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Class I PI 3-Kinases: function and evolution

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Abstract: In many human cell types, the class I phosphoinositide 3-kinases play key roles in the control of diverse cellular processes including growth, proliferation, survival and polarity. This is achieved through their activation by many cell surface receptors, leading to the synthesis of the phosphoinositide lipid signal, PIP₃, which in turn influences the function of numerous direct PIP₃-binding proteins. Here we review PI3K pathway biology and analyze the evolutionary distribution of its components and their functions. The broad phylogenetic distribution of class I PI3Ks in metazoa, amoebozoa and choanoflagellates, implies that these enzymes evolved in single celled organisms and were later co-opted into metazoan intercellular communication. A similar distribution is evident for the AKT and Cytohesin groups of downstream PIP₃-binding proteins, with other effectors and pathway components appearing to evolve later. The genomic and functional phylogeny of regulatory systems such as the PI3K pathway provides a framework to improve our understanding of the mechanisms by which key cellular processes are controlled in humans.

Keywords: Phosphoinositide, PI3K, AKT, PTEN, Signal Transduction, Cancer, Kinase, Phosphatase, Evolution, Phylogeny

Introduction:

All cells use a variety of biochemical mechanisms to control their behaviour in response to changes in their environment, whether they are single cells or those making up a large complex organism. The PI 3-kinase/PTEN signalling network is an important biochemical component in this signal transduction process, and which is best recognized as a regulator of cell growth, proliferation and survival in many cell types. A consequence of these functions is that loss of control of PI3K signalling is a frequent characteristic of human disease, particularly many cancers, in which aberrant activation of PI3K itself or loss of the opposing phosphatase, PTEN, are frequently observed events. In this review, we summarize our current understanding of the pathway, analyze the evolutionary distribution of pathway components and relate this knowledge with its functions in human cells.

The Phosphoinositide 3-Kinases (PI3Ks)

Phosphoinositide 3-kinases (PI3Ks) are enzymes which phosphorylate membrane phosphoinositide lipids at the 3-hydroxyl group of the inositol ring (Engelman et al., 2006, Vanhaesebroeck et al., 1997a, Whitman et al., 1988). Their lipid products act as cellular signals, directly regulating diverse lipid-binding effector molecules and thus cellular processes including membrane trafficking, cell growth, proliferation, metabolism, migration and polarity (Katso et al., 2001, Martin-Belmonte et al., 2008, Vivanco and Sawyers, 2002). Based on substrate preference and sequence similarity of the catalytic subunit, PI3Ks are classified in three groups, classes I, II and III (Engelman, 2006, Vanhaesebroeck et al., 2001, Vanhaesebroeck, 1997a, Wymann and Pirola, 1998). Class II PI3Ks are monomeric multi-domain proteins, which most clearly phosphorylate phosphatidylinositol (PtdIns) to produce PtdIns3P and also may generate PI(3,4)P₂ during endocytosis (Posor et al., 2013, Vanhaesebroeck et al., 2012). Class III PI3Ks are heterodimeric proteins, first identified in yeast. Their preferred substrate *in vivo* is PtdIns and they are mainly responsible for the production of most cellular PtdIns3P. Class I PI3Ks are the most heavily studied of all PI3Ks; they can catalyze the phosphorylation of PtdIns, PtdIns4P or PtdIns(4,5)P₂ *in vitro* but their preferred cellular substrate is PtdIns(4,5)P₂, leading to the synthesis of PtdIns(3,4,5)P₃, often known as PIP₃. Class I PI3K activity can be stimulated rapidly (seconds) by extracellular signals through membrane receptors including RTKs (Receptor Tyrosine Kinases) and GPCRs (G-Protein coupled receptors) and has been heavily implicated in oncogenic transformation (Yuan and Cantley, 2008).

Class I PI3Ks

Class I PI3Ks are heterodimers, which consist of a catalytic subunit, p110 (four variants in humans, p110 α , p110 β , p110 δ and p110 γ) and one of a range of regulatory subunits (p85 α , p85 β , p55 α , p50 α , p55 γ , p101 or p84) (Beck et al., 2014, Engelman, 2006, Stephens and Hawkins, 2011, Vanhaesebroeck, 1997a). Human Class I PI3Ks are further divided into two subclasses based on their subunit composition and mode of activation. In the Class IA enzymes, (p110 α , p110 β and p110 δ , encoded by three different genes, PIK3CA, PIK3CB and PIK3CD respectively), the p110 subunit binds with an SH2 domain-containing regulatory subunit (p85 α , p85 β or p55 γ , encoded by PIK3R1, PIK3R2 and PIK3R3 respectively, or p55 α and p50 α , splice variants of PIK3R1). These phospho-tyrosine binding SH2 domains allow regulation by Receptor Tyrosine Kinases, although p110 β can be activated by either RTKs or G Protein Coupled Receptors (Maier et al., 1999, Tang and Downes, 1997). In contrast, class IB PI3K (p110 γ) has a catalytic p110 subunit which binds to a p101 or p84 regulatory subunit and is activated by GPCRs. All of the p110 isoforms appear to be activated by direct interaction with small GTPases; in the case of p110 α , γ and δ , this appears to be RAS isoforms, whereas p110 β appears to be co-activated by RAC (Fritsch et al., 2013). Comparing genomic data implies evolutionary conservation of the PI3Ks, showing that one class I PI3K gene is present in *D. melanogaster*, *C. elegans* and three in *D. discoideum* (Engelman, 2006). However, the functional expansion provided by the four catalytic subunits appears to be restricted to vertebrates and some other chordates (Brown and Auger, 2011, Kawashima et al., 2003).

Class I PI3Ks are multi-domain proteins. Although all class I catalytic subunits are encoded by separate genes they share a similar domain organization, with, in order from the N-terminus, a p85 binding or Adapter binding domain (ABD specifically conserved in Class IA p110 α , β and δ), a Ras binding domain (RBD), a C2 domain, a helical domain (PIK) and a catalytic kinase domain at the C-terminus. The regulatory subunits of class IA all have a common core structure consists of p110 binding domain (also termed the iSH2) flanked by two Src-homology 2 (SH2) domains which bind to phospho-tyrosine residues in activated RTKs or adapter proteins and are thereby responsible for translocation to and activation of the PI3K holoenzyme at the plasma membrane (Burke and Williams, 2013, Vanhaesebroeck, 2012, Yu et al., 1998). The longer variants i.e. p85 α and p85 β , also contain an N-terminal SH3 domain and a BCR-homology (BH) domain with potential GTPase activating activity, flanked by two proline rich regions (Fruman et al., 1998).

