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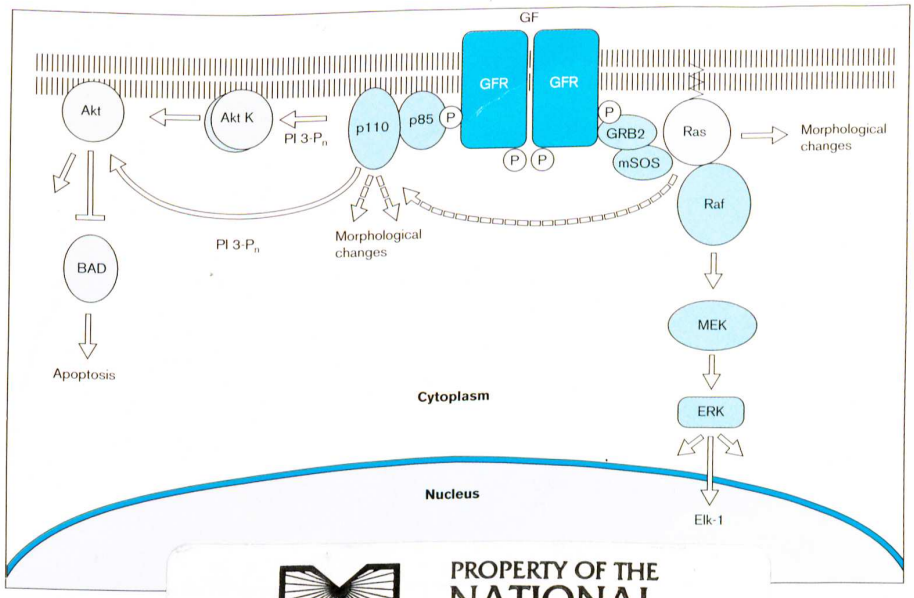

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Mechanisms and consequences of activation of protein kinase B/Akt

Julian Downward

Protein kinase B (PKB)/Akt is a growth-factor-regulated serine/threonine kinase which contains a pleckstrin homology domain. Binding of phosphoinositide 3-OH kinase products to the pleckstrin homology domain results in translocation of PKB/Akt to the plasma membrane where it is activated by phosphorylation by upstream kinases including the phosphoinoside-dependent kinase 1 (PDK1). Activated PKB/Akt provides a survival signal that protects cells from apoptosis induced by various stresses, and also mediates a number of metabolic effects of insulin.

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Abbreviations

BAD	Bcl-X_L/Bcl-2 associated death factor
GSK3	glycogen synthase kinase 3
IGF	insulin-like growth factor
MAP	mitogen activated protein
MEK	MAP kinase kinase
NGF	nerve growth factor
PDGF	platelet-derived growth factor
PDK1	PIP_3-dependent kinase 1
PFK-2	6-phosphofructo-2-kinase
PH	pleckstrin homology
PI 3-K	phosphoinositide 3-OH kinase
$PI(3,4)P_2$	phosphatidylinositol-3,4-bisphosphate
PIP_3	phosphatidylinositol-3,4,5-trisphosphate
PKB	protein kinase B

