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Regulation of Phosphatidylinositol-specific Phospholipase C at the Nuclear Envelope in Cardiac Myocytes

Alan V. Smrcka

Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY, 14642

Alan V. Smrcka: Alan_Smrcka@urmc.rochester.edu

Abstract

Phosphatidylinositol 4,5 bisphosphate hydrolysis at the plasma membrane by phospholipase C is one of the major hormone regulated intracellular signaling systems. The system generates the diffusible second messenger IP₃ and the membrane bound messenger diacylglycerol. Spatial regulation of this system has been thought to be through specific subcellular distributions of the IP₃ receptor or PKC. As is becoming increasingly apparent, receptor-stimulated signaling systems are also found at intracellular membranes. As discussed in this issue, GPCRs have been identified at the nuclear envelope implying intracellular localization of the signaling systems that respond to GPCRs. Here we discuss the evidence for the existence of PLC signals that regulate nuclear processes, as well as the evidence for nuclear and nuclear envelope localization of PLC signaling components, and their implications for cardiac physiology and disease.

Introduction

Hormone-dependent regulation of phosphoinositide turnover is a fundamental process in regulation cell function and homeostasis. The classical paradigm that has developed over the past 40 years began with the original demonstration of hormone-stimulated turnover of phosphoinositides [1, 2] to the now well-understood stimulation-dependent hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP₂) by phospholipase C (PLC) to generate the second messengers inositol 1,4,5 trisphosphate (IP₃) and diacylglycerol (DAG). These second messengers drive cellular functions by binding to IP₃ receptors (IP₃R) to release Ca²⁺ from the endoplasmic reticulum (ER) and through DAG dependent regulation of protein kinase signaling cascades through protein kinase C (PKC) activation [3]. In addition to the classical role of PIP₂ as a precursor for hydrolysis it is an important cellular regulator in its own right serving as a direct regulator of ion channel and cytoskeletal functions [4, 5]. PIP₂ is also a direct substrate for phosphorylation by phosphoinositide 3 kinases (PI3K) that to generate phosphatidylinositol 3,4,5 trisphosphate (PIP₃) an important regulator of Akt signaling and other downstream processes [6].

In most of these scenarios PIP₂ is localized and functions at the plasma membrane [7]. On the other hand PIP₂ has been found inside the nucleus associated with the nuclear matrix, along with all the enzymes and precursors required for its synthesis [8, 9]. Additionally PLCs have been found in the nucleus suggesting a role for PIP₂ hydrolysis in the nucleus [10]. Recently it has been described that the precursor for PI 4,5 P₂, PI4P can be a substrate

for PLC in cardiac cells [11]. PI4P is found on the plasma membrane and intracellular membranes and may represent a novel intracellular substrate for PLC in multiple cell types [12, 13].

In this review we will discuss the some basic concepts in phosphoinositide signaling downstream of GPCRs and receptor tyrosine kinases, and how they may relate to signaling in the nucleus by PLC enzymes localized at, in, or near the nucleus in cardiac cells.

Phospholipase C enzymes

There are multiple isoforms and splice variants that comprise the family of enzymes that hydrolyze PIP₂. A basic outline of the PLC families and their regulation is presented here. For a detailed discussion of this topic please refer to several comprehensive reviews on the subject [14–17]. All PLC isoforms contain conserved domains that comprise the catalytic domain, an EF-hand domain, whose specific function is not well defined, and a C2 domain that binds phospholipids [14]. All PLCs require calcium for function but this does not involve the EF hands. Rather, calcium in the catalytic site is directly involved in catalysis [18]. All of the PLC isoforms except for the sperm specific PLC ζ contain a pleckstrin homology (PH) domain that can either involved in phosphoinositide binding (PLC δ) [19] or binding of protein regulators such as Rac or G $\beta\gamma$ in the PLC β isoforms [20]. The PLC β isoforms are activated directly downstream of GPCRs by virtue of direct binding of either G protein α_q or $\beta\gamma$ subunits, or both [21–24]. Direct binding of the Rho family small G protein the, Rac, also regulates PLC β 's [25]. The PLC γ isoforms are regulated by direct binding to phosphorylated tyrosine residues in receptor and non receptor tyrosine kinases [26]. PLC δ is highly responsive to calcium [27] and may respond to changes in calcium that occur secondarily to IP₃-dependent release initiated by PLC β or γ activity, or in response to calcium influx through other mechanisms [28]. PLC ϵ has the potential to be downstream of multiple stimuli by virtue of its ability to respond to direct binding of multiple proteins of the Ras family, including Ras, Rho and Rap [17, 29]. PLC ϵ can also be activated either directly or indirectly by G $\beta\gamma$ subunits [30].

Role of canonical PIP₂ hydrolysis in cardiac myocytes

Cardiac myocytes contain multiple isoforms described of PLC including PLC β 1 and β 3, PLC δ 1 and PLC γ 1 and PLC ϵ [31, 32]. In cardiac myocytes a role for IP₃ dependent calcium release downstream of PLC has been enigmatic because calcium levels in the cytosol control beat-to-beat contraction of the myocyte. With each heart beat depolarization of the sarcolemma tightly controls calcium- influx through L-type calcium channels coupled to release of calcium from the sarcoplasmic reticulum through the type 2 ryanodine receptor (Ryr2). How an IP₃-dependent increase in calcium would interact with this system is not as straightforward as in other cell types where IP₃ dramatically regulates global changes in cytosolic Ca²⁺. Type 2 IP₃ receptors are the predominant isoform found in cardiac myocytes [33, 34]. These IP₃ receptors are present at very low levels in the SR of ventricular myocytes and somewhat higher levels in atrial myocytes, but in both cases are greatly outnumbered by type 2 ryanodine receptors (Ryr2) [35]. In atrial myocytes IP₃ receptors may cooperate with Ryr2 to regulate the amplitude of calcium signals in response to stimuli that couple to PLC

hydrolysis including angiotensin II (ATII), endothelin 1 (ET-1) and norepinephrine [36, 37]. Recently it has been shown that IP₃Rs in the SR in ventricular myocytes may contribute to action potential prolongation through local regulation of the Na⁺/Ca²⁺ exchanger in the sarcolemma [38].

In part because IP₃ receptors are present in relatively high levels in atrial myocytes and are present in the SR along with Ryr2, PLC activation by Gq/11-coupled GPCRs can enhance inotropic function but can also trigger arrhythmic responses [36, 37, 39, 40]. Normally Calcium release by Ryr2 is triggered by the rhythmic depolarization induced influx of Calcium through L-type calcium channels. The presence of IP₃ receptors in the SR can serve as an alternate calcium source that can trigger ectopic calcium release that is potentially arrhythmogenic. In ventricular myocytes the level of IP₃ receptors in the SR is very low but increase during cardiac failure [41]. The levels of PLCβ and IP₃ generation can also increase during cardiac disease and may contribute to arrhythmias observed during failure [42].

