Chapter 17
Phospholipase C Signaling in Heart Disease

Elizabeth A. Woodcock

Abstract Phospholipase C (PLC) expression and activity have repeatedly been reported to be elevated in cardiomyocytes under pathological conditions, including ischemia/reperfusion, hypertrophy, and chamber dilatation. In recent studies the subtypes of PLC involved have been identified, paving the way for studies of the mechanisms by which PLC may be activated under pathological conditions and how this may contribute to disease progression. PLC subtypes are localized by subtype- and tissue-specific binding to scaffolding proteins providing the possibility of developing cardiac-specific therapies based on inhibition of the localization of particular PLC subtypes in cardiomyocytes.

Keywords Ischemia/reperfusion • Hypertrophy • Dilatation • Scaffolding protein

17.1 Introduction

Phosphatidylinositol-specific phospholipases C (PLCs) are enzymes that cleave the plasma membrane phospholipid, phosphatidylinositol(4,5)*bis*phosphate (PIP_2), to generate inositol(1,4,5)*tris*phosphate ($Ins(1,4,5)P_3$), a Ca^{2+} releasing intracellular messenger, and *sn*-1,2-diacylglyerol (DAG), an activator of conventional subtypes of protein kinase C. The substrate lipid and the two products all have critical roles in regulating cellular responses and therefore PLCs are of central importance in the functioning of all cell types. Furthermore, perturbations in PLC activity may contribute substantially to disease phenotypes in a range of different tissues. As expected from a family of enzymes with such a central role in signaling, PLCs can be

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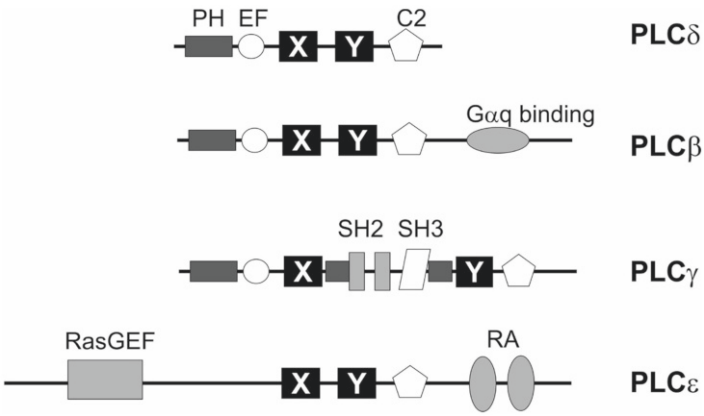

Fig. 17.1 Diagram showing the relationship between the different classes of the PLC family of proteins, emphasizing the structural motifs present

regulated in many different ways. PLCs are classified into six major classes (β, γ, δ, ε, ν, ζ), each of which includes multiple subtypes and splice variants (Fig. 17.1) [1]. PLCβ family members (PLCβ1-4) respond to G protein subunits activated downstream of seven transmembrane spanning receptors (also called G protein-coupled receptors, GPCR) [2]. PLCβ1 and PLCβ3 are expressed in cardiomyocytes, but PLCβ2 is not. PLCβ1 exists as two splice variants that differ only in their extreme C-terminal sequences, PLCβ1a (MW 150kD) and PLCβ1b (MW 140kDa, Fig. 17.2). Whilst both splice variants are expressed in neonatal rat cardiomyocytes [3], only PLCβ1b is expressed in adult human, rat, and mouse heart [4]. PLCγ members (PLCγ1 and PLCγ2) translocate to the plasma membrane subsequent to the activation of receptor tyrosine kinases, following stimulation with the appropriate growth factor [5]. Hearts express primarily PLCγ1 [6]. PLCδ subtypes are more sensitive to activation by Ca^{2+} than other subtypes, and hearts express PLCδ1, but the physiological importance of this has not been firmly established [7, 8]. PLCε regulation is complex involving a variety of activators including monomeric G proteins of the Ras family, as well as heterotrimeric G proteins of the $G_{12/13}$ family and Gβγ [9]. Thus receptor activation can lead to PLCε activation by a variety of signaling mechanisms, often well downstream of receptor activation. There is only a single PLCε gene product, but this is expressed as two N-terminal splice variants [10]. Other PLC subtypes are not expressed in heart and will not be considered further.

