Caenorhabditis elegans PIAK, a Phospholipid-independent Kinase That Activates the AKT/PKB Survival Kinase*

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Phospholipid-dependent kinase 1 (PDK1) is a 3’-phospholipid-responsive serine/threonine kinase that plays a critical role in cell survival by phosphorylating and activating the anti-apoptotic AKT/PKB kinase. While PDK1 is clearly an important component of the cell survival machinery, the potential for phospholipid-independent activation of the AKT/PKB survival pathway has not been extensively examined at the molecular level. We have identified a second form of PDK1 in the nematode Caenorhabditis elegans that we have termed PIAK (phospholipid-independent AKT/PKB kinase). PIAK is highly homologous to C. elegans and mammalian PDK1 with the exception that the novel kinase lacks a phospholipid binding pleckstrin homology domain. The domain structure of PIAK suggests that it might be a phospholipid-independent kinase, and PIAK phosphorylates mammalian AKT/PKB at the activating Thr308 residue in the presence of the phosphatidylinositol (PI) 3-kinase inhibitors as well as in the absence of growth factors. In addition, PIAK is capable of inducing the phospholipid-independent, AKT/PKB-induced phosphorylation of the AFX-type forkhead transcription factor, resulting in its cytoplasmic localization. Because the nuclear localization of this transcription factor induces an apoptotic state, this PIAK-mediated cytoplasmic sequestration allows for cell survival. Finally, PIAK activity appears to be induced by various inhibitors of cell cycle G1 progression. These data suggest an alternate, phosphatidylinositol 3-kinase-independent mechanism for the activation of the AKT/PKB survival pathway that may be utilized during periods of cellular quiescence.

PI3K3-kinases are important conduits, which funnel information from the cell surface to downstream survival pathways by producing various 3’-phosphorylated phospholipids, including phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4)P2 and PtdIns(3,4,5)P3, respectively). Activation of the PI3-kinase pathway by normal (i.e. growth factor) or oncogenic (i.e. HER 2 overexpression) cell surface events results in the production of PtdIns(3,4)P2 and PtdIns(3,4,5)P3 (1). These specific phospholipids can then act as membrane binding sites for the lipid-binding pleckstrin homology (PH) domains of two classes of downstream kinases, 3-phospholipid-dependent kinase 1 (PDK1) and the AKT/PKB kinases (2–8). The PH domain-induced membrane association of these kinases results in physical interactions between these two types of enzymes, so that the PDK1 enzyme mediates the phosphorylation of a specific threonine residue (Thr308) in the activation loop of the AKT/PKB kinases (8–12). The PDK1-activated AKT/PKB can subsequently phosphorylate a number of substrates involved with cell survival, including BAD (13) and a subset of the forkhead transcription factors (14). The phosphorylation of a number of sites in these forkhead transcription factors results in their transport from the nucleus (14). This cytoplasmic localization is an important aspect of PI3-kinase-mediated cell survival, as nuclear localization of the forkhead transcription factors in cultured cells rapidly results in cell death, which is in part mediated by the activation of caspases (15, 14).2 This survival pathway can be down-regulated by the catalytic activity of PTEN/MMAC, a lipid phosphatase with specificity for the 3 position of PtdIns(3,4)P2 and PtdIns(3,4,5)P3 (16). The importance of the PI3-kinase pathway to oncogenesis is emphasized by the finding that PTEN/MMAC is a tumor suppressor gene, which is homozygously lost in a high percentage of a variety of tumors (17, 18). Examination of tumors and cell lines lacking PTEN/MMAC has demonstrated increased AKT/PKB activity, which presumably results in pro-survival signals (19, 20). Thus, the PI3-kinase-mediated activation of AKT/PKB is likely to be a major component of cell survival in both normal as well as oncogenic situations.

