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"New"-Clear Functions of PDK1: Beyond a Master Kinase in the Cytosol?

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Abstract Activation of cytosolic phosphoinositide-3 kinase (PI-3K) signaling pathway has been well established to regulate gene expression, cell cycle, and survival by feeding signals to the nucleus. In addition, strong evidences accumulated over the past few years indicate the presence of an autonomous inositol lipid metabolism and PI-3K signaling within the nucleus. Much less, however, is known about the role and regulation of this nuclear PI-3K pathway. Components of the PI-3K signaling pathway, including PI 3-kinase and its downstream kinase Akt, have been identified at the nuclear level. Consistent with the presence of a complete PI-3K signaling pathway in the nucleus, we have recently found that phosphoinositide-dependent kinase 1 (PDK1), a kinase functioning downstream of PI-3K and upstream of Akt, is a nucleo-cytoplasmic shuttling protein. In the present review, we update our current knowledge on the regulatory mechanisms and the functional roles of PDK1 nuclear translocation. We also summarize some of the kinase-independent activities of PDK1 in cell signaling. J. Cell. Biochem. 96: 1157–1162, 2005.

Key words: nuclear PI-3K signaling pathway; PDK1; insulin; IGF-1; PIP_3

Activation of phosphoinositide-3 kinase (PI-3K) pathway constitutes one of the most important mechanisms that regulate important cellular functions such as gene expression, cell cycle progression, cell growth, and differentiation [Dygas and Baranska, 2001]. While PI-3K signaling in the cytosol has been well documented as an essential pathway for transducing signals from the plasma membrane to the nucleus, we are only beginning to understand the role of an autonomous PI-3K signaling pathway operative within the nucleus [Neri et al., 2002]. Many proteins involved in the PI-3K signaling pathway, such as PI-3K, Akt, and the phosphatase and tensin homolog on chromosome 10 (PTEN), are localized within the nucleus. However, whether these PI-3K signaling pathway components can be solely activated in the nucleus remains largely unknown.

Activation of Akt and other PI-3K downstream kinases in the cytosol is mediated by 3′-phosphoinositide dependent kinase 1 (PDK1), a serine/threonine kinase originally identified as a kinase critical for Akt activation loop phosphorylation and activation [Cohen et al., 1997]. Since then, this kinase has been found to phosphorylate and activate other members of the cAMP-dependent, cGMP-dependent, and protein kinase C (AGC) kinase family of proteins including p70S6K, p90RSK, atypical and novel PKC isoforms, PRK1/2, SGK, and others (reviewed in [Wick and Liu, 2001; Mora et al., 2004]). Activation of these substrates leads to an increase in glucose uptake, protein synthesis, and inhibition of pro-apoptotic proteins.

PDK1, a 63 kDa protein kinase, consists of an N-terminal kinase domain and a C-terminal

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pleckstrin homology (PH) domain. The PH domain of PDK1 has been shown to bind to the PI-3K products phosphoinositol (3, 4, 5) phosphate (PIP_3) and phosphoinositol (3,4) phosphate (PIP_2), which target PDK1 to the plasma membrane where it phosphorylates Akt at Thr^{308} in the activation loop (T-loop) [Anderson et al., 1998; Komander et al., 2004]. Other substrates of PDK1 that do not possess PH domain or targeted to plasma membrane are activated by direct association with PDK1 (Fig. 1) [Biondi, 2004]. PDK1 itself is also activated by phosphorylation in the activation loop (Ser^{241} for human and Ser^{244} for mouse PDK1) [Casamayor et al., 1999; Wick et al., 2002] and this phosphorylation has been shown to be mediated by dimerization and trans-phosphorylation [Wick et al., 2003]. In addition to phosphorylation, PDK1 function has also been shown to be regulated by protein–protein interaction and its subcellular localization [Makris et al., 2002]. The present review focuses on regulation and potential function of PDK1 in the nucleus, which is a missing piece of the chain in the nuclear PI-3K pathway.

