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Phosphoinositide 3-kinase in Health and Disease

Volume 1

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Contents

Introduction 1
Tina L. Yuan and Lewis C. Cantley

PDK1: The Major Transducer of PI 3-Kinase Actions 9
José Ramón Bayascas

Protein Kinase B (PKB/Akt), a Key Mediator of the PI3K Signaling Pathway 31
Elisabeth Fayard, Gongda Xue, Arnaud Parcellier, Lana Bozulic, and Brian A. Hemmings

PI3Ks in Lymphocyte Signaling and Development 57
Klaus Okkenhaug and David A. Fruman

The Regulation of Class IA PI 3-Kinases by Inter-Subunit Interactions ... 87
Jonathan M. Backer

Phosphoinositide Signalling Pathways in Metabolic Regulation 115
Lazaros C. Foukas and Dominic J. Withers

Role of RAS in the Regulation of PI 3-Kinase 143
Esther Castellano and Julian Downward

More Than Just Kinases: The Scaffolding Function of PI3K 171
Carlotta Costa and Emilio Hirsch

PI3K Signaling in Neutrophils 183
Phillip T. Hawkins, Len R. Stephens, Sabine Suire, and Michael Wilson

PI 3-Kinase p110β Regulation of Platelet Integrin $\alpha_{IIb}\beta_3$ 203
Shaun P. Jackson and Simone M. Schoenwaelder

Regulatory Subunits of Class IA PI3K 225
David A. Fruman

The Neurodevelopmental Implications of PI3K Signaling 245
Kathryn Waite and Britta J. Eickholt

PI3 Kinase Regulation of Skeletal Muscle Hypertrophy and Atrophy 267
David J. Glass

Taking PI3Kδ and PI3Kγ One Step Ahead: Dual Active PI3Kδ/γ Inhibitors for the Treatment of Immune-Mediated Inflammatory Diseases 279
Christian Rommel

Index 301

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Introduction

Tina L. Yuan and Lewis C. Cantley

Contents

1 Establishing Order Within the Cell 1
2 Phosphatidylinositol and Phosphoinositides as Ideal Substrates 2
3 Nucleating a Protein Complex at a Target Location 3
4 Coupling PI3K Activity to Extracellular Cues 3
5 Disease Implications 5
References 6

Abstract The phosphoinositide-3-kinase (PI3K) family of lipid kinases has been well conserved from yeast to mammals. In this evolutionary perspective on the PI3K family, we discuss the prototypical properties of PI3Ks: 1) the utilization of sparse but specifically localized lipid substrates; 2) the nucleation signaling complexes at membrane-targeted sites; and 3) the integration of intracellular signaling with extracellular cues. Together, these three core properties serve to establish order within the entropic environment of the cell. Many human diseases, including cancer and diabetes, are the direct result of loss or defects in one or more of these core properties, putting much hope in the clinical use of PI3K inhibitors singly and in combination to restore order within diseased tissues.

1 Establishing Order Within the Cell

The lipid kinase activity of phosphoinositide 3-kinase (PI3K) has been evolutionarily conserved from yeast to mammals and has evolved from a simple means of sorting vacuolar proteins to nucleating large signaling complexes that regulate

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growth, metabolism and survival (Engelman et al. 2006). Here, we reflect on the unique properties of PI3Ks that explain the diverse roles that these enzymes play in cellular regulation and their relevance in multiple human diseases.

A typical mammalian cell is composed of approximately 70% water and 20% proteins. In their textbook example, Lodish and colleagues estimate that for a hepatocyte this translates into roughly 8×10^9 protein molecules, most of which are randomly diffusing within a chaotic 15-μm^3 space (Lodish et al. 2000). In such a disordered environment, order and directionality must be established to successfully transmit growth and survival signals, for example from a membrane-anchored growth factor receptor to a transcription factor in the nucleus. Perhaps the most valuable and thus conserved property of PI3K is the ability to impose such order in a highly entropic environment.

The core properties that allow PI3K to carry out this function have been conserved from unicellular to multicellular organisms. These include (1) having low abundant but highly specific lipid substrates and products; (2) generating membrane-anchored products that nucleate signaling complexes at targeted sites; and (3) having the ability to associate with membrane-bound proteins that sense extracellular stimuli. Over the course of evolution, higher organisms have evolved several classes of PI3Ks that utilize these prototypical properties to regulate a wide range of functions ranging from directional motility to metabolism, growth, and survival. Importantly, it is also the loss of these core properties that result in aberrant signaling and disease.

2 Phosphatidylinositol and Phosphoinositides as Ideal Substrates

Evolving biological systems require simplicity that will not convolute cellular communication or waste resources. Yet there must be enough variability in the system to allow for diversification and selection. Following this model, PI3K has only three lipid substrates: phosphatidylinositol (PtdIns) and two of its phosphoinositide derivatives, PI-4-P and PI-4, 5-P_2. Additionally, these substrates are present at low levels within the cell. While only 5% of the mass of a mammalian cell is comprised of lipids, only 4% of total lipids are PtdIns and less than 1% of total PtnIns is phosphorylated. Importantly, the PI3K products make up only about 1% of the total phosphorylated forms of PtdIns (Mulgrew-Nesbitt et al. 2006). This extreme low abundance of PI3K lipid products ensures that PI3K signaling is deliberate, dynamic, non-promiscuous, and exquisitely localized.

Yet, despite the scarcity of PtdIns in the cell, the inositol head group contains five free hydroxyl groups that could potentially be phosphorylated to generate variability in the phosphoinositide pool. Three of the five hydroxyl groups (D3, D4, and D5 positions) are phosphorylated alone or in combination, yielding seven phosphoinositides, each with unique stereospecificity and charge. At least 10 discreet protein domains have independently evolved the ability to bind one or

more phosphoinositides and have been identified in hundreds of proteins across numerous species (Lemmon 2008; DiNitto et al. 2003). Thus, by modifying a single lipid substrate, the phosphoinositide kinases have evolved the unique ability to regulate numerous proteins while carefully preserving specificity.

3 Nucleating a Protein Complex at a Target Location

The most ancient role of PI3K in unicellular organisms remains arguably its most relevant role in multicellular organisms. This is the role of nucleating a protein complex at a target location within the cell. *Saccharomyces cerevisiae* expresses the most primordial PI3K, the class III Vps34, which generates PI-3-P at sorting endosomes. Proteins containing FYVE domains bind to PI-3-P and form complexes that regulate vacuolar protein sorting (Burd and Emr 1998). The generation of PI-3-P specifically at sorting endosomes ensures that the protein-sorting complexes are carefully localized to this compartment. Proper localization of protein complexes is also critical for directional movement in another unicellular organism, *Dictyostelium discoideum*. The generation of PI-3, 4, 5-P_3 by class I PI3K at the cell's leading edge recruits PH domain containing proteins such as CRAC and AKT that rearrange the cytoskeleton for directed movement towards shallow chemoattractant gradients (Parent et al. 1998; Meili et al. 1999).

