TIMELINE

PI3K signalling: the path to discovery and understanding

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Abstract | Over the past two decades, our understanding of phospoinositide 3-kinases (PI3Ks) has progressed from the identification of an enzymatic activity associated with growth factors, GPCRs and certain oncogene products to a disease target in cancer and inflammation, with PI3K inhibitors currently in clinical trials. Elucidation of PI3K-dependent networks led to the discovery of the phosphoinositide-binding PH, PX and FYVE domains as conduits of intracellular lipid signalling, the determination of the molecular function of the tumour suppressor PTEN and the identification of AKT and mTOR protein kinases as key regulators of cell growth. Here we look back at the main discoveries that shaped the PI3K field.

Phosphoinositide 3-kinases (PI3Ks) generate intracellular lipids that affect a range of cell biological phenomena. They phosphorylate the 3-OH group of inositol membrane lipids to modulate the activity of intracellular protein effectors that regulate many aspects of cell function. The PI3K pathway can be mutationally activated (such as in cancer) or inactivated (such as in some myopathies, neuropathies and ciliopathies) and acts in immunity, metabolism and cardiac function. PI3K inhibitors have recently entered the clinic, and the number of scientists involved in this area has vastly expanded, making it timely to capture the key discoveries that led to the molecular understanding of PI3K signalling and function. This Timeline article summarizes the discovery of 3-phosphorylated inositol lipids and modular lipid-binding domains in PI3K effector proteins, which together defined a new paradigm of membrane-to-cytosol communication. We also describe the path to the understanding that PI3Ks form a family of proteins with distinct substrate specificities and biological functions. Moreover, we discuss how the discovery of mutations affecting the PI3K pathway, together with the delineation of the functions of PI3K isoforms in mice, put these enzymes on the map as targets for therapeutic inhibition (Timeline). Finally, this article reminds us how much there is still to be learned. For example, although every cell is estimated to have 50–100 downstream effectors of PI3K, the field has thus far focused on only a small number of the most tractable effectors, such as AKT. The contribution of most of the PI3K isoforms to organismal function as well as disease also remains unknown.

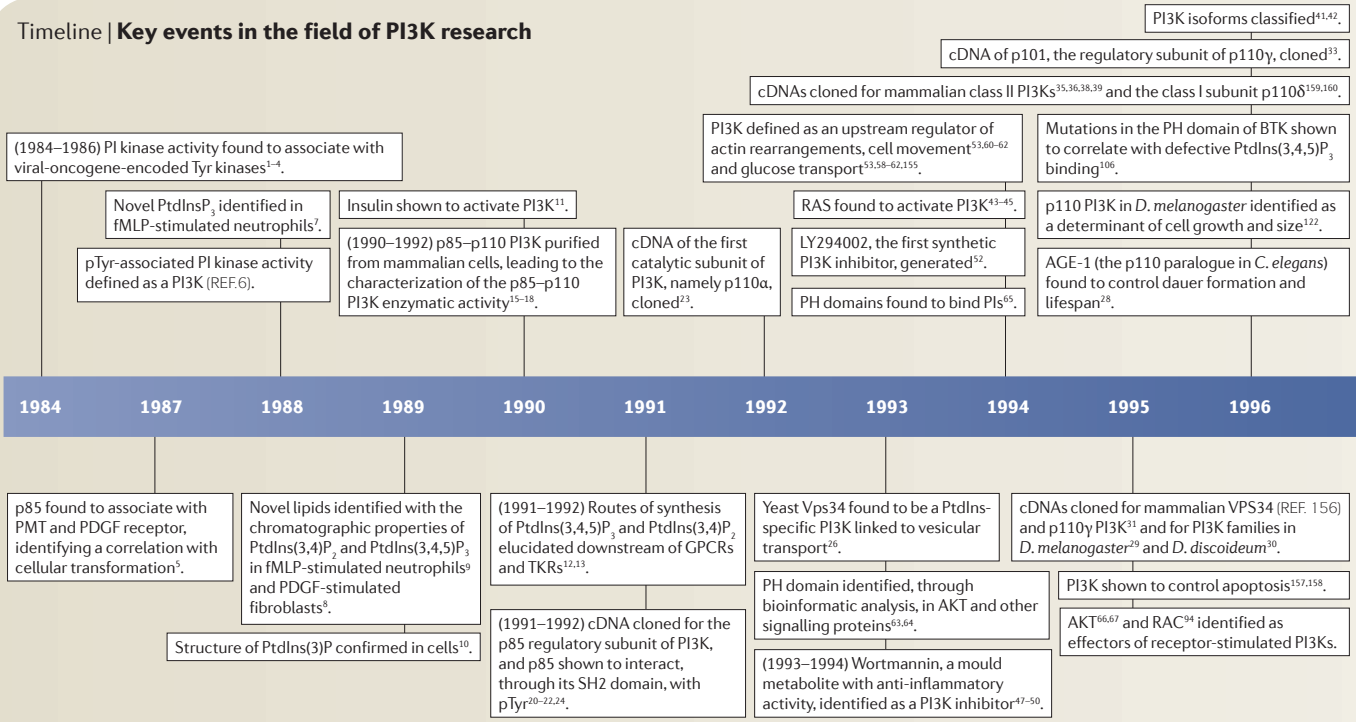
Discovery of PI3K activity

In the mid 1980s, there was a great deal of interest in a cellular signalling system that was based on receptor-stimulated hydrolysis of the membrane lipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) by phospholipase C (PLC) (FIG. 1). This reaction generates the so-called second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3), which propagate signals to the cell interior via the activation of protein kinase C (PKC) and the mobilization of calcium, respectively. During this period several groups also found that some viral oncoproteins (such as the Tyr kinase SRC or polyoma virus middle T antigen (PMT)) associate with a cellular lipid kinase activity that uses PtdIns as a substrate *in vitro*[1–4]. Moreover, transformation of cells in culture by such oncoproteins depends to some extent on the association with this 'PtdIns kinase' activity[4,5].

