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Nuclear GPCRs in cardiomyocytes: an insider's view of β-adrenergic receptor signaling

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Vaniotis G, Allen BG, Hébert TE. Nuclear GPCRs in cardiomyocytes: an insider's view of β-adrenergic receptor signaling. *Am J Physiol Heart Circ Physiol* 301: H1754–H1764, 2011. First published September 2, 2011; doi:10.1152/ajpheart.00657.2011.—In recent years, we have come to appreciate the complexity of G protein-coupled receptor signaling in general and β-adrenergic receptor (β-AR) signaling in particular. Starting originally from three β-AR subtypes expressed in cardiomyocytes with relatively simple, linear signaling cascades, it is now clear that there are large receptor-based networks which provide a rich and diverse set of responses depending on their complement of signaling partners and the physiological state. More recently, it has become clear that subcellular localization of these signaling complexes also enriches the diversity of phenotypic outcomes. Here, we review our understanding of the signaling repertoire controlled by nuclear β-AR subtypes as well our understanding of the novel roles for G proteins themselves in the nucleus, with a special focus, where possible, on their effects in cardiomyocytes. Finally, we discuss the potential pathological implications of alterations in nuclear β-AR signaling.

G protein-coupled receptors; nuclear signaling; oligomerization; signaling complexes

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The three β-adrenergic receptor (β-AR) subtypes were initially believed to comprise rather simple and linear signaling cascades involving the receptor, the G_s heterotrimer and activation of adenylyl cyclase (AC). This organization required nothing but a series of sequential agonist-driven interactions: first between the ligand and the receptor, then the receptor and the G protein, and finally between the activated G protein and the effector. Essentially, this would be similar in all cell types that expressed each receptor. In the case of the cardiomyocyte, the β_1-AR driving this pathway was primarily responsible for the chronotropic and inotropic effects of sympathetic stimulation. However, it has been more recently appreciated that all three receptor subtypes could also interact with other G proteins such as the pertussis toxin (PTX)-sensitive G_i heterotrimer [reviewed in (31)], presumably not simply to provide an inhibitory stimulus to the same effector enzyme, AC, but also to contribute to signaling diversity. Since then, it has become clear that each receptor interacts with a wide array of signaling pathways, some of which depend directly on G protein-dependent signaling and others which involve agonist-dependent recruitment of G protein-coupled receptor (GPCR) kinases and β-arrestins [reviewed in (82)]. In cardiomyocytes, β_1-ARs, localized to the sarcolemma and tubular network, play a predominant role in regulating cardiomyocyte contractility. The β_2-AR, which shares this distribution, plays a more modest role in regulating the inotropic and lusitropic responses. Both receptors signal through G_s and AC with similar efficacy, although the signals are compartmentalized differently, possibly because of localization in distinct membrane microdomains and/or dual coupling of the β_2-AR to G_s and G_i [reviewed in (125, 127, 146)]. β_3-ARs are also found in cardiomyocytes, although their function remains ill defined. In fact, transgenic mice knocked out for both the β_1-AR and β_2-AR show markedly reduced contractile phenotypes despite the presence of the β_3-AR (22). Hence the β-AR subtypes may serve unique functions and play nonredundant roles within the cardiomyocyte.

Signaling from β-AR Localized to the Nuclear Membrane

Once believed to be primarily involved in the desensitization and internalization of GPCRs, β-arrestin-dependent signaling events, it has become clear, enrich the phenotypic diversity of signaling but also deliver receptor-dependent signals to distinct subcellular targets. This second wave of signaling is thought by some authors to be essentially G protein independent [see for example (94, 99)]. Receptor internalization, therefore, is no longer simply a way of desensitizing receptors. Internalization of GPCRs may lead to a switch in signaling pathways by desensitizing the primary, second messenger-based or cell surface-based pathways while simultaneously activating a second wave of signaling in endosomal compartments. However,

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the discovery of G protein-independent, or "post G protein," signaling events still implicates an initial surface targeting event for GPCRs.

Nuclear GPCR-Mediated Signaling

An increasing number of GPCRs have been demonstrated to be targeted to the nuclear membrane, including lysophosphatidic acid receptors (39), metabotropic glutamate receptors [MGluR5 (53, 68, 92)], apelin receptors (73), platelet-activating factor receptors (85), bradykinin B_2 receptors (73), angiotensin II type I and type II receptors (14, 73, 80, 129, 153), prostaglandin receptors (40), endothelin receptors (5), and α_1-ARs [(38, 142), reviewed in (7, 41)]. Also, mutant V_2 vasopressin receptors, which are trapped in intracellular compartments, can signal in response to nonpeptide agonists, indicating that they are in fact functional even when mistrafficked (104).

In addition, a large number of signaling proteins, classically associated with receptor-mediated events at the cell surface including heterotrimeric G proteins [(8, 40, 151), reviewed in (26, 28, 141)], AC isoforms (118, 147), phospholipase A_2 (117), phospholipase Cβ (59) and phospholipase D (35), regulator of G protein signaling (RGS) proteins [reviewed in (11)], β-arrestin-1 (120, 135), GPCR kinases (52, 148, 149), A kinase anchoring proteins, and PKA (113), among others, have been demonstrated to be trafficked to the nucleus and/or nuclear membrane. Interestingly, enzymes involved in the generation and metabolism of phosphoinositides (4) or the processing of peptide ligands such as angiotensin-converting enzyme (81) and endothelin-converting enzyme-1 (51) have also been localized to the nuclei of different cell types. Furthermore, these "intracrine" signaling loops are not restricted to GPCRs and may include a number of other classes of "cell surface" receptors as well, such as activin-like kinase-1 types 4 and 5, TGF-β superfamily receptors responsive to activin A (42), and VEGF receptors [(74), reviewed in (18)]. Although these data, taken together, suggest that these nuclear GPCRs are relevant to cell physiology since they seem to be present constitutively in native cell systems, it remains to be conclusively demonstrated whether they have defined roles in the context of intact cells, rather than isolated nuclei.

Molecular Mechanisms of Nuclear Signaling by β-AR.

Although most nuclear GPCRs seem to regulate proximal signaling pathways (i.e., involving generation of second messengers or activation of ERK1/2 and Akt) similar to those seen at the cell surface [reviewed in (7)], a number of these receptors more directly regulate nuclear events such as DNA synthesis (139), transcription initiation (6) and gene expression (53, 116, 134), and histone modification (100).

We demonstrated that cardiac β_1- and β_3-ARs (6, 134) were targeted to endomembrane locations in cardiomyocytes where they are functional with respect to cellular signaling. Interestingly, subcellular fractionation experiments in adult rat ventricular cardiomyocytes indicated colocalization of β-AR with Nup-62, a marker of the nuclear membrane. To more carefully characterize the distribution and possible physiological relevance of the three receptor subtypes, we complemented these studies with immunocytochemistry, ligand-binding studies and functional assays using primary tissue that has certainly turned out to be a key requirement for convincing journal reviewers and colleagues. Functional β-ARs were localized to the nuclear membrane, and more importantly, this localization was subtype specific. Surprisingly, our experiments showed that β_1-AR and β_3-AR, but not the β_2-AR, distributed to the nuclear membrane and that the former two β-AR isoforms subserve different functions (6). Interestingly, both receptors were differentially coupled to signaling pathways in isolated heart nuclei. The β_1-AR activated AC, presumably through G_s, whereas the β_3-AR modulated transcriptional initiation in a PTX-sensitive manner, presumably through G_i. Furthermore, we showed that both rRNA (18S rRNA) and mRNA (NF-κB and components related to its signaling pathways) levels were modulated by β-AR stimulation (134). All of the transcriptional events mediated by β-AR stimulation in isolated cardiac nuclei were sensitive to inhibitors of ERK1/2, p38, and JNK as well as Akt, suggesting that these signaling systems all impact on the tone of nuclear GPCR signaling.

Our initial findings regarding changes in Akt and ERK status after receptor stimulation in isolated nuclei (134) essentially found that the three MAPK (including upstream regulators such as MEK and Raf1) and Akt (including the downstream target GSKβ) pathways were active in isolated cardiac nuclei treated with isoproterenol (data not shown). In our hands, only Akt was activated by the β-AR in isolated nuclei. Therefore, the other signaling pathways likely modulate nuclear β-AR signaling via molecular cross talk (134) in response to numerous signals. In an effort to understand signaling networks activated by nuclear β-AR, we have recently begun to determine whether receptor stimulation alters the phosphorylation status of nuclear proteins using standard one- and two-dimensional electrophoretic approaches. In these experiments, nuclei were treated with isoproterenol, phosphoproteins enriched using gallium-immobilized metal ion affinity chromatography and resolved on one-dimensional (Fig. 1, *middle*) or two-dimensional gels (immobilized pH gradient gel/SDS-PAGE, Fig. 1, *left* and *right*), and phosphoproteins were visualized with SYPRO ruby fluorescent stain. Changes in the patterns of the nuclear phosphoproteome are evident in both sets of experiments, highlighting the dynamic nature of β-AR signaling in intact nuclei. Further experiments should thus be aimed at identifying proteins where the phosphorylation status has been altered.

