# Protein kinase B/Akt at a glance

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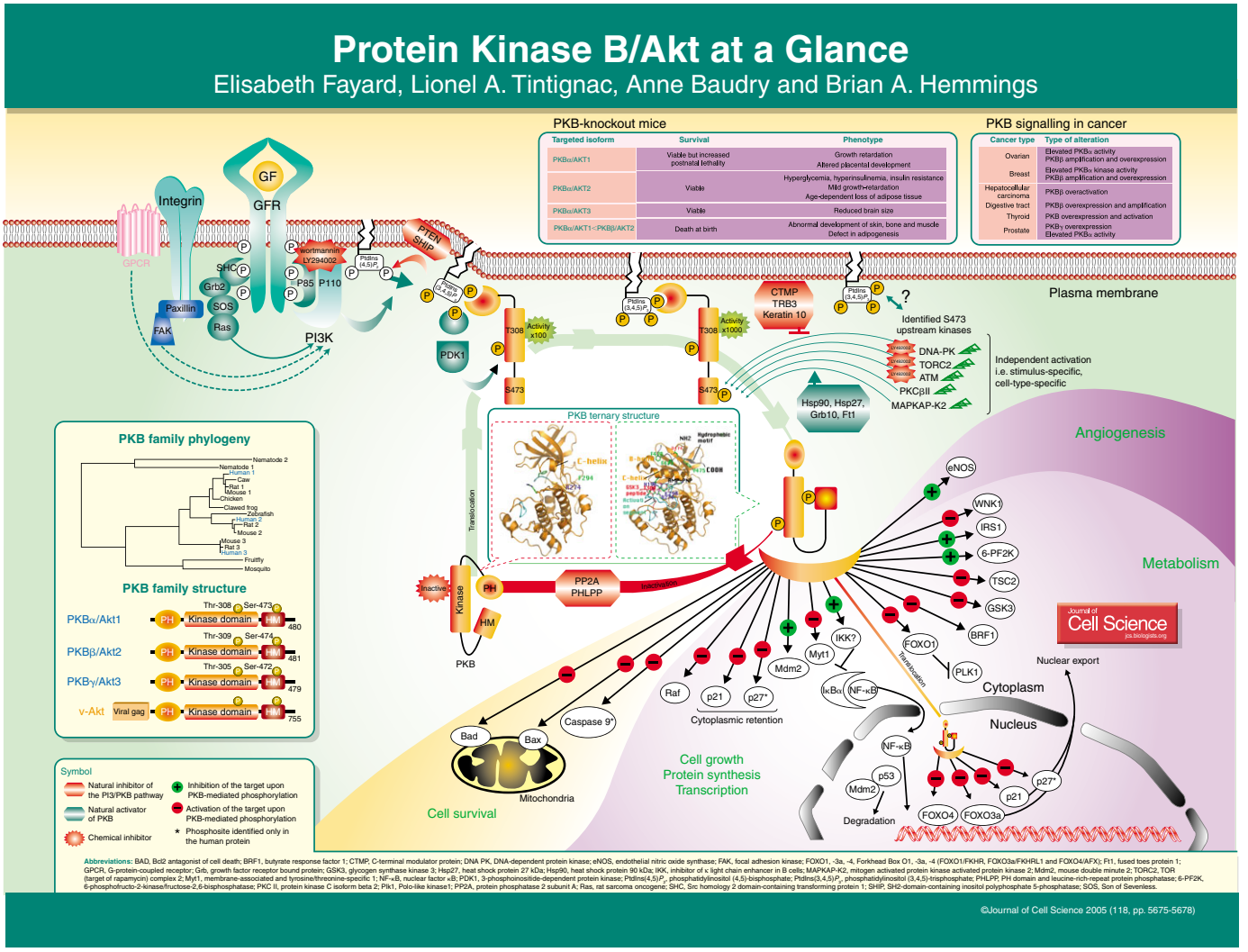
Among the signalling proteins that respond to a large variety of signals, protein kinase B (PKB, also known as Akt) appears to be a central player in regulation of metabolism, cell survival, motility, transcription and cell-cycle progression. Conserved from primitive metazoans to humans, PKB belongs to the AGC subfamily of the protein kinase superfamily, which consists of 518 members in humans (Manning et al., 2002). *PKB* and *v-AKT* were first cloned in 1991 by three independent groups (Brazil and Hemmings, 2001), more than a decade after the original identification of transforming murine leukemia virus Akt8 (Staal et al., 1977). The PKB subfamily comprises three mammalian isoforms, PKBα, PKBβ and PKBγ (Akt1, Akt2 and Akt3, respectively), which are products of distinct genes and share a conserved structure that includes three functional domains: an N-terminal pleckstrin homology (PH) domain, a central kinase domain, and a C-terminal regulatory domain containing the hydrophobic motif (HM) phosphorylation site [FxxF(S/T)Y] (Hanada et al., 2004). This architecture is conserved among species from fly, worm, mouse to man. Both the central kinase domain and the HM are highly conserved among AGC members, such as protein kinase C, ribosomal p90 S6 kinase, serum- and glucocorticoid-induced kinase, and p70 S6 kinase, mediators of responses triggered by insulin and/or other growth factors. Interestingly, the PH domain, essential for binding to lipids such as PtdIns(3,4,5)$P_3$, is absent in the *S. cerevisiae* orthologue (Sch9).