In 1988, Cantley's group, in a collaboration with the team led by Downes, made the surprising discovery that this oncoprotein-associated kinase is actually a 'PtdIns 3-kinase', in that it phosphorylates the 3-OH group of the inositol ring of PtdIns *in vitro*, generating the previously unknown lipid PtdIns(3)P[6]. Crucially, this finding placed this enzyme on a separate pathway to that for PtdIns(4,5)P_2 synthesis and subsequent PLC-dependent signalling (FIG. 1). In the same year, Traynor-Kaplan *et al.* reported that G protein-coupled receptor (GPCR)-stimulated neutrophils contain a lipid with the chromatographic properties of a PtdInsP_3 — that is, a PtdIns carrying three phosphate groups on the inositol ring[7]. These discoveries indicated that novel inositol lipids can be formed in stimulated cells and drove research to elucidate the structures, routes of metabolism and function of these lipids. The following year, Cantley and co-workers reported that proteins immunoprecipitated from platelet-derived growth factor (PDGF)-activated cell lysates with the use of phosphotyrosine (pTyr)-specific antibodies phosphorylated PtdIns, PtdIns(4)P and PtdIns(4,5) P_2 *in vitro* to produce the versions of these inositol lipids that are phosphorylated at the 3-OH group of the inositol ring (that is, PtdIns(3)P, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, respectively)[8]. Furthermore, trace quantities of lipids with the chromatographic properties of these 3-phosphoinositides could be detected in PDGF-stimulated cells[8]. Traynor-Kaplan *et al.* also reported increases in analogous lipids in *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated neutrophils[9], and Stephens *et al.* formally confirmed the presence of PtdIns(3)P in mammalian cells[10]. Insulin was also found to stimulate large increases in PtdIns 3-kinase activity at a time when the elucidation of the insulin signalling pathway represented something of a 'holy grail' for cell signalling researchers[11].

A picture soon began to emerge that cells in the basal state possess low levels of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 but substantial quantities of PtdIns(3)P[9,12,13]. Activation by appropriate Tyr kinase receptors (TKRs) or GPCRs was shown to stimulate the rapid 3-phosphorylation of PtdIns(4,5)P_2 to form PtdIns(3,4,5)P_3

Timeline | **Key events in the field of PI3K research**



AGE-1, ageing alteration protein 1; ARF, ADP-ribosylation factor; BTK, Bruton's Tyr kinase; *C. elegans*, *Caenorhabditis elegans*; *D. discoideum*, *Dictyostelium discoideum*; *D. melanogaster*, *Drosophila melanogaster*; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; FOXO, forkhead box; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; mTORC1, mammalian target of rapamycin complex 1; PDGF, platelet-derived growth factor; PDK1, phosphoinositide-dependent kinase 1; PH, Pleckstrin homology; PI, phosphoinositide; PI3K, phosphoinositide 3-kinase; PMT, polyoma virus middle-T antigen; PtdIns phosphatidylinositol; PTEN, phosphatase and tensin homologue; pTyr, phosphotyrosine; PX, phox; S6K, S6 kinase; SH2, SRC homology 2; TKR,Tyr kinase receptor; TSC2, tuberous sclerosis 2; Tyr, tyrosine; Vps34, vacuolar protein sorting-associated protein 34;

(REFS 12,13), which could be dephosphorylated by the activities of as-yet-unidentified 3- and 5-lipid phosphatases to form PtdIns(4,5)P_2 or PtdIns(3,4)P_2, respectively (FIG. 1). By contrast, the already significant levels of PtdIns(3)P did not change much during equivalent stimulation. This suggested that the *in vitro* 'PtdIns 3-kinase' activity previously found to be associated with receptors and oncoproteins was likely to be a PtdIns(4,5)P_2 3-kinase in cells, responsible for the rapid synthesis of PtdIns(3,4,5)P_3 and, indirectly, PtdIns(3,4)P_2 (REFS 12,13) (FIG. 1). Furthermore, the generation of these two lipids had all the hallmarks of an acute receptor-regulated signalling pathway, with PtdIns(3,4,5)P_3 as the most likely 'second messenger'. The corollary of this conclusion was that there was probably a separate 'housekeeping' PtdIns 3-kinase with distinct functions, which was responsible for PtdIns(3)P synthesis (FIG. 1). This led to the general adoption of the term PI3K for 'phosphoinositide 3-kinase', to refer to any activity capable of phosphorylating one or more inositol lipids at the 3-OH group of the inositol ring *in vitro*, thus encompassing both receptor-activated PI3Ks directed towards PtdIns(4,5)P_2 and non-receptor-activated PI3Ks directed towards PtdIns[14].

