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Signalling by the phosphoinositide 3-kinase family in immune cells

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Abstract

Phosphoinositide 3-kinases control many important aspects of immune cell development, differentiation and function. Mammals have eight PI3K catalytic subunits which are divided into 3 classes based on similarities in structure and function. Specific roles for the class I PI3Ks have been broadly investigated and are relatively well understood as is the function of their corresponding phosphatases. More recently, specific roles for the class II and class III PI3Ks have emerged. Through vertebrate evolution and in parallel with the evolution of adaptive immunity, there has been a dramatic increase not only in the genes for PI3K subunits but also in genes for phosphatases that act on 3-phosphoinositides and on 3-phosphoinositide binding proteins. Our understanding of the PI3Ks in immunity is guided by fundamental discoveries made in simpler model organisms as well as by appreciating new adaptations of this signalling module in mammals in general and immune cells in particular.

Keywords

PI3K; Akt; Foxo; mTOR; signalling; autophagy

Introduction

The phosphoinositides (PtdIns) represent a minor component of the plasma membrane, but play a major role in intracellular signalling. This review will focus on the role of PI3Ks which phosphorylate the inositol ring on the 3 position. The 3-phosphorylated inositol ring creates a docking site for proteins at the inner leaflet of the plasma membrane and on intracellular membranes. 3-phosphorylated phosphoinositides are subjected to the action of inositol phosphatases which either remove the 3-phosphate itself or additional phosphates on the 4 or 5 position. The asymmetry of the phosphorylated inositol ring and the highly regulated phosphorylation and de-phosphorylation of the 3, 4 and 5 positions has created a complex signalling code involving 7 differentially phosphorylated phosphoinositide species (1) (Fig 1).

The kinases and phosphatases, as well as the proteins that bind to phosphoinositides have been under strong selective pressure during vertebrate and mammalian evolution (2). Conserved functions of the PI3Ks include the control of intracellular vesicle trafficking, cellular motility and the regulation of nutrient metabolism. PI3Ks control these functions as

well as more recently evolved and specialised aspects of cell biology necessary for the development and function of immune cells.

The PI3K family

There are 8 catalytic PI3K subunits encoded by vertebrate genomes and these are divided into three classes based on sequence alignment and domain structures (3, 4)(Fig 2). A number of tools have been developed over the last decade to probe the individual functions the PI3K catalytic and regulatory subunits. Gene targeted mice, selective small molecule inhibitors and crystal structures now exist for all the class I PI3Ks, for some of the class II and class III PI3Ks and also for some of the phosphatases that act on 3-phosphorylated phosphoinositides. These tools have contributed to a deep understanding of the isoform-selective functions among the class I PI3Ks and are beginning to unravel specific roles for class II and class III PI3Ks. This article starts by reviewing what is known about the founding member of the family, Vps34, followed by a discussion on the class II PI3Ks. I then describe recent developments in our understanding class I PI3Ks in the latter half of the review, with particular focus on the role of the individual catalytic subunits.

Class III PI3K: the founding member of the family

The class III PI3K Vps34 is the oldest PI3K and is the only PI3K found in yeast and plants as well as in metazoans (2). Vps34 phosphorylates PI to generate PI3P which is the most abundant of the phosphoinositides and which acts as a docking site for proteins that contain PX or FYVE domains (5)(Fig 1). Total PI3P levels do not change dramatically upon cell activation, however, local production can be significantly increased during the early stages of the formation of autophagic vesicles (omegasomes) or during endocytosis and phagocytosis (6-9). Vps34 was originally identified as gene required for protein sorting to the lysosome-like vacuole in yeast and subsequently shown to also control endocytosis, phagocytosis and autophagy in various cell types (10, 11). In yeast, Vps34 exists as part of two distinct complexes (12). A core complex consists of Vps15, Vps34 and Vps30 (also known as Atg6, the mammalian homologue of which is Beclin). Vps15 is a serine-threonine kinase which regulates the activity of Vps34 and which also brings Vps34 to lipid membranes (13). The role of Vps30 is less well understood but it is likely to act as an adapter protein. Vps34 complex I additionally contains Atg14 and is required for autophagy whereas Vps34 complex II contains Vps38 and is required for vacuolar sorting (12, 14). In mammalian cells, the picture is likely more complex, with the existence of three or more distinct Vps34-containing complexes (3, 5). Vps34 is also required for amino acid stimulation of mTOR activity in mammalian cells (15, 16).

Currently, no selective inhibitors are available for Vps34, though 3-methyl adenine (3MA) is often used in conjunction with Wortmannin as an initial approach to implicate or exclude the role of Vps34 in a particular process (5). Such experiment need to be interpreted with caution since both Wortmannin and 3MA inhibits other PI3K isoforms as well (5). A crystal structure of Vps34 was recently published which not only revealed novel insight into regulation of Vps34 activity, but which should guide the development of more selective inhibitors (17). Vps34^{-/-} mice die as embryos; however conditional knockouts are

beginning to shed light on the role of Vps34 in selected mammalian cell types, including cells of the immune system (16, 18-20).

Role of PI3P in Phagocytosis and vesicle trafficking

In macrophages and neutrophils, PI3P accumulates on the phagocytic cup and is required for phagosome maturation (7, 8, 21). Vps34-produced PI3P is also required for the activation of the NOX2 NADPH oxidase which generates reactive oxygen species that contribute to the destruction of bacteria (22, 23). The p40 subunit of NOX2 contains a PI3P-binding PX domain and a mutation in this PX domain abrogates ROS production in responses to bacterial challenge of neutrophils (24). Recently, SLAM has been identified as a receptor on macrophages that can bind bacterial proteins and which is associated with a complex containing Vps15, Vps34 and beclin (25, 26). This is an interesting observation as the mechanism of recruitment of Vps34 to the membrane has been elusive (5). Phagosomes eventually fuse with lysosomes to which they deliver their cargo and therefore share some common features with autophagosomes, including their reliance on PI3P produced by Vps34.