## PKB regulation

The key role of PKB in signalling became obvious when it was shown to be a downstream component in phosphoinositide (PI) 3-kinase signalling, which is activated upon (1) autophosphorylation of receptor tyrosine

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kinases induced by ligands (such as insulin or other growth factors), (2) stimulation of G-protein-coupled receptors, or (3) activation of integrin signalling (Foster et al., 2003; Wymann et al., 2003). PI 3-kinase is the key enzyme in the generation of the second messenger PtdIns(3,4,5)$P_3$ from PtdIns(4,5)$P_2$. This allows the translocation of PKB from the cytoplasm to the plasma membrane, which involves its PH domain. Interestingly, artificial targeting of PKB to the plasma membrane, using a myristoylated/palmitoylated sequence motif fused to the N-terminus of PKB, induces constitutive activation of the kinase. Once recruited to the plasma membrane, PKB is activated by a multi-step process that requires phosphorylation of both Thr308 in the activation loop of the kinase domain and Ser473 within the HM of the regulatory domain. Phosphorylation and subsequent activation are prevented by the PI 3-kinase inhibitors wortmannin and LY294002, both of which are key diagnostic reagents for delineating signalling events associated with this pathway.

The serine/threonine kinase phospho-inositide-dependent kinase 1 (PDK1), once recruited to the plasma membrane by PtdIns(3,4,5)$P_3$ through its PH domain, is the kinase responsible for the phosphorylation of Thr308 (Alessi et al., 1997; Stephens et al., 1998). However, the identity of the Ser473 kinase (S473K, also called PDK2) is still controversial. Several candidates have been proposed, including PKB itself (Toker and Newton, 2000), PDK1 (Balendran et al., 1999), integrin-linked kinase 1 (ILK1) (Persad et al., 2001), mitogen activated protein kinase activated protein kinase 2 (MAPKAP-K2) (Alessi et al., 1996), protein kinase C βII (PKCβII) (Kawakami et al., 2004), and the members of the atypical PI 3-kinase related protein kinase (PIKK) family: DNA-dependent protein kinase (DNA-PK) (Feng et al., 2004), ataxia telangiectasia mutant (ATM) (Viniegra et al., 2005) and, more recently, the rapamycin-insensitive mTOR complex TORC2 (Sarbassov et al., 2005). While their relative physiological significance remains to be determined, S473K activity is probably partially redundant and the identity of the kinase might depend on the cellular and physiological context. Phosphorylation of Ser473 is the key step in the activation of PKB because it stabilizes the active conformation state (Yang et al., 2002). Once activated at the plasma membrane, phosphorylated PKB can translocate to the cytosol or the nucleus (Andjelkovic et al., 1999).

Interestingly, a number of PKB-binding proteins have been shown to modulate PKB activity further in either a positive or negative manner. This suggests transient regulation of the kinase by adaptator proteins (Brazil et al., 2002; Song et al., 2005). Moreover, several phosphatases negatively regulate PKB activity. The tumor suppressor phosphatase and tensin homology deleted on chromosome ten (PTEN) (Stambolic et al., 1998) and the SH2-domain-containing inositol polyphosphate 5-phosphatase (SHIP) (Huber et al., 1999) inhibit PKB activity indirectly by converting PtdIns(3,4,5)$P_3$ to PtdIns(4,5)$P_2$ and PtdIns(3,4)$P_2$. Protein phosphatase 2A (PP2A) and PH domain leucine-rich repeat protein phosphatase (PHLPPα) do so directly by dephosphorylating Ser473 and/or Thr308 on PKB (Andjelkovic et al., 1996; Gao et al., 2005). Although PKB is also proposed to be activated in a PI 3-kinase-independent manner (Vanhaesebroeck and Alessi, 2000), the physiological significance of these findings requires further study.

## PKB in physiology

To understand the role of PKB, tremendous efforts have been made to identify its physiological substrates. PKB has been shown to phosphorylate and regulate the function of many cellular proteins involved in metabolism, survival/apoptosis, differentiation and proliferation (Brazil et al., 2004). Most of the PKB substrates contain the minimal consensus sequence RxRxx(S/T), where x is any amino acid and S/T is the phosphorylation site. Interestingly, many of the pathways modulated by PKB are also subject to regulation by other pathways, further highlighting the extreme complexity of PKB signalling regulation. The interesting issue of the substrate specificities of the different PKB isoforms still needs to be addressed, but recent work on the three knockout mice suggests isoform-specific functions, as well as some functional redundancy (Yang et al., 2004).

