their role at a qualitative level and in understanding how their actions are integrated into the complex physiology of an organism. This has largely been achieved using the modern repertoire of molecular genetic tools to develop animal models into platforms for genetic screening and molecular manipulation. The combination of genetically tractable model organisms with in vivo physiology and imaging provides a powerful system for linking the molecular details of receptor function to physiology. Hence, genetic modifications have added direct manipulation of single receptors at the molecular scale to Ahlquist’s pharmacological strategy to interrogate the function of entire receptor populations.

The most popular animal models that contribute to understanding pieces of the signaling logic of GPCRs and other membrane receptor pathways are the nematode, *Caenorhabditis elegans*, the vinegar fly, *Drosophila melanogaster*, the zebrafish, *Danio rerio*, and the mouse. The 2014 Lorentz Center workshop on “Exploring the Biology of GPCRs from In Vitro to In Vivo” saw several talks highlighting how work in these species provided entry points into delineating the function of individual GPCRs and membrane receptors, which by and large have remained uncharted territory for classic pharmacology. These include projects on the adhesion class of GPCRs and their roles in the nervous system, aminergic and peptidergic GPCRs involved in circadian rhythm, sleep-wake cycle, eclosion, and male mating behavior. Furthermore, several speakers provided an overview on recent technologies to interrogate receptor function in vivo, including genetically encoded probes and optogenetic tools. Here, we briefly present the main aspects of these projects.

Animal Models to Discover and Validate Molecular Concepts

The Ins and Outs of Adhesion GPCR Actions. The adhesion class of G protein-coupled receptors (aGPCRs) was discovered through a genome-wide bioinformatic search for the sequence fingerprint of their heptahelical transmembrane unit (Bjarnadóttir et al., 2004). Like all GPCRs, aGPCRs possess a 7-transmembrane (TM) domain, but the class is defined structurally by a large extracellular N-terminal region that is separated from the 7TM by a GPCR autoproteolysis-inducing

domain (Araç et al., 2012), which encompasses the G protein-coupled receptor proteolysis site (GPS). aGPCRs represent the second largest GPCR class; however, the appreciation of their biologic roles lags behind that of all other GPCR classes at both physiologic and pharmacological levels. Several findings, obtained through phenotypic analyses of null or hypomorphic mutants of aGPCR genes in animal models, have established that these receptors function during developmentally dynamic periods of organogenesis and are involved in cell differentiation, migration, and polarity, similar to Frizzled-type GPCRs (Schulte, 2010; Dijksterhuis et al., 2014). However, whether aGPCRs also function in postmitotic tissue, which signals they read out, and how they transduce these into intracellular messages has only recently begun to unfold (see also Monk et al., 2015). Among others, two aGPCR homologs, Gpr126 (*ADGRG6*) and Latrophilin (*ADGRGL1-3*), have served as model receptors to dissect aGPCR signals.

Analysis of zebrafish *gpr126* mutants uncovered an essential role for this aGPCR in the development of myelinated axons in the peripheral nervous system. In the vertebrate peripheral nervous system, the myelin sheath is made by specialized glial cells called Schwann cells and is required for rapid impulse propagation. Without Gpr126, Schwann cells can ensheath axons but fail to spiral their membranes to generate the myelin sheath (Monk et al., 2009). Thus, animal models served to uncover a critical function of this aGPCR that would have been impossible to decipher in traditional heterologous cell systems. Intriguingly, myelin defects in *gpr126* mutants could be rescued by cAMP elevation, suggestive of G_s coupling. These studies are discussed in more detail in Monk et al. (2015). At the Lorentz Center workshop, more recent advances in understanding how Gpr126 controls Schwann cell development and myelination were presented. The advent of rapid genome editing tools has afforded unprecedented advances in mutant generation to study the function of genes in vivo. Using transcription activator-like effector nucleases, new *gpr126* mutant alleles were generated in zebrafish; their analysis demonstrated a function of the Gpr126 N terminus in early Schwann cell development that is distinct from the signaling function of the C terminus. Moreover, genetic analyses in both mouse and zebrafish supported a model in which interactions between the Gpr126 N terminus and the extracellular matrix (ECM) protein Laminin-211 modulates receptor signaling, perhaps by physical removal of the N terminus, to allow for 7TM signaling of the C terminus and myelination (Fig. 1A) (Petersen et al., 2015).

