

Cell Science at a Glance

Classes of phosphoinositide 3-kinases at a glance

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ABSTRACT

The phosphoinositide 3-kinase (PI3K) family is important to nearly all aspects of cell and tissue biology and central to human cancer, diabetes and aging. PI3Ks are spatially regulated and multifunctional, and together, act at nearly all membranes in the cell to regulate a wide range of signaling, membrane trafficking and metabolic processes. There is a broadening recognition of the importance of distinct roles for each of the three different PI3K classes (I, II and III), as well as for the different isoforms within each

class. Ongoing issues include the need for a better understanding of the *in vivo* complexity of PI3K regulation and cellular functions. This Cell Science at a Glance article and the accompanying poster summarize the biochemical activities, cellular roles and functional requirements for the three classes of PI3Ks. In doing so, we aim to provide an overview of the parallels, the key differences and crucial interplays between the regulation and roles of the three PI3K classes.

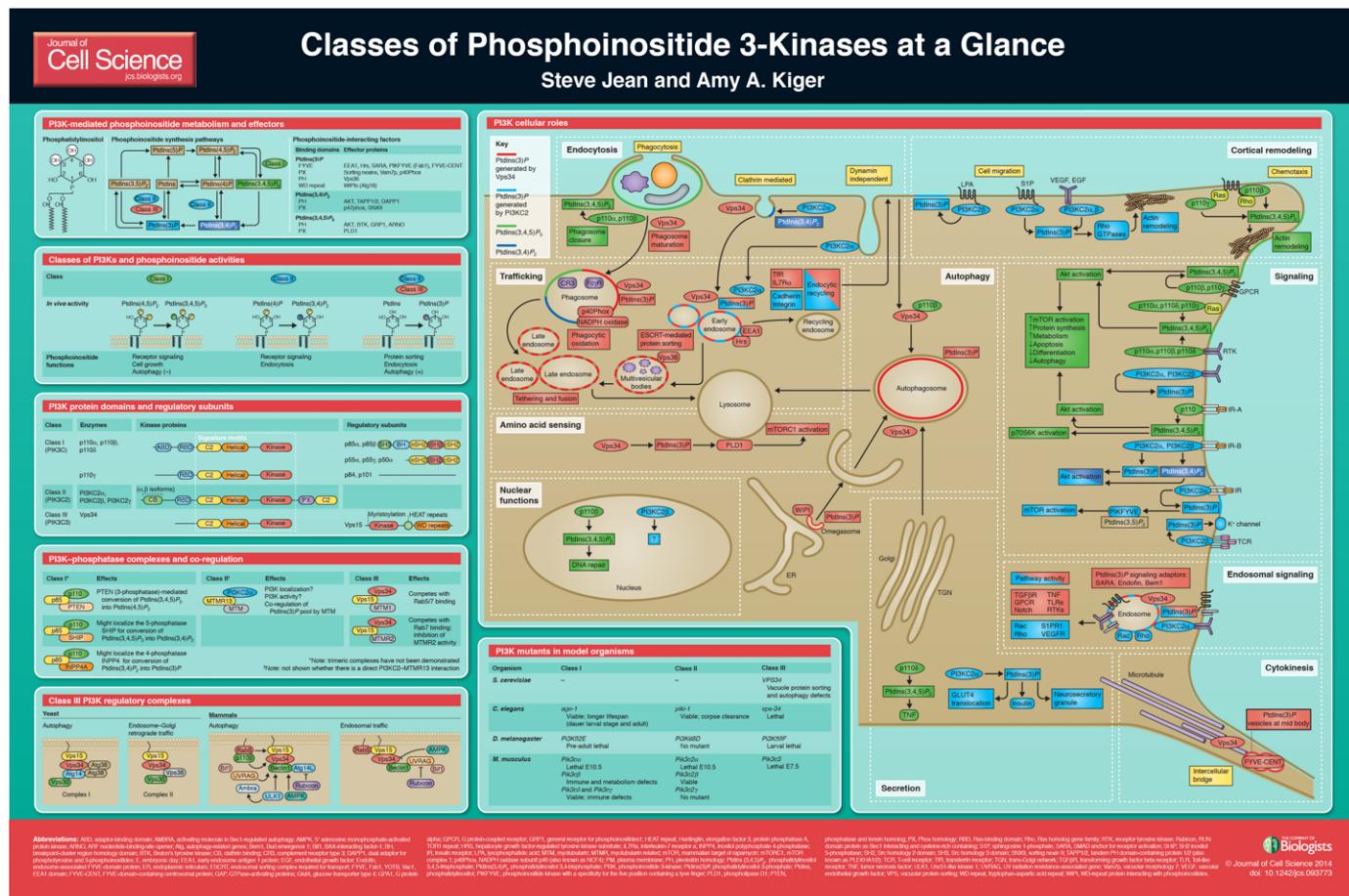
KEY WORDS: Class I PI3K, Class II PI3K, Class III PI3K, PI3-kinases, PI3K, Phosphoinositide

