

MOL49015

Rich tapestry of GPCR signaling and regulatory mechanisms

Vsevolod V. Gurevich and Eugenia V. Gurevich

Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN

37232

MOL49015

Running title: Diverse mechanisms of GPCR regulation

Corresponding author: Vsevolod V. Gurevich,

Vanderbilt University, Department of Pharmacology, PRB 418, Nashville, TN 37232

e-mail Vsevolod.Gurevich@vanderbilt.edu.

MOL49015

Abstract

G protein-coupled receptors (GPCRs) are the largest family of signaling proteins and the most common therapeutic targets. In the last two decades, an impressive progress in the understanding of GPCR function has been achieved, largely driven by the idea of similarity of the molecular mechanisms underlying their signaling and regulation. However, recent comprehensive studies of signaling and trafficking of several GPCR subtypes, including endogenous M3 muscarinic and H1 histamine receptor and expressed cysteinyl leukotriene type 1 receptor in HEK293 cells, clearly demonstrate that each receptor is regulated by a unique set of molecular mechanisms involving different players. These data indicate that the “gold mine” of similarities is nearly exhausted, and that uncritical extrapolation from one receptor to another is more likely to be misleading than illuminating. Further progress in the field requires careful analysis of the regulation of individual GPCR subtypes in defined cellular context.

MOL49015

Striking similarity between the signaling pathways that translate light captured by rhodopsin into the cGMP phosphodiesterase activity in photoreceptors and those converting hormonal activation of β -adrenergic receptor (b2AR) into the adenylyl cyclase activity in other cells was noted in mid-eighties (Bitensky et al., 1984). However, the GPCR field as we know it was born after seminal elucidation of the b2AR structure, which clearly showed that rhodopsin and b2AR belong to the same protein family (Dixon et al., 1986). This discovery suggested that the mechanisms regulating rhodopsin activity, such as phosphorylation and arrestin binding, likely operate in the b2AR-driven signaling pathway. The idea proved remarkably fruitful: the first functional analog of rhodopsin kinase, β -adrenergic receptor kinase (β ARK; now known as G protein-coupled receptor kinase 2, or GRK2) was cloned the same year (Benovic et al., 1986). Subsequent elegant experiments demonstrated that phosphorylation alone does not fully account for b2AR desensitization, suggesting the role for an arrestin analog that binds non-visual GPCRs (Benovic et al., 1987), which was soon discovered and termed β -arrestin (now known as arrestin2 or β -arrestin1) (Lohse et al., 1990). The cloning of additional GPCRs (Frielle et al., 1987; Kobilka et al., 1987), GRKs (Benovic et al., 1989; Benovic and Gomez, 1993; Kunapuli and Benovic, 1993), and arrestins (Attramadal et al., 1992; Sterne-Marr et al., 1993) added further proof of sequence similarity between these proteins and their respective visual counterparts (Shinohara et al., 1987; Lorenz et al., 1991), reinforcing the view that most, if not all, GPCRs signal similarly and are controlled by the same regulatory mechanisms. So, when receptor-bound arrestins were found to act as adaptors linking the receptor to the components of the internalization machinery of the coated pit,

MOL49015

clathrin (Goodman et al., 1996) and AP2 (Laporte et al., 1999), to mobilize and activate c-Src (Luttrell et al., 1999), and to scaffold kinase cascades activating JNK3 (McDonald et al., 2000) and ERK1/2 (Luttrell et al., 2001), it was implicitly assumed that these findings apply to pretty much all GPCRs. The data indicating that this is not necessarily the case were usually swept aside as inconsequential details. Largely due to this tradition of uncritical generalization, beautiful demonstration of the dimeric nature of a small group of class C GPCRs (reviewed in (Pin et al., 2003)), along with bits and pieces of evidence for dimerization of a few class A receptors under certain circumstances, was interpreted by some as proof positive that all GPCRs exist as dimers, and that receptor dimer is necessary to interact with G proteins, arrestins, and other binding partners (e.g., see (Fotiadis et al., 2006)). Although rigorous experimental testing quickly blew large holes in this model (James et al., 2006; Bayburt et al., 2007; Hanson et al., 2007b; Whorton et al., 2007), reviewed in (Gurevich and Gurevich, 2008a; Gurevich and Gurevich, 2008b), in some ways its obvious overreach proved beneficial for the field, forcing us all to see the folly of sweeping generalizations and pay close attention to the particulars of the regulation of individual receptors in defined cellular context.