Autophagosomes are PI3P-rich structures

Autophagy is generally thought of as a mechanism of organelle clearance during prolonged periods of starvation or when the organelles are no longer functional. Autophagosomes can be recognised by electron microscopy as double-membrane structures within the cells. Autophagosomes can also be detected by other methods using antibodies against LC3 (which become lipidated upon autophagy and forms puncta in the cytoplasm) or with probes that recognise PI3P (27, 28). Antigen presenting cells appear to have co-opted autophagy to recognise and destroy invading pathogens (29, 30). This insight was used to explain how viruses, which replicate in the cytoplasm, can be detected by TLRs in the lysosome of plasmacytoid dendritic cells (pDCs) where the autophagy pathway delivers virus particles from the cytoplasm to the lysosome (31). Both wortmannin and 3ME inhibited virus-induced autophagosome induction and TLR-dependent type 1 interferon response to the viruses (31). Moreover, these responses were absent in *Atg5^{-/-}* pDCs suggesting that this process depends on autophagy. Autophagosomes can also target pathogens to MHC class II loading compartments providing a route for antigen cross-presentation (32, 33). A number of other studies have indicated that phagocytes more generally use autophagy to degrade intracellular pathogens and how pathogens have evolved to avoid autophagy (19, 29, 30). However, unequivocal evidence supporting a role for Vps34 in the recognition and destruction of pathogens awaits the development of selective inhibitors or gene-targeting of Vps34 in myeloid cells.

Vps34 is essential for peripheral T cell homeostasis

Autophagosomes can eliminate mitochondria by a process known as mitophagy. Normally, mitochondrial content is dramatically reduced as thymocytes differentiate to peripheral T cells, but T cells deficient in Atg proteins contain higher numbers of mitochondria and this is thought to negatively impact their survival in the periphery (34). Two recent publications have reached opposing conclusions regarding the role of Vps34 in T cell autophagy (19, 20). Both published reports showed relatively normal thymic development, but with fewer CD4

or CD8 single positive thymocytes and reduced numbers of T cells in the spleen and lymph nodes (19, 20). McLeod and colleagues showed stimulation with anti-CD3 and anti-CD28 induced the appearance of LC3 puncta in wild type and Vps34^{-/-} T cells, but not in Atg3^{-/-} T cells, suggesting that autophagosomes were formed independently of Vps34. Moreover, the appearance of LC3 puncta in Vps34^{-/-} T cells could be blocked using the inhibitor 3MA (which also demonstrated that this reagent is not a specific inhibitor for Vps34). The authors argued that Vps34 was not required for autophagy in T cells, but rather for optimal IL-7 expression, as IL-7 was trapped in intracellular vesicles in Vps34^{-/-} T cells (20). However, Willinger and Flavell argued against an intrinsic role for Vps34 in regulating IL-7R expression and instead showed that the reduced expression of IL7R on Vps34 T cells was secondary to the influence of a lymphopenic environment since normal IL7R expression was restored on Vps34^{-/-} T cells in mixed bone marrow chimaeric mice (19). Although Willinger and Flavell detected lipidated LC3 protein in Vps34^{-/-} T cells, they found that autophagic flux was impaired in Vps34^{-/-} T cells. That is, basal autophagy was normal (as judged by appearance of lipidated LC3), but when autophagic vesicles fusion with the phagosomes was blocked by the toxin bafilomycin A, lipidated LC3 accumulated in WT cells to a greater extent than in Vps34^{-/-} cells (19). How could the two reports reach such different conclusions? Part of the answer may involve differences in how the assays were carried out. Jaber and colleagues have shown that LC3 puncta can accumulate in Vps34^{-/-} cells, but that these fail to localise to autophagosomes and instead exist as cytoplasmic protein aggregates (16). Thus Vps34 may be required for the targeting, rather than formation of LC3 puncta within cells. Consistent with this idea, Wipi2, which is a mammalian homologue of Atg18, binds PI3P and is required for the recruitment of LC3 to autophagosomes (35).

A further possibility that could explain differences between the two studies concerns the Vps34 gene targeting strategy. MacLeod et al used mice with Vps34 exon 17 and 18 were conditionally deleted (floxed), whereas Willinger and Flavells had floxed exon 4. Deletion of Exon 4 led to the disappearance of the entire protein whereas deletion of exons 17 and 18 could theoretically lead to the production of a truncated protein that would lack kinase activity, but which could stabilise beclin, for instance. The levels of Beclin were dramatically reduced in the mice with floxed exon 4 which might have had a strong impact on autophagy independently of the Vps34 kinase activity (19). However, to accept this explanation, one would have to argue that Vps34 plays a more important role as a scaffolding protein than as a kinase that decorates early autophagosomes (omegasomes) with PI3P. This notion is inconsistent with our understanding of how Vps34 works as PI3P is considered to be a key membrane tether for proteins involved in autophagy (35).

The most parsimonious explanation therefore seems to be that autophagic components such as LC3 can be assembled in cells in absence of Vps34 activity, but that Vps34 kinase activity is required for the correct targeting of LC3-containing protein complexes to the autophagosome and for autophagosome maturation. However, this interpretation does not exclude a key role for Vps34 in controlling endocytosis or normal trafficking of other membrane compartments and their cargo in T cells.

Regulation of potassium channels by the Class II PI3K C2 β in T cells

Mammals express three class II PI3Ks: C2 α , C2 β and C2 γ . Although the C2 PI3Ks can phosphorylate PI or PI4P *in vitro*, their favoured substrate is thought to be PI (Refs 3, 36). Different receptors, such as insulin receptor, EGF receptor, GPCRs and TNF family receptors have been reported to activate C2 PI3Ks. The precise mechanism coupling these receptors to the C2 PI3K enzyme is unclear, but is likely to involve induced translocation of the enzyme to the plasma membrane where they interact with phospholipids, clathrin and calcium (3, 36). C2 $\alpha^{-/-}$ mice suffer from kidney failure and increased IgA depositions in the kidneys were observed. However, bone marrow reconstitution experiments suggested the kidney failure was not caused by altered immune responses in the C2 $\alpha^{-/-}$ mice (37). C2 $\beta^{-/-}$ have so far not revealed any phenotypic abnormalities (38, 39). Nevertheless, Skolnik and colleagues have shown that C2 β , but not C2 α , can move towards the immune synapse formed by a Jurkat T cell and anti-CD3 antibodies embedded in a lipid bilayer (40). C2 β colocalised with CD3 in the central supramolecular activation cluster suggesting that its translocation was under control of TCR signalling. Moreover, they found that PI3P generated by C2 β could activate the potassium channel KCa3.1 in CD4 T cells (40-44). Potassium channels such as KCa3.1 maintain negative membrane potential by facilitating the efflux of K $^{+}$. This is required for the cells to maximise their intake of Ca $^{2+}$ once the plasma membrane calcium channels have been opened. C2 β regulation of KCa3.1 channels appears to involve the activation of nucleoside diphosphate kinase b (NDPK-b) which is required for the phosphorylation of a histidine residue within KCa3.1 (44). However, NDPK-b does not contain any recognised PI3P binding motive, so exactly how it is activated by C2 β is unclear.

