Nilius B, Prenen J, Voets T, Droogmans G: 2004b. Intracellular nucleotides and polyamines inhibit the Ca^{2+}-activated cation channel TRPM4b. Pflügers Arch 448:70–75.

Nilius B, Prenen J, Tang J, et al.: 2005. Regulation of the Ca^{2+} sensitivity of the nonselective cation channel TRPM4. J Biol Chem 280:6423–6433.

Okada T, Inoue R, Yamazaki K, et al.: 1999. Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem 274:27359–27370.

Perez CA, Huang L, Rong M, et al.: 2002. A transient receptor potential channel expressed in taste receptor cells. Nat Neurosci 5:1169–1176.

Ruocco C, Cerbai E, Failli P, et al.: 1996. Calcium-dependent electrophysiological alterations in hypertrophied rat cardiomyocytes. Biochem Biophys Res Commun 229:425–429.

Teulon J: 2000. Ca^{2+}-activated nonselective cation channels. *In* Endo M, Kurachi Y, Mishina M (Eds.), Pharmacology of ionic channel function: activators and inhibitors-Springer-Verlag, Berlin, 625–649.

Ullrich ND, Voets T, Prenen J, et al.: 2005. Comparison of functional properties of the Ca^{2+}-activated cation channels TRPM4 and TRPM5 from mice. Cell Calcium 37:267–278.

Van den Abbeele T, Tran Ba Huy P, Teulon J: 1996. Modulation by purines of calcium-activated non-selective cation channels in the outer hair cells of the guinea-pig cochlea. J Physiol 494:77–89.

Verkerk AO, Veldkamp MW, Bouman LN, van Ginneken AC: 2000. Calcium-activated Cl^- current contributes to delayed afterdepolarizations in single Purkinje and ventricular myocytes. Circulation 101: 2639–2644.

Verkerk A, Veldkamp MW, Baartscheer A, et al.: 2001. Ionic mechanisms of delayed afterdepolarizations in ventricular cells isolated from human end-stage failing hearts. Circulation 104:2728–2733.

Wu Y, Anderson ME: 2000. Ca^{2+}-activated non-selective cation current in rabbit ventricular myocytes. J Physiol 522:51–57.

Zhainazarov AB: 2003. Ca^{2+}-activated nonselective cation channels in rat neonatal atrial myocytes. J Membr Biol 193:91–98.

Zhang Z, Okawa H, Wang Y, Liman ER: 2005. Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. J Biol Chem 280:39185–39192.

Zygmunt AC: 1994. Intracellular calcium activates a chloride current in canine ventricular myocytes. Am J Physiol 267: H1984–H1995.

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Phosphoinositide 3-kinase γ Regulates Cardiac Contractility by Locally Controlling Cyclic Adenosine Monophosphate Levels

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Class I phosphoinositide 3-kinases (PI3Ks) are enzymes with both protein and lipid kinase activities that regulate important cellular functions in many tissues. In the heart, subclass IA PI3Ks (mainly PI3Kα) regulate cell growth, apoptosis, cell division and cell size, whereas PI3Kγ, the only member of subclass IB, has been shown to regulate cardiac contractility. We have shown that the loss of PI3Kγ (PI3K$\gamma^{-/-}$ mice) enhances cardiac excitation–contraction coupling by modulating cyclic adenosine monophosphate (cAMP) levels in subcellular domains containing the sarcoplasmic reticulum. Specifically, PI3K$\gamma^{-/-}$ mice show enhanced sarcoplasmic reticulum Ca^{2+} cycling in association with increased cAMP. Surprisingly, L-type Ca^{2+} current, a prototypic target of cAMP-dependent protein kinase A phosphorylation, is largely unchanged in PI3K$\gamma^{-/-}$ mice. In this article, we discuss the consequences and implications of cAMP compartmentation in cardiomyocytes. We also review the different roles of PI3Kγ in the heart, particularly as they relate to cardiac contractility, intracellular cAMP levels, and the regulation of β-adrenergic receptor signaling in physiologic and pathologic states. (Trends Cardiovasc Med 2006;16:250–256) © *2006, Elsevier Inc.*

Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes that have the unique capacity to function both as lipid and protein kinases. PI3Ks have been divided into three classes (I, II, and III) based on their substrate specificity, mode of activation, and molecular structure (reviewed in Oudit et al. 2004). Class I PI3Ks convert phosphatidylinositol-4, 5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-triphosphate (PIP_3), which acts as a second messenger by recruiting effectors with pleckstrin homology domains, such as protein kinase B

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Abbreviations: AC, Adenylyl cyclase; AKAP, A-kinase anchoring protein; β-AR, β-Adrenergic receptors; ECC, Excitation–contraction coupling; $I_{Ca,L}$, L-type Ca^{2+} current; ISO, Isoproterenol; PDE, Phosphodiesterase; PI3K, Phosphoinositide 3-kinase; PIP_2, Phosphatidylinositol-4,5-bisphosphate; PIP_3, Phosphatidylinositol-3,4,5-triphosphate; PKA, Protein kinase A; PKB/Akt, Protein kinase B; PLN, Phospholamban; RyR_2, Ryanodine receptor type 2; SERCA-2a, Sarcoendoplasmic reticulum Ca^{2+}-ATPase pump type 2a; SR, Sarcoplasmic reticulum.

