

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

George Vaniotis,^{1,2} Bruce G. Allen,^{1,2,3,4} and Terence E. Hébert⁴

¹Montreal Heart Institute; Departments of ²Biochemistry and ³Medicine, Université de Montréal; and ⁴Department of Pharmacology and Therapeutics, McGill University, Montréal, Québec, Canada

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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,

Address for reprint requests and other correspondence: T. E. Hébert, Dept. of Pharmacology and Therapeutics, McGill Univ., Rm. 1303. McIntyre Medical Sciences Bldg., 3655 Promenade Sir William Osler, Montréal, QC, H3G 1Y6, Canada (e-mail: terence.hebert@mcgill.ca).

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 B₂ 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 V₂ 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 A₂ (117), phospholipase C β (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- κ B 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 GSK β) 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

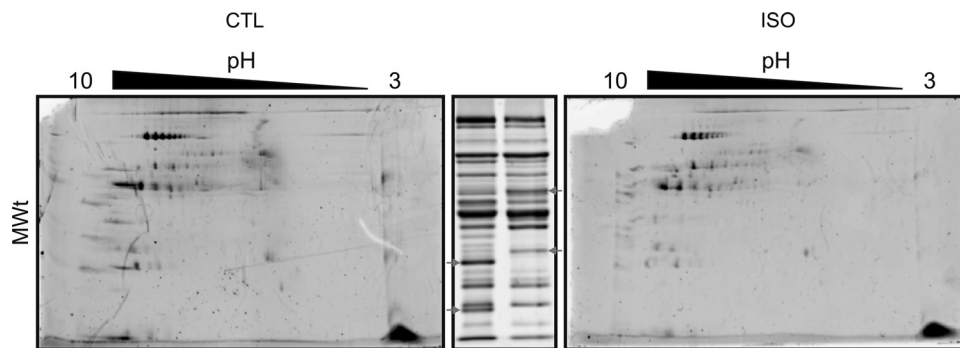


Fig. 1. β -Adrenergic receptor (β -AR) stimulation results in alterations in the nuclear phosphoproteome. Isoproterenol (Iso) alters protein phosphorylation patterns in nuclei isolated from rat ventricular myocardium. Enriched nuclear fractions were resuspended in a buffer comprising 150 mM Tris-HCl (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 μ M 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 [3H]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,

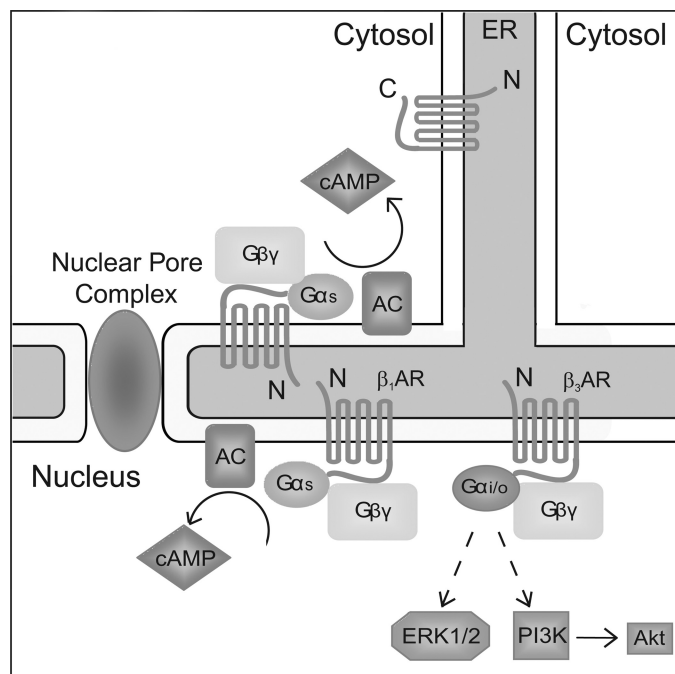


Fig. 2. Possible orientation of nuclear β -AR signaling complexes. Both the β_1 -AR, coupled to G_s , and the β_3 -AR, coupled to G_i , but not the β_2 -AR, are resident on the nuclear membrane, at least in rat and mouse adult ventricular cardiomyocytes (6, 134). How these receptors are trafficked to 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, NH₂-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 [³H]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 $\beta\gamma$ -subunits. $G\alpha$ subunits bind and hydrolyze GTP and were classically believed responsible for most effector activation effects. More recent work has shown that $G\beta\gamma$ 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 $G\beta\gamma$ subunits. $G\beta_1\gamma_2$ 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 $G\beta\gamma$ dimers interacted with HDAC5, releasing MEF2 and allowing it to stimulate transcriptional activity. Both the $G\alpha_{i/o}$ inhibitor PTX and the $G\beta\gamma$ scavenger, β -AR kinase COOH terminus, inhibited MEF2 activity (124). It remains uncertain as to whether cytoplasmic $G\beta\gamma$ dimers sequester HDAC or whether these events exclusively occur in the nucleus.

