Summary

It has long been known that phosphoinositides are present in cellular membranes, but only in the past four decades has our understanding of their importance for proper cell function advanced significantly. Key to determining the biological roles of phosphoinositides is understanding the enzymes involved in their metabolism. Although many such enzymes have now been identified, there is still much to learn about their cellular functions. Phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks) are a group of kinases that catalyse the production of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P$_2$]. As well as being a substrate for the enzymes phospholipase C (PLC) and phosphatidylinositol 3-kinase (PI3K), PtdIns(4,5)P$_2$ acts as a second messenger in its own right, influencing a variety of cellular processes. In this Commentary, we review how PIP5Ks are modulated to achieve regulated PtdIns(4,5)P$_2$ production, and discuss the role of these proteins in different cellular processes.

Key words: PIP5K1, PIP5K, PtdIns(4,5)P$_2$, Phosphatidylinositol, Phosphatidylinositol (4,5)-bisphosphate, Phosphatidylinositol 4-phosphate 5-kinase

Introduction

The phosphoinositide family consists of seven derivatives of phosphatidylinositol (PtdIns) that are formed through the phosphorylation of the 3-, 4- and 5-positions of the inositol ring (Fig. 1A). Despite their low abundance in the cell, phosphoinositides are important regulators of a large variety of cellular processes. The production of the different phosphoinositide species is spatially and temporally regulated through the actions of kinases, phosphatases and phospholipases (Fig. 1A), some of which can be localised in different subcellular compartments.

Among phosphoinositides, phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P$_2$] has been particularly well studied. Although initial interest in PtdIns(4,5)P$_2$ was centred on its role as a substrate for phospholipase C (PLC), which produces inositol (1,4,5)-trisphosphate [Ins(1,4,5)P$_3$] and diacylglycerol (DAG) (Michell, 1975), and phosphatidylinositol 3-kinase (PI3K), which produces phosphatidylinositol (3,4,5)-trisphosphate (Ins(3,4,5)P$_3$), it is now generally acknowledged that PtdIns(4,5)P$_2$ also fulfils an important role as a second messenger itself (Oude Weernink et al., 2004b). As a second messenger, PtdIns(4,5)P$_2$ affects cell migration by influencing actin-fibre formation and the formation and turnover of focal contacts, but also affects cell-cell adhesion, cytokinesis, the stress response, apoptosis, and nuclear processes such as cell-cycle progression and splicing.

PtdIns(4,5)P$_2$ can be synthesised through the action of two distinct but related phosphoinositide kinases. Phosphatidylinositol 5-phosphate 4-kinase (PIP4K) (EC 2.7.1.149) phosphorylates phosphatidylinositol 5-phosphate [PtdIns(5)P] at the 4-position, whereas phosphatidylinositol 4-phosphate 5-kinase (PIP5K) (EC 2.7.1.68) phosphorylates phosphatidylinositol 4-phosphate [PtdIns(4)P] at the 5-position to form PtdIns(4,5)P$_2$. Because the cellular level of PtdIns(4)P is approximately ten times higher than that of PtdIns(5)P, the major synthetic pathway for the formation of PtdIns(4,5)P$_2$ is probably through the activity of PIP5Ks. PIP5Ks can also phosphorylate PtdIns and phosphatidylinositol 3-phosphate [PtdIns(3)P] at the 5-position, albeit to a lesser extent (Zhang et al., 1997; Toliass et al., 1998). Finally, it is important to mention that the phosphorylation of PtdIns(3,4)P$_2$ at the 5-position to generate phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P$_3$] can also be catalysed by PIP5K in vivo (Halstead et al., 2001) (Fig. 1B). The Schizosaccharomyces pombe homologue of PIP5K, Iis3, also has this ability, and can generate both PtdIns(3,4,5)P$_3$ and PtdIns(4,5)P$_2$ (Mitra et al., 2004). As the fission yeast S. pombe does not contain a class-I PI3K, the production of PtdIns(3,4,5)P$_3$ by PIP5K might represent the original pathway for PtdIns(3,4,5)P$_3$ synthesis.

As mentioned above, PtdIns(4,5)P$_2$ itself can act as a second messenger in various cellular processes such as migration, adhesion, cell division and polarity. These are all essentially regulated by the activity and localisation of the kinases that produce PtdIns(4,5)P$_2$ – the PIP5Ks. In this Commentary, we highlight some of the important roles that PIP5Ks and their product, PtdIns(4,5)P$_2$, play in these processes, and highlight the mechanisms that regulate PIP5K activity.

The PIP5K family

Three isoforms of PIP5K have been identified and are known as PIP5K$_{\alpha}$, PIP5K$_{\beta}$ and PIP5K$_{\gamma}$ (Ishihara et al., 1996; Loijens and Anderson, 1996; Oude Weernink et al., 2004b). PIP5K$_{\alpha}$ and PIP5K$_{\beta}$ each have a molecular mass of 64 kDa. Mouse PIP5K$_{\gamma}$ has three different splice variants of 69 or 72 kDa in size (Box 1). The nomenclature of PIP5K isoforms has become somewhat confusing because the nomenclature for mouse genes is opposite to that of human genes (human PIP5K$_{\alpha}$ is similar to mouse PIP5K$_{\beta}$ and vice versa). In addition, several synonyms are currently in use for this protein family, including PIP5K, PIPK1, PIPK1 and PI5Kin. Here, we use the term PIP5K for this family together with the isoform nomenclature that is used for the human proteins, because this terminology is now used by the National Centre for Biotechnology (NCBI) (Box 1). Further variation in the sequence of PIP5Ks is created through the generation of splice variants. In mice, eight ?, two ? and three ? splice variants have been described in the Ensemble database, whereas for humans three ?, four ? and
Fig. 1. The generation of phosphoinositides by PIP5K. (A) Diagram of the seven members of the phosphoinositide family, the pathways that generate the different species and the enzymes involved. (B) PIP5K can generate three different species of phosphoinositide using different substrates [PtdIns(4)P, PtdIns(3,4)P₂ or PtdIns(3)P]. The grey areas represent the hydrophobic region of the membrane in which the acyl chains of phosphoinositides are inserted, whereas the polar head groups are exposed on the membrane surface. Red circles represent a newly added phosphate group. p235PIKfyve, phosphatidylinositol 3-phosphate 5-kinase; SHIP, SH2 domain-containing inositol-5'-phosphatase.
only one γ splice variant have been described (http://xmap.picr.man.ac.uk) (Box 1). A PIP5K isoform called PIP5KH (also known as PIP5KL1) has been identified in humans (Chang et al., 2004). This isoform has a molecular weight of 44 kDa, but lacks the full catalytic domain and does not seem to have catalytic activity. However, when it is expressed in cells, PIP5KH induces PtdIns(3,4,5)$P_3$ synthesis, probably through its interaction with and activation of other PIP5K isoforms. A pseudogene called MGC26597 has also been identified in humans (Hoffmann and Valencia, 2004) (http://www.ihop-net.org/).