Introduction

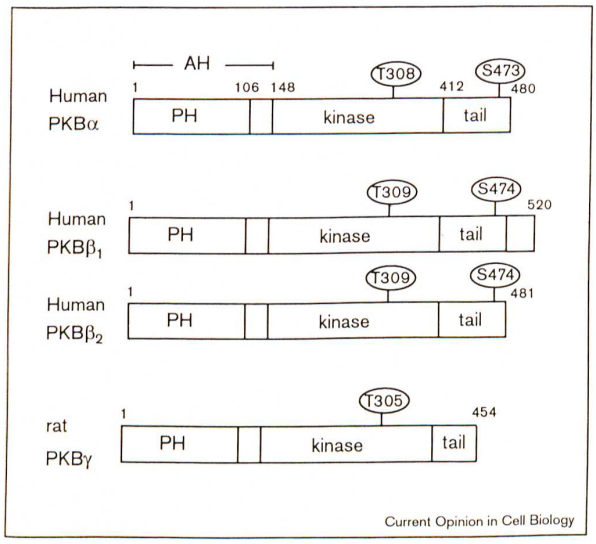
The serine/threonine protein kinase B (PKB)/Akt was identified independently in 1991 by three different groups. Two groups identified the kinase as a result of its homology to both protein kinase C (73% similarity to the kinase domain of PKCε) and protein kinase A (68% similarity to the kinase domain of PKA), giving rise to the names protein kinase B (PKB) [1] and RAC-PK (related to the A and C kinases) [2]. At the same time, this kinase was identified as the product of the oncogene v-*akt* of the acutely transforming retrovirus AKT8 found in a rodent T-cell lymphoma [3]. The retroviral oncogene encoded a fusion of the cellular Akt protein to the viral structural protein Gag. The nomenclature RAC-PK is now avoided to prevent confusion with the unrelated Rho-family GTPase Rac, but both PKB and Akt are widely used. This review will summarise the evidence that PKB/Akt is an important mediator of the physiological effects of insulin and of several growth factors and stimuli, and will address recent progress in understanding how PKB/Akt is regulated by phosphoinositide 3-OH kinase and how it plays a key part in protecting cells from apoptosis.

Regulation of protein kinase B

In addition to having a serine/threonine kinase domain, PKB/Akt contains a pleckstrin homology (PH) domain at its amino-terminal end (amino acids 1–106), which makes up the major part of the amino-terminal regulatory domain (residues 1–147). The kinase domain stretches from amino acid 148 to 411, with the carboxy-terminal tail region (amino acids 412–480) accounting for the remainder of the protein (Figure 1). In addition to the first characterised member of the family (PKBα, Akt1, RAC-PKα), two other very close relatives have now been identified — RAC-PKβ, also termed PKBβ and Akt2 [4], and RAC-PKγ [5]. PKB/Akt is activated in response to treatment of cells with a wide variety of growth stimuli, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, thrombin and nerve growth factor (NGF). Several findings indicate that the lipid kinase phosphoinositide 3-OH kinase (PI 3-K) is involved in regulation of PKB/Akt: growth-factor-induced activation of PKB/Akt is inhibited by the PI 3-K inhibitor wortmannin [6–8], by the expression of a dominant negative form of PI 3-K [7] and, in the case of PDGF, by mutation of PDGF receptor tyrosines 740 and 751, which bind to the PI 3-K regulatory subunit [6,7]. PI 3-K activity is thus required for the regulation of PKB/Akt by growth factors, but it also appears to be sufficient: expression of constitutively activated forms of PI 3-K results in stimulation of PKB/Akt [9,10,11••,12]. As PI 3-K is also stimulated by direct interaction of the small GTPase Ras with the p110 catalytic subunit [13,14], PKB/Akt is also controlled by Ras [9,10,15]. PI 3-K activity, and hence PKB/Akt activity, has also been shown recently to be stimulated by adhesion of epithelial cells to extracellular matrix [16••,17•].

In addition to PI 3-K-mediated regulation of PKB/Akt, another pathway for the regulation of PKB/Akt exists that is not sensitive to PI 3-K inhibitors such as wortmannin. Cellular stresses such as heat shock and hyperosmolarity, both of which activate the p38/HOG1 kinase cascade, are able to stimulate the activity of PKB/Akt [18]. Heat shock induces association of PKCδ with the PH domain of PKB/Akt; previously it had been reported that the kinase also associates with PKCζ [19]. The significance of PKB/Akt in stress signalling is currently unclear.

Figure 1



Schematic representation of the four known PKB/Akt isoforms. PKBβ1 and β2 are alternatively spliced forms derived from the same gene. The sites of insulin-induced phosphorylation are shown. AH, Akt homology domain; PH, pleckstrin homology domain.