The need for proper protein localization for the most basic functions in unicellular organisms suggests that this was the original function of PI3K. Multicellular organisms have conserved this property by utilizing localized phosphoinositides to regulate cellular polarity and migration, particularly in epithelial cells, neutrophils, and macrophages (Gassama-Diagne et al. 2006; Fruman and Bismuth 2009). However, the utility of this enzyme in multicellular organisms extends into far more complex realms of signaling that nevertheless hinge on the ability to nucleate large signaling complexes at cellular membranes.

4 Coupling PI3K Activity to Extracellular Cues

The evolution towards multicellularity was accompanied by the emergence of two additional classes of PI3K. In addition to class III, class I and II PI3Ks are found in *Caenorhabditis elegans*, *Drosophila melanogaster*, and all vertebrates, suggesting that these later evolving classes specialize in mediating cell–cell communication. Extracellular sensing in unicellular organisms is essentially a survey of the local nutrient landscape, which informs the cell whether or not to grow and proliferate. In multicellular organisms, numerous extracellular stimuli instruct cells not just to grow and proliferate, but also to migrate to new location, to activate survival

mechanisms, and to alter gene expression programs to control metabolic needs and other specialized functions of differentiated tissues.

Class I and II PI3Ks evolved to cope with these new and complex demands by targeting the assembly of various protein complexes directly downstream of membrane-bound receptors, thereby integrating intracellular signaling with extracellular cues. Class I PI3Ks are targeted to active receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs). For class IA PI3Ks, localization to RTKs or adaptors downstream of RTKs is facilitated by conserved SH2 domains in the p85 family of regulatory subunits, which bind to Tyr-phosphorylated Y-X-X-M motifs on activated RTKs or adaptor molecules. The class IB PI3K, p110γ, localizes to GPCRs through the interaction of a regulatory subunit, p101 or p84, with Gβγ subunits. The class IA PI3K, p110β can bind to both RTKs and to Gβγ subunits of GPCRs and appears to act as an integrator of signaling through both pathways. Class II PI3Ks are less well studied, but can be activated by RTKs such as the epidermal growth receptor (EGFR) and insulin receptor and play an important role in clathrin-mediated vessel trafficking (Williams et al. 2009).

Due to their specialized role in interpreting extracellular cues, class I PI3Ks have been most extensively studied. It is the only class that generates PI-3, 4, 5-P_3, a potent second messenger, from PI-4, 5-P_2 in the membrane. High local concentrations of PI-3, 4, 5-P_3 in the membrane colocalize signaling molecules containing PH domains, which bind PI-3, 4, 5-P_3 with high specificity. Some of these molecules include guanine nucleotide exchange factors (GEF) such as GRP1, as well as kinases such as the Bruton tyrosine kinase (BTK), other members of the Tec family of non-receptor tyrosine kinases, and the serine/threonine kinases AKT (also known as PKB) and phosphoinositide-dependent kinase 1 (PDK1) (Cantley 2002). Importantly, localized pools of PI-3, 4, 5-P_3 are generated transiently, limited by the local availability of PI-4, 5-P_2 and by rapid degradation due to the presence of nearby phosphoinositide phosphatases. Three families of phosphoinositide phosphatases are important in modulating class I PI3K signaling: (1) PTEN dephosphorylates the 3′ position of PI-3, 4, 5-P_3 to regenerate PI-4, 5-P_2; (2) SHIP family members dephosphorylate the 5′ position of PI-3, 4, 5-P_3 to generate PI-3, 4-P_2; and (3) INPP4A/4B family members dephosphorylate the 4′ position of PI-3, 4-P_2 to generate PI-3-P. Ultimately, disengagement of PI3K from receptors due to various negative feedback loops terminates signaling. Temporal and spatial regulation of PI-3, 4, 5-P_3 (as well as PI-3, 4-P_2) production is unique to class I PI3Ks and highlights the importance of negatively regulating this complex pathway for receptor-mediated signaling (Lemmon 2008).

One PI-3, 4, 5-P_3 binder in particular, AKT, is responsible for much of this complexity. The PH domain of AKT can bind to both PI-3, 4, 5-P_3 and PI-3, 4-P_2, and both lipids appear to contribute to AKT recruitment to membranes (Franke et al. 1997), as well as recruitment of the upstream activating kinase, PDK1. There are more than 100 reported substrates for AKT, identified under varying degrees of stringency (Manning and Cantley 2007). Canonical AKT substrates contain R-X-R-X-X-S/T motifs, and phosphorylation on serine or threonine residues initiates a

complex cascade of signaling events in the cytosol and nucleus. Briefly, AKT promotes proliferation through the inhibitory phosphorylation of FOXO transcription factors and GSK3, promotes protein synthesis by phosphorylating TSC2 and PRAS40, promotes cell survival by phosphorylating BAD and MDM2, and induces glucose uptake in GLUT4-containing cells through phosphorylation of AS160 (Manning and Cantley 2007). Interestingly, by comparing the number, specificity, and location of PI3K and AKT substrates, it is clear that these two well-conserved enzymes serve non-redundant functions as initiator and effector kinases, so to speak. Given the breadth of their cooperative actions, it is important to note that these two kinases are regulated by multiple feedback mechanisms to ensure that the system resets to basal levels following acute cell stimulation by growth factors (Engelman et al. 2006).

5 Disease Implications

As discussed above, though the responsibilities of PI3K in the cell have vastly increased over the course of evolution, its core properties have remained unchanged. Using low abundance, membrane-anchored lipids to organize signaling complexes downstream of extracellular cues persists as an optimal mode of transmitting signals within the cell. It is, thus, not surprising that alterations in these core properties result in evolutionarily unfavorable signaling and disease. Alterations in the PI3K pathway account for at least 30% of all human cancers and have been implicated in type-2 diabetes. To conclude, we briefly review how genetic alterations in PI3K pathway components unhinge the core properties of PI3K.

In normal cells, the low abundance of phosphoinositides ensures deliberate and targeted signaling downstream of PI3K activation. However, through loss of the PI-3, 4, 5-P_3 phosphatase, PTEN, or oncogenic hotspot mutations in p110α that confer constitutive kinase activity, PI-3, 4, 5-P_3 levels and cellular distribution increase dramatically, resulting in promiscuous and prolonged downstream signaling that contributes to tumorigenesis (Engelman et al. 2006). Recent work has also shown that other components of the PI3K signaling network that influence the phosphoinositide pool, including p85α and INPP4B, are frequently mutated or deleted in human cancers (Cancer Genome Atlas Research Network 2008; Gewinner et al. 2009).

