DOCUMENT SUMMARY

This document is a foundational scientific minireview from the journal *Cell*, titled "Cellular Signaling: Pivoting around PDK-1." It focuses on the critical role of the enzyme **phosphoinositide-dependent kinase-1 (PDK-1)** within the **phosphoinositide 3-kinase (PI3K)** signaling pathway. The paper explains that PDK-1 is a master kinase that activates numerous other kinases in the **AGC superfamily** (like Akt/PKB and PKC), and its function is primarily regulated by substrate conformation and its location within the cell, rather than by being switched on or off itself.

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- **Related Docs**: This paper details specific mechanisms within the broader concepts discussed in "Translocation and Reversible Localization of Signaling Proteins" and provides a molecular basis for processes relevant to "The Neuroscience of Autism."

FORMATTED CONTENT

Cellular Signaling: Pivoting around PDK-1

Alex Toker and Alexandra C. Newton *Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School Department of Pharmacology, University of California, San Diego*

The **phosphoinositide 3-kinase (PI3K)** signaling pathway mediates a multitude of cellular responses following extracellular stimulation by peptide growth factors and hormones. Deregulation of this pathway is associated with human diseases such as cancer and diabetes. The importance of this pathway in cell biology is underscored by the fact that **PI3K** signaling influences both cell survival and death, in addition to other fundamental cellular functions such as growth, motility, differentiation and insulin action. It does so by activating multiple distinct secondary signaling cascades, and considerable information exists about the precise biochemical mechanisms by which **PI3K** mediates these events. One group of enzymes that has emerged as a key mediator of the **PI3K** signal is the **AGC superfamily** of serine/threonine protein kinases (so named because it includes protein kinases A, G, and C), long known to be critical components of the signal transduction machinery. Most members of this family require an activating **phosphorylation**, setting off the search for a potential upstream kinase that was linked to the **PI3K** pathway.

> The search for such a kinase culminated with the discovery in 1997 of a novel member of the **AGC** family, the **phosphoinositide-dependent kinase-1 (PDK-1)**. **PDK-1** has now been shown to stand at a pivotal point in signaling, initiating a flurry of studies into understanding how **PDK-1** function is regulated. This review discusses how the primary regulators of **PDK-1** function are substrate conformation and subcellular localization.

Akt/PKB, the Archetypal PDK-1 Substrate

The discovery that activation of the proto-oncogene **Akt**, also known as **protein kinase B (PKB)**, is dependent on 3' phosphoinositides spawned much of the interest in the role of **PI3K** in cell signaling. Both **PtdIns-3,4-P2** and **PtdIns-3,4,5-P3** bind with high affinity to the **pleckstrin homology (PH) domain** of **Akt/PKB**, thus recruiting the kinase to the plasma membrane. However, an additional event is required to fully activate **Akt/PKB**. A common regulatory mechanism of kinases is through **phosphorylation** of a segment near the entrance to the active site, the **activation loop**, and a second phosphorylation site at the carboxyl terminus, in the **hydrophobic motif**. In **Akt**, these sites correspond to **Thr308** in the activation loop and **Ser473** in the hydrophobic site. Extensive biochemical studies have clearly demonstrated that **PDK-1** is the upstream kinase for **Thr308**. Following on the heels of the discovery that **PDK-1** is the **Akt/PKB** upstream kinase came the observation that **PDK-1** also phosphorylates a number of other kinases, including **p70S6-kinase (p70S6-K)** and **protein kinase C (PKC)**.

Substrate Conformation: A Key Regulator of PDK-1 Activity

The phosphorylation of **Akt/PKB** by **PDK-1** is regulated by the conformation of **Akt**. Specifically, the engagement of the **PH domain** on the membrane by binding **PtdIns-3,4,5-P_3/PtdIns-3,4-P_2** relieves autoinhibition of the active site, allowing **PDK-1** to access **Thr308** on the activation loop. Similarly, access of **PDK-1** to the activation loop of **PKC** is conformationally regulated. In this case, the autoinhibitory pseudosubstrate sequence of **PKC** must be removed from the substrate binding cavity in order for **PDK-1** to phosphorylate **PKC**.

> Thus, substrate conformation is a major determinant in allowing **PDK-1** phosphorylation to occur.

PDK-1 activates its substrate kinases by two mechanisms, direct or indirect. For **Akt/PKB** and the atypical **PKC**, phosphorylation at the activation loop serves as a direct "ON/OFF" switch for catalytic activity. In contrast, phosphorylation at the activation loop of conventional **PKC** isozymes does not result in activation but rather "primes" **PKC** for subsequent activation.

The Elusive PDK-2

While the regulation of the activation loop by **PDK-1** is widely accepted, that of the C-terminal hydrophobic site (**Ser473** in **Akt/PKB**) is less clear. This site is also conserved in other **AGC kinases**, and initial studies led to the proposal that an upstream kinase, distinct from **PDK-1**, was responsible. Thus, the name **PDK-2** was coined for the hydrophobic site kinase. Despite extensive biochemical analyses, such an enzyme has remained refractory to identification. Autophosphorylation now appears to account for the mechanism by which the hydrophobic site is regulated in **Akt/PKB**.

PDK-1 and the Hydrophobic Motif

A series of studies provided the first evidence that **PDK-1** interacts with high affinity with sequences corresponding to the C-terminal hydrophobic phosphorylation motif. This interaction appears to mask the autophosphorylation sites. Proteins containing **PDK-1 Interacting Fragment (PIF)** sequences could effectively compete for binding to **PDK-1**, releasing it from the C terminus and unmasking the hydrophobic site for autophosphorylation.

Regulation of PDK-1: Lipids, Location, Phosphorylation, and "PIF"

In contrast to its substrates, no significant switches for the intrinsic kinase activity of **PDK-1** have yet to be defined. Rather, recent studies converge on the idea that **PDK-1** function is regulated primarily by substrate conformation (as discussed above) and by cellular relocalization.

- **Lipids & Location**: The **PH domain** of **PDK-1** selectively binds **PtdIns-3,4-P2** and **PtdIns-3,4,5-P3**, explaining its relocalization from the cytosol to the plasma membrane in stimulated cells.
- **Phosphorylation**: The activation loop of **PDK-1** itself is regulated by autophosphorylation. Additionally, **tyrosine phosphorylation** by **Src** and **Abl** kinases can also activate **PDK-1**.
- **"PIF"**: Binding of **PIF** to **PDK-1** could stabilize the active conformation of **PDK-1**, increasing its activity toward substrates.

Perspectives

PDK-1 is a key enzyme in transducing signals to multiple effector pathways, and thus represents a pivotal point in **PI3K-dependent** and -independent signaling.

> Initial findings that **PDK-1** has a high basal activity even in unstimulated cells led to the notion that it is constitutively active, and that its activity is not critically regulated. However, recent studies have clearly demonstrated that the function of **PDK-1** is under tight control, with phosphorylation depending on substrate conformation and subcellular location.

The regulation by substrate conformation provides an attractive mechanism to allow **PDK-1** to discriminate between one subset of targets over another, leading to a specific cellular response. The challenge remains to attribute true **PI3K-dependent** signaling to specific **PDK-1** targets.