The Regulation and Activities of the Multifunctional Serine/Threonine Kinase Akt/PKB

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The serine/threonine kinase Akt, or protein kinase B (PKB), has recently been a focus of intense research. It appears that Akt/PKB lies in the crossroads of multiple cellular signaling pathways and acts as a transducer of many functions initiated by growth factor receptors that activate phosphatidylinositol 3-kinase (PI 3-kinase). Akt/PKB is particularly important in mediating several metabolic actions of insulin. Another major activity of Akt/PKB is to mediate cell survival. In addition, the recent discovery of the tumor suppressor PTEN as an antagonist of PI 3-kinase and Akt/PKB kinase activity suggests that Akt/PKB is a critical factor in the genesis of cancer. Thus, elucidation of the mechanisms of Akt/PKB regulation and its physiological functions should be important for the understanding of cellular metabolism, apoptosis, and cancer. © 1999 Academic Press

INTRODUCTION AND HISTORICAL PERSPECTIVE

In 1991 two independent lines of research converged on the discovery of a cDNA encoding a novel serine/threonine kinase. One group cloned the cellular homologue of the v-akt oncogene from a transforming retrovirus (AKT8) in spontaneous thymoma of the AKR mouse and its product was called c-Akt [1, 2]. The same cDNA was cloned by two other groups searching for novel members of the protein kinase C (PKC) and protein kinase A (PKA) superfamily as possible participants in signal transduction cascades [3, 4]. Accordingly, the novel kinase was called RAC (related to A and C kinases) or PKB (protein kinase B)—and in this review we will refer to it as Akt/PKB. Eight years of subsequent research has left little doubt that Akt/PKB plays a prominent role in both growth factor signaling and oncogenesis. Currently, close Akt homologues have been identified in a variety of species, including birds, insects, nematodes, slime mold, and yeast and at least

some organisms have more than one gene for similar yet distinct isoforms of this enzyme [5–11].

Interest in Akt/PKB was piqued in 1995 when it was shown to be a direct downstream effector of phosphatidylinositol 3-kinase (PI 3-kinase) [12, 13]. When glycogen synthase kinase 3 (GSK3) was identified as an Akt/PKB target [14], it established the current paradigm for insulin signaling in which Akt/PKB plays the key role. The finding that PI 3-kinase activates Akt/PKB led to studies showing that it is a major participant in growth factor-mediated cell survival [15–18]. It also became clear that Akt/PKB is capable of linking growth factor signaling through PI 3-kinase to basic metabolic functions, such as protein and lipid synthesis, carbohydrate metabolism, and transcription. As the field evolved, new prospectives on the interplay between cell growth, survival, and metabolism, under both normal and pathological conditions, have been established. These concepts and several emerging questions will be discussed in this review.

THE AKT/PKB GENE FAMILY

Three major isoforms of Akt/PKB encoded by three separate genes have been found in mammalian cells. Akt1 or PKBα was the first isolated isoform; Akt2/PKBβ and Akt3/PKBγ were subsequently cloned through homology screen [19–22]. All three genes have greater than 85% sequence identity and their protein products share the same structural organization (Fig. 1). The first amino-terminal 100 amino acids possess a pleckstrin homology (PH) domain that binds phospholipids. A short glycine-rich region that bridges the PH domain to the catalytic domain follows the PH domain. All Akt/PKB isoforms are assumed to have identical or similar substrate specificity but this has never been overtly tested. The last 70 amino acids of the carboxy-terminal tail contain a putative regulatory domain. In v-Akt, a truncated viral group-specific antigen, gag, is fused in frame to the full-length Akt1 coding region through a short 5′ untranslated region of Akt1 [2]. All three Akt/PKB isoforms possess conserved threonine and serine residues (T308 and S473 in Akt1/PKBα) that together with the PH domain are critical for Akt/
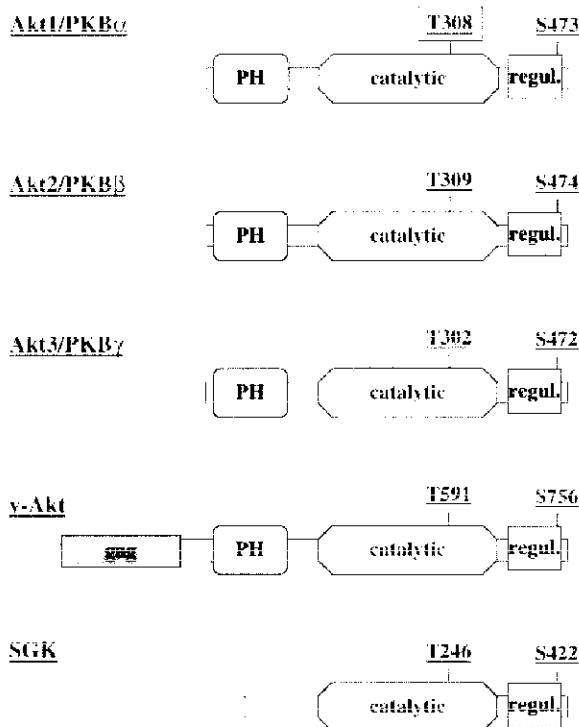
FIG. 1. Structural organization of the three major Akt/PKB isoforms is shown in comparison to virally encoded v-Akt and serum- and glucocorticoid-inducible kinase SGK. All Akt/PKB variants contain a plecsktrin homology domain (PH), a catalytic domain, and a putative regulatory fragment at the C-terminus (regul). SGK has a similar structure and sequence, but lacks a pleckstrin homology domain. v-Akt is an in-frame fusion of Akt-1 with a portion of retroviral group-specific antigen (gag). Amino acid positions are shown for mouse proteins. Threonine and serine residues whose phosphorylation is required to induce activities of the enzymes are indicated. See text for details.

