Minireview

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Mining the Receptorome*[S]

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The prosperity of an organism relies on its ability to respond to its ever changing environment. The mechanism for this adaptive response is simple in theory; external stimuli are received and integrated, thereby eliciting a concerted and appropriate response. This cellular communication depends largely on the transmission of signal couriers (*i.e.* "ligands") from one cell, which are then received via cell surface and intracellular recognition molecules (*i.e.* "receptors") on the recipient cell (1). The interaction of an activating ligand (*i.e.* "agonist") with the receptor ultimately results in transduction of the signal via a complex web of biochemical interactions to produce the required response.

The diversity of physiological responses that occur in multicellular organisms includes the modulation of the central and peripheral nervous system and cardiovascular, gastrointestinal, metabolic, reproductive, genitourinary, and immunological functions and reflects the number of ligand and receptor molecules that elicit them. These ligands can include environmental stimulants such as photons, odorants, tastants, pheromones, and viruses as well as native molecules, including various small molecule neurotransmitters, amino acids, polypeptides, hormones, nucleotides, ions, and lipids. To accommodate the selective recognition of these diverse ligands a number of large receptor superfamilies encompassing both membrane receptors (*e.g.* G protein-coupled receptors, receptor tyrosine kinases, ligand-gated ion channels, and integrins) and intracellular nuclear receptors have evolved (2). Taken in its entirety, that portion of the proteome dedicated to ligand reception has been described as the "receptorome" (3) and encompasses more than ~5% of the human genome (2).

The richness in both number and diversity of physiological responses that receptors control, as well as the relative success in developing clinically active small molecule ligands for them, has made the receptorome the most successful target for therapeutic drug discovery (4). However, the full therapeutic potential of the receptorome remains untapped, especially when considering that native ligands for many "orphan" receptors remain elusive (2, 4). Furthermore, comprehensively screening existing drugs at the receptorome has revealed a previously unanticipated level of complexity in terms of selectivity and diversity in the number of receptors targeted by drugs (5). Together, identifying ligands for orphan receptors (*i.e.* "deorphanization") and profiling drug interactions at deorphanized receptors will offer new insights into disease pathogenesis and provide improved therapeutics.

To facilitate the discovery process, it is necessary to develop means of high throughput screening (HTS)[1] for drug-like compounds against the receptorome. These screens take advantage of receptor properties (*e.g.* sequence content, ligand binding, and signal transduction) that can be screened virtually (*in silico*) or empirically (physical HTS assays). This review will focus on various approaches for mining the G protein-coupled receptor (GPCR) receptorome superfamily. The GPCR superfamily is one of the largest protein constituents of the genome and is a significant portion of current pharmaceutical targets (2, 4, 6). Importantly, the principles described herein toward mining the GPCR receptorome may be applied to other receptorome superfamilies.

The GPCR Receptorome

Analysis of the human genome has revealed the existence of 735–802 GPCR open reading frames, of which ~375 are neither olfactory nor taste receptors (3, 7). Based on sequence homology and, to a lesser extent, pharmacological similarities, human GPCRs fall into one of five families: A (rhodopsin), B (secretin), C (glutamate), adhesion, and Frizzled/Smoothen/Taste2 (7). Family A is the largest family, and its members recognize a diverse array of ligands including odorants, biogenic amines, neuropeptides and peptidergic hormones, lipids, nucleotides, proteases, or in the case of the prototypical family A receptor, rhodopsin, photons (8). The family B receptors are responsive to hormones and peptides. The family C receptors are activated by amino acids, ions, and tastants. Adhesion receptors are hypothesized to interact with extracellular matrix or membrane-bound proteins whereas Frizzled and Taste2 receptors are activated by Wnt proteins and tastants, respectively.

GPCRs, which differ vastly in primary sequence, share a common hydrophobic arrangement indicative of a seven-transmembrane topology and, therefore, are also referred to as seven-transmembrane (7TM) or heptahelical receptors (Fig. 1). The crystal structure of bovine rhodopsin demonstrates, as predicted, 7TM helices oriented such that the N terminus is extracellular and the C terminus of the receptor is intracellular with the 7TM helices being separated by three intracellular and three extracellular loops (9). Common binding sites for endogenous ligands include the N terminus and/or extracellular loops for endogenous peptide ligands or, in the case of small molecules like biogenic amines, a hydrophilic pocket formed between the TMs near the extracellular face (8). Most small molecule drugs are directed toward this hydrophilic pocket that is suitable for controlling the activity of receptors in which native ligands bind within or even outside this pocket (8).