Perhaps most interesting was the observation that the inhibition of Akt "switched" isoproterenol from an agonist to an inverse agonist with respect to transcriptional initiation; that is, in the presence of triciribine, isoproterenol reduced levels of RNA synthesis below that of the unliganded control (134). The ability of different ligands to discriminate between signaling pathways coupled to a given GPCR has been termed "biased" agonism (57). Such biased signaling has been well demonstrated for all three β-AR subtypes. For example, it has been shown that different β-AR agonists have varying capabilities to activate AC or ERK1/2 MAPK signaling pathways downstream of either the β_1-AR or the β_2-AR. It was also noted that certain neutral antagonists and even inverse agonists for the AC pathway turned out to be agonists for the ERK1/2 pathway [(2, 36), reviewed in (31, 37, 94)]. It has been recently shown that certain classical β-blockers, such as carvedilol, act as agonists for a prosurvival pathway in the heart involving the β_1-AR, β-arrestin, and transactivation of the EGFR leading to

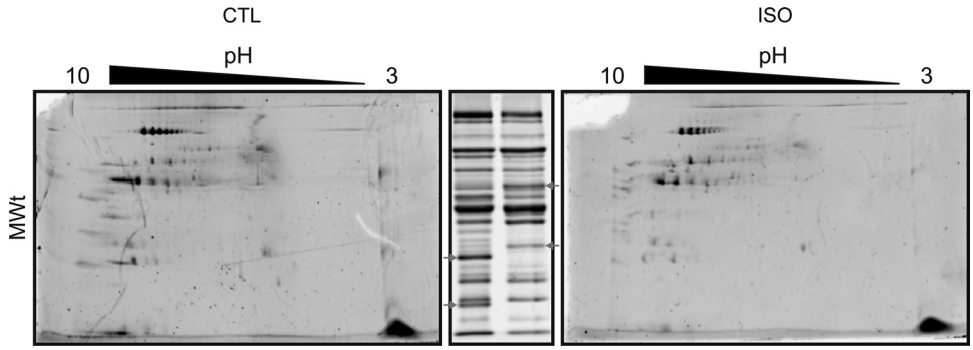

Fig. 1. β-Adrenergic receptor (β-AR) stimulation results in alterations in the nuclear phosphoproteome. Isoproterenol (Iso) alters protein phosphorylation patterns in nuclei isolated from rat ventricular myocardium. Enriched nuclear fractions were resuspended in a buffer comprising 150 mM Tris·HCl (pH 7.9), 450 mM KCl, 3 mM $MnCl_2$, 18 mM $MgCl_2$, 3 mM ATP, 6 mM DTT, and 3 U/ml RNAse inhibitor and incubated in the presence or absence of 1 μM Iso for 30 min. Incubations were terminated by addition of trichloroacetic acid to a final concentration of 10%. Following centrifugation, supernatants were aspirated, pellets were washed with acetone and resuspended using 6 M urea, and the soluble proteins were applied to 1 ml HiTrap Chelating HP columns preequilibrated with $GaCl_3$. Phosphoproteins were eluted using 6 M urea, 50 mM Tris-acetate (pH 7.4), 100 mM EDTA, and 100 mM EGTA, resolved by SDS-PAGE and visualized with SYPRO ruby. *Left* and *right*: changes detected in the abundance of phosphorylated proteins in the absence (*left*) or presence (*right*) of Iso when two-dimensional gels are used. The pH gradient is shown along the top of each gel. *Middle*: arrows indicate selected bands on one-dimensional SDS-PAGE having altered abundance (increased or decreased) as a result of stimulation with Iso. CTL, control; MWt, molecular weight.

MAPK activation (60, 91, 132). These findings are likely to have significant clinical consequences for the development of more appropriate β-blockers (and likely a shift in our use of this term to biased ligands) for use in treating heart failure. Similar patterns of biased agonism have emerged for the β_3-AR (114, 115). Ligands, once classified according to results obtained with a single signaling readout, need to be reassessed according to their ability to act as biased ligands in a pathway-specific manner and, indeed, in a compartment-specific manner.

It has also become clear in recent years that most if not all GPCRs can form dimers and possibly higher order structures [see (10, 46, 87, 98) for review]. Receptor heterooligomerization can alter both signaling profiles and/or receptor trafficking [reviewed in (10, 87, 98, 131)]. Not surprisingly, all three β-AR subtypes have been shown to form heterodimers with each other. The β_2-AR can heterodimerize with both other subtypes (9, 71, 72, 86). Trafficking was altered in both cases. In the β_1-AR/β_2-AR heterodimer, the characteristics of the β_1-AR predominated, such that the heterodimer trafficked and signaled like the β_1-AR expressed alone both in HEK293 cells (72) and in adult mouse ventricular cardiomyocytes (152). The pharmacology of ligand binding was altered in this pair in that ligands for both receptors needed to be present to achieve high-affinity binding of subtype-selective ligands (71). In the case of the β_2-AR/β_3-AR heterodimer, this pair trafficked like the β_3-AR expressed alone and was also unable to couple to G_i, unlike either of the two parent receptors when expressed alone (9). To date, no direct demonstration has been provided for interactions between the β_1-AR and the β_3-AR, which may be important given their unique nuclear distribution in cardiomyocytes. One wonders though if these two receptors can heterodimerize on the nuclear membrane. If so, it will now be necessary to reevaluate the pharmacology of nuclear receptor signaling in that context.

Most studies evaluating the signaling downstream of nuclear GPCRs have obviously relied on isolated nuclei. Studies have been performed measuring the production of second messengers such as cAMP and Ca^{2+} using imaging techniques in isolated cardiomyocytes, but these have all relied on ligands delivered to the cell surface (75, 96). Thus particular contributions of nuclear signaling in this regard need to be evaluated carefully. Since the identification of nuclear GPCRs, many questions have arisen regarding the sources of ligands that activate them. In cases where ligands are synthesized in the same cells as the receptors, such as angiotensin II (129) or endothelin (5), intracrine signaling loops are easily imaginable. Hydrophobic ligands that cross membranes such as prostaglandins are also easily envisaged to attain intracellular receptors. However, for hydrophilic ligands such as epinephrine or norepinephrine, this becomes intrinsically more difficult: one must invoke the presence of some sort of active or passive transport mechanism. Interestingly, it has already been demonstrated that [^{3}H]norepinephrine, incubated with intact cells, can accumulate in the nuclei of neonatal ventricular cardiomyocytes (12). It is also clear that β-AR and other GPCRs can exist in an active state (or states) even in the absence of agonist (16, 112). This constitutive activity led to the identification of the class of receptor ligands known as inverse agonists and to an appreciation that most GPCRs existed in at least two states which could be toggled toward active by agonists and in the opposite direction by inverse agonists [see (37, 56, 58) for review]. This constitutive activity itself may thus be an important component of nuclear GPCR signaling [reviewed in (7)].

A further consideration as to why it will be important to demonstrate the effects of nuclear GPCRs in an intact cell setting is as follows. As can be seen in Fig. 2, there are two possible orientations for nuclear GPCRs, one with the receptor COOH-terminus facing the nucleoplasm and the other facing the cytosol. A number of GPCRs contain nuclear localization sequences (73). It is possible that these sequences allow GPCRs to use the nuclear pore complexes to "turn the corner" and move from the outer to inner nuclear membrane. This suggests that the accumulation of the ligand within the perinuclear space, that compartment between the inner and outer nuclear membrane, might also result in signals delivered in two directions simultaneously. For adrenergic and other small ligand-activated receptors to have intracellular effects, one must consider an uptake mechanism to deliver ligands endomembrane compartments, in contrast to ligands, discussed above,

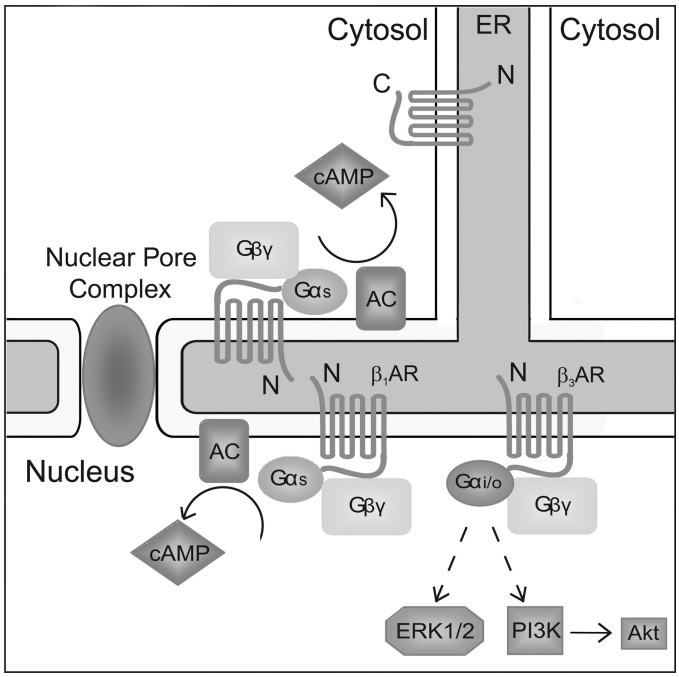

Fig. 2. Possible orientation of nuclear β-AR signaling complexes. Both the β_1-AR, coupled to G_s, and the β_3-AR, coupled to G_i, but not the β_2-AR, are resident on the nuclear membrane, at least in rat and mouse adult ventricular cardiomyocytes (6, 134). How these receptors are trafficked to distinct endomembrane compartments is not well understood and could either be a result of receptor internalization from the cell surface or via de novo delivery from the biosynthetic pathway. The outer nuclear membrane is continuous with both the endoplasmic reticulum (ER) and the inner nuclear membrane, with which it is joined at the level of nuclear pore complex insertion. Ligand must be able to attain the space between the two nuclear membranes to activate these receptors, or receptors must be constitutively active. The possibility that there may be two distinct orientations for nuclear G protein-coupled receptors (GPCRs), i.e., either capable of delivering signals toward the cytosol or the nucleoplasm, is something that can only be explored in an intact cell context. Also, what the role of the nuclear pore complex is in determining the sidedness of nuclear GPCR signaling remains to be determined. C, COOH-terminus; N, NH_2-terminus; AC, adenylyl cyclase; PI3K, phosphatidylinositol 3-kinase.

that are made intracellularly (e.g., angiotensin II, endothelin, or glutamate) or ligands that can cross membranes (e.g., prostaglandins and other lipid mediators). With respect to catecholamines, the classic reuptake system found in neurons is unlikely to operate in cardiomyocytes. However, there are nonselective uptake systems such as extraneuronal monoamine transporter (or organic cation transporter 3) found in several tissues including heart [(30, 43), reviewed in (54)], which could transport catecholamines to intracellular targets in cardiomyocytes. Independent of mechanism, the most important study in this regard found that in neonatal rat cardiomyocytes, extracellular [^{3}H]norepinephrine was taken up, and indeed a large fraction was detected in the nuclear fraction within hours (12). Intriguingly, this is a very hydrophilic ligand, suggesting that optimizing the properties of a subpopulation of the large number of available β-AR ligands might make it possible to design ligands that specifically target the internal receptor pool. Whether this is true or not can only be confirmed in an intact cell context, which is surely the next challenge facing researchers in this area.

