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PDK1, the master regulator of AGC kinase signal transduction

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Abstract

The interaction of insulin and growth factors with their receptors on the outside surface of a cell, leads to the activation of phosphatidylinositol 3-kinase (PI 3-kinase) and generation of the phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) second messenger at the inner surface of the cell membrane. One of the most studied signalling events controlled by PtdIns(3,4,5)P_3, comprises the activation of a group of AGC family protein kinases, including isoforms of protein kinase B (PKB)/Akt, p70 ribosomal S6 kinase (S6K), serum- and glucocorticoid-induced protein kinase (SGK) and protein kinase C (PKC), which play crucial roles in regulating physiological processes relevant to metabolism, growth, proliferation and survival. Here, we review recent biochemical, genetic and structural studies on the 3-phosphoinositide-dependent protein kinase-1 (PDK1), which phosphorylates and activates the AGC kinase members regulated by PI 3-kinase. We also discuss whether inhibitors of PDK1 might have chemotherapeutic potential in the treatment of cancers in which the PDK1-regulated AGC kinases are constitutively activated.

Keywords: AGC kinase; Growth; Proliferation; Apoptosis and insulin signalling

1. Introduction

The interaction of insulin and growth factors with their receptors leads to the activation of phosphatidylinositol 3-kinase (PI 3-kinase) and phosphorylation of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) at the D3 position of the inositol ring to generate the phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) second messenger. It is now well established that many of the diverse metabolic, proliferative and survival effects triggered through activation of PI 3-kinase and generation of PtdIns(3,4,5)P_3, are mediated by the activation of a subgroup of the AGC family of protein kinases. These comprise isoforms of protein kinase B (PKB, also known as Akt) [1], p70 ribosomal S6 kinase (S6K) [2], serum- and glucocorticoid-induced protein kinase (SGK) [3] and atypical isoforms of protein kinase C (PKC) [4]. These enzymes are activated within minutes of insulin- or growth factor-induced stimulation of PI 3-kinase. Once activated, PKB phosphorylates and modulates the function of a number of important regulatory proteins, resulting in inhibition of apoptosis, promotion of cell division and stimulation of glucose uptake and storage [5]. S6K controls a number of different steps of protein synthesis, required for cell growth and storage of amino acids [6]. SGK may play important roles in regulating ion transport [3]. The roles played by atypical PKC isoforms activated downstream of PI 3-kinase are less well defined.

Much research has also focused on understanding the mechanism by which AGC kinases are activated following insulin and growth factor stimulation of PI 3-kinase. These studies have revealed that all agonists that activated PKB [7], S6K [8] and SGK [9–11] isoforms, do so by inducing their phosphorylation at two highly conserved Ser/Thr residues. One is located in the T-loop (also known as activation-loop), whilst the other is C-terminal to the catalytic domain in a region termed the hydrophobic motif. Phosphorylation of both residues is required for the maximal activation of these enzymes. The 3-phosphoinositide-dependent protein kinase-1 (PDK1) was first purified from tissue extracts as an enzyme that could phosphorylate the T-loop of PKBα (Thr308) in the presence of PtdIns(3,4,5)P_3 [12,13]. Cloning of PDK1 revealed that it was a 556-amino acid containing

Abbreviations: mTOR kinase, mammalian target of rapamycin kinase; PDK1, 3-phosphoinositide-dependent protein kinase-1; PFK2, 6-phosphofructo-2-kinase; PH, Pleckstrin homology; PI 3-kinase, phosphatidylinositol 3-kinase; PIF, PDK1-interacting fragment; PKA, cyclic AMP-dependent protein kinase; PKB, protein kinase B; PKC, protein kinase C; PRK2, PKC-related kinase-2; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate; RSK, p90 ribosomal S6 kinase; S6K, p70 ribosomal S6 kinase; SGK, serum- and glucocorticoid-induced protein kinase; UCN-01, 7-hydroxystaurosporine

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enzyme possessing a kinase domain at its N-terminus (residues 70–359) and a Pleckstrin homology (PH) domain at its C-terminus (residues 459–550), which interacted with high affinity with PtdIns(3,4,5)P_3 and one of its immediate breakdown products PtdIns(3,4)P_2 [14–16], also thought to function as a signalling molecule. The three PKB isoforms (PKBα, PKBβ and PKBγ, also known as Akt1, Akt2 and Akt3), unlike all other AGC family kinases, also possess a PtdIns(3,4,5)P_3/PtdIns(3,4)P_2-binding PH domain, which in contrast to PDK1, is located N-terminal to the catalytic domain. PDK1 also efficiently catalyses the phosphorylation of the activation-loop residue of isoforms of S6K [17,18] and SGK [9–11]. Genetic evidence that PDK1 is the major T-loop kinase of PI 3-kinase-regulated AGC kinases in mammalian cells, came from the finding that in embryonic stem cells lacking PDK1, IGF1 failed to activate PKBα and S6K1 [19] as well as SGK1 [20], under conditions where these enzymes were activated in wild-type cells. PDK1 is also thought to be the enzyme which phosphorylates the T-loop of all isoforms of protein kinase C (PKC) (reviewed in Ref. [4]) and p90 ribosomal S6 kinase (RSK) [21,22]. Consistent with this notion, in PDK1-deficient ES cells, RSK cannot be activated [19] and most PKC isoforms are unstable [23], as phosphorylation of their T-loop is required for PKC stability as well as activity (reviewed in Ref. [24]).