The vast majority of 7TM receptors couple to and activate heterotrimeric G proteins to stimulate production of second messengers (8). The heterotrimeric G protein consists of a Gα subunit and the G$\beta\gamma$ complex, both of which can activate effectors. Including splice variants, there are 18 Gα, 6 Gβ, and 12 Gγ subunits in humans (10). The theoretical combinational complexity of receptor and G protein associations is likely constrained through preferential interactions between receptors and G proteins in conjunction with cell type-specific expression patterns (10, 11). GPCRs couple to and activate one or more of the four distinct G protein classes defined by the Gα subunit (Gα_s, G$\alpha_{i/o}$, G$\alpha_{q/11}$, or G$\alpha_{12/13}$) leading to activation of distinct effectors (Fig. 1 and Refs. 8 and 12).

A range of responses (including cell proliferation, differentiation, contraction, and neurotransmitter release) is dependent upon cell type, specificity of G protein coupling, and the duration and intensity of receptor activation (13). Many of these responses are a consequence of modified gene expression resulting from the quasi-universal activation of MAPK pathways by GPCRs (13, 14). A number of receptor-associated proteins, particularly scaffold proteins (*e.g.* PDZ-containing proteins, β-arrestins, caveolins, etc.), are likely to also differentially organize responses (8, 15, 16). Thus, depending on the expression profile of these proteins, different cell types may respond differently to receptor activation by the same ligand-receptor pair.

"Receptor-omics": Computationally Screening the Receptorome

Bioinformatics Approaches to Deorphanize GPCRs—The human genome project and EST data bases have accelerated the identifi-

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[S] The on-line version of this article (available at http://www.jbc.org) contains Figs. 2S and 3S.

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[1] The abbreviations used are: HTS, high throughput screening; GPCR, G protein-coupled receptor; 7TM, seven-transmembrane; MAPK, mitogen-activated protein kinase; oGPCR, orphan GPCR; IP_3, inositol trisphosphate; PLCβ, phospholipase Cβ; AC, adenylate cyclase; KOR, κ opioid receptor; PML, progressive multifocal leukoencephalopathy; JCV, JC virus.