PIKfyve and PI(3,5)P $_2$

PI3P is not only a tether for PX and FYVE domains, but also a substrate for the kinase PIK-FYVE, known as Fab1 in yeast where it was first identified (45). Fab1 phosphorylates PI3P to generate PI(3,5)P $_2$. Fab1 yeast mutants fail to recycle proteins and membranes from the vacuoles to other compartments and consequently have very large vacuoles (46). Little is known about the role of PI(3,5)P $_2$ in immune cells; however a mouse knockout has been generated and these mice die early during embryogenesis (47). Moreover, inhibition of PIKfyve has also been shown to limit infection by *Salmonella* in epithelial cells (48). It would not be surprising if PIKfyve also turns out to play important roles in APCs and other immune cells.

Myotubularins are PI3P phosphatases

PI3P is dephosphorylated by the myotubularins (MTM and the related MTMRs) of which there are 14 members in mammals (49, 50). Intriguingly, 6 of these lack phosphatase activity, but pair up with phosphatase active members to increase their activity and/or target them to the correct compartment. Mutations in genes in the MTM family are the cause of various neuromuscular diseases (49). MTMR6 can inhibit the Kca3.1 channel in T cells (43), but little is known more generally about the role of the MTMRs in immune cells. However, in *Drosophila*, one of the 7 MTMRs, Mtm was found to antagonise the function of In haemocytes (insect immune cells) under circumstances where Vps34 appeared not to

contribute significantly (51). Further work is required to determine whether some MTMRs preferentially antagonise Vps34 signalling and other C2 PI3K signalling, but given the abundance of the MTMR subunits and the number of possible heterodimeric complexes, this will not be a simple task.

The Class I PI3Ks generate PI(3,4,5)P₃ in response to receptor activation

The class I PI3Ks are heterodimeric proteins containing an adapter subunit and a catalytic subunit. *C. elegans* and *Drosophila* have one class I isoform each, whereas in vertebrates, the class I PI3K subunits are further subdivided into class IA (p110 α , p110 β and p110 δ) and class IB (p110 γ) (2, 3). The class IA subunits are associated with SH2 containing regulatory subunit, of which there are several types: p85 α , p55 α , p50 α , p85 β and p55 γ (collectively referred to as p85 in this review; a detailed review on the distinct role of the class IA PI3K-regulatory subunits was published recently (52)). The p85 SH2 domains recruit p110 α , p110 β or p110 δ to proteins that have been phosphorylated on YXXM motifs by tyrosine kinases. By contrast p110 γ is found as a heterodimer with p101 or p84, both of which bind G $\beta\gamma$ -subunits and therefore recruit p110 γ to activated G protein coupled receptors (GPCRs). At the plasma membrane, PI3K activity is further enhanced by the interaction between GTP-Ras and the Ras binding domain in the p110 subunit (53). All the class I PI3Ks have in common that their preferred substrate is PI(4,5)P₂ which is converted to PI(3,4,5)P₃ (simply known as PIP₃ since this is the only tris-phosphorylated phosphoinositide) (1). PIP₃ acts as a membrane-tether for a subset of proteins containing one or more PH domains (described in greater detail below).

Why do immune cells express 4 class I PI3K catalytic subunits?

Individual roles for each of the class I PI3Ks have been described in immune cells using knockout mice, kinase-dead knockin mice and specific inhibitors (54-63). P110 α and p110 β are broadly expressed in most cell types, whereas p110 γ and p110 δ expression is more limited to cells of the immune system (3). It is easy to rationalise why p110 γ plays a unique role in many settings by virtue of its distinct p101 and p84 regulatory subunits (Fig 3). Accordingly, p110 γ is a key signalling protein engaged by chemokine receptors in the immune system (64). The surprising finding, especially for the class IA PI3Ks, is there is often lack of redundancy between these subunits in different cellular contexts. If the class IA PI3Ks bind the same adapter proteins and phosphorylate the same substrate, then what makes them different? Some clues are emerging. By a mechanism that has yet to be elucidated, p110 β can also be activated by GPCRs in many cell types, especially those that do not express high levels of p110 γ (65-67). Furthermore, p110 β was shown to integrate signals from tyrosine-kinase linked Fc γ R receptor and the GPCR coupled leukotriene receptor BLT1 in neutrophils suggesting a specialised function for p110 β as a coincident tyrosine kinase and GPCR signalling detector (56)(Fig 3). Surprisingly, In NK cells and B cells, p110 δ is activated by chemokine receptors (68, 69); however, in general, p110 δ is less effectively activated by GPCRs than are p110 γ and p110 β . If one considers that p110 β compensates for the lack of p110 γ in non-immune cells and in addition is especially adept at integrating tyrosine kinase and GPCR signals then this can be considered as an adaption that distinguishes p110 β from p110 α and p110 δ . What then are the differential roles of p110 α

and p110 δ ? How are these activated differently by different receptors in different cell types? Before we consider this question, it is worth reviewing some recent developments that have shed new light on how class IA PI3Ks are activated.

The unique role of p110 α in human cancers

PIK3CA, the gene for p110 α is the one of the most frequently mutated oncogenes in human cancer (70). The mutations in PIK3CA centre around three hotspots where E542K, E545K and H1047R are the most common amino acid substitutions, respectively (71-73). In the p85-p110 heterodimer, the p85 SH2 and iSH2 domains make several contacts with the p110 kinase domain (72, 74-76). These are inhibitory contacts that are relieved upon engagement of the SH2 domain by a tyrosine phosphorylated peptide (such as would be found on an activated receptor or its associated adapter proteins)(77-79). The PIK3CA E542K and E545K mutants exhibits higher basal kinase activity which is not further increased by the binding of pY peptides to the p85 SH2 domain (80). Corresponding oncogenic mutations in p85 have also been identified which abrogate the inhibitory effect on p110 (81, 82).

The ability of oncogenic mutants of Ras to transform cells depends, at least in part, on their ability to activate p110 α (53, 83). Moreover, the Ras binding domain of p110 α is required for normal development of lymphatic vesicles (83). It is therefore of interest that the H1047R mutation increases the association of p110 α with the plasma membrane and also renders p110 α less dependent on Ras activity (80, 84). The high penetrance of the PIK3CA E545K, H1047R and similar mutations in human cancers, and how these mutations bypass the requirements for upstream activating events, has thus revealed how important dual input of p85 and Ras are for optimal activation of p110 α under normal physiological conditions.