The nucleation of protein complexes not only organizes signaling molecules to one location, but the systematic assembly of the complex ensures that signaling only occurs when all components are primed and ready. This system of checks and balances is disrupted, for example, in tumors with E17K mutations in AKT or p110α hotspot mutations (Carpten et al. 2007). Through enhanced enzymatic activity and membrane localization, cells bearing these mutations are no longer dependent on the step-wise assembly of the signaling complex, and can independently (over)activate downstream pathways that can lead to hyperproliferation.

Lastly, by coupling PI3K activity to activated extracellular receptors in normal cells, intracellular signaling is in harmony with extracellular conditions. However,

in many tumors with RTK mutations or amplifications, such as in ERBB1 and ERBB2, extracellular conditions are exaggerated and can result in unsustainable PI3K signaling. In the other extreme, when PI3K activity is uncoupled from RTK signaling, such as in models of insulin resistance where PI3K is insensitive to insulin receptor activation, type-2 diabetes can arise (Engelman et al. 2006).

Given the large role PI3K plays in tumorigenesis and in the activation of macrophages and lymphocytes, there has been a profound effort by pharmaceutical companies to develop targeted inhibitors of this pathway for treating cancers and for suppressing immune responses. Several PI3K inhibitors, with varying specificities for submembers of the family, have shown great promise in pre-clinical cancer models and are currently in phase I/II clinical trials. AKT catalytic site inhibitors have also entered phase I clinical trials. It is now clear from extensive sequencing of genes from primary human cancers that mutations in the PI3K pathway are very frequent, but that they are invariably combined with mutations in other pathways. Thus, while there is much excitement about the PI3K and AKT inhibitors, it is likely that these compounds will need to be combined with other drugs that target other pathways activated in the same tumors in order to be effective. There is much hope that future "personalized" clinical trials that focus on matching drugs to mutational events in individual tumors will further validate targeted therapy and provide a logical path for conquering the myriad of cancers that have evaded more conventional cancer therapies over the past 40 years. It is likely that PI3K inhibitors will be a significant addition to the arsenal needed for this approach.

References

Burd CG, Emr SD (1998) Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. Mol Cell 2(1):157–162

Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455(7216):1061–1068

Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296(5573):1655–1657

Carpten JD et al (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448(7152):439–444

DiNitto JP, Cronin TC, Lambright DG (2003) Membrane recognition and targeting by lipid-binding domains. Sci STKE 2003(213):re16

Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7(8):606–619

Franke TF et al (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3, 4-bisphosphate. Science 275(5300):665–668

Fruman DA, Bismuth G (2009) Fine tuning the immune response with PI3K. Immunol Rev 228 (1):253–272

Gassama-Diagne A et al (2006) Phosphatidylinositol-3, 4, 5-trisphosphate regulates the formation of the basolateral plasma membrane in epithelial cells. Nat Cell Biol 8(9):963–970

Gewinner C et al (2009) Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. Cancer Cell 16(2):115–125

Lemmon MA (2008) Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol 9(2):99–111

Lodish H et al (2000) Molecular cell biology, 4th edn. Freeman & Co, New York, NY

Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129(7): 1261–1274

Meili R et al (1999) Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in Dictyostelium. EMBO J 18(8): 2092–2105

Mulgrew-Nesbitt A et al (2006) The role of electrostatics in protein-membrane interactions. Biochim Biophys Acta 1761(8):812–826

Parent CA et al (1998) G protein signaling events are activated at the leading edge of chemotactic cells. Cell 95(1):81–91

Williams R et al (2009) Form and flexibility in phosphoinositide 3-kinases. Biochem Soc Trans 37 (Pt 4):615–626

PDK1: The Major Transducer of PI 3-Kinase Actions

José Ramón Bayascas

Contents

1 Introduction 10
2 Mechanism of Activation of the AGC Kinases 10
  2.1 PDK1, the Common T-Loop Kinase 11
  2.2 mTORC1 and mTORC2, the Hydrophobic Motif Kinases 12
  2.3 Two Mechanisms of Regulation by PDK1 14
3 Structure of PDK1 16
4 Genetic Models and Disease 17
  4.1 PDK1 and Diabetes 17
  4.2 PDK1 and T-Cell Development 19
  4.3 PDK1, Growth and Cancer 19
5 PDK1 as a Druggable Target 20
6 Concluding Remarks 22
References 23

Abstract Most of the cellular responses to phosphatidylinositol 3-kinase activation and phosphatidylinositol 3,4,5-trisphosphate production are mediated by the activation of a group of AGC kinases comprising PKB, S6K, RSK, SGK and PKC isoforms, which play essential roles in regulating physiological processes related to cell growth, proliferation, survival and metabolism. All these growth-factor-stimulated AGC kinases possess a common upstream activator, namely PDK1, a master kinase, which, being constitutively active, is still able to phosphorylate and activate its AGC substrates in response to rises in the levels of the PtdIns(3,4,5)P_3 second messenger. In this chapter, the biochemical, structural and genetic data on the mechanism of action and physiological roles of PDK1 are reviewed, and its

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potential as a pharmaceutical target for the design of drugs therapeutically beneficial to treat human disease such us diabetes and cancer is discussed.

1 Introduction

The 3-phosphoinositide-dependent protein kinase-1 (PDK1) was first discovered in the field of the insulin signal transduction research as the protein kinase capable of phosphorylating and activating PKB in a PtdIns(3,4,5)P_3-dependent manner (Alessi et al. 1997b; Stokoe et al. 1997). In the same way as the activation of the phosphatidylinositol 3-kinase (PI 3-kinase) and subsequent production of phosphatidylinositol (3,4,5) trisphosphate, or PtdIns(3,4,5)P_3, is the major apical signalling event triggered by growth factors and hormones, PDK1 plays crucial roles in reading out the increases in PtdIns(3,4,5)P_3 levels and thereby in governing many of the cellular responses to this second messenger.

In fact, most of the physiological effects of PtdIns(3,4,5)P_3 rises in cells are mediated by a particular set of AGC protein kinase family members that controls cell growth, proliferation, survival as well as metabolic responses to insulin. These include protein kinase B (PKB)/akt isoforms, which regulate cell viability and proliferation as well as glucose homeostasis (Whiteman et al. 2002; Dummler and Hemmings 2007; Manning and Cantley 2007); p70 ribosomal S6 kinase isoforms (S6K), implicated in the regulation of protein synthesis and cell growth (Dann et al. 2007); the serum- and glucocorticoid-induced protein kinase isoforms (SGK) that play important roles in regulating ion transport, hormone release, neuroexcitability, cell proliferation and apoptosis (Lang et al. 2006); the p90 ribosomal S6 kinases (RSK) which control survival, proliferation, growth and motility (Hauge and Frodin 2006; Anjum and Blenis 2008); as well as several protein kinase C (PKC) isoforms (Newton 2003, 2010). PDK1, which itself belongs to the AGC family, is precisely the common upstream kinase phosphorylating and activating all these agonist-stimulated AGC kinases (Mora et al. 2004).