The significance of the PI3-kinase pathway to cell survival as well as to the regulation of metabolic processes is emphasized by genetic and molecular studies in the nematode, Caenorhabditis elegans. The dauer larval stage is a reversible larval arrest pathway where worms do not feed, and their metabolism is shifted toward energy storage rather than energy production. Genetic analysis of this pathway, together with molecular analysis of the genes involved with dauer formation, have demonstrated that it appears to be the worm homologue of the mammalian insulin signaling pathway. Activation of a membrane-associated homologue of the insulin/IGF-1 receptor (DAF-2) appears to be the initiator of an inhibitory cascade that blocks dauer formation. Importantly, a worm homologue of the PI3-kinase is found downstream of this receptor tyrosine kinase (21). Constitutive activation of this kinase also inhibits dauer formation. Interestingly, two different AKT/PKB kinases are found to be downstream of the PI3-kinase, and it appears that mutation of these AKT/PKB kinases results in constitutive dauer formation, suggesting that the role of these kinases is to block dauer formation in

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‡ The abbreviations used are: PDK1, phosphatidylinositol; PtdIns(3,4)P2, phosphatidylinositol 3,4-bisphosphate; PtdIns(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PIP3, phosphatidylinositol 1,4,5-trisphosphate; PH, pleckstrin homology; PDK1, phospholipid-dependent kinase 1; GFP, green fluorescent protein; EGFP, enhanced GFP; GST, glutathione S-transferase; IGF, insulin-like growth factor; V5, epitope, Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Arg-Ser-Thr.
response to activation of the PI 3-kinase by the DAF-2 receptor (22). The dauer pathway appears to converge upon the DAF-16 transcription factor, which is the worm homologue of the mammalian forkhead proteins known to be phosphorylated by AKT/PKB (22, 23). Recently, this pathway has been further elucidated with the discovery that DAF-18 mutations result in a constitutive block in dauer formation. Examination of the DAF-18 gene revealed that it is the worm homologue of mammalian PTEN, consistent with the interpretation that loss of PTEN results in irreversible activation of the worm AKT/PKBs and inhibition of the activity of the worm homologue of mammalian forkhead proteins (24). Finally, recent data have shown that a worm homologue corresponding to mammalian PDK 1 appears to be downstream of the DAF 2 receptor and PI 3-kinase but upstream of the AKT/PKB kinases, consistent with biochemical observations in the mammalian system (24). An important finding is that this pathway also appears to be conserved in the fruit fly Drosophila melanogaster, where it appears to regulate cell number and cell size (25). Together, these data suggest that the dauer pathway is a homologue of the mammalian insulin pathway. In addition, because worms with mutations in components of this pathway show body size, egg laying, and neurological phenotypes, the results also suggest that other aspects of cell regulation mediated by the PI 3-kinase/PIK/AKT pathway may also be conserved between worms and mammals.

While the majority of literature suggests that the AKT/PKB pathway is regulated by phospholipids, a number of reports suggest that other mediators, such as isoproteronol (26) and cAMP (27, 28), may also be involved with the activation of this pathway in a PI 3-kinase-independent fashion. In addition, during periods of cellular quiescence, for example when nutrients are limiting and the PI 3-kinase pathway is down-regulated, it seemed likely that additional protective mechanisms might be operative. Examination of the C. elegans genome revealed a kinase, which was homologous to PDK 1, but which

![Fig. 1. Comparison of the primary structures of PIAK, CEPDK1a, and human PDK1. A, alignment of the deduced amino acid sequence of PIAK (GenBank™ accession number T33662) with the human PDK1 sequence (hPDK1, GenBank™ accession number AAC51825) and the C. elegans PDK1 sequence (CEPDK1a, GenBank™ accession number AAD42307), carried out using the CLUSTAL W program. The ATP binding site, the active site, the substrate recognition site, and the PH domain are indicated. The catalytic lysine is indicated by an asterisk. B, schematic diagram of the domain structures of PIK, C. elegans PDK1, and human PDK1.](image-url)
lacked the phospholipid-binding PH domain. Here we report that this novel PDK 1-related enzyme is capable of activating the AKT/PKB kinase in a manner that is consistent with a PI 3-kinase-independent pathway.

EXPERIMENTAL PROCEDURES

Materials—LY 294002 and wortmannin were purchased from Sigma. Olomoucine and roscovitine were obtained from Biomol Research Laboratories. IGF-1 was purchased from Roche Molecular Biochemicals. The anti-AKT, anti-phospho-AKT (Thr308), and anti-phospho-AKT (Ser473) antibodies were from New England Biolabs. The anti-GFP, anti-V5, and anti-phospho-AFX (Ser193) were obtained from CLONTECH, Invitrogen, and Upstate Biotechnology, respectively.

Constructs—Dr. M. Yan and Dr. T. Tang (Genentech, Inc.) kindly provided the FLAG-tagged AKT in PRK5 and the EGFP-AFX vector, respectively. The FLAG-DPH-AKT encodes amino acid residues 118–480 of AKT, thus excluding the NH2-terminal PH domain. Plasmids for expression of GST-AKT in 293E cells were constructed in the vector pEBG-2T. Using standard polymerase chain reaction protocols, full-length cDNA encoding PIAK or CEPDK1 was cloned from a C. elegans cDNA library (Stratagene), and hPDK1 was cloned from a human fetal brain cDNA library. Expression vectors for PIAK, CEPDK1, and hPDK1 were constructed in pCMV3-his/T-v-His TOPO vector (Invitrogen). The V5-Δ PH-CEPDK1 encodes amino acid residues 1–480 of CEPDK1, thus excluding the COOH-terminal PH domain. The PIAK K59M, CEPDK1 K98M, and hPDK1 K111M mutants were made by site-directed mutagenesis using the Quickchange TM kit from Stratagene. The presence of the introduced mutation and fidelity of polymerase chain reaction reactions were confirmed by sequence analysis.

Cell Culture—The 293E human epithelial cell line was cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Life Technologies, Inc.) and antibiotics (50 units/ml penicillin and 50 μg/ml streptomycin). The MCF7 human breast carcinoma cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cells were transfected using the FuGENE TM 6 reagent (Roche Molecular Biochemicals). The T98G human glioblastoma cells were obtained from ATCC and cultured in Eagle’s minimal essential medium supplemented with 10% fetal calf serum (Life Technologies, Inc.), 1.0 mM sodium pyruvate, and 0.1 mM nonessential amino acids.

Expression of GST-AKT in 293E Cells—The 293E cells were transiently transfected with DNA constructs expressing FLAG-AKT with wild type or kinase dead (KA) mutant of V5-CEPDK1, V5-Δ PH-CEPDK1, or V5-PIAK. 36 h after transfection, cell extracts were prepared, immunoprecipitated with anti-AKT antibody, and then immunoblotted with a policlonal antibody (New England Biolabs) to detect the phospho-Thr308 of AKT, the phospho-Ser473 of AKT, or the total AKT. 20 μg of cell lysate proteins was also blotted with anti-V5 antibody (Invitrogen). B, 36 h after transfected with constructs expressing FLAG-Δ PH-AKT with V5-CEPDK1 or V5-PIAK, 293E cells were analyzed for the phosphorylation of AKT at Thr308 as in A.

FIG. 2. PIAK phosphorylates human AKT at Thr308. A, 293E cells were transiently transfected with DNA constructs expressing FLAG-AKT with wild type or kinase dead (KA) mutant of V5-CEPDK1, V5-Δ PH-CEPDK1, or V5-PIAK. 36 h after transfection, cell extracts were prepared, immunoprecipitated with anti-AKT antibody, and then immunoblotted with a policlonal antibody (New England Biolabs) to detect the phospho-Thr308 of AKT, the phospho-Ser473 of AKT, or the total AKT. 20 μg of cell lysate proteins was also blotted with anti-V5 antibody (Invitrogen). B, 36 h after transfected with constructs expressing FLAG-Δ PH-AKT with V5-CEPDK1 or V5-PIAK, 293E cells were analyzed for the phosphorylation of AKT at Thr308 as in A.

FIG. 3. PIAK phosphorylates human AKT at Thr308 in a PI 3-kinase-independent manner. A, 293E cells were transiently transfected with DNA constructs expressing FLAG-AKT with wild type or kinase dead (KA) mutant of V5-hPDK1, -CEPDK1, or -PIAK. 12 h after transfection, cells were starved for 18 h. The starved cells were then left untreated, stimulated with IGF-1 (100 ng/ml) for 10 min, or treated with 200 nM wortmannin for 30 min before the stimulation of IGF-1 (100 ng/ml) for 10 min. Phosphorylation of AKT was detected by immunoprecipitation with AKT antibody, followed by immunoblotting with anti-phospho-AKT (Thr308) or anti-AKT antibodies. B, purified GST-AKT was incubated for 30 min at 30 °C with the immunoprecipitated wild type or kinase dead (KA) mutant of V5-PIAK in the presence of 100 μM ATP and phospholipid vesicles with or without PtdIns(3,4,5)P3. Phosphorylation of AKT was detected by immunoblotting with anti-phospho-AKT (Thr308). C, the assay was repeated with purified PDK 1. Note the lipid dependence of PDK 1 activity versus the lipid independence of PIAK activity.
PIAK interacts with AKT more efficiently in the presence of LY 294002. 293E cells were transiently transfected with plasmids expressing FLAG-AKT with V5-hPDK1, V5-CEPDK1, or V5-PIAK. 12 h after transfection, the cells were left untreated or treated with 20 μM LY 294002 for 16 h. Cell lysate proteins were immunoprecipitated with anti-AKT antibody and then immunoblotted with anti-V5 antibody, anti-phospho-AKT (Thr308) antibody, or anti-AKT antibody. 10% of the lysate used in the immunoprecipitation was blotted with anti-V5 antibody showing the input proteins.

The supernatant was incubated for 2 h with glutathione-Sepharose beads. The beads were collected by centrifugation for 1 min at 3000 × g and then washed three times in buffer A. The beads were further washed five times in buffer B (50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.03% Brij-35, 0.27 M sucrose to remove Triton X-100, which might interfere with the activation of AKT by PIK). GST-AKT was eluted from the beads with buffer B containing 20 mM glutathione, pH 8.0. Immunoprecipitates were washed in lysis buffer, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subsequently analyzed by protein immunoblotting.

In Vitro Phosphorylation of GST-AKT by PIK and PDK 1—In vitro phosphorylation of GST-AKT by PIK and PDK 1 was determined as described in Materials and Methods. The reaction was initiated by the addition of 2 μg of purified GST-AKT. After incubation for 30 min at 30°C, the reaction was terminated by 1% SDS. The assay was performed in a similar manner using PDK 1.

RESULTS AND DISCUSSION

**A Novel C. elegans Kinase That Phosphorylates AKT/PKB on Thr308**—While examining the C. elegans genome for a homologue of the PDK 1 kinase that might be involved with the phosphorylation of the second (Ser473) activation site on AKT/PKB, we observed a kinase that was highly related to both C. elegans and mammalian PDK 1. Fig. 1 illustrates that the kinase (PIAK) contained a kinase domain that was conserved with the kinase domain of mammalian (41% identity, 57% similarity) and C. elegans (32% identity, 56% similarity) PDK 1. In addition, this figure illustrates that the novel kinase lacked the lipid-binding PH domain, which is found in both the PDK 1 as well as the AKT/PKB kinases. In addition, scrutiny of the C. elegans genome did not reveal any phospholipid-binding PH encoding exons 3’ of the gene encoding this novel kinase. Examination of the non-kinase sequences in the novel protein did not demonstrate any known membrane association motifs. These data suggested that the novel C. elegans kinase could have substrate specificity that was similar to PDK 1, but might be regulated in a phospholipid-independent manner.

To determine whether the C. elegans PDK 1 can phosphorylate AKT/PKB at the activating Thr308 residue in a heterologous system, cotransfected the novel kinase together with human AKT/PKB into 293 cells in the presence of serum and examined the phosphorylation of this residue using a phospho-specific antibody. Fig. 2A illustrates that the C. elegans PDK 1 is capable of phosphorylating human AKT/PKB at Thr308, thus confirming the genetic data suggesting a role for this kinase in AKT/PKB activation in the dauer pathway (24). Importantly, removal of the PH domain of this kinase resulted in a loss of Thr308 phosphorylation, suggesting that the AKT/PKB phosphorylating activity of C. elegans PDK 1 kinase requires an interaction with cellular 3'-phospholipids mediated by this domain. This figure also illustrates that the novel kinase is capable of phosphorylating the Thr308 residue as efficiently as the known PDK 1 kinase, despite the fact that the novel kinase lacks a PH domain. In addition, the figure illustrates that the kinase activity of the novel enzyme is required for this phosphorylation event, since the ATP binding mutant (KA) shows little, if any, activity. Finally, Fig. 2B shows that, while the PDK 1 kinase of C. elegans requires a PH domain on the AKT/PKB substrate for Thr308 phosphorylating activity, the novel kinase does not require this domain. These data suggest that, while the C. elegans PDK 1 appears to have fastidious requirements for phospholipid interaction domains, the novel kinase is capable of phosphorylating the activation site of AKT/PKB in the absence of a phospholipid-binding domain on either protein.

**The Novel Kinase Phosphorylates AKT/PKB Thr308 in a PI 3-Kinase-independent Manner**—The above data were consistent with the hypothesis that the novel C. elegans PDK 1-like kinase might be active in the absence of the 3'-phospholipids produced by PI 3-kinase activity. To examine this possibility, human and C. elegans PDK 1 as well as the novel C. elegans kinase were analyzed for AKT/PKB Thr308 phosphorylating activity in the presence of the PI 3-kinase inhibitor, wortmannin. Fig. 3 illustrates that, in contrast to human and C. elegans PDK 1, PIAK is able to phosphorylate AKT at Thr308 in the absence of the IGF-1 growth factor. The figure also illustrates that the IGF-1-mediated activation of human and C. elegans PDK 1 is strongly inhibited by wortmannin, while the constitutive activity of PIAK is not. Fig. 3B further shows that in the presence or absence of PI 3-kinase, purified GST-AKT can be phosphorylated by PIAK in vitro. Fig. 4 illustrates that, as expected, LY 294002 strongly inhibits the ability of both human as well as C. elegans PDK 1 kinases to phosphorylate human AKT/PKB at the Thr308 residue in response to serum. Importantly, this figure also depicts that the activity of the PIAK kinase is not inhibited by LY 294002, but is instead enhanced, suggesting that the activity of this kinase is independent of PI 3-kinase. To further examine the possible mechanism for this enhanced activity, we analyzed the ability of the PDK 1 kinases to interact physically with AKT/PKB in a co-precipitation assay. Fig. 4 illustrates that both human and C. elegans PDK 1 can be coprecipitated with human AKT/PKB, and this interaction appears to depend upon the presence of serum-induced PI 3-kinase activity. One interpretation of these results is that membrane association of the two kinases via PH-mediated binding to 3'-phospholipids is in part responsible...
for their physical interaction (2–7). In contrast to this result, the association between the human AKT/PKB and the novel kinase appears to be strengthened by the inhibition of PI 3-kinase activity. A possible explanation for this result is that loss of 3-phospholipids after inhibition of PI 3-kinase activity by LY 294002 releases the membrane-associated AKT/PKB, thus allowing for an interaction with the presumably nonmembrane-associated novel kinase (29). Together, these data strongly suggest that the novel kinase is able to phosphorylate AKT/PKB in a phospholipid-independent manner. Thus, we have chosen to name the novel kinase PIAK (phospholipid independent AKT/PKB kinase).

PIAK Induces the PI 3-Kinase-independent Cytoplasmic Localization of AFX—While these data suggested that PIAK was capable of inducing the phosphorylation of the AKT/PKB activating site, they did not address the functional implications of this phosphorylation event. Both genetic and biochemical data suggest that the phosphorylation of a subset of forkhead transcription factors by AKT/PKB mediates the cytoplasmic localization of these factors (14, 15, 22, 23). In addition, it has been established that the nuclear localization of these transcription factors induces cell death via apoptosis in cultured cells, presumably because the transcription factors bring about the expression of genes that precipitate the apoptotic cascade (14). Thus, a major activity of AKT/PKB is to sequester these death-inducing transcription factors in the cytoplasm, where they are inactive. We therefore sought to examine the phosphorylation of one of these transcription factors, AFX, in response to human and C. elegans PDK 1 as well as to PIAK in the absence and presence of

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**Fig. 5.** PIAK induces the PI 3-kinase-independent phosphorylation and cytoplasmic localization of AFX. MCF7 cells were transfected with EGFP-AFX, FLAG-AKT, and V5-PDK (hPDK1, CEPDK1, or PIAK). 12 h after transfection, cells were untreated or treated with 20 μM LY 294002 for 16 h. Cell lysate proteins were immunoblotted with an antibody against phospho-AFX (Ser193), GFP, AKT, or V5. Localization of EGFP-AFX was detected by direct fluorescence. Representative pictures are shown in B, and quantification of the fluorescence data is shown in C. For each condition, 100–200 cells were scored. Cells were scored as having fluorescence that was stronger in the nucleus, equal in nucleus and cytoplasm, or stronger in cytoplasm.
A Phospholipid-independent AKT/PKB Kinase

Another specific PI 3-kinase inhibitor, wortmannin.

Fig. 5A illustrates that both human as well as C. elegans PDK 1 are able to stimulate the AKT/PKB-dependent phosphorylation of AFX as detected by a phospho-specific antibody. In addition, this figure shows that this phosphorylation is inhibited in the presence of wortmannin and is dependent upon serum, again consistent with the hypothesis that the phosphorylation of AKT/PKB by these kinases is contingent upon phospholipids produced by PI 3-kinase. Analysis of the ability of PIAK to induce AFX phosphorylation reveals that this occurs both in the absence of serum as well as in the presence of wortmannin, consistent with the suggestion that PIAK is able to induce the activation of AKT/PKB in the absence of phospholipids produced by PI 3-kinase. To examine whether the AFX phosphorylation was of functional importance, we analyzed the subcellular localization of a green fluorescent protein (GFP) expressed in PIAK-transfected cells. Because nuclear localization of AFX is lethal (15, 14),2 these results suggest that PIAK is capable of inhibiting AFX-induced apoptosis in the absence of PI 3-kinase activity.

CDK Inhibitors Induce PIAK Activity—Because LY 294002 is capable of inducing G1 arrest (30), next we decided to examine whether inhibitors of cell cycle progression could induce the AKT/PKB phosphorylating activity of PIAK. Fig. 6A illustrates that olomoucine and roscovitine, specific inhibitors of cyclin-dependent kinases (31), are able to increase the level of AKT/PKB phosphorylation in response to transfection PIAK, but not to either human or C. elegans PDK 1. This increase in AKT/PKB activity was not observed with other types of inhibitors such as nocodazole or aphidicolin (data not shown), suggesting that it may be a specific effect of inhibiting cyclin-dependent kinases. Interestingly, the enhancement of phosphorylation by olomoucine was similar to that observed with the protein phosphatase 2A inhibitor okadaic acid (data not shown), confirming the suggestion that protein phosphatase 2A dephosphorylates the Thr308 site on AKT/PKB in vivo (32) and suggesting that the observed olomoucine-induced enhancement was significant. Finally, Fig. 6B shows that these CDK inhibitors also activate endogenous AKT/PKB in T98G human glioblastoma cells. Together, these data suggest that PIAK may be activated by inhibition of cell cycle progression, particularly by the arrest of G1 progression.

The results reported here are consistent with the proposal that a PI 3-kinase-independent pathway can activate the AKT/PKB survival kinase, at least in the nematode C. elegans. This conclusion is important, because it provides the first detailed molecular evidence that this critical cytoprotective pathway can be regulated by nonphospholipid-dependent mechanisms. Importantly, the results are completely consistent with the domain structure of PI 3-kinase, which lacks the phospholipid binding PH motif found in PDK 1. The results also suggest that the activation of PIAK induces the cytoplasmic localization of the apoptotic transcription factor, AFX. This latter result is significant, because it suggests that the activation of this novel kinase is functionally important to the maintenance of cell survival. Finally, the activation of this kinase by induction of cell cycle arrest, particularly in the G1 phase of the cell cycle, suggests that PIAK may be functionally important during periods of cellular inactivity induced by growth factor starvation.