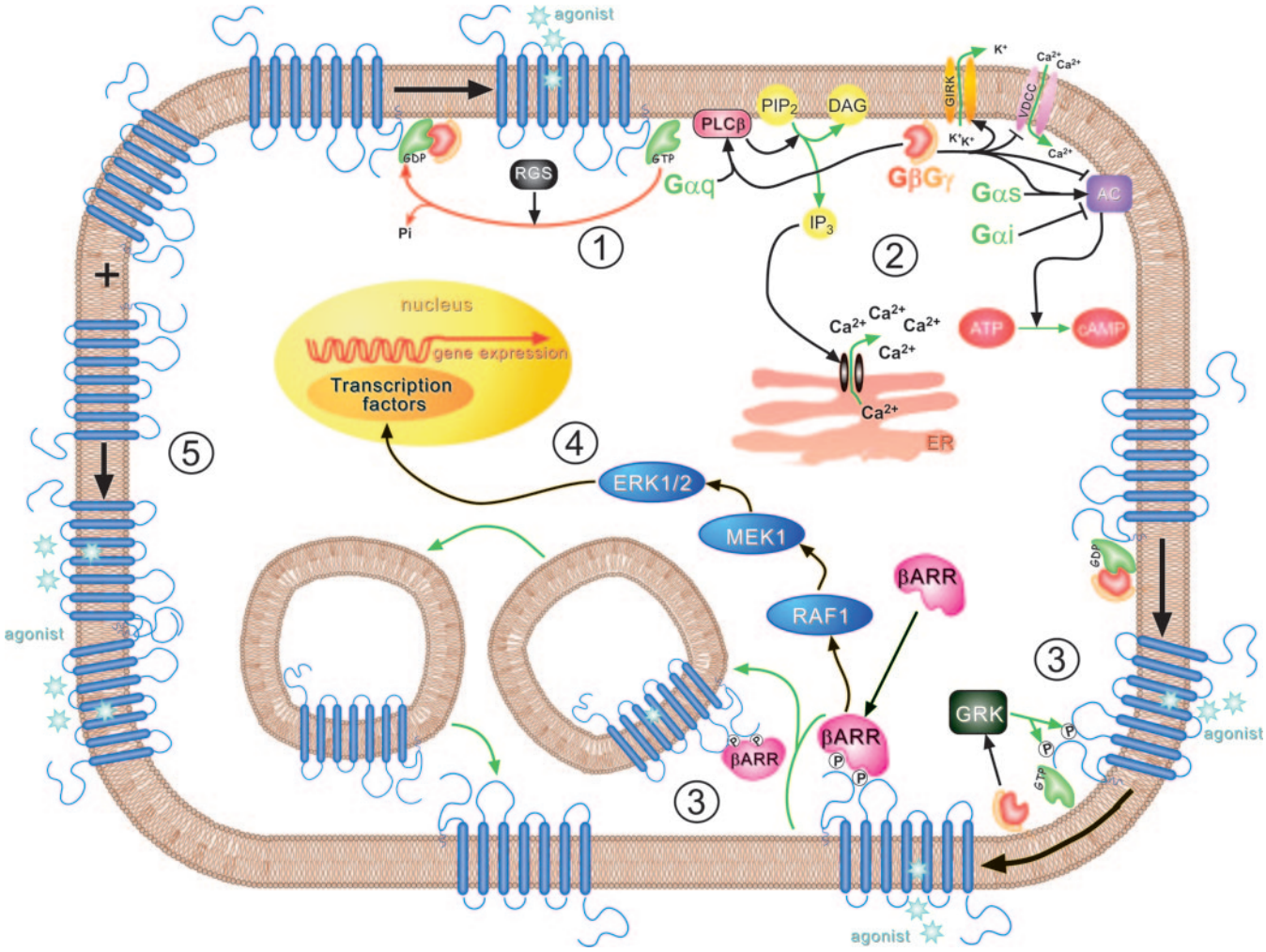

FIG. 1. **Functional assays based on diverse signaling and regulation of GPCRs.** *1*, G protein activation. Agonist-induced GPCR activation results in the exchange of GDP with GTP within Gα and can be monitored using non-hydrolyzable [^{35}S]GTPγS. The intrinsic GTPase activity of Gα (release of $^{32}P_i$ from [γ-^{32}P]GTP), which is enhanced by RGS proteins, has also been assayed. *2*, second messenger production by G protein-activated effectors. The modulation of G protein effectors (*e.g.* $G\alpha_s$ stimulation and $G\alpha_{i/o}$ inhibition of AC-stimulated cAMP production or activation of PLCβ by $G\alpha_{q/11}$) is well established as are the principal assays for examining GPCR activation. Fluorescent measurement of intracellular Ca^{2+} release resulting from PLCβ-driven IP_3 production is a convenient functional assay platform. Similarly, other G protein-activated effectors, such as ion channels, may be monitored by fluorescent dyes that bind to ions and/or respond to changes in membrane potential or pH. *3*, GPCR desensitization and receptor membrane expression. Following agonist activation, GPCRs are desensitized via phosphorylation and internalization into endosomes and are either recycled back to the cell surface or degraded in lysosomes. HTS assays have been designed to monitor transitions in cellular localization of proteins involved in desensitization (*e.g.* transient redistribution of β-arrestins from the cytosol to the membrane following agonist treatment (48)) or enhanced cell surface expression of wild-type and constitutively active GPCRs by inverse agonists (31, 49). *4*, transcriptional activation following GPCR stimulation. A number of reporter assays have been developed to monitor the transcriptional activation of reporter genes and/or cell proliferation in response to GPCR-mediated activation of MAPK pathways (50, 51). *5*, ligand-induced GPCR dimerization. Co-expression of GPCRs differentially tagged with fluorescent markers has been used in combination with resonance energy transfer methods, such as fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET), to identify agonists based on spectrum shifts resulting from ligand-induced receptor dimerization and/or G protein dissociation (52). *GIRK*, G-protein-activated inwardly rectifying K^+ channel; *VDCC*, voltage-dependent Ca^{2+} channel; *GRK*, G-protein-coupled receptor kinase; *βARR*, β-arrestin; *PIP_2*, phosphatidylinositol; *DAG*, diacylglycerol; *RAF1*, *MEK1*, and *ERK1/2*, mitogen-activated protein kinases; *RGS*, regulator of G protein signaling.