2 Mechanism of Activation of the AGC Kinases

AGC kinase family members share structural similarity and a common mechanism of activation relying on the dual phosphorylation of two residues that are each located in two highly conserved motifs: the T-loop or activation loop present in the catalytic domain of the majority of protein kinases and the hydrophobic motif, a structural signature of most AGC kinases that is positioned C-terminal to the kinase domain (Pearce et al. 2010). Phosphorylation of both residues is required for maximal catalytic activity. Some AGC kinases also contain a third phosphorylation site termed the *turn motif* or *zipper site* (Z/TM), which promotes their integrity both by stabilizing the active confirmation of the enzyme and by protecting the

hydrophobic motif from dephosphorylation (Hauge et al. 2007). As mentioned before, PDK1 phosphorylates all these 23 agonist-stimulated AGC kinases at the serine/threonine residues in their T-loop (Mora et al. 2004). By contrast, the regulation of the hydrophobic motif phosphorylation is quite distinct among the different PDK1 targets. The mechanism of regulation of the turn motif phosphorylation, as well as the identity of the kinase/-s phosphorylating this motif, is just being characterized (Alessi et al. 2009).

2.1 *PDK1, the Common T-Loop Kinase*

PDK1 is expressed from a single-copy gene located on human chromosome 16p13.3, which produces a cytosolic protein of 556 amino acids (Alessi et al. 1997a; Stephens et al. 1998). PDK1 consists of two well-characterized functional domains, the N-terminal serine/threonine kinase domain of the AGC family and the C-terminal Pleckstrin homology (PH) domain that interacts with high affinity with both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 as well as other phosphoinositides such as PtdIns(4,5)P_2 (Alessi et al. 1997a; Currie et al. 1999). Interestingly, the three PKB isoforms are, among all the PDK1 substrates characterized, the only ones possessing a PH domain, which in this case is located at the N-terminus of the protein and specifically interacts with PtdIns(3,4,5)P_3. The exclusive presence of a PH domain in the PKB isoforms entails quite a distinctive mechanism of activation when compared to the rest of PDK1 substrates.

After the discovery of PDK1 being the PKB T-loop kinase, many labs reasoned that the high degree of homology shared between different AGC kinase family members within the activation loop might indicate that PDK1 will perhaps also phosphorylate this site in other AGC kinases. This research area entered in the late 1990s onto a vibrating chase, which culminated in the confirmation of PDK1 being also the major T-loop kinase for S6K (Alessi et al. 1998; Pullen et al. 1998), RSK (Richards et al. 1999; Jensen et al. 1999), SGK (Park et al. 1999; Kobayashi and Cohen 1999) and many PKC isoforms (Dutil et al. 1998; Le Good et al. 1998; Chou et al. 1998). The final genetic confirmation that PDK1 was indeed the major T-loop kinase for all these PtdIns(3,4,5)P_3-regulated substrates in mammalian cells came from the finding that, in PDK1 knockout embryonic stem (ES) cells, agonist stimulation failed to activate PKB, S6K, RSK (Williams et al. 2000) and SGK1 (Collins et al. 2003), and the protein stability of a number of PKC isoforms was also compromised (Balendran et al. 2000a, b). All these studies further proved that the so-called "PDK2" hydrophobic motif kinase was distinct from PDK1.

PDK1 is ubiquitously expressed in cells and, surprisingly for an enzyme that regulates as much as 23 agonist-stimulated AGC kinases, its own catalytic activity is not stimulated by these agonists (Alessi et al. 1997a). Although several regulatory mechanisms and phosphorylation sites have been proposed to contribute to the regulation of PDK1 activity (Casamayor et al. 1999; Wick et al. 2003; Riojas et al. 2006; Yang et al. 2008), bacterially expressed PDK1 is fully active and

autophosphorylated at its own Ser241 T-loop residue, perhaps explaining why it is constitutively active in mammalian cells. Much research has focussed on understanding how an enzyme that is always active is then capable of phosphorylating in an inducible manner its myriad of substrates in response to specific stimuli. Biochemical, genetic and structural data from many studies suggested that in the absence of stimuli, the catalytic activity of PDK1 is kept under control by limiting its access to substrates, and only after agonist stimulation, the different PDK1 substrates are converted into forms that can be recognized, phosphorylated and activated by PDK1. In this regard, phosphorylation of the hydrophobic motif becomes a rate-limiting step for the action of PDK1. Despite the importance of this second phosphorylation site for the understanding of the mechanism of activation of the AGC kinases, the identity of the kinase phosphorylating the hydrophobic motif remained elusive for years. Most evidence now demonstrates that at least two different complexes of mTOR function as the PDK2 kinase, as discussed in the next section.

2.2 mTORC1 and mTORC2, the Hydrophobic Motif Kinases

As PDK1 phosphorylated several growth-factor-stimulated AGC kinases at their T-loop, it was reasonable to propose that a common kinase might have also phosphorylated each of this group of AGC kinases at their hydrophobic motifs in an analogous manner. The mammalian target of rapamycin mTOR was the first characterized hydrophobic motif kinase that, in complex with Raptor (Hara et al. 2002; Kim et al. 2002) and mLST8, initially named GβL (Kim et al. 2003), was shown to phosphorylate the S6K hydrophobic motif, thereby contributing to the activation of this enzyme. Because the immunosuppressant rapamycin blocked the phosphorylation and activation of the S6K (Chung et al. 1992; Price et al. 1992) by inhibiting mTOR (Sabatini et al. 1994), while other AGC kinases such as PKB, RSK, SGK and most PKC isoforms were shown to be rapamycin insensitive, the notion of a common hydrophobic motif kinase fell out of favour.

The p90 ribosomal S6 kinase case is quite unusual, as it is a serine–threonine protein kinase containing two catalytic domains. The N-terminal kinase domain belongs to the AGC family and phosphorylates and regulates a number of cellular substrates mediating growth-factor-induced cell survival, proliferation, growth and motility. By contrast, the C-terminal catalytic domain belongs to the CAMK family and accomplishes a regulatory role. Following mitogen stimulation, ERK1/2 phosphorylates and activates the C-terminal catalytic domain of RSK. The activated C-terminal kinase domain then phosphorylates the hydrophobic motif, which in RSK is located in a linker region between the two kinase domains (Dalby et al. 1998). Phosphorylation of the hydrophobic motif is essential for PDK1-mediated activation of the N-terminal kinase domain (Frodin et al. 2000). Hence, the hydrophobic motif kinase activity for RSK relies on an autophosphorylation catalysed by the C-terminal kinase domain.