17.2 The Regulation of PLC Activity in Heart

Early studies showed that activation of α_1-adrenergic receptors [11], M2 muscarinc cholinergic receptors [12] or endothelin receptors [13] resulted in generation of Ins(1,4,5)P_3 and its metabolites. Subsequently, activation via purinergic receptors

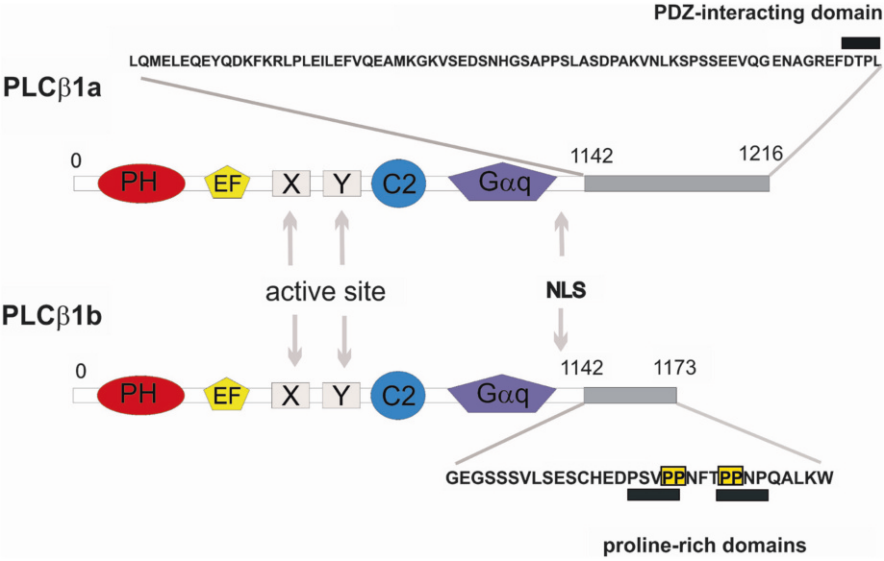

Fig. 17.2 The splice variants of PLCβ1. Diagram showing the structures of PLCβ1a and PLCβ1b outlining the sequence differences in the C-terminal regions of the proteins. Proline-rich domains and PDZ-interacting domains are indicated. NLS is nuclear localization sequence

was reported [3]. All of these factors bind receptors coupled to Gq and would therefore be expected to activate PLCβ family members [14]. There have also been reports of activation via growth factor receptors that would be expected to activate PLCγ subtypes [15]. More recently the novel PLCε subtype has been identified in cardiomyocytes [16] and shown to be activated via thrombin (protease activated receptor 1, PAR1) and sphingosine1-phosphate (S1P) receptors [17]. In addition to activation by hormones and neurotransmitters, PLC in intact hearts and in cardiomyocytes in culture responds to acute stretch [18–21].

17.3 Localization of PLC Subtypes in Heart

To be active PLCs must be located close to their substrate PIP_2, localized primarily or exclusively at the sarcolemma. It is now well recognized that PLC subtypes are specifically localized to particular membrane regions by binding scaffolding proteins. These scaffolds are selective for particular PLC subtypes and, in some cases, are also tissue specific.

In the case of the PLCβ family, such scaffolding interactions generally involve a C-terminal PDZ-interacting domain, present in all PLCβ1 subtypes except PLCβ1b. These PDZ-interacting domains associate with particular PDZ (postsynaptic density protein, Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein domain) proteins. PLCβ3, for instance, binds to cell polarity proteins,