More stringent criteria for the activation of p110 β and p110 δ than for p110 α

Structure function experiments have revealed interesting differences in how p110 α , p110 β and p110 δ are regulated by p85 and Ras. Some of the greatest sequence divergence between the class I PI3K isoforms is found within their Ras binding domains (85). The Ras binding domain of p110 α and p110 γ can bind most members of the Ras family, including H-Ras, K-Ras and N-Ras which are key signal transducers by tyrosine-kinase linked receptors and frequently mutated oncogenes (86). P110 β showed very limited activation by Ras in in vitro assays, whereas P110 δ was activated by R-Ras and TC21 (also known as R-Ras2), but not by the other Ras proteins (86). Indeed, gene targeting studies have shown that TC21 contributes to p110 δ activation in B cells and T cells (87). Whereas the optimal activation of p110 α by p85 requires the engagement of the N terminal SH2 domain of p85 by a tyrosine phosphorylated peptide, both the N-terminal and C-terminal SH2 domains of p85 need to be thus engaged for optimal activation of p110 β or p110 δ (79, 88). Therefore, whether the activation signal comes from p85 or from Ras, the activation requirements for p110 δ and p110 β appear to be more stringent than for p110 α . Indeed, in fibroblasts, insulin responsive tissues, endothelial cells and epithelial tumour cells, p110 α is the main isoform that generates PIP3 in response to activation by tyrosine-kinase linked receptors (89-92). In immature pre-B cells, p110 α and p110 δ show complete redundancy (55). However, p110 α deletion has minimal effect on the phosphorylation of Akt in response to activation of the BCR, whereas p110 δ inhibition nearly completely ablates Akt phosphorylation in mature B

cells in which both isoforms are expressed (54, 55, 61, 93, 94). Similarly, p110 δ is the main isoform activated by various tyrosine kinase-linked receptors on T cells, mast cells, macrophages, and dendritic cells (95). How cells of the immune system evolved such exquisite dependence on p110 δ remains an unresolved conundrum – made even more difficult to understand given that p110 δ in principle should be more difficult to activate than p110 α which is also expressed in cells of the immune system, albeit at slightly lower levels (79, 86, 96). One interesting observation is that in a cellular transformation assay where the oncogenic potential of the p110 units were tested, p110 α was dependent on its RBD to cause transformation, whereas p110 δ was not (80, 84). These observations raise the possibility that p110 δ activity is less dependent on Ras. Consistent with this possibility, the Ras binding domain of p110 δ is differently orientated relative to the kinase domain than in p110 α (97). Additional questions remain about how the p85 SH2 domains are productively engaged in immune cells (95). Bidentate YXXM...YXXM motifs which could simultaneously engage both SH2 domains of p85 and which are deemed to be required for optimal activation of p110 δ (79) are not commonly found in mature immune cells (a notable exception being CD19 in B cells (98)). Indeed, in many contexts, the proteins and the specific tyrosine residues involved in the recruitment of p85-p110 heterodimers in cells of the immune system remain incompletely characterised (95).

PIP3 and PI(3,4)P2 are lipid second messenger signalling molecules

PIP3 is a short lived lipid species that is rapidly degraded by one of two pathways (Fig 1). The lipid phosphatase Pten is the only enzyme known to be able to convert PIP3 back to PI(4,5)P2 (99). Ship1 and Ship2 are members of a larger family of inositol 5 phosphatases that can convert PIP3 to PI(3,4)P2 (99). Ship phosphatases are of particular interest to immunologists as they can be recruited by their SH2 domains to receptors that contain immune tyrosine inhibitory motifs (ITIMs) found in Fc γ RIIB (100). The Akt PH domain binds non-discriminately to PI(3,4)P2 and PIP3, whereas other PH domains bind selectively to either lipid (101-103). As a consequence, PI(3,4)P2 may have unique signalling properties by virtue of its selective recruitment of proteins such as Bam32 and TAP (104). The deletion of Pten in thymocytes leads to aggressive T cell lymphoma and autoimmunity (105, 106). However, Pten can be deleted in mature T cells without causing transformation and instead renders the T cells hypersensitive to stimulation (105, 107, 108). This hypersensitivity can be attenuated by p110 δ inhibition (108). Deletion of Pten alone in B cells prevents the induction of anergy, leads to increased production of natural antibodies, but also prevents the B cells from undergoing class switching (109-111). The latter phenomenon will be described further in a subsequent section. The effect of Pten deletion in B cells is partially restored by simultaneous deletion or inhibition of p110 δ (93, 109). Deletion of Ship leads to lethal autoimmunity caused by the aberrant activation of T cells, B cells and myeloid cells (112). Moreover, the deletion of both Pten and Ship in B cells leads to leukaemic transformation (113). The phosphatase INPP4B removes the 4 phosphate from PI(3,4)P2. Solid tumours lacking both PTEN and INPP4B have found, suggesting that these phosphatases work in concert to extinguish class I PI3K signals (i.e. by degrading PIP3 and PI(3,4)P2, respectively) (114, 115). These results also suggest the PIP3 and PI(3,4)P2 work together for optimal activation of downstream pathways (112). The role of INPP4B or the related INPP4A in immune cells is not yet known.

Signalling by class I PI3Ks

PH domains must have sufficient affinity for PIP3 relative to other phosphoinositides to be selectively regulated by class I PI3Ks. For instance, a PH domain that binds equivalently to PI(4,5)P2 and PIP3 is unlikely to be influenced by PI3K signalling since PI(4,5)P2 is much more abundant in the lipid membrane (116). Instead, such proteins may use phosphoinositides more generally to facilitate their interactions with plasma membranes (117, 118). In mammals, about 40 of the 200+ proteins containing PH domains can potentially be controlled by PIP3 (116, 119, 120). However, most work to date has focused on the regulation of the Akt pathway and its role in controlling the activation of mTOR and transcription by Foxo (Figure)(121). There is a strong rationale for focusing on this pathway given that it is conserved through evolution and because the Foxo transcription factors and mTOR play important roles in immune responses (121-124). Akt can also phosphorylate a plethora of other substrates that can influence the function of immune cells (125). Vertebrates express three Akt isoforms, Akt1, Akt2 and Akt3 (also known as PKB α , PKB β and PKB γ). Akt activity is controlled by the binding of PIP3 or PI3,4P2 to its PH domain and by phosphorylation on Thr308 by Pdk1 and Thr473 by mTORC2 (125). The different Akt isoforms share some common substrates, but evidence is also emerging for isoform selective substrates (126). Each of the Akt isoforms have shown to be required for haematopoietic stem cell survival, T cell development and/or B cell development (127-131).

