Subcellular compartmentalization of proximal Gαq-receptor signaling produces unique hypertrophic phenotypes in adult cardiac myocytes

Erika F. Dahl BSc¹, Steven C. Wu PhD², Chastity L. Healy BSc², Brian A. Harsch MSc³, Gregory C. Shearer³, PhD, and Timothy D. O’Connell, PhD²,⁴

From the ¹Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, U.S.A.; ²Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN 55455, U.S.A.; ³Department of Nutritional Sciences, Pennsylvania State University, University Park, PA 16802, U.S.A.

Running title: Compartmentalized myocyte Gq-receptor signaling

Key words: cell signaling, signaling mechanism, myocardial biology, GPCR, G protein-coupled receptor, intracellular compartmentalization, G protein signaling, adrenergic receptor, angiotensin receptor

⁴Corresponding Author
Timothy D. O’Connell, PhD

Address for Correspondence:
Department of Integrative Biology and Physiology
The University of Minnesota
2231 6th St SE, Cancer and Cardiovascular Research Building, 3-141
Minneapolis, MN 55455
Telephone: 612-625-6750
Fax: 612-301-1543
Email: tdoconn@umn.edu

Word count: 8,745

Funding: This work was funded by start-up funds from the University of Minnesota (Minneapolis, MN) (TDO) and start-up funds from Pennsylvania State University (University Park, PA) (GCS).
ABSTRACT

G protein–coupled receptors that signal through Gαq (Gq receptors), such as α1-adrenergic receptors (α1-ARs) or angiotensin receptors, share a common proximal signaling pathway that activates phospholipase Cβ1 (PLCβ1), which cleaves phosphatidylinositol-4,5-bisphosphate (PIP2) to produce inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. Despite these common proximal signaling mechanisms, Gq receptors produce distinct physiological responses, yet the mechanistic basis for this remains unclear. In the heart, Gq receptors are thought to induce myocyte hypertrophy through a mechanism termed excitation–transcription coupling, which provides a mechanistic basis for compartmentalization of calcium required for contraction versus IP3-dependent intranuclear calcium required for hypertrophy. Here, we identified subcellular compartmentalization of Gq-receptor signaling as a mechanistic basis for unique Gq receptor–induced hypertrophic phenotypes in cardiac myocytes. We show that α1-adrenergic receptors (α1-ARs) co-localize with PLCβ1 and PIP2 at the nuclear membrane. Further, nuclear α1-ARs induced intranuclear PLCβ1 activity, leading to histone deacetylase 5 (HDAC5) export and a robust transcriptional response, i.e. significant up- or down-regulation of 806 genes. Conversely, we found that angiotensin receptors localize to the sarcolemma and induce sarcolemmal PLCβ1 activity, but fail to promote HDAC5 nuclear export, while producing a transcriptional response that is mostly a subset of α1-AR–induced transcription. In summary, these results link Gq-receptor compartmentalization in cardiac myocytes to unique hypertrophic transcription. They suggest a new model of excitation–transcription coupling in adult cardiac myocytes that accounts for differential Gq-receptor localization and better explains distinct physiological functions of Gq receptors.

INTRODUCTION

G-protein coupled receptors that signal through Gαq (Gq-receptors) share a common proximal signaling pathway through the activation of phospholipase Cβ1 (PLCβ1), which cleaves phosphatidylinositol-4,5-bisphosphate (PIP2) to produce inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (1). However, in any cell, simultaneous activation of multiple proximal Gq-receptor signaling events would preclude the cell’s ability to process different signals and produce a unique outcome. Despite this commonality, each Gq-receptor mediates distinct physiologic processes, but how this specificity is achieved is unclear in many cases. Compartmentalization of signaling provides one mechanism through which a cell could handle a multitude of potentially overlapping signals. Cardiac myocytes offer several examples of compartmentalized receptor signaling, for instance, the ability to discriminate β-adrenergic (β-AR)-Gα1-induced calcium signals that augment contractility from Gq-receptor-mediated calcium signals that induce hypertrophy (2). In this case, inositol sensitive calcium release occurs in the nucleus based on the localization of the inositol trisphosphate receptor to the inner nuclear membrane. However, this ultimately raises questions about how Gq-receptors, traditionally thought to localize to the cell surface, might activate a nuclear calcium signal to regulate hypertrophy.

In cardiac myocytes, Gq-receptors, including α1-adrenergic, endothelin, and angiotensin receptors (α1-AR, ET-R, AT-R, respectively), regulate vital signaling pathways controlling hypertrophy, cell survival, and inotropy that impact heart failure (HF) (3). Early studies in cell and animal models established the long-held convention that Gq-signaling is maladaptive and exacerbates HF (4-6). However, several studies have challenged the convention that Gq-receptor signaling universally worsens HF. Clinical studies indicate that antagonists targeting Gq-receptors in human HF do not uniformly improve HF outcomes. Although AT-R antagonists are standard of care (7), α1-AR antagonists worsen HF (3). In mice, 8-fold cardiac myocyte-specific overexpression of Gq induces cardiac myocyte cell death and HF (6). However, Gq levels are increased only 2-fold in human HF (8,9), which in mice produces no obvious phenotype (6). Our lab previously demonstrated that α1-ARs are cardioprotective as defined by their ability to initiate adaptive hypertrophy, survival signaling, and positive inotropy (reviewed in (3)). The finding that α1-ARs are cardioprotective agrees with the clinical data that
α1-antagonists worsen HF. Collectively, these data suggest that Gq-receptors are functionally unique; some protective, like α1-ARs, others maladaptive, like AT-Rs.

How can these apparently divergent data on cardiac Gq-receptor function be reconciled? A potentially important clue is our finding that cardiac α1-ARs primarily localize to and signal at the nucleus unlike other Gq-receptors (reviewed in (3,10)). Using fluorescent ligands and binding assays in fractionated adult cardiac myocytes, we previously demonstrated that endogenous α1-ARs localize primarily to the nucleus. We also found that α1-ARs contain nuclear localization sequences (NLS), and that while reconstitution of wild type α1-ARs in α1-knock out cardiac myocytes restores α1-signaling, reconstitution of α1-NLS mutant receptors does not demonstrating a requirement for α1-nuclear localization (11). Further, we showed that α1-ARs activate intranuclear signaling in adult cardiac myocytes based on our observations that the α1-agonist phenylephrine activates protein kinase C in isolated nuclei, and that blockade of nuclear export inhibits α1-mediated signaling for contractile function (11). Finally, we found that organic cation transporter 3 (OCT3) mediates rapid and specific catecholamine uptake in cardiac myocytes to facilitate α1-signaling, and others have demonstrated that OCT3 knockout mice phenocopy the small heart phenotype seen in α1-AR knockout animals (12,13). In total, we identified an entirely novel model for nuclear α1-cardioprotective signaling in cardiac myocytes distinct from the classical model of maladaptive Gq-receptor signaling.

Based on these findings, we hypothesized that unique Gq-receptor function is dictated by receptor localization. To test this hypothesis, we examined the relationship between subcellular compartmentalization of proximal Gq-receptor signaling and the activation of hypertrophic signaling pathways in adult cardiac myocytes. The current model of Gq-receptor hypertrophic signaling is largely based on ET-R signaling and suggests that sarcolemmal Gq-receptors produce IP3-dependent intranuclear calcium release, activation of calmodulin kinase, phosphorylation and nuclear export of histone deacetylases (HDACs), and de-repression of transcription (2,14). This model is notable for explaining how cytosolic calcium transients required for contraction are segregated from IP3-dependent nuclear calcium signals required for hypertrophic signaling. However, it is not entirely clear how this model might reconcile the data suggesting Gq-receptors induce unique physiology or our model of nuclear α1-cardioprotective signaling. Here, we report for the first time that in adult cardiac myocytes, α1-ARs and AT-Rs localize to and activate PLCβ1 in unique subcellular compartments. Further, we demonstrate for the first time that these compartmentalized proximal signals induce differential activation of nuclear hypertrophic signaling pathways to produce unique hypertrophic transcriptomes. Finally, these data suggest an entirely new model of excitation-transcription coupling that accounts for differential localization of Gq-receptors and better explains the distinct physiologic function of Gq-receptors in adult cardiac myocytes.

**RESULTS**

α1-ARs localize to the nuclei and AT-Rs localize to the sarcolemma in adult cardiac myocytes.