The pleckstrin homology domain of PKB/Akt

The integrity of the pleckstrin homology domain has been found to be essential for activation of PKB/Akt in intact cells in response to various growth factors [6,20], and also in response to expression of activated PI 3-K [9,10,11••,12]. The mechanism behind this has recently been elucidated: the lipids produced by PI 3-K, phosphatidylinositol-3,4,5-trisphosphate (PIP_3) and phosphatidylinositol-3,4-bisphosphate ($PI[3,4]P_2$), are able to bind to the PH domain of PKB/Akt with relatively high affinity and specificity [11••,21••, 22•, 23•]. Binding of PIP_3, the immediate product of PI 3-K in cells, to the PH domain of PKB/Akt does not activate its kinase activity *in vitro*, although $PI(3,4)P_2$ is able to cause a modest increase in the activity of PKB/Akt *in vitro* [11••,22•,23•]. Probably the most important aspect of the function of the PH domain of PKB/Akt is to mediate translocation of the kinase from the cytosol to the plasma membrane following activation of PI 3-K in response to treatment of cells with growth factor [24••]. It appears that this translocation is required in order to present PKB/Akt to upstream activating kinases.

Upstream kinases for PKB/Akt

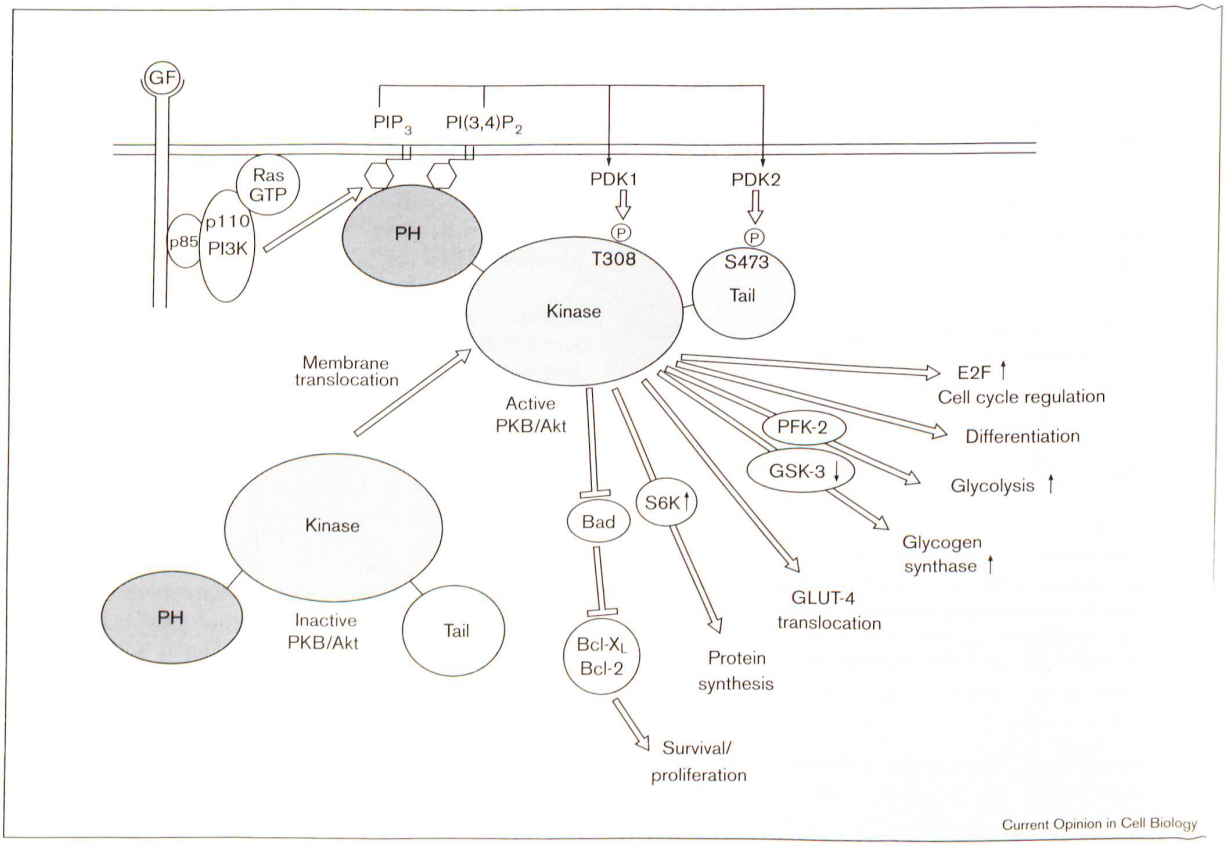
In response to growth factor treatment of cells, PKB/Akt becomes phosphorylated at two major sites, Thr308 in the kinase domain and Ser473 in the carboxy-terminal tail [25••]. As mutants of PKB/Akt, in which the kinase is inactivated, also become efficiently phosphorylated at these sites this is unlikely to represent an intramolecular autophosphorylation reaction. The sequences surrounding the two phosphorylated residues are very different, suggesting that two different kinases are probably involved in phosphorylating these sites. Phosphorylation of the two sites occurs independently and causes strong activation of the kinase activity of PKB/Akt which can be reversed by phosphatase treatment [7,20]. Membrane translocation medited by the PH domain can be constitutively mimicked by addition of a myristoylation sequence to PKB/Akt: this causes its activation due to constitutive phosphorylation [24••,26].