Isoform specific PI3K signalling

The existence and differential expression of functionally diverged catalytic and regulatory subunits in different tissues or cell types suggests isoform specific signalling. p110 α , p110 β , p85 α and p85 β seem to be present ubiquitously with little evidence for selective heterodimeric pairing, but with some variation in levels of expression. p55 γ has been reported to be restricted to liver cells, however expression of p110 γ , p110 δ , p101 and p84 is most evident in blood cells (Stephens and Hawkins, 2011, Vanhaesebroeck, 2001, Vanhaesebroeck et al., 1997b). The generation of isoform specific knock out (KO) mice, kinase dead mutant knock-in (KI) mice, work using microinjection of isoform specific anti-catalytic antibodies and isoform specific inhibitors has given much more insight into isoform specific signalling which has been reviewed well (Vanhaesebroeck et al., 2010a). Briefly, full deletion of either p110 α or p110 β leads to embryonic lethality and both enzymes appear to play important and distinctive roles in growth and development (Jia et al., 2008, Vanhaesebroeck, 2010a). Functional loss of p110 γ or δ on the other hand leads to impaired immune functions and has motivated the targeting of these enzymes for inflammatory disease (Hirsch et al., 2000, Okkenhaug et al., 2002, Sasaki et al., 2000).

Phosphoinositides: ubiquitous eukaryotic signals

PtdIns and its phosphorylated derivatives, including the lipid products of PI3Ks (class I, II and III) or other classes of PI kinases, are collectively called phosphoinositides (PIs) and are found throughout animals, plants, fungi and other eukaryotes that have been investigated (Michell, 2008). Structurally PIs are amphipathic molecules composed of D-*myo* inositol 1-phosphate polar head group, which faces the cytosolic side of the cell, attached via its phosphate group to diacylglycerol (DAG) which sits within the lipid bilayer, including two fatty acid chains which in mammals are most commonly found to correspond to arachidonic acid and stearic acid. The inositol head group can be phosphorylated at the 3rd, 4th or 5th hydroxyl group, whereas the 2nd and 6th positions of inositol ring have not been known to become phosphorylated in phosphoinositide lipids. In metazoans, PIP₃, the lipid product of class I PI3Ks is known to act as an indispensable signal regulating diverse cellular processes with implications in both health and diseases. However, in yeast, although production of PIP₃ has been reported in *S. pombe* (Mitra et al., 2004) it appears to be detrimental to growth of *S. cerevisiae* (Rodriguez-Escudero et al., 2005). However other phosphoinositides including the class I PI3K substrate PI(4,5)P₂, appear to play important roles throughout the eukaryotes, including regulating cell

division and polarity in budding yeast (Bertin et al., 2010, Strahl and Thorner, 2007).

Interpreting the language of lipids: PH domain containing PI3K effectors

The class I PI3Ks influence cellular behavior through a diverse group of PIP₃-binding effector proteins (Fig. 1), in humans numbering at least 30 and possibly as many as 100. These effector proteins contain a lipid-binding domain in their structure with selective affinity for PIP₃, and in some cases causing bulk translocation of the protein onto the plasma membrane when PIP₃ levels rise. The most common lipid binding domain among various downstream effector proteins is the Pleckstrin Homology (PH) domain. The function most commonly attributed to PH domains has been this selective binding to phosphoinositide lipids (Haslam et al., 1993, Mayer et al., 1993), but importantly, analyses suggest that most do not possess this ability and play other roles (Lemmon, 2008, Yu et al., 2004). PH domains are evolutionary conserved (Lemmon, 2008) and approximately 120 amino acid long with, in the human genome, 323 examples of the domain reported in 276 different independent gene products (Leslie et al., 2012) but it is strongly expected that most have functions unrelated to phosphoinositides. The PH domains that have been shown to function in binding PIP₃ include the serine threonine kinases AKT and PDK1, protein tyrosine kinases of Bruton's tyrosine kinases (BTKs) and Tec family, the Cytohesins (GRP1/ARNO), and further more diverse GEFs (Guanine nucleotide Exchange Factors) and GAPs (GTPase Activating Proteins) for GTPases of the ras superfamily (Leslie, 2012, Park et al., 2008, Vanhaesebroeck, 2012).

The AKT and PDK1 kinases

The most heavily studied downstream mediators of PI3K function are the AKT kinases, also known as PKB (Protein Kinase B) which belong to the AGC family of Serine/Threonine kinases. They are activated in response to increased PIP₃ levels by two stable phosphorylation events. First, their PH domain-mediated translocation to the plasma membrane upon stimulated production of PIP₃ brings about a conformational change in the kinase. This in turn allows phosphorylation at the T308 position (numbering from human AKT1) in the activation loop by another PH domain-containing PI3K effector and protein kinase PDK1 (Alessi et al., 1997, Stephens et al., 1998) and at S473 position in the hydrophobic region of the C-terminal regulatory domain by TORC2 (Fayard et al., 2005, Sarbassov et al., 2005) which stabilizes the active conformation (Yang et al., 2002). Once activated, AKT can move into the cytoplasm or nucleus and phosphorylates a large number of substrates, leading to regulation of diverse cellular processes (Manning and Cantley, 2007, Toker and Marmiroli, 2014). In contrast to AKT, PDK1 is often

considered a constitutively active enzyme and it also activates several kinases other than AKT. Accordingly, the higher affinity of its PH domain for PIP₃ combined with its ability to bind to PtdSer ensure that a significant fraction of cellular PDK1 is located at the plasma membrane even in cells with very low levels of PIP₃ (Lucas and Cho, 2011, Mora et al., 2004).