The paper by Luo et al in this issue (Luo et al., 2008) is an excellent example of this type of study. The authors comprehensively explored the signaling by M3 muscarinic acetylcholine receptor endogenously expressed in HEK293 cells by knocking down individual regulatory proteins. Luo and colleagues found that GRK2, GRK3, and GRK6 significantly contribute to desensitization of the M3 receptor, whereas GRK5 does not. In addition, knockdown of either arrestin2 or arrestin3 increased M3-stimulated calcium response, implicating both subtypes in M3 desensitization. The authors confirmed earlier

MOL49015

observations (Budd et al., 2000) that casein kinase-1 β (CKI β) also participates in the suppression of M3-mediated calcium signaling. It is worth noting that the list of kinases other than GRKs that phosphorylate GPCRs and directly regulate their activity is growing. We can expect its further expansion as more receptor subtypes are carefully studied. The role of CKI β in M3 receptor signaling is similar to the recently described the key role of PKC phosphorylation in cysteinyl leukotriene type 1 receptor (CL1R) desensitization (Naik et al., 2005). However, the discovery of the crucial role of PKC in CL1R endocytosis (Naik et al., 2005), which was often thought to be mediated by GRK phosphorylation of the receptor and subsequent arrestin binding, reveals an additional rather unexpected role that phosphorylation of GPCRs by a variety of kinases might play. Interestingly, PKC-induced CL1R endocytosis is arrestin-independent, although CL1R can also internalize in arrestin-dependent manner (Naik et al., 2005). Although this aspect of GPCR trafficking is often overlooked, CL1R is not the only receptor that internalizes via more than one pathway: this phenomenon was reported with M2 muscarinic acetylcholine receptor (Pals-Rylaarsdam et al., 1997) and several other GPCR subtypes (reviewed in (Gurevich and Gurevich, 2006b)). To summarize the study of Luo and colleagues, a large number of regulatory proteins, including three different GRKs, CKI β , and two non-visual arrestins, are required for the normal attenuation of calcium response to endogenous M3 receptor activation in HEK293 cells.

An important point highlighted in this work and several previous studies by the same group is the multi-functionality of GRKs and arrestins. Virtually every protein has multiple functions, all of which are indiscriminately suppressed by its knockdown. Even when a certain GRK or arrestin subtype is identified as a player in the regulation of a

MOL49015

particular receptor, without direct evidence we cannot assume that the GRK in question acts via receptor phosphorylation, or that arrestin affects signaling via its binding to active GRK-phosphorylated receptor. GRKs carry a regulator of G protein signaling (RGS) domain on their N-terminus that binds active GTP-liganded β -subunits of Gq/11 (Carman et al., 1999). Thus, GRKs can inhibit the signaling of Gq/11-coupled receptors, such as M3, via at least two distinct mechanisms: by phosphorylating the receptor to promote arrestin-mediated uncoupling from cognate G proteins (Gurevich and Gurevich, 2004), and by sequestering activated β -subunits of Gq and G11 (Carman et al., 1999). To elucidate the actual mechanism of GRK2-mediated inhibition of M3 receptor signaling, the authors used precisely targeted tools: GRK2 mutants devoid of either kinase activity or the ability to bind Gq/11 β -subunits. Since kinase-dead GRK2-K220R turned out to be as effective as wild type GRK2, whereas both mutants defective in G β q binding had no effect on M3 receptor signaling, the data clearly demonstrate that GRK2 largely mediates M3 desensitization via sequestration of G β q/11 (Luo et al., 2008). In addition to sequestering and silencing Gq/11 (Carman et al., 1999; Iwata et al., 2005; Luo et al., 2008), GRK2 binds G $\beta\gamma$ dimers (Pitcher et al., 1992), blunts ERK1/2 activation by binding MEK1 (Jiménez-Sainz et al., 2006), and phosphorylates quite a few non-GPCR substrates, such as tubulin (Carman et al., 1998) and ezrin (Cant and Pitcher, 2005). In addition, GRKs 2 and 5 phosphorylate several isoforms of synuclein (Pronin et al., 2000). GRKs can phosphorylate these proteins independently of receptor activation in vitro. However, GRK interactions with phospholipids and G $\beta\gamma$ which are promoted by GRK recruitment to active GPCRs in the cell, enhance phosphorylation of non-receptor substrates. In most cases, we do not know whether phosphorylation of these substrates is

MOL49015

actually affected by receptor activation. A well-described example of such a link is the phosphorylation of ezrin by GRK2, which is a necessary step in the receptor activation-dependent reorganization of the actin cytoskeleton (Cant and Pitcher, 2005).