Introduction

This Cell Science at a Glance article and the accompanying poster provide a broad overview of the current knowledge and

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emerging themes related to the important phosphoinositide 3-kinase (PI3K) family. Although widely appreciated for crucial roles in cell signaling or membrane trafficking, new research directions continue to reveal important complexities in specific PI3K class regulation and functions. Here, we will not elaborate on the details of specific pathways or processes that engage individual PI3K subfamily members in normal and human disease states, which have been reviewed in depth elsewhere. We instead will highlight the similarities and differences in the molecular-cellular requirements for each of three PI3K classes (I, II and III).

Class I, II and III PI3Ks synthesize three distinct phosphoinositides

Most PI3K functions are mediated by phosphoinositides, the low-abundance phosphorylated forms of phosphatidylinositol (PtdIns) (see poster) (Di Paolo and De Camilli, 2006; Sasaki et al., 2009). All three PI3K classes (I, II and III) phosphorylate the 3'-position hydroxyl of the D-myo-inositol head group to generate specific phosphoinositide forms (see poster) (Vanhaesebroeck et al., 2010a). *In vitro*, all classes can generate phosphatidylinositol 3-phosphate [PtdIns(3)P], class I and II can synthesize phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)P₂], and only class I can produce phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃]. An understanding of more-selective *in vivo* PI3K activities has been derived from approaches that combine the use of genetic mutants or pharmacological inhibitors with phosphoinositide analysis by chromatography, microscopy imaging of fluorescent biosensors and epistasis with specific phosphoinositide 3-phosphatases. *In vivo*, there is significant support for class I PI3K synthesis of PtdIns(3,4,5)P₃ [and indirectly, PtdIns(3,4)P₂], class III PI3K synthesis of PtdIns(3)P, and to a lesser extent, class II PI3K synthesis of PtdIns(3)P and PtdIns(3,4)P₂ (see poster) (Backer, 2008; Vanhaesebroeck et al., 2010a; Falasca and Maffucci, 2012; Posor et al., 2013; Schink et al., 2013). Despite their overlapping selectivities, the different PI3Ks exhibit non-redundant functions in cells and animals, as discussed below. The PI3K family appears restricted to eukaryotes, and only the class III PI3K is conserved from yeast to human (Brown and Auger, 2011). In contrast, class I and II PI3Ks have evolved conserved multidomains, distinct adaptors and expanded targets that are relevant to multicellular life (Engelman et al., 2006).

PI3K phosphoinositide functions are mediated by effector recruitment

A common role for phosphoinositides is the recruitment of effector proteins through phosphoinositide-binding protein domains. The best-characterized 3-phosphoinositide-binding domains are FYVE and a subset of PX and PH domains (Lemmon, 2008) (see poster). The relatively low phosphoinositide-binding affinity of these domains in combination with 3-phosphoinositide phosphatase activities permits highly reversible effector localization and responses. In addition, ‘coincidence detection’ of both a phosphoinositide and another membrane-localized protein can further promote effector specificity (Di Paolo and De Camilli, 2006; Jean and Kiger, 2012). There is a broad range of known phosphoinositide-regulated effector functions. One category of effectors directs localized membrane remodeling events, such as membrane tubulation, fusion, fission or transport. This is seen with the endosomal sorting and tubulation performed by the PtdIns(3)P-binding PX-domain-containing sorting nexins