Here, we sought to define the subcellular localization of α1-ARs and AT-Rs in adult cardiac myocytes (Figures 1 and 2). Previously, we demonstrated that endogenous α1-ARs localize to the nucleus in adult mouse ventricular myocytes (AMVM) (11,12,15). However, reagents typically employed to localize receptors are generally unreliable, especially α1-AR subtype-specific antibodies, which lack specificity (16), or fluorescent ligands, which are no longer commercially available, but nonetheless had suboptimal binding kinetics (12). To overcome these shortcomings, we developed a novel α1-AR ligand comprised of 2-piperazinyl-4-amino-6,7-dimethoxyquinazoline, the common pharmacophore of α1-AR antagonists such as terazosin and doxazosin, attached to a polyethylene glycol linker with a terminal biotin moiety fused to a streptavidin coated fluorescent quantum dot (QDot) with an emission wavelength of 565 nm (Figure 1A) (17). We validated the α1-QDot-565 by infecting wild-type (WT) AMVM with a green fluorescent protein labeled α1A-AR (α1A-GFP), incubating infected myocytes with
the α1-QDot, and co-localizing the GFP and QDot fluorescent signals as an indication of receptor binding (Figure 1B, nuclei indicated with white arrows). In AMVM expressing α1A-GFP, the α1-QDot-565 fluorescent signal co-localized with the GFP fluorescent signal at the nucleus, and pretreatment with the α1-AR antagonist prazosin diminished QDot fluorescence to nearly undetectable levels, demonstrating specificity (Figure 1C). We employed the same method as above in uninfected WT AMVM, and the α1-QDot-565 bound to endogenous α1-ARs at the nuclei in WT AMVM in the absence of prazosin, but was blocked by pretreatment with prazosin replicating our previous findings (11,12,15) (Figures 1D and 1E, quantified in 1F). Further, the α1-QDot-565 bound endogenous α1-ARs in nuclei isolated from WT AMVM (Figure 1G) and this signal could be blocked by prazosin (Figure 1H), indicating the presence of α1-ARs at the nuclear membrane, again replicating our previous findings (11,12,15). Therefore, the α1-QDot-565 fluorescent ligand identified endogenous α1-ARs at the nuclear membrane in AMVM, and outperformed prior fluorescent α1-ligands by improving kinetics (30 minutes) at lower concentrations (25 nM) (12).

Similar to α1-ARs, the lack of validated antibodies for AT-Rs led us to employ another fluorescent ligand to define the localization of endogenous AT-Rs in AMVM. In this case, we used angiotensin II (AngII), the endogenous ligand of AT-Rs, labeled with the red fluorophore tetramethylrhodamine (TAMRA). We incubated AMVM with AngII-TAMRA in the absence or presence of unlabeled AngII to demonstrate the specificity of AngII-TAMRA (Figures 2A, 2B and 2C). In AMVM, AngII-TAMRA produced a distinct localization in confocal sections from the myocyte surface (Figure 2B, left panel), whereas AngII-TAMRA showed much less specific binding in confocal sections from the middle of the myocyte (Figure 2A, left panel). Pretreatment with unlabeled AngII abolished the AngII-TAMRA signal indicating specificity (Figure 2C). To clarify receptor localization, slices from the top and middle of the cell were deconvolved, and 3D surface plots were created that identified AngII-TAMRA signal predominantly at the myocyte surface (Figure 2A versus 2B, center and right panels, arrows indicate localization). Finally, a volume rendering was created from a confocal stack of a myocyte labeled with AngII-TAMRA demonstrating that the majority of the AngII-TAMRA signal localized to the myocyte surface (Figure 2D).

Previously, a small population of AT-Rs was identified at the nucleus in AMVM based on immunochemical detection and functional assays, although the overall physiologic significance of this population of AT-Rs is still unclear (18). Further, this population of nuclear AT-Rs, which is activated by intracrine AngII, represents only a fraction of total AT-Rs (18). Our results with AngII-TAMRA indicate that the majority of ligand-accessible AT-Rs exist at the sarcolemma, and failure to detect nuclear AT-Rs here is likely due to the fact that neither AngII nor AT-R antagonists readily cross the sarcolemma (19). In summary, AngII-TAMRA identified endogenous AT-Rs on the sarcolemma, and combined with results from α1-QDot-565 identification of endogenous α1-ARs at the nucleus, the data indicate that endogenous α1-ARs and AT-Rs localize to different subcellular compartments in adult cardiac myocytes.

**Phospholipase Cβ1 (PLCβ1) and its substrate phosphatidylinositol-4,5-bisphosphate (PIP2) localize to the nuclei in adult cardiac myocytes.** Proximal Gq-receptor signaling involves activation of PLCβ1 and cleavage of PIP2 into IP3 and DAG. While it is generally accepted that Gq-receptors activate PLCβ1 to hydrolyze PIP2 at the sarcolemma (20), it is not clear if this occurs at the nucleus in AMVM. Although Gq-receptors localize to the nucleus in AMVM, including α1-ARs (Figure 1, (11,12,15)), as well as small populations of AT-Rs (18) and ET-Rs (21), it is not certain that either PLCβ1 or PIP2 localize to the nucleus (22). In other cells, PIP2 hydrolysis is observed in the nuclear matrix, but is reported to be independent of Gq-receptor signaling (23). However, nuclear α1-ARs activate intranuclear PKCδ in nuclei isolated from AMVM (11), suggesting production of DAG from a yet to be defined nuclear phosphatidylinositol species. Further, both AT-Rs and ET-Rs induce intranuclear signaling dependent on the production of inositol phosphates in AMVM (14,18).

Based on prior demonstrations of Gq-receptor localization and signaling at the nucleus,
we sought to clarify whether PLCb1 is localized to the nucleus in adult cardiac myocytes. First, we sought to validate potential PLCb1 antibodies. Preferably, we would employ AMVM from PLCb1 knockout-mice, but these mice exhibit spontaneous seizures and high mortality around 3 weeks of age (24). Alternatively, we attempted to use siRNA technology to knock down PLCb1 in AMVM, but were unable to achieve significant PLCb1 mRNA knockdown by 40 hours, likely due to low turnover of PLCb1 in cultured AMVM. Subsequently, we attempted to validate potential PLCb1 antibodies using siRNA technology in the N38 embryonic mouse hypothalamic cell line due to PLCb1 enrichment in the brain (25). To assess PLCb1 knockdown, N38 cells were transfected with either PLCb1 siRNA or scramble siRNA for 72 hours. We observed knockdown of PLCb1 mRNA (Figure 3C, quantified in Figure 3D) and knockdown of PLCb protein was visualized by decrease in staining of the PLCb1 antibody (Figure 3A, quantified in Figure 3B). Finally, we stained wild type AMVM with the PLCb1 antibody and our results indicate that PLCb1 localizes to the sarcolemma, t-tubules and nuclear envelope (Figure 3E, nuclei indicated with white arrows).

PIP2, the substrate for PLCb1, localizes to the sarcolemma in AMVM (22,26,27). Yet, identifying a population of nuclear PIP2 has been elusive because either 1) PIP2 is not present at the nucleus or 2) the methods used to detect PIP2 have been insufficient. To address this conundrum, we isolated AMVM nuclei using differential centrifugation, and analyzed samples by mass spectrometry to determine if PIP2 is present in nuclear membranes in AMVM. To detect the presence of PIP2 species, we ran two blanks and two AMVM nuclear samples spiking one of each with commercially available 36:2 PIP2, PIP2[4',5'](18:1(9Z)/18:1(9Z)). Using ESI-MS scan with precursor ion m/z 281 (carboxylated anion of oleic acid – C18:1), we were able identify PIP2 36:2 species in all the samples except the non-spiked blank (Figure 4A, left panel). Spectra of the peak showing a parent ion m/z 1021 (Figure 4A, right panel) confirming the identity of the commercially available 36:2 Pi(4,5)P2 in the PIP2 spiked blank sample with ion fragments at m/z 259, 281, 339, and 419 corresponding to the inositol phosphate (IP), the oleic acid backbone, inositol diphosphate (IP2), and inositol triphosphate (IP3) components of the fragmented 36:2 PIP2.