Most relevant to this review, IP3R2 is enriched in the nuclear envelope (NE) of ventricular cardiac myocytes [33]. Other components of the calcium handling machinery are present on the nuclear envelope such as the Serca pump, and Ryr2 [43]. The presence of IP₃ receptors on the nuclear envelope, if localized facing the nuclear matrix, suggests that IP₃ can regulate nuclear calcium levels. Indeed it has been shown that ATII and ET- I treatment of either neonatal or adult cardiac myocytes results in release of calcium into the nuclear matrix without a significant associated rise in cytosolic calcium [44, 45]. This nuclear calcium increase is critical for cell growth and cardiac hypertrophy since blocking the calcium increase by blocking IP₃ receptors or blocking IP₃ itself with an IP₃ sponge blocks hypertrophy development [44–46]. Calcium in the nucleus regulates CamKII-dependent phosphorylation of histone deacetylase (HDAC) [47] and regulates calcineurin/NFAT activity in the heart [44]. Both HDAC and NFAT regulate expression of hypertrophic genes.

A widely accepted model for how the PLC signaling system regulates nuclear calcium is through PM GPCR-dependent activation of Gα_q, which in turn activates PLCβ and PIP₂ hydrolysis at the plasma membrane [48]. The IP₃ generated is diffusible in the cytoplasm [49] and can reach the nucleus to interact with IP₃ receptors to release calcium from the nuclear envelope, which is continuous with the SR. Evidence in favor of this hypothesis is that blocking PLC activation at the PM blocks hypertrophy [50] and blocking cytosolic IP₃ with a cytoplasmic IP₃ sponge blocks hypertrophy [46].

The other arm of the PLC pathway involves production of DAG and subsequent downstream activation of PKC and PKD. PKCs have been shown to play various roles in regulating cardiac function [51]. In the nucleus PKD in particular has been shown to be important for regulation of hypertrophy [52, 53]. Deletion of PKD decreases hypertrophy development in vivo and in vitro. A mechanism for PKD's hypertrophic activity involves phosphorylation of HDAC leading to nuclear export and expression of hypertrophic genes. Unlike IP₃, DAG cannot diffuse through the cytoplasm but rather is retained in the membrane where it was generated. In order to achieve activation of PKD in the nucleus via the classical PIP₂ hydrolysis pathway, PKD would have to be activated at the PM and then diffuse to the nucleus. Indeed such translocation has been demonstrated in cardiac myocytes [54]. Recent

evidence suggests that activation at the PM is not sufficient to maintain activation of PKD in the nucleus or may not be the only location where DAG-dependent PKD activation occurs [11].

Phosphoinositide signaling in the nucleus

Using a variety of biochemical approaches and recently developed fluorescent probes for detection of phosphoinositides, a consensus has emerged that PIP₂ is not present in significant quantity on intracellular membranes in most cells and is enriched in the PM of most cells [7, 12]. On the other hand PIP₂ is found inside the nucleus in an apparently non-membranous form associated with the matrix [8, 9, 55, 56]. This PIP₂ is not extractable with detergents indicating it is not associated with the nuclear membrane [56, 57]. Also found in the nucleus are PLCβ1 [10, 58], δ1 [59], DAG [10, 57], PI and PI4P [10, 57] indicating the presence of all of the components of the phosphoinositide cycle.

Nuclear matrix PIP₂ levels decrease as a result of PLC-dependent hydrolysis in response to stimulation with growth factors such as IGF-1 in Swiss 3T3 fibroblasts leading to nuclear DAG production and translocation of PKC to the nucleus [57, 60]. The mechanism for IGF-1 stimulation is through activation of nuclear MAPK, which phosphorylates PLC in the nucleus increasing its activity [57, 61]. Regulation of hydrolysis of the nuclear matrix pool of PIP₂ through GPCR driven signals has not been clearly demonstrated. However, as discussed in the next section, there is evidence for a GPCR stimulated IP₃ signaling pathway in the nucleus but the location for the PIP₂ substrate is not clear. The nuclear matrix phosphoinositide cycle has not been investigated in cardiac myocytes, but the data discussed above from other cell types, suggest one potential source of substrate for PLC pathways stimulated by GPCRs localized to the nuclear envelope. Theoretically this could represent a mechanism for GPCR-dependent DAG generation in the nucleus that could drive nuclear PKD activation. In this scenario GPCR activation in the inner nuclear envelope could activate PLCβ, but subsequent access of the matrix localized PIP₂ would involve an unconventional activity of this enzyme that would seem to require dissociation from the inner nuclear membrane. Classical G protein regulated activation of PLCβ isoforms occurs on a two-dimensional lipid bilayer surface where collisions between activated G protein and PLC can occur [20]. Additionally PLCβ isoforms prefer a two-dimensional lipid substrate for G protein-dependent activation [62]. The G protein-dependent enhancement of PLCβ catalytic activity requires the continuous engagement with the G protein. For G protein-activated PLC to access PIP₂ associated with in the nuclear matrix would require simultaneous dissociation of PLC and G protein from the NE membrane or access to nuclear PIP₂ while remaining bound to the NE. Additionally, the G protein-PLC complex would have to access PIP₂ in non-planar bilayer configuration.

Evidence for GPCR stimulated PIP₂ hydrolysis in the nucleus

Gq and Gs coupled GPCRs have been found at the nuclear envelope in cardiac myocytes [63–66] as well as other cell types [67] and specific roles for these receptors have been found in regulation of cell physiology [68–70]. A key question is whether Gq coupled receptors at this location couple to PLC to mediate local release of IP₃ and DAG. Evidence

in favor demonstrating existence of this specific pathway in cardiac myocytes relies mainly of pharmacological evidence. Stimulation of nuclear ET1B receptors with caged ET-1 leads to a gradual increase in nuclear Ca^{2+} that is partially inhibited by the IP_3 receptor blocker 2-Aminoethoxydiphenyl borate (2-APB) or completely inhibited by ryanodine [71]. The only partial inhibition by 2-APB and the fact that 2-APB can inhibit Trp and Ori channels casts some doubt on whether the observed calcium release is IP_3 mediated. While the data support the idea that nuclear ET1B signals can cause a nuclear calcium increase, without further evidence it cannot be concluded that ET1B receptors are stimulating PLC-dependent IP_3 production. Indeed recent experiments from our laboratory failed to detect PIP_2 at the nuclear envelope in cardiac myocytes, although it is possible that very low levels of PIP_2 exist or the NE PIP_2 may not be accessible to the GFP-PLC δ -PH probe [11]. While, as discussed in the previous section, there is PIP_2 in the nucleus it is unclear whether a membrane associated PLC system could access this PIP_2 .

In another study examining local nuclear calcium signaling through IGF-1 it was suggested that invaginations of the sarcolemma closely interact with the nuclear envelope to mediate Ca^{2+} signals [72]. While these detailed studies indicate that IGF-1 can initiate a local calcium signal in the nucleus and IGF-1 receptors are localized in invaginations that closely surround the nucleus, whether this system couples through the classical PLC system is not clear. The authors used the GFP-PLC δ -PH domain probe to follow apparent nuclear PIP_2 signaling but these data are not clear and directly contrast with PIP_2 localization studies from our own laboratory using the same cell preparation with GFP-PLC δ -PH domain or Tubby-GFP as cellular PIP_2 detectors.