Isolation of the PI3K gene family

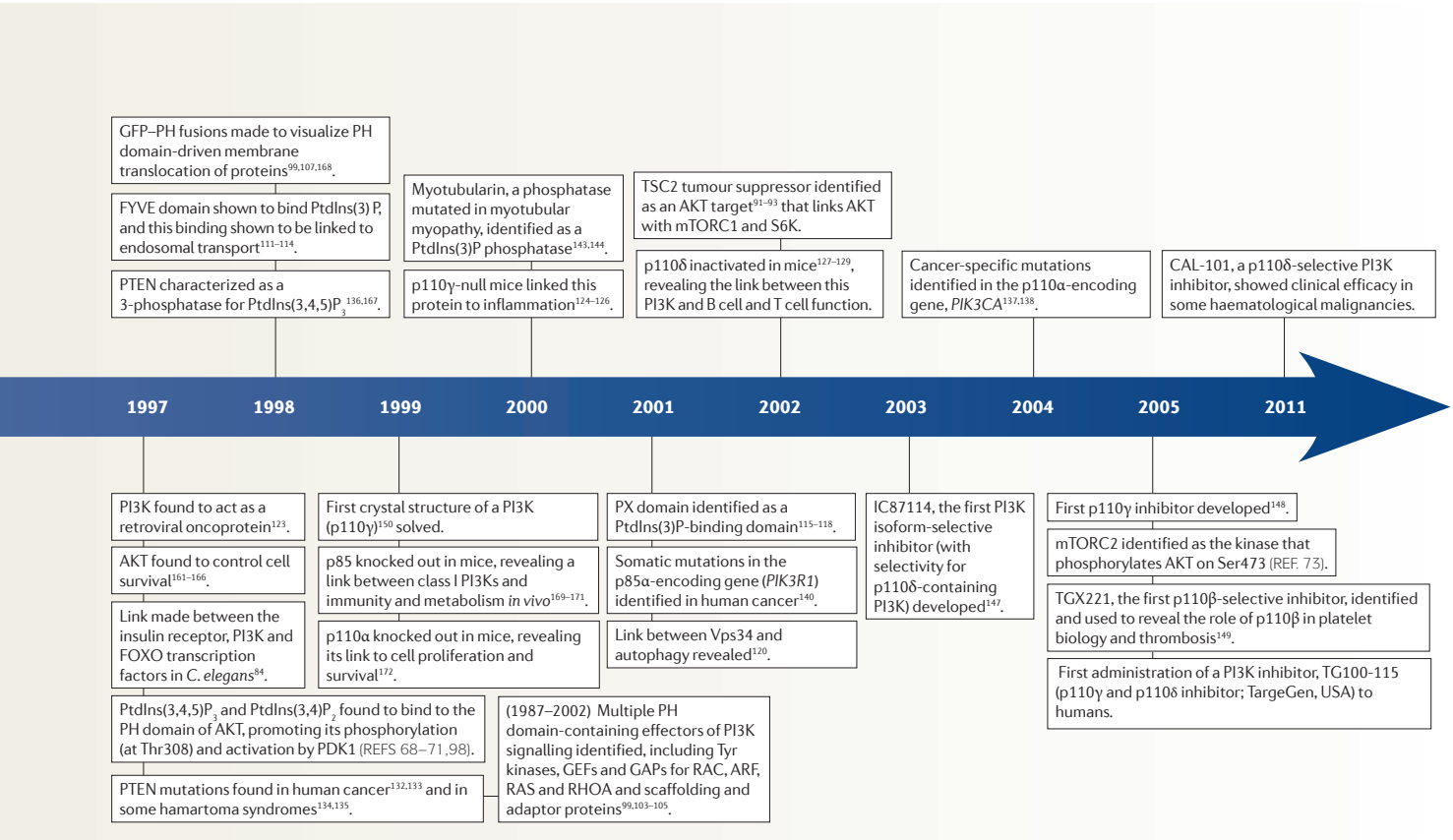
Several groups set out to purify a PI3K from mammalian cells to homogeneity and, in several instances, found that a PI3K activity on chromatography columns correlated with the presence of ~110 kDa (p110) and ~85 kDa (p85) proteins[15–18]. p85 had previously been found to be associated with certain oncoproteins and TKRs[5,19]. Under some circumstances, p85 was also found to be phosphorylated on tyrosine residues[5,19]. This observation made many believe that p85 was the PI3K.

In 1991, cDNA cloning of p85α by three independent groups[20–22] revealed that this protein has no intrinsic PI3K activity but contains an SRC homology 3 (SH3) domain and two SH2 domains. Around the same time, it became clear that SH2 domains recognize pTyr residues in specific peptide contexts. This provided a plausible model for how p85 interacts with pTyr residues on autophosphorylated TKRs, as well as on their direct or indirect substrates, such as the PMT [20–22] (FIG. 2). The role of Tyr phosphorylation on p85 itself is still not clear.

In 1992, protein microsequencing of purified p110 facilitated cloning of the cDNA for the PI3K catalytic subunit p110α[23], formally establishing that p110 carries the PI3K activity in a heterodimeric p85–p110 complex. Further work established regions in which p85 and p110 interact, and suggested a plausible mechanism through which pTyr docking to the SH2 domains of p85 mediates allosteric activation of the complex[24].

The fact that the p85–p110 heterodimer was discovered in the context of growth factor stimulation linked this enzyme to the PtdIns(4,5)P_2-specific PI3K activity. However, cloning of p110α cDNA also revealed a close and unexpected homology with vacuolar protein sorting-associated protein 34 (Vps34), a *Saccharomyces cerevisiae* protein involved in endosomal sorting of proteins towards the vacuole, the yeast equivalent of the mammalian lysosome[25]. Further studies[26] revealed that yeast Vps34 indeed has PI3K activity but with a substrate specificity for PtdIns, rather than PtdIns(4,5)P_2, and that mammalian cells contain PtdIns-specific PI3Ks distinct from the growth factor-sensitive PtdIns(4,5)P_2-specific PI3K[27]. Thus, this work yielded the first clue as to the identity of a PtdIns-specific PI3K activity in cells and its role in vesicular trafficking.