Par3 and Par6, in renal tubular epithelial cells, SH3 domain and ankyrin repeat protein 2 (Shank2) at glutamatergic synapses in neuronal tissues [22], and the sodium hydrogen exchange regulatory protein 2 (NHERF2) in Cos7 cells [23]. All of these interactions require PDZ domain interactions via the C-terminal sequence, NTQL. PLCβ3 is not localized to the sarcolemma, at least in neonatal rat cardiomyocytes [24], suggesting that suitable scaffolding proteins are not expressed, or are not associated with the sarcolemma. The C-terminal PDZ-interacting domain of PLCβ1a (DTPL) binds selectively to the first PDZ domain (nearest the N-terminal) of the scaffolding protein, sodium hydrogen exchange regulatory factor 1 (NHERF1), but not NHERF2, in HEK293 cells [25]. The first PDZ domain of NHERF1 also binds PLCβ2 via the sequence ESRL [26, 27]. NHERF1 is not expressed in heart providing an explanation for the cytoplasmic localization of PLCβ1a when expressed in cardiomyocytes [24]. As noted above, PLCβ1b does not have a C-terminal PDZ-interacting domain and so must target to membranes by a different mechanism from that used by other PLCβ subtypes. The presence of two proline-rich domains at the C-terminal end points to targeting by an SH3 domain- [28] or a WW domain-containing protein [29]. In cardiomyocytes the scaffolding protein for PLCβ1b was identified as Shank3. Shank3 is a high MW protein with multiple protein interaction motifs. Importantly, Shank3 has a type 1 SH3 domain suitable for binding the PPNP (1165–1168 in the human PLCβ1b sequence) proline-rich sequence in the extreme C-terminal region of PLCβ1b [30]. In addition to its SH3 domain, Shank3 has an N-terminal ankyrin-rich repeat sequence that binds α-fodrin, a PDZ domain, a long proline-rich sequence that binds the Homer family of proteins and cortactin, and finally a C-terminal sterile alpha motif (SAM) that facilitates dimerization. Association with fodrin likely localizes Shank3 close to the sarcolemma. Thus, association with Shank3 makes PLCβ1b part of a multi-protein system that may be critical for downstream signaling and cellular responses (Fig. 17.3). Importantly, Shank3 is expressed in only a limited number of tissues, primarily heart and glutamatergic neurons [30], and thus the binding of PLCβ1b (also with limited tissue distribution) to Shank3 provides a possible heart-specific drug target.

PLCδ1 is expressed in heart [4], although no function has unequivocally been ascribed. PLCδ subtypes have a high affinity PH domain that shows high selectivity for PIP_2 and this is sufficient to localize these to the sarcolemma [31].

PLCγ family members are activated following phosphorylation by receptor tyrosine kinases and this facilitates binding to SH2 domains present in growth factor receptors localizing these PLCs close to the plasma membrane and their substrate PIP_2 [5]. As with PLCβ subtypes, localization and activation of PLCγ members may also involve binding to other signaling proteins. PLCγ subtypes have been reported to bind to sodium-hydrogen exchanger 3 (NHE3), a plasma membrane-localized ion exchanger, and regulate its activity [32]. Interestingly, PLCγ1 has been shown to interact directly with canonical transient receptor 3 (TrpC3) to control its cell surface expression [33]. TrpC3 is implicated as contributing to pathological cardiomyocyte hypertrophy [34]; however, PLCγ1 has not been implicated in this response.

As outlined earlier, PLCε is structurally more complex that other PLCs and, as a consequence of this, its regulation also is multifactorial. Like other PLC subtypes,