In terms of known multi-functionality, arrestins are way ahead of GRKs, interacting with an incredible variety of signaling proteins (Gurevich and Gurevich, 2006a; Xiao et al., 2007). Multiple partners preferentially interact with receptor-bound arrestins (Gurevich and Gurevich, 2003; Lefkowitz and Shenoy, 2005), some (e.g., microtubules (Hanson et al., 2007a) and calmodulin (Wu et al., 2006)) exclusively bind free arrestins because their interaction sites overlap with that of the receptor (Vishnivetskiy et al., 2004; Hanson et al., 2006), whereas others simply prefer arrestin in its free “inactive” conformation (Song et al., 2006). Bound arrestin not only covers the cytoplasmic tip of the receptor, “crowding out” G proteins (Krupnick et al., 1997), but often initiates the second round of signaling (Gurevich and Gurevich, 2003; Lefkowitz and Shenoy, 2005), serving as a scaffold for MAP kinase cascades (McDonald et al., 2000; Luttrell et al., 2001). GPCR activation can be translated into ERK1/2 phosphorylation via distinct mechanisms mediated by G proteins or arrestins. After essentially the same story emerged from studies with angiotensin II (Ahn et al., 2004), β_2 -adrenergic (Shenoy et al., 2006) and parathyroid hormone (Gesty-Palmer et al., 2006) receptors, it was accepted as a general rule that G-protein-mediated ERK phosphorylation is very transient, whereas arrestins mediate sustained ERK activation. However, the study of the M3 receptor challenges this “rule”: Luo et al (Luo et al., 2008) demonstrated that non-visual arrestins are key players in M3 receptor desensitization, but detected no arrestin-dependent ERK activation via endogenous M3 receptor. Interestingly, in this case G protein-mediated

MOL49015

ERK phosphorylation was sustained for up to 60 min, and was further enhanced and prolonged by knockdown of GRK2 and arrestins. Obviously, interactions of GRKs and arrestins with multiple non-GPCR partners can significantly affect receptor signaling. As beautifully illustrated by the work of Luo et al (Luo et al., 2008), the actions of GRK2 in HEK293 cells on both the M3 receptor-stimulated calcium mobilization and ERK1/2 activation are mediated by interactions with non-receptor partners, and neither involves receptor phosphorylation. It remains to be elucidated to what extent the fact that this study was performed with endogenous M3 receptor expressed at physiological low level contributed to the observed differences with previous studies where the receptors were overexpressed. It is also unclear how much of the reported similarities of ERK1/2 activation induced by aniotensin II, b2-adrenergic, and parathyroid hormone receptors were brought about by overexpression of these receptors.

M3 is the third receptor comprehensively studied by Dr. Benovic and colleagues in HEK293 cells (Iwata et al., 2005; Naik et al., 2005; Luo et al., 2008). It is important to note that the only unifying conclusion of these studies is that each GPCR subtype has a unique pattern of regulation. M3 receptor is desensitized via phosphorylation by GRKs 3, 6, and CKIa (Luo et al., 2008) and GRK2-mediated sequestration of G α q; histamine H1 receptor is primarily desensitized by GRK2 via both phosphorylation and G α q binding (Iwata et al., 2005); the internalization of both M3 and H1 receptors is mediated by arrestins, whereas desensitization as well as arrestin-independent internalization of CL1R requires receptor phosphorylation by PKC (Naik et al., 2005). Remarkably, GRK5, which is one of the two most abundant GRKs in HEK293 cells, does not appear to participate in the regulation of any of these three receptors. These data bring up an important question

MOL49015

of receptor specificity of GRK isoforms, which is usually considered only in terms of preferential phosphorylation of certain GPCR subtypes by a particular GRK. However, because GRKs have functional capabilities that do not involve the kinase activity, this issue is much more complex. For example, RGS domain of GRKs 2 and 3 sequesters GTP-liganded G α q/11, but not β -subunits of other G proteins. Therefore, this mechanism of GRK2/3 action is specific for Gq/11-dependent signaling pathways. The same two GRKs bind $\beta\gamma$ -dimers suppressing $\beta\gamma$ -mediated signal transduction often important in signaling mediated by a different group of G proteins, Gi/o subfamily. In addition, the ability of GRK2 to inhibit ERK1/2 activation by MEK1 makes this mechanism specific for the pathways that involve the MEK1-ERK1/2 module. In most studies, in contrast to the work of Luo et al (Luo et al., 2008), the actual mechanism of GRK-dependent suppression of receptor signaling was not determined.

In mammals, four ubiquitously expressed GRKs (GRKs 2,3,5, and 6), as well as the more restricted GRK4, are available to regulate more than 700 GPCR subtypes. Obviously, 1:1 specificity for receptors is out of the question. However, it does not mean that GRK isoforms are simply redundant, e.g., can regulate any GPCR in the same manner. *In vitro* experiments using GRK overexpression often show that many GRK isoforms are able to promote desensitization and trafficking of various GPCRs. However, *in vivo* studies with knockout and transgenic mice provided evidence for unexpectedly strict receptor specificity of different GRKs. For example, the elimination of GRK6 causes behavioral supersensitivity to dopaminergic stimulation (Gainetdinov et al., 2003), whereas knockout of its closest relative GRK5 does not alter dopaminergic signaling (Gainetdinov et al., 1999). Instead, the loss of GRK5 enhances central responses to