2. Regulation of PDK1 activity

Unexpectedly, when PDK1 was immunoprecipitated from either unstimulated or growth factor/insulin-stimulated cells, it possessed the same high catalytic activity, suggesting that PDK1 activity was not directly altered by agonist stimulation [15]. Moreover, the binding of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 to PDK1 in vitro did not affect its catalytic activity either [14–16]. PDK1 is also a member of the AGC family of protein kinases and like all other AGC kinases, requires phosphorylation at its own T-loop residue (Ser241) in order to be activated [25]. The finding that bacterially expressed PDK1 was stoichiometrically phosphorylated at Ser241 and fully active, indicated that PDK1 possesses the intrinsic ability to phosphorylate its own T-loop residue, perhaps explaining why it is constitutively active in mammalian cells. Recent analysis indicated that PDK1 autophosphorylation at Ser241, is mediated by an intermolecular (*trans*) reaction, rather than intramolecular (*cis*) reaction [26].

3. Regulation of PKB by PDK1

As PDK1 was constitutively active, research focused on understanding how PtdIns(3,4,5)P_3 production in cells could induce PDK1 to phosphorylate and activate its substrates. In the case of PKB, it appears that the mutual ability of PKB and PDK1 to interact with PtdIns(3,4,5)P_3/PtdIns(3,4)P_2 via their PH domains, plays an important role in co-localising these enzymes at the plasma membrane and enabling PDK1 to phosphorylate, and hence activate PKB. The evidence for this is based on the finding that PDK1 can only phosphorylate PKB efficiently in vitro in the presence of lipid vesicles containing PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 [12,13]. Moreover, agonists that trigger activation of PI 3-kinase induce recruitment of PKB to the plasma membrane, a process that is prevented by treatment of cells with inhibitors of PI 3-kinase [27]. This translocation is dependent upon the PH domain of PKB, as mutants that cannot interact with PtdIns(3,4,5)P_3, are unable to translocate to the membrane [28]. Whether PDK1 also translocates to the plasma membrane following PI 3-kinase activation is controversial, as conflicting results have been reported. Two studies concluded that PDK1 also translocated to the membrane [29,30], whilst another found that a pool of PDK1 was constitutively associated with the plasma membrane in unstimulated cells and the amount of PDK1 in this pool was not further increased by agonists [16]. Attachment of a plasma membrane targeting motif to PKB, induced maximal T-loop phosphorylation in unstimulated cells [28,31], which could only occur if some PDK1 was localised at the membrane under basal conditions.

Importantly, as is the case for PDK1, the binding of PtdIns(3,4,5)P_3 to PKB does not activate it [12,32]. There is, however, strong evidence that binding of PKB to PtdIns(3,4,5)P_3 induces a conformational change that greatly enhances the rate at which it can be phosphorylated by PDK1. The evidence for this is based upon the observation that in the absence of 3-phosphoinositides, PDK1 is unable to phosphorylate wild-type PKB, under conditions where it can efficiently phosphorylate a mutant form of PKB that lacks its PH domain(ΔPH-PKB) [14,15]. Moreover, artificially promoting the interaction of PDK1 with wild-type PKB and ΔPH-PKB by the attachment of a high affinity PDK1 interaction motif to these enzymes, was sufficient to induce maximal phosphorylation of the T-loop in ΔPH-PKB, but not in wild-type PKB in unstimulated cells [33], further indicating that PDK1 cannot phosphorylate wild-type PKB in the absence of PtdIns(3,4,5)P_3. More recently, fluorescence lifetime imaging microscopy has been employed to demonstrate that recruitment of PKB to the plasma membrane in PDGF-stimulated NIH3T3 cells, resulted in a marked conformational change in PKB [34]. Finally, comparison of the high-resolution crystal structure of the PH domain of PKBα in a non-complexed form [35], with the structures of the PH domain of PKBα complexed to Ins(1,3,4,5)P_4 (the head group of PtdIns(3,4,5)P_3) [36], indicated that in contrast to all other PH domains crystallised thus far, binding of 3-phosphoinositide to the PH domain of PKB, induced a large conformational change [35,36]. This is characterised by marked changes in certain residues making up the phosphoinositide-binding site, formation of a short α-helix in one of the variable loops, and a marked movement of another variable loop away from the lipid-binding site [35]. The mechanism of how these conformational changes convert PKB into a substrate for PDK1 is not known, but

could result in the exposure of the T-loop residue and/or creation of a specific PDK1-binding/docking site.