Akt-Foxo

Phosphorylation of Foxo1, Foxo3 and Foxo4 by Akt leads to their nuclear exclusion and degradation (121). Key Foxo target genes in lymphocytes include the Rag recombinases, Ikaros, the Il7r, Foxp3, Ccr7 and Cd62l (121, 132-143). By activating class I PI3Ks, certain Foxo target genes can be turned off. It follows that class I PI3K-Akt signalling needs to be inhibited to turn Foxo target genes on or off. Hence, the temporal activation and inhibition of class I PI3Ks and Akt during immune development is of key importance and this is a theme we will return to in context of B cell development and Treg differentiation. Akt also controls gene expression by regulating mRNA stability. In a human glioma cell line, 40% of genes whose expression was down-regulated upon PI3K inhibition showed reduced mRNA stability (144). mRNA stability is controlled by so-called ARE-binding proteins which bind to AU-rich sequences in the 3' untranslated region of mRNAs (145). Phosphorylation of Foxo proteins and ARE-binding proteins may represent the major mechanisms through which class I PI3Ks control gene expression.

Akt-mTOR

mTOR is a serine threonine kinase with whose catalytic domain shows homology to the PI3Ks, and which along with DNAPK and ATM is sometime referred to as a class IV PI3K (even though neither protein can phosphorylate phosphoinositides) (2). mTOR exists as part of two protein complexes, the rapamycin sensitive complex1 (mTORC1 which contains Raptor) and the rapamycin insensitive complex 2 (mTORC2 which contains Rictor) (122, 146)(Fig 4). mTORC1 activity is controlled by the small GTPase Rheb. The GTPase activating domain of Tuberin (Tsc2) increase the rate of hydrolysis of Rheb-bound GTP, returning Rheb to the inactive GDP-bound form. Thus, by phosphorylating and inhibiting

Tsc2, Akt releases Rheb from the inhibition by Tsc2 and allows GTP-Rheb to activate mTORC1. In addition to Akt, both Erk and Rsk can phosphorylate Tsc2, and hence activate the mTOR pathway (147). Thus while mTOR requires receptor initiated signals (e.g. from the TCR or BCR), there is redundancy in the pathway leading to its activation (132, 148). Moreover mTOR will only be activated if there is sufficient amino acid availability and ATP in the cells (otherwise initiating protein or lipid synthesis, both of which are key consequences of mTOR activation, would be futile). AMPK, which is active when AMP/ATP ratios are high, inhibits mTOR whereas amino acids activate mTOR through a mechanism that has yet to be fully elucidated, but which involves the assembly of proteins of the Rag family at the lysosome (149, 150). Exactly how mTORC2 gets activated is not known. However, its substrate Ser473 on Akt is of key interest for the current discussion on class I PI3K signalling.

The best characterised substrates for mTORC1 are S6K and 4EBP1 (146). These in turn contribute to protein synthesis by phosphorylating ribosomal S6 and by releasing the inhibition of elongation factors, respectively. A more comprehensive analysis of mTORC1 substrates has recently been published, but the function of most of these have yet to be established explicitly in immune cells (151, 152). Among the key genes whose expression is dependent on mTORC1 is HIF1 α which is required to increase glycolysis in lymphocytes (153, 154). mTORC1 can also negatively regulate class I PI3K signalling via different mechanisms including phosphorylation of receptors. Thus acute inhibition mTORC1 can enhance class I PI3K signalling (146). However, persistent administration of Rapamycin has a knock-on effect on mTORC2 and hence leads also to the inhibition of Akt (155). This dual effect of mTORC1 inhibition was recently demonstrated in CD8 T cells where Rapamycin enhanced Akt Ser473 phosphorylation for the first 12 hours, but subsequently suppressed Akt Ser473 phosphorylation (thus enhancing Foxo activity) (156). ATP-analogues that inhibit mTOR regardless of which complex it finds itself in have been developed and these will also inhibit Akt Ser473 phosphorylation regardless of the duration of inhibition. The complexity of this circular pathway, where Akt is both upstream and downstream of mTOR, means that one has to take some care when interpreting experiments using Rapamycin or other mTOR inhibitors as these can also affect Akt substrates, including Foxo (Fig 4).

PIP3-dependent activation of Tec-family tyrosine kinases

Another important family of PIP3-binding proteins are the Tec kinases represented by Itk, Btk and Tec (157). A fourth member, Rlk lacks a PIP3-binding PH domain. *Drosophila* express a Btk orthologue which also has a PIP3-binding PH domain suggesting an evolutionary conserved role for PI3K regulation of Tec kinases (119). It is important to appreciate, however, that Tec kinases also contain SH2 and SH3 domains that can recruit these kinases to protein complexes at the plasma membrane. Thus PI3Ks may not be absolutely required for the recruitment and activation of Tec kinases, but rather make more subtle contributions to their function once recruited by other mechanisms. The Xid mutation in mice is caused by a mutation in the PH domain which renders Btk insensitive to PIP3 (158). This was seen as strong genetic evidence that Btk requires PIP3 for its full activation. However, PI3K and Btk-deficient phenotypes are non-overlapping and subsequent studies by Koyasu and colleagues showed that the Xid mutation causes Btk to be less stable and as

such acts as a hypomorphic mutation (159, 160). Direct evidence that PI3K is required for the other Tec kinases (Tec and Itk) in primary immune cells is lacking and may require the generation of new knockin mutations in their PH domain that do not affect protein stability.

PIP3-dependent activation and inhibition of small GTPases

In many cell types, class PI3Ks control the activation the small GTPase Rac (161). P-Rex1 was purified as a protein that controlled the activation of Rac in neutrophils (162). Sequential activation of p110 γ and p110 δ contributes to many important facets of neutrophil activation, including migration and ROS production (64, 163). P-Rex contains a PH domain which binds PIP3 with high selectivity and a GEF domain that catalyses the exchange of Rac-bound GDP for GDP. P-Rex is in part redundant with Vav proteins in neutrophils where these proteins regulate chemotaxis, ROS production and adhesion (164). The Vav proteins also contain PH domains that can bind PIP3, but the Vav PH domain also binds PIP2 and it seems less clear that Vav activation is acutely regulated by PI3K signalling (117, 118). Rather, increasing evidence suggests that Vav is an important activator of PI3K as both the PH domain of Vav and its catalytic activity are required for optimal PI3K activity (117, 165).

Curiously PI3K is also required for the activation of the Rac-GAP ArhGAP15 which contains a PH domain and which is recruited to the plasma membrane in a PIP3-depedent manner (166, 167). ArhGAP15^{-/-} neutrophils show enhanced chemotaxis, ROS production and phagocytosis (167). A major outstanding question is how the positive and negative effect of PIP3 via P-Rex1 and ArhGAP15, respectively, are balanced. Do they control distinct pools of Rac or are the respective PI3K-dependent GEF and GAP activities separated temporally?

Arap3 is a Rho-GAP which contains a remarkable 5 PH domains, two of which bind PIP3 synergistically (120, 168). In addition, Arap3 is activated by Rap1. ARAP3^{-/-} neutrophils adhere more strongly to integrin ligands, thus interfering with normal chemotaxis (169). Thus emerging evidence suggests that in addition to be controlled by small GTPases of the Ras family, PI3Ks can both positively and negatively control the activation of diverse small GTPases by stimulating GEFs and GAPS.

Role of individual class I PI3K p110 catalytic subunits and opposing phosphatases in the immune system

Several reviews have considered the role of class I PI3K in the immune system more generally (170-175); here I will focus on more recent studies that have elucidated individual roles for each of the catalytic subunits as well as studies that have emphasised the requirement to increase or suppress PI3K activity at particular stages during development and differentiation.

Class I PI3K signalling promotes HSC differentiation

Haematopoiesis in p110 δ and p110 γ deficient mice appears normal, however, mice lacking all class I PI3K activity in HSCs have yet to be reported. The role of PI3K activity in HSCs

has been investigated using complementary approaches, however. Deletion of *Pten* led to increased proliferation of HSCs at the expense of long-term repopulation capacity. There was reduced retention of white blood cells in the bone marrow and, instead, blood and spleens were enriched in myeloid cells, eventually leading to myeloma and T cell lymphomas (176, 177). Remarkably, treatment of the mice with the mTOR inhibitor rapamycin not only depleted the leukemic-initiating cells, but also restored the HSC compartment (177). These experiments therefore revealed a differential dependence on the PI3K pathway for normal HSC maintenance (requiring low flux through class I PI3K to mTOR) and leukaemia initiating cells (favoured by elevated class I PI3K/mTOR signalling). The deletion of *Foxo-1*, *-3* and *-4* also led to depletion of repopulating HSCs and an increase in myelopoiesis (178). The effects could be reversed by the anti-oxidant N-acetyl-L-cysteine (NAC), consistent with the observation that genes that regulate ROS are enriched among those controlled by Foxo transcription factors. By contrast, HSCs lacking *Akt1* and *Akt2* showed increased quiescence, reduced ROS, and again, reduced HSC repopulation capacity (128). Together, these reports emphasise that the class I PI3K/Akt/mTOR/Foxo pathways need to be carefully balanced for optimal HSC renewing capacity and hence homeostasis of the haematopoietic system.

Role of p110 α and p110 δ in B cell development and function

B cell development occurs through several stages characterised by checkpoints for quality control (179). The first such checkpoint occurs after the rearrangement of the immunoglobulin heavy chain. Only pre-B cells which functionally rearranged the *Igh* locus and express the heavy chain in conjunction with surrogate light chains develop further. Mice lacking p110 α and p110 δ activity in lymphocytes show a complete block at this stage of development, whereas p110 β and p110 γ do not contribute significantly (55). The precise reason for this block in development is not clear, but may reflect requirement PI3K to transmit signals from the pre-BCR promoting further differentiation. Defective B cell development in p110 α -p110 δ deficient mice could also be caused by impaired IL7R signalling. Phosphorylation of Tyr449 in the IL7R α chain can lead to recruitment and activation of PI3K via p85; however a Tyr449Phe mutation does not block early B cell development (180, 181). Nevertheless, signalling via the pre-BCR can sensitise the developing B cells to limiting amounts of IL-7. Thus, even if recruitment of p85/p110 to the IL7R itself is not essential, IL7 responsiveness could be affected by defective pre-BCR signalling in absence of p110 α and p110 δ . (182). Indeed, p110 α -p110-deficient pre-B cells showed impaired proliferative responses to IL-7 in culture and addition of antibodies against the pre-BCR failed to rescue development of p110 α -p110 δ deficient pre-B cells in vivo suggesting that both pre-BCR signalling and IL7 responsiveness are compromised in p110 α -p110 δ double deficient pre-B cells (55). Blocked class I PI3K signalling can also lead to the failure to turn the expression of Rag off (133, 134). Attenuation of Rag expression is necessary to allow for clonal expansion before Rag is re-expressed to rearrange the κ or λ light chains (179). Indeed, pre B cells lacking p110 α and p110 δ activity show high expression of Rag, had rearranged *Igh* genes and expressed the heavy chain (55). By contrast, pre B cells that lack Foxo protein fail to express Rag proteins and the IL7-R and are therefore blocked in their development (133, 134). The latter results indicate the

constitutively high PI3K activity would also be detrimental for normal B cell development as it would prevent the expression of important Foxo target genes. Jumaa et al have proposed a model whereby the pre-BCR-associated adapter protein SLP65 eventually leads to the suppression of class I PI3K activity and that this is required for B cells to progress towards a stage of development when the light chains are rearranged to form the mature BCR with Igh (179, 183, 184). After the light chain rearrangement is completed, class I PI3K activity may yet again be required for further development. Basal levels of class I PI3K activity in mature B cells is also required to suppress RAG expression and prevent spurious Ig gene rearrangements in immature and mature B cells (185-187). Because neither p110 α or p110 δ deficiency alone significantly affect B cell development in the bone marrow, yet double deficient cells are blocked at pre-B cell stage, p110 α and p110 δ can be considered redundant with regards to early B cell development (55). In fact, the expression of p110 α from a single allele was sufficient to promote normal B cell development in the bone marrow in absence of p110 δ , though the spleen was nearly devoid of B cells in these mice (55).

The different roles of p110 α and p110 δ in tonic and agonist induced BCR signalling

Tonic class I PI3K signalling by the BCR is required to keep mature B cells alive (188-190). The requirement for tonic signalling generally was initially demonstrated by the loss of B cells after induced deletion of the BCR or the associated Ig α signalling adapter (188, 189). Strikingly, expression of a membrane targeted p110 α transgene, the deletion of Foxo or the deletion of Pten were each able to rescue the survival of B cells that had lost BCR expression (190). Moreover, gradual loss p110 α and p110 δ activities led to a dose-dependent loss of follicular B cells although the loss of p110 δ activity had a more profound affect than the loss of p110 α activity (55). Therefore, in terms of B cell survival, some redundancy between p110 α and p110 δ was evident.

However, BCR-dependent Akt phosphorylation, proliferation, development of marginal zone B cells and the production of natural antibodies were blocked by p110 δ deficiency, but were unaffected by p110 α deficiency (55). Why can p110 α no longer compensate for the loss of p110 δ under these circumstances? Pre B cell selection and follicular B cell survival depend on the pre BCR and mature BCR, respectively, but there is no requirement for crosslinking of the BCR by antigen. The development of MZ B cells, by contrast, is facilitated by self-antigen recognition by the BCR, as do strong phosphorylation of Akt (some Akt phosphorylation can be detected in resting B cells). Indeed, transgenic expression of p110 α only moderately increased phosphorylation of Akt (190). It appears therefore that tonic BCR signalling leading to low levels of Akt phosphorylation can be mediated by either p110 α or p110 δ , whereas agonist induced signalling resulting in high levels of Akt phosphorylation is effectively mediated by p110 δ , but not p110 α . The reason why agonist induced BCR signalling does effectively engage p110 α remains unknown.

GC reaction, class Switching, affinity maturation

During the germinal centre reaction B cells with intermediate affinities for antigen are selected to undergo class switch recombination and somatic hyper-mutation of their Ig genes to generate high affinity IgG antibodies. P110 δ deficient mice show impaired T cell

dependent and T cell independent immune responses (54, 60, 61, 191). These results combined with a requirement for p110 δ to promote B cell proliferation in response to BCR crosslinking, could be interpreted as requirement for p110 δ activity within B cells to promote the differentiation of B cells to antibody producing plasma cells. This is indeed the case for the production of natural antibodies during T cell-independent immune responses. These are mostly of the IgM class, have low to intermediated affinity for self-antigens and common bacterial epitopes. Natural antibodies are much reduced in p110 δ -deficient mice due in large part to the lack of marginal zone B cells (54, 61, 192). By contrast, deletion Pten in B cell increases marginal zone B cells and natural antibody production, both of which can be attenuated by concomitant deletion of p110 δ (93, 111). Surprisingly, however, deletion of Pten in B cells interferes with T cell-dependent Ig class switching and affinity maturation, whereas the deletion of p110 δ in B cells has little impact on these events (109, 111, 193). It appears that PI3K signalling antagonises the expression of key proteins involved in class switching and plasma cell differentiation, including Aid and Bcl6 (109, 111, 194, 195). Therefore, as was the case after heavy chain Ig rearrangements, PI3K signalling needs to be actively suppressed in the germinal centre reaction to produce class-switched IgG producing plasma cells. How p110 δ activity is suppressed at this stage is not clear, but the recent observation that cycling B cells in the GC express high levels of Shp1 protein (196). It is interesting to speculate that Shp1 dephosphorylates proteins that activate p110 δ and hence reduce the production of PIP3. Why do p110 δ -deficient mice produce reduced IgG levels after immunisation? The answer to this question is that follicular helper T cells (T_{FH}) require p110 δ for their development and/or maintenance (193). Therefore, deletion of p85 or p110 δ in T cells reduces the production of high affinity class switched antibodies, whereas deletion of Pten in activated T cells as the opposite effect (193, 197).

Role of T cell development and function

T cell development proceeds through various checkpoints, the first of which is to ensure that the TCR β chain is productively rearranged (the β -checkpoint) during which T cells progress from CD4⁻CD8⁻ double negative to CD4⁺CD8⁺ double positive thymocytes (198). In addition to signals generated by the pre-TCR composed of TCR β and the surrogate preT α chain, the Notch and IL7 receptors are also required for the development of DP thymocytes. However, the requirement for the pre-TCR or IL-7 can be bypassed by deleting Pten which highlights the requirement for PI3K signalling for the development of DP cells (199). By contrast to B cells, mature T cell do develop in mice lacking p110 α and p110 δ in thymocytes (55). Instead, the combined loss of p110 δ and p110 γ leads to a near complete block in T cell development at the β -checkpoint (200-203). This is somewhat of a surprise, because p110 δ and p110 γ are activated by distinct receptors in T cells and hence redundancy would not necessarily be expected. This conundrum was in part resolved through the realisation that signalling via CXCR4 is required at the β -checkpoint (200). Thus while the pre-TCR engages p110 δ , the CXCR4 receptor preferentially activates p110 γ , with some contribution also from p110 δ (200). The observation that some PI3K activity provided either by the pre-TCR or CXCR4 via p110 δ or p110 γ is sufficient for the development of DP T cells raises some interesting questions. If some class I PI3K signalling is sufficient, as suggested by the ability of Pten deletion to bypass β -selection, then what prevents the

CXCR4 receptor from promoting differentiation of DP T cells from progenitors that have not productively rearranged their TCR β chain? One possibility is that pre-TCR signalling is required to render DN thymocytes cells responsive to CXCL12. Notch also requires class I PI3K activity to promote thymocyte development (204, 205). Notch signals by releasing its intracellular domain which acts as a transcription factor in the nucleus. Recent evidence suggest that Notch increases class I PI3K signalling through its target gene Hes1 which suppresses the expression of Pten (206). Notch may also regulate class I PI3K signalling via the interaction between the notch intracellular domain and the mTORc2 associated protein Rictor (207).

Mice lacking two or more Akt isoforms or Pdk1 in T cells show near complete block in T cell development (129, 130, 208, 209). However, caution is needed before one concludes that these are the only PI3K dependent signals required since DP thymocytes do develop when both Pten and Pdk1 are deleted in thymocytes, suggesting that other PIP3 effectors also play a critical role during development (210). The precise nature of these PIP3 effectors is not known, but Pten deletion could not rescue the development of thymocytes in which Rho has been inactivated suggesting that a PIP3-dependent RhoGEF may be important for T cell development (210).

The mature TCR activates p110 δ , and this can be enhanced by CD28 through a mechanism that does not involve direct recruitment of p85 to the CD28 YXXM motif (191). Rather, CD28 may act to boost and sustain signalling by the TCR more generally (211, 212). The related costimulatory receptor ICOS, by contrast, appears to depend entirely on its YXXM motif to signal via PI3K to promote T cell help to B cells (213). ICOS plays a key role in the development and survival of T_{FH} cells and which are virtually absent in mice where the ICOS YXXM motif has been mutated to FXXM or where p110 δ was conditionally deleted (193, 213). P110 γ also plays an important role in mature T cells by promoting the survival of memory T cells (214). While p110 γ is largely dispensable for the chemotaxis of naïve T cells, activated p110 $\gamma^{-/-}$ T cells show reduced chemotaxis towards inflammatory chemokines such as CCL5, CCL22 and LTB4 (69, 215-217). An intriguing possibility is that these chemokines also provide important survival signals to tissues resident memory T cells.