cation of potential members of the various receptor superfamilies, including the GPCR superfamily. As a result, there is an increasing emphasis on "reverse pharmacology"-based approaches to identify endogenous ligands ("deorphanize") and assign physiological functions to the ~160 non-olfactory orphan GPCRs (oGPCRs) (2–4, 7). Several bioinformatic approaches have been employed to identify ligands for oGPCRs. A first approach to narrow possible ligands for oGPCRs is to match oGPCRs with GPCRs with known ligands using sequence homology. BLAST, or more sophisticated and accurate programs designed to decipher relationships between family members based on consensus domain profiling and hidden Markov models, have been used to identify closely related receptors and have even been successful at providing hints to the identity of ligands for some receptors (17, 18). The bioinformatic analysis of receptor sequences has recently revealed the potential to predict G protein associations, which if applied to oGPCRs may help to identify the best experimental platform (see below) for their deorphanization (19). As well as aiding to deorphanize receptors, computational genomics approaches are likely to link receptor dysfunction with disease and provide insight into population variances in therapeutic responsiveness by identifying genetic mutations and polymorphisms (20). In addition to receptor homology, creating a data base of tissue expression profiles for GPCRs, their signaling components, and potential ligand transcripts is one means of determining potential oGPCR ligands and functions or a means to bestow insights into which GPCRs (*e.g.* $GABA_{B(1a)}$ and $GABA_{B\ (2)}$ receptors heterodimerize to form a functional receptor) dimerize *in vivo* (Fig. 2S, *A*) (reviewed in Ref. 21).

Computational Approaches—Molecular modeling is another *in silico* method that has classically been used to study receptor structure-function and, more recently, to virtually screen compound libraries for both deorphanization and drug discovery efforts (Fig. 2S, *B*). Most GPCR molecular models have been created by homology modeling with the crystal structure of inactive bovine rhodopsin (22, 23). These models have been successful in identifying known antagonists and agonists from seeded libraries (24). Furthermore, computational development of ligand pharmacophores to virtually screen drug libraries has successfully identified

lead compounds and assisted the subsequent development of useful analogs (25). As more validated molecular models accumulate, drug candidates may be screened *in silico* against a "virtual receptorome" to identify possible drug interactions.

Data bases, such as the NIMH-PDSP web site (pdsp.cwru.edu/pdsp.htm) that hosts a data base comprising over 29,300 K_i values compiled from the literature and the NIMH-PDSP screening initiative, allow users to quickly identify commonalities among drug-receptor interactions. Such data bases may be used to find receptor-specific drugs, identify lead compounds, and elucidate the structural features of ligands.

Receptoromics: Physically Screening the Receptorome

Various approaches are, at least theoretically, available for physically screening the receptorome. Ideally, receptoromics profiling could be utilized both to identify the molecular targets for endogenous ligands and as a drug discovery tool. These approaches are described in the following sections.

Ligand Binding Screens—Competition ligand binding experiments between a test ligand and a receptor-specific high affinity radiolabeled ligand using either whole cell or cell membrane preparations in multiwell (96+) formats have been used to identify ligand-receptor interactions. A distinct advantage of high throughput binding assays is the ability to use frozen stocks of tissue or cellular membranes as opposed to live specimens (which are required for most functional assays). However, several major drawbacks to binding screens exist: 1) they primarily rely on radiolabeled ligands; 2) they do not distinguish between the functional properties of ligands (*i.e.* agonist, partial agonist, inverse agonist, and antagonist); 3) they are not readily suitable for identifying allosteric ligands that bind distinctly from the primary (orthosteric) binding site (26); and 4) they are not suited for deorphanizing receptors. To date, no commercial or public entities have the resources to completely screen the receptorome, although with the advent of the Molecular Libraries Initiative (nihroadmap.nih.gov/molecularlibraries/index.asp) as part of the NIH Roadmap Initiative, such a capacity may be available in the future. At present, in the public domain, the NIH/NIMH-PDSP has the single largest collection of receptors composed of receptorome superfamilies including GPCRs, transporters, and ligand-gated ion channels for which compounds may be physically screened. A unique property of the NIH/NIMH-PDSP is that many receptors are probed simultaneously against a given ligand (Fig. 3S).