MOL49015

muscarinic stimulation (hypothermia, tremor, salivation, and locomotion) without affecting responsiveness to the μ -opioid or 5-HT_{1A} receptor stimulation (Gainetdinov et al., 1999). Mice lacking GRK3, 5, or 6 have relatively mild phenotypes, indicating that to a certain extent the remaining GRKs can take over the functions of the missing isoform. However, the fact that knockout of GRK2 is embryonic lethal (Jaber et al., 1996) proves that it has functions that cannot be performed by any other member of the family. At the same time, knockout of GRK3, the isoform remarkably similar to GRK2 structurally and biochemically (Arriza et al., 1992; Willets et al., 2003), produces only very mild neuronal phenotype (Gainetdinov et al., 2004). In some cases, apparent receptor specificity is based on specific cellular complement of GRK isoforms. For example, the loss of the odorant receptor desensitization in GRK3 knockout mice (Peppel et al., 1997) is due to the fact that GRK3 is the major, if not the only, isoform expressed in the olfactory epithelium. Nonetheless, even when multiple GRKs are expressed in the same cell, they often only regulate specific receptors and/or regulate the same GPCR via distinct non-overlapping mechanisms. For example, GRKs 2 and 3 are expressed in cardiac myocytes at similar levels, but GRK2 is primarily responsible for regulation of the β -adrenergic and angiotensin receptors (Vinge et al., 2007). In contrast, GRK3 does not seem to regulate β -adrenergic signaling, but appears to control β ₁-adrenergic and endothelin receptors in these cells (Eckhart et al., 2000; Vinge et al., 2007). This receptor specificity of GRKs 2 and 3 defines the biological role of each isoform in different aspects of heart function: GRK2 is the key player in the heart development and heart failure (Jaber et al., 1996; Hansen et al., 2006), whereas GRK3 is important for the control of the cardiac growth and hypertrophy (Vinge et al., 2007; Vinge et al., 2008). The work by Dr. Benovic's

MOL49015

group lends further support for the idea that receptors are preferentially regulated by specific GRK isoforms. Moreover, when multiple GRKs regulate signaling by the same receptor, functional consequences differ depending on the isoform involved. Luo et al (Luo et al., 2008) found that knockdown of GRK2, 3, or 6 similarly enhances calcium mobilization via M3 receptor, whereas ERK activation by the same receptor was not affected by the GRK3 knockdown. It is remarkable that even though GRK2 and GRK5 are the two major isoforms in HEK293 cells, one regulates M3 muscarinic receptor without actually phosphorylating it, whereas the other does not affect it at all. These findings clearly show that when GRKs are expressed at physiological levels, different isoforms demonstrate pronounced receptor specificity. It is also clear that virtually every cell expresses multiple GRKs and many GPCRs. Therefore, the mere fact of coexpression does not mean that specific GRK isoform is in any way involved in the regulation of a particular receptor.

The situation with arrestins is even more intriguing, since there are only two ubiquitous isoforms, arrestin2 and 3, each represented by two splice variants (Sterne-Marr et al., 1993). In some tissues, particularly in the brain, the concentration of arrestin2 is many times higher than that of arrestin3, and this difference becomes more dramatic during development (Gurevich et al., 2002; Gurevich et al., 2004; Ahmed et al., 2008a; Ahmed et al., 2008b). The knockout of arrestin2 causes slightly increased responsiveness to α -adrenergic stimulation in the heart (Conner et al., 1997) and no enhanced behavioral responses to dopaminergic or μ -opioid drugs (Gainetdinov et al., 2004). In contrast, the ablation of arrestin3 elevates antinociceptive and rewarding effects of morphine, reduces tolerance to morphine, and increases μ -opioid receptor coupling to G proteins (Bohn et

MOL49015

al., 1999; Bohn et al., 2000; Bohn et al., 2003). In the study of Lou et al (Luo et al., 2008), knockdown of either arrestin enhanced carbachol-induced calcium mobilization and ERK phosphorylation, although only the knockdown of arrestin3 resulted in prolonged ERK activation. Thus, the two non-visual arrestins are certainly non-redundant. Strong evidence of receptor and functional specificity of different arrestins and GRKs *in vitro* and *in vivo* is rapidly accumulating. It is becoming increasingly clear that, in addition to the nature of the receptor, many other factors contribute to the functional performance of individual arrestins and GRKs. Relative intracellular concentrations and the complement arrestin and GRK isoforms in the cell, the subcellular distribution of the receptor and particular arrestins and GRKs, as well as the expression levels of other signaling proteins all play a role. The precise experimental elucidation of the functional repertoire of each GRK and arrestin will significantly contribute to our ability to unravel the exceedingly complex cellular signaling network.