PDK1 NUCLEAR LOCALIZATION: COMPLETING PI-3K PATHWAY PRESENCE IN THE NUCLEUS

Over the past several years, there have been increasing evidences for the presence of an autonomous nuclear specific PI-3K signaling pathway. It has been shown that the nucleus maintains an independent pool of PIP_3 [Neri et al., 1999, 2002]. In addition, key PI-3K signaling components, including PI-3K and its downstream effectors such as Akt, PKCζ, p70S6KβI, and p70S6KβII, have all been found to be present and activated in the nucleus. An interesting question remains to be addressed is how these PI-3K downstream effectors, which are all substrates of PDK1, are activated in the nucleus. It is possible that these kinases are phosphorylated and activated by PDK1 in the cytosol, which leads to their subsequent nuclear translocation. On the other hand, PDK1 may directly phosphorylate these kinases in the nucleus. We recently found that the treatment of various cell lines with the nuclear export inhibitor, Leptomycin B (LMB), led to constitutive nuclear localization of PDK1, suggesting

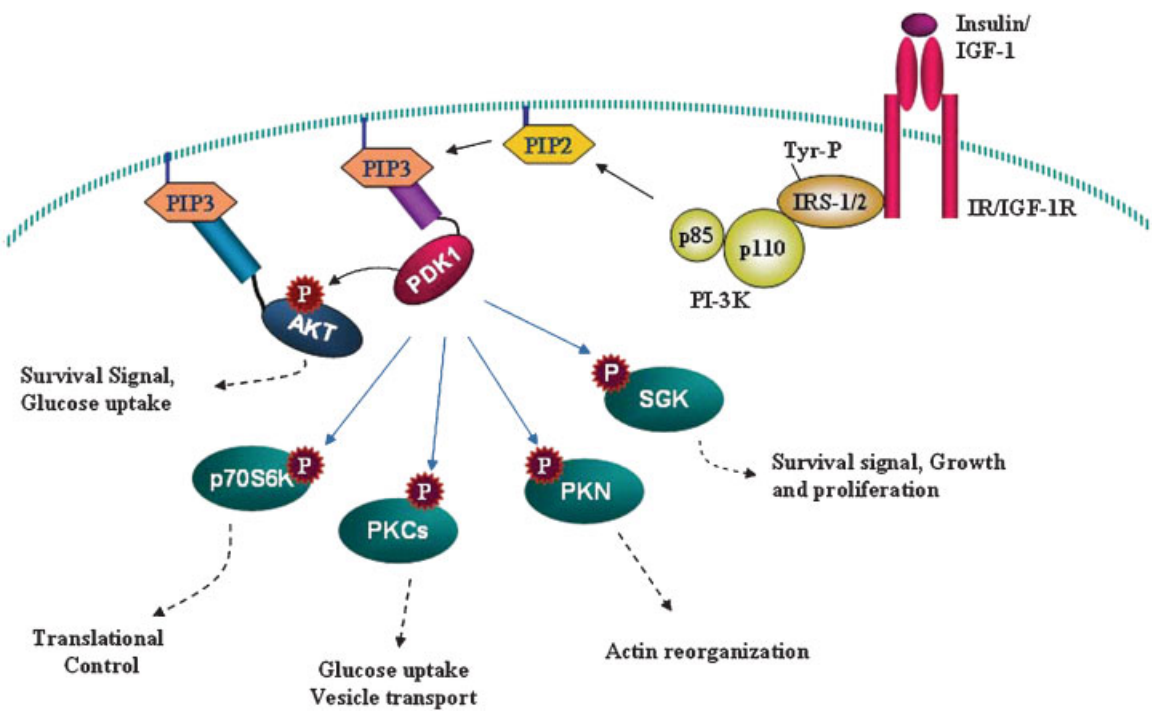

Fig. 1. Role of PDK1 in insulin signaling: Insulin stimulates phosphorylation of its receptor by tyrosine phosphorylation. Activated receptors then recruits and activates insulin receptor substrate 1/2 (IRS1/2), leading to the activation of PI-3K and subsequent generation of inositols lipid, PIP_3. PIP_3 targets PDK1 and Akt on to plasma membrane where PDK1 activates Akt by phosphorylating the kinase in the activation loop.

that PDK1 is indeed a nucleo-cytoplasmic shuttling protein [Lim et al., 2003]. Deletion mapping and mutagenesis studies further revealed a functional nuclear export signal (NES) in mouse PDK1 between the kinase domain and the PH domain (amino acid residues 382–391) [Lim et al., 2003]. These findings suggest a possibility that downstream effectors of nuclear PI-3K may be phosphorylated and activated by PDK1 in the nucleus.