In other cell types nuclear GPCR coupling to nuclear PLC signaling has been examined in some greater detail. As an example, in HEK293 cells and striatal neurons, overexpressed and endogenous, respectively, metabotropic glutamate receptor mGluR5 has been found at the nucleus [67]. Stimulation of nuclei isolated from mGluR5 transfected HEK cells with glutamate led to intranuclear calcium oscillations that were blocked by PLC inhibitors (U73122 and ET18OCH₃), IP_3 receptor inhibitors 2-APB and Xestospongin, and by the ryanodine receptor antagonist, Ryanodine [73]. In intact striatal neurons, transfection of dominant negative $\text{G}\alpha_q$ or PLC β 1 siRNA inhibited mGluR dependent increases in nuclear calcium [73]. Finally, the GFP-PLC δ -PH domain PIP_2 probe seemed to detect PIP_2 in the nuclear envelope in isolated nuclei from HEK cells and in intact striatal neurons. Stimulation of mGluR receptors led to an increase in nuclear fluorescence associated with GFP-PLC δ -PH domain interpreted as IP_3 production from the nuclear envelope [73]. These data support the existence of a mGluR- $\text{G}\alpha_q$ -PLC β 1- PIP_2 - IP_3 cascade in HEK293 cells.

As discussed earlier, the evidence for PIP_2 at membranes other than the PM is sparse. As with most experiments, lack of detection is a negative result, so it is difficult to definitively state that PIP_2 is not present on the NE. With the emergence of multiple GFP based phosphoinositide specific detector probes, most famously the GFP-PLC δ -PH domain as well as GFP-Tubby, multiple laboratories have examined the subcellular localization of PIP_2 in live intact cells [74–76]. The majority of these studies show prominent plasma membrane PIP_2 associated fluorescence and a lack of labeling of internal membranes. Additionally, antibodies raised against PIP_2 detect predominantly stain the plasma membrane [12, 77].

Some caveats to these studies are that GFP-lipid detection domains or PIP₂ antibodies may only detect enriched pools of lipid and while the overwhelming evidence supports enrichment of PIP₂ at the PM, lower levels of PIP₂ in other compartments may not be detected [78]. With GFP-lipid probes there may also be an issue with accessibility to the inner nuclear envelope, although tubby-GFP can be found in the nucleus but is not localized to the nuclear periphery as would be expected for NE association. Antibody based approaches require specific fixation and cell permeabilization protocols that may not preserve NE integrity.

The nuclear envelope is continuous with the endoplasmic reticulum which is enriched in phosphatidylinositol, the precursor for PI4P and PI4, 5P₂ synthesis. Higher level phosphorylated forms of PI have not been detected in the ER which may be in part due to the high levels of PI4P phosphatase activity associated with Sac1 that cycles between the ER and the cisGolgi [79]. As will be discussed later, this may function to preserve a gradient of PI4P between the cisGolgi and the ER that may drive lipid exchange between these compartments. If Sac1 is also present in the nuclear envelope it could explain a lack of PI4, 5P₂ in the NE.

On the other hand there are some reports using other methods that appear to detect NE PIP₂. One report used an immunostaining approach with isolated hepatic nuclei showing apparent peripheral staining consistent with NE localization [77]. Another approach used probed thin cryosections of cultured cells with GST-PLC δ -PH followed by immunoEM with anti-GST immunogold antibodies [80]. Here the majority of the labeling was at the PM but some labeling was observed at intracellular locations including the NE.

Thus while there is some evidence for PIP₂ in the nuclear envelope, more convincing evidence is needed to show that a sufficient pool of NE localized PIP₂ to support receptor-stimulated IP₃ production at this location. Improved nuclear-targeted PIP₂ detectors or improved imaging methods could be developed that might detect PIP₂ at low levels in the NE. Such methods that detect very low levels of lipids could always be subject to questions of specificity or background issues. One possible approach to resolve this more conclusively might be to target a PI5 phosphatase to the inner and/or outer nuclear envelope using a chemical dimerization approach **FKBP-rapamycin binding** domain(FRB)/FK506 binding protein (FKBP) fusion proteins. Here FRB might be fused to Nesprin or another NE specific protein and the PI5 phosphatase would be fused to FKBP. Rapamycin could be used to induce translocation of the PI5 phosphatase to NE and deplete the PIP₂ specifically in this compartment. This approach has been used to specifically deplete PIP₂ at the PM [75]. For this to be definitive it would have to be shown that detectable PIP₂ is removed at the NE by rapamycin treatment and that this eliminates receptor-stimulated responses.

Phosphatidylinositol 4-phosphate (PI4P) as an alternative PLC substrate at the nuclear envelope Golgi interface

Recent studies in our laboratory have focused on understanding the roles for PLC ϵ relative to PLC β isoforms in cardiac myocytes. We found that deletion of PLC ϵ in cardiac myocytes prevents hypertrophy development in response to multiple stimuli [81]. In intact animals,

PLC ϵ deletion inhibits development of pressure overload hypertrophy [11]. In cardiac myocytes PLC ϵ scaffolds to both Ryr2 and to muscle-specific A kinase anchoring protein (mAKAP) [11]. As discussed in this issue, mAKAP is highly enriched in the nuclear envelope and scaffolds to multiple signaling proteins associated with cardiac gene expression and hypertrophy to the nuclear envelope [82–85]. These include participants in the PLC ϵ cascade such as Epac and PKC [86]. In cardiac myocytes disruption of PLC binding to mAKAP inhibits increases in cell size and hypertrophic gene expression driven by various extracellular stimuli [11]. Since PLC ϵ is scaffolded to mAKAP at the nuclear envelope and regulates key myocyte functions that arise in the nucleus, it suggests that the local catalytic activity of PLC ϵ is important at the nuclear envelope. As has been discussed, both products of PLC hydrolysis, IP₃ and DAG, regulate Ca²⁺ and protein kinase activity in the nucleus important for expression of hypertrophic genes.

Similarly to what was discussed in the previous section, in order for PLC ϵ to produce local second messengers a substrate must be present. In our hands two different fluorescent PIP₂ probes, GFP-PLC δ -PH domain and GFP-Tubby domain, were unable to detect PIP₂ associated with the nuclear envelope of neonatal cardiac myocytes, adult cardiac myocytes or HEK 293 cells [11]. In contrast in AVMs and HEK293 cells clear PM localization of PIP₂ was observed. While it is still possible that PIP₂ is present in the membrane, the levels must be very low relative to the PM. On the other hand two different PH domain probes detected strong signals for the precursor to PIP₂, PI4P surrounding the nucleus. Stimulation of PLC ϵ activity via direct Epac stimulation, or extracellular ET-1 led to a time dependent depletion of PI4P associated fluorescence that is dependent on PLC ϵ activity. Pharmacologic or genetic depletion of PI4P, or disruption of PLC ϵ -mAKAP scaffolding, block activation of a nuclear pool of PKD by ET-1, and inhibits hypertrophy development [11].