In the last two decades, the key molecular mechanisms of GPCR signaling and its regulation have been elucidated. In the process, we learned the hard way that there is no such thing as a generic receptor, generic GRK, or a generic cell. The evolution endowed mammals with ~1,000 different GPCRs (Rompler et al., 2007) that are phosphorylated by seven GRKs (Moore et al., 2007) and a number of other kinases (Budd et al., 2000; Naik et al., 2005; Luo et al., 2008) and interact with four arrestin subtypes (Gurevich and Gurevich, 2006b). Each tissue and cell has a unique complement of receptors (Penn et al., 2001), GRKs and arrestins (Penn et al., 2001; Gurevich et al., 2002; Ahmed et al., 2008a; Ahmed et al., 2008b; Bychkov et al., 2008) that changes, sometimes quite dramatically, during development (Gurevich et al., 2002; Gurevich et al., 2004), disease (Ahmed et al.,

MOL49015

2008a; Bychkov et al., 2008), and drug treatment (Ahmed et al., 2008b). To make matters even more complicated, phosphorylation of the same receptor at different sites (Pals-Rylaarsdam et al., 1997; Lee et al., 2000; Key et al., 2003; Jones and Hinkle, 2008), by different GRKs (Kim et al., 2005; Ren et al., 2005; Luo et al., 2008), or even by the same GRK to different levels (Vishnivetskiy et al., 2007) generates functionally distinct receptor species that bind arrestins with different biological consequences. Apparently, thousands of distinct patterns of signaling and regulation generated by this variety are necessary for survival. We have a huge task of elucidating these patterns for each receptor in every cell type where it is endogenously expressed to understand the biological significance of each thread in this incredibly rich tapestry of signaling regulation.

References

- Ahmed MR, Bychkov E, Gurevich VV, Benovic JL and Gurevich EV (2008a) Altered expression and subcellular distribution of GRK subtypes in the dopamine-depleted rat basal ganglia is not normalized by l-DOPA treatment. *J Neurochem* **104**:1622-1636.
- Ahmed MR, Gurevich VV, Dalby KN, Benovic JL and Gurevich EV (2008b) Haloperidol and clozapine differentially affect the expression of arrestins, receptor kinases, and extracellular signal-regulated kinase activation. *J Pharmacol Exp Ther* **325**:276-283.
- Ahn S, Shenoy SK, Wei H and Lefkowitz RJ (2004) Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *J Biol Chem* **279**:35518-35525.
- Arriza JL, Dawson TM, Simerly RB, Martin LJ, Caron MG, Snyder SH and Lefkowitz RJ (1992) The G-protein-coupled receptor kinases bARK1 and bARK2 are widely distributed at synapses in rat brain. *J Neurosci* **12**:4045-4055.
- Attramadal H, Arriza JL, Aoki C, Dawson TM, Codina J, Kwatra MM, Snyder SH, Caron MG and Lefkowitz RJ (1992) Beta-arrestin2, a novel member of the arrestin/beta-arrestin gene family. *J Biol Chem* **267**:17882-17890.
- Bayburt TH, Leitz AJ, Xie G, Oprian DD and Sligar SG (2007) Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. *J Biol Chem* **282**:14875-14881.

MOL49015

- Benovic JL, DeBlasi A, Stone WC, Caron MG and Lefkowitz RJ (1989) Beta-adrenergic receptor kinase: primary structure delineates a multigene family. *Science* **246**:235-240.
- Benovic JL and Gomez J (1993) Molecular cloning and expression of GRK6. A new member of the G protein-coupled receptor kinase family. *J Biol Chem* **268**:19521-19527.
- Benovic JL, Kühn H, Weyand I, Codina J, Caron MG and Lefkowitz RJ (1987) Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proc Natl Acad Sci U S A* **84**:8879-8882.
- Benovic JL, Strasser RH, Caron MG and Lefkowitz RJ (1986) Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc Natl Acad Sci U S A* **83**:2797-2801.
- Bitensky MW, Yamazaki A, Wheeler MA, George JS and Rasenick MM (1984) The mechanism of activation of light-activated phosphodiesterase and evidence for homology with hormone-activated adenylate cyclase. *Adv Cyclic Nucleotide Protein Phosphorylation Res* **17**:227-237.
- Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ and Caron MG (2000) Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. *Nature* **408**:720-723.
- Bohn LM, Gainetdinov RR, Sotnikova TD, Medvedev IO, Lefkowitz RJ, Dykstra LA and Caron MG (2003) Enhanced rewarding properties of morphine, but not cocaine, in beta(arrestin)-2 knock-out mice. *J Neurosci* **23**:10265-10273.