Similar to previous PIP2 analyses (28), we used negative ion MS/MS to analyze the product ions of m/z 1045 (38:4 PIP2), the most abundant PIP2 species in the nuclear fraction sample without the PIP2 spike (Figure 4B). Ions at m/z 259, 283, 303, 339, and 419 correspond to IP, carboxylate anion fatty acyl chains of stearic acid, the arachidonic acid backbone, IP2, and IP3, all portions of the fragmented 38:4 PIP2 spectrum. Our results indicate that PIP2 localizes to membranes within the nuclei of AMVM, which to our knowledge is the first such demonstration. In total, our findings reveal that PLCb1 and its substrate, PIP2, are found in the same subcellular compartments as both the α1-AR (nuclear) and AT-R (sarcolemma), suggesting the potential for compartmentalized Gq-receptor signaling in adult cardiac myocytes.

AT-Rs, but not α1-ARs, activate PLCb1 at the sarcolemma in adult cardiac myocytes.

Based on the observed distinct subcellular compartmentalization of α1-ARs and AT-Rs (Figures 1 and 2), we reasoned that AT-Rs would activate proximal signaling at the sarcolemma and α1-ARs at the nuclei. To test this, we measured the compartmentalization of Gq-receptor activation of PLCb1 with the PLCb1 activity sensor GFP-C1-PLCδ-PH (GFP-PHD, Figure 5A). GFP-PHD is comprised of GFP fused to the N-terminus of the pleckstrin homology domain of PLCδ1, which preferentially binds PIP2 over other membrane phosphatidylinositol in vitro (29). In general, GFP-PHD associates with PIP2 in membranes in the basal state, and upon Gq-receptor stimulation, PLCb1 hydrolyzes PIP2, and as PIP2 is depleted, GFP-PHD dissociates from the membrane as illustrated in Figure 5B. In AMVM expressing GFP-PHD, the probe localized to the sarcolemma and t-tubules in the basal state, in agreement with previous reports (22). More importantly, the α1-agonist phenylephrine (PE), in the absence or presence of prazosin produced no change in the localization of GFP-PHD compared to vehicle (Figure 5C, quantified in D). Conversely, AngII induced a marked dissociation of GFP-PHD from the membrane compared to vehicle, which was blocked by the non-selective...
AT-R antagonist losartan indicating a receptor-specific effect (Figure 5E, quantified in F). These results demonstrate that AT-Rs, but not $\alpha_1$-ARs, activate PLC$\beta$1 at the sarcolemma in adult cardiac myocytes consistent with the subcellular compartmentalization of each receptor.

**$\alpha_1$-ARs, but not AT-Rs, activate PLC$\beta$1 at the nuclear envelope in adult cardiac myocytes.** Interestingly, GFP-PHD was not detected at the nuclear membrane in the basal state (Figure 5). We suggest there are two explanations for this observation: 1) PIP₂ is not present in the nuclear membrane, despite our identification of PIP₂ in nuclear membranes (Figure 4) or 2) GFP-PHD, which lacks a NLS, is unable to target the nucleus to bind nuclear PIP₂. To clarify this and additionally determine if Gq-receptor mediated activation of PLC$\beta$1 possibly occurs at the nucleus, we inserted a NLS sequence at the N-terminus of GFP-PHD to create NLS-GFP-PHD (Figure 6A). Insertion of a NLS promoted nuclear localization of GFP-PHD (Figure 6C and E) suggesting PIP₂ is found in the nucleus, in agreement with detection of nuclear PIP₂ by mass spectrometry (Figure 4). NLS-GFP-PHD associates with PIP₂ in the nuclear membrane in the basal state, and upon nuclear Gq-receptor stimulation, PLC$\beta$1 hydrolyzes PIP₂, and as PIP₂ is depleted, NLS-GFP-PHD dissociates from the nuclear membrane and moves into the nucleoplasm as illustrated in Figure 6B. Using the same experimental conditions as our experiments with GFP-PHD, the $\alpha_1$-agonist PE induced a marked dissociation of NLS-GFP-PHD from the nuclear membrane, which was blocked by prazosin (Figure 6C, quantified in D). Conversely, AngII, in the absence or presence of losartan, produced little change in the localization of NLS-GFP-PHD (Figure 6E, quantified in F). These results demonstrate that $\alpha_1$-ARs, but not AT-Rs, primarily activate PLC$\beta$1 at the nuclear membrane in AMVM. The combined results from experiments using GFP-PHD and NLS-GFP-PHD indicate that $\alpha_1$-AR- and AT-R-mediated proximal signaling is confined to distinct subcellular compartments consistent with receptor localization.

**$\alpha_1$-ARs, but not AT-Rs, induce IP₃-dependent nuclear export of HDAC5 in adult cardiac myocytes.** Gq-receptors are thought to induce hypertrophy through a mechanism known as excitation-transcription coupling (2). Conventionally, sarcolemmal Gq-receptor-mediated production of IP₃ elicits intranuclear calcium release from the nuclear envelope, activation of calmodulin kinase type II, and phosphorylation and nuclear export of HDAC5 (2). Nuclear compartmentalization of IP₃-dependent calcium release allows myocytes to distinguish calcium required for contraction from calcium required for transcriptional signaling. Here, we examined whether differentially localized Gq-receptors would have the same effect on HDAC5 export. In AMVM expressing HDAC5-GFP, PE but not AngII induced a moderate, but significant, export of HDAC5 at 30 minutes (Figure 7A, quantified in 7B). By 1 hour, PE significantly induced HDAC5 nuclear export, whereas AngII did not (Figure 7C, quantified in 7D), consistent with previous reports for PE (30). To determine if PE-induced HDAC5 nuclear export was IP₃-dependent, AMVM expressing HDAC5-GFP were pretreated with the IP₃R inhibitor 2-aminoethoxydiphenyl borate (2-APB). Pretreatment with 2-APB abolished the PE-mediated HDAC5 nuclear export (Figure 7E, quantified in 7F). Taken together, these results indicate that $\alpha_1$-ARs, but not AT-Rs, activate IP₃-dependent HDAC5 nuclear export in AMVM.

**$\alpha_1$-ARs and AT-Rs induce unique transcriptomes in adult cardiac myocytes.** Physiologically, $\alpha_1$-ARs and AT-Rs have diametrically opposed effects on the heart. $\alpha_1$-ARs induce physiologic hypertrophy, survival signaling, positive inotropy, and are not associated with fibrosis (3). AT-Rs induce pathologic hypertrophy, myocyte cell death, negative inotropy, and are pro-fibrotic (31,32). We hypothesized that these differences in the physiologic function of cardiac $\alpha_1$-ARs and AT-Rs would be revealed in their transcriptomes and reflective of their distinct subcellular localization. To evaluate $\alpha_1$-AR and AT-R transcriptomes, we treated mice with vehicle, PE (30 mg/kg/day), or AngII (0.5 mg/kg/day) continually for 3 days using osmotic mini-pumps, at which point, we isolated cardiac myocytes and performed RNASeq.
The doses of PE and AngII we used are known to induce hypertrophy without a concomitant increase in blood pressure (33-36). Consistent with our HDAC5 results and that HDAC5 activation relieves transcriptional repression, α1-ARs induced a larger transcriptional response, with a total of 806 genes changed (increased or decreased) 1.7-fold versus vehicle, whereas AT-Rs induced a much smaller response with only 173 genes changed 1.7-fold versus vehicle. 1.7 fold expression over vehicle was used as the threshold since adult cardiac myocytes are post-mitotic and generally do not induce large transcriptional responses. Interestingly, between α1-ARs and AT-Rs, 155 genes were changed by both agonists, indicating α1-ARs induced 651 unique genes, whereas AT-Rs induced only 18 unique genes (Figure 8A). These results suggest that the AT-R transcriptome is largely a subset of the α1-AR transcriptome in cardiac myocytes.

To parse the RNASeq results further, we initially attempted to utilize the Ingenuity Pathway Analysis (IPA) software, but the majority of the database is derived from oncogenic studies and lacks cardiac myocyte specific pathways. Thus, we derived our own analysis and sorted genes that were regulated by α1-ARs alone, AT-Rs alone, or were in common between the two into gene ontologies corresponding to Gq-receptor biology: hypertrophy, survival signaling, inotropy, and fibrosis (Figure 8B, Supplementary Tables 1-4). α1-ARs most robustly altered genes in all categories as compared to common genes and AT-R only genes. While surprising that α1-ARs altered more fibrotic genes than AT-Rs, these genes are not classically associated with alterations in the extracellular matrix leading to fibrosis (Supplementary Table 4). Finally, principle component analysis (PCA) was performed to determine the degree of difference between vehicle, AngII, and PE treated samples (Figure 8C). Principle component 1 (PC1) accounted for 66% of the variance and aligned with the α1-AR transcriptomes, whereas PC2 accounted for 22% of the variance and aligned with the AT-R transcriptome. The AngII treated samples also closely grouped with the vehicle treated samples indicating that AT-Rs do not induce a highly distinct transcriptome from control, consistent with our gene ontology results (Figure 8A and 8B) The top 25 genes determining PC1 and PC2 are presented in Figure 8D. Taken together, α1-ARs robustly activate transcription in adult cardiac myocytes whereas AT-Rs minimally activate transcription. These results identify distinct differences in the transcriptomes induced by α1-ARs and AT-Rs that align with their distinct subcellular localization and activation of proximal signaling to produce differential activation of nuclear hypertrophic signaling pathways.