The PIP5K proteins have a well-conserved central region that includes the kinase catalytic domain. Outside of this region there is little sequence conservation between the different isoforms. All isoforms contain a subdomain within the catalytic domain called the activation loop, which differs in sequence between the PIP5K and the PIP4K family. This loop determines substrate specificity, because substitution of specific amino acids in this domain of PIP5K to the corresponding amino acids in PIP4K switches its substrate specificity from PtdIns(4)$P_2$ to PtdIns(5)$P_2$. The subcellular localisation of this mutated form of PIP5K is also different, which suggests that the substrate specificity and subcellular localisation of PIP5K are intimately linked (Kunz et al., 2000; Kunz et al., 2002).

The subcellular localisation of the different PIP5K isoforms has been characterised, although visualisation of the endogenous proteins has not always been possible. PIP5Kα localises to the plasma membrane and the Golgi complex, and has also been observed at sites of membrane ruffling induced by the Rho GTPase Rac. Interestingly, PIP5Kα has also been observed in the nucleus in structures known as nuclear speckles (Mellman et al., 2008). In general, PIP5Kβ localises to the plasma membrane but is also found on vesicles in the perinuclear region of the cell (Doughman et al., 2003). Mouse PIP5Kγ661 has a 26-amino-acid region that specifies its localisation to focal adhesions through its ability to interact with the focal-adhesion protein talin (Di Paolo et al., 2002). This isoform is also present at adherens junctions in epithelial cells, where it colocalises with cadherin (Ling et al., 2007). Surprisingly, unlike mouse PIP5Kγ661, rat PIP5Kγ688 (also known as PIP5KγC) is not present at focal contacts, even though it contains the same 26 amino-acid residues that are thought to be important for the targeting of PIP5Kγ661 to this location (Giudici et al., 2006). Although visualisation of endogenous PIP5K suggests that the various isoforms are differentially localised, overexpressed GFP-labelled PIP5Ks localise strongly at the plasma membrane. The reason for this discrepancy is not clear but might be related to the increased PtdIns(4,5)$P_2$ levels as a consequence of the overexpression.

### Box 1. PIP5K isoforms in mice and humans

The isoforms and splice variants of PIP5Ks in humans and mice show subtle but important differences. The differences in PIP5Kγ splice variants is particularly important, because no splice variants have been identified in humans so far, whereas in mice there are three that have been described in several reports to behave differently. The diagram of the splice variants was generated using data from the EMBL database and analysed with Xmap software. The chromosomal locations are shown together with amino-acid number for the PIP5Kγ splice variants. Exons are drawn to scale, with translated regions shown in dark orange and untranslated regions in yellow. All transcripts are orientated from left to right.

Generation of the PIP5Kγ splice variants occurs through inclusion or exclusion of the following fragments:

- PIP5Kγ661 is the longest and most complete splice variant.
- PIP5Kγ635 is generated by deletion of 26 amino acids between amino acids 635-661.
- PIP5Kγ627 is generated by deletion of 60 amino acids between amino acids 341-401 and an insertion of 26 amino acids at amino acid 635.

In the rat, the homologue of mouse PIP5Kγ661 is called PIP5Kγ662, whereas there are two splice variants containing the 26-amino-acid insertion at amino acid 636, called PIP5Kγ688 and PIP5Kγ628. PIP5Kγ628 also has a deletion between amino acids 347-407.
Indeed, overexpressed kinase-inactive PIP5Ks often do not localise strongly to the plasma membrane (Giudici et al., 2006). A notable exception, however, is that overexpressed rat PIP5Kγ688 was reported to localise to an intracellular membrane compartment (Giudici et al., 2006). Therefore, it is clear that there is non-overlapping localisation of the different isoforms, suggesting that they have different cellular functions. The role of different splice variants still remains unclear, but might prove to add an extra layer of complexity to the PIP5K family.

**Regulation of PIP5K activity and localisation**

The local production of Ins(1,4,5)P$_3$ and PtdIns(3,4,5)P$_2$ from PtdIns(4,5)P$_2$, and the second-messenger function of PtdIns(4,5)P$_2$, suggest that the levels of PtdIns(4,5)P$_2$ cannot be uniformly distributed in the cell, but rather that there are different pools of PtdIns(4,5)P$_2$ that are temporally and spatially regulated. The generation of local concentrations of PtdIns(4,5)P$_2$ might be achieved by regulating either its degradation or its synthesis, possibly through specific regulation of the activity of PIP5K isoforms.

**Regulation by Rho and Rac**

PtdIns(4,5)P$_2$ can interact with several proteins that regulate the actin cytoskeleton, such as vinculin, α-actinin, talin and actin-capping proteins, which suggests that PtdIns(4,5)P$_2$ has a role in regulating cytoskeletal dynamics. The small GTPases of the Rho family are also key regulators of the actin cytoskeleton (Etienne-Manneville and Hall, 2002). Regulation of PIP5Ks by Rho-family proteins provides a potential link between these two pathways. In *Saccharomyces cerevisiae*, the activity of PIP5K (Mss4) seems to be regulated upstream of the activation of two Rho guanine-nucleotide exchange factors (GEFs) (Desrivieres et al., 1998). By contrast, in mammalian cells, PIP5K seems to be a downstream effector of Rho activation (Shibasaki et al., 1997). Cell adhesion and the activation of integrins also regulate the levels of PtdIns(4,5)P$_2$, which in turn is required to maintain receptor-mediated Ca$^{2+}$ signalling. The requirement of integrin activation for the stimulation of PtdIns(4,5)P$_2$ synthesis can be bypassed by the overexpression of activated forms of Rho GEFs or of Rho itself (Ren and Schwartz, 1998). Furthermore, recombinant RhoA can interact with and activate PIP5K (Oude Weernink et al., 2004a), although it is not clear whether RhoA regulates a specific isoform of PIP5K.

The interaction between Rho and PIP5K does not depend on the nucleotide loading of Rho. However, in an in vitro cell-lysate assay system, Rho-mediated PIP5K activation was found to depend on Rho-GTP. The activation of PIP5K is probably not due to a direct interaction between PIP5K and Rho, because Rho kinase (ROCK) has been implicated in the activation of PIP5K (Oude Weernink et al., 2000) (Fig. 2A). In platelets, the binding of thrombin to the G-protein-coupled receptor PAR1, or overexpression of a dominant-active G-protein Gzq, leads to the translocation of PIP5Kα from the perinuclear region to the plasma membrane and an increase in its activity. This effect is dependent on the activity of Rho downstream of Rac but upstream of ROCK (Chatah and Abrams, 2001; Yang et al., 2004).