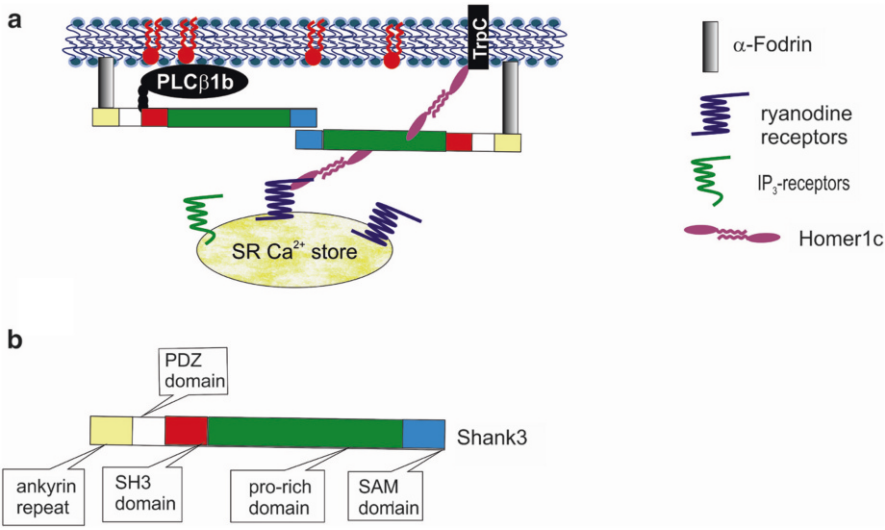

Fig. 17.3 (**a**) PLCβ1b binding to a Shank3 complex localized below the sarcolemmal membrane. Shank3 forms homodimers via its C-terminal SAM domains and is bound to α-fodrin via ank repeats in the N-terminal sequence. Dimeric Homer proteins cross-link Shank3 to TrpC channels and to intracellular Ca^{2+} channels. (**b**) Diagram showing domain structure of Shank3

PLCε binds to a scaffolding protein via sequences in its C-terminal region, in this case its (Ras association 1) RA1 domain. The RA1 domain of PLCε binds to the first spectrin repeat domain of muscle A-kinase-anchoring protein β (mAKAPβ) localizing this PLC subtype principally to the nuclear envelope in cardiomyocytes [35]. mAKAPβ, like Shank and NHERF proteins, is a multidomain scaffold and thus PLCε probably functions as part of a large protein complex.

17.4 Pathological Responses in the Heart

The primary function of the heart is to supply blood to all tissues of the body at sufficient level to optimize their function. The pump function of the heart can be compromised by a loss of contractile function of the muscle that reduces cardiac output resulting in failure to adequately supply blood to the body, a condition known as heart failure. Ineffective pumping can also be caused by a loss in organization of the contraction of the individual muscle cells, a condition known as arrhythmia. Heart failure and arrhythmia often occur together, each worsens the other and both can result from chronic hypertrophic growth of the myocardium. Because of this, there is an interest in developing therapies targeted at reducing pathological hypertrophic cardiomyocyte growth, improving contractile function (inotropic agents), or reducing arrhythmia (anti-arrhythmic agents). Currently used pharmaceuticals commonly

target cell surface receptors or ion channels, their ligands, or the downstream signaling pathways, including drugs that reduce the generation or the receptor binding of angiotensin II, blockers of β-adrenergic receptors, Ca^{2+} channel blockers, and agents that reduce the metabolism of cAMP [36]. There is clearly a need for the development of better tolerated therapies, particularly if they can be made relatively cardiac-specific.

17.5 How Might PLC Activation Contribute to Pathology?

PLC enzymes hydrolyze the sarcolemmal phospholipid, PIP_2, to generate Ins(1,4,5)P_3 that can release Ca^{2+} from intracellular stores [37] and *sn*-1,2-diacylglycerol (DAG), an activator of conventional PKC subtypes [38], PKD [39] and some TrpC channels [40]. Each of these factors, individually and in concert, can have critical effects on cellular responses.

17.5.1 Ins(1,4,5)P_3

Ins(1,4,5)P_3 binds and activates IP_3-R localized on intracellular Ca^{2+} stores [41]. The expression level of IP_3-R in cardiomyocytes is low compared with that in most other tissues and compared with the highly expressed ryanodine receptors [42] that are primarily responsible for the intracellular Ca^{2+} cycling that regulates the heart beat. Furthermore, IP_3-R in ventricular myocytes are localized around the nuclear membrane [43], seemingly distal from the site of generation of Ins(1,4,5)P_3 following activation of cell surface receptors. These nuclear membrane-localized IP_3-R(2) may supply the localized Ca^{2+} signals required to activate calmodulin-activated protein kinases (CaMKII) involved in transcriptional regulation [44]. Ins(1,4,5)P_3 has been suggested to be involved in arrhythmogenesis [45–47] and in hypertrophy [48], although direct evidence for either of these is lacking.