Recently, two groups have independently reported the identification and characterisation of a kinase that phosphorylates Thr308 of PKB/Akt resulting in its activation [27••,28••]. The activity of this kinase, termed PDK1 (PIP_3-dependent kinase 1) by Alessi *et al.*[27••], towards PKB/Akt is strongly stimulated *in vitro* by PIP_3 and $PI(3,4)P_2$ (Figure 2). This points to a dual role for PIP_3 and $PI(3,4)P_2$ in regulating PKB/Akt in the cell: firstly, these PI 3-K products bind to the PH domain of PKB/Akt and cause its translocation to the plasma membrane, possibly thereby promoting its interaction with PDK1. Secondly, they activate the kinase activity of PDK1 itself. The cDNA for PDK1 has now been cloned and encodes a ubiquitous 556-residue kinase from the same family as PKB/Akt, PKA and PKC, which also has a PH domain near its carboxy terminus [29••]. Interestingly, PDK1 is related to the *Drosophila* kinase DSTPK61, which has been implicated in the regulation of sex differentiation, oogenesis and spermatogenesis.

A number of issues remain to be resolved about the function of PDK1. Do PIP_3 and $PI(3,4)P_2$ bind to, and stimulate, PDK1 directly, or is the activation of PDK1 kinase activity towards PKB/Akt substrate driven? In other words, does PIP_3 or $PI(3,4)P_2$ binding to PKB/Akt cause a conformational change that results in its acting as a better substrate for PDK1? Since deletion of the PH domain of PDK1 results in a 30-fold reduction in its ability to activate PKB/Akt *in vitro*, it seems likely that PIP_3 and $PI(3,4)P_2$ do bind to the PH domain of PDK1 and stimulate its kinase activity independently of the substrate, PKB/Akt [29••]. PDK1 lacking a PH domain still retains some capacity to be activated towards PKB/Akt by PIP_3, possibly indicating an effect stemming from PKB/Akt localisation to the lipid vesicles used in this assay. However, deletion of the PH domain of PKB/Akt resulted in an enzyme that was activated *in vitro* by PDK1 in a PIP_3-independent manner. Possibly, the vesicle-based assay system used in these experiments does not adequately mimic the conditions in the cell.

It is still unclear whether or not PDK1 is enriched in the plasma membrane: it was originally isolated from brain cytosol, so membrane localisation is neither constitutive nor complete. Without some form of preferential localisation of PDK1 to the membrane it is unclear why

Figure 2



A model for the activation of PKB/Akt by PI 3-K-dependent mechanisms. Following stimulation of growth factor receptors, PI 3-K is activated, resulting in the production of PIP_3 and $PI(3,4)P_2$. The binding of the pleckstrin homology (PH) domain of PKB/Akt to these phosphoinositides recruits PKB/Akt to the plasma membrane. PKB/Akt is then phosphorylated on Thr308 by PDK1 and on Ser473 by PDK2. The activity, or possibly the localisation, of PDK1, and perhaps PDK2, may also be regulated by PI 3-K products. Activated PKB/Akt phosphorylates substrates, resulting in a variety of biological effects, including suppression of apoptosis and control of metabolism. PI 3-K, phosphatidylinositol 3-OH kinase; S6K, p70 S6 kinase; GSK3, glycogen synthase kinase 3; PDK, PIP_3-dependent kinase; PFK-2, 6-phosphofructo-2-kinase.