Functional Screens—Functional screens based on receptor activation/signaling represent a complementary approach to empirically identifying endogenous and exogenous receptor ligands. In general, functional screening of GPCRs relies on receptor activation of G proteins to modulate effector activity and generate second messengers, which provoke signaling cascades eliciting a cellular response. As shown in Fig. 1, virtually every conceivable signal transduction pathway has been "hijacked" for HTS (96+ well format) functional screening.

Fluorescent measurements of Ca^{2+} release induced by IP_3 produced following activation of $G\alpha_{q/11}$-coupled receptors have become a staple of HTS (27). Thus, there have been many successful efforts to adopt non-$G\alpha_{q/11}$-coupled receptors and oGPCRs to universal signaling through the PLCβ/Ca^{2+} pathway (18, 27). To achieve this, investigators have taken advantage of "promiscuous" $G\alpha_{15}$ and $G\alpha_{16}$ proteins, which nonspecifically couple with a large number of GPCRs to activate PLCβ (28). Additionally, chimeric $G\alpha_{q/11}$ proteins, which either have their extreme C-terminal amino acids removed or exchanged with those of $G\alpha_s$ or $G\alpha_{i/o}$, have been engineered to allow pan-activation of PLCβ by $G\alpha_s$- or $G\alpha_{i/o}$-coupled receptors (18, 27, 28). However, to date no truly "universal coupling" system exists (28). Furthermore, these artificial G protein coupling systems may identify ligand potencies and efficacies that are non-physiological (12, 29). Another concern in developing HTS screens, especially when using heterologous expression systems, is whether a receptor is expressed on the cell surface in a particular cellular setting that may not contain the appropriate chaperone proteins (18, 30). With these concerns in mind, constitutively active GPCRs (*i.e.* mutated receptors that are active in the absence of ligand) have been used to establish which G proteins, cell system, or cellular milieu is acceptable for screening a given receptor (18, 31–34).

A number of heterologous expression systems have also been used to monitor ligand-dependent GPCR activation. GPCR-mediated pheromone signaling in the budding yeast, *Saccharomyces cerevisiae*, has been genetically manipulated to allow activated mammalian GPCRs, heterologously expressed in these yeast, to stimulate a MAPK pathway leading to transcriptional activation of pheromone-responsive promoters to drive reporter gene expression (35). As most mammalian cellular systems express a variety of endogenous GPCRs, yeast offer the unique advantage of a "clean" expression system. *Xenopus laevis* melanophores offer another heterologous expression system to screen for mammalian GPCR activation (18, 31, 34). Melanosomes, organelles containing dark melanin pigment, found within melanophores will aggregate upon inhibition of AC activity or disperse upon stimulation of PLCβ and AC resulting in cell lightening or darkening, respectively.

Validation of Molecular Targets—Assignment of the physiological roles of GPCRs lags behind that of receptor deorphanization. To designate likely physiological roles with oGPCRs, investigators have determined expression profiles (Fig. 2S, *A*) as well as generated transgenic animals to either knock-out the receptor or knock-in a constitutively active one at the native gene locus (31, 36). However, as a number of constitutively activated GPCRs are prone to induce cellular hyperplasia, unexpected phenotypes may arise simply because of cellular transformation *in vivo* (13, 37). Moreover, although some receptors will have similar pharmacology and phenotypes between mice and humans, others frequently differ, which may foster false assumptions about the role of specific receptors in human disease (38). Therefore, studies in lower organisms should be cautiously interpreted.