Model organisms have also been indispensable for understanding the function of Latrophilin/Cir1, an aGPCR conserved from ancient metazoa to humans, which was known for many years only as a biochemical binding target for black widow spider venom at neurons. Concrete evidence for the physiologic role of the receptor emerged through studies in *C. elegans*. Removal of the latrophilin receptor *lat-1* causes severe developmental problems owing to the loss of the polar alignment of neuroblasts along the anterior-posterior body axis of worm embryos, indicating that this aGPCR functions in the control of planar cell polarity signals (Langenhan et al., 2009). Second, *lat-1* mutants proved infertile, but the nature of stimuli perceived through the receptor protein remained elusive (Prömel et al., 2012). Recent findings on the function of the latrophilin homolog dCir1 in *D. melanogaster* have now provided insights into this matter. Latrophilin/CIRL is located in peripheral mechanosensory neurons, which perceive

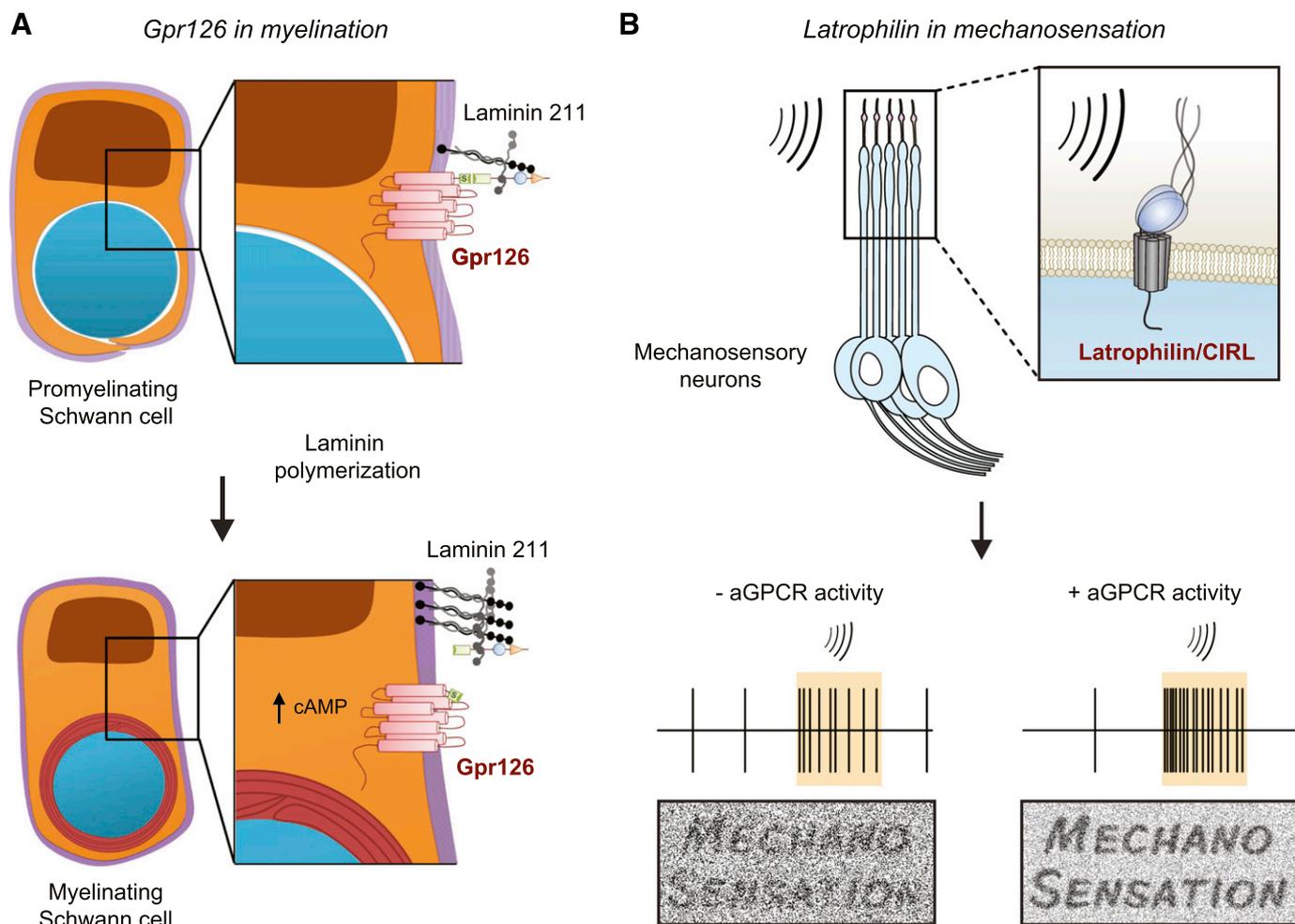


Fig. 1. Adhesion GPCRs as mechanically activated receptors. (A) Early in Schwann cell development, Laminin-211 interacts with the N terminus of *Gpr126* in a conformation that prevents cAMP accumulation through suppression of basal signaling, potentially allowing the Schwann cell to remain immature. After Laminin-211 polymerization in the basal lamina (purple shadow), Laminin-211 facilitates an active conformation of the receptor, perhaps by physical removal of the N terminus, which exposes a cryptic ligand (noted by “S”) (Petersen et al., 2015). (B) The aGPCR *Latrophilin/CiRL* is present in mechanosensory neurons of *Drosophila*, where it regulates their sensitivity toward mechanical stimulation and maintains a physiological signal-to-noise ratio. Loss of *Latrophilin/CiRL* results in numbness, amblyacousia, and proprioception deficits (Monk et al., 2015; Scholz et al., 2015).

mechanical signals such as sound, touch, and muscle stretch. A genomically engineered *Latrophilin/dCiRL* null mutant exhibited a much reduced sensitivity toward these sensory inputs implicating them (similar to *Gpr126*) as mechanosensors (Fig. 1B) (Scholz et al., 2015). Genetic experiments further demonstrated that *Latrophilin/dCiRL* activity may regulate the input-output function of mechanosensory nerve cells through the modulation of transient receptor potential channels, which ultimately govern the electrical response of these neurons (Scholz et al., 2015).