17.5.2 DAG

The other product generated by PLC, DAG, has a complex spectrum of activities, all of which could contribute to pathology. DAG was initially discovered as an activator of PKC [38], particularly the “conventional” PKC subtypes (PKC α, β, γ, δ ε, η, θ) [49]. DAG also activates some TrpC channels [50] and protein kinase D directly [39], in addition to actions dependent on PKC. In contrast to the controversy surrounding the contribution of Ins(1,4,5)P_3 and IP_3-R to cardiac physiology/pathophysiology, DAG and the PKC family are well accepted as a contributor to cardiac regulation. The contribution of PKC to regulation in the heart is complex,

varying with the PKC subtype, the stage of development, and the mechanism of activation. PKCα activation serves to suppress contractility [51], but can have profound pathological consequences when the regulatory domain that limits catalytic activity is removed by calpain cleavage under conditions of ischemia and reperfusion [52]. PKCβ subtypes have been shown to be involved in diabetic cardiomyopathy [53]. PKCδ has been considered an important contributor to cardiac pathology and cardiac remodeling, apparently related to activation of mitochondrial apoptotic responses [54]. PKCε primarily has a protective role in heart and is a component of preconditioning mechanism that reduces subsequent ischemic damage, discussed in more detail subsequently [55]. A recent review provides detailed information about PKC contribution to cardiac signaling under physiological and pathological conditions [56].

17.5.3 PIP_2

The process of PLC activation depletes PIP_2 as it generates $Ins(1,4,5)P_3$ and DAG. Reductions in PIP_2 are often localized and transient with the PIP_2 being replaced immediately, presumably by phosphorylation of PIP [57, 58]. However, PLC-induced localized changes in PIP_2 regulate ion channels and exchangers that are critical in maintaining heart rhythm [59], for a review see [40]. PIP_2 is also critical for maintaining the cytoskeleton via its association with actin-binding proteins [60] and PIP_2 is essential for localizing proteins to the plasma membrane [61].

17.6 PLC Involvement in Ischemia and Post-ischemia Reperfusion

Cardiac ischemia occurs when there is an interruption in the blood supply to the heart, depriving it of oxygen and nutrients, a condition associated with arrhythmia and cardiomyocyte death. The reintroduction of flow, reperfusion, also is associated with arrhythmia, cell death and contractile dysfunction. A number of studies have reported increased activity of PLC in animal models of acute cardiac ischemia [62–64]. Substantially increased PLC activation has been reported in early post-ischemic reperfusion following a brief period of ischemia [65–67], and inhibition of PLC under these conditions successfully prevents reperfusion arrhythmias [45, 46, 68] in addition to improving functional recovery [69]. However, the subtypes of PLC activated by ischemia/reperfusion are unknown as are the mechanisms leading to the heightened PLC response.

Increased expression of PLCβ, as well as of activating G proteins, has been reported in border zone and remote myocardium following myocardial infarction in humans, suggesting the likelihood of enhanced PLC activation [70] and pointing to a possible involvement in the heart's responses to chronic ischemia.

Other studies reported that protection from chronic ischemic damage by ethanol is mediated by elevation of PLC activity, but the subtype of PLC was not identified [71].

Defining contributions of PLC, its substrate and products, to ischemic or reperfusion responses is confounded by the likelihood that one or other of these might contribute to preconditioning, a phenomenon that can provide protection from arrhythmia and infarction following an ischemic insult [72]. Preconditioning involves subjecting hearts to brief periods of ischemia and reperfusion prior to the main ischemia/reperfusion procedure. This pretreatment procedure is sufficient to limit PLC activation in early post-ischemic reperfusion [73]. Preconditioning protection can be mimicked by activation of some of the PKC subtypes that are activated downstream of PLC, and to further complicate the situation, different PKC subtypes can have opposing effects on preconditioning [74]. Overexpression of either subtype of α_1-adrenergic receptors (α_{1A}- or α_{1B}-) results in heightened PLC responses to endogenous or exogenous norepinephrine. However, whilst PLC activity in these overexpressing transgenic strains was heightened in normoxia, the exaggerated response during early reperfusion was eliminated, along with the reperfusion arrhythmias [75, 76]. Presumably, this apparent contradiction is related to activation of preconditioning pathways possibly initiated by PKC activation. Taken together, these studies imply that factors downstream of PLC, most likely PKC-initiated responses, effectively precondition the myocardium, and that preconditioning reduces PLC activation.