Molecular biology approaches (such as degenerate PCR based on p110α and Vps34 sequence homology), as well as biochemical purification strategies, then allowed the



isolation of genes for multiple PI3K isoforms in mammals, *Caenorhabditis elegans*[28], *Drosophila melanogaster*[29], *Dictyostelium discoideum*[30] and plants. A notable highlight during the isolation of PI3Ks was the finding that the p110γ isoform of PI3K signals downstream of GPCRs[31,32] (FIG. 2). In addition, it was revealed that p110γ does not occur in a complex with p85 but instead interacts with p101, an unrelated regulatory subunit that facilitates the activation of p110γ by Gβγ subunits released upon GPCR activation[33] (FIG. 2). Also, Vps34 was found to be in a heterodimeric complex with a putative protein kinase, namely Vps15 in yeast[34] (known as p150 in mammals) (FIG. 2). Another group of PI3Ks cloned on the basis of sequence homology turned out to be larger monomeric enzymes with a carboxy-terminal C2 domain (therefore called PI3K-C2 kinases[35–39]) (FIG. 2), the *in vivo* lipid substrates of which remain unknown to date, although there is some evidence that they can produce PtdIns(3)P[40] and possibly PtdIns(3,4)P_2 in cells.

This large body of work identified an evolutionarily conserved family of PI3K enzymes, which, on the basis of structural and biochemical characteristics, can be divided into three main classes[41,42] (FIG. 2): class I enzymes are receptor-regulated PtdIns(4,5)P_2 kinases; class II enzymes are PI3K-C2 kinases; and the class III enzyme is the PtdIns-specific enzyme Vps34. Mammals have eight isoforms of PI3K (four class I isoforms, three class II isoforms and one class III isoform). A single representative of each of the three PI3K classes is present in *C. elegans* and *D. melanogaster*, whereas yeast and plants have only a sole class III PI3K.

Early clues to cellular PI3K functions

The almost ubiquitous association of growth factor receptors with PI3K activation, including the discovery that the oncoprotein RAS can also activate this pathway[43–45], suggested an important link between PI3K activation and the regulation of cell growth and proliferation.

A key discovery in 1993–94 was that wortmannin, a mould metabolite with anti-inflammatory activities that was first described in 1974 (REF. 46), is a highly potent, selective and cell-permeable PI3K inhibitor[47–51]. Around the same time, researchers at Eli Lilly generated LY294002, the first synthetic inhibitor of PI3K[52], and made it freely available to the research community. These pharmacological tools, which inhibit all PI3K isoforms (known as pan-PI3K inhibitors), were used in conjunction with other, targeted PI3K inhibition strategies (such as cell-based studies with dominant-negative mutants of p85 (REF. 53), microinjection of neutralizing PI3K-specific antibodies[54], and the expression of mutant receptors that can no longer bind specific cytosolic signalling molecules[55,56], including PI3Ks) to probe for PI3K functions.

These experiments positioned various cellular responses downstream of PI3K signalling, including mitogenesis[56,57], glucose uptake[58,59], actin rearrangements and membrane ruffling[53,60–62], chemotaxis[61], respiratory burst[47] and secretion[48]. This led to an explosion of PI3K research and the identification of many biological processes in which PI3K has a role. The molecular effectors of this pathway, however, remained obscure.

AKT and other PH domain effectors

By the early 1990s, bioinformatics approaches had begun to identify several conserved modular protein domains in signalling proteins. One of these, namely the pleckstrin homology (PH) domain, a loosely conserved modular domain of about 120 amino acids[63,64], would turn out to be common to class I PI3K effectors.