(SNXs) (Cullen, 2008). A second category of effectors mediates membrane-localized signaling. Examples of this are signal transduction at the plasma membrane via the PtdIns(3,4,5)P₃-binding PH-domain-containing proteins Akt and Grp1 (Hawkins et al., 2006). PI3K phosphoinositide activities can also serve other roles by locally modifying the biophysical properties of membranes, such as electrostatic interactions or a less well-understood function in regulating membrane curvature (McLaughlin and Murray, 2005; Lemmon, 2008).

PI3K protein domains and regulatory subunits

All PI3Ks possess a ‘PI3K signature motif’ that is composed of a C2 domain, which likely binds membranes, a helical domain and the catalytic kinase domain (see poster) (Vanhaesebroeck et al., 2010a). The classification of PI3Ks into the three different classes is based mainly on the presence of additional protein domains and their interactions with regulatory subunits. The nomenclature of the PI3K subunits is shown in Table 1.

The class I PI3K subfamily comprises four members in vertebrates (see poster), only one member in worm and fly, and there are none in yeast (Hawkins et al., 2006; Brown and Auger, 2011). Class I PI3Ks function as heterodimers consisting of one of four catalytic p110 subunits (p110 α , β , δ or γ) and a regulatory subunit [p85 α (or its splice variants p55 α and p50 α), p85 β , p55 γ , p101 or p84]. (Vanhaesebroeck et al., 2010a; Vadas et al., 2011). There are two major classes of regulatory subunits, each represented by alternative isoforms. Alternative p85 regulatory subunits (p85, p55, p50), which each harbor two Src homology 2 domains (nSH2, cSH2) and an intervening p110-binding region (iSH2), constitutively interact with the p110 α , p110 β and p110 δ catalytic subunits through an N-terminal adaptor-binding domain (ABD). Acting as an adaptor, p85 recruits the complex to phosphorylated tyrosine commonly downstream of activated receptor tyrosine kinases (RTKs). p85 also negatively regulates the kinase activity of p110 α through a helical domain interaction, with important effects in cancer (Vadas et al., 2011). In contrast, p110 γ does not have a clear p85-binding domain. Instead, p110 γ heterodimers form with the regulatory subunits p101 or p84 that are devoid of SH2 domains and are almost exclusively activated by G protein-coupled receptors (GPCRs). Class I PI3Ks also harbor a Ras-binding domain (RBD) in the N-terminal extension,

Table 1. Nomenclature of PI3K subunits.

Subunit	Protein	Gene name (human)
Class 1		
Catalytic	p110 α p110 β p110 δ p110 γ	PIK3CA PIK3CB PIK3CD PIK3CG
Regulatory	p85 α , p55 α , p50 α p85 β p55 γ p101 p84, p87	PIK3R1 PIK3R2 PIK3R3 PIK3R5 PIK3R6
Class 2		
Catalytic	PI3KC2 α PI3KC2 β PI3KC2 γ	PIK3C2A PIK3C2B PIK3C2G
Class 3		
Catalytic	Vps34	PIK3C3
Regulatory	Vps15	PIK3R4

and p110 α , p110 δ and p110 γ are each stimulus-dependent Ras effectors (Vanhaesebroeck et al., 2010a). In contrast, the p110 β RBD interacts with Rab5 GTPase (Christoforidis et al., 1999) and Rho GTPase family members (Fritsch et al., 2013), and specifically, Rac potentiates p110 β GPCR responses (Guillermet-Guibert et al., 2008; Dbouk et al., 2012; Fritsch et al., 2013).