Regulation of the PKC hydrophobic motif phosphorylation encompasses diverse mechanisms among the members of this rather intricate family of AGC kinases. The conventional PKC isoforms (PKCα, β and γ) and the novel PKC isoforms (PKCδ, ε, η and θ) are primed for allosteric activation by both diacylglycerol and calcium (conventional PKCs) or diacylglycerol (novel PKCs) following phosphorylation of both the T-loop and the hydrophobic motif (Mellor and Parker 1998). For the conventional forms, phosphorylation of the T-loop occurs first by PDK1, followed by autophosphorylation of the hydrophobic motif, at least in PKC βII (Newton 2001). For the novel forms, a role of mTOR as the hydrophobic motif kinase was suggested by the observation that phosphorylation of the hydrophobic motif site in PKC δ and ε isoforms was shown to be sensitive to both rapamycin and nutrients (Parekh et al. 1999). The atypical isoforms (PKC ζ and λ) and the PKC-related kinases (PRK1 and PRK2) are not regulated by diacylglycerol or calcium. These members possess a negatively charged amino acid at the position equivalent to the hydrophobic motif phosphorylation site present in other AGC kinases (Balendran et al. 1999, 2000a) and, when expressed in cells, they are constitutively active. A distinct and major role of the turn motif phosphorylation in regulating the docking interaction with PDK1 and the activation of PRK2 has been recently demonstrated (Dettori et al. 2009). Hence, hydrophobic motif auto-phosphorylation, regulation by mTOR and presence of aspartic/glutamic acid residues mimicking the negative charge of a phosphate group in the hydrophobic motif are all mechanisms found to contribute to the activation of the different PKC isoforms.

The identity of the PKB hydrophobic motif kinase attracted for years the attention of many investigators, and several kinases were miscalled to mediate this phosphorylation (Bayascas and Alessi 2005). As mentioned before, since phosphorylation of PKB at Ser473 was not sensitive to rapamycin, mTOR was never considered a convincing candidate, until Sabatini and co-workers demonstrated in a seminal paper that mTOR, as part of a rapamycin insensitive complex with Rictor and mLST8, was indeed the PKB hydrophobic motif kinase (Sarbassov et al. 2005). This rapamycin-insensitive complex of mTOR, which also consisted of Sin1 (Frias et al. 2006; Jacinto et al. 2006; Yang et al. 2006) and Protor (Pearce et al. 2007), was termed mTORC2, whereas the original mTOR: raptor:mLST8 rapamycin-sensitive complex, which phosphorylated the hydrophobic motif site of S6K, has been thereafter termed mTORC1 (Guertin and Sabatini 2007). Ablation in mice of different mTORC2 components such as Rictor or mLST8 demonstrated that mTORC2 was, in addition, the hydrophobic motif kinase for PKCα (Sarbassov et al. 2004; Guertin et al. 2006). It was only very recently that mTORC2 was reportedly shown to phosphorylate the hydrophobic motif of the SGK isoforms (Garcia-Martinez and Alessi 2008), completing our understanding on the identity of the hydrophobic motif kinases for the PDK1-regulated kinases (Fig. 1). However, the existence of mTOR complexes others than mTORC1 and mTORC2 has been also suggested (Garcia-Martinez et al. 2009).

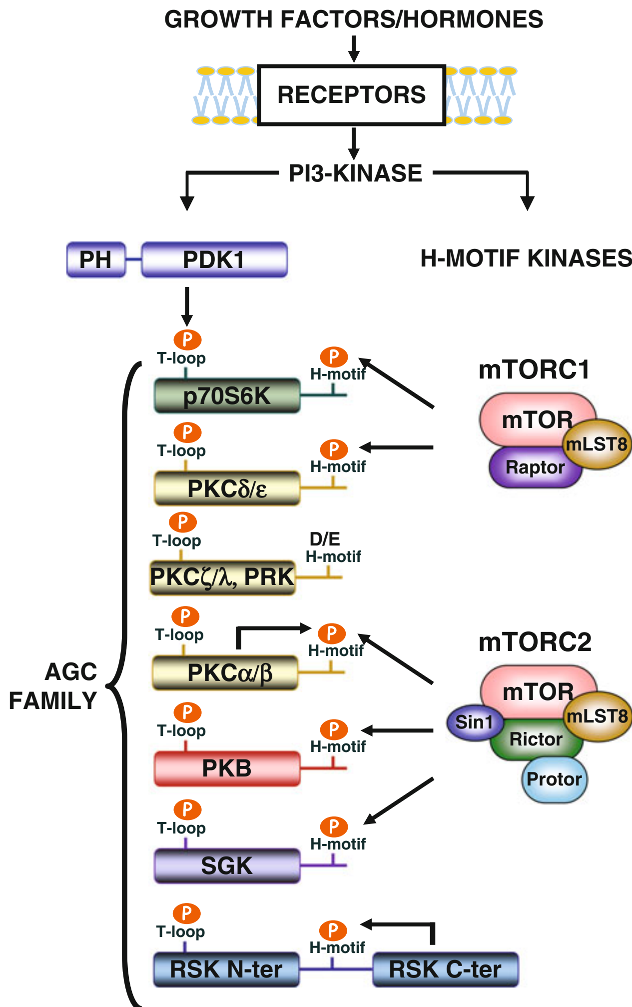

Fig. 1 Mechanism of activation of the agonist-stimulated AGC kinases by dual T-loop and hydrophobic motif phosphorylation. Following agonist stimulation and PI 3-kinase activation, the mTOR complexes 1 and 2 play a major role phosphorylating the hydrophobic motif, while PDK1 is the common kinase phosphorylating the T-loop, of all these kinases

2.3 Two Mechanisms of Regulation by PDK1

The phosphorylated hydrophobic motif is meant to play a dual role for the activation of many PDK1-regulated kinases. First, it functions as a docking site for the binding of PDK1, enabling the phosphorylation of the substrate at its T-loop by PDK1; in addition, once phosphorylated, the hydrophobic motif interacts with a groove in the catalytic domain of the same molecule, promoting in that way the

transition of the corresponding AGC kinase to an active conformation. Interestingly, PDK1 itself does not possess any hydrophobic motif, but presents a hydrophobic motif binding pocket, termed the PIF pocket, which is precisely the domain in PDK1 that recognizes the docking site presented by the substrate. Moreover, interaction of the PIF pocket of PDK1 with the phosphorylated hydrophobic motif in the substrate induces the allosteric activation of PDK1 (Mora et al. 2004; Biondi 2004). The final demonstration that this was the mechanism that operates in vivo for the vast majority of PDK1 substrates came from the finding that in ES cells expressing PIF pocket mutant forms of PDK1 incapable of recognizing the phosphorylated hydrophobic motif, activation of S6K, RSK and SGK by growth factors was severely impaired, as it was the PDK1-dependent stabilization of a number of PKC isoforms (Collins et al. 2003, 2005). By contrast, activation of PKB isoforms proceeded normally, further highlighting the existence of a docking-site-independent mechanism regulating PKB isoforms activation.