PKB activation (see below). Equivalent threonine and serine residues in a similar amino acid context are also present in p70 S6 kinase and in all PKC isoforms. It is noteworthy that the distance between the two phosphorylated residues (~160–170 aa) is also conserved in these different protein kinases. Two additional Akt isoforms have been described and represent minor splice variants of human Akt2 and rat Akt3 [19, 23]. These isoforms exhibit a carboxy-therminal insertion of 40 aa and a partial deletion of 25 aa in the carboxy-terminal regulatory domain (including S473), respectively. The biological significance of these isoforms remains unclear.

A close relative of Akt/PKB is serum- and glucocorticoid-inducible kinase (SGK) that was shown to have a substrate specificity similar to that of Akt/PKB [24, 25]. SGK has extensive sequence homology to Akt/PKB in the catalytic domain and possesses residues equivalent to T308 and S473 of Akt1, but lacks a PH domain. SGK is more similar to the Akt homologues, Ypk1 and Ypk2/Ykr2, in budding yeast [26], suggesting that SGK might be closer to the ancestral prototype of the Akt/PKB family.

All three Akt/PKB isoforms are ubiquitously expressed in mammals, although the levels of expression vary among tissues [19–23, 27]. Akt1/PKBα is the predominant isoform in most tissues. The highest expression of Akt2/PKBβ was observed in the insulin-responsive tissues: skeletal muscle, heart, liver, and kidney [20], suggesting that this isoform is important for insulin signaling. This is further substantiated by the observation that Akt2/PKBβ expression in developing embryos is also highest in the insulin-responsive tissues, including liver, brown fat, and skeletal muscle [28]. A peculiar pattern of Akt1/PKBα expression was detected in brain, where it is markedly increased in regenerating neurons [29]. Akt1/PKBα is also the predominant isoform in mouse embryo fibroblasts (W. Chen and N.H., unpublished). Unlike two other isoforms, Akt3/PBKγ shows a more restricted pattern of expression. Higher levels of Akt3/PKBγ were detected in testis and brain and low levels in the adult pancreas, heart, and kidney [21–23]. The expression pattern of the three isoforms may not always reflect their activities. Different levels of kinase activities of the different isoforms have been observed in certain tissues and during differentiation, which is not necessarily correlated with their level of expression [30, 31].

THE MECHANISMS OF AKT/PKB ACTIVATION

Activation by PI 3-Kinase

The viral gag domain in v-Akt possesses a myristoylation signal that mediates its targeting to the plasma membrane and renders the enzyme constitutively active. This suggested that membrane association might be important in the activation process of c-Akt and subsequent studies bore out this hypothesis. Indeed, while c-Akt is localized primarily to the cytoplasm, a large proportion of v-Akt is localized to the plasma membrane [32]. Only upon stimulation does a fraction of c-Akt migrate to the membrane and attach there via its PH domain [33]. The presence of a PH domain together with the observation that the kinase activities of both Akt1/PKBα and Akt2/PKBβ can be rapidly activated by PDGF in rodent fibroblasts led to studies showing that Akt/PKB is a direct target of PI 3-kinase [14, 13]. PI 3-kinase is activated by growth factor receptors through binding of its regulatory subunit to phosphotyrosine residues in the receptor. Upon activation the catalytic subunit of PI 3-kinase phosphorylates phosphoinositides (PI) at the 3-position of the inositol ring to generate PI3P, PI(3,4)P2, and PI(3,4,5)P3 (see review by B. van Haesebroeke and M. Waterfield in this issue). PI 3-kinases are classified