The class II PI3K (PI3KC2) subfamily has three members in vertebrates (see poster), only one member in worm and fly, and also none in yeast (Brown and Auger, 2011; Falasca and Maffucci, 2012). This class has additional domains in both N- and C-terminal extensions. There is no known obligatory regulatory subunit, but the class II enzymes interact with proteins that could serve adaptor functions. PI3KC2 α and PI3KC2 β contain an N-terminal clathrin-binding (CB) region, suggesting a link with clathrin-coated vesicles. The PI3KC2 α N-terminal region appears to inhibit kinase activity, which can be released by clathrin binding (Gaidarov et al., 2001), and PI3KC2 α has been implicated in clathrin-mediated endocytosis (Posor et al., 2013). The PI3KC2 β N-terminus also binds the scaffold protein intersectin, which promotes increased PtdIns(3) P synthesis (Das et al., 2007). Unlike the PI3KC2 α and - β isoforms, PI3KC2 γ protein interactions have not been tested. We identified a scaffold for the 3-phosphoinositide phosphatase myotubularin (MTM) as a possible PI3KC2 adaptor, as discussed below (Jean et al., 2012). Class II PI3Ks also harbor a Ras-binding domain (RBD), although these signaling inputs are not well characterized. All three PI3KC2 subfamily members possess a unique C-terminal extension that carries a C2 domain and a PX domain that preferentially binds to phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5) P_2] (Stahelin et al., 2006).

A single class III PI3K is conserved in all eukaryotes (see poster) (Backer, 2008). This enzyme was first identified as vacuolar protein sorting 34 (Vps34), the sole PI3K in yeast (Schu et al., 1993). Vps34 binds to the adaptor protein Vps15 which is N-terminally myristoylated and regulates the intracellular membrane localization of Vps34 and its activity (Backer, 2008). Vps15 also engages with other key membrane proteins, such as the Rab5 GTPase to coordinate Vps34 activity at endosomes (Christoforidis et al., 1999). Vps34 does not contain known protein domains outside of the ‘PI3K signature motif’, but it engages in a growing list of protein interactions that regulate distinct PtdIns(3) P pools (see below) (Simonsen and Tooze, 2009; Kim et al., 2013).

PI3K and phosphatase co-regulation

An emerging theme is the co-regulation of specific PI3Ks and PtdIns-phosphatases through shared adaptor protein interactions (see poster) (Jean and Kiger, 2012). Although first identified as a p110 regulatory subunit, p85 has since been shown to also bind, regulate share phenotypes with the antagonizing phosphatase and tensin homologue (PTEN) 3-phosphatase (Chagpar et al., 2010). In this way, p85 reversibly regulates the conversion of PtdIns(4,5) P_2 to PtdIns(3,4,5) P_3 . p85 also interacts with the 5-phosphatase SHIP (Jackson et al., 1995) and with the 4-phosphatase INPP4 (Munday et al., 1999), which have possible significance in the successive endosomal conversion of class I PI3K PtdIns(3,4,5) P_3 to PtdIns(3,4) P_2 and PtdIns(3) P , respectively (Ivetac et al., 2005; Shin et al., 2005). Likewise, we identified a conserved physical interaction between class II PI3K and MTMR13/Sbf, an adaptor for 3-phosphoinositide phosphatase MTMR2/Mtm, and we also found that all these

components functionally co-regulate a PtdIns(3) P pool in endosomal trafficking (Jean et al., 2012). Finally, the Vps15 adaptor for class III Vps34 was identified to exist in a trimeric complex with the 3-phosphatases MTM1 or MTMR2 that compete with Rab5 and Rab7 for Vps15 binding (Cao et al., 2007). These parallels suggest that shared kinase–phosphatase adaptor interactions provide a tight spatiotemporal control of distinct phosphoinositide pools and thus of their specific cellular functions.

PI3K cellular functions and pathways

The collective cellular and organismal functions for the PI3K family extend into all parts of the cell, cell-types and developmental stages (see poster). Each PI3K class has multiple cellular roles through the regulation of distinct phosphoinositide pools. The direct roles of PI3Ks can be categorized predominantly as acting in cell signaling (class I, II) or membrane trafficking (class II, III). Although not yet widely addressed, members of different PI3K classes can act at successive steps in shared pathways and processes (Dou et al., 2010; Lu et al., 2012). There are also emerging descriptions of PI3K localization and/or roles in the nucleus (Kumar et al., 2011). In addition, there are many indirect consequences of these diverse PI3K functions. Below is a brief overview of the cellular roles and pathways depicted on the poster and as reviewed elsewhere.