REGULATION OF PDK1 NUCLEAR LOCALIZATION

In unstimulated cells, endogenous PDK1 resides mainly in the cytoplasm with a very low percentage of plasma membrane localization. PDK1 nuclear localization becomes evident and statistically significant only when cells were treated with LMB, suggesting that PDK1 shuttles between nucleus and cytoplasm, with the export rate significantly higher than the import rate. While physiological factors that stimulate PDK1 nuclear localization are currently unknown, we have observed a significant increase in PDK1 nuclear localization in $PTEN^{-/-}$ cells, suggesting an involvement of PI-3K-mediated signaling in regulating PDK1 nuclear localization [Lim et al., 2003]. Interestingly, insulin or IGF-1 treatment also causes a moderate increase in PDK1 nuclear localization [Lim et al., 2003].

Little is known about the molecular mechansim underlying PI-3K-dependent PDK1 nuclear translocation. Scheid et al. recently demonstrated that IGF-1 stimulated human PDK1 phosphorylation at Ser^{396} and that Ser^{396} to Ala mutation reduced IGF-1-stimulated PDK1 nuclear translocation [Scheid et al., 2005]. These results suggest that phosphorylation at Ser^{396} may provide a mechanism by which IGF-1 regulates PDK1 nuclear localization. It is interesting to note that Ser^{396} is in close proximity to the NES of PDK1. Since the association of NES with nuclear export machinery containing chromosomal region maintenance protein 1 (CRM1) is essential for the nuclear exporting of NES-containing proteins [Kutay and Guttinger, 2005], it is possible that phosphorylation at Ser^{396} may interfere with the CRM1-dependent nuclear export of PDK1, leading to increased PDK1 nuclear localization [Scheid et al., 2005]. However, replacing Ser^{396} with Ala failed to prevent LMB-stimulated nuclear accumulation of PDK1, suggesting that at least the basal nucleo-cytoplasmic shuttling of PDK1 is independent of Ser^{396} phosphorylation. Furthermore, it is unknown how IGF-1 stimulates human PDK1 phosphorylation at Ser^{396}, since phosphorylation of Ser^{396} corresponding site in mouse PDK1 (Ser^{399}) is mediated by *cis*-autophosphorylation mechanism [Wick et al., 2002, 2003]. Apart from regulating the export of PDK1 from the nucleus, growth factors may promote PDK1 nuclear localization by enhancing its nuclear import rate. As mentioned earlier, PDK1 nuclear localization becomes evident approximately 30 min after insulin or IGF-1 treatment [Lim et al., 2003; Scheid et al., 2005]. Interestingly, this time course coincides with the nuclear translocation observed with other components of the PI-3K pathway, such as PI-3K and Akt [Andjelkovic et al., 1997; Meier and Hemmings, 1999]. These findings raise the possibility that a unified mechanism might exist for nuclear translocation of components in the PI-3K signaling pathway.

POTENTIAL ROLES OF NUCLEAR PDK1

Growth factor-stimulated activation of the PI-3K signaling pathway has been shown to regulate many important events such as transcription, survival, and cell cycle. Since plasma membrane translocation is necessary for PDK1 to phosphorylate and activate cytosolic kinases such as Akt, nuclear translocation may result in the sequestration of PDK1 from the cytosol and subsequent downregulation of PI-3K-mediated cellular events. Consistent with this, we found that the cell lines stably expressing wild type, but not constitutively nuclear localized PDK1, displayed increased resistance to UV induced apoptosis. In addition, nuclear PDK1 had a lower potential to induce anchorage-independent cell growth compared to wild-type PDK1 [Lim et al., 2003].