Nuclear G Protein-Mediated Signaling

Heterotrimeric G proteins transmit numerous stimuli from cell surface GPCRs to various intracellular effector molecules as enzymes and ion channels. The heterotrimer is composed of α- and βγ-subunits. Gα subunits bind and hydrolyze GTP and were classically believed responsible for most effector activation effects. More recent work has shown that Gβγ subunits are also key regulators of cellular signaling events but also serve a broader role in organizing the assembly and trafficking of receptor-based complexes in intracellular compartments such as the endoplasmic reticulum (ER) and Golgi apparatus [(108), see (28) for review]. Recently, a number of studies have indicated a direct nuclear impact for Gβγ subunits. Gβ1γ2 dimers can interact directly with histone deacetylase 5 (HDAC5) and possibly other HDAC isoforms as well (124). In the basal state, HDAC5 interacts with the muscle differentiation factor, myocyte enhancer factor 2 (MEF2), resulting in reduced transcriptional activity. Following stimulation of the α_{2A}-AR, activated Gβγ dimers interacted with HDAC5, releasing MEF2 and allowing it to stimulate transcriptional activity. Both the $G\alpha_{i/o}$ inhibitor PTX and the Gβγ scavenger, β-AR kinase COOH terminus, inhibited MEF2 activity (124). It remains uncertain as to whether cytoplasmic Gβγ dimers sequester HDAC or whether these events exclusively occur in the nucleus.

Gβ5 subunits interact with a number of RGS proteins. One RGS class, the R7 subfamily, is enriched in brain and functions as part of a stable RGS-Gβ5 complex, which is localized to both the cytosol and the nucleus (151). The R7BP protein interacts with the R7-Gβ5 pair and potentiates the capacity of this complex to modulate inwardly rectifying K^+ (Kir)3 channels in response to M_2 muscarinic receptor stimulation (24). R7BP is palmitoylated, and this interaction allows anchoring of RGS7-Gβ5 at the plasma membrane to regulate GPCR signaling. However, the addition of palmitate is a transient and tightly regulated process (123). In this case, the loss of the palmitate moiety on R7BP releases the R7BP-RGS7-Gβ5 complex from the plasma membrane and shuttles it to the nucleus. Other RGS proteins that are also localized to the nucleus include RGS6, which can regulate transcription in mammalian cells (76). The precise role of these proteins in the nucleus remains uncertain at present. These authors proposed this as a novel mechanism for transmitting neurotransmitter signals from receptors at the plasma membrane directly to the nucleus [for review (47)]. Interestingly, mutant Gβ5 subunits unable to form a complex with RGS7 but still capable of interacting with Gγ2 were not found in the nucleus of either HEK293 or PC12 cells, suggesting the importance of the RGS protein in the nuclear localization of Gβ5 (109).

It has been shown that Gβγ subunits containing the other Gβ isoforms can interact with the transcriptional repressor known as the adipocyte enhancer-binding protein (AEBP1) (93). AEBP1 specifically forms a complex with Gβγ subunits containing Gγ5 in nuclei of 3T3-L1 but interestingly not NIH 3T3 cells. The Gβγ5/AEBP1 interaction attenuates its transcriptional repression activity. Another Gβγ effector is the glucocorticoid receptor (GR) localized in the cytoplasm and translocated to the nucleus in response to ligand binding, where several target genes are transcriptionally regulated. Both Gβ1- and Gβ2 subunits directly interact with the GR and translocate

with it to the nucleus following treatment with the agonist dexamethasone (61, 62). The interaction of Gβγ with GR suppresses transcriptional activity most likely by associating with transcriptional complexes formed on GR-responsive promoters. Gβ2 mutants unable to bind Gγ cannot suppress GR transcriptional activity. These studies begin to highlight a central role of Gβγ in numerous subcellular compartments, directly regulating fundamental processes as diverse as transcription and protein trafficking in the ER and Golgi. Gβγ subunits are more than simply signaling molecules responsive to GPCR stimulation.

We have recently shown that the coexpression of Gβγ decreased PMA-stimulated activating protein-1 (AP-1) gene reporter activity in different cell lines (107). We identified Fos transcription factors as novel interactors of the Gβγ subunits. Gβγ did not interfere with the dimerization of Fos and Jun or the ability of AP-1 complexes to bind DNA. Rather, Gβγ colocalized with the AP-1 complex in the nucleus and recruited HDACs to inhibit AP-1 transcriptional activity as determined using chromatin immunoprecipitation in contrast to their effect on MEF2 (124). This novel role for Gβγ subunits as transcriptional regulators may be potentially independent of their classical functions as mediators of GPCR signaling.

Recently, a number of novel interactors of Gβγ have been identified, as have Gβγ-dependent signaling events, some of which are independent of the receptor per se [for review, see (28)]. Interestingly, a number of these events occur at subcellular sites distinct from the plasma membrane. Among these, a number of nuclear targets for Gβγ have been identified. The modulation of prenylation status has been shown to increase the amount of Gβγ in the nucleus associated with the GR (61, 62). Recent reports have indicated that a subpopulation of Gγ subunits may escape from being prenylated and thus remain soluble (19). This may suggest that some of the transcriptional effects of Gβγ may in fact be receptor independent and depend on protein complexes formed with "free" Gβγ or G protein heterotrimers (Table 1). In fact, under basal conditions, we noted the presence of Gβγ subunits in many cell types include cardiomyocytes, suggesting they may be resident there (101). These observations suggest that Gβγ subunits may be more general transcriptional regulators. It is known that cFos transcription is activated by several GPCRs. M_2 muscarinic receptor stimulation leads to activation of the cFos promoter, and this event is mediated through Gβγ and is dependent on ERK and JNK (128). On one hand, the activation of heterotrimeric G proteins leads to the activation of cFos transcription, whereas on the other hand, a subsequent interaction of Gβγ with AP-1 proteins decreases transcriptional activity, providing, in effect, a negative feedback loop. STAT3 is another transcription factor that may be a target for dual GPCR and Gβγ regulation (150). The source of Gβγ for these two classes of events may in fact be different; i.e., the formation of Gβγ/transcription factor complexes may not necessarily be receptor dependent and these proteins may interact directly following their biosynthesis or there may be a pool of "free" Gβγ in the cell. In preliminary experiments, we noted that an overexpression of Gαq did not alter the response of an AP-1 reporter gene to the presence of Gβγ, suggesting that these latter two possibilities must be considered (S. Gora and T. E. Hébert, unpublished study). Interestingly, the Gβ1 promoter contains several putative AP-1 response elements (63). De novo synthesis or release of Gβγ may therefore result from increased levels of Fos synthesis. All five Gβ subunits inhibited AP-1 activity in reporter assays, suggesting that this is a common feature of Gβγ signaling (107).

Ontogeny of GPCR and G Protein Signaling Systems: How Do They Get to the Nucleus?

Little is known at present as to how GPCRs and their attendant signaling partners traffic to distinct subcellular locations such as the nuclear membrane. It is possible that de novo complexes of GPCRs and their signaling partners assembled along the biosynthetic pathway might also be delivered to endomembrane locations in addition to the cell surface. Transient receptor/G protein/effector interactions, which explain G protein-mediated signal transduction in the mammalian visual system, cannot account for the exquisite signaling specificity seen in cells such as cardiomyocytes or neurons. These latter cell types, which may express dozens of possible receptor/G protein heterotrimer/effector combinations, exhibit high signaling fidelity in vivo from one receptor activation cycle to the next. In vitro studies, where promiscuous coupling is often seen, have not reflected this [reviewed in (44, 45)]. Particular combinations of heterotrimeric G proteins have been demonstrated to couple GPCRs to particular effectors (1, 55, 64–66, 105, 106, 119, 136–138). The possibility that receptors and G proteins might be associated before receptor activation has

Table 1. *Are Gβγ interactors strictly dependent on GPCR activation?*

Nuclear Effectors	Subcellular Location (Site of Action)	References
RGS-R7 proteins (Gβ5)	Nucleus	47, 151
AEBP1 (Gβγ5)	Nucleus	93
Glucocorticoid receptor (Gβ1 and Gβ2), possibly GPCR independent	Cytosol/nucleus	61, 62
HDAC5 (Gβ1-5, Gγ2)	Cytosol/nucleus	107, 124
AP-1 complex (Gβ1-5, Gγ2), possibly GPCR independent	Nucleus	107
STAT3 (multiple Gβγ combinations), possibly GPCR independent	Nucleus	150
ERK1/2 MAPK	Nucleus	78, 79

Canonical Gβγ-interacting proteins may initially interact with Gβγ subunits in the endoplasmic reticulum during biosynthesis (reviewed in Ref. 28) but are usually associated with G protein-coupled rceptor (GPCR) signaling at the plasma membrane. However, a number of transcriptional regulators also interact with Gβγ subunits in the cytosol or in the nucleus. Studies described in the text suggest that if Gγ subunits in a Gβγ dimer are not prenylated, and presumably not targeted to the plasma membrane, they accumulate in the nucleus. This suggests the possibility that some such Gβγ subunits may signal in a GPCR-independent manner. How the cell organizes these different possible signaling complexes is unclear at present. Specific Gβ or Gγ subunits known to interact with particular transcription factors are shown in parentheses. RGS, regulator of G protein signaling; AEBP, adipocyte enhancer-binding protein; HDAC5, histone deacetylase 5; AP-1, activating protein-1.

been incorporated into models of G protein signaling for some time (140), but experimental evidence that stable "precoupled" complexes exist in living cells has been obtained only relatively recently. A large number of studies have demonstrated association, copurification, or coimmunoprecipitation of receptors with G proteins [reviewed in (95, 102)].