There is strong evidence from genetics in mice, flies and worms and extensive characterization of signalling pathways in mammalian cells showing the key role of the AKT kinases in mediating many of the cellular effects of PI3K activation, particularly those on cell growth, proliferation and metabolism (Manning and Cantley, 2007, Toker and Marmiroli, 2014). However, many effects of PI3K on other processes, particularly migration and polarity, appear to be AKT independent (Charest and Firtel, 2007, Leslie et al., 2008, Liu et al., 2004). Perhaps the strongest evidence for the importance of AKT in mediating effects downstream of PI3K/PTEN is the demonstrated rescue of lethality caused by PTEN loss in drosophila that can be achieved with a mutation in dAKT which reduces the affinity of its PH domain for PIP₃ and which supports viability despite highly elevated PIP₃ levels (Stocker et al., 2002). However, flies appear to have far fewer PIP₃-binding PH domains than mammals (Park, 2008) and the relative significance of AKT downstream of PI3K/PTEN in humans is controversial, particularly given the commitment put into developing drugs targeting the AKT/TOR signalling axis to treat cancers with activated PI3K or lacking PTEN.

The Cytohesin family of ARF-GAP proteins

The established PH domain containing PIP₃-binding proteins also include the cytohesin family of proteins, consisting of four members: cytohesin-1, cytohesin-2 (ARNO, Arf nucleotide binding site opener), cytohesin-3 (GRP1, general receptor for phosphoinositides) and cytohesin-4. All the members of the cytohesin family contain a N-terminal coiled coil (CC) domain, a central Sec7 domain and a C-terminal PH domain. The Sec7 domain possesses a highly conserved Arf-GEF function which mediates the activation of Arf GTPases (Chardin et al., 1996). All the cytohesin protein PH domains appear to mediate recruitment to the plasma membrane and thus can allow regulation by PI3K (Klarlund et al., 1997, Nagel et al., 1998, Venkateswarlu et al., 1998).

Other PI3K effectors

Many further class I PI3K effector proteins, such as FGD6, were identified by functional and/or bioinformatic screening of PH-domains (Dowler et al., 2000, Park, 2008). However some unbiased proteomic approaches to identify other phosphoinositide lipid binding domains have been highly successful, leading to the discovery of further PH domain containing proteins, such as the ARAP

proteins (Jungmichel et al., 2014, Krugmann et al., 2002), and others, with apparently novel PIP₃ and PI(3,4)P₂ binding domains (Dixon et al., 2011, Dixon et al., 2012, Lee et al., 2005, Lemmon, 2008, Premkumar et al., 2010).

Phosphoinositide phosphatases

The major cellular lipid product of class I PI3Ks, PIP₃ is metabolized by various 3-, and 5- phosphatases at these positions of the inositol ring respectively. A major antagonist of PI3K signaling is the 3-phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) which converts PIP₃ back to its more abundant precursor PI(4,5)P₂ (Cantley and Neel, 1999, Downes et al., 2001, Stambolic et al., 1998). The 5-phosphatases SHIP1/2 (and probably also SHIP) also metabolize PIP₃, converting it to the alternate signal PI(3,4)P₂ which itself contributes to mediating the outcomes of PI3K activation (Leslie, 2012). The contribution of PI(3,4)P₂ in mediating the outcomes of PI3K activation is currently unclear but some evidence, such as the tumour suppressor status of INPP4B, a 4-phosphatase which dephosphorylates PI(3,4)P₂ (Gewinner et al., 2009), implies important roles.

PTEN: a phosphoinositide 3-phosphatase and tumour suppressor

In 1997, two independent research groups first identified PTEN as a candidate tumor suppressor (Li et al., 1997, Steck et al., 1997). It exists predominantly as a 403 amino acid protein as well as a very interesting but only recently identified 576 amino acid secreted isoform, is a member of PTP (Protein Tyrosine Phosphatase) family and in vitro has phosphatase activity against both protein and lipid substrates (Hopkins et al., 2013, Worby and Dixon, 2014). However, its principle substrate appears to be PIP₃ (McConnachie et al., 2003, Salmena et al., 2008). A key determinant of the tumour suppressor activity of PTEN seems to be that under normal cellular conditions, the PTEN protein pool has substantial constitutive activity keeping PIP₃ levels tightly regulated and maintaining homeostasis (Leslie and Foti, 2011, Myers et al., 1998, Stambolic, 1998). The *PTEN* gene has long been known to be the subject of mutation and deletion in cancer, causing loss of tumour suppressor function (Bonneau and Longy, 2000, Salmena, 2008). However, PTEN levels and its activity are controlled at the transcriptional, translational and post-translational levels (Bertrand et al., 2014, Leslie, 2008) and significantly, evidence has more recently accumulated showing that the physiological mechanisms that control PTEN activity are themselves dysregulated in cancer, leading indirectly to loss of PTEN function (Leslie and Foti, 2011, Poliseno et al., 2010, Silva et al., 2008).

5-phosphatase SHIP proteins

The phosphoinositide 5-phosphatases are a large family in mammals of 10 Mg²⁺-dependent phosphatases including OCRL, INPP5E, SKIP, SHIP1 and SHIP2. Many of them are catalytically active and capable of metabolizing PIP₃ (Astle et al., 2007, Ooms et al., 2009), however SHIP1 (hematopoietic restricted) and SHIP2 (more widely expressed) have most clearly been demonstrated to regulate PI3K-dependent signalling in cells. Unlike PTEN which appears to provide constant activity, the activity of both SHIP1 and SHIP2 is increased by tyrosine phosphorylation and regulated membrane recruitment (Batty et al., 2007, Rohrschneider et al., 2000). The large 145kDa SHIP proteins contain a N-terminal SH2 domain, which helps bind SHIP to tyrosine phosphorylated forms of Shc, SHP-2, Doks, certain ITIMs (immunoreceptor tyrosine based inhibitory motifs) and ITAMs (immunoreceptor tyrosine based activation motifs). In addition, SHIP proteins contain a centrally located catalytic phosphatase domain, followed by a C2 domain, potentially involved in allosteric regulation by PtdIns(3,4)P₂ and two NPXY motifs and four PxxP motifs at C-terminus which are involved in protein-protein interactions (Dyson et al., 2012, Rohrschneider, 2000).