While these results argue for a significant role for PIAK in cytoprotection, a number of questions remain. The mechanism by which PIAK interacts with and activates AKT/PKB remains unresolved. The fact that the two proteins can be efficiently coimmunoprecipitated, especially in the absence of PIP3 phospholipids, suggests that these proteins are directly associated. In addition, while previous data suggested that the AKT/PKB kinase maintains a structure that is resistant to Thr308 phosphorylation in the absence of 3-phospholipids (2–7), the current data suggest that PIAK may interact with AKT/PKB in a manner that exposes this critical residue to the enzyme’s kinase activity. A second question concerns the conservation of this kinase in other species. While examination of mammalian data bases has not revealed an obvious homologue of PIAK, examination of the D. melanogaster genome revealed that the inaC (33) protein kinase, a homologue of protein kinase C, was the kinase most closely related to PIAK. While it is unlikely that the inaC kinase is a functional homologue of PIAK, it is of course possible that a more distantly related kinase is in fact the functional homologue of the nematode kinase. No apparent homologue of PIAK in Homo sapiens has yet been found, although a definitive answer awaits the release of the complete human genome sequence. In one human glioblastoma cell line, T98G, the phosphorylation of endogenous AKT at Thr308 was found to be stimulated by Cdk and the inhibitors olomoucine and roscovitine (Fig. 6B). Because only PIAK, but not PDK 1, was found to be stimulated by Cdk inhibitors, this observation suggests that there is at least a functional homologue of PIAK in humans. Alternatively, it is possible that PIAK functions in a pathway that is specific for C. elegans. For example, PIAK might be activated during the dauer stage of the larval nematode to enable cytoprotection under low nutrient conditions where cells are presumably quiescent. It will of course be important to examine the effects of mutations of PIAK in worms to more...

Fig. 5C. C. elegans PI 3-kinase interacts with and activates AKT/PKB. (A) Lysates of PIAK-transfected 293E cells were immunoprecipitated with antibodies to V5 epitope, and then immunoblotted with anti-AKT antibody or anti-phospho-AKT antibody and then immunoblotted with anti-phospho-AKT (Thr308) antibody or anti-AKT antibody. (B) Immunoprecipitated lysates were left untreated or treated with 200 μg of the lysate protein was also blotted with anti-V5 antibody to show the expressed PDKs. B. The inhibitors of CDKs stimulate the phosphorylation of endogenous AKT at Thr308 in the T98G human glioblastoma cells. 3 × 105 T98G cells were left untreated or treated with 200 μg olomoucine or 30 μg roscovitine for 2 h. Cell lysates were prepared, immunoprecipitated with anti-AKT antibody and then immunoblotted with anti-phospho-AKT (Thr308) antibody or anti-AKT antibody.

Fig. 6. PIAK is activated by the inhibitors of CDKs. A. 293E cells were transiently transfected with plasmids expressing FLAG-PIAK with V5-PIPK1, V5-CEPDK1, or V5-PIAK. 24 h after transfection, the cells were left untreated or treated with 200 μM olomoucine or 30 μM roscovitine for 2 h. Cell lysates were immunoprecipitated with anti-AKT antibody and then immunoblotted with anti-phospho-AKT (Thr308) antibody or anti-AKT antibody. 20 μg of the lysate protein was also blotted with anti-V5 antibody to show the expressed PDKs. B. The inhibitors of CDKs stimulate the phosphorylation of endogenous AKT at Thr308 in the T98G human glioblastoma cells. 3 × 105 T98G cells were left untreated or treated with 200 μg olomoucine or 30 μg roscovitine for 2 h. Cell lysates were prepared, immunoprecipitated with anti-AKT antibody and then immunoblotted with anti-phospho-AKT (Thr308) antibody or anti-AKT antibody.
under diverse stressful conditions. In addition, it will be of significant interest to examine the ability of PIAK to phosphorylate other PDK 1 substrates, such as SGK, P70S6K, P90RSK, and various PKC isoforms (8). Finally, while the exact mechanism by which PIAK is itself activated remains to be elucidated, the fact that inhibitors of GI progression result in PIAK-induced Th308 phosphorylation is also consistent with the hypothesis that this activation pathway may be operable during periods of cell quiescence. One possibility is that low PIP3 levels during cellular quiescence might release membrane-associated AKT/PKB, thus allowing for an enhanced interaction with PIAK. Together, these data suggest that disparate mechanisms may operate to ensure that the apoptotic cascade initiated by forkhead transcription factors is inhibited under diverse stressful conditions.

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