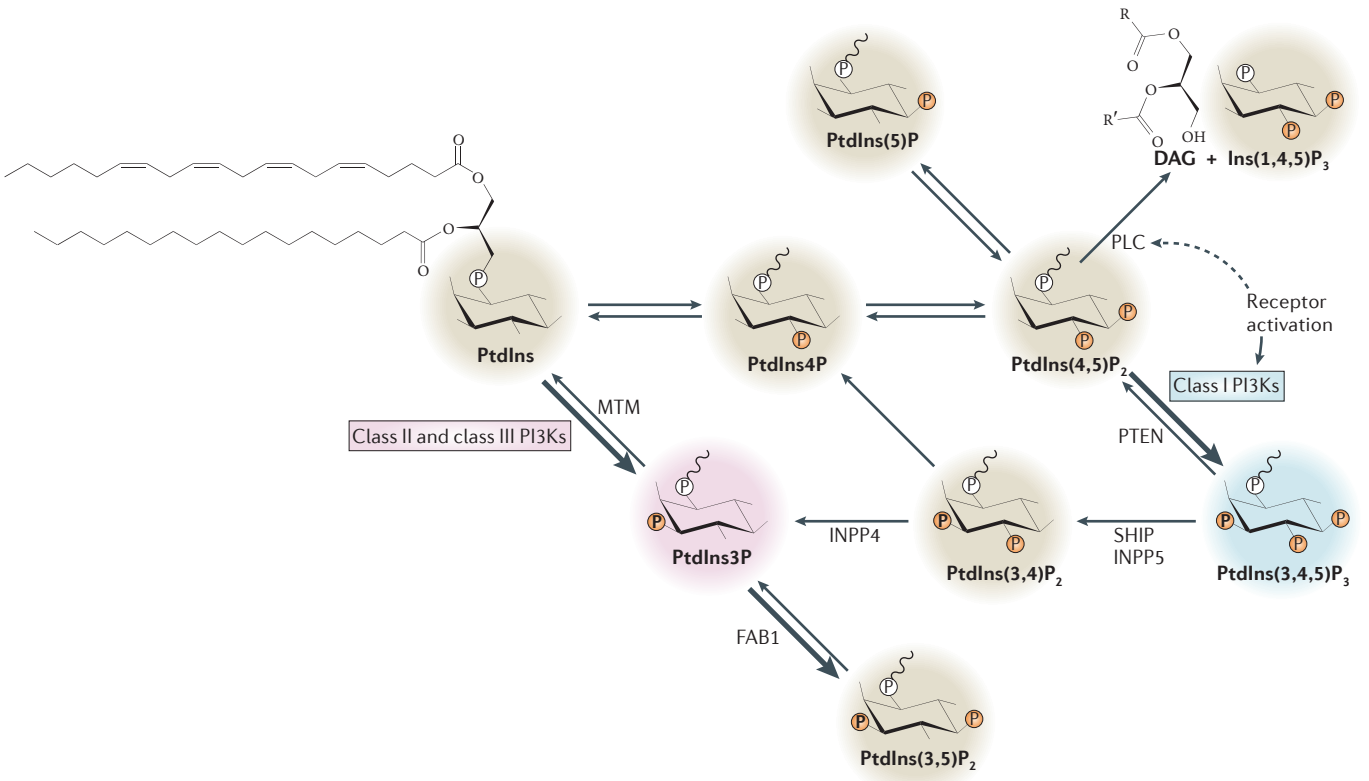


Figure 1 | **Reactions catalysed by PI3Ks in cells.** The main metabolic pathways that interconvert inositol phospholipids in eukaryotic cells are shown. Class I phosphoinositide 3-kinases (PI3Ks) are activated by cell surface receptors to make phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and, indirectly, PtdIns(3,4)P_2; these two lipids are intracellular signals that carry important information through the receptor signalling network. Class III and probably class II PI3Ks make PtdIns(3)P and play a key part in regulatory networks controlling vesicular trafficking through the endosome–lysosome system. DAG, diacylglycerol; FAB1, PtdIns(3)P 5-kinase; INPP5, inositol polyphosphate 5-phosphatase; MTM, myotubularin; PLC, phospholipase C; PTEN, phosphatase and tensin homologue; SHIP, SH2 domain-containing inositol 5′-phosphatase.

AKT as a key PI3K effector. In 1993, a Ser/Thr kinase known as AKT (the mammalian homologue of the retroviral transforming protein v-Akt; AKT is also known as PKB, as it is distantly related to both PKA and PKC) was one of several proteins found to contain a PH domain[63,64]. Furthermore, in the following year, a PH domain was shown to bind PtdIns(4,5)P_2 (REF. 65), raising the prospect that AKT could be a direct effector of phosphoinositide lipids. In 1995, Franke *et al.*, as well as Burgering and Coffer, made the key discovery that AKT can be rapidly activated by growth factors in a PI3K-dependent manner[66,67].

However, the link between PtdIns(3,4,5)P_3 and AKT activation was not simple to elucidate. It was not until 1997 that chemists could synthesize significant quantities of the relevant lipids, and two groups showed in parallel that PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 bind directly to the PH domain of AKT, thereby promoting its phosphorylation on Thr308 by an upstream kinase called phosphoinositide-dependent kinase 1 (PDK1)[68–71] (FIG. 3). Although PI3K-dependent phosphorylation of AKT on Thr308 is the critical step in AKT activation, additional phosphorylation on Ser473 also has a major impact on AKT activity[72]. However, it took another 8 years to identify mammalian target of rapamycin complex 2 (mTORC2) as the kinase complex that phosphorylates this site[73] (FIG. 3).

AKT turned out to have a central role in the regulation of cell function. Several laboratories started to place AKT upstream of other signalling proteins, either by genetic studies (mainly in *C. elegans*) or by biochemical approaches, facilitated by the identification of the consensus sequence for phosphorylation by AKT[74]. The AKT substrates identified in this manner are glycogen synthase kinase 3 (GSK3) in the insulin signalling pathway[75], the pro-apoptotic protein BCL-2 antagonist of cell death (BAD)[76,77], the cell cycle regulators p21 (REF. 78) and p27 (REFS 79–81), and AS160 (AKT substrate of 160 kDa)[82,83], which is a RAB GTPase-activating protein (GAP) that regulates insulin-stimulated exocytosis of glucose transporter type 4 (GLUT4) (FIG. 3). Studies in *C. elegans* further uncovered the functional links between PI3K and forkhead box O (FOXO) transcription factors[84] and between AKT and FOXOs[85], followed by identification of FOXOs as direct AKT substrates in mammalian cells[86–90]. More than 100 AKT substrates have now been identified, one of the most recent ones being the tuberous sclerosis 2 (TSC2) tumour suppressor[91–93], which is a GAP for the monomeric GTPase that antagonizes mTORC1 signalling through the inhibition of RAS homologue enriched in brain (RHEB) (FIG. 3). This work showed that AKT phosphorylates and inhibits TSC2, allowing RHEB to activate mTORC1 such that it can phosphorylate its downstream target, S6 kinase (S6K). The AKT–mTORC1 pathway is now known to regulate cellular growth and autophagy (FIG. 3).

Other PI3K effectors with PH domains. In parallel with the progress in placing AKT downstream of class I PI3Ks, several other molecules were identified in this pathway, such as RAC[94], S6K[95,96] and GSK3 (REFS 75,97). However, it was the emerging