17.7 PLC in Acute and Chronic Dilatation of the Myocardium

The myocardium responds to acute stretch by increasing cardiac output in order to manage the increase in blood volume. Thus, acute stretch results in increased rate and force of contraction. Acute stretch of the right atrium causes substantial release of atrial natriuretic peptide, possibly to facilitate a lowering of blood volume [77]. As noted earlier, in addition to activation by ligand receptor binding, PLC in heart can be activated acutely by stretch [18–21]. In perfused rat heart preparations, right atrial stretch caused PLC activation that correlated with release of atrial natriuretic peptide [78]. Stretch activation of PLC requires Gq and may involve angiotensin II receptors (AT1) acting in a ligand-independent manner [21]. The involvement of Gq and AT1 receptors implicates PLCβ subtypes as major contributors to the response to acute stretch.

Chronically increased wall tension results in chamber dilatation and wall thinning that eventually limit contractile performance and these are the hallmarks of dilated cardiomyopathies. Dilatation of the atria is observed in patients with valve diseases and is also seen in association with ventricular failure. Interestingly, substantially heightened PLC activity was observed in the dilated atria of patients suffering from valvular heart disease, as well as in atria from a mouse model of dilated cardiomyopathy that has severe atrial enlargement together with conduction

block and a sensitivity to atrial fibrillation [4, 79]. Furthermore, in both humans and mice, PLC activity correlated with atrial volume, suggesting that PLC activation was either a cause or a consequence of dilatation. Dilated atrial tissue from both humans and mice showed increased expression of only one PLC subtype, PLCβ1b, providing suggestive evidence that PLCβ1b is selectively involved in the response to chronic dilatation. There were no changes in expression of PLCβ3, PLCδ1, or PLCγ1 associated with atrial dilatation [4]. PLCε was not measured in these studies and a role for this subtype, therefore, cannot be discounted. PLCβ1a, although expressed in neonatal rat cardiomyocytes, was not expressed at measurable levels in adult human myocardium. The two splice variants of PLCβ1, PLCβ1a and PLCβ1b, differ only in their extreme C-terminal sequences as shown in Fig. 17.2. Whilst the catalytic domains and the Gαq-binding regions are identical, the differences in the C-terminal sequences would be expected to result in different localization, and consequently different activities.

Overexpression of a constitutively active Gαq is sufficient to cause severe chamber enlargement together with heightened PLC activity [80], but there are conflicting opinions about the role of PLC in promoting atrial dilatation in these Gαq-overexpressing models. Overexpression of a Gαq mutant with reduced ability to activate PLCβ, unlike the wild-type, did not result in chamber dilatation [81], providing powerful evidence for a requirement for PLC activity for the pathological responses initiated by Gq. Other studies showed that atrial remodeling in Gαq-overexpressing mice was reversed by co-expression of diacylglycerol (DAG) kinase ζ, an enzyme that depletes DAG, one of the immediate products of PLC activation [82], supporting a critical role for PLC and its immediate product, DAG, in atrial dilatation. However, in contrast to these findings, studies comparing two different Gαq-expressing transgenic lines reported that the degree of dilatation did not correlate with the extent of PLC activation [83]. These apparent discrepancies might be accounted for if there was a maximal level of PLC activation, above which further increases produced no greater effect on chamber dilatation.

At the cellular level, chamber dilatation and wall thinning are thought to involve loss of functional myocytes by apoptotic and non-apoptotic mechanisms. The ability of activated mutants of Gαq to induce apoptosis in cardiomyocytes is well documented [84], and more recently overexpression of wild-type PLCβ1b has also been shown to cause cardiomyocyte apoptosis [85]. Thus, heightened PLCβ1b activity could contribute to a dilated phenotype by promoting apoptotic death of cardiomyocytes. In summary, there is evidence for an involvement of PLC, and in particular PLCβ1b, in responses to acute and chronic dilatation of the myocardium, but the mechanisms involved remain to be established.