The evolution of the Class I PI 3-Kinase signalling network

Class I PI 3-Kinases were discovered and characterized in vertebrates acting at the inner leaflet of the plasma membrane, allowing the cells of these multicellular organisms to interpret intercellular signals such as hormones, growth factors and neurotransmitters (Downes and Carter, 1991, Parker and Waterfield, 1992). Since then, the roles of these PI3Ks in the regulation of cell proliferation, growth and survival in response to intercellular communication has been intensely studied and conserved functions in invertebrates such as flies and worms have been established (Engelman, 2006). However, genome sequence analysis can help identify key components of the signalling network, including class I PI3K enzymes themselves, in many single celled organisms. The results imply that the network must have existed in a single celled common ancestor in which it fulfilled somewhat different functions. Notably, in animals, the identification of localised pools of PIP₃ in the leading edge of cells such as neutrophils undergoing chemotaxis, in axonal growth cones during neuronal polarization and in the basolateral membranes of some
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 and Downward, 1999, Weiner, 2002). Similar polarization of PI3K signalling has been observed during directed cell migration in the protozoan Dictyostelium discoideum (Charest and Firtel, 2006, Iijima et al., 2002, Weiner, 2002). Therefore, it is tempting to speculate that the ancestral role for

class I PI3K may perhaps relate to chemotaxis and feeding and the linked remodeling of metabolism in response to nutrient availability. Here we present and discuss an analysis of recent available genome sequence information.

Eukaryotic conservation of PI kinase and phosphatases

The genomes of most groups of single celled organisms and perhaps all multicellular organisms contain both phosphoinositide kinases with similarity to the class I PI3Ks and cysteine dependent phosphoinositide phosphatases related to PTEN. These extensively conserved enzymes are most similar to the related human phosphoinositide 4-kinases and class III PI 3-kinases (the latter also known as PIK3C3 or VPS34) and to the Myotubularin and Sac groups of PI phosphatases. In terms of apparent evolutionary distance from humans, each of the enzyme groups is observed in most eukaryotic genomes, including fungi, amoebozoa, plants, the chromalveolata group (including alveolates and stramenopiles and rhizaria) and excavates such as giardia.

Enzymes of PIP₃ synthesis and metabolism

Class I PI3K catalytic subunits sequentially contain a Ras-binding domain, a C2 domain and a C-terminal PI-kinase domain and proteins with this domain structure and sequence similarity closest to class I enzymes are found throughout many unikonts, including amoebozoa, fonticula, choanoflagellates and animals, with the notable exception of the fungi. Functional studies provide confidence that these unikont enzymes represent true class I PI3Ks (Clark et al., 2014) supported by phylogenetic analyses (Brown and Auger, 2011) and are shown in Fig. 2. Class I PI3Ks synthesize PIP₃ from PI(4,5)P₂ and are related to the class II PI3K enzymes which synthesize PI3P and in some cases PI(3,4)P₂ (Posor, 2013, Vanhaesebroeck, 2012). However, the Class I and II PI3K catalytic subunits display extensive structural similarity, with the class II enzymes also sequentially containing a Ras-binding domain, a C2 domain and a C-terminal PI-kinase domain, making the two groups difficult to distinguish from amino acid sequence without functional data. In more distantly related phylogenetic groups, although such class I/II PI3Ks appear to be absent from prokaryotes, from many excavates and stramenopiles and from almost all plants, they are present in many other diverse eukaryotic groups. These PI3Ks are found in several parasitic excavates such as kinetoplastid trypanosomes, trichomonas, naegleria and giardia (Cox et al., 2006) and examples are also identifiable in the genomes of several alveolates (eg *Tetrahymena thermophila*, XP_001022838; *Ichthyophthirius multifiliis*, XP_004025156; *Paramecium tetraurelia*, XP_001451414) and a few stramenopiles (eg. *Albugo candida*, CCI1092; *Phytophthora infestans*, XP_002905219). Without functional data, their firm functional classification into class I or class II is difficult, but comparisons with

class I enzyme sequences including *Dictyostelium discoideum* PI3Ks finding modestly greater similarity to these divergent eukaryote PI3Ks and the acutely regulated synthesis of PIP₃ in *Dictyostelium* (Clark, 2014) imply that class I PI3K signalling may be more broadly distributed than previously proposed. It is difficult to distinguish whether this broad distribution of Class I/II enzymes in more distant eukaryotes indicates loss from specific groups (as seems likely for the fungi) or (perhaps less likely given the diversity of groups identified) gain by horizontal transfer into specific groups such as the excavate parasites (See Fig. 2).

The Class IA regulatory subunits with some similarity to p85, defined here by tandem phosphotyrosine-binding SH2 domains and p110-binding inter-SH2 region, are only evident in animals and close relatives, with the most evolutionarily distant protein with this retained domain architecture currently identified in the choanoflagellate *Salpingoeca rosetta*. This is in accordance with the distribution of tyrosine kinase based regulatory systems which are absent from many other eukaryotic groups. The expansion of the catalytic p110 gene family into class 1A and 1B enzymes is evident only in the vertebrates and other chordates (eg *Ciona intestinalis* (Kawashima, 2003)), coinciding with the appearance of the class 1B adaptors p101 and p87 which lack clear relatives in nematodes or insects.

Phosphatases similar to the human PIP₃ 3-phosphatase, PTEN, sharing its tightly associated Phosphatase-C2 domain architecture are found throughout eukaryotes, including fungi, plants, stramenopiles, alveolates and rhizaria and euglenozoa. Since some of these organisms lack class I PI3Ks, it would appear that some PTEN-like enzymes may function to utilize an alternate substrate, or possibly to ensure PIP₃ produced by other routes does not accumulate, as in some organisms, such as *Saccharomyces cerevisiae*, PI3K activity is strongly deleterious (Rodriguez-Escudero, 2005). On this point it is also relevant that in chordate genomes, the closest protein relative of PTEN, VSP/TPIP is able to metabolise several phosphoinositides, acting both as a 3- and 5-phosphatase (Kurokawa et al., 2012, Walker et al., 2001). Additionally, the SHIP group of PIP₃ 5-phosphatases, including an N-terminal SH2 domain, show conservation within animals, choanoflagellates and the related *Capsaspora*, but are not evident in other groups (most of which lack phosphotyrosine based signalling mechanisms). Given the existence of many phosphoinositide 5-phosphatases with substrates other than PIP₃, it is difficult from sequence analysis to predict whether other phosphotyrosine-independent PIP₃ 5-phosphatases may function in more distantly related organisms.

Functional conservation of PIP₃ signalling – the evolution of PIP₃-binding proteins

Attempts to analyse the evolutionary diversity of PIP₃-binding proteins are not simple, as the family of domains most commonly known to function as PIP₃-binders, the pleckstrin homology (PH) domains, also contains many examples that lack this capability and in some cases bind to other lipids. In the absence of solid functional data, prediction of binding characteristics has proved difficult. Therefore, in analyses based on simple sequence similarity and retained domain architecture, both the AKT and Cytohesin groups of PIP₃-binding effector proteins show a similar evolutionary distribution to the class I PI3K enzymes in unikonts but not fungi, implying highly conserved functions for these proteins (Fig. 3). Although these conserved functions are well recognized for the AKT kinases (Manning and Cantley, 2007, Paradis and Ruvkun, 1998, Stocker, 2002), the functions of the cytohesins are less clearly defined.