However, it is possible that the nuclear translocation of PDK1 might have distinct roles in cell function. Activated nuclear PI-3K is shown to be an important mediator of the anti-apoptotic action of NGF [Ahn et al., 2004]. Nuclear translocation and activation of PI-3K has also been found to play a significant role in NGF-mediated PC12 cell differentiation [Martelli et al., 2003]. Recent findings on the presence of an autonomous nuclear inositol lipid metabolism [Neri et al., 2002] raise the

possibility that PI-3K-PDK1-Akt forms a signaling center within the nucleus to regulate specific nuclear events (Fig. 2). In support of this idea, nuclear PI-3K has been found to be activated by a nucleus-specific GTPase PI-3-kinase enhancer (PIKE) [Ye et al., 2000]. Our recent studies revealed that the nuclear localized PDK1 is kinase active and overexpression of a constitutively nuclear PDK1 indeed led to increased phosphorylation of p70S6KβI.

Very recently, Bimbo et al. showed the involvement of Pdk1p, a yeast homolog of mammalian PDK1, in cell cycle [Bimbo et al., 2005]. Pdk1p was found to be associated with the spindle checkpoint and its deficiency led to multi-nucleated cells arrested in cytokinesis. These results suggest a potential role of PDK1 in mitosis and cytokinesis. Interestingly, deficiency in another fission yeast homolog of mammalian PDK1, Ksg1p, also shows defects in G2 phase of cell cycle [Niederberger and Schweingruber, 1999]. Additional evidences suggest that PDK1 may play similar role in regulating cell cycle in mammalian cells. For example, antisense mediated depletion of PDK1 caused significant reduction in proliferation and the number of cells in mitosis phase of glioblastoma cells [Flynn et al., 2000]. However, it is currently unknown whether nuclear localization of PDK1 and subsequent activation of the nuclear PI-3K pathway play any roles in regulating cell cycle.

KINASE INDEPENDENT FUNCTIONS OF PDK1

In addition to its pivotal role as a master kinase in receptor tyrosine kinase signaling, PDK1 has recently been found as an adaptor protein in several signaling events independent of its kinase activity. First, PDK1 has been shown to interact with the N-terminal region of the Ras effector protein Ral guanine nucleotide dissociation stimulator (RalGDS) [Tian et al., 2002]. This interaction relieves the auto-inhibitory effect of RalGDS catalytic domain, leading to enhanced GEF activity of RalGDS. Further support for a kinase-independent role of PDK1 in cell signaling was obtained from recent studies on T-cell development [Hinton et al., 2004; Lee et al., 2005]. In stimulated T cells, PDK1 interacts and recruits PKCθ in the lipid raft. PDK1 also interacts and recruits the scaffold protein, CARD11 into lipid rafts along with Bcl10-MALT1. This complex then ubiquitinates and degrades the regulatory subunit of IKK complex, NEMO (IKKγ). Thus, PDK1 plays an essential role in T-cell development not only through its kinase activity (by activating PKCθ)