To devise a solid model on receptor functionality, the in vivo findings on the mechanosensitive nature of aGPCRs will require testing how mechanical stimuli translate into metabotropic signals under in vitro conditions.

Polycystin Proteins as Atypical aGPCRs: Lessons from *C. elegans*. *C. elegans* is a powerful model for neurobiology. GPCRs constitute about 7% of the *C. elegans* genome, most encoding chemoreceptors (Bargmann, 1998; Fredriksson and Schiöth, 2005; Thomas and Robertson, 2008). With the exception of the neuropeptide GPCRs (Frooninckx et al., 2012), much of our knowledge of *C. elegans* GPCR function comes from forward screens to identify genes regulating animal behavior.

ODR-10, the first bona fide olfactory GPCR, was identified in a screen for mutants with a specific defect in chemotaxis to diacetyl (Sengupta et al., 1996). A natural variation in the neuropeptide Y receptor *npr-1* gene regulates social feeding behaviors (de Bono and Bargmann, 1998). Genetic analysis of *C. elegans* male mating behavior identified two types of TM spanning receptors. The Secretin-like class 2 GPCR pigment dispersing factor receptor (PDFR)-1 modulates the neural circuit that promotes mate searching and male sex drive (Barrios et al., 2012).

Intriguingly, LOV-1 and PKD-2, the nematode homologs of the 11TM spanning molecule polycystin-1 (PC1) and the transient receptor potential channel homolog polycystin-2 (PC2), are required for mate searching, response to hermaphrodite contact, and location of the hermaphrodite’s vulva (Barr and Sternberg, 1999; Barr et al., 2001; Barrios et al., 2008). In both *C. elegans* and mammals, the polycystins localize to cilia and ciliary extracellular vesicles, where they are thought to act in a signaling capacity (O’Hagan et al., 2014; Wood and Rosenbaum, 2015). In humans, abnormalities in polycystin trafficking or stability may underlie autosomal dominant polycystic kidney disease (Cai et al., 2014). The Barr laboratory

has been using *C. elegans* as a model for studying mechanisms regulating the localization and functions of the polycystins in cilia and extracellular vesicles (O'Hagan et al., 2014; Wang et al., 2014). PC1 proteins, like aGPCRs, contain a GPCR autoproteolysis-inducing domain, undergo autoproteolytic cleavage at a GPS into N-terminal and C-terminal regions, and can activate G protein second messengers, endowing them with GPCR-like properties (Delmas et al., 2004; Yu et al., 2007; Prömel et al., 2013). LOV-1 possesses a GPS, and an N-terminal region lacking TM domains remains associated with cilia and extracellular vesicles, suggesting that LOV-1 may be an atypical aGPCR (Barr and Sternberg, 1999; O'Hagan et al., 2014; Wang et al., 2014).