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(PKB/Akt). PI3Kα and PI3Kγ, which are members of PI3K subclass IA and IB, respectively, are the two main isoforms expressed in cardiomyocytes (Prasad et al. 2003). While PI3Kα regulates heart growth (Luo et al. 2005), PI3Kγ has emerged as an important regulator of cardiac contractility (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Alloatti et al. 2005, Kerfant et al. 2005) because of its ability to modulate cyclic adenosine monophosphate (cAMP) metabolism (Kerfant et al. 2005). The available data indicate that PI3Kγ regulates cAMP in compartmentalized microdomains within ventricular cardiomyocytes (Kerfant et al. 2005). This review will focus on the compartmentalized regulation of cAMP metabolism and cardiac ECC by PI3Kγ. We begin with a general description of ECC and cAMP compartmentation in the heart.

Cardiomyocyte contraction is controlled by a series of events in which electrical signals generated at the plasma membrane of the cardiomyocyte lead to contraction of the myofilaments (Bers 2002), a process called cardiac-excitation-contraction coupling (ECC). Specifically, during the cardiac action potential, sarcolemmal Na^+ and L-type Ca^{2+} channels open in response to changes in membrane voltage. Ca^{2+} influx through L-type Ca^{2+} channels induces local elevations of intracellular Ca^{2+} ($[Ca^{2+}]_i$), leading to the opening Ca^{2+} release channels (called "ryanodine receptors") in the sarcoplasmic reticulum (SR), by the process of Ca^{2+}-induced Ca^{2+} release. The Ca^{2+} released from the SR, in combination with the influx of Ca^{2+} from L-type Ca^{2+} channels, raises $[Ca^{2+}]_i$ to a level (~600 nM) sufficient to activate myofilament contraction. To maintain Ca^{2+} homeostasis, cytosolic Ca^{2+} is returned to the SR via the sarcoendoplasmic reticulum Ca^{2+}-ATPase pump type 2a (SERCA-2a) and extruded from the cell primarily by sarcolemmal Na^+–Ca^{2+} exchangers as well as secondarily by plasmalemmal Ca^{2+} pumps (reviewed in Bers 2002).

Several of the proteins involved in cardiac ECC are sensitive to phosphorylation and dephosphorylation events by kinases and phosphatases. The best-characterized example of this type of regulation is the positive inotropic and lusitropic response of cardiomyocytes to β-adrenergic receptor (β-AR) activation. Agonist stimulation of β-ARs (mainly β_1-ARs) activates the heterotrimeric guanosine triphosphate-binding protein, G_s, causing the activation of adenylyl cyclase (AC) enzymes. Adenylyl cyclase stimulates cAMP production, which activates the cAMP-dependent protein kinase A (PKA), leading to the phosphorylation of proteins such as L-type Ca^{2+} channels, phospholamban (PLN), ryanodine receptor type 2 (RyR_2), myosin-binding protein C, and troponin I (Bers 2002). Thus, the regulation of intracellular cAMP levels is a critical determinant of the heart's contractile function.

• cAMP Compartmentation in the Heart

In addition to the β-ARs, there are several other hormones and compounds that bind to G-protein-coupled receptors and increase cAMP; however, not all of these hormones produce the inotropic and lusitropic responses seen with β-AR stimulation in the heart. For example, both glucagon-like peptide-1 and prostaglandin E_1 increase cAMP to a level comparable with that elicited by the β-AR agonist isoproterenol (ISO), but neither of these hormones elicit the contractile responses observed with ISO (Keely 1979, Vila Petroff et al. 2001). These observations suggest that cAMP signaling may be compartmentalized in cardiomyocytes and that different hormones activate distinct pools of downstream signaling molecules (reviewed in Steinberg and Brunton 2001, Bers and Ziolo 2001).

Phosphodiesterases (PDEs), the enzymes responsible for the hydrolysis of cAMP, appear to be critical for cAMP compartmentation (Jurevicius and Fischmeister 1996, reviewed in Baillie et al. 2005). For example, in studies measuring L-type Ca^{2+} current ($I_{Ca,L}$) with two electrodes, the application of forskolin (a direct activator of AC) to a selected region of the cell membrane increased $I_{Ca,L}$ throughout the cell, whereas local application of ISO only increased $I_{Ca,L}$ locally. However, the same local application of ISO increased $I_{Ca,L}$ throughout the entire cell when PDEs were inhibited. These results establish that PDEs restrict cAMP signaling and PKA-dependent phosphorylation in cardiomyocytes to local cellular compartments surrounding β-ARs. In a separate study, mice with cardiac-specific overexpression of human AC type 8 showed increased cardiac contractility in association with elevated Ca^{2+} transients and accelerated relaxation but without alterations of the $I_{Ca,L}$ amplitude (Georget et al. 2003). These compartmentation effects were linked to the reorganization of specific PDEs within cardiomyocytes (Georget et al. 2003). A more recent study has shown that PDE4D3 binds to RyR_2 in adult cardiomyocytes and locally regulates cAMP levels at the Z-line region of the sarcomere (Lehnart et al. 2005), suggesting the PDE4 family may be important in cAMP compartmentation in SR regions containing the RyR_2 proteins in cardiomyocytes.

Additional molecules that contribute to the compartmentation of cAMP signaling are anchoring proteins for PKA and phosphatases (Bers and Ziolo 2001, Bauman and Scott 2002, Wong and Scott 2004). For example, A-kinase anchoring proteins (AKAPs) play a critical role in the organization and localization of macromolecular complexes of signaling molecules in microdomains of cells. A-kinase anchoring proteins often bind directly to proteins that are targets for PKA-dependent phosphorylation. One such molecule is muscle-specific A-kinase anchoring protein (mAKAP), which dynamically associates with both PKA and PDE4D3 and functions to locally control PKA activity by controlling cAMP levels in microdomains (Bauman and Scott 2002, Wong and Scott 2004). In summary, protein–protein interactions between AKAPs, PKA, and PDEs (as well as other proteins) result in the formation of dynamic microdomains for spatial and temporal control of cAMP signaling in cardiomyocytes.