Class I PI3Ks

There is a rich literature on the functions for this founding class of the PI3K family. Numerous growth factor pathways are under the control of activated RTKs or GPCRs that recruit p85–p110 complexes to the plasma membrane, where upon relief of p85 inhibition, p110 converts PtdIns(4,5) P_2 in to PtdIns(3,4,5) P_3 to elicit signaling responses (Vanhaesebroeck et al., 2010a). Notably, PtdIns(3,4,5) P_3 recruits the kinase Akt, which controls multiple pathways (including activation of mTORC1, FOXO and others), to regulate cell growth, proliferation, survival, metabolism and autophagy (Vanhaesebroeck et al., 2012). Localized class I PI3K activity also plays a role in cortical F-actin dynamics, which underlies chemotaxis and phagocytosis of large particles (Leverrier et al., 2003; Tamura et al., 2009; Hawkins et al., 2010; Flanagan et al., 2012; Weiger and Parent, 2012). For example, at the neutrophil leading edge, p110 γ -induced PtdIns(3,4,5) P_3 formation results in the recruitment of Rac GTPase, which promotes F-actin polymerization, lamellipodia formation and cell migration (Yoo et al., 2010). Consistent with a central role for PtdIns(3,4,5) P_3 in all of these cellular processes, the 3-phosphatase PTEN downregulates PtdIns(3,4,5) P_3 and these class I PI3K-activated pathways (Song et al., 2012).

The four class I catalytic isoforms share overlapping but distinct functions. The p110 γ and p110 δ isoforms are mainly restricted to functions in immune cells where they are expressed, whereas p110 α and p110 β are ubiquitous, but also exhibit isoform-specific cell-type- and context-dependent requirements. Most class I PI3K functions are related to their catalytic properties; however, there is growing evidence for kinase-independent scaffolding roles for p110 γ and p110 β (Patrucco et al., 2004; Hirsch et al., 2009; Dou et al., 2010; Rauch et al., 2011; Dou et al., 2013). Less well understood are the roles for the numerous regulatory adaptor isoforms in the regulation of class I p110 functions. Given the importance of class I PI3K function in cellular homeostasis and regulation, mutations in class I PI3Ks or

in p85 adaptors are associated with a multitude of human diseases ranging from diabetes to cancer. In addition, *PIK3CA*-activating mutations have recently been linked to congenital lipomatous overgrowth with vascular, epidermal and skeletal anomalies, or CLOVES syndrome (Kurek et al., 2012). We refer readers to reviews that discuss the involvement of class I PI3K in human disease (Vicinanza et al., 2008; Kok et al., 2009; Vanhaesebroeck et al., 2010b; Wong et al., 2010).

Class II PI3Ks

Class II PI3Ks have received less research attention to date, so it is more difficult to generalize on their functions. One emerging theme is the requirement for class II PI3K functions at the cell cortex, as seen by its disparate roles in cell migration, cortical remodeling, glucose transport, insulin signaling, channel regulation, endocytosis and exocytosis (Mazza and Maffucci, 2011; Falasca and Maffucci, 2012). Class II PI3K functions have been mostly (although controversially) attributed to PtdIns(3)*P*, which has roles in either cell signaling or intracellular membrane trafficking. Growing evidence suggests that these two mechanisms could be interrelated at the level of ‘endosomal signaling’ (Yoshioka et al., 2012; Biswas et al., 2013), or endosome platforms for the formation of specific signal transduction complexes and responses (Platta and Stenmark, 2011). With regard to cell signaling roles, PI3KC2-induced PtdIns(3)*P* appears to mediate immune cell K⁺ channel activity (in the case of PI3KC2β), growth factor receptor responses, and activation of Rho GTPases in cell contraction and migration (in the cases of PI3KC2α and -β) (Bridges et al., 2012; Falasca and Maffucci, 2012; Yoshioka et al., 2012), whereas PI3KC2α-mediated PtdIns(3,4)*P*₂ activity has been associated with insulin-induced Akt stimulation (Leibiger et al., 2010). With regard to membrane trafficking roles, PI3KC2α-induced PtdIns(3)*P* activity appears to direct endosomal trafficking in endocytic recycling (Krag et al., 2010; Jean et al., 2012; Yoshioka et al., 2012), phagosome maturation (Lu et al., 2012), late steps in exocytosis (Mazza and Maffucci, 2011; Falasca and Maffucci, 2012) and autophagy (Behrends et al., 2010; Devereaux et al., 2013), whereas PtdIns(3,4)*P*₂ activity has been recently implicated with a central role in clathrin-mediated endocytosis (Posor et al., 2013).