membrane binding of PKB/Akt, for example by artificial myristoylation, should be stimulatory. Other proteins could be involved in directing the localisation of PDK1 which would be missing from *in vitro* reconstitution experiments. The issue of whether PDK1 is constitutively active or stimulated by growth factors is one of the biggest unresolved questions: when overexpressed in serum-starved 293 cells, PDK1 does not appear to be stimulated by treatment of cells with insulin-like growth factor-1 (IGF-1) [29••]. It is, however, possible that this activity is due to the basal levels of PIP_3 in the cells, as the phosphorylation of even membrane-targeted PKB/Akt is reduced by long-term exposure of cells to PI 3-K inhibitors [24••].

Phosphorylation of PKB/Akt at Thr308 does not result in phosphorylation at Ser473, suggesting that this site is phosphorylated by a kinase other than PDK1 or PKB/Akt. This unidentified kinase, referred to as PDK2 by Alessi *et al.*, also appears to be under the control of PI 3-K as phosphorylation of Ser473 in response to insulin is sensitive to wortmannin [25••]. A direct target of p38/HOG1, mitogen activated protein kinase activated protein (MAPKAP) kinase 2, is able to phosphorylate Ser473 *in vitro* [25••], and may therefore be able to contribute to the activation of PKB/Akt. However, MAPKAP kinase 2 is not generally activated by stimuli that activate PKB/Akt, and vice versa, so it is very unlikely that MAPKAP kinase 2 is responsible for the phosphorylation of Ser473 of PKB/Akt.

Consequences of PKB/Akt activation

There is considerable interest in the substrates of PKB/Akt. The preferred substrate sequence for PKB/Akt to phosphorylate in a synthetic peptide is RxRyz(S/T)(hy), where x is any amino acid, y and z are small residues other than glycine, and hy is a bulky hydrophobic group [30]. The first direct *in vivo* substrate to be identified was glycogen synthase kinase 3 (GSK3) [31]. Stimulation

of 293 cells with insulin or IGF-1 leads to PKB/Akt mediated phosphorylation of GSK3 at Ser9, resulting in its inactivation and consequent activation of glycogen synthesis [32]. GSK3 is involved in regulation of several intracellular signalling pathways including control of the transcription factors AP1 and CREB (the cyclic AMP response element binding protein), the tumour suppressor gene product APC, the *wingless* developmental pathway in flies and dorsoventral patterning in frogs [33]. GSK3 is regulated by several different mechanisms. In addition to GSK3, another metabolic regulatory enzyme that is phosphorylated by PKB/Akt is 6-phosphofructo-2-kinase (PFK-2) [34•]. Another target for PKB/Akt is the ribosomal protein S6 kinase, $p70^{S6K}$, although in this case there is no evidence that it is a direct substrate [7]. $p70^{S6K}$ is responsible for altering the pattern of protein synthesis following mitogenic stimulation of cells. In addition, PKB/Akt has been found to stimulate glucose uptake and GLUT4 translocation (GLUT4 is the insulin-responsive glucose transporter isoform. It is translocated from intracellular compartments to the plasma mambrane in response to insulin) [35], and to induce adipocyte differentiation [36] in 3T3-L1 cells. It is likely that PKB/Akt is responsible for transducing a considerable number of the metabolic effects of insulin.