• PI3Kγ and cAMP Compartmentation in Ventricular Cardiomyocytes

Two separate mouse strains with targeted deletion of the PI3Kγ gene (PI3K$\gamma^{-/-}$) have been created (Hirsch et al. 2000, Sasaki et al. 2000). These transgenic mice are viable and fertile, with normal heart rates and normal mean arterial pressures (Crackower et al. 2002). PI3K$\gamma^{-/-}$ mice show significantly enhanced cardiac function and increased cardiomyocyte contractility in association with elevated basal intracellular cAMP levels (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004,

Alloatti et al. 2005), but without alterations in PKB/Akt activity. Consistent with increased levels of cAMP, PI3K$\gamma^{-/-}$ mice display elevated phosphorylation of PLN, which correlates closely with the increases in cardiac contractility and lusitropy (Crackower et al. 2002, Patrucco et al. 2004). These data suggest that PI3Kγ negatively regulates cAMP levels. Remarkably, the cAMP changes observed in PI3K$\gamma^{-/-}$ mice were eliminated by overexpression of a kinase-dead PI3Kγ gene in the hearts of PI3K$\gamma^{-/-}$ mice, supporting the conclusion (although see below) that the inhibition of cAMP by PI3Kγ is not dependent on alterations in PIP_2/PIP_3 levels (Patrucco et al. 2004).

We have demonstrated that cAMP/PKA activity is enhanced in subcellular compartments of PI3K$\gamma^{-/-}$ ventricular myocytes containing the SR, but not in the vicinity of the sarcolemma (Kerfant et al. 2005). Specifically, we found that cardiomyocytes lacking PI3Kγ have enhanced contractility due to increased SR Ca^{2+} release (Figure 1) and SR Ca^{2+} load, which were eliminated after PKA inhibition (Kerfant et al. 2005). These findings suggest that the effects of PI3Kγ deletion were related to PKA-dependent phosphorylation of PLN. Indeed, PLN phosphorylation is increased in PI3K$\gamma^{-/-}$ mice without obvious changes in the level of SERCA-2a or PLN expression (Crackower et al. 2002, Patrucco et al. 2004). Despite enhanced cAMP levels in PI3K$\gamma^{-/-}$ cardiomyocytes (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Alloatti et al. 2005), and although it has been shown that $I_{Ca,L}$ was modestly elevated (~20%) in another PI3K$\gamma^{-/-}$ mice model (Alloatti et al. 2005), we did not detect differences in $I_{Ca,L}$ amplitude between PI3K$\gamma^{-/-}$ and control cardiomyocytes under conditions where Ca^{2+} transients were present (Figure 1). This was somewhat surprising because $I_{Ca,L}$ is profoundly regulated by cAMP-dependent PKA, which enhances the open channel probability resulting in larger $I_{Ca,L}$ density (Bers 2002). Although $I_{Ca,L}$ amplitude was unaffected by PI3Kγ ablation, the Ca^{2+}-dependent phase of $I_{Ca,L}$ inactivation was accelerated in PI3K$\gamma^{-/-}$ cardiomyocytes, consistent with increased SR Ca^{2+} release. Protein kinase A inhibition did not affect $I_{Ca,L}$ amplitude in PI3K$\gamma^{-/-}$ or control mice (Kerfant et al. 2005, Sun et al. 2006), but it did normalize the Ca^{2+}-dependent inactivation phase of $I_{Ca,L}$ between PI3K$\gamma^{-/-}$ and wild-type mice.

- **Implications and Possible Mechanism(s) of PI3Kγ Regulation of cAMP**

Intracellular cAMP levels are highly regulated in heart and other tissues by the β-AR signaling pathways (Bers 2002).