DISCUSSION

Here, we identified an entirely novel mechanistic explanation for unique Gq-receptor function in adult cardiac myocytes predicated upon subcellular compartmentalization of proximal Gq-receptor signaling and propose a novel model of excitation-transcription coupling. We found that α1-ARs localize to the nucleus and induce intranuclear activation of PLCβ1, stimulate IP3-dependent nuclear export of HDAC5, and activate a robust and unique transcriptome associated with hypertrophic, survival, inotropic, and (anti)-fibrotic gene programs. Conversely, we observed that AT-Rs primarily localize to and activate PLCβ1 at the sarcolemma, but have little effect on nuclear export of HDAC5, and induce a small transcriptome that is a subset of the α1-transcriptome. More importantly, these findings are consistent with our hypothesis that Gq-receptor localization dictates function by showing compartmentalization of proximal Gq-signaling is correlated with phenotypic outcome in adult cardiac myocytes.

The excitation-transcription model of Gq-receptor mediated hypertrophic signaling in adult cardiac myocytes proposes that sarcolemmal Gq-receptors induce IP3 production and IP3-sensitive intranuclear calcium release from perinuclear calcium stores to activate calmodulin kinase, phosphorylate and induce nuclear export of HDAC5, and thereby activate transcription (Figure 9A). While the current model of excitation-transcription coupling suggests that Gq-receptors induce IP3 production at the sarcolemma leading to activation of IP3-dependent calcium release at the nucleus to induce HDAC5-export and promote gene transcription, it fails to explain...
how Gq-receptors might produce unique physiological function in cardiac myocytes.

Both ET-Rs and insulin-like growth factor receptors conform to the traditional excitation-
transcription model (2,14,37). Our data indicate that α1-ARs might support this model as well, but interestingly, AT-Rs do not. Although we observed AT-R mediated activation of PLCβ1, we failed to detect AT-R mediated nuclear export of HDAC5, and found a much smaller transcriptional response. One interpretation of this result is that close proximity to the nucleus is required for Gq-receptor mediated activation of IP3-dependent hypertrophic signaling. In support of this interpretation, α1-ARs localize to the inner nuclear membrane (11), and ET-Rs and insulin-like growth factor receptors localize to the bottom of t-tubules in close apposition to the nucleus in adult cardiac myocytes (37,38). Further, the failure of AT-Rs to induce nuclear export of HDAC5 suggests that AT-R-mediated activation of PLCβ1 at the sarcolemma either fails to generate enough IP3 to reach the nucleus or that IP3 is degraded before it reaches the nucleus. The potential degradation of IP3 prior to reaching the nucleus might be analogous to the compartmentalization of cAMP signaling in cardiac myocytes (39).

Here, we propose a new model of excitation-transcription coupling that is based on compartmentalization of Gq-receptors that explains distinct physiologic function of Gq-receptors in adult cardiac myocytes. Our model suggests Gq-receptor induced IP3 production is compartmentalized and that IP3 produced inside the nucleus (or possibly in close proximity to the nucleus) induces HDAC5 export to promote gene transcription, whereas IP3 produced at a distance from the nucleus (at the sarcolemma) has a different, and smaller effect on transcriptional regulation (Figure 9B). In summary, we suggest that Gq-receptor compartmentalization has a large influence on the transcriptomes induced by differentially localized Gq-receptors, illustrating the fundamental physiologic importance of Gq-receptor compartmentalization.

With regard to our model of nuclear α1-induced HDAC5 export, previous work indicated that HDAC5 export downstream of α1-ARs occurs mainly through activation of protein kinase D (PKD), and our results do not exclude activation of this signaling cascade (40). Yet, in agreement with our results, Luo et. al. demonstrated that α1-ARs activate IP3-dependent nuclear calcium transients in cardiac myocytes (41), consistent with our finding that α1-ARs induce IP3-sensitive HDAC nuclear export. At this time, the discrepancy between these findings is not entirely clear.

The consensus view of cardiac Gq-receptor function has been that Gq-receptors mediate pathologic remodeling, promoting maladaptive hypertrophy, myocyte cell death, and negative inotropic responses (4). However, in clinical trials of hypertension (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial, ALLHAT) and HF (Vasodilator Heart Failure Trial, V-HeFT), α1-AR antagonists worsened outcomes (42,43). Further, our studies indicate that α1-ARs are cardioprotective, promoting adaptive or physiologic hypertrophy, prevention of cell death, and positive inotropic effects identifying a mechanistic basis for the negative results of α1-AR antagonists in clinical trials (3). In short, the cardioprotective nature of α1-ARs stands in stark contrast to the consensus view of maladaptive Gq-receptor function. Here, our data suggests that compartmentalization of Gq-receptors could explain differences in Gq-receptor function. We demonstrated that although both α1-ARs and AT-Rs activate PLCβ1, they do so in different subcellular compartments which has a profound effect on activation of intranuclear hypertrophic signaling and transcriptional activation. Therefore, we suggest that nuclear Gq-receptor signaling, typified by α1-ARs, is cardioprotective. Aside from α1-ARs, a small population of functional ET-Rs and AT-Rs localize to the nucleus as well (18,21), although their physiologic significance is unclear. Conversely, we observed AT-Rs primarily at the sarcolemma, but not in close proximity to the nucleus, which might suggest that Gq-receptor signaling at the sarcolemma is pathologic, which is supported by studies with AT-Rs (31,32). In support of this concept, adenoviral mediated expression of the PLCβ1b at the sarcolemma induces contractile dysfunction (44). In summary, our hypothetical model of compartmentalized Gq-receptor signaling, where nuclear Gq-receptor signaling is cardioprotective, suggests a more
nuanced view of Gq-receptor function in cardiac myocytes.

The concept of GPCR compartmentalization in cardiac myocytes is not without precedence. In co-cultures of sympathetic ganglionic neurons and neonatal rat cardiac myocytes, β1-ARs localize to regions of axonal contact rich in SAP97, AKAP97, catenins, and cadherins, whereas β2-ARs are excluded from these domains (45). In adult cardiac myocytes, β1-ARs are distributed over the entire sarcolemma, whereas β2-ARs are restricted deep within T-tubules (46). Finally, platelet-derived growth factor receptors, which also signal through Gs, localize to caveolae in cardiac myocytes and do not induce an inotropic response (47). These examples demonstrate that cardiac myocytes compartmentalize Gs-mediated GPCR signaling, analogous to our findings with Gq-receptors.

In conclusion, our findings support a model of compartmentalized Gq-receptor signaling in adult cardiac myocytes and suggest a revision of the classic model of excitation-transcription coupling. Our new model, largely based on our identification of cardioprotective nuclear α1-AR signaling, provides a plausible mechanistic basis to explain the unique function of cardiac Gq-receptors. Additionally, this model suggests a re-examination of the classic paradigm of maladaptive Gq signaling in cardiac myocytes in favor of a more nuanced view of compartmentalized Gq-receptor signaling.

Experimental Procedures

Experimental Models
Mice: In this study, male and female C57BL/6J mice (10-15 weeks of age) were used for primary adult cardiac myocytes. Male FVB/NJ (10-11 weeks of age) mice were used for infusion of agonist to measure Gq-receptor induced hypertrophic transcriptional responses. All animals were sourced from Jackson Laboratories. The use of all animals in this study conformed to the PHS Guide for Care and Use of Laboratory Animals and was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Method Details
Isolation and Culture of AMVM: was carried out as described previously (48).
Isolation and labeling of Nuclei from AMVM: was carried out as described previously (49). Labeling is described in the online supplement.
Adenoviral Production: The α1A-GFP adenovirus was previously described (50). Production of the GFP-C1-PLCdelta-PH (GFP-PHD) and NLS-GFP-PHD adenoviruses are described in the online supplement. The HDAC5-GFP adenovirus was a gift from Timothy McKinsey, Ph.D. (51). For all experiments, cultured AMVM were counted and infected with adeno viruses at the following multiplicity of infection α1A-GFP: 1000 (titer: 5.9 x 10^10); GFP-PHD 100 (titer: 2.4 x 10^8); NLS-GFP-PHD (titer: 1.4 x 10^10) and HDAC5-GFP (titer: 6.1 x 10^15).