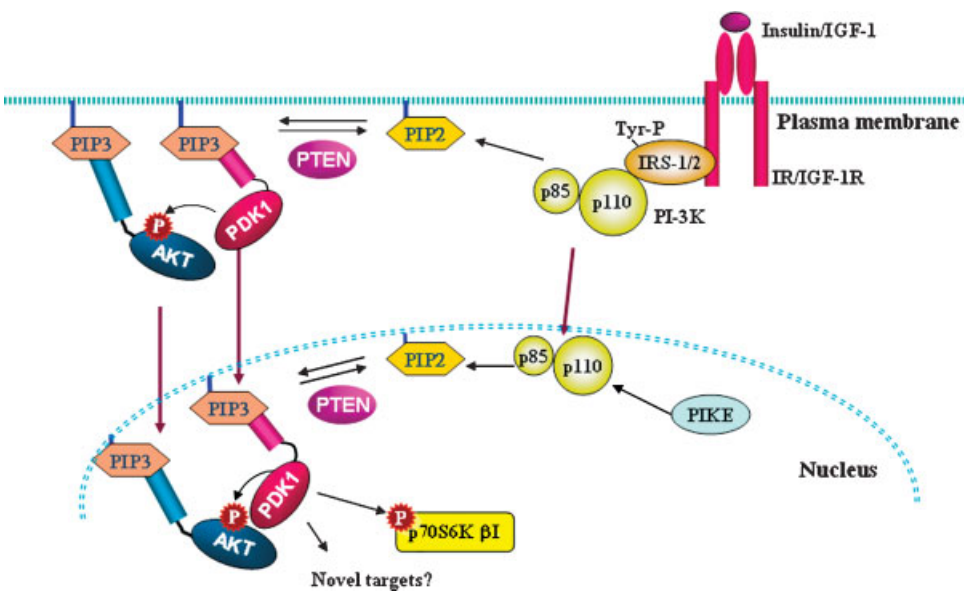

Fig. 2. A proposed model for nuclear PDK1 activation and its role in nuclear PI-3K signaling. Activation of nuclear PI-3K by PIKE leads to increased nuclear PIP_3 concentration. PDK1 and its substrates such as Akt are targeted to the nuclear membrane by nuclear PIP_3, leading to the activation of these kinases. PDK1 may also activate its nuclear specific substrates such as p70S6K β1 and other yet to be identified substrates.

but also by acting as an adaptor in recruiting PKCθ and CARD11 to regulate IKK and NF-κB [Lee et al., 2005]. This novel and kinase-independent role of PDK1 demonstrate that PDK1 may function in more diverse signaling pathways and its role may not be limited to a kinase activator. It would be interesting to determine whether PDK1 plays a similar role in the nucleus.

CONCLUSION AND FUTURE PERSPECTIVE

Evidences accumulated over the past several years suggest the presence of a phospholipid pool inside the nucleus distinct from those in the cytosol. In addition, kinases that participate in the PI-3K signaling family, including PI-3K and Akt, have also been reported to be in the nucleus. The recent finding that PDK1, a kinase functioning downstream of PI-3K and upstream of Akt in the cytosol, is located also in the nucleus provide further evidence that the nucleus possesses a complete PI-3K-PDK1-Akt signaling pathway.

Several important questions regarding nuclear localization of PDK1 remain to be answered. First, it is unclear whether and how nuclear PDK1 is activated and what role it may play in nuclear PI-3K signaling. We found that kinase-inactive PDK1 also underwent nuclear translocation [Lim et al., 2003], suggesting that the kinase activity of PDK1 is dispensable for its nuclear translocation process. The presence of nuclear phospholipids and PI-3K further suggests that PDK1 could potentially be activated in the nucleus. Thus, activation of PDK1 in the nucleus may be specifically regulated and have functions that are distinct from those operative in the cytosol.

Second, the regulation of PDK1 nuclear localization remains unclear. While increase in the level of PIP_3 precedes the nuclear translocation of some of the PI-3K components [Neri et al., 1999], the precise role of PIP_3 signaling in regulating nuclear import remains unknown. One of the general mechanisms of protein nuclear translocation involves nuclear localization signal (NLS)-importin dependent nuclear import [Pemberton and Paschal, 2005]. Although no NLS has been identified in Akt, PDK1 appears to possess a bi-partitate NLS (Kikani C, Dong L.Q, Liu F, unpublished). However, it remains to be established whether these sequences function as a bona fide NLS for PDK1 nuclear translocation in cells and if so, how they regulate PDK1 nuclear import.

Finally, what are the functional roles of PDK1 nuclear translocation? Does nuclear translocation of PDK1 play a role in sequestrating its function away from the cytosol or is the translocation necessary for completing the nuclear PI-3K signaling pathway, or both? Such a scenario has been suggested for p42/p44 MAP kinases, where chronic stimulation results in its nuclear sequestration, dephosphorylation, and loss of kinase activity [Volmat et al., 2001]. It is unclear whether a similar mechanism is operating for regulating PDK1. On the other hand, nuclear translocation of PDK1 may be essential for nuclear PI-3K signaling events. Consistent with this, some of the PDK1 substrates such as p70S6Kβ1 and β2 have been found to be predominantly localized in the nucleus [Minami et al., 2001], and are better substrates of nuclear PDK1 compared to wild-type PDK1, which is predominantly cytoplasmic. Taken together, these results suggest that nuclear PDK1 may participate in the nuclear PI-3K pathway. Since cytosolic and nuclear PI-3K signaling may play distinct roles in regulating specific events such as energy homeostasis, cell growth, differentiation, and apoptosis, answers to all these questions will not only shed new light on the regulatory mechanism and function of PDK1, but also provide important information for developing therapeutic drugs that can target specific cellular signaling modules.

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