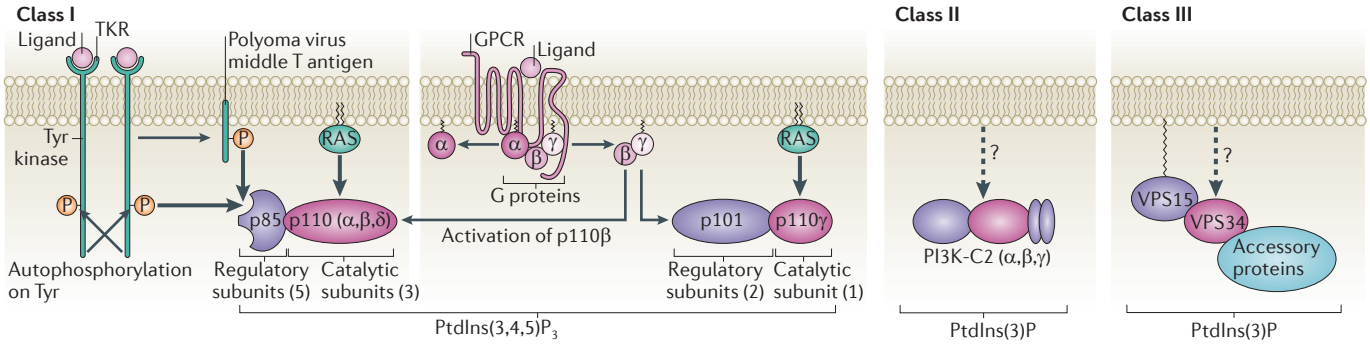


Figure 2 | **The distinct classes of mammalian PI3Ks.** Class I phosphoinositide 3-kinases (PI3Ks) are receptor-regulated phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) kinases that generate PtdIns(3,4,5)P_3. The p110α, p110β and p110δ isoforms of the catalytic subunit are constitutively bound to a p85 regulatory subunit, of which five isoforms exist. All p85 isoforms have SRC homology 2 (SH2) domains, which bind to phosphorylated Tyr (pTyr) in a specific amino acid sequence context in membrane-associated proteins, thereby bringing class I PI3Ks into contact with their lipid substrates. The p110γ isoform does not bind to a p85 subunit but binds to the unrelated p101 or p84 (not shown) regulatory subunits, which link p110γ to Gβγ subunits released from heterotrimeric G proteins downstream of G protein-coupled receptors (GPCRs). p110β is also activated by Gβγ subunits, through a mechanism that is not yet clear. Class II PI3Ks (PI3K-C2α, PI3K-C2β or PI3K-C2γ) do not constitutively associate with regulatory subunits, and activation of these kinases might be relayed through their amino and carboxyl termini, which are extended compared to those of class I and class III PI3Ks to include an additional C-terminal C2 domain and that is the defining feature of this class of PI3K. Class II PI3Ks are thought to produce PtdIns(3)P in cells. The sole class III PI3K is the PtdIns-specific vacuolar protein sorting-associated protein 34 (VPS34), which forms a constitutive heterodimer with the myristoylated VPS15 protein; the VPS15–VPS34 complex is tethered to intracellular membranes. VPS34 is found in distinct multiprotein complexes that define its biological roles.

realization that a subset of PH domains can selectively bind PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2, causing 'translocation' of target proteins from the cytosol to the inner leaflet of the plasma membrane[65], that really defined the modus operandi of this signalling system[98,99]. This work led directly to the identification of several other PH domain-containing effectors in the class I PI3K signalling network, including guanine nucleotide exchange factors (GEFs) and GAPs for small GTPases of the RHO, RAC, RAS and ADP-ribosylation factor (ARF) families, together with several protein kinases and signalling adaptors[99–105] (key examples are listed in FIG. 4). Notably, certain mutations in the PH domain of Bruton's Tyr kinase (BTK) cause X-linked agammaglobulinaemia (XLA), and these mutations were found to correlate with defective PtdIns(3,4,5)P_3 binding[106], providing an example of how the failure of a PH domain to bind PtdIns(3,4,5)P_3 can lead to disease. These mutations also highlighted the importance of conserved basic residues in the PH domain, which allowed the lipid-binding properties of this domain to be investigated in other effectors. Furthermore, the emerging use of fluorescent protein reporters allowed the plasma membrane translocation of these PI3K effectors to be followed in real time with good spatial and temporal resolution[99,107], leading to the discovery that PI3K signalling becomes highly polarized towards the leading edge in motile cells[108,109].

A large number of proteins with PH domains have now been identified (1,689 sequences in mammalian proteins, according to the Sanger Institute Pfam database in May 2011). However, only a small fraction of PH domains bind phosphoinositides, and only a minor subset of these specifically interact with 3-phosphoinositides, with varying selectivity. Nonetheless, current estimates suggest that approximately 20–50 proteins in a typical mammalian cell are regulated, at some level, by direct binding to PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2, although in most cases the extent of this regulation and whether it is driven by translocation and/or conformational activation are still unknown. This complexity clearly explains the wide input of this pathway into several important cellular responses.

PI3K effectors with FYVE or PX domains

In 1998, several years after the demonstration that some PH domains bind 3-phosphoinositides, the FYVE domain[110] was shown to bind PtdIns(3)P in studies that functionally linked inositol lipids to endosomal transport[111–114]. This was followed in 2001 by the identification of the phox (PX) domain as a PtdIns(3)P-binding module in various proteins, including many involved in vesicular transport[115–118]. As with PH domain-containing molecules, the interaction of FYVE and PX domain-containing proteins with membrane-bound PtdIns(3)P can allow their membrane recruitment and biological activity (key examples are listed in FIG. 4), although the roles of these domains in many proteins have not yet been defined. Indeed, many FYVE and PX domain-containing sequences have now been identified (226 and 490, respectively, in mammalian proteins, according to Pfam in May 2011), illustrating the potential diversity of signalling downstream of PtdIns(3)P. However, it should be stressed that the impact of 3-phosphoinositide binding on most of these proteins has not been investigated, and it is possible that, as with PH domains, only a small subset of these FYVE or PX domain-containing proteins are bona fide effectors of 3-phosphoinositide signalling.