A surprising caveat to this model is that the PI4P fluorescence that surrounds the nuclear envelope in cardiac myocytes is associated with the Golgi apparatus, not the nuclear envelope [11]. This is based on: 1) Treatment with an ADP ribosylation factor 1 (ARF1) inhibitor that disrupts the Golgi apparatus, brefeldin A, eliminates Golgi PI4P associated fluorescence, but not nuclear envelope mAKAP staining. 2) PI4P associated fluorescence co-localizes with Golgi markers but not ER markers in myocytes. 3) PI4P in most cell types is enriched in the Golgi apparatus where it plays an important role in vesicle trafficking and lipid transport [7]. In a standard confocal fluorescence microscope, Golgi associated PI4P fluorescence colocalizes with mAKAP staining (Malik and Smrcka, unpublished data), even though these are clearly in different membrane compartments. Thus the separation between these compartments in myocytes is at the limits or below the resolution limits of light microscopy. Indeed early EM ultrastructural analysis of skeletal and cardiac muscle myocytes indicates an unusual conformation of the Golgi apparatus where it tightly surrounds the outer nuclear envelope with a consistent distance of approximately 100–200 nm [87, 88].

Exactly how mAKAP-scaffolded PLC ϵ on the nuclear envelope accesses Golgi PI4P is unclear. Some possibilities are diagrammed in Figure 1. Recent data suggests an active PI4P transport system at the ER-Golgi interface that transports PI4P from the *trans* Golgi to the ER at specialized interaction surfaces where the distance between the Golgi and the ER is

10–20 nm [89]. Since the nuclear envelope is an extension of the ER, the architecture of the Golgi in cardiac myocytes yields a large surface of ER-Golgi interactions with potential for formation of close contact sites. PI4P could be transported from the Golgi to the NE for where it could be hydrolyzed by PLC ϵ (Fig. 1C), although this would have to occur at the *cis* face of the Golgi rather than *trans*, and no contact sites with dimensions less than 100 nm have yet been observed in myocytes. Another possibility is that PLC ϵ in an extended conformation, bound to an extended conformation of mAKAP, which is in turn bound to an extended conformation of Nesprin 1 α , could span NE-Golgi junctions to access PI4P for hydrolysis at the *cis* Golgi (Fig. 1A). This accessibility would depend on the dimensions of the proteins involved and the distance between the two membranes in cardiac myocytes. These dimensions should be carefully reexamined in cardiac myocytes to determine the viability of such models. A final possibility is that the K_d for PLC ϵ binding to mAKAP could be in a range that could allow local transient dissociation from mAKAP such that it can access the local Golgi pool through a local diffusion based mechanism (Fig. 1B).

The consequences of PI4P as a substrate localized in close proximity to the NE are multiple. First, most obviously, IP₃ would not be generated from the hydrolysis reaction and thus this reaction would not be directly involved in regulation of nuclear calcium through IP₃ receptors. The only bioactive product produced would be DAG, which is consistent with a role for the PLC ϵ in regulating nuclear PKD activation. This potentially makes sense because nuclear Ca²⁺ release in cardiac myocytes can be controlled by the restricted localization of the IP₃ receptor and diffusion of IP₃ from PLC dependent PIP₂ hydrolysis at the PM. DAG regulation of nuclear PKD is more problematic because DAG is not diffusible through the cytoplasm. It has been shown that some agonists stimulate transient PKD activation at the PM followed by subsequent diffusion to the nucleus [54]. On the other hand we showed that PLC ϵ -dependent PI4P hydrolysis was required for nuclear PKD activity stimulated by ET-1 [11]. While there are some discrepancies to be resolved in these studies, a partial reconciliation can be proposed if initial PKD activation occurs at the PM but for this signal to be maintained in the nucleus, DAG has to be generated at or near the nucleus.

Implications of PI4P hydrolysis at the perinuclear Golgi for NE GPCR-stimulated PLC activation

As has been discussed here and in other articles in this issue, there is significant evidence for the existence of GPCRs in the nuclear envelope in cardiac myocytes. Caution should be taken with these results because GPCRs traffic through the Golgi in the biosynthetic maturation process as the receptors are transported to the cell surface. Since it is impossible to distinguish NE from Golgi by light microscopy in cardiac myocytes, and likely difficult to remove Golgi contamination from isolated nuclei, it remains a possibility that some apparently NE GPCRs in cardiac myocytes are indeed receptors in the process of being transported through the Golgi to the cell surface. Most of the cytochemical studies in cardiac myocytes demonstrating NE GPCRs have not examined potential Golgi localization.

Arguing in favor of GPCR localization at the NE are studies with α 1-adrenergic receptor (α 1-AR) showing that mutation of a nuclear localization sequence in the C terminal tail (α 1A-AR-NLSmut) leads to localization away from the nucleus [90]. In myocytes isolated

from $\alpha 1\text{AB-AR}$ knockout mice, wild type $\alpha 1\text{A-AR}$ rescues phenylephrine induced contraction while $\alpha 1\text{A-AR-NLS}$ does not. These studies would strongly argue against Golgi localization for $\alpha 1\text{-AR}$. The authors go on to show that stimulation of $\alpha 1\text{-ARs}$ in isolated nuclei from cardiac myocytes leads to activation of $\text{PKC}\delta$. These results could be consistent with either PI4P or PIP_2 as a substrate for PLC pathway activation.

In another example, stimulation of intracellular ET1B receptors stimulates an elevation of intranuclear calcium, NO and alterations in gene expression in adult cardiac myocytes [69, 71]. As was discussed earlier, this could be the result of activation of $\text{PLC}\beta$ inside the nucleus to hydrolyze either PIP_2 in the nuclear matrix (Fig. 2A) or a difficult to detect PIP_2 substrate in the nuclear envelope (Fig. 2B). Alternatively one could imagine GPCRs at the NE stimulating $\text{PLC}\epsilon$ scaffolded to mAKAP , or $\text{PLC}\beta$ that could be at the nuclear envelope to hydrolyze PI4P to generate local DAG (Fig. 2C and D). Such hydrolysis would not be expected to stimulate IP_3 -dependent Ca^{2+} release but could play a very important role in local PKC or PKD activation. Indeed experiments with ET1B stimulated a slow Ca^{2+} release that is not typical of IP_3 dependent release and is only weakly blocked with an IP_3 receptor blocker [71] suggesting perhaps another mechanism for ET1B stimulated Ca^{2+} release.

Whether $\text{PLC}\beta$ or $\text{PLC}\epsilon$ is involved in NE GPCR -dependent regulation has not been demonstrated but either is theoretically possible. $\text{PLC}\epsilon$ has been definitively identified as being involved in cardiac myocyte NE localized PI4P hydrolysis while $\text{PLC}\beta$ has not been clearly identified at this location. On the other hand, $\text{PLC}\epsilon$ is not directly regulated by $\text{G}\alpha_q$ but rather is regulated by small GTPases or $\text{G}\beta\gamma$ [91]. Thus Gq coupled receptors would rely on $\text{G}\beta\gamma$ released from Gq or a more complex cascade involving small GTPases to activate $\text{PLC}\epsilon$. Many Gq coupled receptors such as ET1 receptors couple to more than one G protein so $\text{Gi/G}\beta\gamma$ responses are also possible. Gs coupled receptors localized at the NE could also potentially regulate the $\text{PLC}\epsilon$ PI4P /hydrolysis signaling system. The β -adrenergic receptor has been identified at the NE and adenylyl cyclase has been found associated with mAKAP [65, 92]. Locally generated cAMP could activate Epac , also found in the mAKAP signaling complex, which could activate $\text{PLC}\epsilon$ through Rap activation as has been previously demonstrated [93, 94].