In neuronal cells, the activation of Rho in response to guidance signals leads to neurite remodelling. Moreover, neurite retraction requires both the RhoA-ROCK pathway and PIP5Kβ activation (Yamazaki et al., 2002; van Horck et al., 2002). Whether PIP5Kβ activation occurs directly downstream of Rho signalling is not clear. However, Rac-regulated localisation of PIP5Kβ at the plasma membrane is also required for neurite retraction, suggesting that there is crosstalk between the Rho and Rac pathways during this process. Rac can interact directly with all PIP5K isoforms in a GTP-independent manner (Oude Weernink et al., 2004a). Unlike the interaction of Rac with most other effectors, the interaction between Rac and PIP5K requires the C-terminal polybasic region (PBR) of Rac. Importantly, a point mutation in the C-terminus of Rac that attenuates PIP5K binding also disrupts Rac1-induced actin polymerisation in permeabilised platelets, whereas the interaction between PIP5K and Rac modestly increases (1.5-fold) the activity of PIP5K (Tolias et al., 2000). However, our own studies of neuroblastoma cells suggest that the major function of Rac is to regulate the localisation of PIP5Kβ, as a mutant of PIP5Kβ that does not bind to Rac is no longer localised at the plasma membrane although its activity remains unchanged (I.v.d.B., N.D., Jonathan R. Halstead and Nicolai E. Savaskan, unpublished data.)

PIPKβ interacts with Ajuba, a LIM-domain-containing protein that targets the Rac activator p130Cas to focal-adhesion sites (Pratt et al., 2005). The interaction between Ajuba and PIP5Kβ results in the localisation of these proteins at the leading edge of the cell, where increased levels of PtdIns(4,5)P$_2$ are observed (Kisseleva et al., 2005). Therefore, Ajuba targets PIP5Kβ to regions at which Rac is present, which allows Rac to activate PIP5K and leads to PtdIns(4,5)P$_2$ synthesis, which in turn induces the branching and reorganisation of actin fibres through the activation of proteins such as neural Wiskott-Aldrich syndrome protein (N-WASP) (Fig. 2B). Interestingly, the PBR of Rac also interacts with PtdIns(4,5)P$_2$ and this interaction is important for Rac plasma-membrane localisation. Therefore, Rac seems to regulate PIP5K localisation and activity, but the product of PIP5K activity, PtdIns(4,5)P$_2$, might also influence the localisation and activity of Rac.

**Regulation by ARF**

ADP-ribosylation factors (ARFs) are a family of small GTPases that control membrane trafficking and actin cytoskeletal dynamics (Aikawa and Martin, 2005), and have also been shown to influence PIP5K activity. Overexpression of ARF6 increases PtdIns(4,5)P$_2$ levels at the plasma membrane, and ARF1 and ARF6 can interact with and activate PIP5K in the presence of phosphatidic acid (PA) (Martin et al., 1996; Honda et al., 1999). Overexpression of a constitutively active form of ARF6 (Q67L) induces the formation of large internal vesicle structures, probably through the fusion of endocytic vesicles that are prevented from recycling back to the plasma membrane (Aikawa and Martin, 2005). These vesicles are rich in PtdIns(4,5)P$_2$ and are coated in actin. Under normal conditions, PtdIns(4,5)P$_2$ is rarely found on internal vesicles. A similar phenotype to that seen upon ARF6 (Q67L) overexpression is observed when PIP5K is overexpressed in cells and, because PIP5K-induced vesicle formation is not blocked by a dominant-negative form of ARF6, PIP5K probably acts downstream of ARF6 to induce non-clathrin-mediated endocytosis. Data also suggest that the removal of PtdIns(4,5)P$_2$ from vesicles (either by the action of phosphatases or by phospholipases) is required for the efficient recycling of membrane components back to the plasma membrane (Brown et al., 2001; Aikawa and Martin, 2003). ARFs have also been implicated in the regulation of cytoskeletal dynamics and have been shown to regulate axonal growth. Overexpression of a dominant-negative mutant of the ARF-GEF ARNO leads to an increase in axonal length and increased arborisation, but both processes are attenuated by the co-overexpression of PIP5K. Therefore, active ARF is important for PIP5K activation and
Regulation and functions of PIP5K

PtdIns(4,5)P$_2$ synthesis, which in turn inhibits axonal growth (Hernandez-Deviez et al., 2004). This is in line with previous data showing that the overexpression of PIP5K can attenuate neurite outgrowth (van Horck et al., 2002; Yamazaki et al., 2002).

**Regulation by PA and phospholipase D**

PA seems to be an important activator of PIP5K, because the activity of purified PIP5K is stimulated by PA (Moritz et al., 1992). Furthermore, it has been proposed that PA can regulate the affinity of PIP5K for PtdIns(4)P (Jarquin-Pardo et al., 2007). PA is generated through the hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) or through the phosphorylation of DAG by diacylglycerol kinase (DGK) (Kanaho et al., 2007). In support of a role for PA in PIP5K activation, both PLD2 and DGKζ show extensive colocalisation and interaction with PIP5K (Divecha et al., 2000; Luo et al., 2004). Interestingly, PtdIns(4,5)P$_2$ is required as
a cofactor for PLD activation, and DGKζ has a pleckstrin-homology (PH) domain that can bind to PtdIns(4,5)P₂ (N.D., unpublished data). A model has been proposed whereby ARF6 activates both PLD and PIP5K to generate PA and PtdIns(4,5)P₂. PA generated by PLD activates PIP5K, and PtdIns(4,5)P₂ generated by PIP5K activates PLD. Thus, ARF6 might act as a switch for the initial activation of both enzymes, which drives a feed-forward loop resulting in the increased synthesis of PA and PtdIns(4,5)P₂ (Fig. 3). Changes in the membrane composition of PtdIns(4,5)P₂ and PA, which might be influenced by the PLD-PIP5K loop, can drive clathrin- and non-clathrin-mediated endocytosis (Arneson et al., 1999; Brown et al., 2001). This loop might also be important for integrin-mediated adhesion, because the loss of PLD abrogates cell adhesion that can be rescued by the addition of PA or PtdIns(4,5)P₂, whereas a dominant-negative form of PIP5K also attenuates cell adhesion (Powner et al., 2005).

Regulation by ARF6
Talin is an integrin-binding protein that can alter the affinity of integrins for their ligand and so affect cell signalling and adhesion. PIP5Kγ can bind to talin in focal contacts (Ling et al., 2002; Di Paolo et al., 2002). The mouse PIP5Kγ splice variant PIP5Kγ661 contains an exon coding for 26 amino acids at the C-terminus that is not present in PIP5Kγ635 and that is essential for the localisation of PIP5Kγ to focal contacts (Ling et al., 2002). Overexpression of PIP5Kγ661, but not PIP5Kγ635, induces the loss of talin from focal adhesions independently of the lipid-kinase activity of PIP5Kγ661 (Box 1). Moreover, the expression of the 26-amino-acid C-terminal region of PIP5Kγ661 can induce the loss of talin from focal contacts. Talin localises to focal adhesions through its interaction with β-integrins and, because PIP5Kγ661 competes for the same binding site on talin, it can negatively regulate the talin–β-integrin interaction (Di Paolo et al., 2002; Barsukov et al., 2003). The frequency of interaction between talin and PIP5Kγ661 is dramatically increased by the phosphorylation of the Y649 residue in PIP5Kγ661 by the Src tyrosine kinase, and phosphorylation at this site seems to antagonise the phosphorylation of the adjacent S650 residue. S650 can be phosphorylated by cyclin-dependent kinase 5 (Cdk5) and its activator p35; such phosphorylation negatively regulates the interaction between talin and PIP5Kγ661 (Ling et al., 2003; Lee et al., 2005). Therefore, phosphorylation of PIP5Kγ661 regulates its interaction with talin and, through competitive binding, the PIP5Kγ661-talin interaction can regulate the interaction of talin with β-integrins.

