The phosphoinositide (PI) 3-kinase family

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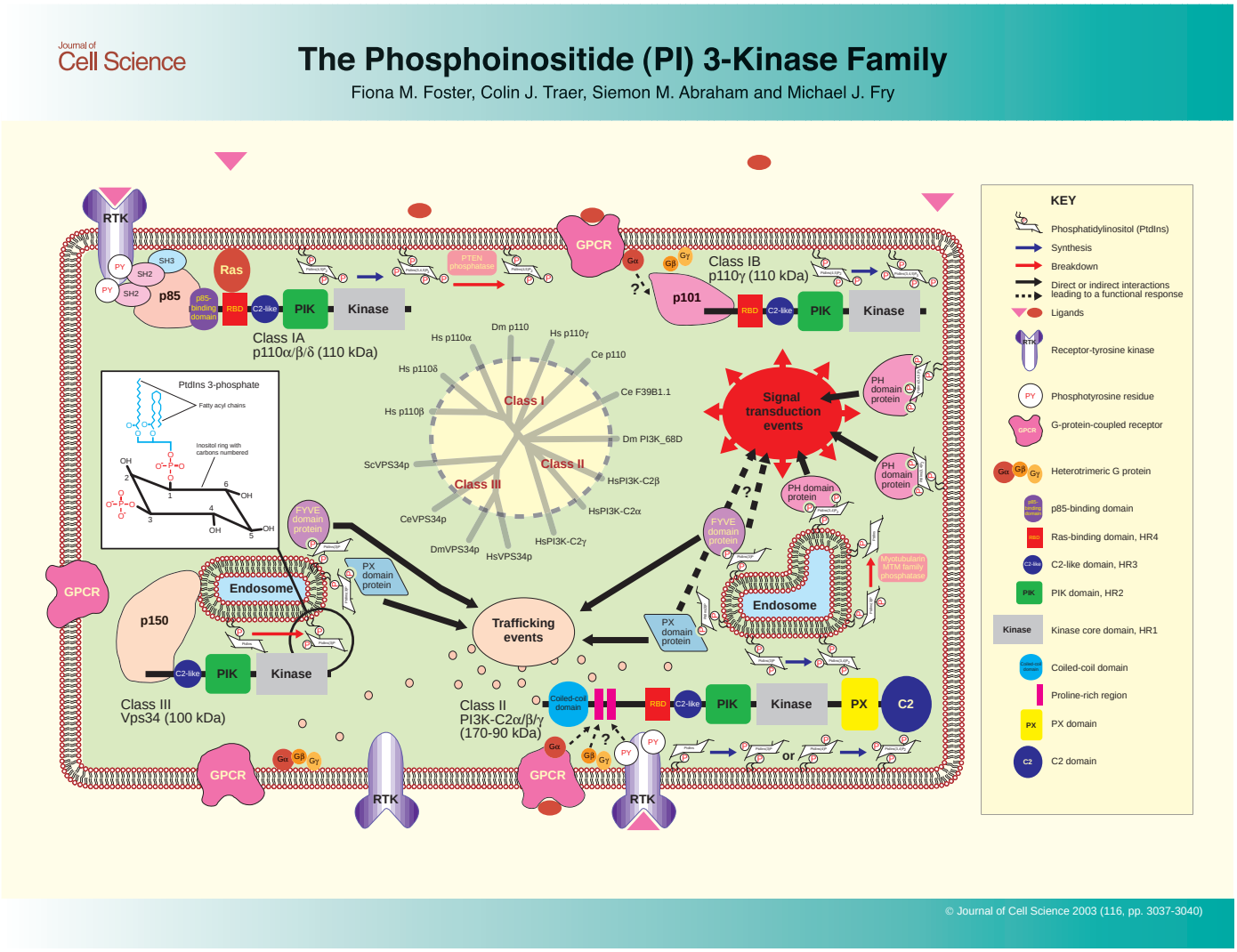
Phosphoinositide (PI) 3-kinase was first observed in 1984 as a minor inositol lipid kinase activity associated with immunoprecipitated oncogene products (e.g. Src, Abl and polyoma mT antigen) and present in activated growth factor receptor complexes (e.g. PDGF receptor). In 1988, the enzyme associated with this activity was found to have the novel ability to phosphorylate the 3 position hydroxyl group of the inositol ring (see poster) of phosphatidylinositol (PtdIns). PI 3-kinase activities have been subsequently found in all eukaryotic cell types examined (Fry, 1994; Katso et al., 2001) and are linked to an incredibly diverse set of key cellular functions, including cell growth, proliferation, motility, differentiation, survival and intracellular trafficking (Fry, 1994; Rameh and Cantley, 1999; Fry, 2001; Katso et al., 2001). The emerging links between PI 3-kinase activity and many human maladies, including allergy, inflammation, heart disease and cancer, has made them the focus of intense study, and inhibitors of these enzymes are considered potential therapeutic agents (Stein and Waterfield, 2000).