Summary

Clearly PLCs can regulate nuclear signaling processes. Mechanisms where PLC activity at the plasma membrane can generate signals that diffuse to the nucleus are well described. Emerging ideas based on the discovery of PLCs and GPCRs at or near the nucleus are changing our concepts about how PLC may regulate gene expression. Despite this emerging data, the details of how GPCRs may couple to PLCs at the nucleus remain to be dissected. One particular issue that must be better understood is what is the substrate and what are the products of PLC reactions that are stimulated by NE GPCRs or other receptors. This will require more sensitive probes to detect phosphoinositides at the nuclear envelope as well as more sophisticated imaging techniques to visualize possible PIP_2 in the NE assuming that there is actually PIP_2 present in the NE or that barely detectable levels of PIP_2 are able to support IP_3 production at a level that would activate IP_3 receptors.

The recent discovery that PLC at the nuclear envelope can access PI4P instead of PIP₂ suggests one possible alternative to the classical PIP₂ hydrolysis reactions that may occur at or near the nucleus. It is possible that the role of GPCR coupling to PLCs at the nuclear envelope is to generate local DAG rather than IP₃, obviating the need to find a PIP₂ substrate to support the physiological function of NE PLCs.

References

1. Hokin LE, Hokin MR. Effects of acetylcholine on the turnover of phosphoryl units in individual phospholipids of pancreas slices and brain cortex slices. *Biochim Biophys Acta*. 1955; 18(1):102–10. [PubMed: 13260248]
2. Hokin MR, Hokin LE. Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. *J Biol Chem*. 1953; 203(2):967–77. [PubMed: 13084667]
3. Berridge MJ. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Ann Rev Biochem*. 1987; 56(1):159–193. [PubMed: 3304132]
4. Hilgemann DW, Ball R. Regulation of Cardiac Na⁺, Ca²⁺ Exchange and K_{ATP} Potassium Channels by PIP₂. *Science*. 1996; 273(5277):956–959. [PubMed: 8688080]
5. Suh BC, Hille B. Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. *Curr Opin Neurobiol*. 2005; 15(3):370–378. [PubMed: 15922587]
6. Carpenter CL, Cantley LC. Phosphoinositide 3-kinase and the regulation of cell growth. *Biochim Biophys Acta*. 1996; 1288(1):M11–6. [PubMed: 8764841]
7. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol Rev*. 2013; 93(3):1019–137. [PubMed: 23899561]
8. Keune, Wj; Bultsma, Y.; Sommer, L.; Jones, D.; Divecha, N. Phosphoinositide signalling in the nucleus. *Advances in Enzyme Regulation*. 2011; 51(1):91–99. [PubMed: 21035491]
9. Ramazzotti G, Faenza I, Fiume R, Matteucci A, Piazzini M, Folio MY, Cocco L. The physiology and pathology of inositide signaling in the nucleus. *J Cell Physiol*. 2011; 226(1):14–20. [PubMed: 20658523]
10. Martelli AM, Gilmour RS, Bertagnolo V, Neri L, Manzoli L, Cocco L. Nuclear localization and signalling activity of phosphoinositidase C β in Swiss 3T3 cells. *Nature*. 1992; 358(6383):242–245. [PubMed: 1321347]
11. Zhang L, Malik S, Pang J, Wang H, Park K, Yule D, Blaxall B, Smrcka A. Phospholipase C ϵ Hydrolyzes Perinuclear Phosphatidylinositol 4-Phosphate to Regulate Cardiac Hypertrophy. *Cell*. 2013; 153(1):216–227. [PubMed: 23540699]
12. Hammond GR, Schiavo G, Irvine RF. Immunocytochemical techniques reveal multiple, distinct cellular pools of PtdIns4P and PtdIns(4,5)P₂. *Biochem J*. 2009; 422(1):23–35. [PubMed: 19508231]
13. Hammond GR, Machner MP, Balla T. A novel probe for phosphatidylinositol 4-phosphate reveals multiple pools beyond the Golgi. *J Cell Biol*. 2014; 205(1):113–26. [PubMed: 24711504]
14. Harden TK, Waldo GL, Hicks SN, Sondek J. Mechanism of Activation and Inactivation of Gq/Phospholipase C- β Signaling Nodes. *Chem Rev*. 2011; 111(10):6120–6129. [PubMed: 21988240]
15. Harden TK, Sondek J. Regulation of phospholipase C isozymes by ras superfamily GTPases. *Ann Rev Pharmacol Toxicol*. 2006; 46(1):355–379. [PubMed: 16402909]
16. Rhee SG. Regulation of Phospho-Specific Phospholipase C. *Ann Rev Biochem*. 2001; 70(1):281–312. [PubMed: 11395409]
17. Smrcka AV, Brown JH, Holz GG. Role of phospholipase C ϵ in physiological phosphoinositide signaling networks. *Cell Signal*. 2012; 24(6):1333–43. [PubMed: 22286105]
18. Essen LO, Perisic O, Cheung R, Katan M, Williams RL. Crystal structure of a mammalian phosphoinositide-specific phospholipase C δ . *Nature*. 1996; 380(6575):595–602. [PubMed: 8602259]
19. Garcia P, Gupta R, Shah S, Morris AJ, Rudge SA, Scarlata S, Petrova V, McLaughlin S, Rebecchi MJ. The pleckstrin homology domain of phospholipase C δ 1 binds with high affinity to