Genetic manipulation of *Pkb* genes in mice, either by transgenic or knockout approaches, has brought novel insights into PKB signalling (Brazil et al., 2004; Yang et al., 2004 and references therein). These studies identified a wide range of in vivo functions of PKB, revealing tissue-specific requirements for the different PKB isoforms in metabolism and embryonic development. Various transgenic mice expressing constitutively active PKB under the control of tissue-specific promoters have been generated. From the analysis of these, it appears that PKB plays a major role in cell growth: overexpression of active PKB leads to cardiac or skeletal muscle hypertrophy and an increase in β-cell mass and size, as well as potentially promoting the progression of tumours such as T cell lymphoma or prostate intraepithelial neoplasia (Lai et al., 2004; Yang et al., 2004). Interestingly, recent results from transgenic mice expressing a kinase-dead mutant of PKBα in β cells suggest that it is also involved in the regulation of the insulin secretory pathway (Bernal-Mizrachi et al., 2004). Expression of activated PKBα in the mammary gland results in delayed involution and the induction of lipid synthesis, leading to an increase in milk fat content and lactation defects (Schwertfeger et al., 2001; Schwertfeger et al., 2003). Finally, PKB has been shown to exert a neuroprotective effect against ischemic brain damage in genetically modified mice expressing an active form of PKB in neuronal cells (Ohba et al., 2004).

The *Pkbα/Akt1*, *Pkbβ/Akt2* or *Pkbγ/Akt3* genes in mice have been targeted by homologous recombination. *Pkbα/Akt1* deficiency results in significant neonatal mortality (Cho et al., 2001; Yang et al., 2003) and growth retardation due to a defect in placental development (Chen et al., 2001; Cho et al., 2001; Yang et al., 2003). By contrast, *Pkbβ/Akt2*$^{-/-}$ mice display insulin resistance and a type-II-diabetes-like syndrome. Furthermore, the absence of *Pkbβ/Akt2* is accompanied by mild growth-retardation

and age-dependent loss of adipose tissue. Both *Pkbα/Akt1* and *Pkbβ/Akt2* are required for normal platelet aggregation (Chen et al., 2004; Woulfe et al., 2004).

Recently, PKBγ has been shown to play an essential role in postnatal brain development; *Pkbγ/Akt3*$^{-/-}$ mice are characterized by a 20-25% reduction in brain size and weight, which is partially due to a decrease in cell size and cell number (Easton et al., 2005; Tschopp et al., 2005). Simultaneous inactivation of two PKB isoforms in mice leads to a more severe phenotype: for instance, *Pkbα/Akt1-Pkbβ/Akt2* double-knockout mutants die immediately after birth and exhibit dwarfism, impairment of adipogenesis and defects in skin, skeletal muscle and bone development (Peng et al., 2003). Overall, the phenotypic analysis of these mouse models highlights the specific functions of each PKB isoform in vivo and the necessity for cells to preserve a crucial level of PKB activity for normal growth, metabolism and differentiation. Additional combinations of *Pkb/Akt* knockout mice should provide further insights in the physiological role of PKB.

## PKB deregulation and human diseases

A precise understanding of upstream regulators, downstream targets, and *in vivo* functions of PKB should benefit treatment of diseases in which PKB signalling is deregulated, such as cancer and diabetes as well as schizophrenia (Nicholson and Anderson, 2002; Whiteman et al., 2002; Emamian et al., 2004). In cancer, constitutive high levels of PKB activity occur through a variety of mechanisms, including amplification of *Pkb/Akt* genes and mutations in components of the PI 3-kinase signalling pathway, such as PTEN (Luo et al., 2003) and the PI 3-kinase subunits. However, constitutive activation of PKB alone is believed to be insufficient for tumorigenesis. It is more likely to contribute to cancer progression by promoting the proliferation, cell survival and metabolic capacity of cells.

PKB is an attractive therapeutic target for treatment of cancer, and novel PH-domain-dependent inhibitors have recently been reported to exhibit PKB specificity (Barnett et al., 2005). However, only a subset of the cellular processes regulated by the PI-3-kinase–PKB pathway are involved in cancer progression. Therefore, the choice of drug targets should also take into account the adverse effects that could result from the inhibition of other PKB-regulated cellular processes, such as glucose metabolism, in which inhibition of PKB potentially leads to a diabetes-like syndrome (Luo et al., 2005). Furthermore, it is also crucial to keep in mind the potentially opposing effects of different PKB isoforms in cancer progression. Whereas a reduction in metastasis is observed upon Pkbα/Akt1 expression owing to increased differentiation (Hutchinson et al., 2004) overexpression of Pkbβ/Akt2 has been associated with invasion and metastasis (Arboleda et al., 2003). Therefore, pharmaceutical inhibitors specific for each PKB isoform will be crucial for treatment of diseases that exhibit abnormal PKB signalling.

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