Because phosphorylation of PKB by PDK1 is PtdIns(3,4,5)P_3 dependent (Alessi et al. 1997b; Stokoe et al. 1997), both PDK1 and PKB contain PtdIns(3,4,5)P_3 interacting PH domains (Alessi et al. 1997a) and agonist stimulation induces the recruitment of PKB to the plasma membrane (Andjelkovic et al. 1997), a model was proposed by which, upon growth factor stimulation and PtdIns(3,4,5)P_3 production, both PKB and PDK1 translocated to the plasma membrane via the specific interaction of their PH domains with newly generated PtdIns(3,4,5)P_3, where PDK1 could then readily phosphorylate and activate PKB (Lizcano and Alessi 2002). In vivo disruption of the interaction of the PDK1 PH domain with PtdIns(3,4,5)P_3 by knock-in mutation has been shown to severely affect the activation of PKB, without altering the intrinsic PDK1 catalytic activity, in ES cells (McManus et al. 2004). A pool of PDK1 constitutively associated with the plasma membrane has been previously observed (Currie et al. 1999) that could account for the marginal activation of PKB detected in mice expressing a mutant form of PDK1 incapable of interacting with PtdIns(3,4,5)P_3 (Bayascas et al. 2008). How PDK1 is constitutively anchored to the plasma membrane and how interaction of PtdIns(3,4,5)P_3 with the PH domain of PDK1 enhances the rate of phosphorylation and activation of PKB are aspects that still need to be clarified. Phosphorylation of PKB at Ser473 might as well positively influence phosphorylation of Thr308 by PDK1, as suggested by the fact that mTOR-specific inhibitors not only prevented Ser473 phosphorylation but also affected, although to a lesser extent, Thr308 phosphorylation (Garcia-Martinez et al. 2009). The interaction of PtdIns(3,4,5)P_3 with the PH domain of PKB not only triggers the translocation of PKB to the plasma membrane but also induces a large conformational change in the PKB protein, which is thought to create a PDK1 interacting site or to expose the T-loop phosphorylation residue (Thomas et al. 2002; Milburn et al. 2003). More recently, a comprehensive model accounting for the inactive conformer of PKB has been suggested, in which both the PH domain and the C-terminal hydrophobic motif are folded in together with the kinase domain, preventing in this way the phosphorylation of both the Thr308 by PDK1 and the Ser473 by mTORC2 (Calleja et al. 2007, 2009). Binding to PtdIns (3,4,5)P_3 would disrupt the interaction of the PKB PH domain with its own kinase

domain, allowing the phosphorylation of the two regulatory residues. Moreover, in vivo analysis with fluorescent reporters provided strong evidence that PDK1 might form in cells a complex with the inactive form of its substrate PKB, and that this interaction might play a role in the recruitment of PDK1 to the plasma membrane by agonists (Calleja et al. 2007).

3 Structure of PDK1

High-resolution crystal structures of both the kinase domain (Biondi et al. 2002) and the Pleckstrin homology domain (Komander et al. 2004) of PDK1 have been reported and provided insightful information regarding the mechanism of action of this enzyme.

The catalytic domain of PDK1 assumes the classical bilobal kinase fold and is more similar to other AGC kinases crystallized, such as PKA or PKB. As in other kinases, the αC-helix is a key element in the kinase core, linking together the hydrophobic and phosphopeptide pockets as well as the serine 241 in the T-loop of PDK1 (Biondi et al. 2002). In contrast to PKBβ, in which phosphorylation of the T-loop is essential for the stabilization of both the αC-helix and the hydrophobic pocket (Yang et al. 2002a, b), T-loop phosphorylation of PDK1 is not required for the structural integrity of the αC-helix or the hydrophobic pocket, but contributes to the catalytic activity of PDK1 upon substrate binding (Komander et al. 2005).

A close examination of the small lobe of the catalytic domain of PDK1 confirmed the existence of an already postulated hydrophobic groove, named PIF pocket, involved in the interaction with the hydrophobic motif in the substrates (Biondi et al. 2000; 2001) and permitted the definition of the residues that form this domain. Among them, Leu155, the amino acid that was mutated to glutamic acid for knock-in analysis (Collins et al. 2003), was shown to be located at the core of that hydrophobic pocket. Interestingly, a second pocket occupied by a sulfate ion in the crystal was also observed next to the PIF pocket, which was hypothesized to be the phosphate-binding site for the phosphorylated residue in the hydrophobic motif. Mutation of Arg131 to Ala in this second pocket severely affected the ability of PDK1 to recognize the phosphorylated hydrophobic motif of S6K, and, as a consequence, the S6K T-loop was poorly phosphorylated (Biondi et al. 2002; Frodin et al. 2002). Accordingly, in knock-in ES cells expressing the Arg131Ala mutant form of the phosphate pocket of PDK1, agonist stimulation failed to efficiently activate S6K, RSK and SGK isoforms, whereas PKB was normally activated (Collins et al. 2005).

The crystal structure of the isolated PDK1 PH domain showed, when compared to the standard PH domain fold, an unusual N-terminal extension of unknown function. The phosphoinositide-binding pocket is a rather shallow and positively charged surface and is significantly more spacious than other PH domains, thus providing an explanation for the ability of PDK1 to efficiently bind different phosphoinositides. Furthermore, this larger ligand binding site might account for

the ability of PDK1 to interact with Ins(1,3,4,5,6)P_5 and InsP_6 with high affinity, a mechanism that could serve to anchor PDK1 in the cytosol, thereby creating a complementary pool to the one interacting with phosphoinositides in the membrane. This second interaction might be involved in the regulation of substrates that do not translocate to the plasma membrane (Komander et al. 2004). In contrast to PKBα (Milburn et al. 2003), the PDK1 PH domain does not undergo any ligand-induced conformational change when it binds PtdIns(3,4,5)P_3, as suggested by the fact that both the non-complexed and phosphoinositide-complexed structures of the PDK1 PH domain superimposed well, and no remarkable conformational changes were detected. Mutational analysis of the different residues defining the phosphoinositide binding site revealed that Lys465, which established hydrogen bonds with the D3 and D4 phosphates, was an essential residue for phosphoinositide binding. Accordingly, mutation of this residue to glutamic acid completely abrogated binding of PDK1 to phosphoinositides as well as translocation of PDK1 to the plasma membrane in cells (Komander et al. 2004), and severely affected the activation of PKB, but not that of other AGC kinases, in mouse tissues (Bayascas et al. 2008).