Regulation by Brutons tyrosine kinase
In activated B cells, Brutons tyrosine kinase (BTK) binds to PIP5K and induces the translocation of PIP5K to the plasma membrane (Saito et al., 2003). The translocation of PIP5K is independent of the kinase activity of BTK but requires the association of the PH domain of BTK with PtdIns(3,4,5)P₃ (Saito et al., 2003; Carpenter, 2004). Enhanced localisation of PIP5K at the plasma membrane stimulates increased PtdInsP₂ and PtdIns(3,4,5)P₃ synthesis in response to B-cell-receptor stimulation, which in turn enhances Ca²⁺ signalling through an increase in Ins(3,4,5)P₃ synthesis (Carpenter, 2004).

Regulation of PIP5Kβ during Wnt signalling
PtdIns(4,5)P₂ regulates the aggregation and subsequent phosphorylation of low-density lipoprotein receptor-related protein 6 (LRP6), which is part of the cell-surface receptor complex activated by Wnt3A (Pan et al., 2008). More specifically, Wnt3A activates PIP5Kβ through Frizzled and Dishevelled, which are also part of the Wnt-receptor complex, leading to an increase in PtdIns(4,5)P₂ at the plasma membrane. Direct binding between Dishevelled and PIP5Kβ or PIP5Kα has been detected in vitro, where the interaction between Dishevelled and PIP5Kβ was found to stimulate PIP5Kβ activity. The effect of PIP5Kβ knockdown on LRP6 phosphorylation was much greater than observed after knockdown of either PIP5Kα or PIP5Kγ, suggesting that PIP5Kβ is the main isoform involved in this pathway (Pan et al., 2008). It is not yet clear, however, how the increased PtdIns(4,5)P₂ levels regulate LRP6 phosphorylation.

Regulation by the retinoblastoma-susceptibility gene product
PIP5K activity is present in the nucleus, where it can phosphorylate PtdIns(4)P to generate PtdIns(4,5)P₂. Fractionation studies have demonstrated that PIP5K activity associates with the inner matrix of the nucleus (Payrastre et al., 1992), and further studies using antibodies specific for PIP5K suggest that it is localised in nuclear speckles, which are highly enriched in splicing factors (Boronenvok et al., 1998). How PIP5K is regulated in the nucleus is not clear, but we presume that there are nucleus-specific upstream regulators of its activity. Nuclear PIP5K activity is highly upregulated as cells progress through the cell cycle, during differentiation and under conditions of oxidative stress (Clarke et al., 2001). The retinoblastoma-susceptibility gene product (pRB) is a master regulator of cell-cycle progression through G1 and into S-phase, and acts as a gatekeeper to ensure that conditions are favourable for cell division. PIP5K can associate with pRB, and this association induces the activation of PIP5K activity and PtdIns(4,5)P₂ synthesis (Divacheva et al., 2002).

Phosphorylation of PIP5Ks
As discussed above, several proteins can regulate the activity of the PIP5K family. A common regulatory mechanism is the phosphorylation of specific sites in the protein that allows binding partners to interact with or dissociate from PIP5K, or that leads to conformational changes resulting in changes in its kinase activity. For example, PIP5Kβ is phosphorylated at S214 by the cAMP-dependent protein kinase PKA, which leads to a modest reduction in the lipid-kinase activity of PIP5Kβ (Park et al., 2001). Following stimulation with either lysophosphatidic acid (LPA) or phorbol 12-myristate 13-acetate (PMA), overexpressed PIP5Kβ is dephosphorylated and there is a modest increase in its activity. The authors of this study suggested that crosstalk between the

Fig. 3. A feed-forward loop regulates PA and PtdIns(4,5)P₂ production. After ARF6 has activated PIP5K and/or PLD, a feed-forward loop is activated in which PLD-dependent PA production leads to the activation of PIP5K, PtdIns(4,5)P₂ and PLD. Lipid enzymes are shown in orange and lipid products in green. Black arrows denote activation of a downstream protein or process, and green arrows denote conversion to a lipid product.
cAMP pathway and PKC activation might regulate PIP5Kβ activity.

PIP5Kγ661 is phosphorylated at two adjoining residues in its C-terminal tail: Y649 and S650 (Ling et al., 2002; Di Paolo et al., 2002). It was suggested that phosphorylation of Y649 by Src directly increases the affinity of PIP5Kγ for talin (Ling et al., 2002). However, it is likely that phosphorylation of Y649 modulates phosphorylation at S650, which subsequently regulates talin binding (as discussed above) (Lee et al., 2005).

Interestingly, PIP5K is not only a lipid kinase but can also autophosphorylate itself. PIP5K autophosphorylation is greatly stimulated in vitro by the addition of PtdInsPs and results in a decrease in the activity of PIP5K (Itoh et al., 2000). Whether autophosphorylation plays a role in regulating PIP5K activity in vivo is unclear.

Physiological functions of PIP5Ks

Links to disease in humans

A recent report has linked the mutation of human PIP5Kγ to a lethal congenital contracture syndrome type 3 (LCCS3) characterised by multiple joint contractures, micrognathia and anterior-horn atrophy in the spinal cord (Narkis et al., 2007). The origin of this disease was traced back to a mutation (G757A) in the kinase domain of PIP5Kγ that renders the protein unable to phosphorylate PtdIns(4)P (Narkis et al., 2007). PIP5Kγ is highly expressed in the brain and the symptoms are linked to major neurological defects. In neurons, PtdIns(4,5)P2 is important for different processes, including synaptic-vesicle endocytosis and neurite outgrowth. A lack of PtdIns(4,5)P2 might impinge on these processes, resulting in the neurological defects found in LCCS3.

The activity of phosphatidylinositol 4-kinase (PI4K) and of PIP5K is increased in different hepatoma cell lines compared with that in normal liver cells (Singhal et al., 1994). Together with the fact that PtdIns(3,4,5)P3 levels are altered in many cancers because of mutations of the phosphatase and tensin homolog (PTEN) (which negatively regulates the levels of PtdIns(3,4,5)P3), we suggest that increased PtdIns(3,4,5)P3 levels (through changes in PIP5K activity) might also be important to sustain increased PtdIns(3,4,5)P3 production during cancer progression.