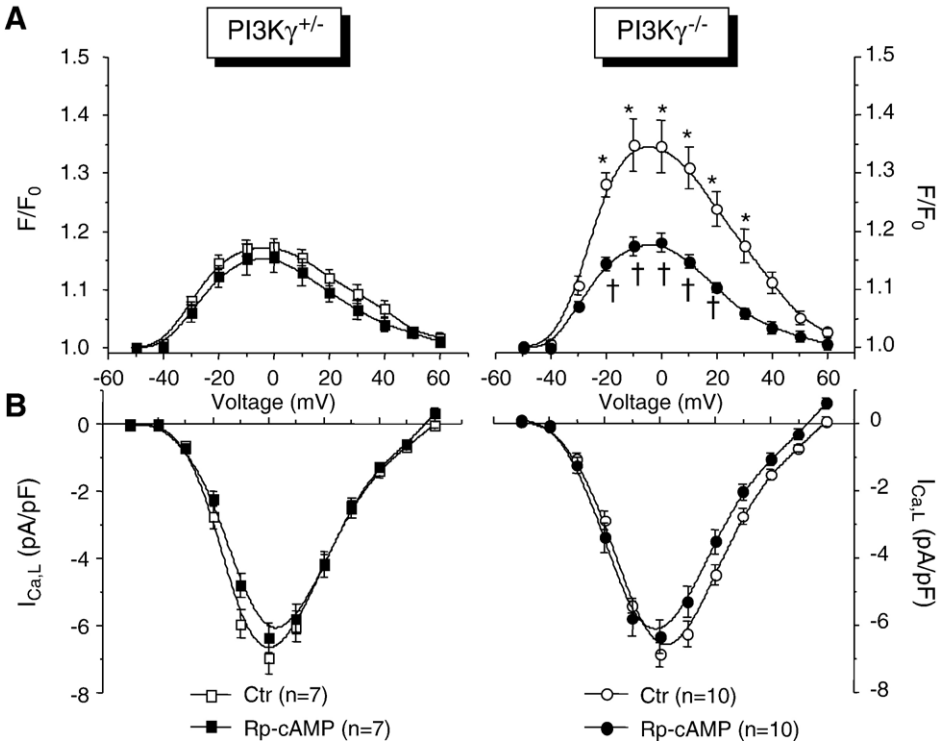

Figure 1. Sarcoplasmic reticulum Ca^{2+} release is elevated in a cAMP-dependent manner, whereas $I_{Ca,L}$ is unchanged in PI3K$\gamma^{-/-}$ cardiomyocytes. (**A**) Mean fluorescence-voltage relationship of Ca^{2+} transients recorded in PI3K$\gamma^{-/-}$ (*circles*) and PI3K$\gamma^{+/-}$ (*squares*) cardiomyocytes before (*open symbols*) and after (*closed symbols*) cell dialysis with the cAMP antagonist Rp-cAMP (100μM). After subtraction of the background fluorescence, the fluorescence signal (F) was normalized to the fluorescence signal before depolarization (F_0) (adapted with permission from *Circ Res.* 2005;96:1079-1086). (**B**) Current-voltage relationship of mean $I_{Ca,L}$ densities recorded simultaneously with Ca^{2+} transients in PI3K$\gamma^{-/-}$ and PI3K$\gamma^{+/-}$ cardiomyocytes. *Asterisk* indicates a significant difference ($P < .01$) between genotypes; *dagger*, a significant difference between control and drug treatment within the same genotype. $I_{Ca,L}$ was elicited, simultaneously to Ca^{2+} transient, by applying 100-millisecond voltage clamp steps between -50 and +60 mV (0.1 Hz) from a holding potential of -80 mV. A 500-millisecond voltage ramp to -50 mV was applied before the voltage steps to inactivate the Na^+ current (I_{Na}). The superfusate contained the following (in mM): 140 NaCl, 0.5 $MgCl_2$, 5 CsCl, 5.5 glucose, 5 HEPES, and 1.8 $CaCl_2$ (pH adjusted to 7.4 with NaOH); the pipette solution contained the following (in mM): 130 CsCl, 1 $MgCl_2$, 1 NaH_2PO_4, 3.6 Na_2-phosphocreatine, 2 KCl, 5 MgATP, 0.05 fluo-3 pentapotassium salt, and 10 HEPES (pH adjusted to 7.2 with CsOH).

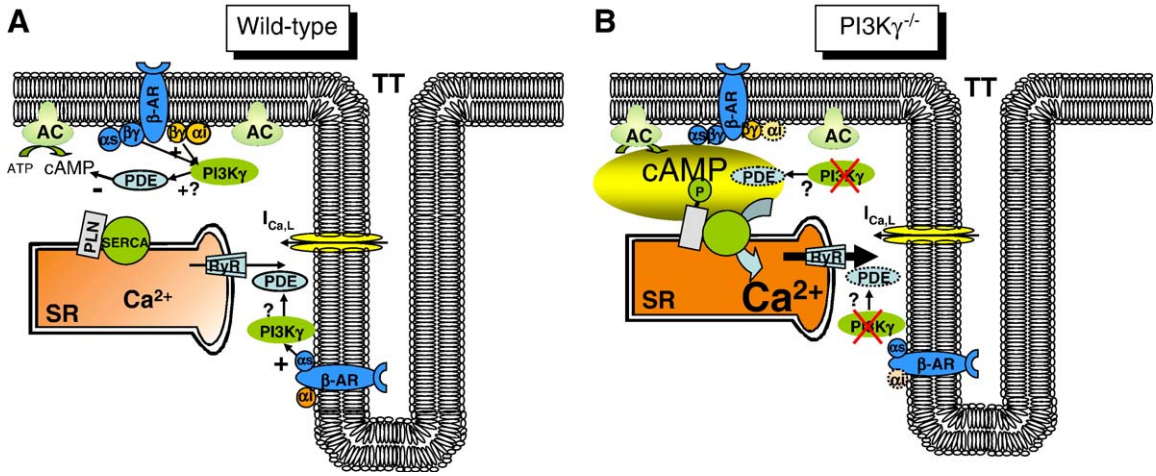

Figure 2. Phosphoinositide 3-kinase γ regulates cAMP within cardiomyocytes. Schematic representation of cAMP regulation by β-AR signaling and PDE activity in wild-type (**A**) and PI3K$\gamma^{-/-}$ (**B**) cardiomyocytes. Phosphoinositide 3-kinase *?* is activated by $\beta\gamma$ subunits of G-proteins. The regulation of specific PDE activity by PI3Kγ at the level of the SR and the L-type Ca^{2+} channels is still unclear, as indicated by *question marks*. Genetic ablation of PI3Kγ results in increased intracellular local cAMP levels, enhanced phosphorylation of PLN, enhanced SERCA-2a activity (indicated by larger arrows), increased SR Ca^{2+} load (darker color), and decreased PDE and G_i (dashed circle) activities. In our PI3Kγ knockout model, Ca^{2+} release from the SR is significantly enhanced without change in $I_{Ca,L}$ levels (note arrow sizes in figure).