$G\beta_5$ 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- $G\beta_5$ complex, which is localized to both the cytosol and the nucleus (151). The R7BP protein interacts with the R7- $G\beta_5$ pair and potentiates the capacity of this complex to modulate inwardly rectifying K⁺ (Kir)3 channels in response to M₂ muscarinic receptor stimulation (24). R7BP is palmitoylated, and this interaction allows anchoring of RGS7- $G\beta_5$ 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- $G\beta_5$ 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 $G\beta_5$ subunits unable to form a complex with RGS7 but still capable of interacting with $G\gamma_2$ were not found in the nucleus of either HEK293 or PC12 cells, suggesting the importance of the RGS protein in the nuclear localization of $G\beta_5$ (109).

It has been shown that $G\beta\gamma$ subunits containing the other $G\beta$ isoforms can interact with the transcriptional repressor known as the adipocyte enhancer-binding protein (AEBP1) (93). AEBP1 specifically forms a complex with $G\beta\gamma$ subunits containing $G\gamma_5$ in nuclei of 3T3-L1 but interestingly not NIH 3T3 cells. The $G\beta\gamma_5$ /AEBP1 interaction attenuates its transcriptional repression activity. Another $G\beta\gamma$ 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 $G\beta_1$ - and $G\beta_2$ subunits directly interact with the GR and translocate

with it to the nucleus following treatment with the agonist dexamethasone (61, 62). The interaction of G $\beta\gamma$ with GR suppresses transcriptional activity most likely by associating with transcriptional complexes formed on GR-responsive promoters. G $\beta 2$ mutants unable to bind G γ cannot suppress GR transcriptional activity. These studies begin to highlight a central role of G $\beta\gamma$ in numerous subcellular compartments, directly regulating fundamental processes as diverse as transcription and protein trafficking in the ER and Golgi. G $\beta\gamma$ subunits are more than simply signaling molecules responsive to GPCR stimulation.

We have recently shown that the coexpression of G $\beta\gamma$ 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 G $\beta\gamma$ subunits. G $\beta\gamma$ did not interfere with the dimerization of Fos and Jun or the ability of AP-1 complexes to bind DNA. Rather, G $\beta\gamma$ 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 G $\beta\gamma$ subunits as transcriptional regulators may be potentially independent of their classical functions as mediators of GPCR signaling.

Recently, a number of novel interactors of G $\beta\gamma$ have been identified, as have G $\beta\gamma$ -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 G $\beta\gamma$ have been identified. The modulation of prenylation status has been shown to increase the amount of G $\beta\gamma$ in the nucleus associated with the GR (61, 62). Recent reports have indicated that a subpopulation of G γ subunits may escape from being prenylated and thus remain soluble (19). This may suggest that some of the transcriptional effects of G $\beta\gamma$ may in fact be receptor independent and depend on protein complexes formed with “free” G $\beta\gamma$ or G protein heterotrimers (Table 1). In fact, under basal conditions, we noted the presence of G $\beta\gamma$ subunits in many cell types include cardiomyocytes, suggesting they may be resident there (101). These observations suggest that G $\beta\gamma$ 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 G $\beta\gamma$ 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 G $\beta\gamma$ 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 G $\beta\gamma$ regulation (150). The source of G $\beta\gamma$ for these two classes of events may in fact be different; i.e., the formation of G $\beta\gamma$ /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” G $\beta\gamma$ in the cell. In preliminary experiments, we noted that an overexpression of G α_q did not alter the response of an AP-1 reporter gene to the presence of G $\beta\gamma$, suggesting that these latter two possibilities must be considered (S. Gora and T. E. Hébert, unpublished study). Interestingly, the G $\beta 1$ promoter contains several putative AP-1 response elements (63). De novo synthesis or release of G $\beta\gamma$ may therefore result from increased levels of Fos synthesis. All five G β subunits inhibited AP-1 activity in reporter assays, suggesting that this is a common feature of G $\beta\gamma$ 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 G $\beta\gamma$ interactors strictly dependent on GPCR activation?