Evidence from gene deletions in mice

Knockout mice have been generated for all three isoforms of PIP5K, with the effects on their phenotype varying between each knockout. A genetrap knockout mouse, in which a target gene is disrupted by the insertion of a marker gene containing a stop codon, was generated for PIP5Kα. These mice had a normal phenotype except for poorer breeding capacity than their wild-type counterparts (Wang et al., 2008b). However, platelet aggregation in these animals was inhibited by the PAR4 thrombin-receptor-agonist peptide, thromboxane-receptor agonist or ADP. Interestingly, platelets from PIP5Kα-knockout mice showed diminished PtdIns(4,5)P2 production, PLC activation and Ins(1,4,5)P3 production after thrombin treatment. This suggests that PIP5Kα is the major isoform responsible for the replenishment of PtdIns(4,5)P2 after stimulation of G-protein-coupled receptors. PIP5Kβ-knockout mice develop normally but show increased degranulation and cytokine production by mast cells when they are activated via the Fcε receptor, suggesting that PIP5Kβ is a negative regulator of Fcε-receptor signalling. PIP5Kβ-knockout mice also show an increased susceptibility to type-I-hypersensitivity allergic reactions, in which mast cells are involved (Sasaki et al., 2005). Finally, two knockout mice for PIP5Kγ have been generated. In the first PIP5Kγ knockout, the majority of the kinase domain was deleted, giving rise to mice that were born without any obvious abnormalities but that died within 24 hours of birth. PIP5Kγ is highly expressed in the brain, and these mutant mice showed a reduction in clathrin-coated endocytosis and a reduction in the exocytosis of a small recyclable pool of synaptic vesicles (Di Paolo et al., 2004). Another PIP5Kγ-knockout mouse strain has been generated using genetrap methodology. These mutant mice had a severe phenotype with embryonic lethality at the organogenesis stage, and abnormalities in the cardiovascular and nervous systems (Wang et al., 2007). The reason for the very different phenotypes of the two PIP5Kγ-knockout mice is not known.

It is clear from studies of the various knockout mice that different isoforms of PIP5K function in different capacities in an organism. However, how this relates to the cellular function of different PIP5Ks is not clear. For instance, in platelets derived from mice, Ins(1,4,5)P3 production in response to thrombin, a G-protein-coupled-receptor agonist, requires the activity of PIP5Kα and PIP5Kβ, but not PIP5Kγ (Sasaki et al., 2005). This conflicts with data obtained in HeLa cells, in which Ins(1,4,5)P3 production in response to another G-protein-coupled-receptor agonist, histamine, seemed to be dependent on PIP5Kγ (Wang et al., 2004). Knockdown of PIP5Kγ only reduced the levels of PtdIns(4,5)P2 by approximately 13%, yet this decrease attenuated Ins(1,4,5)P3 generation to almost basal levels. How a drop in the levels of PtdIns(4,5)P3 by 13% can attenuate Ins(1,4,5)P3 signalling is not clear, but these results suggest the presence and maintenance of pools of PtdIns(4,5)P3 that are dedicated for specific cellular functions. Because studies with fluorescently labelled PtdIns(4,5)P3 suggest that its lateral diffusion is very rapid, isoform-specific recruitment and activation of PIP5Ks to localised areas of receptor signalling at the plasma membrane probably occurs to specifically supply the PtdIns(4,5)P3 needed for Ins(1,4,5)P3 generation in response to histamine or thrombin. It is not clear why the other 87% of PtdIns(4,5)P3 present in the cell cannot be used by PLC to generate Ins(1,4,5)P3. It is conceivable that this pool of PtdIns(4,5)P3 might be sequestered by interactions with cytoskeletal elements and therefore might not be available for breakdown induced by G-protein-coupled receptors. Alternatively, this pool could be present in other intracellular organelles such as the nucleus.

The role of PIP5K in other model organisms

Caenorhabditis elegans expresses only one PIP5K homologue, called PPK-1. RNA interference (RNAi)-mediated depletion of PPK-1 causes a defect in ovulation, reduced gonadal-sheath contractility and sterility (Xu et al., 2007). Increased Ins(1,4,5)P3 signalling can compensate for these defects, suggesting that PPK-1 plays an essential role in Ins(1,4,5)P3 signalling (Xu et al., 2007). PPK-1 also seems to be important during the first asymmetric embryonic cell division, when it localises at the posterior end of the embryo through the action of casein kinase 1 gamma (CSNK-1) (Panbianco et al., 2008). In turn, CSNK-1 activity is regulated by anterior PAR proteins. CSNK-1 regulates not only the localisation of PPK-1 but also its activity. Knockdown of CSNK-1 causes uniformly increased PPK-1 levels and increased symmetric cortical levels of the receptor-independent activators of Gα – GPR-1 and GPR-2 (collectively referred to as GPR-1/2), and LIN-5 – which leads to increased spindle-pulling forces and, in turn, symmetric cell division (Fig. 4). As expected for a negative regulator of PPK-1, loss of CSNK-1 leads to increased levels of PtdIns(4,5)P2. The data
Regulating the actin cytoskeleton and focal adhesions
The capacity of PIP5K to regulate the actin cytoskeleton is important for many of its cellular functions. This role for PIP5K and PtdIns(4,5)P_2 was initially suggested with the observation that PtdIns(4,5)P_2 can interact with profilin, rendering profilin inactive and unable to bind to actin (Lassing and Lindberg, 1985). Since this observation was made, a large number of PtdIns(4,5)P_2-interacting proteins that regulate the actin cytoskeleton have been identified (Gervais et al., 2008; Yin and Janmey, 2003; Oude Weernink et al., 2004b). The functional link between PIP5K and the actin cytoskeleton was established when it was shown that the overexpression of PIP5K altered actin dynamics, leading to the dissolution of stress fibres (Shibasaki et al., 1997), the formation of motile actin comets (Rozelle et al., 2000), cell rounding (van Horck et al., 2002) and increased cell migration (Kisseleva et al., 2005). The effect of PIP5K on actin dynamics is thought to result from the local increase in the levels of PtdIns(4,5)P_2 at the plasma membrane. The increased amount of PtdIns(4,5)P_2 causes the release of actin-capping proteins, resulting in rapid branching of actin (Tolias et al., 2000; Yin and Janmey, 2003) (Fig. 2B). In addition, ezrin is activated after the binding of PtdIns(4,5)P_2, leading to its phosphorylation and thereby unmasking its membrane- and actin-binding sites. This allows ezrin to couple the actin fibres to the plasma membrane (Fig. 2B). This process depends on PIP5K activity, which is regulated by Rac (Matsui et al., 1999; Auvinen et al., 2007; Fievet et al., 2004). Changes in PtdIns(4,5)P_2 levels can also regulate cofilin function, which enhances actin severing to stimulate branching and polymerisation (van Rheenen et al., 2007). In platelets, thrombin stimulation was shown to induce a translocation of PIP5K to the plasma membrane that was dependent on RhoA activation, which is correlated with platelet aggregation (Chatah and Abrams, 2001; Yang et al., 2004). Moreover, a recent study showed that PIP5Kα-expressing mice had decreased thrombus formation, suggesting that PIP5Kα has a function in this process in vivo (Wang et al., 2008a).