Previous studies have indicated that PI3K activity is enhanced by β_1-ARs (Leblais et al. 2004) or β_2-ARs (Chesley et al. 2000, Zhu et al. 2001, Jo et al. 2002) (or both), as well as by AC activation (Leblais et al. 2004). Although it appears that PI3Kγ is not able to directly hydrolyze cAMP (Patrucco et al. 2004), genetic ablation of PI3Kγ clearly increases cAMP levels in cardiomyocytes (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Alloatti et al. 2005). Despite elevated cAMP levels in PI3Kγ-deficient cardiomyocytes, combined β_1-AR and β_2-AR stimulation with ISO increased $I_{Ca,L}$ and Ca^{2+} transients as well as SR Ca^{2+} release equally (~2-fold) in PI3K$\gamma^{-/-}$ and control mice (Kerfant et al. 2005), although baseline Ca^{2+} transients were larger in PI3K$\gamma^{-/-}$ mice. These findings demonstrate that, although cAMP levels and signaling are enhanced in the region of the SR (but not in sarcolemmal regions containing L-type Ca^{2+} channels) under baseline conditions in PI3K$\gamma^{-/-}$ mice, β-AR stimulation can induce further increases in SR Ca^{2+} release and Ca^{2+} transients, possibly because of increases in $I_{Ca,L}$. Although a cAMP-dependent acceleration of Ca^{2+} transient relaxation was observed in PI3K$\gamma^{-/-}$ compared with control myocytes under baseline conditions, the Ca^{2+} transient relaxation was further accelerated in PI3K$\gamma^{-/-}$ myocytes after ISO application. This observation suggests that cAMP levels and cAMP-dependent signaling in the SR region are not saturated by the loss of PI3Kγ. Our results do, however, contrast somewhat with another study that used a different mouse strain reporting that ISO increases contractile force in papillary muscles to a greater extent in PI3K$\gamma^{-/-}$ mice than in wild-type mice (Alloatti et al. 2005). The basis for the strain differences in PI3Kγ-deficient mice will require further investigation.

Although the findings discussed above establish direct modulation of cAMP levels by PI3Kγ under baseline conditions, inhibition of PI3Kγ can also influence cAMP-dependent signaling by β-AR and may contribute to the (poorly understood) differences in signaling between β_1-ARs and β_2-ARs. For example, activation of β_2-ARs with zinterol increases cAMP levels to a greater extent in PI3K$\gamma^{-/-}$ hearts compared with control (Crackower et al. 2002). Because β_2-AR stimulation activates both G_i and G_s proteins, the enhanced cAMP in PI3K$\gamma^{-/-}$ hearts in response to zinterol may result from reductions in $G_{\alpha i}$-dependent signaling in PI3K$\gamma^{-/-}$ mice (Kerfant et al. 2004, Alloatti et al. 2005). Altered $G_{\alpha i}$ in PI3K$\gamma^{-/-}$ hearts may be mediated by changes in the β-arrestin-dependent recruitment of PDE4D that is linked to switching G_s signaling to G_i signaling after β_2-AR stimulation (Baillie et al. 2003), a process that might depend on the PI3Kγ-dependent internalization of β_2-AR by β-arrestin (Naga Prasad et al. 2001, 2005). Thus, it is conceivable that β_2-ARs, PI3Kγ, G_i proteins, PKA, and selected PDEs are components of macromolecular complexes that colocalize in specific intracellular compartments. It has been suggested that caveolae might represent such an intracellular compartment (Steinberg and Brunton 2001, Pavoine and Defer 2005). Interestingly, caveolae are hypothesized to exist within sarcolemmal invaginations that are contiguous with t-tubules, thereby giving them preferential access to SR regions of the myocyte (Pavoine and Defer 2005). These observations are consistent with the alterations in cAMP signaling and increases in SR Ca^{2+} release, without differences in basal $I_{Ca,L}$, observed in mice overexpressing β_2-ARs (Zhou et al. 1999), which are remarkably similar to the changes seen in PI3K$\gamma^{-/-}$ mice (Kerfant et al. 2005). Compartmentation of β_2-AR signaling is further supported by rat cardiomyocyte studies, showing that β_2-AR stimulation results in localized increases in cAMP and $I_{Ca,L}$ without altering PLN phosphorylation (Kuschel et al. 1999, Chen-Izu et al. 2000). However, after nonselective inhibition of PI3K (with wortmannin and LY294002), β_2-ARs stimulation increased PLN phosphorylation and enhanced the rate of myocyte relaxation. Furthermore, after G_i inhibition with pertussis toxin, β_2-AR stimulation leads to increased $I_{Ca,L}$ and enhanced PLN phosphorylation (Jo et al. 2002),

suggesting a possible direct link between G_i and PI3K. Consistent with this suggestion, β_2-AR stimulation in cat myocytes activates PKB/Akt in a PI3K- and G_i-dependent manner which involves nitric oxide synthase (Wang et al. 2002), possibly by nitric oxide-dependent stimulation of guanylyl cyclase leading to cyclic guanosine monophosphate discharge, inhibition of PDE3, and local elevations of cAMP (Dedkova et al. 2002, Wang et al. 2002). In contrast to the effects of PI3K on β_2-AR signaling, PI3K inhibition potentiates the increases in $I_{Ca,L}$ and contractility induced by β_1-AR stimulation while having no effect on the extent of PLN phosphorylation (Leblais et al. 2004). These actions of PI3K inhibitors on the effects of β_1-AR stimulation were unaffected by $G_{\alpha i}$ (in contrast to β_2-AR stimulation) but were linked to $G\beta_\gamma$ and AC activation (Leblais et al. 2004). Thus, it is clear that, in addition to altering cAMP levels by regulating PDE activity, PI3Ks can also influence cAMP signaling in the heart by modulating β-AR receptor in a subtype-specific manner. It seems likely that PI3Kγ is the PI3K isoform involved in coupling between $\beta_{1/2}$-ARs and PDEs because only PI3Kγ is directly regulated by G-protein-coupled receptors (Oudit et al. 2004). Clearly, further work is needed both to clarify the role of PI3Kγ in molecular complexes containing various PDE, heterotrimeric G-proteins, and β-ARs in cardiomyocytes, and to determine the PI3K isozyme dependence in β-AR signaling.