Synthesis of α1-QDot-565: The synthesis of the α1-QDot-565 was performed as described in (17) with few modifications that are described in the online supplement.

Localization of α1-ARs, AT-Rs, and PLCβ1 in AMVM: Methods used to define the localization of α1-ARs, AT-Rs, and PLCβ1 are described in the online supplement.

Identification of Nuclear PIP2: PIP2 extraction was carried out as described by (52) with some modifications. The electrospray mass spectra of each sample was analyzed on a hybrid quadrupole triple ion trap mass spectrometer (Triple TOF 5600, AB Scieix Instrument). All scans were performed in negative ionization mode and a mass-to-charge (m/z) range from 50 to 1200. PIP2[4',5'][18:1(9Z)/18:1(9Z)] was obtained from Avanti Polar Lipids.

Quantification and Statistical Analysis
The methods for the analysis of phospholipase Cβ1 activity, HDAC5 nuclear export, and hypertrophic transcriptional profiles adenoviruses are described in the online supplement. Details and methods used for statistical analysis can be found in the online supplement.

Data Availability
RNA-Seq Data Set is available at http://dx.doi.org/10.17632/pbw8ww4k55.1
ACKNOWLEDGEMENTS
The authors would like to acknowledge Dr. Timothy McKinsey, University of Colorado, for his generous gift of the HDAC5 virus, Dr. Alessandro Bartolomucci, University of Minnesota, for his generous gift of N38 cells, Christy Long for her effort in making the GFP-PHD virus, Dr. Jop Van Berlo for his assistance in conceptualizing this project, and the University of Minnesota Genomics Core.

CONFLICT OF INTEREST
The authors have no conflict of interest.
REFERENCES

1. Kamato, D., Mitra, P., Davis, F., Osman, N., Chaplin, R., Cabot, P. J., Afroz, R., Thomas, W., Zheng, W., Kaur, H., Brimble, M., and Little, P. J. (2017) Gaq proteins: molecular pharmacology and therapeutic potential. *Cellular and molecular life sciences : CMLS* **74**, 1379-1390

2. Wu, X., Zhang, T., Bossuyt, J., Li, X., McKinsey, T. A., Dedman, J. R., Olson, E. N., Chen, J., Brown, J. H., and Bers, D. M. (2006) Local InsP3-dependent perinuclear Ca2+ signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest* **116**, 675-682

3. O'Connell, T. D., Jensen, B. C., Baker, A. J., and Simpson, P. C. (2014) Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. *Pharmacol Rev* **66**, 308-333

4. Dorn, G. W., 2nd, and Brown, J. H. (1999) Gq signaling in cardiac adaptation and maladaptation. *Trends Cardiovasc Med* **9**, 26-34

5. Adams, J. W., Sakata, Y., Davis, M. G., Sah, V. P., Wang, Y., Liggett, S. B., Chien, K. R., Brown, J. H., and Dorn, G. W. 2nd. (1998) Enhanced Galphaq signaling: a common pathway mediates cardiac hypertrophy and apoptotic heart failure. *Proc Natl Acad Sci U S A* **95**, 10140-10145

6. D'Angelo, D. D., Sakata, Y., Lorenz, J. N., Boivin, G. P., Walsh, R. A., Liggett, S. B., and Dorn, G. W., 2nd. (1997) Transgenic Galphaq overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci U S A* **94**, 8121-8126

7. Chrysant, S. G. (2008) Angiotensin II receptor blockers in the treatment of the cardiovascular disease continuum. *Clin Ther* **30 Pt 2**, 2181-2190

8. Jalili, T., Takeishi, Y., Song, G., Ball, N. A., Howles, G., and Walsh, R. A. (1999) PKC translocation without changes in Galphaq and PLC-beta protein abundance in cardiac hypertrophy and failure. *Am J Physiol* **277**, H2298-2304

9. Ponicke, K., Vogelsang, M., Heinroth, M., Becker, K., Zolk, O., Bohm, M., Zerkowski, H. R., and Brodde, O. E. (1998) Endothelin receptors in the failing and nonfailing human heart. *Circulation* **97**, 744-751

10. Wu, S. C., and O'Connell, T. D. (2015) Nuclear compartmentalization of alpha1-adrenergic receptor signaling in adult cardiac myocytes. *J Cardiovasc Pharmacol* **65**, 91-100

11. Wu, S. C., Dahl, E. F., Wright, C. D., Cypher, A. L., Healy, C. L., and O'Connell, T. D. (2014) Nuclear localization of a1A-adrenergic receptors is required for signaling in cardiac myocytes: an "inside-out" a1-AR signaling pathway. *J Am Heart Assoc* **3**, e000145

12. Wright, C. D., Chen, Q., Baye, N. L., Huang, Y., Healy, C. L., Kasinathan, S., and O'Connell, T. D. (2008) Nuclear alpha1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. *Circ Res* **103**, 992-1000

13. Zwart, R., Verhaagh, S., Buitelaar, M., Popp-Snijders, C., and Barlow, D. P. (2001) Impaired activity of the extraneuronal monoamine transporter system known as uptake-2 in Orc3/Sle22a3-deficient mice. *Mol Cell Biol* **21**, 4188-4196

14. Higazi, D. R., Fearnley, C. J., Drawnel, F. M., Talasila, A., Corps, E. M., Ritter, O., McDonald, F., Mikoshiba, K., Bootman, M. D., and Roderick, H. L. (2009) Endothelin-1-stimulated InsP3-induced Ca2+ release is a nexus for hypertrophic signaling in cardiac myocytes. *Mol Cell* **33**, 472-482

15. Wright, C. D., Wu, S. C., Dahl, E. F., Sazama, A. J., and O'Connell, T. D. (2012) Nuclear localization drives alpha1-adrenergic receptor oligomerization and signaling in cardiac myocytes. *Cell Signal* **24**, 794-802

16. Jensen, B. C., Swigart, P. M., and Simpson, P. C. (2009) Ten commercial antibodies for alpha1-adrenergic receptor subtypes are nonspecific. *Naunyn Schmiedebergs Arch Pharmacol* **379**, 409-412

17. Zhou, G., Wang, L., Ma, Y., Wang, L., Zhang, Y., and Jiang, W. (2011) Synthesis of a quinazoline derivative: a new alpha(1)-adrenoceptor ligand for conjugation to quantum dots to study alpha(1)-adrenoceptors in living cells. *Bioorg Med Chem Lett* **21**, 5905-5909
18. Tadevosyan, A., Maguy, A., Villeneuve, L. R., Babin, J., Bonnefoy, A., Allen, B. G., and Nattel, S. (2010) Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J Biol Chem* **285**, 22338-22349

19. Vaniotis, G., Glazkova, I., Merlen, C., Smith, C., Villeneuve, L. R., Chatenet, D., Therien, M., Fournier, A., Tadevosyan, A., Trieu, P., Nattel, S., Hebert, T. E., and Allen, B. G. (2013) Regulation of cardiac nitric oxide signaling by nuclear beta-adrenergic and endothelin receptors. *J Mol Cell Cardiol* **62C**, 58-68

20. Grubb, D. R., Vasilevski, O., Huynh, H., and Woodcock, E. A. (2008) The extreme C-terminal region of phospholipase C beta1 determines subcellular localization and function; the "b" splice variant mediates alpha1-adrenergic receptor responses in cardiomyocytes. *FASEB J* **22**, 2768-2774

21. Boivin, B., Chevalier, D., Villeneuve, L. R., Rousseau, E., and Allen, B. G. (2003) Functional endothelin receptors are present on nuclei in cardiac ventricular myocytes. *J Biol Chem* **278**, 29153-29163

22. Zhang, L., Malik, S., Pang, J., Wang, H., Park, K. M., Yule, D. I., Blaxall, B. C., and Smrcka, A. V. (2013) Phospholipase cepsonil hydrolyzes perinuclear phosphatidylinositol 4-phosphate to regulate cardiac hypertrophy. *Cell* **153**, 216-227

23. Ramazzotti, G., Faenza, I., Fiume, R., Matteucci, A., Piazzii, M., Follo, M. Y., and Coco, L. (2011) The physiology and pathology of inositide signaling in the nucleus. *J Cell Physiol* **226**, 14-20