Early work implicated PtdIns(3)P produced by Vps34 in several aspects of endosome-to-lysosome trafficking[26], and the identification of individual PtdIns(3)P effectors started to suggest how this lipid is involved in vesicle tethering[111] or protein sorting[119]. In 2001, it was discovered that Vps34, in addition to its role in endosomal trafficking[25], has a role in autophagy[120], with Vps34–Vps15 being present in several distinct complexes (reviewed in REF. 121). It would now seem that PtdIns(3)P is widely used to regulate key proteins in endosomal, lysosomal and phagosomal systems.

Organismal roles of PI3Ks

Following cell-based studies, which implicated PI3K in various signalling pathways and cell biological processes, studies in model organisms uncovered the physiological

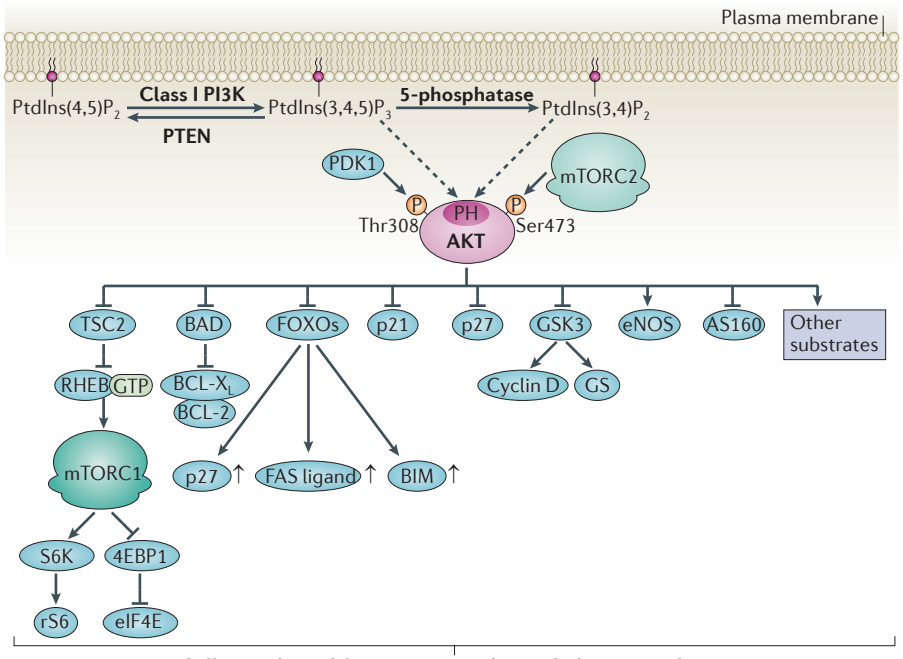


Figure 3 | **AKT as an example of a PI3K effector.** Receptor-stimulated class I phosphoinositide 3-kinases (PI3Ks) generate phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and, indirectly, PtdIns(3,4)P_2, which bind directly to the pleckstrin homology (PH) domain of AKT, driving translocation of AKT to the plasma membrane and allowing its phosphorylation on Thr308 by phosphoinositide-dependent kinase 1 (PDK1). Additional phosphorylation on Ser473 by mammalian target of rapamycin complex 2 (mTORC2) leads to full activation, driving the phosphorylation of a plethora of downstream protein targets, a selection of which is shown. These ultimately regulate cell growth, proliferation, survival, metabolism and autophagy. 4EBP1, eIF4E-binding protein 1; AS160, AKT substrate of 160 kDa; BAD, BCL-2 antagonist of cell death; BIM, BCL-2-interacting mediator of cell death; eIF4E, eukaryotic translation-initiation factor 4E; eNOS, epithelial nitric oxide synthase; FOXO, forkhead box O; GS, glycogen synthase; GSK3, GS kinase 3; GTP, guanosine triphosphate; PTEN, phosphatase and tensin homologue; RHEB, RAS homologue enriched in brain; rS6, ribosomal S6 protein; S6K, S6 kinase; TSC, tuberous sclerosis 2.

functions of PI3Ks. These include control of organismal lifespan by ageing alteration protein 1 (AGE-1; the *C. elegans* paralogue of p110 PI3K[28]); regulation of cell growth and size, rather than cell proliferation, by the p110 PI3K homologue in *Drosophila melanogaster* [122]; and the discovery of PI3K as a retroviral oncoprotein in chicken[123]. Genetic studies in mice revealed roles for p110γ[124–126] and p110δ[127–129] (which are enriched in leukocytes) in inflammation and immunity and a key role for p110α in insulin signalling[130,131].

A major breakthrough in the context of PI3Ks and disease was the realization in 1998 that PTEN (phosphatase and tensin homologue), one of the most frequently inactivated tumour suppressors in many cancers[132,133] and in some hamartoma syndromes[134,135], is a 3-phosphatase for PtdIns(3,4,5)P_3 (REF. 136) (FIG. 1), and PTEN inactivation leads to constitutive activation of the class I PI3K pathway. Equally influential were the reports in 2004 showing that many solid tumours have somatic activating mutations in *PIK3CA*[137,138], the gene encoding p110α. Somatic mutations of the p85α-encoding gene in cancer had been reported several years earlier in mice[139] and in human cancer samples[140]. These data were later validated by human cancer genome sequencing, which additionally uncovered somatic mutations in the genes encoding p85β and p85γ[141,142].

Inactivating mutations were also found in other lipid phosphatases, including myotubularin, which is inactivated in some myotubular myopathies and was shown in 2000 to be a PtdIns(3)P phosphatase[143,144]. Recently, inactivation of inositol polyphosphate 5-phosphatase E (INPP5E), which dephosphorylates PtdIns(3,4,5)P_3, was reported to be involved in ciliopathies[145,146]. In addition, INPP4B has emerged as a potential tumor suppressor in several cancer types[173,174].