The mechanism whereby PI3Kγ spatially regulates cAMP signaling under basal conditions in myocytes is unclear but could involve effects on several different PDEs involved in cardiomyocyte cAMP compartmentation (Steinberg and Brunton 2001, Georget et al. 2003, Lehnart et al. 2005). There are five PDEs isoforms in adult cardiomyocytes, with PDE3 and PDE4 representing the dominant subtypes involved in the regulation of $I_{Ca,L}$ in basal conditions as well as after activation of β-AR signaling (Verde et al. 1999, Mongillo et al. 2004). Recently, it has been shown that PDE3 activity was altered in PI3K$\gamma^{-/-}$ mice (Patrucco et al. 2004, Alloatti et al. 2005) and that PI3Kγ is able to bind to PDE3B but not PDE3A (Patrucco et al. 2004). This observation, combined with the ability of PI3Kγ kinase-dead overexpression mice (PI3K$\gamma^{KD/KD}$) to eliminate elevated contractility in mice lacking endogenous PI3Kγ, supported the conclusion that PI3Kγ activates PDE3B by direct protein–protein interactions (Marcantoni et al. 2006, Patrucco et al. 2004), independent of PIP_2/PIP_3 levels. However, the regulation of cAMP and myocyte contractility by PI3Kγ may be more complex for several reasons. First, PDE3A is the most abundant PDE isoform in cardiomyocytes, whereas PDE3B is expressed almost exclusively in vascular smooth muscle in the heart (Beavo 1995, Movsesian 2002, Maurice et al. 2003). Second, in HEK293 cells transfected with PDE3B, coexpression of PI3Kγ subunits does not inhibit PDE3B activity (Voigt et al. 2006), implying that additional factors or proteins are required. Several candidate proteins that interact with PI3Kγ and/or PDEs, such as the PI3Kγ regulatory subunit P101 (Oudit et al. 2004) or AKAPs (Bauman and Scott 2002, Wong and Scott 2004), may be involved in mediating the inhibition of PDE3B activity by PI3Kγ in heart. One protein of particular interest is the novel regulatory subunit of PI3Kγ called "P87Pikap," which physically interacts with both PDE3B and PI3Kγ and which is highly expressed in heart, although coexpression of PI3Kγ and P87Pikap with PDE3B did not highly inhibit PDE3 activity (Voigt et al. 2006). Third, elimination of the activity of phosphatase and tensin homologue deleted on chromosome 10 (a lipid phosphatase which counteracts PI3Kγ) causes a reduction in myocardial contractility that was reversed by simultaneous loss of PI3Kγ, supporting a role for kinase-dependent PIP_3 changes in the regulation of cAMP by PI3Kγ (Crackower et al. 2002). PIP_2/PIP_3 regulation of cAMP levels and contraction in myocytes is also supported by the ability of PI3K inhibition, with wortmannin or LY 294002, to alter β_1-AR and β_2-AR signaling, as mentioned above. In addition, the activation of PI3K, as opposed to the mere physical presence of PI3Kγ, has been shown to stimulate PDE3B activity, thereby reducing cAMP in other tissues such as the hypothalamus (Sahu and Metlakunta 2005).

Overall, the mechanism for the regulation of cAMP by PI3Kγ seems complex (Figure 2). Further investigations are clearly needed to discriminate between kinase-dependent and kinase-independent actions of PI3Kγ on cAMP metabolism. It also remains unclear whether PI3Kγ regulates cAMP activity by activating the enzymatic activity of specific PDE isoforms (in both kinase-dependent and kinase-independent fashions) or whether PI3Kγ acts as a scaffold protein in a macromolecular complex to physically localize PDE(s) to appropriate specific microdomains in ventricular myocytes, possibly in conjunction with other proteins such as AKAPs. Some of these issues could conceivably be addressed by making use of the recently described selective PI3Kγ inhibitor, AS-604850 (Camps et al. 2005).

• Perspectives and Significance

A prominent feature of diseased myocardium is reduced Ca^{2+} transient amplitude resulting from decreased SR Ca^{2+} uptake without changes in $I_{Ca,L}$ density (Gwathmey et al. 1987, Beuckelmann et al. 1992, Gomez et al. 1997, Benitah et al. 2002, Bers et al. 2003). These observations suggest that alterations in the regulation of cAMP by PI3Kγ may contribute to the functional changes observed in heart disease. Consistent with this idea, PI3Kγ activity and expression is increased in cardiac disease (Naga Prasad et al. 2000, Patrucco et al. 2004), along with elevated activity of selected PDEs (Takahashi et al. 2002) and G_i (Bohm et al. 1997). Thus, enhanced PI3Kγ effects could work in conjunction with reduced SERCA-2a expression (Netticadan et al. 2000) to decrease SR Ca^{2+} uptake, thereby impairing Ca^{2+} handling and contractility, as observed in heart disease. These observations suggest that reducing PI3Kγ levels or function may be beneficial in heart disease. Consistent with this, PI3K$\gamma^{-/-}$ mice are protected from ISO-induced heart failure (Oudit et al. 2003). However, chronic pressure overload induced by transverse aortic constriction in PI3K$\gamma^{-/-}$ mice leads to more extensive dysfunction and myocardial damage than in control mice (Patrucco et al. 2004), suggesting that the role of PI3Kγ may vary depending on the nature of disease and that elevated cAMP levels may be detrimental in the response of the heart to certain challenges. Future studies will clearly be required to more fully assess the role of PI3Kγ and local cAMP signaling in normal and diseased hearts.