The ligands activating PC1 and LOV-1 in the mammalian kidney and *C. elegans* male sensory neurons remain a mystery. As noted above, aGPCRs may function in some capacity as mechanosensors; perhaps PC1/LOV-1 and PC2/PKD-2 act in a similar manner. The N and C termini of aGPCRs may have distinct functions, based on studies of Latrophilins and Gpr126 in several model systems (Prömel et al., 2012; Patra et al., 2013; Petersen et al., 2015; Scholz et al., 2015). Ligands for aGPCRs are often ECM or membrane-associated proteins (Langenhan et al., 2013), which bind the N terminus and may unmask a binding site in the C terminus (Liebscher et al., 2014; Stoveken et al., 2015). In this model, the N terminus of PC1 and LOV-1 may bind to an ECM molecule, which would prime the polycystin complex for signaling. Consistent with this hypothesis, the PC1 N terminus interacts with ECM proteins *in vitro* (Weston et al., 2001), and in zebrafish, the polycystins genetically interact to regulate ECM formation (Mangos et al., 2010). Future work using *C. elegans* can draw from aGPCR studies in model organisms, allowing us to determine whether PC1/LOV-1 is an atypical aGPCR.

GPCR Signaling in the Circadian Clock: The Functional Importance of GPCR Coupling Diversity *In Vivo*. Circadian behavior requires an oscillating neuronal circuit that is both stable and flexible. In *Drosophila*, this circuit consists of approximately 150 neurons, organized into a number of distinct groups, which are kept in synchrony by the actions of pigment dispersing factor (PDF), a peptide released by a population of approximately 16 ventrolateral clock neurons (LNvs).

The effects of PDF are mediated by a single GPCR, the *Drosophila* PDFR, a member of the Secretin receptor (B1) family most closely related to mammalian receptors for calcitonin and calcitonin gene-related peptide (Hewes and Taghert, 2001; Mertens et al., 2004; Hyun et al., 2005; Lear et al., 2005). Interestingly, PDFR is not expressed in all clock neurons (Fig. 2). Indeed, not all members of a particular subset of clock neurons express the receptor (Im and Taghert, 2010) or respond to PDF application (Shafer et al., 2008). Given the behavioral importance of this peptide and the impracticality of biochemical experiments on such small subsets of cells, investigators have used genetic and behavioral strategies *in vivo* to examine PDFR signaling mechanisms. These studies have confirmed, and importantly, modified *in vitro* findings, and the differences between *in vitro* and *in vivo* conclusions illustrate the power and significance of studying GPCR signaling in its native context.

Functional expression of PDFR in mammalian and insect cell lines provided a provisional description of its signaling capabilities. As expected of a family B1 GPCR, PDFR activation elevates cAMP and calcium levels (Hyun et al., 2005; Mertens et al., 2005). RNA interference studies revealed that protein

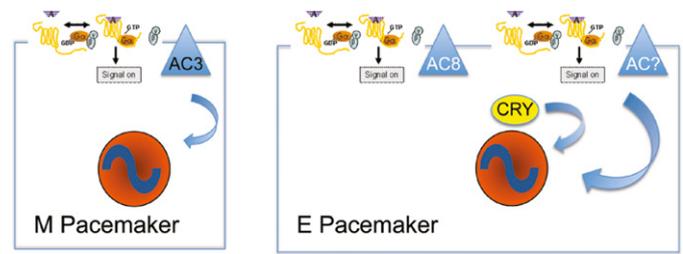


Fig. 2. Cellular context dictates the nature of GPCR signaling. The neuropeptide PDF activates a GPCR in target neurons of the circadian neural circuits in the *Drosophila* brain. M and E pacemakers refer to neurons that are biased to involvement in either a morning or evening bout of locomotor activity. *In vivo* observations indicate that PDFR signalsome components differ in the different target neurons: PDFR associates with AC3 in M cells, but in the E cell subgroup, PDF signaling relies on AC78C (AC8) and at least one other (currently unidentified) AC. This figure was originally published by Duvall and Taghert (2013) and is reproduced here with permission from the *Journal of Biological Rhythms*.