In vivo, the loss of PIP5Kβ in mice leads to enhanced anaphylaxis due to decreased filamentous actin and increased degranulation in mast cells (Sasaki et al., 2005). In PIP5Kγ-knockout mice, megakaryocytes (the producers of platelets) showed extensive membrane blebbing and a reduction in the association of the plasma membrane with the actin cytoskeleton. This phenotype was rescued by the introduction of recombinant PIP5Kγ661 but not by the expression of PIP5Kβ (Wang et al., 2008b). Moreover, the loss of talin had a similar effect, which suggests that PIP5Kγ661, together with talin, plays a role in anchoring actin to the plasma membrane.

Overexpression of PIP5Kβ and PIP5Kγ661 can also regulate the stability of focal adhesions, although the mechanism by which this is mediated is not fully understood. The loss of focal adhesions after PIP5Kγ661 overexpression has been linked to the mutually exclusive interactions that occur between talin and PIP5Kγ or talin and the β-integrin receptor (Di Paolo et al., 2002; Barsukov et al., 2003). In this case, the loss of focal adhesions does not seem to depend on the synthesis of PtdIns(4,5)P_2. By contrast, PIP5Kβ overexpression and its effect on focal adhesions is tightly linked to the synthesis of PtdIns(4,5)P_2, because the expression of the kinase-inactive enzyme does not induce focal-adhesion loss. The ability of PIP5Ks to induce focal-adhesion dissolution suggests that PtdIns(4,5)P_2 synthesis might induce the loss of cell-matrix interactions at the rear of migrating cells. Indeed, in neutrophils, both PIP5Kβ and PIP5Kγ are located in the uropod, a structure that suggests the polarised synthesis of PtdIns(4,5)P_2 controls the localisation of GRP-1 and LIN-5 (Panbianco et al., 2008). Overexpression of PIP5K1 leads to an uncoordinated phenotype in C. elegans adults, possibly owing to aberrant neuronal growth. It was shown that PIP5K1 overexpression induces growth-cone collapse when the neurons engage the neuromuscular junction, resulting in normal neurite projections that eventually lead to increased neuronal tangling and aberrant signalling (Weinkove et al., 2008). In Drosophila melanogaster, it has been shown that the PIP5K homologue Skittles (Sktl) is required for chromatin-mediated gene regulation (Cheng and Shearn, 2004) and is important in germline development (Hassan et al., 1998). Mutations in Sktl also prevent the maintenance of polarity in the developing oocyte, and cause defects in actin and microtubule organisation. Loss of polarity is characterised by mislocalisation of polarity components such as Bazooka, Lrg and the PAR proteins (Gervais et al., 2008). In addition, mRNAs that are normally polarised, such as Oskar and Staufen, are also mislocalised in Sktl mutants (Perdigoto et al., 2008).
formed at the rear of a migrating neutrophil (Lacalle et al., 2007; Lokuta et al., 2007). PIP5κβ is targeted to the uropod through its C-terminal tail and binds to the uropod-based complex of ERM and EBPs (Fig. 2B). Interestingly, when it is active, this complex recruits Rho GDP-dissociation inhibitor (RhoGDI) and thereby allows RhoA to be activated, and this induces contraction and uropod release from the substrate. Although ERM proteins can be activated by interacting with PtdIns(4,5)P_2, the kinase activity of PIP5κβ is not essential for uropod retraction (Lacalle et al., 2007). By contrast, PIP5κγ-localisation to the uropod and its kinase activity were found to be important for neutrophil chemotaxis (Lokuta et al., 2007). Therefore, it is possible that both PIP5κβ and PIP5κγ have distinct functions in uropod retraction. In conclusion, it is clear that PtdIns(4,5)P_2 levels—the regulation of PIP5κ activity and localisation—are essential for the regulation of focal adhesions and the actin cytoskeleton.

Cell-cell adhesion
It is clear that PtdIns(4,5)P_2 and PIP5κ play an important role in regulating the actin cytoskeleton and focal-adhesion dynamics. Another structure that is closely linked to the actin cytoskeleton, the adherens junction, can also be influenced by PIP5κs. PIP5κγ661 binds to the cytoplasmic tail of the major adherens-junction component E-cadherin (Ling et al., 2007), resulting in the recycling of E-cadherin to the plasma membrane. The clathrin adaptor complex AP-1 and, more specifically, the adaptin subunit, interacts with PIP5κ to facilitate the recycling of E-cadherin (Ling et al., 2007). Moreover, PIP5κγ-mediated PtdIns(4,5)P_2 synthesis strengthens adherens junctions by competing with actin for the actin-binding protein gelsolin, resulting in an increase in actin-fibre formation (El Sayegh et al., 2007). In vivo, the deletion of PIP5κγ resulted in the disruption of the fascia adherens between cardiomyocytes and the disorganisation of their actin cables. This led to defects in neural tube closure that were attributed to a decrease in the formation of adherens junctions between neuroepithelial cells (Wang et al., 2007). In a different report, it was shown that, in keratinocytes undergoing external Ca^{2+}-induced differentiation, PIP5κα is recruited to the plasma membrane by the E-cadherin–β-catenin complex (Xie et al., 2009). At the plasma membrane, PIP5κα produces the PtdIns(4,5)P_2 that serves as a PI3K substrate for the production of PtdIns(3,4,5)P_3, which in turn activates PLCγ1. Activated PLCγ1 uses the same pool of PtdIns(4,5)P_2 to produce Ins(1,4,5)P_3, which mobilises internal Ca^{2+} stores and drives keratinocyte differentiation (Fig. 5). This paper suggested that cell-cell adhesion molecules regulate the production of a specific pool of PtdIns(4,5)P_2 that is essential for the maintenance of internal Ca^{2+} levels that drive keratinocyte differentiation.

Endo-, exo- and phagocytosis
Synaptic and dense-core vesicles (DCVs) are responsible for the release of neurotransmitters through exocytosis. These vesicles are docked near the plasma membrane and are primed for rapid Ca^{2+}-induced fusion with the plasma membrane (Robinson and Martin, 1998). This process can be recapitulated in vitro using permeabilised cells, and has been shown to depend on PIP5κ and ATP (Hay et al., 1995). In vivo, expression of the dominant-negative Q76L ARF6 mutant inhibits DCV exocytosis, possibly because it induces the relocation of PIP5κ from the plasma membrane to endosomal membranes (Aikawa and Martin, 2003) with a concomitant loss of plasma-membrane PtdIns(4,5)P_2. Overexpression of PIP5κ together with the dominant-negative ARF6 mutant results in the restoration of both PtdIns(4,5)P_2 synthesis and DCV exocytosis (Aikawa and Martin, 2003). PtdIns(4,5)P_2 synthesis might influence the function of synaptotagmin, which interacts with SNARE complexes and can induce the fusion of vesicles with the plasma membrane in a Ca^{2+}-dependent manner (Takenawa and Itoh, 2001). In vivo, the deletion of PIP5κγ leads to early postnatal death and defects in synaptic transmission as a consequence of enhanced synaptic depression, delayed endocytosis and slower recycling (Di Paolo et al., 2004).