- phosphatidylinositol 4,5-bisphosphate in bilayer membranes. *Biochemistry*. 1995; 34(49):16228–34. [PubMed: 8519781]
20. Runnels LW, Scarlata SF. Determination of the affinities between heterotrimeric G protein subunits and their phospholipase C- β effectors. *Biochemistry*. 1999; 38(5):1488–96. [PubMed: 9931014]
 21. Smrcka AV, Hepler JR, Brown KO, Sternweis PC. Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. *Science*. 1991; 251(4995):804–807. [PubMed: 1846707]
 22. Smrcka AV, Sternweis PC. Regulation of purified subtypes of phosphatidylinositol specific phospholipase C β by G protein α and $\beta\gamma$ subunits. *J Biol Chem*. 1993; 268(13):9667–9674. [PubMed: 8387502]
 23. Waldo GL, Boyer JL, Morris AJ, Harden TK. Purification of an AIF and G-protein $\beta\gamma$ -subunit-regulated phospholipase C-activating protein. *J Biol Chem*. 1991; 266(22):14217–14225. [PubMed: 1650351]
 24. Camps M, Hou C, Sidiropoulos D, Stock JB, Jakobs KH, Gierschik P. Stimulation of phospholipase C by G-protein $\beta\gamma$ subunits. *Eur J Biochem*. 1992; 206(3):821–831. [PubMed: 1606965]
 25. Illenberger D, Walliser C, Nurnberg B, Lorente MD, Gierschik P. Specificity and Structural Requirements of Phospholipase C- β Stimulation by Rho GTPases Versus G Protein $\beta\gamma$ Dimers. *J Biol Chem*. 2003; 278(5):3006–3014. [PubMed: 12441352]
 26. Carpenter G, Hernandez-Sotomayor T, Jones G. Tyrosine phosphorylation of phospholipase C γ 1. *Adv Second Messenger Phosphoprotein Res*. 1993; 28(1):179–85. [PubMed: 8398401]
 27. Rebecchi MJ, Pentyala SN. Structure, Function, and Control of Phosphoinositide-Specific Phospholipase C. *Physiol Rev*. 2000; 80(4):1291–1335. [PubMed: 11015615]
 28. Thompson JL, Shuttleworth TJ. Orai channel-dependent activation of phospholipase C- δ : a novel mechanism for the effects of calcium entry on calcium oscillations. *J Physiol*. 2011; 589(21):5057–5069. [PubMed: 21878525]
 29. Wing MR, Snyder JT, Sondek J, Harden TK. Direct Activation of Phospholipase C- ϵ by Rho. *J Biol Chem*. 2003; 278(42):41253–41258. [PubMed: 12900402]
 30. Wing MR, Houston D, Kelley GG, Der CJ, Siderovski DP, Harden TK. Activation of phospholipase C- ϵ by heterotrimeric G protein $\beta\gamma$ -subunits. *J Biol Chem*. 2001; 276(51):48257–48261. [PubMed: 11641393]
 31. Wang H, Oestreich EA, Maekawa N, Bullard TA, Vikstrom KL, Dirksen RT, Kelley GG, Blaxall BC, Smrcka AV. Phospholipase C ϵ Modulates β -Adrenergic Receptor-Dependent Cardiac Contraction and Inhibits Cardiac Hypertrophy. *Circ Res*. 2005; 97(12):1305–1313. [PubMed: 16293787]
 32. Arthur JF, Matkovich SJ, Mitchell CJ, Biden TJ, Woodcock EA. Evidence for selective coupling of α 1-adrenergic receptors to phospholipase C- β 1 in rat neonatal cardiomyocytes. *J Biol Chem*. 2001; 276(40):37341–6. [PubMed: 11489909]
 33. Bare DJ, Kettlun CS, Liang M, Bers DM, Mignery GA. Cardiac Type 2 Inositol 1,4,5-Trisphosphate Receptor. *J Biol Chem*. 2005; 280(16):15912–15920. [PubMed: 15710625]
 34. Perez PJ, Ramos-Franco J, Fill M, Mignery GA. Identification and functional reconstitution of the type 2 inositol 1,4,5-trisphosphate receptor from ventricular cardiac myocytes. *J Biol Chem*. 1997; 272(38):23961–9. [PubMed: 9295347]
 35. Moschella MC, Marks AR. Inositol 1,4,5-trisphosphate receptor expression in cardiac myocytes. *J Cell Biol*. 1993; 120(5):1137–1146. [PubMed: 8382205]
 36. Kockskamper J, Zima AV, Roderick HL, Pieske B, Blatter LA, Bootman MD. Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. *J Mol Cell Cardiol*. 2008; 45(2):128–147. [PubMed: 18603259]
 37. Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-Induced Arrhythmogenic Ca²⁺ Signaling is Abolished in Atrial Myocytes of Inositol-1,4,5-Trisphosphate (IP₃)-Receptor Deficient Mice. *Circ Res*. 2005; 96(12):1274–1281. [PubMed: 15933266]
 38. Signore S, Sorrentino A, Ferreira-Martins J, Kannappan R, Shafaie M, Del Ben F, Isobe K, Arranto C, Wybieralska E, Webster A, Sanada F, Ogórek B, Zheng H, Liu X, del Monte F, D'Alessandro DA, Wunimenghe O, Michler RE, Hosoda T, Goichberg P, Leri A, Kajstura J, Anversa P, Rota M.

- Inositol 1, 4, 5-Trisphosphate Receptors and Human Left Ventricular Myocytes. *Circulation*. 2013; 128(12):1286–1297. [PubMed: 23983250]
39. Burrell KM, Molenaar P, Dawson PJ, Kaumann AJ. Contractile and arrhythmic effects of endothelin receptor agonists in human heart in vitro: blockade with SB 209670. *J Pharmacol Exp Ther*. 2000; 292(1):449–59. [PubMed: 10604982]
 40. von Lewinski D, Kocksamper J, Rubertus SU, Zhu D, Schmitto JD, Schondube FA, Hasenfuss G, Pieske B. Direct pro-arrhythmogenic effects of angiotensin II can be suppressed by AT1 receptor blockade in human atrial myocardium. *Eur J Heart Fail*. 2008; 10(12):1172–6. [PubMed: 18993115]
 41. Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca^{2+} /Calmodulin-Dependent Protein Kinase Modulates Cardiac Ryanodine Receptor Phosphorylation and Sarcoplasmic Reticulum Ca^{2+} Leak in Heart Failure. *Circ Res*. 2005; 97(12):1314–1322. [PubMed: 16269653]
 42. Woodcock EA, Grubb DR, Filtz TM, Marasco S, Luo J, McLeod-Dryden TJ, Kaye DM, Sadoshima J, Du X-J, Wong C, McMullen JR, Dart AM. Selective activation of the “b” splice variant of phospholipase C β 1 in chronically dilated human and mouse atria. *J Mol Cell Cardiol*. 2009; 47(5):676–683. [PubMed: 19729020]
 43. Ljubojević S, Walther S, Asgarzoei M, Sedej S, Pieske B, Kocksamper J. In Situ Calibration of Nucleoplasmic versus Cytoplasmic Ca^{2+} Concentration in Adult Cardiomyocytes. *Biophys J*. 2011; 100(10):2356–2366. [PubMed: 21575569]
 44. Higazi DR, Fearnley CJ, Drawnel FM, Talasila A, Corps EM, Ritter O, McDonald F, Mikoshiba K, Bootman MD, Roderick HL. Endothelin-1-Stimulated InsP_3 -Induced Ca^{2+} Release Is a Nexus for Hypertrophic Signaling in Cardiac Myocytes. *Mol Cell*. 2009; 33(4):472–482. [PubMed: 19250908]
 45. Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH, Bers DM. Local InsP_3 -dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest*. 2006; 116(3):675–682. [PubMed: 16511602]
 46. Nakayama H, Bodi I, Maillet M, DeSantiago J, Domeier TL, Mikoshiba K, Lorenz JN, Blatter LA, Bers DM, Molkentin JD. The IP_3 Receptor Regulates Cardiac Hypertrophy in Response to Select Stimuli/Novelty and Significance. *Circ Res*. 2010; 107(5):659–666. [PubMed: 20616315]
 47. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol*. 2003; 65(1):45–79. [PubMed: 12524460]
 48. Mishra S, Ling H, Grimm M, Zhang T, Bers DM, Brown JH. Cardiac Hypertrophy and Heart Failure Development Through Gq and CaM Kinase II Signaling. *J Cardiovasc Pharmacol*. 2010; 56(6):598–603. [PubMed: 20531218]
 49. Allbritton NL, Meyer T, Stryer L. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science*. 1992; 258(5089):1812–5. [PubMed: 1465619]
 50. Grubb DR, Iliades P, Cooley N, Yu YL, Luo J, Filtz TM, Woodcock EA. Phospholipase C β 1b associates with a Shank3 complex at the cardiac sarcolemma. *FASEB J*. 2011; 25(3):1040–7. [PubMed: 21148417]
 51. Dorn GW II, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest*. 2005; 115(3):527–537. [PubMed: 15765134]
 52. Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN, McKinsey TA. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol Cell Biol*. 2004; 24(19):8374–8385. [PubMed: 15367659]
 53. Fielitz J, Kim M-S, Shelton JM, Qi X, Hill JA, Richardson JA, Bassel-Duby R, Olson EN. Requirement of protein kinase D1 for pathological cardiac remodeling. *Proc Natl Acad Sci*. 2008; 105(8):3059–3063. [PubMed: 18287012]
 54. Bossuyt J, Chang CW, Helmstadter K, Kunkel MT, Newton AC, Campbell KS, Martin JL, Bossuyt S, Robia SL, Bers DM. Spatiotemporally distinct protein kinase D activation in adult cardiomyocytes in response to phenylephrine and endothelin. *J Biol Chem*. 2011; 286(38):33390–400. [PubMed: 21795686]
 55. Cocco L, Gilmour RS, Ognibene A, Letcher AJ, Manzoli FA, Irvine RF. Synthesis of polyphosphoinositides in nuclei of Friend cells. Evidence for polyphosphoinositide metabolism