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References

Alloatti G, Marcantoni A, Levi R, et al.: 2005. Phosphoinositide 3-kinase gamma controls autonomic regulation of the mouse heart through Gi-independent downregulation of cAMP level. FEBS Lett 579:133–140.

Baillie GS, Sood A, McPhee I, et al.: 2003. beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from G_s to G_i. Proc Natl Acad Sci U S A 100:940–945.

Baillie GS, Scott JD, Houslay MD: 2005. Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract. FEBS Lett 579:3264–3270.

Bauman AL, Scott JD: 2002. Kinase- and phosphatase-anchoring proteins: harnessing the dynamic duo. Nat Cell Biol 4: E203–E206.

Beavo JA: 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol Rev 75:725–748.

Benitah JP, Gomez AM, Fauconnier J, et al.: 2002. Voltage-gated Ca^{2+} currents in the human pathophysiologic heart: a review. Basic Res Cardiol 97 (Suppl 1):1/11–1/18.

Bers DM: 2002. Cardiac excitation–contraction coupling. Nature 415:198–205.

Bers DM, Ziolo MT: 2001. When is cAMP not cAMP? Effects of compartmentalization. Circ Res 89:373–375.

Bers DM, Eisner DA, Valdivia HH: 2003. Sarcoplasmic reticulum Ca^{2+} and heart failure: roles of diastolic leak and Ca^{2+} transport. Circ Res 93:487–490.

Beuckelmann DJ, Nabauer M, Erdmann E: 1992. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation 85:1046–1055.

Bohm M, Flesch M, Schnabel P: 1997. Beta-adrenergic signal transduction in the failing and hypertrophied myocardium. J Mol Med 75:842–848.

Camps M, Ruckle T, Ji H, et al.: 2005. Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. Nat Med 11:936–943.

Chen-Izu Y, Xiao RP, Izu LT, et al.: 2000. G(i)-dependent localization of beta(2)-adrenergic receptor signaling to L-type Ca(2+) channels. Biophys J 79:2547–2556.

Chesley A, Lundberg MS, Asai T, et al.: 2000. The beta(2)-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G(i)-dependent coupling to phosphatidylinositol 3'-kinase. Circ Res 87: 1172–1179.

Crackower MA, Oudit GY, Kozieradzki I, et al.: 2002. Regulation of myocardial contractility and cell size by distinct PI3K–PTEN signaling pathways. Cell 110: 737–749.

Dedkova EN, Wang YG, Blatter LA, et al.: 2002. Nitric oxide signalling by selective beta(2)-adrenoceptor stimulation prevents ACh-induced inhibition of beta(2)-stimulated Ca(2+) current in cat atrial myocytes. J Physiol 542 (Pt. 3):711–723.

Georget M, Mateo P, Vandecasteele G, et al.: 2003. Cyclic AMP compartmentation due to increased cAMP-phosphodiesterase activity in transgenic mice with a cardiac-directed expression of the human adenylyl cyclase type 8 (AC8). FASEB J 17:1380–1391.

Gomez AM, Valdivia HH, Cheng H, et al.: 1997. Defective excitation–contraction coupling in experimental cardiac hypertrophy and heart failure. Science 276: 800–806.

Gwathmey JK, Copelas L, MacKinnon R, et al.: 1987. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 61:70–76.

Hirsch E, Katanaev VL, Garlanda C, et al.: 2000. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. Science 287:1049–1053.

Jo SH, Leblais V, Wang PH, et al.: 2002. Phosphatidylinositol 3-kinase functionally compartmentalizes the concurrent G(s) signaling during beta2-adrenergic stimulation. Circ Res 91:46–53.

Jurevicius J, Fischmeister R: 1996. cAMP compartmentation is responsible for a local activation of cardiac Ca^{2+} channels by beta-adrenergic agonists. Proc Natl Acad Sci U S A 93:295–299.

Keely SL: 1979. Prostaglandin E1 activation of heart cAMP-dependent protein kinase: apparent dissociation of protein kinase activation from increases in phosphorylase activity and contractile force. Mol Pharmacol 15:235–245.

Kerfant BG, Oudit GY, Backx PH: 2004. Pi3Kgamma alters Gi activity while maintaining ICa-L and IKAch densities. 48th Annual Meeting of the Biophysical Society. Biophys J 86 (1, Pt2):296a.

Kerfant BG, Gidrewicz D, Sun H, et al.: 2005. Cardiac sarcoplasmic reticulum calcium release and load are enhanced by subcellular cAMP elevations in PI3Kgamma-deficient mice. Circ Res 96:1079–1086.

Kuschel M, Zhou YY, Cheng H, et al.: 1999. G(i) protein-mediated functional compartmentalization of cardiac beta(2)-adrenergic signaling. J Biol Chem 274: 22048–22052.

Leblais V, Jo SH, Chakir K, et al.: 2004. Phosphatidylinositol 3-kinase offsets cAMP-mediated positive inotropic effect via inhibiting Ca^{2+} influx in cardiomyocytes. Circ Res 95:1183–1190.