4. Regulation of S6K and SGK by PDK1

As S6K and SGK lack a PH domain and are phosphorylated by PDK1 at the same rate in the presence or absence of PtdIns(3,4,5)P_3, the mechanism by which phosphorylation is controlled by PtdIns(3,4,5)P_3, was initially less well understood. The first evidence relating to this came from the finding in a yeast two-hybrid screen that the catalytic domain of PDK1 interacted with high affinity with a C-terminal fragment of the PKC-related kinase-2 (PRK2), encompassing the hydrophobic motif of this enzyme. This fragment was termed the PDK1-interacting fragment (PIF). As discussed above, S6K and SGK have a Ser/Thr residue at the hydrophobic motif site, whose phosphorylation in addition to phosphorylation of the T-loop, is essential for maximal activation of these enzymes. All hydrophobic motifs lie in a conserved Phe-Xaa-Xaa-Phe/Tyr-Ser/Thr-Phe/Tyr sequence, in which the underlined Ser/Thr represents the site of phosphorylation. In contrast to S6K and SGK, PRK2 possesses an acidic Asp at a position equivalent to the phosphorylated Ser/Thr, perhaps mimicking the effect that phosphorylation induces in S6K and SGK. Mutagenesis analysis of the hydrophobic motif of PRK2, revealed that mutation of the Asp residue to Ala or mutation of any of the conserved hydrophobic residues, abolished or greatly reduced the ability of PDK1 to interact with either PIF [37] or full length PRK2 [38]. The atypical PKC isoform PKCζ, also possesses an acidic Glu residue at its hydrophobic motif and mutation of this residue to Ala or mutation of the conserved hydrophobic residues, substantially reduced the ability of PDK1 to interact with PKCζ as well as markedly inhibiting phosphorylation of PKCζ at its T-loop residue in cells [38]. These findings indicated that the hydrophobic motif of AGC kinases could serve as a substrate-docking site, enabling PDK1 to dock and then phosphorylate their substrates. Moreover, they also indicated that phosphorylation of the hydrophobic motif of AGC kinases, might enhance the ability of PDK1 to interact with their substrates.

The cyclic AMP-dependent protein kinase (PKA), the founding member of the AGC family of protein, terminates in the sequence Phe-Xaa-Xaa-Phe, which bears resemblance to first part of the hydrophobic motif of PDK1 substrates [39]. Although one study has demonstrated that PDK1 can phosphorylate the T-loop of PKA leading to its activation [40], as PKA is normally active in PDK1-deficient ES cells [19], it is likely that PDK1 is not rate-limiting in vivo for the activation of PKA. In the crystal structure of PKA [41], the C-terminal Phe-Xaa-Xaa-Phe motif occupies a solvent-exposed hydrophobic pocket in the small lobe of the catalytic domain (Fig. 1A). Interestingly, PDK1 is the only AGC kinase member not to possess the characteristic C-terminal hydrophobic motif. Despite this, sequence alignments and molecular modelling indicated that the residues in PKA that make up the hydrophobic pocket which binds to the Phe-Xaa-Xaa-Phe residues are conserved in PDK1 [39]. As PDK1 does not possess a hydrophobic motif, it was speculated that if it still possessed a hydrophobic pocket, it would be empty and thus potentially available to interact with hydrophobic motifs present in its substrates. This notion was supported by mutational analysis of the residues predicted to line the hydrophobic pocket of PDK1, which revealed that these mutations abolished the ability of PDK1 to interact not only with PIF [39], but also PRK2 and PKCζ [38], as well as S6K and SGK1 [33]. In addition, peptides that encompass the hydrophobic motif of PRK2 [39], S6K [42] and RSK [43], induced a four- to sixfold activation of PDK1, indicating that occupancy of the hydrophobic pocket on PDK1 enhanced its activity. It should be noted that the