Endocytosis of released neurotransmitters is also a crucial step for maintaining synaptic function, and PIP5κγ661 seems to play an important role in this process through its interaction with the β-subunit of the clathrin adaptor complex AP-2. A general role for PIP5κ in the regulation of AP complexes is beginning to emerge, although the molecular details are still not clear. AP complexes consist of two large subunits (α, β1–β4, γ, δ or ε), one medium subunit (μ1–μ4) and one small subunit (σ1–σ4). One of the large subunits mediates an interaction with the membrane, whereas the other recruits clathrin. The μ-subunit is involved in cargo selection. Different AP complexes help to generate the clathrin vesicle coat and determine the protein cargo that is included in the vesicle at various different subcellular membrane compartments (Ohno, 2006). In response to depolarisation, PIP5κγ661 is dephosphorylated by calcineurin, which enhances its interaction with the β-subunit of the AP complex. The interaction between PIP5κγ and AP-2 stimulates PIP5κγ activity, whereas inhibiting this interaction suppresses depolarisation-induced endocytosis (Nakano-Kobayashi et al., 2007) (Fig. 6A). By contrast, PIP5κγ661 has also been shown to interact with the μ1 subunit of the AP-1 complex, where it regulates E-cadherin recycling back to the plasma membrane (Ling et al., 2007). Finally, it has been shown that all three isoforms of PIP5κ interact with the μ2 subunit of the AP-2 complex in vitro. This interaction is mediated by the catalytic domain of PIP5κ (Kraus et al., 2006). Functionally, overexpression of PIP5κα or PIP5κβ, but not PIP5κγ, enhances endocytosis and results in an increase in plasma-membrane-associated AP-2 (Padron et al., 2003).

Two recent papers have described how PIP5κα and PIP5κγ are involved in the regulation of Fcγ-receptor-mediated phagocytosis (Fig. 6B). First, it was shown that PIP5κα is recruited to detergent-resistant membrane fractions containing the bound and clustered Fcγ receptor. Interestingly, this study reported that PIP5κα associates with PtdIns(4,5)P_2 in these clusters (Szymanska et al., 2009). A more detailed study of Fcγ-receptor-induced phagocytosis.
revealed that the α and γ isoforms of PIP5K play distinct roles during this process (Mao et al., 2009). PIP5Kγ-knockout macrophages displayed defective adhesion to IgG partly due to the increased activity of RhoA in these cells, which inhibits the ability of the receptor to cluster. Conversely, the loss of PIP5Kα had no effect on the initial adhesion of IgG but inhibited the subsequent ingestion of IgG due to a decrease in WASP activity, which resulted in the impairment of Arp2/3-dependent actin-fibre formation at the phagocytic cup. Together, these data indicate that different PIP5K isoforms regulate different parts of the same process.

Role of PIP5K and PtdIns(4,5)P₂ in stress responses and apoptosis

The regulation of PtdIns(4,5)P₂ levels have been linked to cell survival and apoptosis because PtdIns(4,5)P₂ is able to bind to and inactivate caspase-3 and consequently protect cells against apoptosis (Azuma et al., 2000). Furthermore, apoptotic stimuli such as hydrogen-peroxide treatment or UV irradiation lead to a depletion in PtdIns(4,5)P₂ levels prior to and independently of caspase activation (Halstead et al., 2006). Overexpression of PIP5Kβ can partially suppress this stress-induced PtdIns(4,5)P₂ depletion and inhibit apoptosis. The depletion of PtdIns(4,5)P₂ in response to stress seems to be the result of the translocation of PIP5Kβ away from the plasma membrane, which also occurs independently of caspase activation (Halstead et al., 2006). Other studies have indicated that PIP5Kα can be cleaved by caspase-3 (Mejillano et al., 2001). It is therefore possible that caspase-independent inhibition of PIP5Kβ might initially induce a reversible decrease in PtdIns(4,5)P₂ levels, whereas longer-term caspase-dependent cleavage of PIP5Kα maintains the depletion of cellular PtdIns(4,5)P₂ levels.

Finally, as mentioned earlier, PIP5Kβ not only generates PtdIns(4,5)P₂ but also PtdIns(3,4,5)P₃. PtdIns(3,4,5)P₃ production is initiated by the stress response after UV radiation or oxidative stress while, at the same time, PtdIns(4,5)P₂ levels drop (Halstead et al., 2001). The ability of PIP5K to generate not only PtdIns(4,5)P₂ but also PtdIns(3,4,5)P₃ suggests that PIP5K is a key regulator of the pathways downstream of both of these phosphoinositides. Although the exact role for increased PtdIns(3,4,5)P₃ and decreased PtdIns(4,5)P₂ during the stress response is not currently understood, these observations might be linked to the fact that many stress-dependent kinases, such as p38 MAPK, are regulated downstream of PtdIns(3,4,5)P₃.

PIPK5, PtdIns(4,5)P₂ and cytokinesis

Similar to migrating cells, dividing cells need to be polarised during cell division. The final step in cell division is cytokinesis, when the two daughter cells separate from one another in an actin-dependent manner. The region of contraction between these two dividing cells is called the cleavage furrow. Active RhoA in this furrow, together with actin, drives the separation of the two cells through the contraction of an actin ring. In addition to RhoA, high levels of PtdIns(4,5)P₂ have been observed in the furrow (Emoto et al., 2005), and depletion of PtdIns(4,5)P₂ leads to delays and defects in cytokinesis (Emoto et al., 2005). In a study of the fission yeast S. pombe, it was shown that a mutation in the PIP5K homologue, Its3, leads to a reduction in its activity and its mislocalisation, which

Fig. 6. PIP5K is an important mediator of endocytosis and phagocytosis. (A) During endocytosis, PIP5K is recruited to the AP-2 complex and undergoes dephosphorylation. Dephosphorylated PIP5K is active and produces PtdIns(4,5)P₂ to increase the binding and clustering of AP-2 complexes, and clathrin is also recruited. Endocytic cups are formed. After fission of the endocytic vesicle, PtdIns(4,5)P₂ levels are reduced on the vesicle, and the AP-2 complex and clathrin coat dissociate. (B) PIP5Kα and PIP5Kγ play distinct roles during Fcγ-receptor-induced phagocytosis. Experiments with knockout cells showed that PIP5Kγ plays a role in regulating the actin cytoskeleton and RhoA activity, allowing Fcγ receptors to cluster. Subsequently, PIP5Kα plays a role during ingestion, during which it regulates WASP and Arp2/3 to allow actin branching.
results in an enhanced septum index (the percentage of cells containing a septum), possibly because of a delay in cytokinesis (Zhang et al., 2000).