kinase A is normally activated by PDF activity and likely promotes stability and cycling of the essential clock proteins TIMELESS (Seluzicki et al., 2014) and PERIOD (Li et al., 2014). Furthermore, GW182, which mediates microRNA-dependent gene silencing through its interaction with AGO1, modulates PDFR signaling by silencing the expression of DUNCE, a cAMP phosphodiesterase (Zhang and Emery, 2013). Zhang and Emery argue that GW182 is a novel light-dependent rheostat modulating the amount of PDF GPCR signaling. Using an RNA interference screen of GPCRs coupled to inositol phosphate-stimulated calcium elevation, PDFR appears to be G_q coupled in some neuronal subsets (Agrawal et al., 2013). To measure cyclic nucleotide levels, a number of investigators have used a fluorescence resonance energy transfer (FRET) reporter built around the cAMP-binding domain of the molecule EPAC (de Rooij et al., 1998; Nikolaev et al., 2004). Shafer et al. (2008) used this reporter as a transgene and reported that most, but not all, circadian pacemaker neuron groups in the brain respond to PDF with elevations of cyclic nucleotides. PDFR couples to different adenylyl cyclases (ACs) in different pacemaker neuron populations (Duvall and Taghert, 2012, 2013). Specifically, PDFR stimulates the ortholog of mammalian AC3 in small LNvs (which express a PDFR autoreceptor), whereas PDFR in dorsolateral neurons couples to the ortholog of mammalian AC8 and at least one other (currently unidentified) AC. Furthermore, within small LNvs, PDFR is coupled to G_{α_s} and AC3, whereas other small LNv G_{α_s} -coupled GPCRs (those sensitive to dopamine or other neuropeptides) are coupled to ACs different from AC3 (Duvall and Taghert, 2012). The differential pairing of peptide GPCRs to distinct AC isoforms in different neurons, and even within single identified neurons, provides a striking example of the localized subcellular domains within which GPCR signaling complexes must be assembled as multiprotein clusters, permitting discrete spatiotemporal communication (Dessauer, 2009).

The ability to use genetic tools with imaging and behavioral endpoints in *Drosophila* provides the opportunity to assess the importance of GPCR function *in vivo*. As *in vitro* biochemical and structural studies provide more information on the mechanisms by which GPCRs couple to different output pathways, genetic model systems can be used to confirm results in native contexts and provide functional relevance for the amazing diversity of GPCR functions.

Insect Ecdysis and the Complexities of In Vivo Studies on GPCRs

One system that exemplifies the complexities of GPCR action is the control of insect ecdysis behavior, the shedding of the old exoskeleton during arthropod molting. This behavior consists of a sequence of behavioral routines and is typically followed by the inflation and hardening of the new exoskeleton (Ewer and Reynolds, 2002). Insects must perform this complex sequence of movements relatively flawlessly to survive and reproduce. While normally stereotyped, ecdysis sequences can also include checkpoints at which sensory inputs can delay the shedding process if needed.

Ecdysis is controlled by numerous peptide hormones (Ewer and Reynolds, 2002; Zitnan and Adams, 2012) that act on identified GPCRs. One of these, ecdysis triggering hormone (ETH), acts as the principal trigger of the ecdysial sequence. Strikingly, the ETH receptor is expressed in all neurons that produce neuropeptides associated with the control of ecdysis, and exposing excised *Drosophila* nervous systems to ETH activates these different targets at different times (Kim et al., 2006). The parallel activation of a GABAergic inhibitory pathway may explain how ETH activation of neuronal elements occurs in succession rather than synchronously (W. Mena and J. Ewer, unpublished data). These findings illustrate one way in which the simultaneous activation of the ETH receptor GPCR can cause the expression of a complex sequence of neuronal activity.

In addition to such ETH-mediated inhibitory delays, the ecdysis system is also subject to delays mediated by sensory inputs (White and Ewer, 2014). For instance, adult flies will postpone wing inflation up to several hours if they find themselves physically constrained (Peabody et al., 2009). This inhibition is neurally mediated and prevents the release of the hormone Bursicon, which appears to feed back to modulate the sensory-mediated delay pathways (Luan et al., 2012). Novel tools to identify and manipulate neurons that express the Bursicon receptor have provided insight into this process (Diao and White, 2012). The receptor uses cAMP as a second messenger, and as shown at the Lorentz Center workshop, optogenetic activation of Bursicon receptor-expressing neurons using a photoactivatable AC overcomes the sensory-mediated delays (F. Diao and B. H. White, unpublished data).

