

Primer

More Paths to PI3K γ

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In the huge collection of molecules that underpin mammalian biology, only a small number stand out as targets for drug development. PI3K γ is one such molecule that has received substantial investments to assess whether inhibitors can be developed as potential therapeutics. Although many studies have addressed the structure and regulation of PI3K γ , our understanding of the enzyme is far from complete. A publication in *PLoS Biology* from the Wymann group provides new insights into this process by identifying an alternate route to PI3K γ activation, and these results enable us to understand how PI3K γ signaling is regulated in mast cells and is thus important in so many cell, tissue, and disease settings.

Roles for PI3K γ in Health and Disease

PI3K γ is expressed strongly in a number of immune cells, including mast cells, neutrophils, and eosinophils. In these cell types, it sits at a key, early, near-receptor stage in pro-inflammatory, intracellular signaling pathways. This is, in part, why genetic loss or selective inhibition of PI3K γ has little effect on normal mouse development and function but can suppress inflammation in a number of mouse models of disease, including rheumatoid arthritis [1], anaphylaxis [2], atherosclerosis [3], and glomerulonephritis [4]. Interestingly, however, PI3K γ seems to perform a variety of important roles in other cell types/systems where it is often barely detectable—for example, in the heart, where it suppresses cAMP signaling and contractility (and hence may be a useful therapeutic target in certain types of cardiac failure) [5–7], and in fat metabolism, where its activity supports fat deposition [8]. Finally, there is evidence that PI3K γ may support tumour growth and progression [9,10].

Class I PI3K Signaling

PI3K γ (phosphoinositide 3-kinase) belongs to the class I PI3K family of signaling enzymes (along with PI3Ks α , β , and δ) that 3-phosphorylate the membrane phospholipid PtdIns(4,5)P₂ to yield the signaling lipid PtdIns(3,4,5)P₃. The class I PI3Ks can all be activated by cell surface receptors to drive accumulation of PtdIns(3,4,5)P₃ in the inner leaflet of the plasma membrane. This acts as a signal enabling proteins capable of binding PtdIns(3,4,5)P₃, typically via PH domains, to concentrate at the cytosolic interface of the plasma membrane. The classic example of a PH domain-containing PtdIns(3,4,5)P₃ effector is PKB (Akt), but there are possibly as many as 60–70 in a mammalian genome and up to at least 20 can be expressed in the same cell. This family of PtdIns(3,4,5)P₃ effectors transduce the lipid signals into many forms, including changes in protein kinase, Rac-family-GEF and Rho-family-GAP activity, and/or distribution.

Primers provide a concise introduction into an important aspect of biology highlighted by a current *PLoS Biology* research article.

PI3K γ —The Detail

PI3K γ is made up of a p110 γ catalytic subunit [11] combined with either a p84 (also called p87^{PIKAP}) [12–14] or a p101 regulatory subunit [15]. Interestingly, the three proteins are well conserved in eukaryotes from humans to fish; p84 and p101 have even retained their neighbouring genetic location. PI3K γ is thought to exist as a dimer *in vivo*, although this is only based on the lack of any evidence for the existence of free p110 γ . It appears that despite immune cells like neutrophils, expressing p101 and p84, mast cells, the subject of the study reported by the Wymann group, only express p84 [16].

The p101 Regulatory Subunit

Our current understanding of PI3K γ activation centers on how the p101 subunit allows the complex to be substantially activated by G-protein $\beta\gamma$ subunits (G $\beta\gamma$ s) liberated from G proteins upon activation of GPCRs. This mechanism is thought to explain why PI3K γ is typically (but not universally) activated by GPCRs. Frustratingly, we do not know the residues/domains in either p101 or p110 γ involved in the interaction with G $\beta\gamma$ s. It appears the interaction between p101 and p110 γ is very tight and unlikely to have a significant on/off rate under physiological conditions. There is some evidence supporting direct interactions between G $\beta\gamma$ s and both p101 and p110 γ [15,17].

The p84 Regulatory Subunit

But the involvement of the p84 in PI3K γ has been less clear. There is anecdotal evidence in the field that the interaction between p84 and p110 γ is less tight than that between p101 and p110 γ . Despite this, once bound, p84/p110 γ complexes are able to survive gel-filtration chromatography or multiple cycles of washing with traditional detergent-containing lysis buffers through pull-down protocols without significant dissociation, suggesting that their association is not rapidly reversible. It is clear, however, that the p84/p110 γ heterodimer is substantially less sensitive to G $\beta\gamma$ s (at least to a limited subset of G $\beta\gamma$ s that have been tested) than the p101/p110 γ complex [14,18].