Interestingly, receptor dimerization has been demonstrated to be required for efficient surface localization of a number of GPCRs, including the β_2-AR (27, 111) and the α_{1B}-AR [(77) reviewed in (88)]. In fact, significant evidence has accumulated that the assembly of GPCR signaling complexes occurs during their biosynthetic journey, rather than in response to agonist stimulation at the plasma membrane. We have studied the ontogeny of GPCR signaling complexes [initially focusing on β_1-AR and β_2-AR (70) as well as AC (3, 25) and Kir3 channels (21, 103, 108)]. Our data suggested that these complexes form during biosynthesis rather than through random, agonist-induced interactions at the plasma membrane. First, these interactions occur in the absence of receptor agonists, suggesting that signaling complexes are preassembled (25, 27, 103), and many of these proteins interact initially in the ER, including monomer equivalents in receptor dimers, receptor and Gβγ subunits as well as effectors such as Kir3 channels, and AC with nascent Gβγ. These interactions were all insensitive to dominant negative Rab1 or Sar1 (DN Rab1 and Sar1, but not Rabs 2, 6 or 11) constructs (25, 27), which regulate anterograde receptor trafficking [reviewed in (23, 26)]. It has recently been demonstrated that different Rab isoforms are important for both the initial membrane targeting of GPCRs [Rab1 (29, 33)] as well as for their internalization and recycling to the plasma membrane in response to agonist stimulation [Rabs 4, 5, 7 and 11 (20, 121, 122)]. This has implications for the trafficking of GPCR signaling complexes to the nuclear membrane, although no studies to date have assessed the roles of the Rabs or for that matter the cytoskeletal trafficking machinery in targeting these signaling systems. Although all of the receptors discussed in this article have been shown to be present constitutively in isolated nuclear membranes, it remains an open question as to how they are trafficked there or whether they can be internalized from the cell surface to nuclear compartments (Fig. 3, *left*).

Implications of Nuclear Signaling in Cardiac Health and Disease

Cardiac hypertrophy is induced by elevated hemodynamic load in vivo and by a number of neurohumoral factors (including angiotensin II, α-adrenergic agonists, endothelin-1, and growth factors) in vitro. Many of the receptors for these ligands have in fact been localized to the nucleus, but this has been never generally considered in studies of cardiac hypertrophy or heart disease. β_1-AR and β_2-AR are known to differentially regulate contractile function as well as other signaling pathways in the cardiomyocyte. Both receptor subtypes modulate L-type channel activity and mediate the positive inotropic effects, but only the β_1-AR seems to modulate the relaxation phase of the contractile cycle [see (126, 144) for review]. This is due to both differential subcellular distributions of each receptor and also to distinct G protein coupling. Recent studies have shown that the β_1-AR is exclusively coupled to G_s,

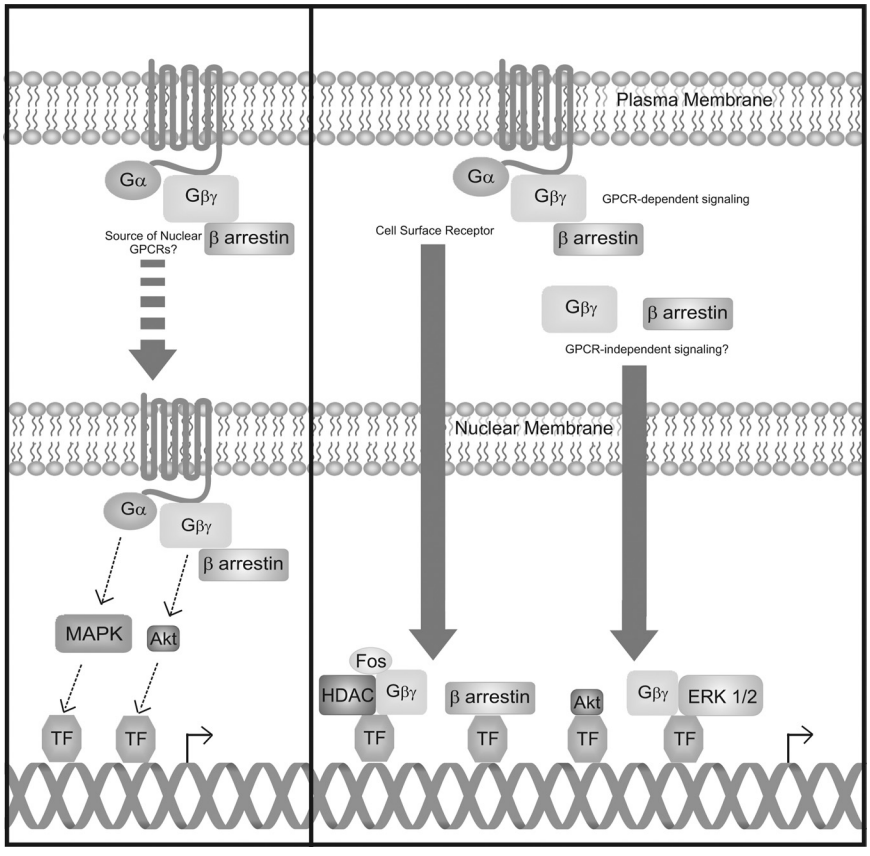

Fig. 3. Novel transcriptional complexes containing transcription factors (TFs), proteins kinases, and Gβγ subunits. *Left*: both cell surface and nuclear GPCRs may activate local signaling proteins including G proteins, β-arrestin, and nuclear protein kinases that modulate the activity of TFs. However, it remains to be determined what the links are between these two pools of receptor. Are nuclear GPCRs trafficked from the cell surface or de novo during their biosynthesis? *Right*: as described in the text, a number of novel chromatin interacting complexes have been identified that translate signaling events into changes in gene expression. Recent studies have shown that Akt, MAPK isoforms, and Gβγ subunits all interact with TFs and associated chromatin regulatory molecules such as histone deacetylase (HDAC) isoforms and actually interact with targets on the genome as well. Whether or not all of these intracellular events require nuclear or cell surface GPCRs or Gα subunits remains to be determined. As discussed, we need to move these studies into an intact cell context to find out.

whereas the β_2-AR is coupled to both G_s and G_i (69, 143, 145). G_i coupling serves to limit the physical distance the G_s signal can diffuse in the cytosol after β_2-AR activation, thus phospholamban, which is critical for the lusitropic response, is not phosphorylated. This differential coupling may also be reflected in the proapoptotic effects of β_1-AR stimulation and the antiapoptotic effects of β_2-AR stimulation in the context of the development of heart failure (15, 17). A clear role for β_3-AR has not been established in the progression to heart failure, but a recent study showed that mice deficient in this receptor are more sensitive to pressure overload induced by transverse aortic constriction (90). Interestingly, the expression of β-AR kinase COOH-terminal domain, the Gβγ inhibitor, in the adrenal gland has already shown promise as a strategy to mitigate the progression of heart failure (83), as have small molecule inhibitors of Gβγ signaling delivered intraperitoneally in a mouse model of heart failure (13). Also, all studies to date in this regard have focused on cell surface receptors, although evidence is emerging that intracrine signaling loops involving internal pools or GPCRs such as the angiotensin II receptor and their ligands are important in cardiovascular disease (101, 129). However, these events remain unexplored at the molecular level. Both Akt (50) and ERK1/2 (79) provide survival signals to the myocardium during the progression from compensated hypertrophy to end-stage heart failure. Interestingly, one novel hypertrophic pathway mediated by ERK1/2 signaling in the heart involved Gβγ subunits, which facilitate phosphorylation of ERK1/2 at sites distinct from those activated by canonical upstream kinases (78). We have shown that β-AR activation in isolated heart nuclei leads to a decrease in the transcription of NF-κB and some of its signaling partners (134). Interestingly, the activation of this pathway is linked with the development of heart failure (34, 110). Thus nuclear β-AR-mediated regulation of this pathway at the transcriptional level might be compensatory and involved in its antiapoptotic action. The links between the development of cardiac hypertrophy and changes in β-AR- and/or Gβγ-dependent nuclear signaling need to be explored in greater detail in future studies.

Integrating Signaling Events Driven by Cell Surface and Nuclear GPCRs and G Proteins

The connections between nuclear GPCRs and nuclear G proteins (which likely function as independent proteins that may be regulated by mechanisms distinct from receptors) or between cell surface and nuclear receptors remain to be disentangled. It is clear that G proteins may impact nuclear function downstream of both cell surface and nuclear-localized GPCRs. Although the nuclear localization of Gβγ dimers has been described, the molecular mechanisms involved in controlling interactions between Gβγ and AP-1 (or other transcriptional regulators) remain to be elucidated. cFos is known to shuttle between the nucleus and cytoplasm (48, 84, 130). A recent study has demonstrated an interaction between ERK1/2 and Gβγ, leading to an autophosphorylation of ERK1/2 on Thr188 and a subsequent accumulation of ERK1/2 in the nucleus (78). Whether ERK1/2 and Gβγ translocate to the nucleus as part of a complex remains unknown (Fig. 3). Interestingly, both Akt and ERK have numerous nuclear effects [reviewed in (67, 89, 133)], including being part of transcriptional complexes that sit on different sites in chromatin in different types of transcriptional complexes [(49, 97), as does p38 (32), see Fig. 3, *right*]. We do not yet know whether the Gβγ effects depend on prior activation of cell surface or nuclear GPCRs or are dependent on pools of "free" Gβγ (Fig. 3, *right*). The development of specific ligands that can discriminate between surface and nuclear GPCRs will be of great utility in dissecting these events.

Conclusions

Taken together, these studies suggest that GPCRs do not have to reach the cell surface to act as signaling entities as a distinction from receptors that continue to signal (even activating different signaling pathways) after they are internalized. An important current focus for molecular pharmacologists is to target single pathways associated with a given GPCR. The current focus on pathway-selective, biased ligands is providing optimism that these approaches may actually work. However, until recently, we have focused both on orthosteric ligand binding sites and cell surface-localized signaling systems, which may not provide the necessary level of discrimination. We would argue that targeting the assembly or trafficking of signaling complexes to different subcellular destinations might actually provide an even more "selective" set of biased "assembly modulators." However, much work remains to identify the molecular determinants of signaling complex assembly in these different cell organelles before this particular strategy can yield drug candidates for the treatment of heart disease and other maladies and/or interesting tool compounds.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G. V., B. G. A., and T. E. H., conception and design of research; G. V. performed experiments; G. V., B. G. A., and T. E. H. analyzed data; G. V., B. G. A., and T. E. H. interpreted results of experiments; G. V., B. G. A., and T. E. H. prepared figures; G. V., B. G. A., and T. E. H. drafted manuscript; G. V., B. G. A., and T. E. H. edited and revised manuscript; and G. V., B. G. A., and T. E. H. approved final version of manuscript.

REFERENCES

1. **Albert PR, Robillard L.** G protein specificity: traffic direction required. *Cell Signal* 14: 407–418, 2002.
2. **Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, Pineyro G.** β-Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci USA* 100: 11406–11411, 2003.
3. **Baragli A, Grieco ML, Trieu P, Villeneuve LR, Hébert TE.** Heterodimers of adenylyl cyclases 2 and 5 show enhanced functional responses in the presence of Gαs. *Cell Signal* 20: 480–492, 2008.
4. **Barlow CA, Laishram RS, Anderson RA.** Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum. *Trends Cell Biol* 20: 25–35, 2010.

5. **Boivin B, Chevalier D, Villeneuve LR, Rousseau E, Allen BG.** Functional endothelin receptors are present on nuclei in cardiac ventricular myocytes. *J Biol Chem* 278: 29153–29163, 2003.
6. **Boivin B, Lavoie C, Vaniotis G, Baragli A, Villeneuve LR, Ethier N, Trieu P, Allen BG, Hébert TE.** Functional β-adrenergic receptor signalling on nuclear membranes in adult rat and mouse ventricular cardiomyocytes. *Cardiovasc Res* 71: 69–78, 2006.
7. **Boivin B, Vaniotis G, Allen BG, Hébert TE.** G protein-coupled receptors in and on the cell nucleus: a new signaling paradigm? *J Recept Signal Transduct Res* 28: 15–28, 2008.
8. **Boivin B, Villeneuve LR, Farhat N, Chevalier D, Allen BG.** Subcellular distribution of endothelin signaling pathway components in ventricular myocytes and heart: lack of preformed caveolar signalosomes. *J Mol Cell Cardiol* 38: 665–676, 2005.
9. **Breit A, Lagace M, Bouvier M.** Hetero-oligomerization between β_2- and β_3-adrenergic receptors generates a β-adrenergic signaling unit with distinct functional properties. *J Biol Chem* 279: 28756–28765, 2004.
10. **Bulenger S, Marullo S, Bouvier M.** Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci* 26: 131–137, 2005.
11. **Burchett SA.** In through the out door: nuclear localization of the regulators of G protein signaling. *J Neurochem* 87: 551–559, 2003.
12. **Buu NT, Hui R, Falardeau P.** Norepinephrine in neonatal rat ventricular myocytes: association with the cell nucleus and binding to nuclear α_1- and β-adrenergic receptors. *J Mol Cell Cardiol* 25: 1037–1046, 1993.
13. **Casey LM, Pistner AR, Belmonte SL, Migdalovich D, Stolpnik O, Nwakanma FE, Vorobiof G, Dunaevsky O, Matavel A, Lopes CM, Smrcka AV, Blaxall BC.** Small molecule disruption of Gβγ signaling inhibits the progression of heart failure. *Circ Res* 107: 532–539, 2010.
14. **Chen R, Mukhin YV, Garnovskaya MN, Thielen TE, Iijima Y, Huang C, Raymond JR, Ullian ME, Paul RV.** A functional angiotensin II receptor-GFP fusion protein: evidence for agonist-dependent nuclear translocation. *Am J Physiol Renal Physiol* 279: F440–F448, 2000.
15. **Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG, Crow MT.** The β_2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G_i-dependent coupling to phosphatidylinositol 3′-kinase. *Circ Res* 87: 1172–1179, 2000.
16. **Chidiac P, Hébert TE, Valiquette M, Dennis M, Bouvier M.** Inverse agonist activity of β-adrenergic antagonists. *Mol Pharmacol* 45: 490–499, 1994.
17. **Communal C, Singh K, Pimentel DR, Colucci WS.** Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the β-adrenergic pathway. *Circulation* 98: 1329–1334, 1998.
18. **Cook JL, Re RN.** Intracellular accumulation and nuclear trafficking of angiotensin II and the angiotensin II type I receptor. In: *The Local Cardiac Renin-Angiotensin Aldosterone System*, edited by Frohlich ED and Re RN. New York: Springer, 2007, p. 29–41.
19. **Cook LA, Schey KL, Wilcox MD, Dingus J, Ettling R, Nelson T, Knapp DR, Hildebrandt JD.** Proteomic analysis of bovine brain G protein γ subunit processing heterogeneity. *Mol Cell Proteomics* 5: 671–685, 2006.
20. **Dale LB, Seachrist JL, Babwah AV, Ferguson SS.** Regulation of angiotensin II type 1A receptor intracellular retention, degradation, and recycling by Rab5, Rab7, and Rab11 GTPases. *J Biol Chem* 279: 13110–13118, 2004.
21. **David M, Richer M, Mamarbachi AM, Villeneuve LR, Dupré DJ, Hébert TE.** Interactions between GABA-B_1 receptors and Kir 3 inwardly rectifying potassium channels. *Cell Signal* 18: 2172–2181, 2006.
22. **Devic E, Xiang Y, Gould D, Kobilka B.** β-Adrenergic receptor subtype-specific signaling in cardiac myocytes from β_1 and β_2 adrenoceptor knockout mice. *Mol Pharmacol* 60: 577–583, 2001.
23. **Dong C, Filipeanu CM, Duvernay MT, Wu G.** Regulation of G protein-coupled receptor export trafficking. *Biochim Biophys Acta* 1768: 853–870, 2007.
24. **Drenan RM, Doupnik CA, Boyle MP, Muglia LJ, Huettner JE, Linder ME, Blumer KJ.** Palmitoylation regulates plasma membrane-nuclear shuttling of R7BP, a novel membrane anchor for the RGS7 family. *J Cell Biol* 169: 623–633, 2005.
25. **Dupré DJ, Baragli A, Rebois RV, Ethier N, Hébert TE.** Signalling complexes associated with adenylyl cyclase II are assembled during their biosynthesis. *Cell Signal* 19: 481–489, 2007.
26. **Dupré DJ, Hébert TE.** Biosynthesis and trafficking of seven transmembrane receptor signalling complexes. *Cell Signal* 18: 1549–1559, 2006.
27. **Dupré DJ, Robitaille M, Ethier N, Villeneuve LR, Mamarbachi AM, Hébert TE.** Seven transmembrane receptor core signalling complexes are assembled prior to plasma membrane trafficking. *J Biol Chem* 281: 34561–34573, 2006.
28. **Dupré DJ, Robitaille M, Rebois RV, Hébert TE.** The role of Gβγ subunits in the organization, assembly, and function of GPCR signaling complexes. *Annu Rev Pharmacol Toxicol* 49: 31–56, 2009.
29. **Duvernay MT, Zhou F, Wu G.** A conserved motif for the transport of G protein-coupled receptors from the endoplasmic reticulum to the cell surface. *J Biol Chem* 279: 30741–30750, 2004.
30. **Eisenhofer G.** The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol Ther* 91: 35–62, 2001.
31. **Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ.** Ligand-directed signalling at β-adrenoceptors. *Br J Pharmacol* 159: 1022–1038, 2010.
32. **Ferreiro I, Barragan M, Gubern A, Ballestar E, Joaquin M, Posas F.** The p38 SAPK is recruited to chromatin via its interaction with transcription factors. *J Biol Chem* 285: 31819–31828, 2010.
33. **Filipeanu CM, Zhou F, Claycomb WC, Wu G.** Regulation of the cell-surface expression and function of angiotensin II type 1 receptor by Rab1-mediated ER-to-Golgi transport in cardiac myocytes. *J Biol Chem* 279: 41077–41084, 2004.
34. **Frantz S, Fraccarollo D, Wagner H, Behr TM, Jung P, Angermann CE, Ertl G, Bauersachs J.** Sustained activation of nuclear factor κB and activator protein 1 in chronic heart failure. *Cardiovasc Res* 57: 749–756, 2003.
35. **Freyberg Z, Sweeney D, Siddhanta A, Bourgoin S, Frohman M, Shields D.** Intracellular localization of phospholipase D1 in mammalian cells. *Mol Biol Cell* 12: 943–955, 2001.
36. **Galandrin S, Bouvier M.** Distinct signaling profiles of β_1 and β_2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol Pharmacol* 70: 1575–1584, 2006.
37. **Galandrin S, Oligny-Longpre G, Bouvier M.** The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci* 28: 423–430, 2007.
38. **Garcia-Cazarin ML, Smith JL, Olszewski KA, McCune DF, Simmerman LA, Hadley RW, Kraner SD, Piascik MT.** The α_{1D}-adrenergic receptor is expressed intracellularly and coupled to increases in intracellular calcium and reactive oxygen species in human aortic smooth muscle cells. *J Mol Signal* 3: 6, 2008.
39. **Gobeil F Jr, Bernier SG, Vazquez-Tello A, Brault S, Beauchamp MH, Quiniou C, Marrache AM, Checchin D, Sennlaub F, Hou X, Nader M, Bkaily G, Ribeiro-da-Silva A, Goetzl EJ, Chemtob S.** Modulation of pro-inflammatory gene expression by nuclear lysophosphatidic acid receptor type-1. *J Biol Chem* 278: 38875–38883, 2003.
40. **Gobeil F Jr, Dumont I, Marrache AM, Vazquez-Tello A, Bernier SG, Abran D, Hou X, Beauchamp MH, Quiniou C, Bouayad A, Choufani S, Bhattacharya M, Molotchnikoff S, Ribeiro-Da-Silva A, Varma DR, Bkaily G, and Chemtob S.** Regulation of eNOS expression in brain endothelial cells by perinuclear EP_3 receptors. *Circ Res* 90: 682–689, 2002.
41. **Goetzl EJ.** Diverse pathways for nuclear signaling by G protein-coupled receptors and their ligands. *FASEB J* 21: 638–642, 2007.
42. **Gressner OA, Lahme B, Siluschek M, Rehbein K, Weiskirchen R, Gressner AM.** Intracrine signalling of activin A in hepatocytes upregulates connective tissue growth factor (CTGF/CCN2) expression. *Liver Int* 28: 1207–1216, 2008.
43. **Grundemann D, Schechinger B, Rappold GA, Schomig E.** Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat Neurosci* 1: 349–351, 1998.
44. **Gudermann T, Kalkbrenner F, Schultz G.** Diversity and selectivity of receptor-G protein interactions. *Annu Rev Pharmacol Toxicol* 36: 429–459, 1996.
45. **Gudermann T, Schoneberg T, Schultz G.** Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu Rev Neurosci* 20: 399–427, 1997.
46. **Hébert TE, Bouvier M.** Structural and functional aspects of G protein-coupled receptor oligomerization. *Biochem Cell Biol* 76: 1–11, 1998.
47. **Hepler JR.** R7BP: a surprising new link between G proteins, RGS proteins, and nuclear signaling in the brain. *Sci STKE* 2005: pe38, 2005.
48. **Higashi N, Kunimoto H, Kaneko S, Sasaki T, Ishii M, Kojima H, Nakajima K.** Cytoplasmic c-Fos induced by the YXXQ-derived STAT3

signal requires the co-operative MEK/ERK signal for its nuclear translocation. *Genes Cells* 9: 233–242, 2004.

49. **Hu S, Xie Z, Onishi A, Yu X, Jiang L, Lin J, Rho HS, Woodard C, Wang H, Jeong JS, Long S, He X, Wade H, Blackshaw S, Qian J, Zhu H.** Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. *Cell* 139: 610–622, 2009.
50. **Ito K, Akazawa H, Tamagawa M, Furukawa K, Ogawa W, Yasuda N, Kudo Y, Liao CH, Yamamoto R, Sato T, Molkentin JD, Kasuga M, Noda T, Nakaya H, Komuro I.** PDK1 coordinates survival pathways and β-adrenergic response in the heart. *Proc Natl Acad Sci USA* 106: 8689–8694, 2009.
51. **Jafri F, Ergul A.** Nuclear localization of endothelin-converting enzyme-1: subisoform specificity. *Arterioscler Thromb Vasc Biol* 23: 2192–2196, 2003.
52. **Johnson LR, Scott MG, Pitcher JA.** G protein-coupled receptor kinase 5 contains a DNA-binding nuclear localization sequence. *Mol Cell Biol* 24: 10169–10179, 2004.
53. **Jong YJ, Kumar V, O'Malley KL.** Intracellular metabotropic glutamate receptor 5 (mGluR5) activates signaling cascades distinct from cell surface counterparts. *J Biol Chem* 284: 35827–35838, 2009.
54. **Jonker JW, Schinkel AH.** Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1–3). *J Pharmacol Exp Ther* 308: 2–9, 2004.
55. **Kalkbrenner F, Degtiar VE, Schenker M, Brendel S, Zobel A, Heschler J, Wittig B, Schultz G.** Subunit composition of G_o proteins functionally coupling galanin receptors to voltage-gated calcium channels. *EMBO J* 14: 4728–4737, 1995.
56. **Kenakin T.** Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. *Trends Pharmacol Sci* 28: 407–415, 2007.
57. **Kenakin T.** Functional selectivity and biased receptor signaling. *J Pharmacol Exp Ther* 336: 296–302, 2011.
58. **Kenakin T.** Functional selectivity through protean and biased agonism: who steers the ship? *Mol Pharmacol* 72: 1393–1401, 2007.
59. **Kim CG, Park D, Rhee SG.** The role of carboxyl-terminal basic amino acids in Gqα-dependent activation, particulate association, and nuclear localization of phospholipase C-β1. *J Biol Chem* 271: 21187–21192, 1996.
60. **Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, Rockman HA.** β-Blockers alprenolol and carvedilol stimulate β-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci USA* 105: 14555–14560, 2008.
61. **Kino T, Kozasa T, Chrousos GP.** Statin-induced blockade of prenylation alters nucleocytoplasmic shuttling of GTP-binding proteins γ2 and β2 and enhances their suppressive effect on glucocorticoid receptor transcriptional activity. *Eur J Clin Invest* 35: 508–513, 2005.
62. **Kino T, Tiulpakov A, Ichijo T, Chheng L, Kozasa T, Chrousos GP.** G protein β interacts with the glucocorticoid receptor and suppresses its transcriptional activity in the nucleus. *J Cell Biol* 169: 885–896, 2005.
63. **Kitanaka J, Kitanaka N, Takemura M, Wang XB, Hembree CM, Goodman NL, Uhl GR.** Isolation and sequencing of a putative promoter region of the murine G protein β_1 subunit (GNB1) gene. *DNA Seq* 13: 39–45, 2002.
64. **Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G, Wittig B.** Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature* 353: 43–48, 1991.
65. **Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B.** Different β-subunits determine G-protein interaction with transmembrane receptors. *Nature* 358: 424–426, 1992.
66. **Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B.** Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. *Science* 259: 832–834, 1993.
67. **Kolch W, Pitt A.** Functional proteomics to dissect tyrosine kinase signalling pathways in cancer. *Nat Rev Cancer* 10: 618–629, 2010.
68. **Kumar V, Jong YJ, O'Malley KL.** Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca^{2+} release. *J Biol Chem* 283: 14072–14083, 2008.
69. **Kuschel M, Zhou YY, Cheng H, Zhang SJ, Chen Y, Lakatta EG, Xiao RP.** G_i protein-mediated functional compartmentalization of cardiac β_2-adrenergic signaling. *J Biol Chem* 274: 22048–22052, 1999.
70. **Lavine N, Ethier N, Oak JN, Pei L, Liu F, Trieu P, Rebois RV, Bouvier M, Hébert TE, Van Tol HH.** G protein-coupled receptors form stable complexes with inwardly rectifying potassium channels and adenylyl cyclase. *J Biol Chem* 277: 46010–46019, 2002.
71. **Lavoie C, Hébert TE.** Pharmacological characterization of putative β_1-β_2-adrenergic receptor heterodimers. *Can J Physiol Pharmacol* 81: 186–195, 2003.
72. **Lavoie C, Mercier JF, Salahpour A, Umapathy D, Breit A, Villeneuve LR, Zhu WZ, Xiao RP, Lakatta EG, Bouvier M, Hébert TE.** β_1/β_2-adrenergic receptor heterodimerization regulates β_2-adrenergic receptor internalization and ERK signaling efficacy. *J Biol Chem* 277: 35402–35410, 2002.
73. **Lee DK, Lanca AJ, Cheng R, Nguyen T, Ji XD, Gobeil F Jr, Chemtob S, George SR, O'Dowd BF.** Agonist-independent nuclear localization of the apelin, angiotensin AT1, and bradykinin B2 receptors. *J Biol Chem* 279: 7901–7908, 2004.
74. **Lee TH, Seng S, Sekine M, Hinton C, Fu Y, Avraham HK, Avraham S.** Vascular endothelial growth factor mediates intracrine survival in human breast carcinoma cells through internally expressed VEGFR1/FLT1. *PLoS Med* 4: e186, 2007.
75. **Leroy J, Abi-Gerges A, Nikolaev VO, Richter W, Lechene P, Mazet JL, Conti M, Fischmeister R, Vandecasteele G.** Spatiotemporal dynamics of β-adrenergic cAMP signals and L-type Ca^{2+} channel regulation in adult rat ventricular myocytes: role of phosphodiesterases. *Circ Res* 102: 1091–1100, 2008.
76. **Liu Z, Fisher RA.** RGS6 interacts with DMAP1 and DNMT1 and inhibits DMAP1 transcriptional repressor activity. *J Biol Chem* 279: 14120–14128, 2004.
77. **Lopez-Gimenez JF, Canals M, Pediani JD, Milligan G.** The α_{1b}-adrenoceptor exists as a higher-order oligomer: effective oligomerization is required for receptor maturation, surface delivery, and function. *Mol Pharmacol* 71: 1015–1029, 2007.
78. **Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ.** A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. *Nat Med* 15: 75–83, 2009.
79. **Lorenz K, Schmitt JP, Vidal M, Lohse MJ.** Cardiac hypertrophy: targeting Raf/MEK/ERK1/2-signaling. *Int J Biochem Cell Biol* 41: 2351–2355, 2009.
80. **Lu D, Yang H, Shaw G, Raizada MK.** Angiotensin II-induced nuclear targeting of the angiotensin type 1 (AT1) receptor in brain neurons. *Endocrinology* 139: 365–375, 1998.
81. **Lucero HA, Kintsurashvili E, Marketou ME, Gavras H.** Cell signaling, internalization, and nuclear localization of the angiotensin converting enzyme in smooth muscle and endothelial cells. *J Biol Chem* 285: 5555–5568, 2010.
82. **Luttrell LM, Gesty-Palmer D.** Beyond desensitization: physiological relevance of arrestin-dependent signaling. *Pharmacol Rev* 62: 305–330, 2010.
83. **Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ.** Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. *Nat Med* 13: 315–323, 2007.
84. **Malnou CE, Salem T, Brockly F, Wodrich H, Piechaczyk M, Jariel-Encontre I.** Heterodimerization with Jun family members regulates c-Fos nucleocytoplasmic traffic. *J Biol Chem* 282: 31046–31059, 2007.
85. **Marrache AM, Gobeil F Jr, Bernier SG, Stankova J, Rola-Pleszczynski M, Choufani S, Bkaily G, Bourdeau A, Sirois MG, Vazquez-Tello A, Fan L, Joyal JS, Filep JG, Varma DR, Ribeiro-Da-Silva A, Chemtob S.** Proinflammatory gene induction by platelet-activating factor mediated via its cognate nuclear receptor. *J Immunol* 169: 6474–6481, 2002.
86. **Mercier JF, Salahpour A, Angers S, Breit A, Bouvier M.** Quantitative assessment of β_1- and β_2-adrenergic receptor homo- and heterodimerization by bioluminescence resonance energy transfer. *J Biol Chem* 277: 44925–44931, 2002.
87. **Milligan G.** G protein-coupled receptor hetero-dimerization: contribution to pharmacology and function. *Br J Pharmacol* 158: 5–14, 2009.
88. **Milligan G.** The role of dimerisation in the cellular trafficking of G-protein-coupled receptors. *Curr Opin Pharmacol* 10: 23–29, 2010.
89. **Miyamoto S, Rubio M, Sussman MA.** Nuclear and mitochondrial signalling Akts in cardiomyocytes. *Cardiovasc Res* 82: 272–285, 2009.
90. **Moens AL, Leyton-Mange JS, Niu X, Yang R, Cingolani O, Arkenbout EK, Champion HC, Bedja D, Gabrielson KL, Chen J, Xia Y, Hale AB, Channon KM, Halushka MK, Barker N, Wuyts FL, Kaminski PM, Wolin MS, Kass DA, Barouch LA.** Adverse ventricular remodeling and exacerbated NOS uncoupling from pressure-overload in

mice lacking the β3-adrenoreceptor. *J Mol Cell Cardiol* 47: 576–585, 2009.
91. **Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, Le Corvoisier P, Violin JD, Wei H, Lefkowitz RJ, Rockman HA.** β-Arrestin-mediated β_1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest* 117: 2445–2458, 2007.
92. **O'Malley KL, Jong YJ, Gonchar Y, Burkhalter A, Romano C.** Activation of metabotropic glutamate receptor mGlu5 on nuclear membranes mediates intranuclear Ca^{2+} changes in heterologous cell types and neurons. *J Biol Chem* 278: 28210–28219, 2003.
93. **Park JG, Muise A, He GP, Kim SW, Ro HS.** Transcriptional regulation by the γ5 subunit of a heterotrimeric G protein during adipogenesis. *EMBO J* 18: 4004–4012, 1999.
94. **Patel CB, Noor N, Rockman HA.** Functional selectivity in adrenergic and angiotensin signaling systems. *Mol Pharmacol* 78: 983–992, 2010.
95. **Pétrin D, Hébert TE.** Imaging-based approaches to understanding G protein-coupled receptor signalling complexes. *Methods Mol Biol* 756: 37–60, 2011.
96. **Picht E, Zima AV, Shannon TR, Duncan AM, Blatter LA, Bers DM.** Dynamic calcium movement inside cardiac sarcoplasmic reticulum during release. *Circ Res* 108: 847–856, 2011.
97. **Pokholok DK, Zeitlinger J, Hannett NM, Reynolds DB, Young RA.** Activated signal transduction kinases frequently occupy target genes. *Science* 313: 533–536, 2006.
98. **Prinster SC, Hague C, Hall RA.** Heterodimerization of G protein-coupled receptors: specificity and functional significance. *Pharmacol Rev* 57: 289–298, 2005.
99. **Rajagopal K, Lefkowitz RJ, Rockman HA.** When 7 transmembrane receptors are not G protein-coupled receptors. *J Clin Invest* 115: 2971–2974, 2005.
100. **Re M, Pampillo M, Savard M, Dubuc C, McArdle CA, Millar RP, Conn PM, Gobeil F Jr, Bhattacharya M, Babwah AV.** The human gonadotropin releasing hormone type I receptor is a functional intracellular GPCR expressed on the nuclear membrane. *PLoS One* 5: e11489, 2010.
101. **Re R.** Intracellular renin-angiotensin system: the tip of the intracrine physiology iceberg. *Am J Physiol Heart Circ Physiol* 293: H905–H906, 2007.
102. **Rebois RV, Hébert TE.** Protein complexes involved in heptahelical receptor-mediated signal transduction. *Receptors Channels* 9: 169–194, 2003.
103. **Rebois RV, Robitaille M, Gales C, Dupré DJ, Baragli A, Trieu P, Ethier N, Bouvier M, Hébert TE.** Heterotrimeric G proteins form stable complexes with adenylyl cyclase and Kir3.1 channels in living cells. *J Cell Sci* 119: 2807–2818, 2006.
104. **Robben JH, Kortenoeven ML, Sze M, Yae C, Milligan G, Oorschot VM, Klumperman J, Knoers NV, Deen PM.** Intracellular activation of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus by nonpeptide agonists. *Proc Natl Acad Sci USA* 106: 12195–12200, 2009.
105. **Robillard L, Ethier N, Lachance M, Hébert TE.** Gβγ subunit combinations differentially modulate receptor and effector coupling in vivo. *Cell Signal* 12: 673–682, 2000.
106. **Robishaw JD, Guo ZP, Wang Q.** Ribozymes as tools for suppression of G protein γ subunits. *Methods Mol Biol* 237: 169–180, 2003.
107. **Robitaille M, Gora S, Wang Y, Goupil E, Pétrin D, Del Duca D, Villeneuve LR, Allen BG, Laporte SA, Bernard DJ, Hébert TE.** Gβγ is a negative regulator of AP-1 mediated transcription. *Cell Signal* 22: 1254–1266, 2010.
108. **Robitaille M, Ramakrishnan N, Baragli A, Hébert TE.** Intracellular trafficking and assembly of specific Kir3 channel/G protein complexes. *Cell Signal* 21: 488–501, 2009.
109. **Rojkova AM, Woodard GE, Huang TC, Combs CA, Zhang JH, Simonds WF.** Gγ subunit-selective G protein β5 mutant defines regulators of G protein signaling protein binding requirement for nuclear localization. *J Biol Chem* 278: 12507–12512, 2003.
110. **Saito T, Giaid A.** Cyclooxygenase-2 and nuclear factor-κB in myocardium of end stage human heart failure. *Congest Heart Fail* 5: 222–227, 1999.
111. **Salahpour A, Angers S, Mercier JF, Lagace M, Marullo S, Bouvier M.** Homodimerization of the β_2-adrenergic receptor as a prerequisite for cell surface targeting. *J Biol Chem* 279: 33390–33397, 2004.
112. **Samama P, Pei G, Costa T, Cotecchia S, Lefkowitz RJ.** Negative antagonists promote an inactive conformation of the β_2-adrenergic receptor. *Mol Pharmacol* 45: 390–394, 1994.
113. **Sastri M, Barraclough DM, Carmichael PT, Taylor SS.** A-kinase-interacting protein localizes protein kinase A in the nucleus. *Proc Natl Acad Sci USA* 102: 349–354, 2005.
114. **Sato M, Horinouchi T, Hutchinson DS, Evans BA, Summers RJ.** Ligand-directed signaling at the β_3-adrenoceptor produced by 3-(2-Ethylphenoxy)-1-[(1,S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propan ol oxalate (SR59230A) relative to receptor agonists. *Mol Pharmacol* 72: 1359–1368, 2007.
115. **Sato M, Hutchinson DS, Evans BA, Summers RJ.** The β_3-adrenoceptor agonist 4-[[(Hexylamino)carbonyl]amino]-N-[4-[2-[[(2S)-2-hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl]-phenyl]-benzenesulfonamide (L755507) and antagonist (S)-N-[4-[2-[[3-[3-(acetamidomethyl)phenoxy]-2-hydroxypropyl]amino]-ethyl] phenyl]benzenesulfonamide (L748337) activate different signaling pathways in Chinese hamster ovary-K1 cells stably expressing the human β_3-adrenoceptor. *Mol Pharmacol* 74: 1417–1428, 2008.
116. **Savard M, Barbaz D, Belanger S, Muller-Esterl W, Bkaily G, D'Orleans-Juste P, Cote J, Bovenzi V, Gobeil F Jr.** Expression of endogenous nuclear bradykinin B2 receptors mediating signaling in immediate early gene activation. *J Cell Physiol* 216: 234–244, 2008.
117. **Schievella AR, Regier MK, Smith WL, Lin LL.** Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* 270: 30749–30754, 1995.
118. **Schulze W, Buchwalow IB.** Adenylyl cyclase in the heart: an enzymocytochemical and immunocytochemical approach. *Microsc Res Tech* 40: 473–478, 1998.
119. **Schwindinger WF, Betz KS, Giger KE, Sabol A, Bronson SK, Robishaw JD.** Loss of G protein γ7 alters behavior and reduces striatal α(olf) level and cAMP production. *J Biol Chem* 278: 6575–6579, 2003.
120. **Scott MG, Le Rouzic E, Perianin A, Pierotti V, Enslen H, Benichou S, Marullo S, Benmerah A.** Differential nucleocytoplasmic shuttling of β-arrestins. Characterization of a leucine-rich nuclear export signal in β-arrestin2. *J Biol Chem* 277: 37693–37701, 2002.
121. **Seachrist JL, Anborgh PH, Ferguson SS.** β_2-Adrenergic receptor internalization, endosomal sorting, and plasma membrane recycling are regulated by rab GTPases. *J Biol Chem* 275: 27221–27228, 2000.
122. **Seachrist JL, Laporte SA, Dale LB, Babwah AV, Caron MG, Anborgh PH, Ferguson SS.** Rab5 association with the angiotensin II type 1A receptor promotes Rab5 GTP binding and vesicular fusion. *J Biol Chem* 277: 679–685, 2002.
123. **Smotrys JE, Linder ME.** Palmitoylation of intracellular signaling proteins: regulation and function. *Annu Rev Biochem* 73: 559–587, 2004.
124. **Spiegelberg BD, Hamm HE.** Gβγ binds histone deacetylase 5 (HDAC5) and inhibits its transcriptional co-repression activity. *J Biol Chem* 280: 41769–41776, 2005.
125. **Steinberg SF.** β_2-Adrenergic receptor signaling complexes in cardiomyocyte caveolae/lipid rafts. *J Mol Cell Cardiol* 37: 407–415, 2004.
126. **Steinberg SF.** The molecular basis for distinct β-adrenergic receptor subtype actions in cardiomyocytes. *Circ Res* 85: 1101–1111, 1999.
127. **Steinberg SF, Brunton LL.** Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol* 41: 751–773, 2001.
128. **Sun Y, Yamauchi J, Kaziro Y, Itoh H.** Activation of c-fos promoter by Gβγ-mediated signaling: involvement of Rho and c-Jun N-terminal kinase. *J Biochem* 125: 515–521, 1999.
129. **Tadevosyan A, Maguy A, Villeneuve LR, Babin J, Bonnefoy A, Allen BG, Nattel S.** Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J Biol Chem* 285: 22338–22349, 2010.
130. **Tanos T, Marinissen MJ, Leskow FC, Hochbaum D, Martinetto H, Gutkind JS, Coso OA.** Phosphorylation of c-Fos by members of the p38 MAPK family. Role in the AP-1 response to UV light. *J Biol Chem* 280: 18842–18852, 2005.
131. **Terrillon S, Bouvier M.** Roles of G-protein-coupled receptor dimerization. *EMBO Rep* 5: 30–34, 2004.
132. **Tilley DG, Kim IM, Patel PA, Violin JD, Rockman HA.** β-Arrestin mediates β_1-adrenergic receptor-epidermal growth factor receptor interaction and downstream signaling. *J Biol Chem* 284: 20375–20386, 2009.
133. **Turjanski AG, Vaque JP, Gutkind JS.** MAP kinases and the control of nuclear events. *Oncogene* 26: 3240–3253, 2007.