MOL49015

- Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG and Lin FT (1999) Enhanced morphine analgesia in mice lacking beta-arrestin2. *Science* **286**:2495-2498.
- Budd DC, McDonald JE and Tobin AB (2000) Phosphorylation and regulation of a Gq/11-coupled receptor by casein kinase 1alpha. *J Biol Chem* **275**:19667-19675.
- Bychkov ER, Gurevich VV, Joyce JN, Benovic JL and Gurevich EV (2008) Arrestins and two receptor kinases are upregulated in Parkinson's disease with dementia. *Neurobiol Aging* **29**:379-396.
- Cant SH and Pitcher JA (2005) G protein-coupled receptor kinase 2-mediated phosphorylation of ezrin is required for G protein-coupled receptor-dependent reorganization of the actin cytoskeleton. *Mol Biol Cell* **16**:3088-3099.
- Carman CV, Parent JL, Day PW, Pronin AN, Sternweis PM, Wedegaertner PB, Gilman AG, Benovic JL and Kozasa T (1999) Selective regulation of Galpha(q/11) by an RGS domain in the G protein-coupled receptor kinase, GRK2. *J Biol Chem* **274**:34483-34492.
- Carman CV, Som T, Kim CM and Benovic JL (1998) Binding and phosphorylation of tubulin by G protein-coupled receptor kinases. *J Biol Chem* **273**:20308-20316.
- Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE and Seidman JG (1997) b-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to b-adrenergic stimulation. *Circ Res* **81**:1021-1026.
- Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ and Strader CD (1986) Cloning of the gene and cDNA for

MOL49015

- mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature* **321**:75-79.
- Eckhart AD, Duncan SJ, Penn RB, Benovic JL, Lefkowitz RJ and Koch WJ (2000)
Hybrid transgenic mice reveal in vivo specificity of G protein-coupled receptor kinases in the heart. *Circ Res* **86**:43-50.
- Fotiadis D, Jastrzebska B, Philippsen A, Muller DJ, Palczewski K and Engel A (2006)
Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr Opin Struct Biol* **16**:252-259.
- Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ and Kobilka BK (1987)
Cloning of the cDNA for the human beta 1-adrenergic receptor. *Proc Natl Acad Sci U S A* **84**:7920-7924.
- Gainetdinov RR, Bohn LM, Sotnikova TD, Cyr M, Laakso A, Macrae AD, Torres GE, Kim KM, Lefkowitz RJ, Caron MG and Premont RT (2003) Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. *Neuron* **38**:291-303.
- Gainetdinov RR, Bohn LM, Walker JK, Laporte SA, Macrae AD, Caron MG, Lefkowitz RJ and Premont RT (1999) Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. *Neuron* **24**:1029-1036.
- Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ and Caron MG (2004)
Desensitization of G protein-coupled receptors and neuronal function. *Annual Review of Neuroscience* **27**(1):107-144.

MOL49015

- Gesty-Palmer D, Chen M, Reiter E, Ahn S, Nelson CD, Wang S, Eckhardt AE, Cowan CL, Spurney RF, Luttrell LM and Lefkowitz RJ (2006) Distinct beta-arrestin- and G protein-dependent pathways for parathyroid hormone receptor-stimulated ERK1/2 activation. *J Biol Chem* **281**:10856-10864.
- Goodman OB, Jr., Krupnick JG, Santini F, Gurevich VV, Penn RB, Gagnon AW, Keen JH and Benovic JL (1996) Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature* **383**:447-450.
- Gurevich EV, Benovic JL and Gurevich VV (2002) Arrestin2 and arrestin3 are differentially expressed in the rat brain during postnatal development. *Neuroscience* **109**:421-436.
- Gurevich EV, Benovic JL and Gurevich VV (2004) Arrestin2 expression selectively increases during neural differentiation. *J Neurochem* **91**:1404-1416.
- Gurevich EV and Gurevich VV (2006a) Arrestins are ubiquitous regulators of cellular signaling pathways. *Genome Biol* **7**:236.
- Gurevich VV and Gurevich EV (2003) The new face of active receptor bound arrestin attracts new partners. *Structure* **11**:1037-1042.
- Gurevich VV and Gurevich EV (2004) The molecular acrobatics of arrestin activation. *Trends Pharmacol Sci* **25**:59-112.
- Gurevich VV and Gurevich EV (2006b) The structural basis of arrestin-mediated regulation of G protein-coupled receptors. *Pharm Ther* **110**:465-502.
- Gurevich VV and Gurevich EV (2008a) GPCR monomers and oligomers: it takes all kinds. *Trends Neurosci* **31**:74-81.