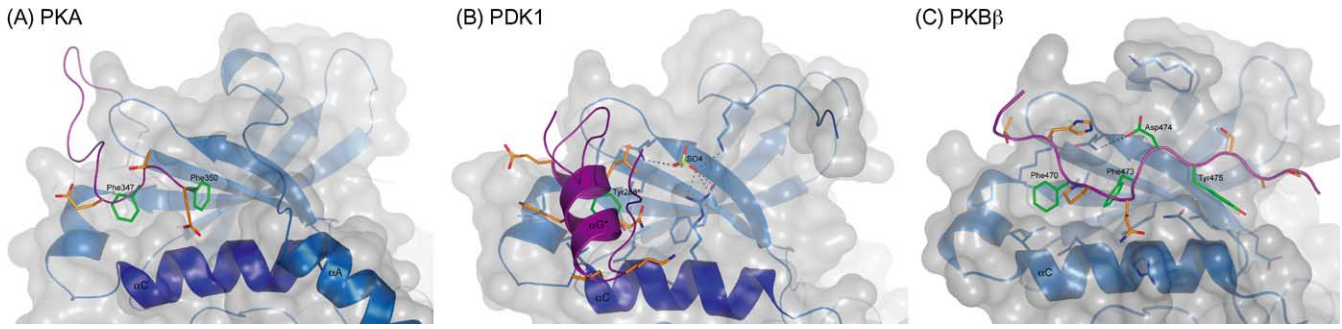

Fig. 1. The hydrophobic motif pocket of AGC kinases. AGC family kinases possess a pocket to accommodate their own C-terminal hydrophobic motif in the N-lobe of the kinase domain. The N-lobe is shown with blue secondary structure elements under a grey transparent surface. The corresponding hydrophobic motif is shown as purple string, with orange and green residues. (A) The kinase domain of PKA terminates with Phe-Thr-Glu-Phe, and the two aromatic side chains (in green) are buried in distinct hydrophobic pockets. The pocket is closed by the N-terminal αA-helix of the kinase, which is missing in PKB and PDK1. (B) In PDK1, Tyr288 of the αG-helix of a symmetry-related molecule lies in the PIF-pocket, which as discussed in the main text is a crystallographic phenomenon. In close proximity, lies the phosphopeptide-binding site, which is occupied by a sulfate molecule. Several basic residues are located within hydrogen bonding distance to the anion. (C) In PKBβ, the C-terminal Phe residues are deeply buried in the hydrophobic pocket, remarkably similar to PKA. Asp474, mimicking the hydrophobic motif phosphorylation, displays one hydrogen bond to Gln220, which is conserved in PDK1 (Gln150). Tyr475, the C-terminal residue of the hydrophobic motif, also displays specific hydrophobic interactions and hydrogen bonds.

hydrophobic pocket on PDK1 is frequently referred to as the "PIF-pocket".

The idea that phosphorylation of the hydrophobic motif of AGC kinases could create a PDK1-docking site and hence promote their activation by PDK1, was also supported by the finding that mutation of the Ser/Thr residue in the hydrophobic motif of either S6K1 [17,18] or SGK1 [9,11] to an acidic residue to mimic phosphorylation, greatly enhanced the phosphorylation of these enzymes by PDK1 in vitro. Moreover, when these acidic mutants were overexpressed in cells, they were found to be constitutively activated and their activity or T-loop phosphorylation was not affected by incubation of cells with inhibitors of PI 3-kinase, indicating that these enzymes were no longer under the control of PtdIns(3,4,5)P_3 [9,11,17,18]. These results support the notion that PI 3-kinase promotes the activation of S6K and SGK by controlling hydrophobic motif phosphorylation of these enzymes. This phosphorylation does not directly activate S6K or SGK but regulates their interaction with PDK1 and hence activation.

Hydrophobic pocket PDK1 mutants, such as PDK1-[L155E], were incapable of binding or phosphorylating S6K and SGK [33], thus confirming the importance of the PIF-pocket of PDK1 as a substrate-docking site. In contrast, PKB in the presence of PtdIns(3,4,5)P_3, was activated normally in vitro, by the PDK1[L155E] mutant [33], suggesting that the PIF-pocket was not required for the activation of PKB. This conclusion was supported in the study of ES cells in which both copies of the PDK1 gene were altered by knock-in mutation to express the PDK1[L155E] mutant. In the knock-in ES cells, IGF1 induced normal activation of PKB and phosphorylation of the PKB substrates glycogen synthase kinase-3 and the FOXO1 transcription factor. In contrast, S6K and SGK were not activated, nor were physiological substrates of S6K phosphorylated [20]. These experiments establish that PDK1 recognises PKB and S6K/SGK by different mechanisms and emphasises the physiological importance of the PIF-pocket. It should be noted that one study showed that in cells, the PDK1[L155E] mutant was unable to trigger the activation of ΔPH-PKBα [44]. Although it was concluded in this study that the PDK1 PIF-pocket may play a role in regulating PKB in vivo, it is our opinion that truncation of the PH domain of PKB, converts PKB into an SGK-like substrate which requires binding of PDK1 PIF-pocket, to the ΔPH-PKBα hydrophobic motif to become activated. Consistent with this idea, phosphorylation of ΔPH-PKBα but not wild-type PKB by PDK1, is inhibited by incubation of PDK1 with a peptide that encompasses the hydrophobic motif of PRK2, that interacts strongly with the hydrophobic pocket of PDK1 [33].

A key missing link in our understanding of how PI 3-kinase regulates the activation of PKB, S6K and SGK isoforms, concerns the identity of the upstream hydrophobic motif kinase(s), which despite much research, remain elusive. In the case of S6K, there is evidence that the mammalian target of rapamycin (mTOR) kinase may directly phosphorylate the hydrophobic motif of S6K and that PKB, tuberous sclerosis complex and the Rheb GTPase may also be involved in regulating this phosphorylation (reviewed in Refs. [45,46]). As rapamycin does not inhibit the phosphorylation of the hydrophobic motifs of SGK and PKB, it is likely that a kinase(s) other than mTOR mediate the phosphorylation of these residues. In contrast, RSK isoforms appear to carry their own hydrophobic motif kinase as a second kinase catalytic domain located C-terminal to the AGC kinase domain [47]. The C-terminal RSK hydrophobic motif kinase is activated through phosphorylation by the ERK1/ERK2 MAP kinases in response to stimuli, such as growth factors and phorbol esters. The C-terminal RSK kinase then phosphorylates the hydrophobic motif of the RSK N-terminal AGC kinase domain, thereby allowing PDK1 to dock and phosphorylate the T-loop residue, resulting in the activation of RSK [43].

5. Structural studies on PDK1

Recently a high-resolution 2.0 Å crystal structure of the PDK1 kinase domain in complex with ATP was reported [42]. PDK1 assumes the classic bilobal kinase fold and is similar in overall structure to PKA (Fig. 2). Inspection of the region of the N-terminal kinase domain of PDK1 in which the hydrophobic pocket was predicted to reside, revealed a 5 Å deep pocket lined with hydrophobic residues, present in a similar location to the Phe-Xaa-Xaa-Phe-binding pocket in PKA (Fig. 1B). Leu155 is located at the centre of the hydrophobic pocket, which in the crystal structure is occupied by Tyr288 from a symmetry-related molecule, which makes hydrophobic contacts, similar to the interactions of the phenylalanine residues in the Phe-Xaa-Xaa-Phe motif of PKA (Fig. 1A and B). However, this observation is a crystallographic phenomenon, as gel filtration studies suggest that PDK1 is monomeric. As outlined above, substrates, such as S6K1, interact with PDK1 with higher affinity when they are phosphorylated at their hydrophobic motif. This suggested that a regulatory phosphate-docking site would be located close to the hydrophobic pocket. In fact, in close proximity to the hydrophobic pocket in PDK1, another small pocket lined with basic residues was identified. In the crystal structure, this pocket was occupied by a sulfate-anion, which presumably originated from the crystallisation buffer (Fig. 1B). The sulfate interacts with four residues lining the small pocket, namely, Lys76, Arg131, Thr148 and Gln150. Because of its close proximity to the hydrophobic pocket (Fig. 1B), it was likely that this represented the binding site for the hydrophobic motif phosphorylation site. Mutagenesis analysis revealed that mutation of Lys76, Arg131 and Gln150 vastly reduced the ability of PDK1 to interact with a peptide that encompassed the hydrophobic motif of S6K in which the hydrophobic motif was phosphorylated. Frodin et al. [48], through molecular modelling of the PDK1 catalytic domain, also identified Lys76 and Arg131 as potential