134. **Vaniotis G, Del Duca D, Trieu P, Rohlicek CV, Hébert TE, Allen BG.** Nuclear β-adrenergic receptors modulate gene expression in adult rat heart. *Cell Signal* 23: 89–98, 2011.
135. **Wang P, Wu Y, Ge X, Ma L, Pei G.** Subcellular localization of β-arrestins is determined by their intact N domain and the nuclear export signal at the C terminus. *J Biol Chem* 278: 11648–11653, 2003.
136. **Wang Q, Jolly JP, Surmeier JD, Mullah BM, Lidow MS, Bergson CM, Robishaw JD.** Differential dependence of the D1 and D5 dopamine receptors on the G protein γ7 subunit for activation of adenylylcyclase. *J Biol Chem* 276: 39386–39393, 2001.
137. **Wang Q, Mullah B, Hansen C, Asundi J, Robishaw JD.** Ribozyme-mediated suppression of the G protein γ7 subunit suggests a role in hormone regulation of adenylyl cyclase activity. *J Biol Chem* 272: 26040–26048, 1997.
138. **Wang Q, Mullah BK, Robishaw JD.** Ribozyme approach identifies a functional association between the G protein β1γ7 subunits in the β-adrenergic receptor signaling pathway. *J Biol Chem* 274: 17365–17371, 1999.
139. **Watson PH, Fraher LJ, Natale BV, Kisiel M, Hendy GN, Hodsman AB.** Nuclear localization of the type 1 parathyroid hormone/parathyroid hormone-related peptide receptor in MC3T3-E1 cells: association with serum-induced cell proliferation. *Bone* 26: 221–225, 2000.
140. **Weiss JM, Morgan PH, Lutz MW, Kenakin TP.** The cubic ternary complex receptor-occupancy model. I. Model description. *J Theor Biol* 178: 151–167, 1996.
141. **Willard FS, Crouch MF.** Nuclear and cytoskeletal translocation and localization of heterotrimeric G-proteins. *Immunol Cell Biol* 78: 387–394, 2000.
142. **Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, O'Connell TD.** Nuclear α_1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. *Circ Res* 103: 992–1000, 2008.
143. **Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T, Lefkowitz RJ, Koch WJ, Lakatta EG.** Coupling of β_2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes. *Circ Res* 84: 43–52, 1999.
144. **Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG.** Recent advances in cardiac β_2-adrenergic signal transduction. *Circ Res* 85: 1092–1100, 1999.
145. **Xiao RP, Ji X, Lakatta EG.** Functional coupling of the β_2-adrenoceptor to a pertussis toxin- sensitive G protein in cardiac myocytes. *Mol Pharmacol* 47: 322–329, 1995.
146. **Xiao RP, Zhu W, Zheng M, Chakir K, Bond R, Lakatta EG, Cheng H.** Subtype-specific β-adrenoceptor signaling pathways in the heart and their potential clinical implications. *Trends Pharmacol Sci* 25: 358–365, 2004.
147. **Yamamoto S, Kawamura K, James TN.** Intracellular distribution of adenylate cyclase in human cardiocytes determined by electron microscopic cytochemistry. *Microsc Res Tech* 40: 479–487, 1998.
148. **Yi XP, Gerdes AM, Li F.** Myocyte redistribution of GRK2 and GRK5 in hypertensive, heart-failure-prone rats. *Hypertension* 39: 1058–1063, 2002.
149. **Yi XP, Zhou J, Baker J, Wang X, Gerdes AM, Li F.** Myocardial expression and redistribution of GRKs in hypertensive hypertrophy and failure. *Anat Rec A Discov Mol Cell Evol Biol* 282: 13–23, 2005.
150. **Yuen JW, Poon LS, Chan AS, Yu FW, Lo RK, Wong YH.** Activation of STAT3 by specific Gα subunits and multiple Gβγ dimers. *Int J Biochem Cell Biol* 42: 1052–1059, 2010.
151. **Zhang JH, Barr VA, Mo Y, Rojkova AM, Liu S, Simonds WF.** Nuclear localization of G protein β5 and regulator of G protein signaling 7 in neurons and brain. *J Biol Chem* 276: 10284–10289, 2001.
152. **Zhu WZ, Chakir K, Zhang S, Yang D, Lavoie C, Bouvier M, Hebert TE, Lakatta EG, Cheng H, Xiao RP.** Heterodimerization of β_1- and β_2-adrenergic receptor subtypes optimizes β-adrenergic modulation of cardiac contractility. *Circ Res* 97: 244–251, 2005.
153. **Zhuo JL, Imig JD, Hammond TG, Orengo S, Benes E, Navar LG.** Ang II accumulation in rat renal endosomes during Ang II-induced hypertension: role of AT(1) receptor. *Hypertension* 39: 116–121, 2002.