24. Kim, D., Jun, K. S., Lee, S. B., Kang, N. G., Min, D. S., Kim, Y. H., Ryu, S. H., Suh, P. G., and Shin, H. S. (1997) Phospholipase C isozymes selectively couple to specific neurotransmitter receptors. *Nature* **389**, 290-293

25. Sekerkova, G., Watanabe, M., Martina, M., and Mugnaini, E. (2014) Differential distribution of phospholipase C beta isoforms and diacylglycerol kinase-beta in rodents cerebella corroborates the division of unipolar brush cells into two major subtypes. *Brain Struct Funct* **219**, 719-749

26. Ziegelhoffer, A., Tappia, P. S., Mesaeli, N., Sahi, N., Dhalla, N. S., and Panagia, V. (2001) Low level of sarcolemmal phosphatidylinositol 4,5-bisphosphate in cardiomyopathic hamster (UM-X7.1) heart. *Cardiovasc Res* **49**, 118-126

27. Mesaeli, N., Tappia, P. S., Suzuki, S., Dhalla, N. S., and Panagia, V. (2000) Oxidants depress the synthesis of phosphatidylinositol 4,5-bisphosphate in heart sarcolemma. *Arch Biochem Biophys* **382**, 48-56

28. Wenk, M. R., LuCast, L., Di Paolo, G., Romanelli, A. J., Suchy, S. F., Nussbaum, R. L., Cline, G. W., Shulman, G. I. , McMurray, W., and De Camilli, P. (2003) Phosphoinositide profiling in complex lipid mixtures using electrospray ionization mass spectrometry. *Nat Biotechnol* **21**, 813-817

29. Stauffer, T. P., Ahn, S., and Meyer, T. (1998) Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P2 concentration monitored in living cells. *Curr Biol* **8**, 343-346

30. Chang, C. W., Lee, L., Yu, D., Dao, K., Bossuyt, J., and Bers, D. M. (2013) Acute beta-adrenergic activation triggers nuclear import of histone deacetylase 5 and delays G(q)-induced transcriptional activation. *J Biol Chem* **288**, 192-204

31. Hein, L., Stevens, M. E., Barsh, G. S., Pratt, R. E., Kobilka, B. K., and Dzau, V. J. (1997) Overexpression of angiotensin II type 1 receptor transgene in the mouse myocardium produces a lethal phenotype associated with myocyte hyperplasia and heart block. *Proc Natl Acad Sci U S A* **94**, 6391-6396

32. Paradis, P., Dali-Youcef, N., Paradis, F. W., Thibault, G., and Nemer, M. (2000) Overexpression of angiotensin II type 1 receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proc Natl Acad Sci U S A* **97**, 931-936

33. Bendall, J. K., Cave, A. C., Heymes, C., Gall, N., and Shah, A. M. (2002) Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation* **105**, 293-296
34. Braz, J. C., Bueno, O. F., Liang, Q., Wilkins, B. J., Dai, Y. S., Parsons, S., Braunwart, J., Glascock, B. J., Klevitsky, R., Kimball, T. F., Hewett, T. E., and Molkentin, J. D. (2003) Targeted inhibition of p38 MAPK promotes hypertrophic cardiomyopathy through upregulation of calcineurin-NFAT signaling. *J Clin Invest* **111**, 1475-1486

35. Bueno, O. F., Wilkins, B. J., Tymitz, K. M., Glascock, B. J., Kimball, T. F., Lorenz, J. N., and Molkentin, J. D. (2002) Impaired cardiac hypertrophic response in Calcineurin Abeta-deficient mice. *Proc Natl Acad Sci U S A* **99**, 4586-4591

36. Kawada, N., Imai, E., Karber, A., Welch, W. J., and Wilcox, C. S. (2002) A mouse model of angiotensin II slow pressor response: role of oxidative stress. *J Am Soc Nephrol* **13**, 2860-2868

37. Ibarra, C., Vicencio, J. M., Estrada, M., Lin, Y., Rocco, P., Rebellato, P., Munoz, J. P., Garcia-Prieto, J., Quest, A. F., Chiong, M., Davidson, S. M., Bulatovic, I., Grinnemo, K. H., Larsson, O., Szabadkai, G., Uhlen, P., Jaimovich, E., and Lavandero, S. (2013) Local control of nuclear calcium signaling in cardiac myocytes by perinuclear microdomains of sarcolemmal insulin-like growth factor 1 receptors. *Circ Res* **112**, 236-245

38. Robu, V. G., Pfeiffer, E. S., Robia, S. L., Balijepalli, R. C., Pi, Y., Kamp, T. J., and Walker, J. W. (2003) Localization of functional endothelin receptor signaling complexes in cardiac transverse tubules. *J Biol Chem* **278**, 48154-48161

39. Zaccolo, M. (2009) cAMP signal transduction in the heart: understanding spatial control for the development of novel therapeutic strategies. *Br J Pharmacol* **158**, 50-60

40. Bossuyt, J., Chang, C. W., Helmstadter, K., Kunkel, M. T., Newton, A. C., Campbell, K. S., Martin, J. L., Bossuyt, S., Robia, S. L., and Bers, D. M. (2011) Spatiotemporally distinct protein kinase D activation in adult cardiomyocytes in response to phenylephrine and endothelin. *J Biol Chem* **286**, 33390-33400

41. Luo, D., Yang, D., Lan, X., Li, K., Li, X., Chen, J., Zhang, Y., Xiao, R. P., Han, Q., and Cheng, H. (2008) Nuclear Ca2+ sparks and waves mediated by inositol 1,4,5-trisphosphate receptors in neonatal rat cardiomyocytes. *Cell Calcium* **43**, 165-174

42. ALLHAT, C. R. G. (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). [see comments]. *Jama* **283**, 1967-1977

43. Cohn, J. N. (1993) The Vasodilator-Heart Failure Trials (V-HeFT). Mechanistic data from the VA Cooperative Studies. *Circulation* **87**, V11-4

44. Grubb, D. R., Crook, B., Ma, Y., Luo, J., Qian, H. W., Gao, X. M., Kiriazis, H., Du, X. J., Gregorevic, P., and Woodcock, E. A. (2015) The atypical b' splice variant of phospholipase Cbeta1 promotes cardiac contractile dysfunction. *J Mol Cell Cardiol* **84**, 95-103

45. Shcherbakova, O. G., Hurt, C. M., Xiang, Y., Dell'Acqua, M. L., Zhang, Q., Tsien, R. W., and Kobilka, B. K. (2007) Organization of beta-adrenoceptor signaling compartments by sympathetic innervation of cardiac myocytes. *J Cell Biol* **176**, 521-533

46. Nikolaev, V. O., Moshkov, A., Lyon, A. R., Miragoli, M., Novak, P., Paur, H., Lohse, M. J., Korchev, Y. E., Harding, S. E., and Gorelik, J. (2010) Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentalization. *Science* **327**, 1653-1657

47. Liu, P., Ying, Y., Ko, Y. G., and Anderson, R. G. (1996) Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. *J Biol Chem* **271**, 10299-10303

48. O'Connell, T. D., Rodrigo, M. C., and Simpson, P. C. (2007) Isolation and culture of adult mouse cardiac myocytes. *Methods Mol Biol* **357**, 271-296

49. Boheler, K. R., Chassagne, C., Martin, X., Wisnewsky, C., and Schwartz, K. (1992) Cardiac expressions of a- and b-myosin heavy chains and sarcomeric a-actins are regulated through transcriptional mechanisms. *J Biol. Chem.* **367**, 12979-12985

50. Huang, Y., Wright, C. D., Merkwan, C. L., Baye, N. L., Liang, Q., Simpson, P. C., and O'Connell, T. D. (2007) An alpha1A-adrenergic-extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. *Circulation* **115**, 763-772
51. McKinsey, T. A., Zhang, C. L., Lu, J., and Olson, E. N. (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106-111
52. Cajka, T., and Fiehn, O. (2014) Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. *Trends Analyt Chem* **61**, 192-206
**ABBREVIATIONS**

| Abbreviation | Description                           |
|--------------|---------------------------------------|
| α1-AR        | α1-adrenergic receptor                |
| AMVM         | adult mouse ventricular myocytes      |
| AngII        | angiotensin II                        |
| AT-R         | angiotensin receptor type 1 and 2     |
| β-AR         | β-adrenergic receptor                 |
| DAG          | diacylglycerol                        |
| ET-R         | endothelin receptor                   |
| Gq-receptors | Gq-coupled G-protein coupled receptor |
| HF           | heart failure                         |
| HDAC         | histone deacetylase                   |
| IP           | inositol phosphate                    |
| IP₂          | inositol diphosphate                  |
| IP₃          | inositol-1,4,5-trisphosphate          |
| NLS          | nuclear localization sequence         |
| OCT3         | organic cation transporter 3          |
| PE           | phenylephrine                         |
| PIP₂         | phosphatidylinositol-4,5-bisphosphate |
| PLCβ1        | phospholipase Cβ1                     |
| QDot         | quantum dot                           |
| TAMRA        | tetramethylrhodamine                  |
| WT           | wild-type                             |
Figure 1. Endogenous α1-ARs localize to the nucleus in adult cardiac myocytes