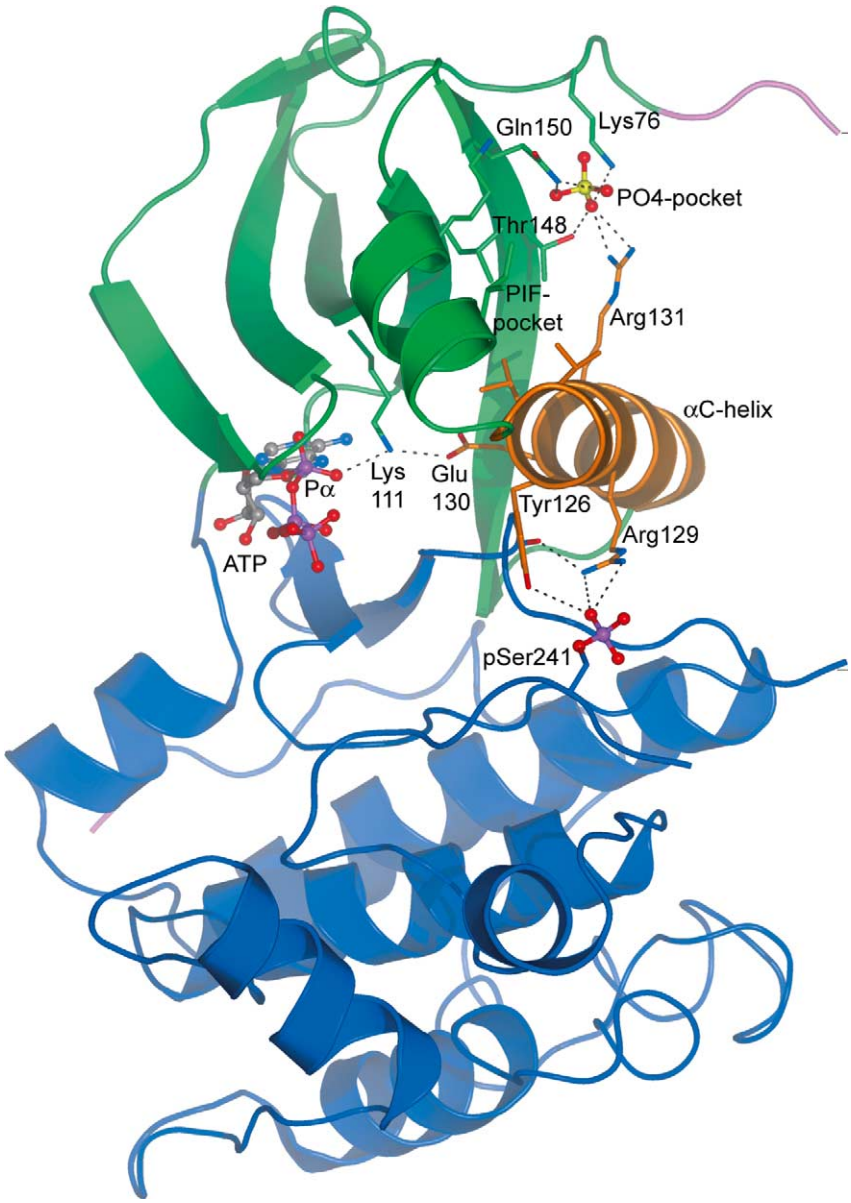

Fig. 2. The αC-helix of PDK1 links the N- and the C-terminal kinase lobes in a phosphate-dependent manner. The smaller N-terminal kinase lobe (in green) and the larger C-terminal lobe (in blue) are connected by Arg131 and Arg129 of the αC-helix (in orange), respectively. Arg131 is coordinated by the presence of phosphorylated hydrophobic motif peptide (represented by the sulfate with yellow/red spheres), whereas Arg129 contacts the phosphorylated Ser241 in the T-loop (represented by purple/red spheres). Glu130, which in turn is coordinated by these residues, is part of the strictly conserved Glu-Lys-ATP hydrogen-bonding network coordinating the alpha phosphate (with ATP drawn in ball and stick representation).

phosphate-binding residues and demonstrated that mutation of Arg131 significantly inhibited PDK1 from phosphorylating S6K.

Recent structural studies of the kinase domain of PKBβ, demonstrate that it possesses a hydrophobic pocket for interaction with its own phosphorylated hydrophobic motif. This pocket is located at an equivalent position, and structurally very similar to the PIF-pocket on PDK1 and PKA (Fig. 1C) [49,50]. This intramolecular binding of the phosphorylated hydrophobic motif of PKBβ to the pocket on its own kinase domain was shown to enhance the kinase activity of PKBβ in a similar fashion by which PDK1 is activated by PIF [49,50]. Sequence alignments indicate that all AGC kinase members that are activated by PDK1 are likely to possess equivalent hydrophobic grooves and phosphate-binding sites in the small lobe of their kinase domains, whose expected role is to bind to their own phosphorylated hydrophobic motif [42]. Recently mutagenesis studies supported the existence of such a hydrophobic motif-binding pocket in the kinase domains of RSK, S6K and SGK, and indicated that occupancy of these pockets activated these enzymes [48].

The PDK1 structure shows that, as in other protein kinases [51], the αC-helix (residues 124–136) is a key element in the kinase core. One turn of the PDK1 αC-helix

(residues 129–131), links together the N-terminal lobe, the C-terminal lobe and the active site. Arg129 points towards the T-loop and forms two hydrogen bonds with the phosphorylated Ser241, whereas Arg131 forms two hydrogen bonds with the sulfate in the phosphate pocket (Fig. 2). Glu130 coordinates Lys111, which forms a hydrogen bond with the α-phosphate of the bound ATP. This interaction is conserved in all protein kinases and crucial for their activation [51]. An additional residue, Tyr126, forms a third hydrogen bond from the αC-helix with the phosphorylated Ser241. Val124 and Val127 on the αC-helix are involved in formation of the PIF-pocket (Figs. 1B and 2). The αC-helix provides a structural link between the hydrophobic and phosphopeptide pockets and the phosphoserine in the T-loop. It is interesting to speculate that phosphorylation in T-loop of PDK1 plays a key role in stabilising and positioning the αC-helix, thereby modulating the overall conformation of the hydrophobic and phosphate pockets. This is supported by structural analysis of PKBβ where, phosphorylation of the T-loop is essential for stabilisation of the αC-helix, and more interestingly, induces and formation of the equivalent hydrophobic motif-binding pocket [49,50].

Based on these results, in Fig. 3, we summarize the mechanism by which we believe that PDK1 phosphorylates and activates its substrates. Thus, far no structural information is available for S6K or SGK isoforms. However, based on the PKB model, it is likely that in the non-phosphorylated inactive forms of these enzymes, the hydrophobic motif is exposed. Following phosphorylation of the hydrophobic motif, PDK1 would bind to this site and phosphorylate the T-loop residue of these enzymes. This would result in stabilisation of the αC-helix and formation of the hydrophobic/phosphate pocket, which would then interact with its own phosphorylated hydrophobic motif, displacing PDK1 from this site.