Lehnart SE, Wehrens XH, Reiken S, et al.: 2005. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. Cell 123:25–35.

Luo J, McMullen JR, Sobkiw CL, et al.: 2005. Class IA phosphoinositide 3-kinase regulates heart size and physiological cardiac hypertrophy. Mol Cell Biol 25:9491–9502.

Marcantoni A, Levi RC, Gallo MP, et al.: 2006. Phosphoinositide 3-kinase γ (PI3Kγ) controls L-type calcium current (ICa,L) through its positive modulation of type-3 phosphodiesterase (PDE3). J Cell Physiol 206:329–336.

Maurice DH, Palmer D, Tilley DG, et al.: 2003. Cyclic nucleotide phosphodiesterase activity, expression, and targeting in cells of the cardiovascular system. Mol Pharmacol 64:533–546.

Mongillo M, McSorley T, Evellin S, et al.: 2004. Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. Circ Res 95:67–75.

Movsesian MA: 2002. PDE3 cyclic nucleotide phosphodiesterases and the compartmentation of cyclic nucleotide-mediated signalling in cardiac myocytes. Basic Res Cardiol 97 (Suppl 1):I83–I90.

Naga Prasad SV, Esposito G, Mao L, et al.: 2000. Gbetagamma-dependent phosphoinositide 3-kinase activation in hearts with in vivo pressure overload hypertrophy. J Biol Chem 275:4693–4698.

Naga Prasad SV, Barak LS, Rapacciuolo A, et al.: 2001. Agonist-dependent recruitment of phosphoinositide 3-kinase to the membrane by beta-adrenergic receptor kinase 1. A role in receptor sequestration. J Biol Chem 276:18953–18959.

Naga Prasad SV, Jayatilleke A, Madamanchi A, et al.: 2005. Protein kinase activity of phosphoinositide 3-kinase regulates beta-adrenergic receptor endocytosis. Nat Cell Biol 7:785–796.

Netticadan T, Temsah RM, Kawabata K, et al.: 2000. Sarcoplasmic reticulum

Ca(2+)/calmodulin-dependent protein kinase is altered in heart failure. Circ Res 86:596–605.

Nienaber JJ, Tachibana H, Naga Prasad SV, et al.: 2003. Inhibition of receptor-localized PI3K preserves cardiac {beta}-adrenergic receptor function and ameliorates pressure overload heart failure. J Clin Invest 112: 1067–1079.

Oudit GY, Crackower MA, Eriksson U, et al.: 2003. Phosphoinositide 3-kinase gamma-deficient mice are protected from isoproterenol-induced heart failure. Circulation 108:2147–2152.

Oudit GY, Sun H, Kerfant BG, et al.: 2004. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. J Mol Cell Cardiol 37: 449–471.

Patrucco E, Notte A, Barberis L, et al.: 2004. PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. Cell 118:375–387.

Pavoine C, Defer N: 2005. The cardiac beta2-adrenergic signalling a new role for the cPLA2. Cell Signal 17:141–152.

Prasad SV, Perrino C, Rockman HA: 2003. Role of phosphoinositide 3-kinase in cardiac function and heart failure. Trends Cardiovasc Med 13:206–212.

Sahu A, Metlakunta AS: 2005. Hypothalamic phosphatidylinositol 3-kinase-phosphodiesterase 3B-cyclic AMP pathway of leptin signalling is impaired following chronic central leptin infusion. J Neuroendocrinol 17:720–726.

Sasaki T, Irie-Sasaki J, Jones RG, et al.: 2000. Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. Science 287:1040–1046.

Steinberg SF, Brunton LL: 2001. Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. Annu Rev Pharmacol Toxicol 41:751–773.

Sun H, Kerfant BG, Zhao D, et al.: 2006. Insulin-like growth factor-1 and PTEN deletion enhance cardiac L-type Ca2+ currents via increased PI3Kα/PKB signaling. Circ Res. doi: 10.1161/01.RES.0000223321. 34482.8c.

Takahashi K, Osanai T, Nakano T, et al.: 2002. Enhanced activities and gene expression of phosphodiesterase types 3 and 4 in pressure-induced congestive heart failure. Heart Vessels 16:249–256.

Verde I, Vandecasteele G, Lezoualc'h F, et al.: 1999. Characterization of the cyclic nucleotide phosphodiesterase subtypes involved in the regulation of the L-type Ca^{2+} current in rat ventricular myocytes. Br J Pharmacol 127:65–74.

Vila Petroff MG, Egan JM, Wang X, et al.: 2001. Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. Circ Res 89: 445–452.

Voigt P, Dorner MB, Schaefer M: 2006. Characterization of p87PIKAP, a novel regulatory subunit of phosphoinositide 3-kinase gamma that is highly expressed in heart and interacts with PDE3B. J Biol Chem 281:9977–9986.

Wang YG, Dedkova EN, Steinberg SF, et al.: 2002. Beta 2-adrenergic receptor signaling acts via NO release to mediate ACh-induced activation of ATP-sensitive K^+ current in cat atrial myocytes. J Gen Physiol 119:69–82.

Wong W, Scott JD: 2004. AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5:959–970.

Zhou YY, Song LS, Lakatta EG, et al.: 1999. Constitutive beta2-adrenergic signalling enhances sarcoplasmic reticulum Ca^{2+} cycling to augment contraction in mouse heart. J Physiol 521 (Pt 2):351–361.

Zhu WZ, Zheng M, Koch WJ, et al.: 2001. Dual modulation of cell survival and cell death by beta(2)-adrenergic signaling in adult mouse cardiac myocytes. Proc Natl Acad Sci U S A 98:1607–1612.

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