MOL49015

- Gurevich VV and Gurevich EV (2008b) How and why do GPCRs dimerize? *Trends Pharmacol Sci* **29**:234-240.
- Hansen JL, Theilade J, Aplin M and Sheikh SrP (2006) Role of G-protein-coupled receptor kinase 2 in the heart-Do regulatory mechanisms open novel therapeutic perspectives? *Trends Cardiovasc Med* **16**:169.
- Hanson SM, Cleghorn WM, Francis DJ, Vishnivetskiy SA, Raman D, Song S, Nair KS, Slepak VZ, Klug CS and Gurevich VV (2007a) Arrestin mobilizes signaling proteins to the cytoskeleton and redirects their activity. *J Mol Biol* **368**:375-387.
- Hanson SM, Francis DJ, Vishnivetskiy SA, Kolobova EA, Hubbell WL, Klug CS and Gurevich VV (2006) Differential interaction of spin-labeled arrestin with inactive and active phosphorhodopsin. *Proc Natl Acad Sci U S A* **103**:4900-4905.
- Hanson SM, Gurevich EV, Vishnivetskiy SA, Ahmed MR, Song X and Gurevich VV (2007b) Each rhodopsin molecule binds its own arrestin. *Proc Nat Acad Sci USA* **104**:3125-3128.
- Iwata K, Luo J, Penn RB and Benovic JL (2005) Bimodal regulation of the human H1 histamine receptor by G protein-coupled receptor kinase 2. *J Biol Chem* **280**:2197-2204.
- Jaber M, Koch WJ, Rockman H, Smith B, Bond RA, Sulik KK, Ross J, Jr., Lefkowitz RJ, Caron MG and Giros B (1996) Essential role of beta -adrenergic receptor kinase 1 in cardiac development and function. *Proc Natl Acad Sci USA* **93**:12974-12979.
- James JR, Oliveira MI, Carmo AM, Iaboni A and Davis SJ (2006) A rigorous experimental framework for detecting protein oligomerization using bioluminescence resonance energy transfer. *Nat Methods* **3**:1001-1006.

MOL49015

- Jiménez-Sainz MC, Murga C, Kavelaars A, Jurado-Pueyo M, Krakstad BF, Heijnen CJ, Mayor FJ and Aragay AM (2006) G protein-coupled receptor kinase 2 negatively regulates chemokine signaling at a level downstream from G protein subunits. *Mol Biol Cell* **17**:25-31.
- Jones BW and Hinkle P (2008) Arrestin binds to different phosphorylated regions of the TRH receptor with distinct functional consequences. *Mol Pharmacol* **in press**.
- Key TA, Foutz TD, Gurevich VV, Sklar LA and Prossnitz ER (2003) N-formyl peptide receptor phosphorylation domains differentially regulate arrestin and agonist affinity. *J Biol Chem* **278**:4041-4047.
- Kim J, Ahn S, Ren XR, Whalen EJ, Reiter E, Wei H and Lefkowitz RJ (2005) Functional antagonism of different G protein-coupled receptor kinases for beta-arrestin-mediated angiotensin II receptor signaling. *Proc Nat Acad Sci USA* **102**:1442-1447.
- Kobilka BK, Matsui H, Kobilka TS, Yang-Feng TL, Francke U, Caron MG, Lefkowitz RJ and Regan JW (1987) Cloning, sequencing, and expression of the gene coding for the human platelet alpha 2-adrenergic receptor. *Science* **238**:650-656.
- Krupnick JG, Gurevich VV and Benovic JL (1997) Mechanism of quenching of phototransduction. Binding competition between arrestin and transducin for phosphorhodopsin. *J Biol Chem* **272**:18125-18131.
- Kunapuli P and Benovic JL (1993) Cloning and expression of GRK5: a member of the G protein-coupled receptor kinase family. *Proc Natl Acad Sci U S A* **90**:5588-5592.

MOL49015

- Laporte SA, Oakley RH, Zhang J, Holt JA, Ferguson SG, Caron MG and Barak LS (1999) The 2-adrenergic receptor/arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Nat Acad Sci USA* **96**:3712-3717.
- Lee KB, Ptasienski JA, Pals-Rylaarsdam R, Gurevich VV and Hosey MM (2000) Arrestin binding to the M2 muscarinic acetylcholine receptor is precluded by an inhibitory element in the third intracellular loop of the receptor. *J Biol Chem* **275**:9284-9289.
- Lefkowitz RJ and Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. *Science* **308**:512-517.
- Lohse MJ, Benovic JL, Codina J, Caron MG and Lefkowitz RJ (1990) beta-Arrestin: a protein that regulates beta-adrenergic receptor function. *Science* **248**:1547-1550.
- Lorenz W, Inglese J, Palczewski K, Onorato JJ, Caron MG and Lefkowitz RJ (1991) The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the beta-adrenergic receptor kinase. *Proc Natl Acad Sci U S A* **88**:8715-8719.
- Luo J, Busillo JM and Benovic JL (2008) M3 muscarinic acetylcholine receptor-mediated signaling is regulated by distinct mechanisms. *Mol Pharmacol*.
- Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG and Lefkowitz RJ (1999) Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* **283**:655-661.
- Luttrell LM, Roudabush FL, Choy EW, Miller WE, Field ME, Pierce KL and Lefkowitz RJ (2001) Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc Nat Acad Sci USA* **98**:2449-2454.