4 Genetic Models and Disease

The first attempts to study the physiological roles of PDK1 in regulating the metabolic responses to insulin at the organism level came up against the early embryonic lethality described for the PDK1 knockout mice (Lawlor et al. 2002). Two PDK1 knock-in mice expressing mutant forms of PDK1 affecting either the function of the PIF pocket (PDK1 Leu155Glu) (Collins et al. 2003) or the PH domain (PDK1 Arg472,473,474Leu) (McManus et al. 2004) were also shown to be embryonic lethal, thus highlighting the essential roles that the PDK1-regulated signalling pathways play during development (McManus et al. 2004). As mentioned before, ES cells derived from all these three genetic models have been proved priceless biological tools to elucidate the physiological substrates of PDK1 and to investigate the mechanisms of action of this master regulatory kinase. To circumvent the prenatal lethality and explore the role of PDK1 in vivo in differentiated cells, tissue-specific conditional knockout and knock-in strategies were employed, which are summarized in Table 1.

4.1 PDK1 and Diabetes

Cre/Lox methodology was exploited to generate a series of tissue-specific conditional knockout mice lacking PDK1 in different insulin-responsive tissues. Mice lacking PDK1 specifically in muscle were first generated (Mora et al. 2003). These mice died from heart failure between 5 and 11 weeks of age as a consequence of the genetic deficiency affecting the cardiac muscle. PDK1-deficient cardiomyocytes

Table 1 PDK1 genetically modified mouse models

Mutation	Tissue	Viability	Other phenotypes	References
PDK1 −/−	Whole organism	Embryonic lethal E9.5	Lack of somites, forebrain and neural crest derived tissues	Lawlor et al. (2002)
PDK1 −/−	Muscle	Lethal at 5–11 weeks	Dilated cardiomyopathy	Mora et al. (2003)
PDK1 −/−	Liver	Lethal at 4–16 weeks	Defective postprandial glucose disposal and liver failure	Mora et al. (2005) and Okamoto et al. (2007)
PDK1 −/−	Pancreas	Viable	Diabetes resulting from loss of beta cell mass	Hashimoto et al. (2006)
PDK1 −/−	T-cell	Viable	Impaired T-cell differentiation	Hinton et al. (2004)
PDK1 −/−	Nervous system	Viable	Microcephaly	Chalhoub et al. (2009)
PDK1 fl/fl	Whole organism	Reduced viability	Reduced organ and body size	Lawlor et al. (2002)
			Protected from PTEN-induced tumourogenesis	Bayascas et al. (2005)
			Defective T-cell proliferative expansion	Kelly et al. (2006)
			Impaired electrolyte intestinal transport	Sandu et al. (2006)
			Impaired intestinal and renal amino acid absorption	Rexhepaj et al. (2006)
			Reduced erythrocyte cell death	Foller et al. (2008)
			Increased gastric acid secretion	Rotte et al. (2008)
			Defective phagocytosis of dendritic cells	Zaru et al. (2008)
PDK1 L155E	Whole organism	Embryonic lethal E12	Forebrain and body axis development defects	Collins et al. (2003)
PDK1 L155E	Muscle	Viable	Normal glucose homeostasis	Bayascas et al. (2006)
PDK1 L155E	T-cell	Viable	Defective T-cell proliferative expansion	Kelly et al. (2007)
PDK1 R131E	Whole organism	Embryonic lethal E19.5	Growth retardation and craniofacial defects	Collins et al. (2005)
PDK1 RRR/LLL	Whole organism	Embryonic lethal E10.5	Head blood vessel and placental defects	McManus et al. (2004)
PDK1 K465E	Whole organism	Viable	Reduced organ and body size, insulin resistance and hyperinsulinemia with normoglycemia	Bayascas et al. (2008)
			Deficient T-cell migration	Waugh et al. (2009)

obtained from these mice were shown to be more sensitive to hypoxia, which may have caused reduced cell viability and ultimately the organ failure (Mora et al. 2003). The PDK1 liver-specific knockout mice were next generated, which also died between 4 and 16 weeks of age as a result of liver failure accompanied by large-interstitial oedema. Before dying, they exhibited glucose intolerance caused by the inability of glucose to silence the expression of the gluconeogenic genes (Mora et al. 2005), a deficit that can be rescued by re-expression of liver glucokinase (Okamoto et al. 2007). The pancreas-specific PDK1 knockout mice were shown to be diabetic due to a deficit in β-cell mass than can be restored by Foxo1 haploinsufficiency (Hashimoto et al. 2006). Moreover, PDK1 PH domain knock-in mice carrying an improved version of the original triple arginine to leucine mutation, namely PDK1 Lys465Glu, were shown to be viable and exhibited some hallmarks of diabetes such us glucose intolerance, insulin resistance and hyperinsulinemia (Bayascas et al. 2008). By contrast, tissue-specific conditional knock-in mice expressing the PIF pocket PDK1 Leu155Glu mutation in muscle were shown to be of normal phenotype regarding glucose homeostasis (Bayascas et al. 2006). All together, these genetic models confirmed both the implication of PDK1 in mediating metabolic responses to insulin as well as in promoting cell viability.

4.2 PDK1 and T-Cell Development

The role of PDK1 in T-cell development has been also thoroughly investigated by using these series of tissue-specific conditional mice models. Deletion of PDK1 in the thymus completely blocked T-cell differentiation (Hinton et al. 2004), whereas reducing the expression of PDK1 by using hypomorphic alleles was shown to be permissive for T-cell differentiation, but blocked proliferative expansion of alpha/beta (α/β) and not gamma delta (γ/δ) T lymphocytes (Hinton et al. 2004; Kelly et al. 2006). Moreover, T-cell-specific conditional knock-in mice expressing the PIF pocket PDK1 Leu155Glu mutation were shown to undergo normal differentiation, but they were deficient for proliferative expansion, thus delimitating the importance of the PKB branch for the initial T-cell differentiation and that of the PIF pocket-dependent branch for the proliferative expansion (Kelly et al. 2007). More recently, a specific role of PDK1 in regulating T-cell migration has been suggested, which required binding of PDK1 to PtdIns(3,4,5)P_3, optimal PKB activation and maximal Foxo phosphorylation (Waugh et al. 2009). A role for PDK1 in modulating the pathogen-mediated activation of dendritic cells has been also demonstrated (Zaru et al. 2008).