Although the majority of published studies have focused on the classical p110-p85 (now known as class I) PI 3-kinases, it has emerged over the past 10 years that the PI 3-kinase superfamily (EC 2.7.1.137) is made up of a large family of structurally related enzymes, with differing PI substrate requirements and modes of regulation, which probably accounts for the reported diversity of function (Rameh and Cantley, 1999; Fry, 2001; Katso et al., 2001). PCR cloning strategies and data mining of genome sequencing projects would seem to set the family limit at eight distinct PI 3-kinase catalytic subunits that are capable of phosphorylating inositol lipids. These eight isoforms have been divided into three functional classes on the basis of



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their protein domain structure, lipid substrate specificity and associated regulatory subunits: namely, the class I enzymes, p110α, p110β, p110δ and p110γ; the class II enzymes, PI3K-C2α, PI3K-C2β and PI3K-C2γ; and the sole class III enzyme, Vps34 (Fry, 2001; Katso et al., 2001).

The relationships between the kinase domains of the different enzymes identified in the human, fly, worm and yeast genomes are indicated in the poster by the non-rooted phylogenetic tree, which was prepared using ClustalX (hs, *Homo sapien*; dm, *Drosophila melanogaster*; ce, *Caenorhabditis elegans*; sp, *Schizosaccharomyces pombe*; sc, *Saccharomyces cerivisiae*). Simple eukaryotes, such as yeasts, and all plant species investigated to date seem to possess only a single class III PI 3-kinase. Multicellular invertebrate organisms, exemplified by *C. elegans* and *D. melanogaster*, have a single representative member of each of the three functional classes. Vertebrate genomes (human, mouse) contain eight distinct PI 3-kinase genes, with some of the family members being widely or ubiquitously expressed (e.g. p110α, p110β, PI3K-C2α, PI3K-C2β and Vps34), while others are more restricted to specific cell and tissue types (e.g. p110δ, p110γ and PI3K-C2γ). To add to this complexity there is a class IV group of PI-3-kinase-related protein serine/threonine kinases found in all eukaryotes (Kastan and Lim, 2000). Mammals have four such protein kinases: TOR (the target of the drug rapamycin), ATM (Ataxia telangiectasia mutated), ATR (Ataxia telangiectasia mutated related) and DNA-PK (DNA-dependent protein kinase). Here, we focus solely on the three classes of true PI 3-kinase.

The various 3-phosphorylated lipid products that are produced by these enzymes [PtdIns(3)*P*, PtdIns(3,4)P_2, PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3] function as part of the mechanism by which a diverse set of signalling molecules, containing pleckstrin homology (PH), FYVE, Phox (PX) and other lipid-binding domains, are recruited to various cellular membranes (Rameh and Cantley, 1999; Wurmser et al., 1999; Ellson et al., 2002). Cellular PI 3-kinase activities are balanced by phosphoinositide 3-phosphatase activities, found in the tumour suppressor protein PTEN and in members of the myotubularin (MTM) family (Maehama et al., 2001).

Functional analysis of PI 3-kinases

Two selective, chemically unrelated PI 3-kinase inhibitors are in wide use: namely, wortmannin and LY294002. Unfortunately, the use of these inhibitors is limited by the fact that they inhibit all known PI 3-kinase isoforms, including the class IV PI-3-kinase-like enzymes. This has led to many cellular processes being linked with PI 3-kinase activity, but considerable confusion as to which PI 3-kinase isoforms control specific cellular functions. In mammalian cells, wortmannin suppresses class I, class II PI3K-C2β and PI3K-C2γ, and class III PI 3-kinase activity with an IC_{50} in the 1-10 nM range, while it inhibits the class II PI3K-C2α isoform with an approximate IC_{50} of 400 nM, and class IV PI-3-kinase-related enzymes in the 100-300 nM range (data summarising many sources). Similarly, LY294002 inhibits all PI 3-kinases with an IC_{50} in the 1-50 μM range. Thus, at low concentrations, these two inhibitors (preferably used in parallel) can implicate a PI 3-kinase activity in a cellular process of interest, but are not suitable for dissecting the involvement of individual PI 3-kinase species. Many pharmaceutical companies are working on isoform-specific PI 3-kinase inhibitors and it is hoped that in time these will become available to the research community. Currently alternative approaches, such as the use of dominant negative mutants, knockout mice or RNA interference, are necessary to attribute a function to a specific PI 3-kinase class or isoform unambiguously.