Regulation by Ras

P110 γ contains a classical Ras-binding domain (RBD) that has been demonstrated to bind selectively to Ras-GTP. Ras-GTP can

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activate p110 γ , p101/p110 γ , and p84/p110 γ *in vitro* or in transfected cells [19]. Furthermore, this interaction is important *in vivo*, as demonstrated by the effect of knocking-in a Ras-insensitive, but G $\beta\gamma$ -sensitive, allele of p110 γ in mice (p110 $\gamma^{DASAA/DASAA}$) [20]. There are lines of evidence which suggest that Ras-GTP (and not G $\beta\gamma$ s) regulates p84/p110 γ complexes in transfected cells, while p101/p110 γ complexes are only regulated by G $\beta\gamma$ s [18]. This view suggests that p84/p110 γ complexes may be less exclusively controlled by GPCRs but rather by any ligand capable of activating Ras, including receptor tyrosine kinase pathways.

Mast Cells

Mast cells are tissue-resident immune cells that express high-affinity receptors for IgE (Fc ϵ RI). Mast cell Fc ϵ RI are effectively permanently bound with IgE. These Fc ϵ RI/IgE complexes are activated by ligands that are specific targets for the variable regions of IgEs, including allergens. Once activated, mast cells can release a huge range of inflammatory mediators, including histamine, LTB₄, PAF, and PGD₂. The intracellular signals generated by activated Fc ϵ RI are primarily based on protein tyrosine kinase-mediated mechanisms that would naturally lead to activation of the class IA PI3Ks such as PI3K δ [21,22]; however, PI3K γ has been shown to have an important role in mast cell activation [2]. The role for PI3K γ is through an autocrine/paracrine mechanism, involving among other things released adenosine working back on A₃ (A₃-Ars) GPCRs, that synergizes with the primary effects of Fc ϵ RI activation.

Insights into Mast Cell Regulation of PI3K γ Activation

A recent study by Walser et al. in *PLOS Biology* began with several threads, a key one of which was the ability of thapsigargin (a drug that causes a very selective receptor-independent increase in cytosolic Ca²⁺ by slowly releasing intracellular ER Ca²⁺ stores into the cytosol and activating Ca²⁺ influx via the store-operated-Ca²⁺-entry (SOCE) route) to stimulate phosphorylation of PKB in a PI3K γ -dependent manner. That result was very unexpected and a variety of controls suggested it was unlikely to be dependent on release of the mast cell paracrine/autocrine factor adenosine acting back on A₃ GPCRs.

Wide-ranging experiments support a model (Figure 1) in which Fc ϵ RI cross-linking leads to a Ca²⁺ signal, involving SOCE, that drives activation of PKC β . Through an interaction between PKC β and the helical domain of p110 γ , S582-p110 γ is phosphorylated. This leads to both activation of the kinase activity of PI3K γ (which leads to increased PIP₃ and downstream signaling) and a reduction in the affinity of p110 γ for p84. The outcome of these events is argued to be a rebalancing in the amount of GPCR-sensitive p84/p110 γ and free, GPCR-insensitive p110 γ , thus reducing the sensitivity of PI3K γ to GPCRs. These conclusions represent major conceptual advances for the field. Beyond their major impact on our understanding of mast cell biology, they offer potential molecular explanations for the roles of PI3K γ in a number of important, but ill-understood, physiological and disease settings. Furthermore, the paper introduces beautiful structural data from deuterium-exchange protection assays that reveal where the presence of p84 masks the surface of p110 γ in its helical domain. Further biochemical experiments demonstrate the potential power of interactions with, or post translational