The identification of class II PI3K effectors and pathways will be important for a broader understanding of their functions. Although not yet causally linked to human disease, PI3KC2 polymorphisms and studies in cell lines suggest that it might be potentially involved in diabetes (PI3KC2α and -γ) and cancer (PI3KC2α and -β) (Falasca and Maffucci, 2012). Recent mouse knockout results also suggest a possible role for human PI3KC2α in blood vessel formation and integrity (Yoshioka et al., 2012). Interestingly, phenotypes that result from an inactivation of specific MTM 3-phosphoinositide phosphatases are suppressed by a co-knockdown of class II PI3K in flies and worms (Lu et al., 2012). This suggests that class II PI3K inhibition might be a therapeutic avenue for treatment of MTM-associated myopathy and neuropathy disorders in humans (Vicinanza et al., 2008).

Class III PI3K

Vps34 predominantly regulates membrane trafficking, with central roles in endosomal protein sorting, endosome–lysosome maturation, autophagosome formation, autophagy flux and cytokinesis (Backer, 2008; Simonsen and Tooze, 2009; Nezis et al., 2010; Raiborg et al., 2013). Vps34 is found in an increasing

variety of protein regulatory complexes that specify the synthesis of PtdIns(3)*P* pools at distinct intracellular membranes (see poster) (Backer, 2008). The three core Vps34 regulatory complex components include the Vps34 catalytic subunit, the Vps15 membrane adaptor (see above), and Vps30 (known as Beclin 1 in mammals, also called Atg6). In yeast, the complex I [Vps34, Vps15, Vps30, Atg14 and Atg38 (Araki et al., 2013)] is implicated in autophagy, whereas complex II (Vps34, Vps15, Vps30 and Vps38) is involved in membrane trafficking for vacuolar protein sorting, although not all Vps34 endosomal functions seem to require Vps30/Beclin 1. Other associated proteins – the extent and identity of which continues to emerge – specify Vps34 localization, activity and membrane accessibility (Backer, 2008; Simonsen and Tooze, 2009; Funderburk et al., 2010; Kim et al., 2013; Russell et al., 2013).

On early endosomes, Rab5 GTPase activates specific Vps34 complexes for endosomal maturation (see poster). The synthesis of endosomal PtdIns(3)*P* leads to the recruitment of effectors, such as endosomal sorting complex required for transport (ESCRT) components that are involved in sorting of protein cargo, and the homotypic fusion and protein sorting (HOPS) complex that mediates endosome fusion and trafficking to lysosomes (Raiborg et al., 2013). In a similar fashion, Rab5 and Vps34 are required for phagosome maturation (Flannagan et al., 2012). Distinct Vps34 complexes that contain the autophagy core factor Atg14 have been associated with autophagy. PtdIns(3)*P* synthesis at autophagosome precursor membranes – potentially at omegasomes that arise at the endoplasmic reticulum (ER) and on nascent autophagosomes – recruits the protein WD-repeat protein interacting with phosphoinositides 1 (WIPI1; Atg18 in yeast) and elicits a hierarchical cascade that directs autophagosome formation (Simonsen and Tooze, 2009; Jaber et al., 2012; Schink et al., 2013). A growing list of regulators controls the activity of the Vps34 complex in autophagy (see poster), including the direct and multi-faceted roles for AMPK that promote Vps34 activity in response to nutrient stress (Kim et al., 2013). Signaling functions are also becoming more broadly established for Vps34, with roles in yeast pheromone signaling (Backer, 2008), regulation of various developmental receptor pathways (von Zastrow and Sorkin, 2007; Platta and Stenmark, 2011; Wada and Sun-Wada, 2013) and amino acid sensing in mTORC1 activation (Yoon et al., 2011; Zoncu et al., 2011; Jaber et al., 2012). In cytokinesis, Vps34 activity at the midbody leads to the synthesis of a PtdIns(3)*P* pool that recruits FYVE-CENT and associated proteins that regulate the role of ESCRT-III in abscission (Nezis et al., 2010; Schink et al., 2013).