The role of PIP5Ks and PtdIns(4,5)P_2 in nuclear function

PIP5Kα has been found to be present on nuclear speckles that are highly enriched in splicing factors. The targeting of PIP5Kα to these speckles depends on the interaction between the C-terminal region of PIP5Kα and nuclear-speckle-targeted PIP5Kα-regulated poly(A) polymerase (Star-PAP) (Mellman et al., 2008). Star-PAP is a poly(A) polymerase that can control the stability of a subset of mRNAs that are regulated in response to oxidative stress. Interestingly, oxidative stress induces an interaction between Star-PAP and PIP5Kα, and Star-PAP activity is highly upregulated by PtdIns(4,5)P_2. These data suggest that changes in nuclear PIP5K activity might coordinate a genetic response to changes in the oxidative environment of the cell (Mellman et al., 2008).

In Drosophila, the PIP5K homologue Sktl also localises to the nucleus (Cheng and Shearn, 2004) where it binds to ASH2, a PHD-domain-containing protein that is involved in chromatin modification. In ash2 mutants, Sktl no longer binds to chromosomes, whereas in both ash2 and sktl mutants, histone H1 is hyperphosphorylated, suggesting that Sktl is targeted to chromosomes to assist in transcriptional regulation (Cheng and Shearn, 2004).

Perhaps the best characterised role for PtdIns(4,5)P_2 in the nucleus is as a substrate for nuclear PLC. In Swiss 3T3 cells, insulin growth factor 1 (IGF-1) stimulates the hydrolysis of nuclear PtdIns(4,5)P_2 to generate DAG, which regulates the function and localisation of protein kinase C (Divecha et al., 1991; Cocco et al., 1988). The predominant nuclear isoform of PLC is PLCβ1, and the activity of this isoform in the nucleus is regulated by MAPK-mediated phosphorylation in response to IGF-1 signalling (Xu et al., 2001; Martelli et al., 1992). Enhanced PtdIns(4,5)P_2 hydrolysis in the nucleus has been linked to cell-cycle progression in Swiss 3T3 cells (Maraldi et al., 1997), attenuation of differentiation in murine erythroleukaemia cells (Matteucci et al., 1998) and enhanced myogenic differentiation of C2C12 cells (Faenza et al., 2003). Hydrolysis of nuclear PtdIns(4,5)P_2 also leads to the generation of Ins(1,4,5)P_3, which might regulate intranuclear levels of Ca^{2+} independently from cytosolic Ca^{2+}. Nuclear Ca^{2+} has been shown to be important in the regulation of gene transcription (Hardingham et al., 1997; Bading, 2000), but whether nuclear Ins(1,4,5)P_3 can specifically regulate Ca^{2+} flux in the nucleus has been a contentious issue (Divecha et al., 1994). Receptors for Ins(1,4,5)P_3 are present in the inner nuclear envelope and application of Ins(1,4,5)P_3 to isolated nuclei induces the release of Ca^{2+} from the nuclear envelope into the nucleus (Malviya et al., 1990; Matter et al., 1993). Recently, agonist-stimulated changes in nuclear Ca^{2+} acting downstream of a nuclear PLC pathway have been convincingly demonstrated (Kumar et al., 2008; Rodrigues et al., 2008). Ins(1,4,5)P_3 can also be further phosphorylated to higher inositol phosphates that, in yeast, have functions in regulating mRNA export (York et al., 1999) and gene transcription (Odom et al., 2000; Jones and Divecha, 2004).

Additional specific roles for nuclear PtdIns(4,5)P_2 have been associated with regulation of a SWI/SNF-like chromatin-remodelling complex, known as BAF, during T-cell-receptor signalling (Zhao et al., 1998), with histone H1 regulation (Cheng and Shearn, 2004; Yu et al., 1998) and with the regulation of splicing of a specific subset of oxidative-stress-regulated mRNAs (Mellman et al., 2008).

Perspectives

PIP5Ks are essential generators of PtdIns(4,5)P_2 that can control myriad cellular processes, including endocytosis, exocytosis, the establishment of cell polarity, cytoskeletal dynamics and apoptosis. The different isoforms of PIP5K can have different and/or non-overlapping effects on these processes. Dramatic changes in total cellular PtdIns(4,5)P_2 levels rarely occur during normal cell signalling, suggesting that cells can establish and maintain specific subcellular pools of PtdIns(4,5)P_2. The lateral diffusion of PtdIns(4,5)P_2 within the plasma membrane is too rapid to allow the generation of such pools solely by the separation of enzymes that generate or degrade PtdIns(4,5)P_2. Rather, the restriction of PtdIns(4,5)P_2 diffusion within the plasma membrane might be a more efficient regulator of its localisation. For example, epithelial cells maintain different PtdIns(4,5)P_2 concentrations at apical and basolateral membranes owing to the movement and/or synthesis of both PtdIns(4,5)P_2 in the different regions and epithelial tight junctions that restrict the diffusion of PtdIns(4,5)P_2. Restriction of phosphoinositide diffusion by components of the actin cytoskeleton, or by the clathrin scaffold, might also be important in phagocytosis and endocytosis, respectively. Proteins that sequester PtdIns(4,5)P_2, such as PIP modulins, can also restrict and concentrate PtdIns(4,5)P_2 locally. Alteration of the binding characteristics of PIP modulins, perhaps through post-translational modifications such as phosphorylation and acetylation, could release PtdIns(4,5)P_2 and permit its interaction with downstream targets. Specific interactions between PIP5Ks and their downstream targets probably enable different PIP5K isoforms to regulate certain signalling processes, and might allow newly synthesised PtdIns(4,5)P_2 to be directly supplied to the targets. If the interaction between a downstream target and PIP5K is a prerequisite for target binding to PtdIns(4,5)P_2, this could explain how some target proteins avoid being constitutively bound by the bulk of cellular PtdIns(4,5)P_2. The binding of PtdIns(4,5)P_2 to AP adaptor proteins and to Star-PAP are both regulated in this manner.

From a clinical perspective, PIP5Ks are interesting molecular targets. PtdIns(4,5)P_2 sits at the heart of two signalling pathways – PI3K and PLC – both of which are upregulated in tumours, and increase cell survival, attenuate apoptosis and stimulate cell migration. Maintenance of these pathways in tumour cells probably requires enhanced PtdIns(4,5)P_2 synthesis compared with normal quiescent cells – that is, tumour cells might have a sort of PtdIns(4,5)P_2 ‘addiction’, which could provide a window for inhibiting PIP5K activity as a strategy for tumour therapy. Pharmacological inhibition of PIP5K might also be useful following neuronal injury. PIP5K activity is essential for Rhod-mediated neuronal retraction, which inhibits neurite outgrowth (Kubo et al., 2007). Therefore, further understanding of the regulation of PIP5K, its interacting partners and how PtdIns(4,5)P_2 is translated into changes in cellular function is likely to yield new and more specific targets for therapy in many different clinical areas.

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