Proteins structurally related to the human PIP₃ binding kinase PDK1 are found in many more distant groups of eukaryotes, including plants and fungi which lack class I PI3Ks. However the recognized binding of the PDK1 PH domain to phosphatidylserine (Lucas and Cho, 2011) and the enzyme's role phosphorylating and activating many PIP₃-independent protein kinases implies PI3K-independent conserved functions. Similarly, the DH-PH domain pair, shared by several PIP₃-binding Guanine nucleotide exchange factors for the Rac/cdc42 group of GTPases, is also found in many other proteins without apparent PIP₃-binding capability and is found in many branches of eukaryotes.

The apparent evolutionary conservation of PI3K pathway components seems likely to reflect conserved functions for these proteins. Studies in invertebrate model organisms, particularly flies and worms, have already contributed greatly to our understanding of the regulation of cell growth, proliferation and movement but further fundamental questions remain unanswered regarding the regulation of these processes and the roles of the PI3K network. Further motivation for such studies is provided by the availability of drugs to inhibit specific PI3K isoforms and some downstream PI3K-activated proteins such as AKT and TOR (Martelli et al., 2014, Vanhaesebroeck et al., 2010b). A deeper understanding of the underlying biology should help greatly in the successful trialing and application of these new therapies.

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References:

- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, et al. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B. *Curr Biol.* 1997;7:261-9.
- Astle MV, Horan KA, Ooms LM, Mitchell CA. The inositol polyphosphate 5-phosphatases: traffic controllers, waistline watchers and tumour suppressors? *Biochemical Society symposium.* 2007:161-81.
- Batty IH, van der Kaay J, Gray A, Telfer JF, Dixon MJ, Downes CP. The control of phosphatidylinositol 3,4-bisphosphate concentrations by activation of the Src homology 2 domain containing inositol polyphosphate 5-phosphatase 2, SHIP2. *Biochem J.* 2007;407:255-66.
- Beck JT, Ismail A, Tolomeo C. Targeting the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway: an emerging treatment strategy for squamous cell lung carcinoma. *Cancer treatment reviews.* 2014;40:980-9.
- Bertin A, McMurray MA, Thai L, Garcia G, 3rd, Votin V, Grob P, et al. Phosphatidylinositol-4,5-bisphosphate promotes budding yeast septin filament assembly and organization. *Journal of molecular biology.* 2010;404:711-31.
- Bertrand FE, McCubrey JA, Angus CW, Nutter JM, Sigounas G. NOTCH and PTEN in prostate cancer. *Advances in biological regulation.* 2014;56:51-65.
- Bonneau D, Longy M. Mutations of the human PTEN gene. *Hum Mutat.* 2000;16:109-22.
- Brown JR, Auger KR. Phylogenomics of phosphoinositide lipid kinases: perspectives on the evolution of second messenger signaling and drug discovery. *BMC evolutionary biology.* 2011;11:4.
- Burke JE, Williams RL. Dynamic steps in receptor tyrosine kinase mediated activation of class IA phosphoinositide 3-kinases (PI3K) captured by H/D exchange (HDX-MS). *Advances in biological regulation.* 2013;53:97-110.
- Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A.* 1999;96:4240-5.
- Chardin P, Paris S, Antonny B, Robineau S, Beraud-Dufour S, Jackson CL, et al. A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. *Nature.* 1996;384:481-4.
- Charest PG, Firtel RA. Feedback signaling controls leading-edge formation during chemotaxis. *Curr Opin Genet Dev.* 2006;16:339-47.
- Charest PG, Firtel RA. Big roles for small GTPases in the control of directed cell movement. *Biochem J.* 2007;401:377-90.
- Clark J, Kay RR, Kielkowska A, Niewczas I, Fets L, Oxley D, et al. Dictyostelium uses ether-linked inositol phospholipids for intracellular signalling. *The EMBO journal.* 2014;33:2188-200.
- Cox SS, van der Giezen M, Tarr SJ, Crompton MR, Tovar J. Evidence from bioinformatics, expression and inhibition studies of phosphoinositide-3 kinase signalling in *Giardia intestinalis*. *BMC microbiology.* 2006;6:45.
- Dixon MJ, Gray A, Boisvert FM, Agacan M, Morrice NA, Gourlay R, et al. A screen for novel phosphoinositide 3-kinase effector proteins. *Mol Cell Proteomics.* 2011;10:M110 003178.
- Dixon MJ, Gray A, Schenning M, Agacan M, Tempel W, Tong Y, et al. IQGAP proteins reveal an atypical phosphoinositide (aPI) binding domain with a pseudo C2 domain fold. *J Biol Chem.* 2012;287:22483-96.
- Dowler S, Currie RA, Campbell DG, Deak M, Kular G, Downes CP, et al. Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *Biochem J.* 2000;351:19-31.