6. PDK1-deficient mice and cells

PDK1 is required for normal embryo development, as mice embryos lacking PDK1 died at day E9.5, displaying multiple abnormalities, including lack of somites, forebrain and neural crest derived tissues [52]. PDK1 hypomorphic mice in which a neomycin resistance gene was inserted into an intron of the PDK1 gene, that resulted in a 90% reduction of PDK1 expression in all tissues, have been generated [52]. These animals were viable and fertile but were 40–50% smaller than control animals and their organ volumes were

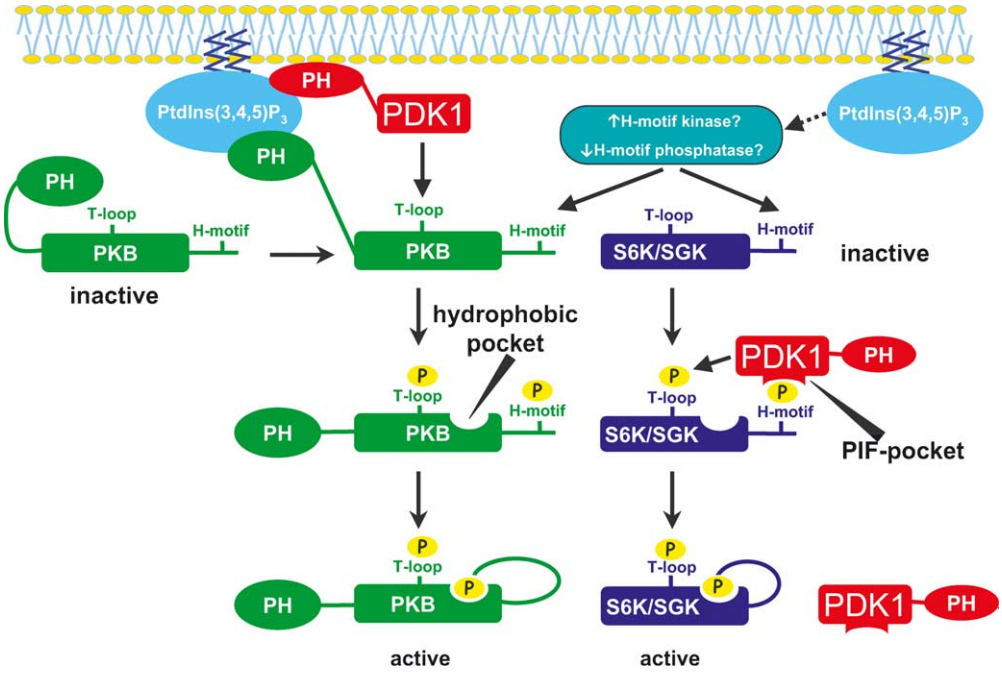

Fig. 3. Mechanism of activation of PKB, S6K and SGK by PDK1. PKB is activated following its recruitment to the plasma membrane through binding of its PH domain to PtdIns(3,4,5)P_3, where it is phosphorylated at its T-loop by PDK1 and its hydrophobic motif by a distinct unknown kinase. Once PKB is phosphorylated at its T-loop, a hydrophobic motif-binding site is formed in the catalytic domain through stabilisation of the αC-helix [49,50], resulting in the intramolecular binding of the phosphorylated hydrophobic motif to this site. This is the step that locks PKB into its maximally active conformation. In contrast, for S6K and SGK, it is the phosphorylation of these enzymes at their hydrophobic motif that enables PDK1 to interact with these enzymes through its PIF-pocket, hence permitting the T-loop phosphorylation of these substrates. PI 3-kinase appears to regulate the phosphorylation of S6K and SGK1 at their hydrophobic motif by a poorly characterised mechanism. This could either involve activation (↑) of a hydrophobic motif kinase and/or inhibition of a hydrophobic motif phosphatase (↓). We speculate that phosphorylation of the T-loop of S6K and SGK like that of PKB, promotes the formation of a binding site within the kinase domain of these enzymes, for their own phosphorylated hydrophobic motif. Binding of the phosphorylated hydrophobic motif residues to this pocket is predicted to result in the activation of these enzymes.

also proportionately reduced. Quantitative non-biased stereological analysis of several organs of these animals, indicated that reduction in animal size was due to smaller cells rather than a reduction in cell number. Similar findings had previously been made in *Drosophila*, where genetic analysis indicated that PDK1 and the kinases it regulates, play central roles in regulating cell volume (reviewed in Refs. [53,54]). Interestingly, activation of PKBα and S6K1 by insulin was normal in the PDK1 hypomorphic mice, showing that regulation of cell size by PDK1 is independent of insulin's ability to activate PKB and S6K. The mechanism by which PDK1 regulates cell size is not known.