MOL49015

- McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT, Davis RJ and Lefkowitz RJ (2000) Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* **290**:1515-1518.
- Moore CA, Milano SK and Benovic JL (2007) Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol* **69**:451-482.
- Naik S, Billington CK, Pascual RM, Deshpande DA, Stefano FP, Kohout TA, Eckman DM, Benovic JL and Penn RB (2005) Regulation of cysteinyl leukotriene type 1 receptor internalization and signaling. *J Biol Chem* **280**:8722-8732.
- Pals-Rylandsdam R, Gurevich VV, Lee KB, Ptasienski J, Benovic JL and Hosey MM (1997) Internalization of the m2 muscarinic acetylcholine receptor: arrestin-independent and -dependent pathways. *J Biol Chem* **272**:23682-23689.
- Penn RB, Pascual RM, Kim YM, Mundell SJ, Krymskaya VP, Panettieri RAJ and Benovic JL (2001) Arrestin specificity for G protein-coupled receptors in human airway smooth muscle. *J Biol Chem* **276**:32648-32656.
- Peppel K, Boekhoff I, McDonald P, Breer H, Caron MG and Lefkowitz RJ (1997) G Protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of odorant receptor desensitization. *J Biol Chem* **272**:25425-25428.
- Pin JP, Galvez T and Prezeau L (2003) Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol Ther* **98**:325-354.
- Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C, Benovic JL, Kwatra MM, Caron MG and Lefkowitz RJ (1992) Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. *Science* **257**:1264-1267.

MOL49015

- Pronin AN, Morris AJ, Surguchov A and Benovic JL (2000) Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *J Biol Chem* **275**:26515-26522.
- Ren XR, Reiter E, Ahn S, Kim J, Chen W and Lefkowitz RJ (2005) Different G protein-coupled receptor kinases govern G protein and beta-arrestin mediated signaling of V2 vasopressin receptor. *Proc Nat Acad Sci USA* **102**:1448-1453.
- Rompler H, Staubert C, Thor D, Schulz A, Hofreiter M and Schoneberg T (2007) G protein-coupled time travel: evolutionary aspects of GPCR research. *Mol Interv* **7**:17-25.
- Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O and Lefkowitz RJ (2006) beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J Biol Chem* **281**:1261-1273.
- Shinohara T, Dietzschold B, Craft CM, Wistow G, Early JJ, Donoso LA, Horwitz J and Tao R (1987) Primary and secondary structure of bovine retinal S antigen (48-kDa protein). *Proc Nat Acad Sci USA* **84**:6975-6979.
- Song X, Raman D, Gurevich EV, Vishnivetskiy SA and Gurevich VV (2006) Visual and both non-visual arrestins in their "inactive" conformation bind JNK3 and Mdm2 and relocalize them from the nucleus to the cytoplasm. *J Biol Chem* **281**:21491-21499.
- Sterne-Marr R, Gurevich VV, Goldsmith P, Bodine RC, Sanders C, Donoso LA and Benovic JL (1993) Polypeptide variants of beta-arrestin and arrestin3. *J Biol Chem* **268**:15640-15648.

MOL49015

- Vinge LE, Andressen KW, Attramadal T, Andersen GO, Ahmed MS, Peppel K, Koch WJ, Freedman NJ, Levy FO, Skomedal T, Osnes J-B and Attramadal H (2007) Substrate specificities of G protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in regulation of myocardial function. *Mol Pharmacol* **72**:582-591.
- Vinge LE, von Lueder TG, Aasum E, Qvigstad E, Gravning JA, How O-J, Edvardsen T, Bjornerheim R, Ahmed MS, Mikkelsen BW, Oie E, Attramadal T, Skomedal T, Smiseth OA, Koch WJ, Larsen TS and Attramadal H (2008) Cardiac-restricted expression of the carboxyl-terminal fragment of GRK3 uncovers distinct functions of GRK3 in regulation of cardiac contractility and growth: GRK3 controls cardiac β_1 -adrenergic receptor responsiveness. *J Biol Chem* **283**:10601-10610.
- Vishnivetskiy SA, Hosey MM, Benovic JL and Gurevich VV (2004) Mapping the arrestin-receptor interface: structural elements responsible for receptor specificity of arrestin proteins. *J Biol Chem* **279**(2):1262-1268.
- Vishnivetskiy SA, Raman D, Wei J, Kennedy MJ, Hurley JB and Gurevich VV (2007) Regulation of arrestin binding by rhodopsin phosphorylation level. *J Biol Chem* **282**:32075-32083.
- Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, Kobilka BK and Sunahara RK (2007) A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc Natl Acad Sci U S A* **104**:7682-7687.

MOL49015

Willets JM, Challiss RA and Nahorski SR (2003) Non-visual GRKs: are we seeing the whole picture? *Trends Pharmacol Sci* **24**:626-633.

Wu N, Hanson SM, Francis DJ, Vishnivetskiy SA, Thibonnier M, Klug CS, Shoham M and Gurevich VV (2006) Arrestin binding to calmodulin: a direct interaction between two ubiquitous signaling proteins. *J Mol Biol* **364**:955-963.

Xiao K, McClatchy DB, Shukla AK, Zhao Y, Chen M, Shenoy SK, Yates JRr and Lefkowitz RJ (2007) Functional specialization of beta-arrestin interactions revealed by proteomic analysis. *Proc Natl Acad Sci U S A* **104**:12011-12016.