PI3K contributes to the differentiation of T cell subsets

PI3K signalling is required for the differentiation of native CD4 T cells towards either of the main T helper (Th) subsets, Th1, Th2, Th17 and T_{FH} (108, 193, 218-221). Recent studies have indicated that Foxo proteins can positively regulate the Ifn γ gene and that PI3K can control IL17 transcription via mTOR-dependent reciprocal regulation of Gfi1 and Ror γ (132, 222). Another possibility is that Foxo prevents T cells from differentiation such that class I PI3K-dependent removal of Foxo from the nucleus is a pre-requisite for further differentiation. The ability of p110 δ inhibitors to attenuate Th differentiation and production offers therapeutic opportunities to alleviate autoimmune and inflammatory diseases using small molecule inhibitors (223, 224). However, this needs to be balanced against the effect of p110 δ inhibition on Treg and the effect of inhibiting p110 δ in dendritic cells (171, 225, 226). Class I PI3Ks can both promote and inhibit the development of Treg (227). Accordingly, there are increased numbers of Treg in the thymus of p110 δ -deficient mice, but

reduced numbers in the spleen and lymph nodes (226). The dual effect of p110 δ inhibition on Treg numbers may be explained in part by the observation that delayed PI3K and mTOR inhibition after T cell activation supports enhanced Treg differentiation (228). Rapamycin and derivatives are some of the most widely used inhibitors in kidney transplantation. Yet, Rapamycin has recently been shown to enhance the generation of memory CD8 T cells (229). This is thought to reflect a change in the balance between the transcription factors T-bet and Eomesodermin (156, 230, 231). mTOR activity is required for T-bet induction, but not for emomesodermin and whereas effector CD8 T cells express high levels of T-bet, memory T cells express higher levels of emomesodermin. By shifting the balance between the two, Rapamycin promotes memory T cell development. This implies that at or just before the peak of the immune response there must be a mechanism for shutting of class I PI3K and/or mTOR signalling in a subset of cells to promote the formation of memory T cells precursor. This is reminiscent of the attenuation of PI3K signalling required during the germinal centre reaction in B cells.

PI3K inhibition in dendritic cells enhances type I immune responses

The deletion of p85 α resulted in increased IL-12 production by dendritic cells, and consequently enhanced type I immune responses against *Leishmania major* characterised by increased T cell-derived IFN γ production (225). P110 δ -deficient mice, by contrast, show impaired T cell IFN γ responses in response to *Leishmania* infection despite elevated IL-12 production by DCs and macrophages (232). These differences may be explained by the different effect of targeting the p110 δ subunit and targeting the p85 α subunit. Deletion of p85 α alone has little effect on T cell activation, whereas deletion of both p85 α and p85b in T cell ablates TCR-induced Akt phosphorylation, similar to that seen in p110 δ -deficient T cells (197). Therefore, in the p85 α ^{-/-} mice, DCs provided an excess of IL-12 to phenotypically normal T cells, whereas in p110 δ ^{D910A} mice, the T cell were unable to produce high levels of IFN γ , despite the excess IL-12 produced by the myeloid cells. Interestingly, p110 δ ^{D910A} mice controlled *Leishmania* infection more effectively than did wild type mice (232). This is likely to be due, at least in part, to defective Treg function in p110 δ mice (218, 227).

Therapeutic use of PI3K inhibitors

At the time of writing, the p110 δ inhibitors GS-1101 is entering phase III clinical trials in to test their efficacy in CLL and iNHL after showing promising results in phase I and II trials, where a large proportion of patients responded by showing a shrinkage of the lymph nodes (233-235). The effectiveness of p110 δ inhibitors alone in B cell leukaemias may be explained in part by the lack of involvement of p110 γ in GPCR signalling which instead is dependent on p110 δ in B cells. By contrast, in mouse a mouse model of T cell leukaemia and in human T-ALL cells, the p110 δ -p110 γ dual inhibitor CAL-263 was much more potent than inhibition of p110 γ or p110 δ alone, perhaps reflecting the redundancy between these subunits in T cell precursors which give rise to T cell leukaemias (236).

Perspectives

The roles played by the class II and III PI3Ks in immune cells are only beginning to be uncovered. Further development of gene targeted mice and small molecule inhibitors will no doubts accelerate these efforts, as they did for the class I PI3Ks. Such studies are likely to provide further insights into how PI3P-dependent pathways control the uptake and killing of pathogens by antigen presenting cells and how PI3P promotes immune cell homeostasis more generally.

The role of the individual PI3K isoforms in lymphocyte biology is still incompletely understood, yet rapid developments are supported by gene targeted mouse lines and the development of isoform selective inhibitors. However, one concept observed in B cell development, germinal centre reactions and Treg differentiation, is that transient suppression of class I PI3K can be essential to facilitate expression of Foxo and possibly other genes. Once these genes have been induced, PI3K signalling may again be required to promote growth and proliferation. The requirement for temporal suppression of a signalling pathway is not always readily appreciated by knockout studies and can also be difficult to probe using small molecule inhibitors, especially in vivo. Methods to accurately monitor signalling output over time are also lacking. Nevertheless, this dual nature of PI3K signalling may have led authors to reach opposite conclusions about the role of the pathway, depending on the experimental model system they used and manner they used to inhibit or boost PI3K signalling. Key challenges in this area include tools to switch pathways on and off at will, a deeper understanding of how this happens within cells (e.g. removal of ligands, dephosphorylation of proteins at the membrane or activation of phosphatases).

Finally, elucidating the role of non-canonical PIP3-binding proteins which have shown great increase in numbers and diversity during vertebrate evolution, will no-doubt continue to reveal new and important insights into the role of class I PI3Ks in immunity.

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Figure 2.
A. List of the genes and protein products of the mammalian PI3K family.
B. Phylogenetic tree of the 8 mammalian PI3K catalytic subunits. The tree was generated using ClustalW (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/)
C. The domain structures of the class III, II and I PI3Ks are represented by Vps34, CIIα and p110α, respectively. The domain structure of p85α is also shown. Not shown are class IB regulatory subunits and the Vps34 regulatory subunits. The Illustrations were generated using Prosite (<http://prosite.expasy.org/scanprosite>).

Regulation of PI3Ks by tyrosine kinases and GPCRs

Figure 3. Schematic illustrating the recruitment of class I PI3Ks to tyrosine kinase-linked receptors and GPCRs. P110β is illustrated as a coincidence detector for tyrosine kinase and GPCR signalling.

The PI3K-Akt-Foxo-mTOR regulatory network

Figure 4.

Diagram illustrating the circular network that connects PI3K signalling to MTOR activation. Regulatory subunits are not shown. How Vps34 contributes to amino acid –dependent mTOR activity is incompletely understood. PI3Ki indicates various PI3K inhibitors, some of which can also inhibit mTOR. TORins describes ATP-competitive mTOR inhibitors. Rapamycin blocks mTORC1 specifically via its association with FKBP12 (not shown).

Figure 5.