- Downes CP, Bennett D, McConnachie G, Leslie NR, Pass I, MacPhee C, et al. Antagonism of PI 3-kinase-dependent signalling pathways by the tumour suppressor protein, PTEN. *Biochem Soc Trans.* 2001;29:846-51.
- Downes CP, Carter AN. Phosphoinositide 3-kinase: a new effector in signal transduction? *Cell Signal.* 1991;3:501-13.
- Dyson JM, Fedele CG, Davies EM, Becanovic J, Mitchell CA. Phosphoinositide phosphatases: just as important as the kinases. *Sub-cellular biochemistry.* 2012;58:215-79.
- Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet.* 2006;7:606-19.
- Fayard E, Tintignac LA, Baudry A, Hemmings BA. Protein kinase B/Akt at a glance. *Journal of cell science.* 2005;118:5675-8.
- Fritsch R, de Krijger I, Fritsch K, George R, Reason B, Kumar MS, et al. RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell.* 2013;153:1050-63.
- Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Annual review of biochemistry.* 1998;67:481-507.
- Gewinner C, Wang ZC, Richardson A, Teruya-Feldstein J, Etemadmoghadam D, Bowtell D, et al. Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell.* 2009;16:115-25.
- Haslam RJ, Koide HB, Hemmings BA. Pleckstrin domain homology. *Nature.* 1993;363:309-10.
- Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, et al. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science (New York, NY).* 2000;287:1049-53.
- Hopkins BD, Fine B, Steinbach N, Dendy M, Rapp Z, Shaw J, et al. A secreted PTEN phosphatase that enters cells to alter signaling and survival. *Science (New York, NY).* 2013;341:399-402.
- Iijima M, Huang YE, Devreotes P. Temporal and spatial regulation of chemotaxis. *Developmental cell.* 2002;3:469-78.
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, et al. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature.* 2008;454:776-9.
- Jungmichel S, Sylvestersen KB, Choudhary C, Nguyen S, Mann M, Nielsen ML. Specificity and commonality of the phosphoinositide-binding proteome analyzed by quantitative mass spectrometry. *Cell reports.* 2014;6:578-91.
- Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol.* 2001;17:615-75.
- Kawashima T, Tokuoka M, Awazu S, Satoh N, Satou Y. A genomewide survey of developmentally relevant genes in *Ciona intestinalis*. VIII. Genes for PI3K signaling and cell cycle. *Development genes and evolution.* 2003;213:284-90.
- Klarlund JK, Guilherme A, Holik JJ, Virbasius JV, Chawla A, Czech MP. Signaling by phosphoinositide-3,4,5-trisphosphate through proteins containing pleckstrin and Sec7 homology domains. *Science.* 1997;275:1927-30.
- Krugmann S, Anderson KE, Ridley SH, Risso N, McGregor A, Coadwell J, et al. Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. *Molecular cell.* 2002;9:95-108.
- Kurokawa T, Takasuga S, Sakata S, Yamaguchi S, Horie S, Homma KJ, et al. 3' Phosphatase activity toward phosphatidylinositol 3,4-bisphosphate [PI(3,4)P₂] by voltage-sensing phosphatase (VSP). *Proc Natl Acad Sci U S A.* 2012;109:10089-94.
- Lee JS, Kim JH, Jang IH, Kim HS, Han JM, Kazlauskas A, et al. Phosphatidylinositol (3,4,5)-trisphosphate specifically interacts with the phox homology domain of phospholipase D1 and stimulates its activity. *Journal of cell science.* 2005;118:4405-13.

- Lemmon MA. Membrane recognition by phospholipid-binding domains. *Nature reviews Molecular cell biology*. 2008;9:99-111.
- Leslie NR, Batty IH, Maccario H, Davidson L, Downes CP. Understanding PTEN regulation: PIP2, polarity and protein stability. *Oncogene*. 2008;27:5464-76.
- Leslie NR, Dixon MJ, Schenning M, Gray A, Batty IH. Distinct inactivation of PI3K signalling by PTEN and 5-phosphatases. *Advances in biological regulation*. 2012;52:205-13.
- Leslie NR, Foti M. Non-genomic loss of PTEN function in cancer: not in my genes. *Trends Pharmacol Sci*. 2011;32:131-40.
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science (New York, NY)*. 1997;275:1943-7.
- Liu H, Radisky DC, Wang F, Bissell MJ. Polarity and proliferation are controlled by distinct signaling pathways downstream of PI3-kinase in breast epithelial tumor cells. *The Journal of cell biology*. 2004;164:603-12.
- Lucas N, Cho W. Phosphatidylserine binding is essential for plasma membrane recruitment and signaling function of 3-phosphoinositide-dependent kinase-1. *J Biol Chem*. 2011;286:41265-72.
- Maier U, Babich A, Nurnberg B. Roles of non-catalytic subunits in gbetagamma-induced activation of class I phosphoinositide 3-kinase isoforms beta and gamma. *The Journal of biological chemistry*. 1999;274:29311-7.
- Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell*. 2007;129:1261-74.
- Martelli AM, Lonetti A, Buontempo F, Ricci F, Tazzari PL, Evangelisti C, et al. Targeting signaling pathways in T-cell acute lymphoblastic leukemia initiating cells. *Advances in biological regulation*. 2014;56:6-21.
- Martin-Belmonte F, Yu W, Rodriguez-Fraticelli AE, Ewald AJ, Werb Z, Alonso MA, et al. Cell-polarity dynamics controls the mechanism of lumen formation in epithelial morphogenesis. *Current biology : CB*. 2008;18:507-13.
- Mayer BJ, Ren R, Clark KL, Baltimore D. A putative modular domain present in diverse signaling proteins. *Cell*. 1993;73:629-30.
- McConnachie G, Pass I, Walker SM, Downes CP. Interfacial kinetic analysis of the tumour suppressor phosphatase, PTEN: evidence for activation by anionic phospholipids. *Biochem J*. 2003;371:947-55.
- Michell RH. Inositol derivatives: evolution and functions. *Nature reviews Molecular cell biology*. 2008;9:151-61.
- Mitra P, Zhang Y, Rameh LE, Ivshina MP, McCollum D, Nunnari JJ, et al. A novel phosphatidylinositol(3,4,5)P3 pathway in fission yeast. *The Journal of cell biology*. 2004;166:205-11.
- Mora A, Komander D, van Aalten DM, Alessi DR. PDK1, the master regulator of AGC kinase signal transduction. *Semin Cell Dev Biol*. 2004;15:161-70.
- Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, et al. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proc Natl Acad Sci U S A*. 1998;95:13513-8.
- Nagel W, Zeitlmann L, Schilcher P, Geiger C, Kolanus J, Kolanus W. Phosphoinositide 3-OH kinase activates the beta2 integrin adhesion pathway and induces membrane recruitment of cytohesin-1. *The Journal of biological chemistry*. 1998;273:14853-61.
- Nozaki H, Maruyama S, Matsuzaki M, Nakada T, Kato S, Misawa K. Phylogenetic positions of Glaucophyta, green plants (Archaeplastida) and Haptophyta (Chromalveolata) as deduced from slowly evolving nuclear genes. *Molecular phylogenetics and evolution*. 2009;53:872-80.
- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science (New York, NY)*. 2002;297:1031-4.

- Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, et al. The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease. *The Biochemical journal*. 2009;419:29-49.
- Paradis S, Ruvkun G. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev*. 1998;12:2488-98.
- Parfrey LW, Grant J, Tekle YI, Lasek-Nesselquist E, Morrison HG, Sogin ML, et al. Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. *Systematic biology*. 2010;59:518-33.
- Park WS, Heo WD, Whalen JH, O'Rourke NA, Bryan HM, Meyer T, et al. Comprehensive identification of PIP3-regulated PH domains from *C. elegans* to *H. sapiens* by model prediction and live imaging. *Molecular cell*. 2008;30:381-92.
- Parker PJ, Waterfield MD. Phosphatidylinositol 3-kinase: a novel effector. *Cell Growth Differ*. 1992;3:747-52.
- Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature*. 2010;465:1033-8.
- Posor Y, Eichhorn-Gruenig M, Puchkov D, Schoneberg J, Ullrich A, Lampe A, et al. Spatiotemporal control of endocytosis by phosphatidylinositol-3,4-bisphosphate. *Nature*. 2013;499:233-7.
- Premkumar L, Bobkov AA, Patel M, Jaroszewski L, Bankston LA, Stec B, et al. Structural basis of membrane targeting by the Dock180 family of Rho family guanine exchange factors (Rho-GEFs). *The Journal of biological chemistry*. 2010;285:13211-22.
- Rodriguez-Escudero I, Roelants FM, Thorner J, Nombela C, Molina M, Cid VJ. Reconstitution of the mammalian PI3K/PTEN/Akt pathway in yeast. *Biochem J*. 2005;390:613-23.
- Rogozin IB, Basu MK, Csuros M, Koonin EV. Analysis of rare genomic changes does not support the unikont-bikont phylogeny and suggests cyanobacterial symbiosis as the point of primary radiation of eukaryotes. *Genome biology and evolution*. 2009;1:99-113.
- Rohrschneider LR, Fuller JF, Wolf I, Liu Y, Lucas DM. Structure, function, and biology of SHIP proteins. *Genes Dev*. 2000;14:505-20.
- Salmena L, Carracedo A, Pandolfi PP. Tenets of PTEN tumor suppression. *Cell*. 2008;133:403-14.
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science (New York, NY)*. 2005;307:1098-101.
- Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, et al. Function of PI3K γ in thymocyte development, T cell activation, and neutrophil migration. *Science (New York, NY)*. 2000;287:1040-6.
- Shi SH, Jan LY, Jan YN. Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell*. 2003;112:63-75.
- Silva A, Yunes JA, Cardoso BA, Martins LR, Jotta PY, Abecasis M, et al. PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest*. 2008;118:3762-74.
- Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*. 1998;95:29-39.
- Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nature genetics*. 1997;15:356-62.
- Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, et al. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science*. 1998;279:710-4.

- Stephens L, Hawkins P. Signalling via class IA PI3Ks. *Advances in enzyme regulation*. 2011;51:27-36.
- Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, Hemmings BA, et al. Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science (New York, NY)*. 2002;295:2088-91.
- Strahl T, Thorner J. Synthesis and function of membrane phosphoinositides in budding yeast, *Saccharomyces cerevisiae*. *Biochimica et biophysica acta*. 2007;1771:353-404.
- Tang X, Downes CP. Purification and characterization of Gbetagamma-responsive phosphoinositide 3-kinases from pig platelet cytosol. *The Journal of biological chemistry*. 1997;272:14193-9.
- Toker A, Marmiroli S. Signaling specificity in the Akt pathway in biology and disease. *Advances in biological regulation*. 2014.
- Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol*. 2010a;11:329-41.
- Vanhaesebroeck B, Leever SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, et al. Synthesis and Function of 3-Phosphorylated Inositol Lipids. *Annu Rev Biochem*. 2001;70:535-602.
- Vanhaesebroeck B, Leever SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci*. 1997a;22:267-72.
- Vanhaesebroeck B, Stephens L, Hawkins P. PI3K signalling: the path to discovery and understanding. *Nature reviews Molecular cell biology*. 2012;13:195-203.
- Vanhaesebroeck B, Vogt PK, Rommel C. PI3K: From the Bench to the Clinic and Back. *Curr Top Microbiol Immunol*. 2010b.
- Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, et al. P110delta, a novel phosphoinositide 3-kinase in leukocytes. *Proc Natl Acad Sci U S A*. 1997b;94:4330-5.
- Venkateswarlu K, Oatey PB, Tavares JM, Cullen PJ. Insulin-dependent translocation of ARNO to the plasma membrane of adipocytes requires phosphatidylinositol 3-kinase. *Current biology : CB*. 1998;8:463-6.
- Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2:489-501.
- Walker SM, Downes CP, Leslie NR. TPIP: a novel phosphoinositide 3-phosphatase. *Biochem J*. 2001;360:277-83.
- Watton SJ, Downward J. Akt/PKB localisation and 3' phosphoinositide generation at sites of epithelial cell-matrix and cell-cell interaction. *Curr Biol*. 1999;9:433-6.
- Weiner OD. Regulation of cell polarity during eukaryotic chemotaxis: the chemotactic compass. *Current opinion in cell biology*. 2002;14:196-202.
- Whitman M, Downes CP, Keeler M, Keller T, Cantley L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature*. 1988;332:644-6.
- Worby CA, Dixon JE. Pten. *Annu Rev Biochem*. 2014;83:641-69.
- Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta*. 1998;1436:127-50.
- Yang J, Cron P, Good VM, Thompson V, Hemmings BA, Barford D. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat Struct Biol*. 2002;9:940-4.
- Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. *Molecular and cellular biology*. 1998;18:1379-87.

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Yu JW, Mendrola JM, Audhya A, Singh S, Keleti D, DeWald DB, et al. Genome-wide analysis of membrane targeting by *S. cerevisiae* pleckstrin homology domains. *Molecular cell*. 2004;13:677-88.

Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008;27:5497-510.

Figure Legends

Fig. 1. Class I PI3K function. The diagram shows a model of PI3K function, activated by a receptor at the plasma membrane. Upon receptor engagement PtdIns(4,5)P₂ (PI(4,5)P₂) is phosphorylated by activated PI3K and ATP to form PtdIns(3,4,5)P₃ (PIP₃). and the reverse conversion catalyzed by PTEN, reproducing PI(4,5)P₂ and releasing free phosphate. Once generated PIP₃ is able to influence diverse cellular processes through a large number of direct PIP₃-binding proteins, including AKT, activating Guanine nucleotide exchange factors (GEFs) for small GTPases, PDK1, the Cytohesins and many others.