inside the nucleus which changes with cell differentiation. *Biochem J.* 1987; 248(3):765–70. [PubMed: 2829840]

56. Vann LR, Wooding FB, Irvine RF, Divecha N. Metabolism and possible compartmentalization of inositol lipids in isolated rat-liver nuclei. *Biochem J.* 1997; 327(Pt 2):569–76. [PubMed: 9359431]
57. Divecha N, Banfic H, Irvine RF. The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *EMBO J.* 1991; 10(11):3207–14. [PubMed: 1655412]
58. Divecha N, Rhee SG, Letcher AJ, Irvine RF. Phosphoinositide signalling enzymes in rat liver nuclei: phosphoinositidase C isoform β 1 is specifically, but not predominantly, located in the nucleus. *Biochem J.* 1993; 289(Pt 3):617–20. [PubMed: 8382043]
59. Stallings JD, Tall EG, Pentyala S, Rebecchi MJ. Nuclear translocation of phospholipase C- δ 1 is linked to the cell cycle and nuclear phosphatidylinositol 4,5-bisphosphate. *J Biol Chem.* 2005; 280(23):22060–9. [PubMed: 15809301]
60. Cocco L, Martelli AM, Gilmour RS, Ognibene A, Manzoli FA, Irvine RF. Changes in nuclear inositol phospholipids induced in intact cells by insulin-like growth factor I. *Biochem Biophys Res Commun.* 1989; 159(2):720–5. [PubMed: 2539123]
61. Xu A, Suh P-G, Marmy-Conus N, Pearson RB, Seok OY, Cocco L, Gilmour RS. Phosphorylation of Nuclear Phospholipase C β 1 by Extracellular Signal-Regulated Kinase Mediates the Mitogenic Action of Insulin-Like Growth Factor I. *Mol Cell Biol.* 2001; 21(9):2981–2990. [PubMed: 11287604]
62. Charpentier TH, Waldo GL, Barrett MO, Huang W, Zhang Q, Harden TK, Sondek J. Membrane-induced Allosteric Control of Phospholipase C- β Isozymes. *J Biol Chem.* 2014; 289(43):29545–57. [PubMed: 25193662]
63. Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, O'Connell TD. Nuclear α 1-Adrenergic Receptors Signal Activated ERK Localization to Caveolae in Adult Cardiac Myocytes. *Circ Res.* 2008; 103(9):992–1000. [PubMed: 18802028]
64. Huang Y, Wright CD, Merkwand CL, Baye NL, Liang Q, Simpson PC, O'Connell TD. An α 1A-Adrenergic–Extracellular Signal-Regulated Kinase Survival Signaling Pathway in Cardiac Myocytes. *Circulation.* 2007; 115(6):763–772. [PubMed: 17283256]
65. Boivin B, Lavoie C, Vaniotis G, Baragli A, Villeneuve L-R, Ethier N, Trieu P, Allen BG, Hébert TE. Functional β -adrenergic receptor signalling on nuclear membranes in adult rat and mouse ventricular cardiomyocytes. *Cardiovascular Res.* 2006; 71(1):69–78.
66. Boivin B, Chevalier D, Villeneuve LR, Rousseau É, Allen BG. Functional Endothelin Receptors Are Present on Nuclei in Cardiac Ventricular Myocytes. *J Biol Chem.* 2003; 278(31):29153–29163. [PubMed: 12756260]
67. O'Malley KL, Jong YJ, Gonchar Y, Burkhalter A, Romano C. Activation of metabotropic glutamate receptor mGlu5 on nuclear membranes mediates intranuclear Ca^{2+} changes in heterologous cell types and neurons. *J Biol Chem.* 2003; 278(30):28210–9. [PubMed: 12736269]
68. Purgert CA, Izumi Y, Jong YJ, Kumar V, Zorumski CF, O'Malley KL. Intracellular mGluR5 can mediate synaptic plasticity in the hippocampus. *J Neurosci.* 2014; 34(13):4589–98. [PubMed: 24672004]
69. Vaniotis G, Glazkova I, Merlen C, Smith C, Villeneuve LR, Chatenet D, Therien M, Fournier A, Tadevosyan A, Trieu P, Nattel S, Hébert TE, Allen BG. Regulation of cardiac nitric oxide signaling by nuclear β -adrenergic and endothelin receptors. *J Mol Cell Cardiol.* 2013; 62(0):58–68. [PubMed: 23684854]
70. Tadevosyan A, Vaniotis G, Allen BG, Hébert TE, Nattel S. G protein-coupled receptor signalling in the cardiac nuclear membrane: evidence and possible roles in physiological and pathophysiological function. *J Physiol.* 2012; 590(6):1313–1330. [PubMed: 22183719]
71. Merlen C, Farhat N, Luo X, Chatenet D, Tadevosyan A, Villeneuve LR, Gillis M-A, Nattel S, Thorin E, Fournier A, Allen BG. Intracrine endothelin signaling evokes IP3-dependent increases in nucleoplasmic Ca^{2+} in adult cardiac myocytes. *J Mol Cell Cardiol.* 2013; 62(0):189–202. [PubMed: 23756157]