A. Synthesis of α1-AR-QDot-565. The structural backbone of the piperazole class of α1-AR antagonists, 2-Piperazinyl-4-amino-6,7-dimethoxyquinazoline, was attached to biotinylated PEG (Poly(ethylene glycol) (N-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino)ethane). The resulting α1-antagonist-PEG-biotinylated molecule was then coupled to streptavidin conjugated QDot-565 to make...
α1-QDot-565. **B. Validation of α1-QDot-565.** To define the specificity of the α1-QDot-565, WT AMVM were infected with an adenovirus expressing α1A-GFP and then treated with 25 nM α1AR-QDot-565. Cells were fixed and imaged by confocal microscopy. α1A-GFP signal (green), QDot-565 signal (yellow), and co-localization determined in post-processing (white). Scale bar = 10 µm. **C. Specificity of α1-QDot-565.** Myocytes expressing α1A-GFP were pretreated with an excess of unlabeled praz (5 µM) then treated with 25 nM α1AR-QDot-565. α1A-GFP signal (green), QDot-565 signal (yellow) and co-localization determined in post-processing (white). Scale bar = 10 µm. **D. α1-ARs localize to the nuclei in AMVM.** α1-QDot-565 was added to cultured WT AMVM (25 nM, 30 min, 37°C). Myocytes were fixed and imaged by confocal microscopy (60X oil immersion). A representative myocyte is shown, arrows indicate nuclei. Inset is intentionally overexposed for visualization of whole cell. Scale bar = 10 µm. **E. Specificity of α1-QDot-565 in WT AMVM.** To demonstrate the specificity of the α1-QDot-565, WT AMVM were pretreated with an excess prazosin (5 µM, 30 min, 37°C) then incubated with α1-AR-QDot-565 (25 nM, 30 min, 37°C). One representative myocyte is shown. Scale bar = 10 µm. **F. Quantification of α1-QDot-565 localization.** The QDot fluorescent intensity from all cardiac myocytes was quantified using FIJI (number of cardiac myocytes, n, indicated in figure). QDot-565 alone images not shown. Praz = prazosin. Data are presented as mean ± SEM. Data were analyzed by one-way ANOVA, and P<0.05 was considered significant. α1-QDot 565 (n=12 myocytes from 4 hearts); α1-QDot 565 + praz (n=3 myocytes from 2 hearts); QDot 565 alone (n=4 myocytes from 1 heart). Scale bar = 10 µm. **G. α1-QDot-565 labels nuclei isolated from AMVM.** Nuclei were isolated from WT AMVM and incubated with α1-AR-QDot-565 (25 nM, 15 min, 37°C). Nuclei were stained with the nuclear marker DRAQ5, fixed, and imaged as in **B.** One representative nuclei is shown. Scale bar = 10 µm. **H. Specificity of α1-QDot-565 in nuclei isolated from AMVM.** To demonstrate the specificity of the α1-QDot-565, nuclei from WT AMVM were pretreated with an excess prazosin (5 µM, 15 min, 37°C) then incubated with α1-AR-QDot-565 (25 nM, 15 min, 37°C). Nuclei were stained with DRAQ5, fixed, and imaged as in **B.** One representative nuclei is shown. Scale bar = 10 µm.
Figure 2. AT-Rs localize to the sarcolemma in adult cardiac myocytes

A and B. AT-Rs localize to the sarcolemma in AMVM. AngII-TAMRA (1 μM, 5 min, 37°C) was added to WT AMVM. Raw optical sections of the middle (top, left) and top (bottom, left) of the myocytes were captured by confocal microscopy (60X oil immersion). Deconvolution of optical sections from the middle (top, center) and surface (bottom, center) was achieved using AutoQuant X3. Arrows indicate AT-R localization at the sarcolemma. 3D surface images of deconvolved images middle (top, right) and surface (bottom, right) were created using FIJI to demonstrate receptor density. Scale bar = 10 μm. C. AngII-TAMRA is specific for AT-Rs in AMVM. WT AMVM were pretreated with an excess of unlabeled AngII (50 μM, 2 min, 37°C) then incubated with AngII-TAMRA (1 μM, 5 min, 37°C). Signal was undetectable indicating specificity. Scale bar = 10 μm. D. Volume plot of AngII-TAMRA treated AMVM showing surface localization. 3D volume plot was created using FIJI.
Figure 3. PLCβ1 localizes to the sarcolemma and nuclear membrane in adult cardiac myocytes

A. Visualization of siRNA-mediated PLCβ1 protein knockdown. N38 cells (embryonic mouse hypothalamic cells) were transfected with either 80 pmol PLCβ1 siRNA or scrambled siRNA using Lipofectamine RNAiMAX for 72 hours at 37°C. Cells were fixed and stained with a primary antibody against PLCβ1. Secondary antibody was conjugated to AlexaFluor594. Cells were imaged using confocal microscopy (20X). Scale bar = 10 µm. B. Quantification of siRNA-mediated PLCβ1 protein knockdown. Fluorescence intensity was measured using FIJI (n=2 cultures, 4 optical areas were measured for scramble and 8 optical areas were measured for PLCβ1 siRNA). Data are represented as mean ± SEM. Data were analyzed by paired student’s t-test, and P<0.05 was considered significant. C. siRNA-mediated PLCβ1 mRNA knockdown. N38 cells were transfected as in A. After 72 hours, RNA was harvested using RNAEasy kit, and PLCβ1 mRNA levels were measured by RT-PCR. Results of densitometry analysis of PLCβ1 mRNA levels are shown in D. D. Quantification of siRNA-mediated PLCβ1 mRNA knockdown. Ratio of GAPDH to PLCβ1 was quantified using densitometry (n=3 cultures). Data are represented as mean ± SEM. E. Endogenous PLCβ1 localizes to the sarcolemma and nuclear membrane in AMVM. WT AMVM were isolated, cultured for 24 hours, fixed, permeabilized, and stained with the primary antibody against PLCβ1. Secondary antibody was conjugated to AlexaFluor488. Myocytes were imaged by confocal microscopy (60X oil immersion). Arrows indicate nuclear membrane. Images were cropped (white lines indicate cropped area) and horizontally aligned. Two representative images are shown. Scale bar = 10 µm.
Figure 4. PIP2 localizes to the nuclear membrane in adult cardiac myocytes

A. Detection and identification of PIP2[4',5'](18:1(9Z)/18:1(9Z)) in AMVM nuclear fractions. Blank and nuclear extracts were analyzed with and without PIP2[4',5'](18:1(9Z)/18:1(9Z)). The yellow peak (not detected) represents blank sample with no PIP2 spike, the green peak represents the blank sample with PIP2 spike, the blue peak represents the nuclear fraction with no PIP2 spike and the red peak represents the nuclear fraction with PIP2 spike (left panel). ESI-MS scans of the PIP2 spiked blank sample with precursor ion m/z 281 showing m/z 1021 identifying the compound as 36:2 PIP2 (right panel).

B. Identification of PIP2 species in AMVM nuclear fractions. Product ions of m/z 1045 (38:4 PIP2) of the non-spiked nuclear fraction sample.
Figure 5. AT-Rs, but not α1-ARS, activate PLCβ at the sarcolemma.