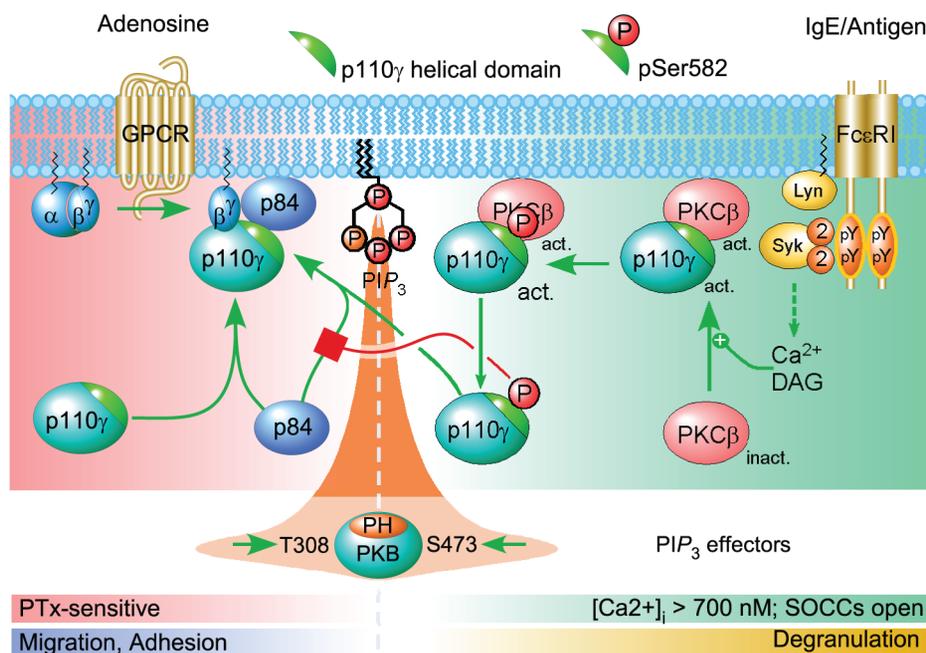


Figure 1. Mast cell environment regulates PI3K γ activation. Coincident with migration and adhesion of mast cells, adaptor protein p84 relays the GPCR signal from GPCR-mediated dissociation of trimeric G proteins to activate the PI3K γ complex. However, when mast cells degranulate, Fc ϵ RI receptors are clustered via IgE/antigen complexes, and a signaling cascade triggers intracellular Ca²⁺ store depletion and the opening of store-operated Ca²⁺ channels. The resulting increase in calcium ion concentration and PLC γ -derived diacylglycerol activates PKC β , which binds to p110 γ and subsequently phosphorylates Ser582. This phosphorylated p110 γ can no longer interact with p84, and the PI3K γ complex is therefore unresponsive to GPCR inputs. Image credit: Walser et al., 10.1371/journal.pbio.1001587 doi:10.1371/journal.pbio.1001594.g001

modifications of, the helical domain of p110s to regulate class I PI3K function both in terms of changes in catalytic activity and binding of adaptor subunits. This type of mechanism may be a widely important mode of regulation in class I PI3K signaling.

As ever with first steps, many questions remain. The role of Ras in the activation of PI3K γ in mast cells is unclear. Given that RasGrp1 (and RasGrp4) is relatively abundant in mast cells, and activated by a combination of PKC-mediated phosphorylation and diacylglycerol (DAG) binding, and in lymphocytes is the primary mechanism driving activation of Ras downstream of antibody receptor activation [23,24], it might have been expected to be involved in this pathway, but does not appear to be. This will be clarified by testing the impact of knocking-in a Ras-insensitive version of p110 γ on mast cell responsiveness. The apparent contradiction between the simple model described above and the dogma that p84 is tightly bound to p110 γ (that is supported by the data from the deuterium-exchange protection assays) needs to be resolved. It currently leaves it difficult to understand the pattern of events; does PKC β need to compete with p84 to enable significant stoichiometries of phos-

phorylation to occur? As always, a high-resolution view of the p84/p110 γ complex would be immensely useful.

Concluding Remarks

The *PLOS Biology* paper from Walser et al. makes many important contributions. It reveals how intracellular signals from mast cell Fc ϵ RI, classically thought to engage class IA PI3K signaling, can control p110 γ directly via the PLC effectors Ca²⁺ and DAG/PKC. These effects are mediated by an interaction between PKC β and the helical domain of p110 γ that enables S582-p110 γ to be phosphorylated and activate the lipid kinase activity of PI3K γ . The outcome is the emergence of a new mechanism by which class I PI3Ks can be activated. The paper also provides a first insight into the interactions between p110 γ and p84, hopefully the first step in the exploitation of deuterium-exchange methodologies to reveal more about the interactions of p101, p84, p110 γ , and G β γ s. Finally, the work may offer molecular explanations for some of the many poorly understood roles that PI3K γ fulfills outside of the immune system.

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