Fig. 2. Eukaryotic phylogenetic tree and the PI3K system. A simplified representation of eukaryotic evolutionary relationships is presented. Several aspects, particularly the relationships of the deepest branches are uncertain (Nozaki et al., 2009, Parfrey et al., 2010, Rogozin et al., 2009). The evolutionary distances represented in the tree by branch lengths are indicative but do not represent a single quantified analysis. Proteins structurally and functionally resembling the human class I PI3Ks are present throughout the Unikonts (Episthokonts and Amoebozoa) with the notable exception of fungi. PI3K enzymes are also present in the genomes of several more divergent eukaryotes (eg some excavate parasites, including trypanosomes and giardia, and some alveolates and stramenopiles) but without functional characterisation they are difficult to classify into the class I or class II PI3K groups. Both the regulatory p85 PI3K subunits and SHIP 5-phosphatases appear to be a more recent evolutionary development, appearing only in metazoa and chaonnoflagellates.

Fig. 3. Occurrence of PI3K pathway components in eukaryotic groups. The patterns of occurrence of individual components of the PI3K signalling system is shown, relating to the phylogenetic tree shown in Fig. 2. Lineages or species are shown in pink shading, in which each protein represented below is evident. It should be noted (*) that proteins with similarity to class I p110 PI3K are present in some species from the more diverse lineages noted, but it is not clear whether these represent class I or class II enzymes. Otherwise a pattern of distribution is shred between p110, AKT and the cytohesins, being found in unikont groups other than fungi. Also, the *Giardia intestinalis* genome contains a phosphatase with sequence similarity to the PTEN catalytic domain but lacking evident amino acid sequence or 3D structural alignment to the adjacent C2 domain, which has been used here as the defining feature of the PTEN enzyme group.

Fig. 2

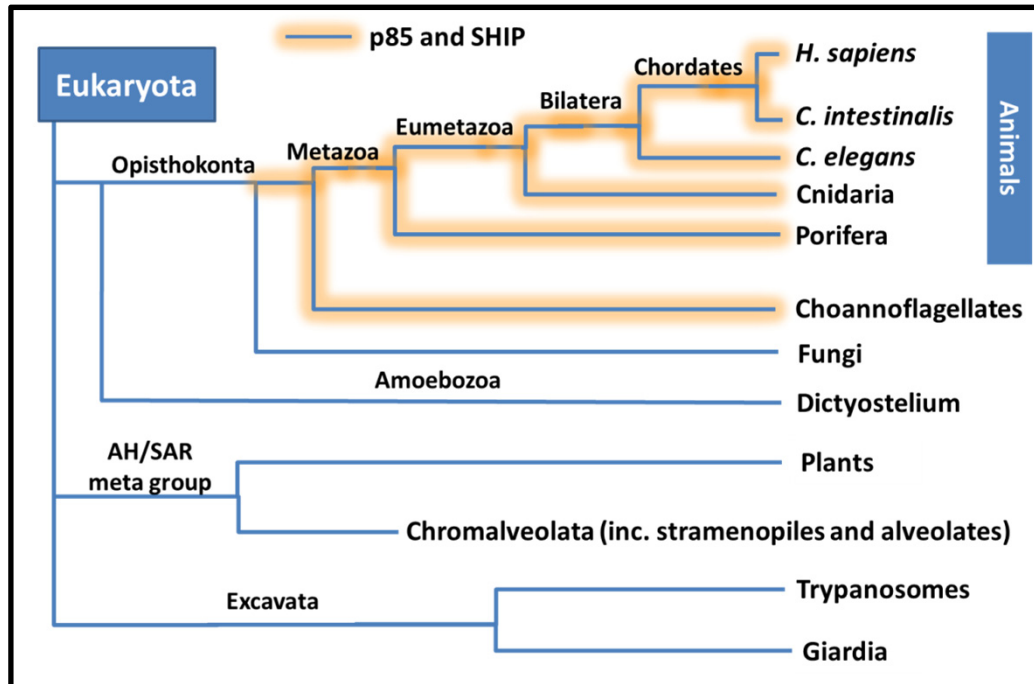
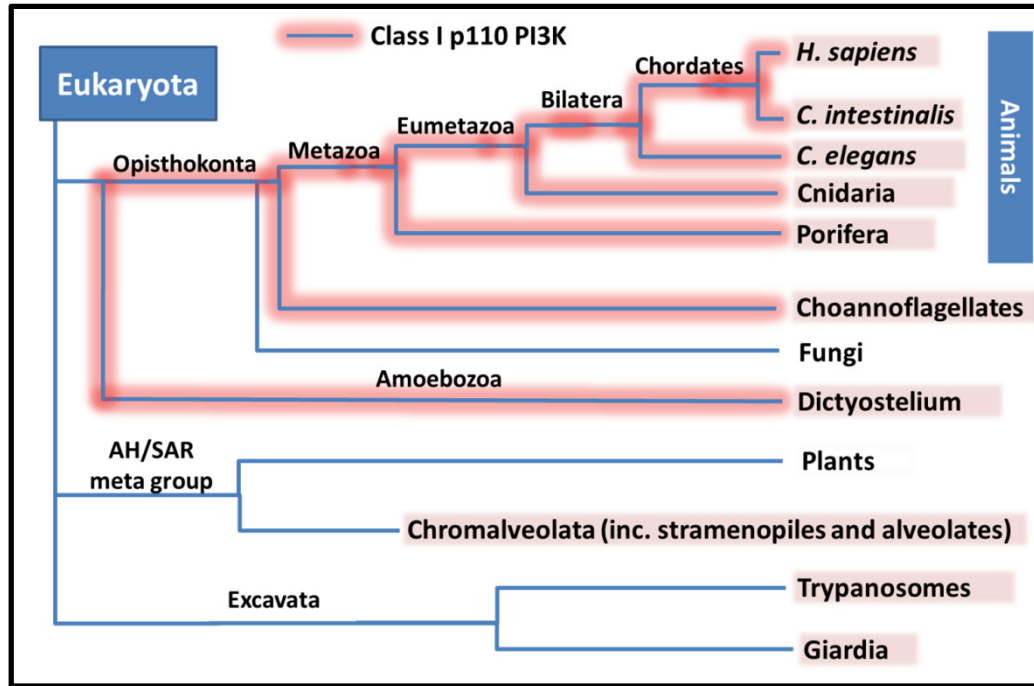


Fig. 3