A. PLCβ1 activity probe GFP-PHD

Sarcolemma

Cytoplasm

B. Basal

Activated

Agonist

D. % of Cells

Vehicle
n=30

PE
n=24

P = ns

Responders

Non-Responders

E. Vehicle

AngII

AngII + Losartan

Whole Cell

Zoom

3D Surface

F. % of Cells

Vehicle
n=30

AngII
n=29

P < 0.0001

Responders

Non-Responders

α1-ARS (C) do not activate PLCβ1 at the sarcolemma, but AT-Rs do (E). WT AMVM expressing GFP-PHD were treated with vehicle (left panels) or PE (20 μM, 5 min, 37°C) in the absence and presence of prazosin (1 mM, 30 min pretreatment, 37°C, middle panels and right panels respectively), or AngII (100 nM, 5 min, 37°C) in the absence and presence of losartan (5 μM, 30 min pretreatment, 37°C, middle and right panels respectively). Images were cropped (white lines indicate cropped area) and horizontally aligned. 3D Surface images were created with FIJI to demonstrate GFP-PHD fluorescence intensity. Scale bar = 10 μm. D and F. Quantification of sarcolemmal PLCβ1 activity downstream of α1-ARS (D) and AT-Rs (F). Myocytes were classified by another investigator blinded to treatment group as responders defined by GFP-PHD movement off the sarcolemma or non-responders defined by no movement of GFP-PHD either at baseline or following agonist treatment. Data were analyzed for cells treated with vehicle or drug (PE/AngII) by Chi Square and P<0.05 was considered significant. Data are represented as mean ± SEM. Vehicle (n=30 myocytes from 7 hearts); PE (n=24 myocytes from 4 hearts); AngII (n=29 myocytes from 4 hearts).
Figure 6. α1-ARs, but not AT-Rs, activate PLCβ at the nucleus

A. PLCβ1 activity probe NLS-GFP-PH

B. Basal

C. Vehicle PE PE+Praz

D. 

E. Vehicle AngII AngII + Losart

F. 

expressing NLS- GFP-PH were treated with either vehicle (left panels) or PE (20 μM, 5 min, 37°C) in the absence or presence of prazosin (1 mM, 30 min pretreatment, 37°C, middle and right panels respectively) or AngII (100 nM, 5 min, 37°C) in the absence and presence of losartan (5 μM, 30 min pretreatment, 37°C, middle and right panels respectively).). Arrows indicate nuclear membrane activation of PLCβ1 by α1-ARs. Images were cropped (white lines indicate cropped area) and horizontally aligned. 3D Surface images were created with FIJI to demonstrate NLS-GFP-PH fluorescence intensity. Scale bar = 10 μm. **D and F.** Quantification of nuclear PLCβ1 activity downstream of α1-ARs (D) and AT-Rs (F). Myocytes were classified by another investigator blinded to treatment group as responders defined by NLS-GFP-PH movement off the nuclear membrane or non-responders defined by no movement of NLS-GFP-PH either at baseline or following agonist treatment. Data were analyzed for cells treated with vehicle or drug (PE/AngII) by Chi Square and P<0.05 was considered significant. Data are represented as mean ± SEM. Vehicle (n=30 myocytes from 7 hearts); PE (n=14 myocytes from 4 hearts); AngII (n=16 myocytes from 4 hearts).
Figure 7. α1-ARs induce IP₃R sensitive HDAC5 export in adult cardiac myocytes

A. Vehicle PE AngII

30 min

B. 

P < 0.05

Ratio Cytoplasmic:Nuclear GFP

n = 12

Vehicle PE 30 min

n = 12

AngII 30 min

C. Vehicle PE AngII

1 hr

D. 

P < 0.05

Ratio Cytoplasmic:Nuclear GFP

n = 12

Vehicle PE 1 hr

n = 12

AngII 1 hr

E. Vehicle 2-APB

F. 

P < 0.05

Ratio Cytoplasmic:Nuclear GFP

n = 12

Vehicle PE

n = 12

2-APB

n = 12

2-APB + PE

Data are represented as mean ± SEM. Data were analyzed by one-way ANOVA, and P < 0.05 was considered significant. Vehicle (n=12 myocytes from 6 hearts); PE (n=12 myocytes from 6 hearts); AngII (n=12 myocytes from 6 hearts) E. HDAC5 export is inhibited in the presence of IP₃R inhibitor, 2-APB. WT AMVM expressing HDAC5-GFP were treated with 2-APB (2 µM, 30 minutes at 37°C) prior to treatment with PE (1 hour at 37°C, middle panel). Scale bar = 10 µm. F. Quantification of HDAC5 export in the presence of IP₃R inhibitor, 2-APB. The ratio of cytoplasmic to nuclear GFP was calculated and plotted. Data are represented as mean ± SEM. Data were analyzed by one-way ANOVA, and P < 0.05 was considered significant. Vehicle (n=12 myocytes from 6 hearts); 2-APB (n=12 myocytes from 6 hearts); PE (n=12 myocytes from 6 hearts); PE+2-APB (n=12 myocytes from 6 hearts).
RNA was isolated, and RNASeq was performed. The gene list generated by RNASeq was filtered based on a minimum 1.7X absolute fold change and FDR corrected $P<0.05$. PE treatment altered 801 transcripts (655 unique) by at least 1.7 fold, whereas AngII only altered 173 (18 unique), with 155 common between the two treatments. B. Gene ontology analysis $\alpha 1$-AR- and AT-R-induced transcriptomes. Gq-receptors regulate hypertrophy, survival signaling, inotropy, and fibrosis. Therefore, using non-overlapping search terms unique to these different biologic functions, genes were sorted into subclasses of hypertrophy, survival signaling, inotropy, and fibrosis. The total number of genes altered (increased and decreased) in common or by PE or AngII alone are plotted in the graph. Search terms used to sort genes associated with hypertrophy: NF-kappa B, GATA4, MEF2, NFAT, ANF, TGF, hypertrophy, myosin heavy chain, MHC, c-myc, c-fos, cell growth, natriuretic; Search terms for survival signaling: ERK, MAPK, apoptotic, apoptosis, survival, cell death; Search terms for inotropy: calcium, contraction, sarcoplasmic reticulum, troponin, myosin, actin, ryanodine, protein kinase C, and sarcomere; Search terms for fibrosis: collagen, matrix metalloprotease, fibrosis, fibroblast, extracellular matrix, matrix. C. Principle Component Analysis of $\alpha 1$-AR and AT-R induced transcriptomes. Principle component analysis of fragments per kilobase of exon per million reads mapped (FPKM). PC1 determined 66% of all variance between samples. PC2 determined 22% of all variance between samples. D. Top 25 genes determining PC1 and PC2. Top 25 genes for PC1 and PC2 were determined using FPKM loadings.
Figure 9. Novel model of excitation-transcription coupling in adult cardiac myocytes. A. Conventional model of excitation-transcription coupling in adult cardiac myocytes. All Gαq-receptors localize to the sarcolemma in adult cardiac myocytes. Upon ligand binding, sarcolemmal PLCβ1 is activated and cleaves PIP2 into DAG and IP3. DAG goes on to activate protein kinase C isoforms (PKCs) and induce contraction. IP3 traverses the myocyte and binds to the IP3R on the inner nuclear membrane inducing intranuclear calcium release. Calcium activates calmodulin (CaM) and calcium-calmodulin dependent protein kinase II (CaMKII), which phosphorylates HDAC5. HDAC5 phosphorylation triggers HDAC5 nuclear export and derepression of transcription.

B. Updated model of excitation-transcription coupling in adult cardiac myocytes. α1-ARs (nuclear) and AT-Rs (sarcolemmal) are differentially localized in adult cardiac myocytes (Figures 1 and 2). Upon ligand binding to either α1-ARs or AT-Rs, PLCβ1 is activated either at the nucleus (α1AR, Figure 6) or at the sarcolemma (AT-R, Figure 5). AT-R induced PLCβ1 activation at the sarcolemma fails to induce HDAC5 nuclear export, whereas α1AR induced PLCβ1 activation at the nucleus induces HDAC5 nuclear export. Furthermore, α1-AR induced HDAC5 nuclear export is IP3-dependent (blocked by 2-APB) (Figure 7). Consistent with α1-induced, IP3-dependent HDAC5 nuclear export, α1-ARs induce a robust transcriptional response, whereas AT-Rs, which fail to induce HDAC5 export, produce a transcriptional response that is largely a subset of α1-AR induced transcriptional responses (potentially suggesting a different non-HDAC dependent mechanism of transcription) (Figure 8).
Subcellular Compartmentalization of proximal Gαq-receptor signaling produces unique hypertrophic phenotypes in adult cardiac myocytes
Erika F. Dahl, Steven C. Wu, Chastity L. Healy, Brian A. Harsch, Gregory C. Shearer and Timothy D. O'Connell

J. Biol. Chem. published online April 2, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.002283

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts