

## PKD1 – PKB/Akt Signaling

### Key References

- Chan, T.O. et al. "AKT/PKB and other D<sub>3</sub> phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation." *Annu. Rev. Biochem.* **68**, 965-1014 (1999).
- Coffer, P.J. et al. "Protein kinase B (c-Akt): A multifunctional mediator of phosphatidylinositol 3-kinase activation." *Biochem. J.* **335**, 1-13 (1998).
- Coghlan, M.P. et al. "Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription." *Chem. Biol.* **7**, 793-803 (2000).
- Datta, S.R. et al. "Cellular survival: A play in three Akts." *Genes Dev.* **13**, 2905-2927 (1999).
- Davies, S.P. et al. "Specificity and mechanism of action of some commonly used protein kinase inhibitors." *Biochem. J.* **351**, 95-105 (2000).
- Dennis, P.B. et al. "Target of rapamycin (TOR): Balancing the opposing forces of protein synthesis and degradation." *Curr. Opin. Gen. Dev.* **9**, 49-54 (1999).
- Downward, J. "Mechanisms and consequences of activation of protein kinase B/Akt." *Curr. Opin. Cell Biol.* **10**, 262-267 (1998).
- Dufner, A., Thomas, G. "Ribosomal S6 kinase signaling and the control of translation." *Exp. Cell Res.* **253**, 100-109 (1999).
- Kandel, E.S., Hay, N. "The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB." *Exp. Cell Res.* **253**, 210-229 (1999).
- Okano, J. et al. "Akt/protein kinase B isoforms are differentially regulated by epidermal growth factor stimulation." *J. Biol. Chem.* **275**, 30934-30942 (2000).
- Vandromme, M. et al. "Protein kinase B beta/Akt2 plays a specific role in muscle differentiation." *J. Biol. Chem.* **276**, 8173-8179 (2001).
- Vanhaesebroeck, B., Alessi, D.R. "The PI3K-PDK1 connection: more than just a road to PKB." *Biochem. J.* **346**, 561-576 (2000).

### Overview

The PDK1–PKB/Akt axis represents one of the most actively researched signaling pathways. This protein kinase cascade is known to play a central role in the actions of diverse stimuli (insulin, growth factors, integrins and GPCRs etc.) and the regulation of cell survival, metabolism (including insulin-stimulated glucose transport and glycogen synthesis), gene expression, cell cycle entry and protein synthesis, to name but a few.

All the kinases associated with this pathway lie in the protein serine/threonine kinase family and form a single highly branching protein kinase cascade (hence their grouping together). Several of these kinases contain pleckstrin homology (PH) domains that bind specific phosphoinositide lipids (e.g. phosphoinositide-3,4,5-trisphosphate (PIP<sub>3</sub>)) which are generated in the plasma membrane in response to agonist activity. As a result, the kinases are activated in a phosphoinositide 3-OH-kinase (PI3-kinase) dependent manner.

3-Phosphoinositide-dependent protein kinase-1 (PDK1) stands at the head of this important signaling pathway. Whether extracellular stimuli directly activate PDK1 (perhaps via the generation of plasma membrane-localized PIP<sub>3</sub>), or whether they simply induce the translocation of PDK1 to its substrate proteins within the plasma membrane, is not known. PDK1 activates a number of AGC-family protein kinases (named after their homology to protein kinases A, G and C) by phosphorylation, including protein kinase B (PKB or Akt) via phosphorylation of the T-loop Thr-308. The full activation of PKB/Akt also involves the binding of PIP<sub>3</sub> to the PH domain of PKB/Akt and the phosphorylation of an

additional residue, Ser 473, either by autophosphorylation, by PDK1, or by an as yet unidentified kinase called "PDK2".

There is a great deal of functional overlap between PKB/Akt isoforms; all phosphorylate the same RXRXXS/T motif and all are capable of transforming a cell when rendered constitutively active by the introduction of a myristolation signal sequence.

Thr 308 and Ser 473 lie within regions of PKB/Akt that are conserved throughout the AGC family kinases. Hence, PDK1 also phosphorylates and activates several other AGC-family kinases, including the serum and glucocorticoid-induced kinases (SGK), atypical forms of protein kinase C (e.g. PKCζ and PKCι/λ), p70S6-kinase and p90RSK. In conclusion, PDK1 is a central controller of multiple signaling pathways.

Once phosphorylated and activated, PKB/Akt phosphorylates and inhibits glycogen synthase kinase 3 (GSK3) leading to a decreased phosphorylation and activation of glycogen synthase. PKB/Akt also phosphorylates the mammalian target of rapamycin (mTOR, also known as FRAP and RAFT) although the role of this is not known. GSK3 continues to grow in importance as it also plays a role in the regulation of β-catenin stability and thus gene expression.

mTOR, which can be phosphorylated by PKB/Akt, is unusual in that it has both serine/threonine protein kinase as well as lipid kinase activities. It is a large complex molecule that is a receptor for the immunosuppressant, rapamycin. mTOR, along with PDK1, then plays an as yet ill-defined role in the activation of p70S6K which is important in the control of protein synthesis, development and growth

control. Thus, at least in part, the immunosuppressive activity of rapamycin is due to its actions on mTOR.

Other than rapamycin, there are few if any highly specific pharmacological inhibitors of this collection of protein kinases. This has often made understanding the role of these protein kinases in any action of an extracellular stimulus very difficult to ascertain. Furthermore, while in some cases kinase-dead derivatives of these kinases have been reported to act as dominant-negatives, this has often been highly controversial, largely because of their complex domain structures and abilities to interact with other proteins and signaling lipids. However, given the central importance of these protein kinases in numerous disease states (e.g. cancer and diabetes), the identification of specific inhibitors remains a very important goal.

## PDK1 – PKB/Akt Signaling

KINASE	PDK1	PKB/Akt	SGK	GSK3 ( <a href="#">G 1663</a> )	mTOR	p70S6K
<b>STRUCTURAL INFORMATION (HUMAN)</b>	556 aa Monomer	α 480 aa β 481 aa γ 479 aa All monomers	1 431 aa 2α 367 aa 2β 427 aa 3 429 aa All monomers	α 483 aa β 420 aa All monomers	2549 aa Monomer	α1 525 aa α2 502 aa β1 495 aa β2 482 aa All monomers
<b>DOMAIN STRUCTURE</b>	1 PH domain binds PtdIns(3,4,5)P <sub>3</sub>	1 PH domain binds PtdIns(3,4,5)P <sub>3</sub> and PtdIns(3,4)P <sub>2</sub>	—	—	16 HEAT domains 1 PI3-kinase homology domain 1 FKBP/rapamycin binding domain	1 autoinhibitory domain 1 nuclear localization signal sequence on α1 and β1
<b>FAMILY</b>	AGC kinase	AGC kinase	AGC kinase		PI 3-kinase	AGC kinase
<b>ACTIVATION</b>	Complex-see Overview	Phosphorylation by PDK1 and "PDK2" and binding of PIP <sub>3</sub> glucocorticoids	Phosphorylation by PDK1 (SGK1 expression induced by glucocorticoids)	Tyrosine phosphorylation (constitutive)	Complex, but may include phosphorylation by PKB	Phosphorylation by PDK1, mTOR, and atypical PKCs
<b>INACTIVATION</b>	—	—	—	Phosphorylation by PKB/Akt	—	—
<b>TARGET SUBSTRATE PHOSPHORYLATION MOTIF</b>	(S/T)FCGTXXYXAPE	RXRXX(S/T)	RXRXX(S/T)	(S/T)XXXSP	(S/T)P	(K/R)XRXX (S/T)
<b>SUBSTRATES</b>	PKA ( <a href="#">P 5511</a> , <a href="#">C 8482</a> , <a href="#">P 2645</a> ), PKG, PKB/Akts, MSKs, atypical PKCs, p70S6Ks, p90RSKs (all are protein kinases)	GSK3, mTOR, Raf, IKK, BAD, eNOS, hCaspase-8 ( <a href="#">C 1099</a> ), hTERT, BRCA1, IRS-1, PDK2, FKHR transcription factors	FKHR transcription factors	Glycogen synthase ( <a href="#">G 1399</a> ), ATP-citrate lyase, eIF-2B, c-Jun, Myc, Myb, PKA ( <a href="#">P 5511</a> , <a href="#">C 8482</a> , <a href="#">P 2645</a> ), CREB, IκB, PP1 ( <a href="#">P 7937</a> ), tau ( <a href="#">T 7675</a> , <a href="#">T 9392</a> )	4E-BP1, p70S6K	Ribosomal S6 subunit
<b>SPECIFIC INHIBITORS <sup>a</sup></b>	(UCN-1)	—	—	SB-216763 SB-415286 (LiCl) ( <a href="#">L 0505</a> ) (Ro 31-8220) ( <a href="#">R-136</a> )	Rapamycin ( <a href="#">R 0395</a> )	(H89) ( <a href="#">B 1427</a> )

### ABBREVIATIONS

**GSK3:** Glycogen synthase kinase 3

**H89:** N-(2-[p-Bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide

**MSK:** Mitogen- and stress-activated kinase

**PKA:** Protein kinase A

**PKC:** Protein Kinase C

**PKG:** Protein kinase G

**PtdIns(3,4)P<sub>2</sub>:** Phosphatidylinositol 3,4-bisphosphate

**PtdIns(3,4,5)P<sub>3</sub>:** Phosphatidylinositol 3,4,5-trisphosphate

**Ro 31-8220:** 2-(1-[3-Amidinothio]propyl)-1H-indol-3-yl)-3-(1-methylindol-3-yl)-maleimide

**SB-216763:** 3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indole-3-yl)-1H-pyrrole-2,5-dione

**SB-415286:** 3-(3-Chloro-4-hydroxyphenylamino)-4-(2-nitrophenyl)-1H-pyrrole-2,5-dione

**SGK:** Serum and glucocorticoid induced kinase

**UCN-1:** 7-Hydroxystaurosporine

### FOOTNOTES

<sup>a</sup> Inhibitors in parentheses are non-selective.