The technique used to target Bursicon receptor-expressing neurons has recently been adapted for use with other genes and can now be used to quickly gain genetic access to cells that express other GPCRs in the intact fly (Diao et al., 2015). This will help determine the spatial relationships between known ligand release to corresponding receptor-carrying cell populations. Specifically in the case of the Bursicon receptor, the results should prove interesting. Indeed, some neurons produce both subunits of Bursicon (and therefore presumably make the heterodimeric hormone), but others synthesize only one subunit. Activation of the Bursicon receptor by homodimers has not been observed, but the only signal transduction assayed thus far is cAMP. If the receptor exhibits bias and uses different downstream transduction pathways for different ligands, homodimeric activation may occur. Colocalization of the Bursicon receptor with release sites for putative homodimers would lend support for the hypothesis of homodimeric activation.

This brief exposition demonstrates some of the advantages of the ecdysis system in exploring how GPCRs and their

ligands orchestrate with great precision, yet with some flexibility, complex sequences of behaviors and physiologic changes. Nonetheless, much remains to be learned about signaling within the ecdysis network. In particular, the signal transduction mechanisms employed by the GPCRs and their effects on neuronal physiology have only been superficially characterized. The use of novel genome engineering tools, most notably clustered regularly interspaced short palindromic repeats (Doudna and Charpentier, 2014), will render more animals amenable to genetic manipulation and recruit new models. This will allow for direct comparisons between GPCR action in *Drosophila* versus other insects, paving the way for an understanding of the evolution of GPCR action.

Tools for In Vivo GPCR Research

Metabotropic Signal Sensors for In Vivo Studies. The two main intracellular messengers modulated through G protein activation are calcium and cAMP. Several FRET-based genetically encoded sensors for both messengers are available, which allow the monitoring of intracellular concentration changes in living cells with high spatial and temporal resolution (van Unen et al., 2015). cAMP sensors based on EPAC (Nikolaev et al., 2004; Ponsioen et al., 2004; Calebiro and Maiellaro, 2014) and genetically encoded calcium indicators D3cpV (Palmer and Tsien, 2006) and GCaMPs (Tian et al., 2009; Akerboom et al., 2013) are popular examples.

Several mouse lines with transgenic FRET sensor expression have been generated (Hara et al., 2004; Calebiro et al., 2009; Grienberger and Konnerth, 2012). However, their utility in monitoring GPCR activity in vivo is still limited by low signal-to-noise ratios and the requisite isolation of organs or cells before experimentation. Moreover, GPCR ligand delivery in intact animals remains a challenge for these investigations.

By contrast, genetically encoded cAMP and calcium probes can be targeted to specific cell populations with subcellular specificity in some animal models, allowing the study of how receptor localization and presence of partner proteins contribute to GPCR function. Furthermore, the optical isolation of the structure of interest for calcium or cAMP level monitoring dampens noise levels and allows for more precise kinetic measurements of GPCR messenger activation even under in vivo conditions.

An emerging animal model for such visualization of GPCR activity in vivo is *Drosophila* (Shafer et al., 2008; Shang et al., 2011; Duvall and Taghert, 2012; Pérez et al., 2013). Through the use of binary expression systems such as UAS/GAL4, probe expression can be confined to individual tissues and an ever enlarging collection of small cell clusters and single cells (del Valle Rodríguez et al., 2012) (Fig. 3, A and B). Indeed, GAL4-directed expression of an aequorin transgene in *Drosophila* was the first example of a calcium reporter animal (Rosay et al., 1997). More recent studies have expressed genetically encoded fluorescent reporters in *Drosophila* LNV clock neurons (Cao et al., 2013). Other studies in *Drosophila*, which have also exploited the power of genetically encoded sensors of GPCR activity, elucidated the role of dopamine and octopamine receptors in olfactory memory formation by monitoring calcium and cAMP signals in the mushroom body (MB), the part of the fly brain where olfactory memory is processed (Tomchik and Davis, 2009; Gervasi et al., 2010). Interestingly, depending on which GPCR was stimulated through its cognate ligand, MB

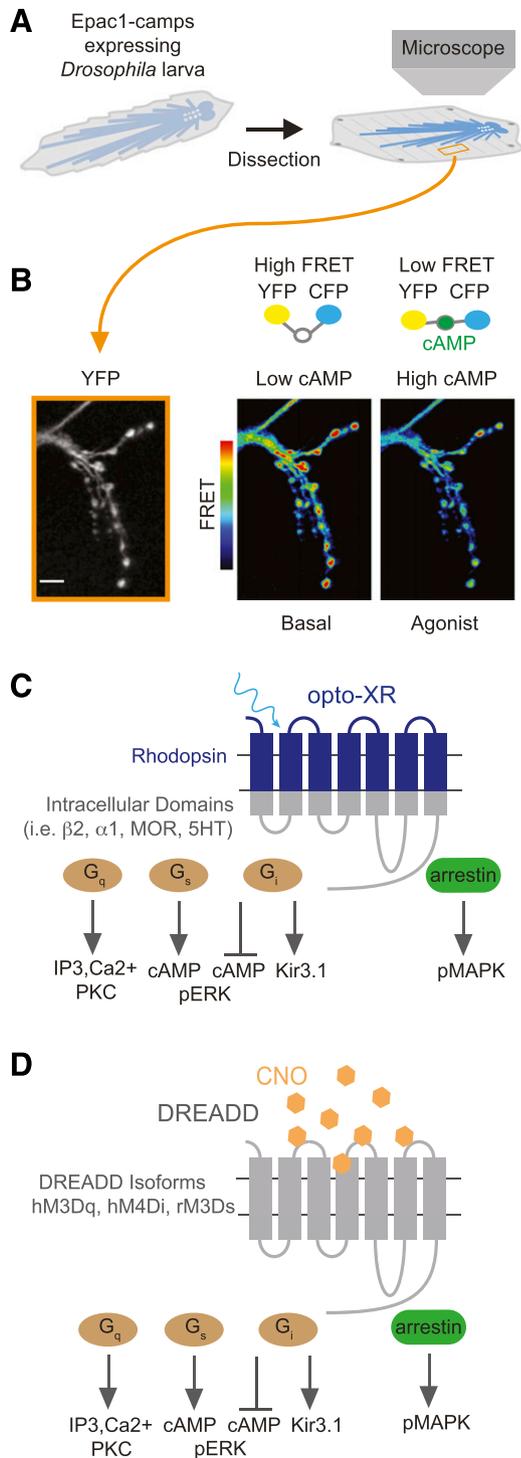


Fig. 3. Modern tools for investigating GPCR signaling in vivo. (A) Representation of intact (right) and dissected (left) *Drosophila* larva expressing the FRET-based sensor for monitoring cAMP changes, Epac1-camps. (B) YFP and time-resolved pseudocolor FRET images of *Drosophila* motoneurons expressing Epac1-camps. The application of agonist mediates the activation of the endogenous receptors, which results in production of cAMP. This cAMP increase is revealed by a loss in FRET caused by a conformational rearrangement of the sensor, induced by cAMP binding. (C) Cartoon depicting the chimeric “Opto-XR” approach, whereby rhodopsin cDNA is fused with wild-type GPCR cDNA intracellular loops and tail to generate a photosensitive receptor system capable of spatiotemporal engagement of canonical GPCR signaling pathways such as G_q, G_s, and G_i or arrestin recruitment in selected cell types when combined with viral and genetic approaches in vivo. (D) Cartoon representing chemogenetic GPCRs termed DREADDs, which selectively respond to the ligand CNO to

metabotropic activity differed substantially. Activation of octopamine receptors by octopamine elicited a generalized cAMP signal in the region of the MB involved in appetite modulation. By contrast, dopamine stimulation triggered a localized cAMP signal in parts of the MB that modulate aversive learning. This demonstrates how knowledge of cellular location and dynamics of GPCR-generated signals in the nervous system helps to unravel complex brain functions, unlocking an organ- and behavior-specific context to GPCR function.

Optogenetics and Designer Receptors Exclusively Activated by Designer Drugs for Studying GPCR Function In Vivo. The advent of optogenetics has transformed the ability of biologists to selectively interrogate neural circuits, cell types, and pathways critical for behavior and disease. Several recent advances are underway to use the advantages of light’s spatial-temporal characteristics to selectively engage GPCR signaling in a cell-type selective manner in vivo.

Investigators have turned to utilizing vertebrate and non-mammalian rhodopsin receptors to mimic G protein signaling both in vitro and in vivo using a variety of approaches (Zemelman et al., 2002; Schroll et al., 2006; Zhang et al., 2007). A recent study showed that bovine rhodopsin and class A GPCRs could be made into receptor chimeras, composed of extracellular and hydrophobic light-sensitive rhodopsin domains and intracellular loops targeted to couple to specific G-protein pathways (Airan et al., 2009). The authors used β₂-adrenergic and α₁-adrenergic receptor intracellular loop and carboxy tail components fused to bovine rhodopsin to achieve Gα_s and Gα_q signaling, respectively (Fig. 3C). This technique allows experimenters to use fiber optic or wireless light-emitting diode technology (Yizhar et al., 2011; Kim et al., 2013) to activate GPCR signaling within selected cell types in the mammalian brain of awake behaving animals. Elegant extensions of this approach have also been used in modifications of vertebrate rhodopsin with components of the 5HT1a or 5HT2c serotonin receptor for examining neural circuits and signaling in anxiety behavior (Masseck et al., 2014; Spoida et al., 2014). Recent work has also shown that chimeric opsin–wild-type receptors can be used to optically mimic opioid receptor signaling (Siuda et al., 2015) (Fig. 3C). Other studies used opsin GPCRs in cellular models for achieving remarkable spatial-temporal control of signaling gradients and cell migration (Karunarathne et al., 2015). These approaches allow the experimenter to precisely control the spatial components of subcellular GPCR signaling without activating receptor signaling in other microdomains or at different cellular stations (O’Neill and Gautam, 2014). Using plant cryptochrome domains (CRY2/CIB1), one can light-trigger recruitment of regulators of G protein signaling or Gbg, allowing for the selected sequestering of GPCR signaling. Similar approaches have used short-wavelength opsins and invertebrate opsins from jellyfish to achieve subcellular spatial-temporal control.

Another line of technology to interrogate GPCR signals in vivo regards the recent wide adoption of designer receptors

engage canonical GPCR signaling pathways in selected cell types when also combined with viral or mouse genetic approaches. CFP, cyan fluorescent protein; CNO, clozapine–nitrous oxide; ERK, extracellular signal-regulated kinase; IP₃, inositol trisphosphate; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; YFP, yellow fluorescent protein. Bar, 10 μm.

exclusively activated by designer drugs (DREADDs) (Sternson and Roth, 2014). DREADDs are activated by a pharmacologically inert ligand, cloxipine–nitric oxide, which displays low or no affinity for endogenous GPCRs and can engage $G\alpha_s$, $G\alpha_i$, and $G\alpha_q$ pathways (Fig. 3D). Additional variants are currently being explored for synaptic targeting (Stachniak et al., 2014) and more selective spatial control and multiplexing using additional chemogenetic substrates based on reagents used with κ -opioid receptors (Vardy et al., 2015). DREADD knock-in animal models, including *Drosophila* and mice, are currently being used by the GPCR research community for engaging cell-type selective G protein signaling. For example, the selective expression of DREADDs in the *Drosophila* heart clarified the signal pathway of the 5-HT receptor in the control of heartbeat (Becnel et al., 2013; Majeed et al., 2013). DREADD/cloxipine–nitric oxide pharmacology does not provide spatial-temporal control; however, there is no need for complex fiber/light-emitting diode systems or expensive microscopy, and this approach has therefore gained a wide user base.

Future work in developing these tools focuses on how we can use opsin and DREADD techniques to mimic endogenous receptor function. Given that they are artificial constructs being introduced via viral or genetic means, it is generally unknown how well they recapitulate the spatial-temporal dynamics of the endogenous receptor systems experimenters are dissecting. Comparing kinetics, signaling pathway efficacy, and expression profiles of these tools in vivo will be needed as the field grows and matures. Nevertheless, as animal models become more tractable, using rhodopsin and DREADD receptors in vivo, in conjunction with side-by-side pharmacological analyses, will allow for a clearer dissection of GPCR signaling as it relates to physiology and behavior.

Conclusions

Classic GPCR investigations have traditionally used elegant, reductionist in vitro approaches. These studies are enhanced and provided with a physiologic context when considered along with in vivo analyses. As genome editing becomes routine, model organisms can give clear answers regarding biologic functions of GPCRs in question. Moreover, continued advances in imaging and receptor manipulation will further our understanding of receptor function—even at subcellular resolution—in a living organism. The deepest understanding of GPCRs can come from concomitant investigations using in vivo and in vitro approaches, and future work can be directed to solving current outstanding problems, including biologic relevance of receptor dimerization, biased signaling, and questions of GPCR structure/function.

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Wrote or contributed to the writing of the manuscript: Langenhan, Barr, Bruchas, Ewer, Griffith, Maiellaro, Taghert, White, Monk.

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