So far, no human diseases are associated with mutations in Vps34, although genetic linkage studies found a correlation between Vps34 promoter variants and schizophrenia (Backer, 2008). In addition, low levels of PtdIns(3)*P* in brain have been found in humans affected with Alzheimer’s disease (Morel et al., 2013), and co-expression of Vps15 and Vps34 could suppress aspects of Danon autophagic vacuolar myopathy (AVM) disease in human patient muscle cells (Nemazany et al., 2013).

PI3K studies in model organisms

PI3K mutants isolated in model organisms have been instrumental in describing cellular roles for PI3Ks (Vanhaesebroeck et al., 2012) (see poster). Roles for class I PI3K in insulin-signaling-mediated regulation of cell size, growth and lifespan were first shown in *Drosophila* and

Caenorhabditis elegans (Oldham and Hafen, 2003; Kenyon, 2005), the class II PI3K subfamily was first identified and studied in flies (MacDougall et al., 1995), and class III PI3K functions in trafficking were identified through its mutant phenotypes in yeast (Schu et al., 1993; Raiborg et al., 2013). Nearly all of the PI3Ks and regulatory subunits have now been targeted by gene deletions in mouse, with extensive phenotypic variation depending on the expression pattern and genetic redundancy of the targeted isoform (Vanhaesebroeck et al., 2005; Falasca and Maffucci, 2012; Jaber et al., 2012; Yoshioka et al., 2012). Although most PI3K isoforms are required for mouse viability, tissue-specific knockout and knock-in strategies are now commonly used to facilitate the *in vivo* characterization of PI3K function. Care must be taken in interpreting PI3K isoform knockouts, owing to both the possible compensation by the non-targeted isoforms in animals with multigene families and the potential for the involvement of kinase-dependent and -independent functions. The use of compound inhibitors has advanced our understanding of class I and class III PI3K functions (Vanhaesebroeck et al., 2012). The development of isoform-specific class I and class III PI3K inhibitors are becoming valuable tools both for use in research and as the focus of clinical trials for use as chemotherapeutics (Wu et al., 2009; Wong et al., 2010). However, class II PI3Ks are generally less sensitive to the first-generation pan-inhibitors, and no specific class II PI3K compounds have been described to date.

Perspectives

A vast amount of knowledge on PI3Ks has been acquired since their discovery nearly 30 years ago (Vanhaesebroeck et al., 2012). Class I PI3K isoforms represent important druggable targets for multiple human diseases, and key lessons such as on feedback loops and crosstalk within and between PI3K and other signaling pathways have been uncovered in pursuit of class I-related therapies (Carracedo and Pandolfi, 2008; Castellano and Downward, 2011). The central role for class III PI3K in autophagy, along with the increasing relevance of autophagy to human disease, has sparked basic and applied research aimed at better understanding the regulation and function of the Vps34 complex. There are still many basic functions that remain to be better illuminated: a deeper understanding of the structure, generalized roles and isolation of selective compounds for PI3KC2; composition and *in vivo* regulation of PI3K protein complex assemblies; and the direct or indirect effects of phosphoinositide phosphatases on PI3K functions. The expanding knowledge on PI3Ks clearly continues to raise new questions with central relevance to both basic biology and human health.

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Competing interests

The authors declare no competing interests.

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