Class I PI 3-kinases

The class I family of PI 3-kinase enzymes in vertebrates comprises four distinct protein species of approximately 110 kDa (p110α, p110β, p110δ and p110γ). All class I enzymes share the majority of their structural features and a common substrate specificity (Rameh and Cantley, 1999; Fry, 2001; Katso et al., 2001). In vitro, all class I PI 3-kinases are capable of phosphorylating PtdIns to PtdIns(3)*P*, PtdIns(4)*P* to PtdIns(3,4)P_2 and PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3, with PtdIns(4,5)P_2 being considered the preferred lipid substrate in vivo. Class I PI 3-kinases are largely cytosolic in resting cells, but upon stimulation are recruited to membranes via interactions with receptors or adaptor proteins. They are thought to function primarily at the plasma membrane, but there have been reports of class I PI 3-kinases associated with vesicular and nuclear membranes (Rameh and Cantley, 1999; Fry, 2001; Katso et al., 2001). The cellular roles of class I PI 3-kinases are diverse, with evidence linking them to cell size, motility, survival and proliferation in response to numerous signalling systems in many different cell types (Fry, 2001; Katso et al., 2001). The class I family is further subdivided into two groups on the basis of their regulatory partners and mechanisms of activation.

Class IA

There are three class IA catalytic subunits: p110α (human gene designation PIK3CA – all subsequent gene names and chromosomal locations listed refer to humans), which maps to chromosome 3 at 3q26.3; p110β (PIK3CB at 3q23); and p110δ (PIK3CD at 1p36.2). All of these PI 3-kinases physically interact with a family of Src homology 2 (SH2)-domain-containing regulatory adaptor proteins. Three distinct genes encode the p85α (PIK3R1 at 5q12-q13), p85β (PIK3R2 at 19q13.2-q13.4) and p55γ (PIK3R3 at 1p34.1) adaptors, each with a number of possible splice variants. All members of this ‘p85’ family of adaptors bind to the N-terminal 100 amino acids (shown in purple) of the class IA PI 3-kinases and mediate their activation by growth factor receptors (mainly of the protein-tyrosine kinase family) through the two SH2 domains that bind to sequence-specific phosphorylated tyrosine residues either on autophosphorylated receptors or on substrate adaptor proteins. Some of the p85α and p85β splice forms also possess an SH3 domain, which can bind to proline-rich ligands in other proteins instead of, or in addition to, SH2-domain-mediated recruitment (Fry,

1994; Katso et al., 2001). A class IA PI 3-kinase was the first to be identified and cloned and thus this class are best understood. Links between interesting biological questions and class I PI 3-kinases are starting to emerge suggesting that the individual isoforms have distinct, but possibly overlapping, roles, which may vary between cell types. Class I PI 3-kinases are linked to cell size in *Drosophila* and in mammals the p110α isoform has been shown to regulate the size of the adult heart (Crackower et al., 2002). Murine knockouts of the p110α and p110β genes result in embryonic lethality, pointing to their essential nature. Reports suggest that p110α may play a role in cell survival, whereas p110β may be more important in promoting cell proliferation (Benistant et al., 2000). Data implicating p110α in numerous cancers continues to mount (Fry, 2001), while studies with knock-in mice bearing catalytically inactive p110δ would seem to suggest that this isoform is critical for full B- and T-cell antigen receptor signalling (Okkenhaug et al., 2002).

Class IB

The p110γ (PIK3CG at 7q21.11) catalytic subunit is the sole class IB member and differs from the class IA enzymes in its extreme N-terminus (lacking a p85 binding site) and in its adaptor partner, p101 (PI 3-kinase regulatory subunit gene, at 17p13.1), which lacks domains found in any other proteins. Whereas class IA enzymes are preferentially activated by tyrosine-kinase-mediated signals, the class IB enzyme is linked to G-protein-coupled receptor (GPCR) systems. Activation of class IB seems to predominantly involve interactions with Gβγ subunits and also possibly Gα subunits (Katso et al., 2001). Recent results from knockout mouse models suggest that p110γ plays a key role as a modulator of inflammation and allergy (Wymann et al., 2003), and also in the regulation of cardiac contractility (Crackower et al., 2002).