PI3K inhibition towards the clinic

Together, the findings described above implicated isoforms or classes of PI3Ks in organismal signalling and disease and provided a rationale for the development of PI3K inhibitors. The first isoform-selective inhibitor to be reported in the public domain, in 2003, was IC87114, which is an ATP-competitive inhibitor with selectivity for p110δ[147]. This was followed by the development of p110γ inhibitors[148] and p110β inhibitors[149], the latter of which were used to identify the role of p110β in platelet biology and thrombosis[149]. The development of PI3K inhibitors was greatly facilitated by the report of the first crystal structure of a PI3K subunit in 1999, namely that of p110γ[150], and more recently by the publication of structures for p110α[151], p110β[152], p110δ[153] and VPS34 (REF. 154).

In 2005 a PI3K inhibitor (TG100-115, a p110γ and p110δ inhibitor from TargeGen) was administered to humans for the first time. Multiple PI3K inhibitors, with varying isoform selectivity, are now progressing through Phase I and Phase II trials for treatment of cancer and inflammation. Early reports released in 2011 indicated that CAL101 (now called GS1101), which is a p110δ-selective PI3K inhibitor, shows promising clinical efficacy in some haematological malignancies.

Concluding remarks

More than 20 years after its emergence from an academic niche, the PI3K pathway has now become a focus of basic, preclinical and clinical research. The discovery of PH, FYVE and PX domain interactions with 3-phosphorylated inositol lipids, and the analogies between these interactions in different PH-, FYVE- or PX-containing proteins, has heralded a new paradigm for understanding the role of phospholipids in cellular regulation. The discovery of germline and somatic mutations in genes encoding PI3K pathway components in humans has underpinned the implication of PI3K in disease. Although much progress has been made in understanding the signalling roles of some PI3K isoforms, many questions about their physiological and pathological roles remain, in particular for the class II and class III PI3Ks (reviewed in REF. 121). The trialing of PI3K inhibitors in human disease is anticipated to increase efforts to address unresolved questions in the field — for example, it is not clear why there seems to be no good correlation between the *PIK3CA* and/or *PTEN* mutation status in cancer cells and the sensitivity of these cells to PI3K inhibitors. It is also surprising that AKT can be inactivated in cancer cells without affecting cell proliferation and survival, indicating the potential importance of AKT-independent PI3K pathways or negative feedback loops. It is anticipated that answering questions such as these will be a

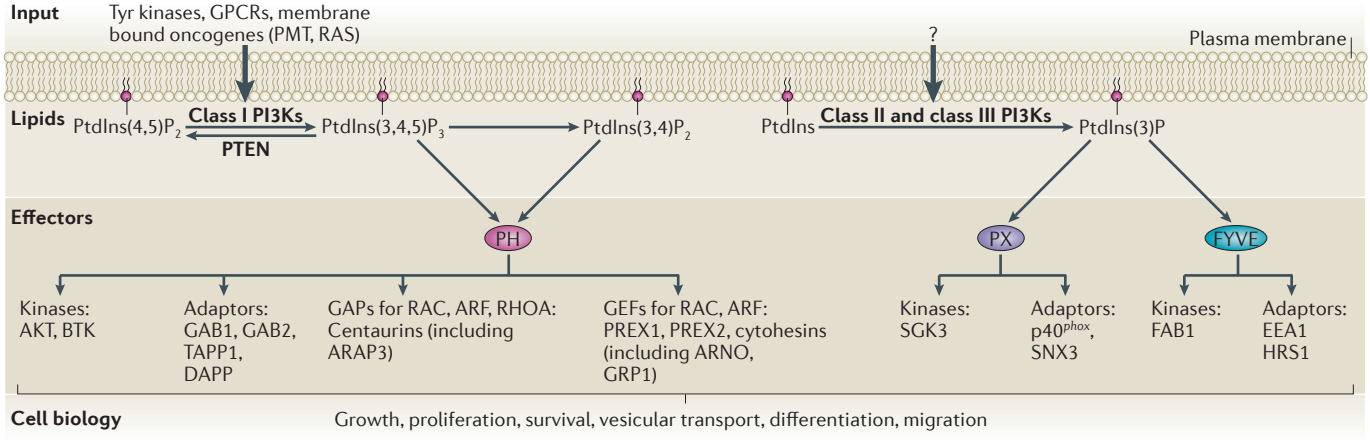


Figure 4 | **The 3-phosphoinositide signalling network.** Phosphatidylinositol -3,4,5 -trisphosphate ($PtdIns(3,4,5)P_3$), $PtdIns(3,4)P_2$ and PtdIns(3)P interact with specific lipid-binding domains in phosphoinositide 3-kinase (PI3K) effector proteins, changing their localization and/or activity, ultimately affecting several important cell biological processes. ARAP3, ARFGAP with RHOGAP domain, ANK repeat and PH domain-containing protein 3; ARF, ADP-ribosylation factor; ARNO, ARF nucleotide-binding site opener; BTK, Bruton's Tyr kinase; DAPP, dual adapter for phosphotyrosine and phosphoinositide; EEA1, early-endosome antigen 1; FAB1, PtdIns(3)P 5-kinase (also known as PIKFYVE); GAB, GRB2-associated binder; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factors; GPCR, G protein-coupled receptor; GRP1, general receptor or phosphoinositides 1; HRS1, hepatocyte-growth factor-regulated Tyr-kinase substrate 1; PMT, polyoma virus middle T antigen; PREX, PtdIns (3,4,5)-dependent RAC exchanger; PTEN, phosphatase and tensin homologue; SGK3, serum and glucocorticoid-regulated kinase 3; SNX3, sorting nexin 3; TAPP1, tandem PH domain-containing protein 1.

fertile ground for discovery in the next decade, hopefully leading to therapeutic benefit in humans.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

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