Another emerging tool to decipher receptor physiology *in vivo* is receptors activated solely by synthetic ligands (RASSLs) (39). These are receptors engineered to be non-responsive to their endogenous ligands and, instead, are selectively activated by foreign small molecules. In combination with advanced transgenic techniques (*e.g.* temporal and tissue-specific expression) these receptors will be convenient molecular "switches" to turn on and off cellular signaling. Additionally, ligand-activated chimeric receptors consisting of the extracellular side (ligand binding) of one receptor and intracellular side (G protein coupling) of another have been made from divergent receptors, suggesting that these chimeras may be used to deorphanize oGPCRs or as RASSLs (40, 41).

Recent Receptorome-based Discoveries

Fen-Phen and the Valvulopathic Receptor—The anorectic drug fenfluramine, found in the dietary supplement "fen-phen," was found to increase the risk of developing potentially fatal conditions of pulmonary hypertension and valvular heart disease in individuals prescribed these medications to treat obesity (42). The discoveries that 5-HT_{2B} receptors were 1) abundantly expressed in heart valves 2) activated by fenfluramine and its metabolite, norfenfluramine, and 3) activated by other valvulopathic drugs suggested that 5-HT_{2B} receptors were involved in valvulopathy etiology (43, 44). Subsequently several other 5-HT_{2B} agonists were also found to be valvulopathogenic (Fig. 3S) (45). As 5-HT_{2B} agonists are therapeutically liable, we have suggested all pharmaceuticals should be screened for activity at 5-HT_{2B} receptors prior to further commercial development (44, 45).

The "Magic Mint" Hallucinogen—The benefit of broad receptorome screening can be appreciated in the case of identification of the molecular target of salvinorin A, a naturally occurring and potent hallucinogen from the sage *Salvia divinorum* (46). Interestingly, salvinorin A, a non-nitrogenous diterpenoid, is structurally distinct from other hallucinogens (46). When salvinorin A was profiled, it was found to have high affinity and be a potent agonist for the κ opioid receptor (KOR) (46). This interaction was specific for KOR, as salvinorin A did not have appreciable affinity for either μ or δ opioid receptors, and unlike lysergic acid diethylamide (LSD), salvinorin A has no affinity for 5-HT_{2A} receptors, which are classically associated with hallucinations and psychosis (46). This finding suggests that the disease states associated with alterations in perception (*e.g.* schizophrenia, Alzheimer disease, etc.) could potentially be treated with KOR-specific antagonists or modification of traditional antipsychotics to include inhibition of KOR (46).

The Human Polyomavirus, JCV, Co-receptor—A recent study by

Elphick *et al.* (47) investigating the inhibition of JCV infection by antipsychotics highlights the importance of pharmacological profiling in discovering roles of receptors in diseases. Infection of oligodendrocytes by the human polyomavirus, JCV, results in neuronal demyelenation, which is responsible for the fatal disease of progressive multifocal leukoencephalopathy (PML). Although persistent JCV infection is common in the general population, spread of the infection to the central nervous system, leading to PML, occurs primarily in immunosuppressed AIDS or cancer patients. It was appreciated that the α2,6-linked sialic acid glycoprotein is a component of the JCV receptor; however, the recent identification that antipsychotics, such as clozapine, inhibit JCV infection suggested that one of the several receptors having an affinity for such drugs may be a JCV co-receptor. In the initial attempt to decipher which of these receptors is the JCV co-receptor, the investigators used a combination of glial cell receptor expression profiling as well as receptor-specific small molecules and antibodies. These and further experiments pointed to a subclass of serotonin receptors, which help to internalize JCV bound to the α2,6-linked sialic acid protein. Importantly, by identifying novel receptors involved in the infection of JCV this study may offer the first available preventive treatment for PML (47).

Conclusion

The wealth of sequence information compiled from the human genome project has allowed for the identification of most components of the receptorome. The challenge, as for most proteome superfamilies, is to use this information as a launching point to better understand the physiological roles of each receptor. Broad, unbiased receptorome screens, such as those described above that simultaneously identify ligand interactions for a panel of receptors, have led to the discovery of novel drug-receptor interactions. Deciphering these interactions will identify additional therapeutic targets and receptors responsible for the side effects of currently marketed drugs. Finally, receptorome screening frequently refutes the notion that the "one drug-one receptor" approach is appropriate for designing drugs for complex diseases (5).