PI 3-kinase core structure

PI 3-kinase family classification is largely based on sequence alignments that define four major blocks of sequence similarity in members of the class I family, termed homology regions (HR) 1-4. X-ray crystal structures of these four domains from p110γ, alone and in complex with Ras or PI 3-kinase inhibitors, have been determined. This provides a basis for understanding this family of kinases at a molecular level (Walker et al., 1999; Djordjevic and Driscoll, 2002). HR1 (shown in grey) is the kinase core domain and is the only domain that is found in all four PI 3-kinase classes. This domain exhibits weak homology to protein kinases. HR2 (also known as the PIK domain – shown in green) seems, from the available structural data, to play a scaffolding role for the other domains. HR3 is a C2-like domain (small blue circle). C2 domains in other proteins mediate interactions with lipids or with other proteins in either a calcium-dependent or calcium-independent manner. The position of this domain in the PI 3-kinase structure suggests a role in general membrane binding and possibly substrate targeting (Walker et al., 1999). HR4 (shown in red) is a putative Ras-binding domain or small G-protein-binding domain, which has only been found in class I and II PI 3-kinases. To date, the HR4 domain has only been shown to have functional effects through interactions with Ras in class I PI 3-kinases (Katso et al., 2001).

Class II PI 3-kinases

Class II PI 3-kinases were identified through homology and PCR cloning approaches and for this reason are the least well understood class of PI 3-kinase (Fry, 2001). Invertebrates have a single representative of this class of PI 3-kinase, whereas mammals and fish (C.J.T. and M.J.F., unpublished) have three class II PI 3-kinases. In humans these class II PI 3-kinases are termed PI3K-C2α (PIK3C2A at 11p15.5-p14), PI3K-C2β (PIK3C2B at 1q32) and PI3K-C2γ (PIK3C2G at 12p12). Whereas class I PI 3-kinases reside mainly in the cytoplasm until recruited to active signalling complexes, the class II PI 3-kinases are largely constitutively associated with membrane structures, including plasma membrane, intracellular membranes and somewhat surprisingly with nuclei (Fry, 2001). Extracellular signals, including integrin engagement, growth factors (e.g. insulin, EGF, SCF and HGF) and chemokines, have all been reported to stimulate class II PI 3-kinase activity (Brown and Shepherd, 2001). No clear mechanism of activation has emerged, with tyrosine phosphorylation, proteolysis and recruitment by adaptor proteins (e.g. Grb2, clathrin) all suggested to play a role in different cell types or receptor systems. Currently there is no clearly defined cellular role for these enzymes or any clear consensus on their in vivo products. In vitro, class II PI 3-kinases can phosphorylate PtdIns and PtdIns(4)*P*, but, unlike class I PI 3-kinases, not PtdIns(4,5)P_2. Class II PI 3-kinases have not been isolated in association with a regulatory subunit, but they possess extended N- and C-termini relative to the class I PI 3-kinases, which may serve this function. The N-terminus lacks defined structural domains, but has both coiled-coil (pale blue) and proline-rich (mauve) motifs, which may mediate protein-protein interactions. The extended C-terminus has tandem PX (in yellow) and C2 (large blue circle) domains, the functions of which have yet to be established in class II PI 3-kinases. Based on structural and functional studies on their roles in other proteins, these domains are likely to mediate binding to membrane lipids or protein-protein interactions (Djordjevic and Driscoll, 2002).

Class III PI 3-kinases

Class III PI 3-kinases are exemplified by the sole *Saccharomyces cerivisiae* PI 3-kinase, Vps34 (Fry, 1994; Wurmser et al., 1999; Katso et al., 2001). Unlike class I and II PI 3-kinases, this enzyme can phosphorylate only PtdIns and is thus a PtdIns 3-kinase. All eukaryotes investigated have a single Vps34 homologue. The human gene (PIK3C3) is located on chromosome 18 at 18q12.3. A single 150 kDa regulatory subunit (in yeast VPS15, in humans PIK3R4, located at 3q22.1) possesses intrinsic protein-serine kinase activity that is required for Vps34 function (Wurmser et al., 1999). Originally isolated as a mutant of vesicle-mediated vacuolar protein sorting in yeast, class III PI 3-kinases are implicated in endosome fusion during intracellular trafficking events and are located mainly on

intracellular membranes (Wurmser et al., 1999). Recent studies suggest that this class of PI 3-kinase is involved in diverse intracellular trafficking events including autophagy (Kihara et al., 2001) and phagosome formation (Vieira et al., 2001), internal vesicle formation within multivesicular endosomes (Futter et al., 2001), retrograde endosome to golgi transport (Burda et al., 2002) and transport at the nuclear membrane (Roggo et al., 2002).

This is an exciting time in PI 3-kinase research with various members of this family being linked to key cellular processes and to many human diseases. The major players are now all known and what remains is for us to tease out the individual functions of the isoforms.

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