Recently, immortalised brown adipocyte cell line in which PDK1 could be deleted employing Cre-loxP technology have been studied [55]. In adipocytes lacking PDK1, insulin-induced activation of PKB and of S6K as well as glucose uptake and translocation of the GLUT4 glucose transporter to the plasma membrane was markedly inhibited. This indicates that PDK1 plays and essential role regulating insulin-induced glucose uptake in adipocytes, consistent with numerous studies indicating that PKB activation is essential for insulin-induced recruitment of GLUT4 to the plasma membrane [56–58]. The Cre-loxP technology has also been employed to generate mice that lack PDK1 in heart muscle. Although these mice were born at the expected Mendelian frequency and initially appeared healthy, they developed heart failure and died at early adulthood (6–10 weeks of age) [59]. Echocardiographic and other analysis indicated that the heart-deficient PDK1 mice developed a form of heart failure similar to that of dilated cardiomyopathy in humans. The PDK1-deficient hearts were markedly smaller and stereological analysis suggested that this was due to a reduction in cardiomyocyte size rather than number [59], consistent with the findings generated with the PDK1 hypomorphic mice [52]. One of the major effects of insulin in the heart is to stimulate glycolysis by activating the 6-phosphofructo-2-kinase (PFK2) [60]. Activation of PFK2 by insulin regulates utilisation of fuel hierarchy in the heart, favouring use of glucose rather than fatty acids, the main energy source in the heart. Previous studies have indicated that PKB [61] or another PDK1-regulated protein kinase [62], triggers the activation of PFK2 by phosphorylating this enzyme at Ser466 and Ser483. Consistent with this notion, in PDK1-deficient hearts, insulin failed to stimulate activation of PFK2 [59]. Another finding relevant to the development of heart failure was that cardiomyocytes lacking PDK1, were markedly more sensitive to hypoxia [59]. These results are consistent with other pharmacological and genetic studies indicating that the PI 3-kinase/PDK1/AGC signalling network plays a key role in regulating cardiac viability and preventing heart failure [63]. Thus, it is possible that a deficiency in the PDK1/AGC kinase pathway could contribute to the development of cardiomyopathy in humans as well as decreasing the ability of cardiomyocytes to withstand conditions associated with oxygen deprivation.

7. PDK1 inhibitors for the treatment of cancer

A significant number of cancers possess mutations in genes that result in elevation of cellular levels of PtdIns(3,4,5)P_3, with one of the most common mutations occurring in the PtdIns(3,4,5)P_3 3-phosphatase PTEN gene (reviewed in Refs. [64,65]). This results in inappropriate elevation of PKB and S6K activity, which are thought to function as major driving forces in promoting the uncontrolled proliferation and enhanced survival of these cells. Antisense-mediated depletion of PDK1 in glioblastomas lacking expression of PTEN, was shown to markedly reduce their proliferation and survival [66]. Moreover, overexpression of PDK1 in mammary epithelial cells induced their transformation by permitting anchorage-independent growth in soft agar [67,68]. Taken together, these observations suggest that an inhibitor of PDK1 might be beneficial for treatment of cancer cells possessing constitutively activated AGC kinase(s). The development of such inhibitors would be also facilitated by the presence of only a single PDK1 isoform in humans. Moreover, the observation that PDK1 hypomorphic mice expressing only 10% of the normal levels of this enzyme, are viable, fertile and display no obvious harmful phenotype [52], indicates that an inhibitor of PDK1 would not be highly toxic or harmful.

The most potent PDK1 inhibitor reported to date is 7-hydroxystaurosporine (UCN-01), which inhibits PDK1 with an IC_{50} of 5 nM [69]. UCN-01, which has been employed in over 300 studies, inhibits growth and induces apoptosis in many cancer cell lines, and is also in clinical trials for cancer with positive results reported in Phase 1 (reviewed in Refs. [70,71]). Unfortunately, UCN-01 is a non-specific kinase inhibitor inhibiting many other kinases, such as Chk1 [72,73], with similar potency to PDK1. Due to the low selectivity of UCN-01, it is unclear which inhibited targets are suppressing growth and survival of cancer in the many studies it has been deployed. Recently, the high-resolution structures of UCN-01 and staurosporine in complex with the kinase domain of PDK1 have been reported [74]. UCN-01 and staurosporine bind to PDK1 in a similar fashion, but the 7-hydroxy group in UCN-01, which is not present on staurosporine, generates additional hydrogen bonds with active site residues [74]. These, inhibitor complexes, help define ATP-binding site properties, which might be useful for specific inhibitor design. However, thus far no sufficiently sensitive or specific inhibitor of PDK1 suitable for in vivo studies has been reported.

8. Conclusions and perspective

Six years have now elapsed since PDK1 was originally identified. Considerable progress has been made in characterising its function, structure and regulation. These studies have firmly established that PDK1 works as a master upstream kinase controlling the activation of numerous AGC

kinase members. The ability of PDK1 to phosphorylate its substrates rather than being governed by activation of PDK1, involves its substrate being converted into forms that can readily interact and become phosphorylated by PDK1. This unusual substrate-directed mechanism of regulation, explains how PDK1 is able to control the activity of a diverse set of AGC kinase members that regulate different physiological processes. Thus, PKB and S6K can be activated downstream of PI 3-kinase by PDK1, independently from RSK and conventional PKC isoforms that require ERK activation and diacylglycerol production, respectively, prior to phosphorylation by PDK1. This mechanism of regulation, contrasts with other signalling pathways such as the MAP kinase pathway, in which all downstream components become activated following the activation of the upstream MAP kinase kinase kinase or MAP kinase kinase enzymes. In the future it will be important not only to develop specific PDK1 inhibitors but also to generate mouse cancer genetic models to test whether PDK1 is a suitable therapeutic anti-cancer target. The use of tissue specific PDK1 knockout mice or PIF-pocket or PH domain knock-in mice in which only a subset of the downstream AGC kinases can be activated, should also allow a better definition of the roles that the PDK1-AGC kinase pathway plays in mediating diverse cellular processes in different tissues.

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