17.8 PLC Involvement in Cardiac Hypertrophy

Early studies using isolated cardiomyocytes or genetically modified mice pointed to a role for Gq family members in pathological growth and remodeling of the heart. Overexpression of Gαq, either the wild-type [86] or a constitutively active mutant [80],

was sufficient to cause cardiomyocyte hypertrophy, and when expressed in vivo, Gαq promoted hypertrophy and heart failure [84]. More importantly, Gq inhibitors expressed in the heart were found to substantially reduce hypertrophic growth in response to the clinically relevant challenges of pressure or volume overload [87–89]. The apparent central role of Gq in these pathological responses suggests mediation by PLCβ subtypes, as these are the best understood effectors of Gq [90]. However, members of the Rho family of monomeric G proteins are activated downstream of Gq [91] and these may also contribute to hypertrophic responses [92].

Of the PLCβ family, only PLCβ1b causes hypertrophy when overexpressed in cardiomyocytes, and this selectivity depends on its sarcolemmal localization facilitated by selective association of the splice variant-specific C-terminal sequence with the scaffolding protein Shank3 [24, 85]. Furthermore, inhibition of PLCβ1b binding to Shank3 prevented hypertrophy in response to Gq activation [85], suggesting that the sarcolemmal targeting of PLCβ1b might provide a novel target to limit hypertrophy and chamber dilatation. Both PLCβ1b and Shank3 have a limited tissue distribution opening up the possibility of cardiac-specific therapy. In addition to cardiomyocytes, Shank3 is expressed primarily in postsynaptic density fractions from central glutamatergic neurons [30], where PLCβ1b is not expressed. In neurons, Shank3 acts as a scaffold facilitating interactions between receptors and early signaling proteins [93]. In heart, Shank3 appears to function similarly, binding fodrin [94] and Homer1c [95] in addition to its association with the C-terminal sequence of PLCβ1b. Homer1c forms homodimers that can cross-link Shank3 to form large molecular scaffolds [96]. Homers promote crosstalk between intracellular Ca^{2+} channels, IP_3-R and ryanodine receptors, and cell surface canonical transient receptor potential channels (TrpC) and thus are regulators of local Ca^{2+} responses [97]. Expression of PLCβ1b in cardiomyocytes results in increased expression of Homer1c as well as its translocation to the Shank3/PLCβ1b complex [95]. The mechanisms involved in these responses are unknown, but they appear to be critical for the hypertrophic response.

The possibility that PLCε was involved in cardiac pathology was first suggested when elevated expression was reported in failed human left ventricle [16]. This idea was supported by studies showing that PLCε−/− mice exhibited exacerbated hypertrophic responses leading to the idea that PLCε, in contrast to PLCβ1b, was protective to the myocardium by inhibiting hypertrophic signaling. However, subsequent studies in isolated cardiomyocytes have questioned this conclusion. These studies found that treatment with si-RNA to knockdown PLCε inhibited hypertrophy in response to endothelin or α_1-adrenergic agonists [35], implying an involvement in Gq-initiated hypertrophy that other studies have shown involves PLCβ1b [85]. Importantly, PLC activity was absolutely required for this contribution of PLCε to hypertrophy, an important finding given the multiple functions of this complex PLC subtype. In cardiomyocytes, PLCε is localized onto the nuclear membrane by association with muscle A-kinase-activating protein (mAKAPβ, AKAP5) [35]. Such localization is suggestive of a role downstream of early signaling responses, such as initiated by PLCβ1b. In agreement with this, knockdown of PLCε inhibited hypertrophy in response to multiple stimuli, including both Gq hypertrophy that models

pathological hypertrophy and hypertrophy caused by IGF treatment, considered a model of physiological hypertrophy that is independent of Gq [35]. This contrasts to PLCβ1b, where inhibition selectively prevented Gq-mediated hypertrophy [85]. There is clearly substantial evidence for an involvement of PLC in hypertrophy of the myocardium, with current data supporting roles for PLCβ1b and PLCε, most likely at different stages in the signaling response.

17.9 Conclusions

Under physiological conditions the functioning of the heart is regulated primarily by pathways that are independent of PLC activation. However, PLC expression and activity have been shown to increase under a range of pathological conditions, including ischemia/reperfusion, hypertrophy, and dilatation and it is likely that PLC contribute to the progression of these diseases.

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