4.3 PDK1, Growth and Cancer

Generation of PDK1 hypomorphic alleles was meant to be also a good genetic strategy to overcome the embryonic lethal periods caused by a complete PDK1

deficiency. That idea was proven right when PDK1 hypomorphic mice expressing reduced levels of PDK1 were obtained and were shown to be viable. These mice exhibited no overt phenotype other than being smaller because of a reduction in cell size rather than cell number, in spite of the fact that no detectable defects in the PDK1 downstream signalling pathways were observed (Lawlor et al. 2002). Interestingly, the PDK1 Lys465Glu knock-in mice described before were shown to be similarly smaller when compared to the PDK1 hypomorphic mice, a phenotype that might be due to a deficiency in PKB activation (Bayascas et al. 2008).

The PDK1 hypomorphic mice became a very fruitful model to explore different aspects of the physiological roles of PDK1, for example in regulating the intestinal electrolyte transport by activating the Na+/H+ exchanger (Sandu et al. 2006); reduction of PDK1 expression leads also to impairment of intestinal absorption and renal re-absorption of amino acids (Rexhepaj et al. 2006), calcium influx and erythrocyte cell death (Foller et al. 2008), as well as gastric acid secretion (Rotte et al. 2008). The epistatic relationships between PTEN and PDK1 in migration and malignant transformation of lymphocytes (Finlay et al. 2009) and in regulating nervous system development (Chalhoub et al. 2009) have been also recently addressed. PTEN, the lipid phosphatase that antagonise PI 3-kinase activity by degrading PtdIns(3,4,5)P_3, functions in cells as a tumour suppressor that is frequently mutated in human cancer. PTEN haploinsufficient mice develop a variety of tumours and have become a widely accepted mouse model prone to develop cancer resulting from elevated PtdIns(3,4,5)P_3 levels. Strikingly, when the PDK1 hypomorphic mice were crossed with the PTEN $+/-$ mice, those PTEN heterozygous mice expressing reduced levels of PDK1 were markedly protected from developing a wide range of tumours, indicating that PDK1 is a key effector in mediating tumorigenesis resulting from loss of PTEN and further validating PDK1 as a valuable anticancer target (Bayascas et al. 2005), as discussed next.

5 PDK1 as a Druggable Target

PDK1 governs, in physiological conditions, a critical signalling node whose deregulation has dramatic consequences in pathologies such us diabetes and cancer. Impairment of the insulin responses leads to glucose intolerance and insulin resistance that normally precede the onset of type II diabetes (Biddinger and Kahn 2006). By contrast, hyperactivation of the same signalling pathway by extracellular growth factors leads to deregulation of growth, survival, proliferation, apoptosis and ultimately to transformation (Vanhaesebroeck et al. 2001). Thus, it seems reasonable to propose that selective activation of PDK1 might be a good strategy for the treatment of diabetes. Likewise, developing specific PDK1 inhibitors appears to be a good approach to treat cancer. It should be taken into account that positive modulators of PDK1 for the treatment of diabetes might be detrimental for cancer and vice versa. Also, the approach to develop therapeutically beneficial compounds targeting PDK1 is completely different in cancer or diabetes research.

Deregulation of the PI 3-kinase pathway is common in human cancer, and many human tumours of quite different origin possess elevated levels of PtdIns(3,4,5)P_3, which are meant to arise from mutations in different apical elements of this signalling pathway, encompassing tyrosine kinase receptors, PI 3-kinase or PTEN (Li et al. 1997; Vanhaesebroeck et al. 2001; Cantley 2002; Yuan and Cantley 2008). Accordingly, mice model in which one of the two copies of the PTEN tumour suppressor gene was disrupted developed a variety of tumours with high incidence and is nowadays considered as one of the best models to study PtdIns(3,4,5)P_3-driven tumorigenesis (Suzuki et al. 1998; Di et al. 1998; Podsypanina et al. 1999). Although PDK1 was shown to be itself oncogenic when expressed in mammary epithelial cells (Zeng et al. 2002; Xie et al. 2003), there are, however, not many reports showing PDK1 alterations in human disease. For example, increased PDK1 expression has been reported in invasive breast cancers, suggesting its importance in the metastatic process (Xie et al. 2006). Overexpression of PDK1 has been reported in 45% of patients with acute myeloid leukaemia, which is closely associated with hyperphosphorylation of PKC isoforms (Pearn et al. 2007). A role of PDK1 in ovarian carcinoma progression has been also proposed (Ahmed et al. 2008). PDK1 overexpression and amplification of the PDK1 gene are common occurrences in breast cancer harbouring upstream lesions on the PI 3-kinase pathway, thus potentiating the oncogenic effect of having elevated PtdIns (3,4,5)P_3 levels (Maurer et al. 2009; Vasudevan et al. 2009). The finding that hypomorphic alleles expressing reduced levels of the PDK1 protein greatly rescued the PTEN heterozygous mice suffering from cancer was considered as the first in vivo evidence of PDK1 mediating neoplasia and convincingly validated PDK1 as a promising anticancer target (Bayascas et al. 2005). The PDK1 hypomorphic mice express 10–20% of PDK1 protein when compared to control littermates, a situation that might be equivalent to the administration of a drug which would reduce the endogenous PDK1 activity by 80–90%. In contrast to the PDK1 PH domain knock-in mice, which suffered from significant insulin resistance presumably due to defective PKB activation, the PDK1 hypomorphic mice did not exhibit either signalling lesions or deleterious phenotypes, which strongly suggests that undesirable side effects of such kind of inhibitory compound would not be expected. Moreover, a role of the PDK1 signalling pathway in mediating resistance of breast cancer cells to tamoxifen has been recently reported, which suggests that PDK1 inhibitors are likely to have additional utility in sensitizing breast tumours to this broadly used anticancer drug (Iorns et al. 2009). In recent years, a number of ATP-competitive PDK1 inhibitors have been suggested to be therapeutically beneficial in inhibiting cancer progression. While more work is needed to design improved, more effective and selective PDK1 inhibitors, validation of these compounds as drugs that could be employed in clinical trials to treat cancers becomes an attractive prospect that might not be a long way off (Peifer and Alessi 2008).

Although most of the PDK1 drug discovery activity has been focussed on developing inhibitors targeting the PDK1 ATP-binding site, biochemical and structural data on the mechanism of action of PDK1 indicate that both the PH domain and the PIF pocket might as well be exploited as druggable targets. While genetic