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

Canonical G $\beta\gamma$ -interacting proteins may initially interact with G $\beta\gamma$ subunits in the endoplasmic reticulum during biosynthesis (reviewed in Ref. 28) but are usually associated with G protein-coupled receptor (GPCR) signaling at the plasma membrane. However, a number of transcriptional regulators also interact with G $\beta\gamma$ subunits in the cytosol or in the nucleus. Studies described in the text suggest that if G γ subunits in a G $\beta\gamma$ dimer are not prenylated, and presumably not targeted to the plasma membrane, they accumulate in the nucleus. This suggests the possibility that some such G $\beta\gamma$ subunits may signal in a GPCR-independent manner. How the cell organizes these different possible signaling complexes is unclear at present. Specific G β or G γ 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 $G\beta\gamma$ subunits as well as effectors such as Kir3 channels, and AC with nascent $G\beta\gamma$. 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 ,

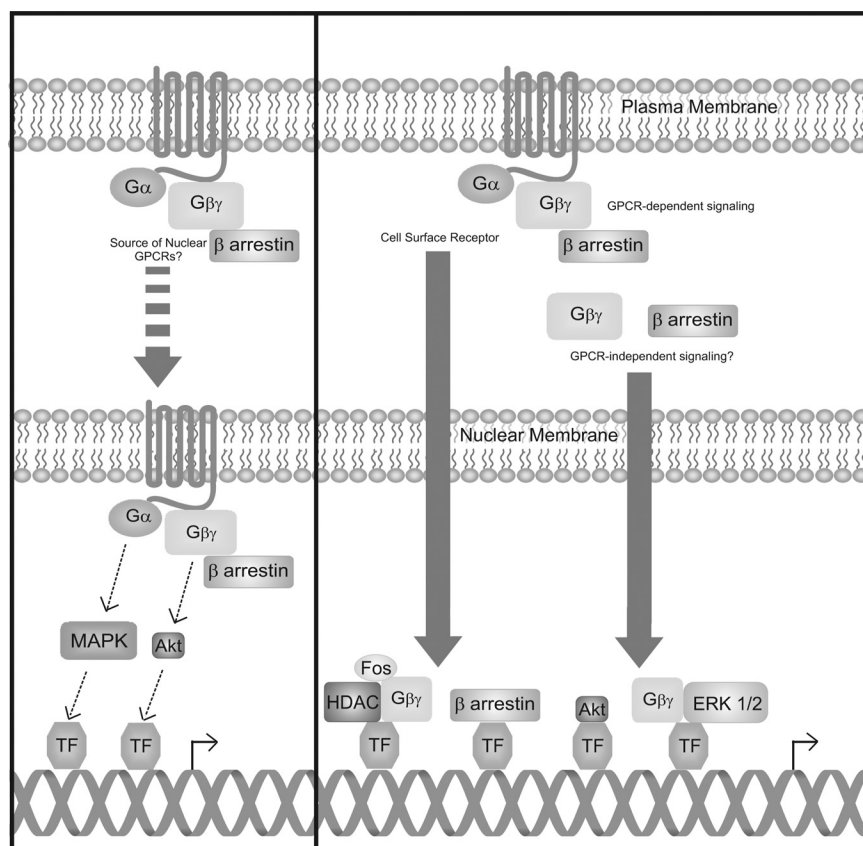


Fig. 3. Novel transcriptional complexes containing transcription factors (TFs), protein kinases, and $G\beta\gamma$ subunits. *Left*: both cell surface and nuclear GPCRs may activate local signaling proteins including G proteins, β -arrestin, and nuclear protein kinases that modulate the activity of 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 $G\beta\gamma$ 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 $G\alpha$ 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 $G\beta\gamma$ 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 $G\beta\gamma$ 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 $G\beta\gamma$ 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- κ B 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 $G\beta\gamma$ -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 $G\beta\gamma$ dimers has been described, the molecular mechanisms involved in controlling interactions between $G\beta\gamma$ 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 $G\beta\gamma$, leading to an autophosphorylation of ERK1/2 on Thr188 and a subsequent accumulation of ERK1/2 in the nucleus (78). Whether ERK1/2 and $G\beta\gamma$ 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 $G\beta\gamma$ effects depend on prior activation of cell surface or nuclear GPCRs or are dependent on pools of “free” $G\beta\gamma$ (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.

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