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REFERENCES

1. Stoddard, B. L., Biemann, H. P., and Koshland, D. E., Jr. (1992) *Cold Spring Harbor Symp. Quant. Biol.* **57,** 1–15
2. Venter, J. C. *et al*. (2001) *Science* **291,** 1304–1351
3. Kroeze, W. K., Sheffler, D. J., and Roth, B. L. (2003) *J. Cell Sci.* **116,** 4867–4869
4. Hopkins, A. L., and Groom, C. R. (2002) *Nat. Rev. Drug Discov.* **1,** 727–730
5. Roth, B. L., Sheffler, D. J., and Kroeze, W. K. (2004) *Nat. Rev. Drug Discov.* **3,** 353–359
6. Lander, E. S. *et al*. (2001) *Nature* **409,** 860–921
7. Fredriksson, R., Lagerstrom, M. C., Lundin, L. G., and Schioth, H. B. (2003) *Mol. Pharmacol.* **63,** 1256–1272
8. Kristiansen, K. (2004) *Pharmacol. Ther.* **103,** 21–80
9. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000) *Science* **289,** 739–745
10. Cabrera-Vera, T. M., Vanhauwe, J., Thomas, T. O., Medkova, M., Preininger, A., Mazzoni, M. R., and Hamm, H. E. (2003) *Endocr. Rev.* **24,** 765–781
11. Albert, P. R., and Robillard, L. (2002) *Cell Signalling* **14,** 407–418
12. Wong, S. K. (2003) *Neurosignals* **12,** 1–12
13. Marinissen, M. J., and Gutkind, J. S. (2001) *Trends Pharmacol. Sci.* **22,** 368–376
14. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) *Nat. Rev. Mol. Cell. Biol.* **3,** 639–650
15. Hall, R. A., and Lefkowitz, R. J. (2002) *Circ. Res.* **91,** 672–680
16. Bhatnagar, A., Sheffler, D. J., Kroeze, W. K., Compton-Toth, B., and Roth, B. L. (2004) *J. Biol. Chem.* **279,** 34614–34623
17. Gaulton, A., and Attwood, T. K. (2003) *Curr. Opin. Pharmacol.* **3,** 114–120
18. Wise, A., Jupe, S. C., and Rees, S. (2004) *Annu. Rev. Pharmacol. Toxicol.* **44,** 43–66
19. Sreekumar, K. R., Huang, Y., Pausch, M. H., and Gulukota, K. (2004) *Bioinformatics* **20,** 3490–3499
20. Small, K. M., McGraw, D. W., and Liggett, S. B. (2003) *Annu. Rev. Pharmacol. Toxicol.* **43,** 381–411
21. George, S. R., O'Dowd, B. F., and Lee, S. P. (2002) *Nat. Rev. Drug Discov.* **1,** 808–820
22. Ballesteros, J. A., Shi, L., and Javitch, J. A. (2001) *Mol. Pharmacol.* **60,** 1–19
23. Shapiro, D. A., Kristiansen, K., Weiner, D. M., Kroeze, W. K., and Roth, B. L. (2002) *J. Biol. Chem.* **277,** 11441–11449
24. Schapira, M., Raaka, B. M., Das, S., Fan, L., Totrov, M., Zhou, Z., Wilson, S. R., Abagyan, R., and Samuels, H. H. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 7354–7359
25. Klabunde, T., and Hessler, G. (2002) *ChemBioChem* **3,** 928–944
26. Christopoulos, A., and Kenakin, T. (2002) *Pharmacol. Rev.* **54,** 323–374
27. Coward, P., Chan, S. D., Wada, H. G., Humphries, G. M., and Conklin, B. R. (1999) *Anal. Biochem.* **270,** 242–248
28. Kostenis, E. (2001) *Trends Pharmacol. Sci.* **22,** 560–564
29. Hermans, E. (2003) *Pharmacol. Ther.* **99,** 25–44
30. Morfis, M., Christopoulos, A., and Sexton, P. M. (2003) *Trends Pharmacol. Sci.* **24,** 596–601
31. Menzaghi, F., Behan, D. P., and Chalmers, D. T. (2002) *Curr. Drug Target CNS Neurol. Disord.* **1,** 105–121
