Identification of the macromolecular complex responsible for PI3Kγ-dependent regulation of cAMP levels

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Abstract

PI3Kγ is a phosphoinositide 3-kinase characterized by both lipid and protein kinase activity. It is activated by G-protein-coupled receptors and is predominantly expressed in leucocytes; in addition, recent work showed its presence in the heart and its involvement in regulating cardiac functions. In this tissue, PI3Kγ acts as a negative modulator of contractility, by decreasing cAMP concentration through a kinase-independent mechanism. Indeed, whereas PI3Kγ-deficient mice show an abnormal cAMP elevation, cAMP levels in knock-in mouse mutants, expressing a kinase-dead PI3Kγ, are comparable with wild-type controls. PI3Kγ regulates cardiac cAMP homoeostasis by forming a macromolecular complex containing PDE3B (phosphodiesterase 3B). In this complex, PI3Kγ could regulate PDE3B activity through protein kinase A, a PDE activator.

PI3Ks (phosphoinositide 3-kinases) are best known as a family of lipid and protein kinases involved in a large variety of cellular functions, ranging from regulation of metabolism, proliferation and survival to cytoskeletal organization and cell migration. Depending on their substrate and their structural properties, PI3Ks are organized in at least three different classes. Among these, the best characterized is the class I, which comprises four members that, based on their activation mechanisms, are further subdivided into two subgroups, A and B. Whereas the three class IA PI3Ks, named α, β and δ, bind an adaptor subunit of the p85 family and are activated by tyrosine kinase receptors, the only class IB PI3K, PI3Kγ, is triggered by G-protein-coupled receptors and binds adaptor proteins of the p101 family [1]. Recent evidence suggests that this latter PI3K isoenzyme differs from class IA enzymes also in its ability to modulate cAMP homoeostasis. Indeed, the first indications that PI3Kγ is involved in cAMP metabolism came from the study of mice lacking PI3Kγ, which are viable and fertile but show reduced inflammatory reactions and a wide range of subtle cardiovascular phenotypes [2]. The first indication of the involvement of PI3Kγ in cAMP homoeostasis came from the characterization of PI3Kγ-deficient platelets, which show a basal increase in cAMP contents under resting conditions and a PGE_1 (prostaglandin E_1)-induced accumulation of cAMP stronger than wild-type controls [3]. Nonetheless, although elevation of cAMP has an anti-aggregant effect and PI3Kγ-deficient platelets show a reduced aggregation response after ADP stimulation, whether the alterations in cAMP contents have a functional effect still remains to be determined.

Key words: cAMP, compartmentalization, heart failure, phosphodiesterase 3B (PDE3B), phosphoinositide 3-kinase γ (PI3Kγ), signal transduction.

Abbreviations used: PDE, phosphodiesterase; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A.