Recent reports reveal that PKB/Akt is involved in regulating cell survival. It has been demonstrated previously that inhibition of PI 3-K blocks the ability of survival factors to protect various cell types from programmed cell death [37,38]. Expression of activated forms of PI 3-K or PKB/Akt protects Cos-7 epithelial cells from apoptosis induced by ultraviolet irradiation [39•], protects neuronal cells from cell death induced by withdrawal of the survival factors IGF-I [40•] or NGF [41•], and protects haematopoietic cells from death induced by removal of interleukin-3 [42•]. Activated PI 3-K and PKB/Akt have also been shown to protect MDCK epithelial cells from apoptosis induced by detachment of adherent cells from their extracellular matrix ('anoikis') [16••] and to protect Rat-1 fibroblasts from apoptosis induced by withdrawal of serum factors while c-*myc* is being artificially expressed [43•,44•]. Dominant-negative forms of PKB/Akt were capable of inducing apoptosis in some of these systems, and other pathways acting downstream of PI 3-K, such as $p70^{S6K}$ and the Rho-family GTPase Rac, were found not to be involved in signalling cell survival. The Raf–MEK–mitogen activated protein (MAP) kinase pathway was also shown not to promote cell survival in epithelial cells [16••] or in fibroblasts [39•,44•]; in fact, the MAP kinase pathway induced apoptosis in *myc*-expressing serum-deprived fibroblasts [44•].

The mechanism by which PKB/Akt protects cells from programmed cell death has been the subject of much investigation recently. PKB/Akt can phosphorylate the pro-apoptotic Bcl-2 family member BAD [45••,46••], both *in vitro* and in intact cells. The phosphorylation occurs at Ser136 of BAD [46••], creating a binding site for 14-3-3 (a family of ubiquitous highly expressed adaptor proteins): when BAD is bound to 14-3-3 it is unable to heterodimerise with and inhibit the survival activity of the proteins Bcl-2 or Bcl-X_L [47]. In both haematopoietic precursor cells and fibroblasts, overexpression of BAD will induce cell death, and this effect is reversed by the expression of activated forms of PKB/Akt. Death induced by non-phosphorylatable BAD with a serine to alanine mutation at codon 136 is not suppressed by activated PKB/Akt. The evidence is good, therefore, that PKB/Akt can act to reverse the death-inducing activity of BAD. However, it is far from clear that this is the only, or even the primary, way in which the survival signal from PKB/Akt is mediated. BAD is only expressed in a limited range of tissues and cell lines, and almost all the work reported to date on BAD has used overexpressed protein, often in cell lines that do not normally express it at all. At the very least, a relative of BAD must be postulated to explain the ability of PKB/Akt to protect cells from apoptosis in the absence of BAD expression, and it is quite possible that PKB/Akt also acts on other unrelated components of apoptosis signalling pathways. It may be significant that PKB/Akt has been reported to translocate to the nucleus following stimulation of cells with growth factor, suggesting that important substrates could exist in this compartment [24••,48]. It is not possible to say whether putative nuclear targets for PKB/Akt are involved in protection from apoptosis or other functions of PKB/Akt.

The ability of PKB/Akt to promote cell survival in the absence of normal protective cues may be critical to its function as an oncogene in the AKT8 retrovirus [3]. PKB–Akt synergises strongly with the Raf/MAP kinase pathway to cause transformation of NIH 3T3 cells [10] and may also be able to drive cell-cycle progression through induction of E2F transcriptional activity [49]. In *ras*-transformed epithelial cells, the ability of the *ras* oncogene to promote cell survival in suspension is mediated through a PI 3-K–PKB/Akt pathway and not through the Raf–MAP kinase pathway [16••]. Roughly a quarter of all human malignancies are carcinomas that have undergone *ras* activation by mutation. It is likely that a major component of their ability to grow in the absence of adhesion is due to the survival signal triggered by Ras acting through PI 3-K to stimulate PKB/Akt. Anchorage-independent growth is one of the key hallmarks of transformation. It might be expected, therefore, that PKB/Akt undergoes activation in some human tumours; this has indeed been found for PKBβ/Akt2, which is amplified in 12% of ovarian carcinomas, 3% of breast carcinomas and 10% of pancreatic carcinomas [50,51].

Conclusions

PKB/Akt has emerged as a critical downstream target for PI 3-K, being regulated by a combination of direct binding of PI 3-K-produced lipids to its pleckstrin homology

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