32. Milligan, G. (2002) *Biochem. Soc. Trans.* **30,** 789–793
33. Rauser, L., Savage, J. E., Meltzer, H. Y., and Roth, B. L. (2001) *J. Pharmacol. Exp. Ther.* **299,** 83–89
34. Chalmers, D. T., and Behan, D. P. (2002) *Nat. Rev. Drug Discov.* **1,** 599–608
35. Pausch, M. H. (1997) *Trends Biotechnol.* **15,** 487–494
36. Zuscik, M. J., Sands, S., Ross, S. A., Waugh, D. J., Gaivin, R. J., Morilak, D., and Perez, D. M. (2000) *Nat. Med.* **6,** 1388–1394
37. Julius, D., Livelli, T. J., Jessell, T. M., and Axel, R. (1989) *Science* **244,** 1057–1062
38. Setola, V., and Roth, B. L. (2003) *Mol. Pharmacol.* **64,** 1277–1278
39. Scearce-Levie, K., Coward, P., Redfern, C. H., and Conklin, B. R. (2001) *Trends Pharmacol. Sci.* **22,** 414–420
40. Gearing, K. L., Barnes, A., Barnett, J., Brown, A., Cousens, D., Dowell, S., Green, A., Patel, K., Thomas, P., Volpe, F., and Marshall, F. (2003) *Protein Eng.* **16,** 365–372
41. Yin, D., Gavi, S., Wang, H. Y., and Malbon, C. C. (2004) *Mol. Pharmacol.* **65,** 1323–1332
42. Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D., and Schaff, H. V. (1997) *N. Engl. J. Med.* **337,** 581–588
43. Fitzgerald, L. W., Burn, T. C., Brown, B. S., Patterson, J. P., Corjay, M. H., Valentine, P. A., Sun, J. H., Link, J. R., Abbaszade, I., Hollis, J. M., Largent, B. L., Hartig, P. R., Hollis, G. F., Meunier, P. C., Robichaud, A. J., and Robertson, D. W. (2000) *Mol. Pharmacol.* **57,** 75–81
44. Rothman, R. B., Baumann, M. H., Savage, J. E., Rauser, L., McBride, A., Hufeisen, S. J., and Roth, B. L. (2000) *Circulation* **102,** 2836–2841
45. Setola, V., Hufeisen, S. J., Grande-Allen, K. J., Vesely, I., Glennon, R. A., Blough, B., Rothman, R. B., and Roth, B. L. (2003) *Mol. Pharmacol.* **63,** 1223–1229
46. Roth, B. L., Baner, K., Westkaemper, R., Siebert, D., Rice, K. C., Steinberg, S., Ernsberger, P., and Rothman, R. B. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 11934–11939
47. Elphick, G. F., Querbes, W., Jordan, J. A., Gee, G. V., Eash, S., Manley, K., Dugan, A., Stanifer, M., Bhatnagar, A., Kroeze, W. K., Roth, B. L., and Atwood, W. J. (2004) *Science* **306,** 1380–1383
48. Ferguson, S. S., and Caron, M. G. (2004) *Methods Mol. Biol.* **237,** 121–126
49. Parnot, C., Miserey-Lenkei, S., Bardin, S., Corvol, P., and Clauser, E. (2002) *Trends Endocrinol. Metab.* **13,** 336–343
50. Weiner, D. M., Burstein, E. S., Nash, N., Croston, G. E., Currier, E. A., Vanover, K. E., Harvey, S. C., Donohue, E., Hansen, H. C., Andersson, C. M., Spalding, T. A., Gibson, D. F., Krebs-Thomson, K., Powell, S. B., Geyer, M. A., Hacksell, U., and Brann, M. R. (2001) *J. Pharmacol. Exp. Ther.* **299,** 268–276
51. Burstein, E. S., Spalding, T. A., Hill-Eubanks, D., and Brann, M. R. (1995) J. *Biol. Chem.* **270,** 3141–3146
52. Janetopoulos, C., Jin, T., and Devreotes, P. (2001) *Science* **291,** 2408–2411

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