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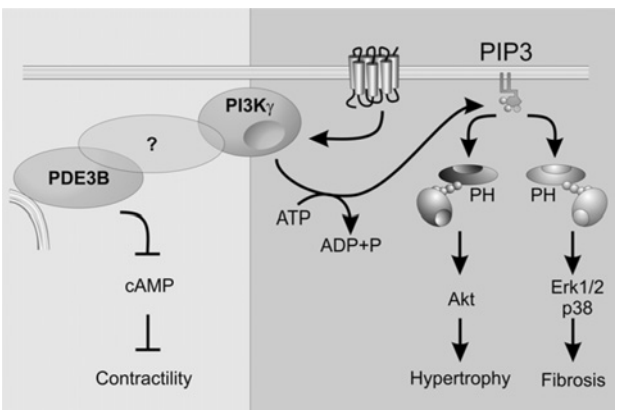
Similarly to platelets, PI3Kγ-deficient mice show increased cAMP content also in the heart and in cultured cardiomyocytes [4]. This observation is supported by the finding that hearts lacking PI3Kγ show increased contractility under basal conditions and after catecholamine stimulation [5]. This further confirmed the view that involvement of PI3Kγ in cAMP metabolism is specific for this isoenzyme, as perturbation of the function of class IA PI3Ks in the myocardium never affected contractility [6]. In agreement with a role of PI3Kγ in decreasing intracellular cAMP concentration, cardiomyocytes lacking PI3Kγ show increased PKA (protein kinase A) activity and an enhanced basal phosphorylation of PKA targets such as phospholamban and L-type Ca^{2+} channels [5,7,8]. These effects all have a profound impact on cardiac reactions to mechanical stress: mice lacking PI3Kγ show increased mortality to aortic constriction accompanied by cardiomyocyte necrosis and heart failure [5]. Interestingly, these responses appeared to be linked to cAMP elevation, caused by the lack of PI3Kγ, as cardiac damage is significantly reduced in PI3Kγ-deficient mice subjected to β-blocker administration capable of decreasing myocardial cAMP levels. The finding that, in the absence of PI3Kγ, only contractility is enhanced but frequency is normal [7], together with the observation that β_2-selective agonists induce stronger cAMP accumulation in mutant cardiomyocytes [4], suggests that PI3Kγ might be involved in a mechanism controlling subcellular compartmentalization of cAMP, but the details of such activity are still elusive.

A major breakthrough in the understanding of the biochemical role of PI3Kγ in cAMP homoeostasis came from the observation that knock-in mice, where the expression of the endogenous PI3Kγ gene was replaced with that of a mutant lacking the catalytic activity, did not show abnormalities in cAMP levels. These mutant mice that express an inactive PI3Kγ, where a single lysine residue in the

Figure 1 | Double role of PI3Kγ in the heart

PI3Kγ is a lipid kinase involved in the activation of the PKB/Akt and MAPK (mitogen-activated protein kinase) signalling cascades (right-hand panel). Nonetheless, PI3Kγ is also able to interact with other unknown proteins and be a crucial part of a multimolecular complex that regulates PDE3B activity and cAMP homoeostasis independently of its catalytic activity (left-hand panel). Erk1/2, extracellular-signal-regulated kinase 1/2; PH, pleckstrin homology domain; PIP3, PtdInsP_3.



catalytic pocket has been replaced with an arginine residue, do not show increased cardiac contractility under basal conditions and respond normally to aortic banding [5]. This observation suggests that PI3Kγ is involved in a protein–protein interaction that modulates cAMP concentration independently of PI3Kγ lipid and protein kinase activity (see Figure 1). Indeed, PI3Kγ was found to co-immunoprecipitate with a PDE (phosphodiesterase) activity that could be inhibited by enoximone, an inhibitor of PDE3. Western-blot analysis with antibodies selective for the different PDE3 enzymes revealed that PI3Kγ can interact with PDE3B and that this interaction, although probably not direct, is able to trigger the activation of this PDE3 isoform. Indeed, measurement of PDE3B activity from PI3Kγ-deficient hearts revealed that this enzyme is less active and that this defect can be rescued by the addition of recombinant PI3Kγ [5]. Recently, the presence of a PDE3B–PI3Kγ complex has been confirmed *in vitro* in HEK-293 cells (human embryonic kidney cells) overexpressing PDE3B and PI3Kγ [9]. PDE3B was also found to couple with a novel PI3Kγ adaptor protein of the p101 family, termed p87^{pikap} [9]. Despite these findings, the presence of PI3Kγ was not sufficient to increase PDE3B activity, further suggesting that the complex might contain other members crucial to trigger a PI3Kγ-dependent activation of PDE3B.

How PI3Kγ controls PDE3B activity thus remains mainly undefined. It has been previously described that PDE activity can be regulated by different kinases such as PKA and Akt/PKB and that activating cAMP hydrolysis forms a negative feedback loop restoring basal cAMP levels [10,11]. Studies in progress are focusing on the identification in the PDE3B–PI3Kγ complex of similar enzymes that activating PDE3B might thus explain the kinase-independent function of PI3Kγ.

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