72. Ibarra C, Vicencio JM, Estrada M, Lin Y, Rocco P, Rebellato P, Munoz JP, Garcia-Prieto J, Quest AF, Chiong M, Davidson SM, Bulatovic I, Grinnemo KH, Larsson O, Szabadkai G, Uhlen P, Jaimovich E, Lavandero S. Local control of nuclear calcium signaling in cardiac myocytes by perinuclear microdomains of sarcolemmal insulin-like growth factor 1 receptors. *Circ Res*. 2013; 112(2):236–45. [PubMed: 23118311]
73. Kumar V, Jong YJ, O'Malley KL. Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca^{2+} release. *J Biol Chem*. 2008; 283(20):14072–83. [PubMed: 18337251]
74. Stauffer TP, Ahn S, Meyer T. Receptor-induced transient reduction in plasma membrane $\text{PtdIns}(4,5)\text{P}_2$ concentration monitored in living cells. *Current Biology*. 1998; 8(6):343–346. [PubMed: 9512420]
75. Hammond GRV, Fischer MJ, Anderson KE, Holdich J, Koteci A, Balla T, Irvine RF. PI4P and $\text{PI}(4,5)\text{P}_2$ Are Essential But Independent Lipid Determinants of Membrane Identity. *Science*. 2012; 337(6095):727–730. [PubMed: 22722250]
76. Várnai P, Balla T. Visualization of Phosphoinositides That Bind Pleckstrin Homology Domains: Calcium- and Agonist-induced Dynamic Changes and Relationship to Myo-[^3H]inositol-labeled Phosphoinositide Pools. *The Journal of Cell Biology*. 1998; 143(2):501–510. [PubMed: 9786958]
77. Tran D, Gascard P, Berthon B, Fukami K, Takenawa T, Giraud F, Claret M. Cellular distribution of polyphosphoinositides in rat hepatocytes. *Cell Signal*. 1993; 5(5):565–81. [PubMed: 8312134]
78. Balla T, Bondeva T, Várnai P. How accurately can we image inositol lipids in living cells? *Trends in Pharmacological Sciences*. 2000; 21(7):238–241. [PubMed: 10871889]
79. Blagoveshchenskaya A, Cheong FY, Rohde HM, Glover G, Knödler A, Nicolson T, Boehmelt G, Mayinger P. Integration of Golgi trafficking and growth factor signaling by the lipid phosphatase SAC1. *The Journal of Cell Biology*. 2008; 180(4):803–812. [PubMed: 18299350]
80. Watt SA, Kular G, Fleming IN, Downes CP, Lucocq JM. Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C $\delta 1$. *Biochem J*. 2002; 363(Pt 3):657–66. [PubMed: 11964166]
81. Zhang L, Malik S, Kelley GG, Kapiloff MS, Smrcka AV. Phospholipase $\text{C}\epsilon$ scaffolds to muscle-specific A kinase anchoring protein (mAKAP β) and integrates multiple hypertrophic stimuli in cardiac myocytes. *J Biol Chem*. 2011; 286(26):23012–21. [PubMed: 21550986]
82. Pare GC, Easlick JL, Mislow JM, McNally EM, Kapiloff MS. Nesprin-1 α contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp Cell Res*. 2005; 303(2):388–399. [PubMed: 15652351]
83. Pare GC, Bauman AL, McHenry M, Michel JJC, Dodge-Kafka KL, Kapiloff MS. The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling. *J Cell Sci*. 2005; 118(23):5637–5646. [PubMed: 16306226]
84. Kapiloff MS, Jackson N, Airhart N. mAKAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope. *J Cell Sci*. 2001; 114(17):3167–3176. [PubMed: 11590243]
85. Kapiloff MS, Schillace RV, Westphal AM, Scott JD. mAKAP: an A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J Cell Sci*. 1999; 112(16):2725–2736. [PubMed: 10413680]
86. Dodge-Kafka KL, Soughayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, Scott JD. The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature*. 2005; 437(7058):574–578. [PubMed: 16177794]
87. Tassin AM, Paintrand M, Berger EG, Bornens M. The Golgi apparatus remains associated with microtubule organizing centers during myogenesis. *J Cell Biol*. 1985; 101(2):630–638. [PubMed: 3894380]
88. Kronebusch PJ, Singer SJ. The microtubule-organizing complex and the Golgi apparatus are co-localized around the entire nuclear envelope of interphase cardiac myocytes. *J Cell Sci*. 1987; 88(1):25–34. [PubMed: 3327863]
89. Mesmin B, Bigay J, Moser von Filseck J, Lacas-Gervais S, Drin G, Antonny B. A four-step cycle driven by $\text{PI}(4)\text{P}$ hydrolysis directs sterol/ $\text{PI}(4)\text{P}$ exchange by the ER-Golgi tether OSBP. *Cell*. 2013; 155(4):830–43. [PubMed: 24209621]

90. Wu SC, Dahl EF, Wright CD, Cypher AL, Healy CL, O'Connell TD. Nuclear Localization of α_1 A-Adrenergic Receptors Is Required for Signaling in Cardiac Myocytes: An "Inside-Out" α_1 -AR Signaling Pathway. *J Amer Heart Assoc.* 2014; 3(2):e000145. [PubMed: 24772522]
91. Smrcka AV, Brown JH, Holz GG. Role of phospholipase C ϵ in physiological phosphoinositide signaling networks. *Cell Signal.* 2012;29:1333–1343.
92. Kapiloff MS, Piggott LA, Sadana R, Li J, Heredia LA, Henson E, Efendiev R, Dessauer CW. An Adenylyl Cyclase-mAKAP β Signaling Complex Regulates cAMP Levels in Cardiac Myocytes. *J Biol Chem.* 2009; 284(35):23540–23546. [PubMed: 19574217]
93. Oestreich EA, Wang H, Malik S, Kaproth-Joslin KA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV. Epac-mediated Activation of Phospholipase C ϵ Plays a Critical Role in β -Adrenergic Receptor-dependent Enhancement of Ca²⁺ Mobilization in Cardiac Myocytes. *J Biol Chem.* 2007; 282(8):5488–5495. [PubMed: 17178726]
94. Schmidt M, Evellin S, Weernink P, von Dorp F, Rehmann H, Lomasney J, Jakobs K. A new phospholipase-C-calcium signalling pathway mediated by cyclic AMP and a Rap GTPase. *Nat Cell Biol.* 2001; 3(11):1020–1024. [PubMed: 11715024]

Figure 1.

Possible mechanisms for nuclear envelope scaffolded PLC ϵ to access and hydrolyze PI4P found in the Golgi. A) An extended scaffold involving PLC ϵ scaffolded to mAKAP, which is in turn scaffolded to nesprin-1 α at the nuclear envelope may span the space between the outer nuclear envelope and the *cis* Golgi to hydrolyze PI4P in trans. B. Scaffolding of PLC ϵ to mAKAP may bind PLC ϵ in a way that allows dissociation and local action at the *cis* Golgi. Binding of PLC ϵ to mAKAP would serve to increase the local concentration of PLC ϵ in the space between the NE and the *cis* Golgi. C. Phospholipid transfer proteins (PLTP) such as oxysterol binding protein (OSBP) have recently been shown to transport PI4P from the Golgi to the ER in exchange for cholesterol at Golgi-ER membrane contact sites. Since the NE is contiguous with the ER it is possible that this process could occur at the large Golgi-ER interface to supply PI4P to the NE where it could be accessed by mAKAP associated PLC ϵ .

Figure 2.

Scenarios for access to phosphoinositide substrates by PLCs downstream of GPCRs. A)

GPCRs on the inner NE could activate PLC β that could theoretically transiently dissociate to

access PIP₂ in the nuclear matrix. PIP₂ has been found in the nuclear matrix. B) Similar to A

but the PIP₂ substrate would be present in the nuclear envelope. This would be a preferred

substrate for a PLC activated on the nuclear envelope but the evidence for the presence of

PIP₂ at this location is lacking. C) As in Figure 1C, GPCR activated PLC on the outer

nuclear envelope could access PI4P supplied to the nuclear envelope from the Golgi by

PLTPs. D) PLCs activated by GPCRs on the outer nuclear envelope could transiently

dissociate to access PI4P in the Golgi.