into three major groups. In this review we will use the term PI 3-kinase to refer to the heterodimeric enzyme composed of p85 regulatory subunit and p110 catalytic subunit. Two major observations strongly suggested that the activation of Akt/PKB is dependent on PI 3-kinase. First, activation of Akt/PKB was shown to depend on tyrosines Y740 and Y751 in the PDGF receptor that had been identified as the binding sites for the p85 regulatory subunit of PI 3-kinase [13]. Second, the PI 3-kinase inhibitors wortmannin and LY94002 could diminish the activation of Akt/PKB by growth factors [12–14, 34]. These initial observations were followed by experiments showing that overexpression of a constitutively activated p110 catalytic subunit of PI 3-kinase can activate Akt/PKB [35]. In addition, it was shown that point mutations in the PH domain that reduce phospholipid binding abrogate the ability of Akt/PKB to be activated by growth factors, and a mutation that increases phospholipid binding superactivates the enzyme [13, 36]. Further studies showed that both PI(3,4)P2 and PI(3,4,5)P3 bind with high affinity to the PH domain of Akt/PKB [37–39]. However, the relative contribution of each 3-phosphorylated phosphoinositide species to Akt/PKB activation in vivo remains unclear. Exposure of cells to synthetic phospholipids showed that PI(3,4)P2 is a better activator in some experiments [38, 39], whereas other experiments showed higher binding affinity and better activation by PI(3,4,5)P3 [37, 40, 41]. The latter experiments were corroborated by observations that the SH2-containing inositol 5-phosphatase (SHIP), which converts PI(3,4,5)P3 to PI(3,4)P2, is a potent inhibitor of Akt/PKB activity in vivo ([42, 43], and see below).

Upon binding of 3-phosphorylated phosphoinositides, the PH domain of Akt/PKB facilitates dimerization of the enzyme [44, 38]. Experimental evidence suggests that Akt/PKB exists in vivo as a dimer or a trimer and this multimerization is required for the regulation of Akt/PKB activity. The interaction between monomers within such a complex may well explain the behavior of at least some dominant-negative forms of the enzyme (reviewed in [45]).

Because Akt/PKB activity is dependent on PI 3-kinase, any mechanism that activates PI 3-kinase can theoretically lead to stimulation of Akt/PKB activity. Indeed, activation of Akt/PKB through PI 3-kinase is not restricted to growth factors. For example, Akt/PKB is activated by integrins through activation of focal adhesion kinase, which in turn binds and activates PI 3-kinase and subsequently Akt/PKB [46–48]. Other cell surface receptors that activate Akt/PKB via PI 3-kinase include CD28 and CD5 in T cells, B cell receptor (BCR) in B cells, G-protein-coupled receptors, and the μ-opioid receptor [42, 49–54]. Angiotensin II and hydrogen peroxide were also reported to activate Akt/PKB through PI 3-kinase [55–57]. Among viral

proteins that activate Akt/PKB via PI 3-kinase are polyomavirus middle-T antigen and HIV Tat protein [58, 59]. In addition, Akt/PKB was shown to be activated by the oncogenic Ras through PI 3-kinase (reviewed in [60]). The GTP-bound Ras binds and activates the catalytic subunit p110 of PI 3-kinase and Ras mutant that is not able to bind p110 could not activate Akt/PKB [61]. It is not clear, however, whether the activation of Akt/PKB by activated Ras is as strong as the activation by growth factors and activated p110, and whether it is universal or dependent on the cell type.

Finally, as discussed below, there is some evidence of PI 3-kinase-independent mechanisms of Akt/PKB activation.

Activation by Phosphorylation: The PDKs

Activation of Akt/PKB in vivo by exposure to growth factors or synthetic phospholipids is preceded by an increase in serine and threonine phosphorylation of the kinase itself. Some residues such as serine 124 and threonine 450 in the mouse Akt1/PKBα are constitutively phosphorylated in a growth factor-independent manner and were predicted to render the protein responsive to subsequent activation events [36]. Two other residues that are rapidly phosphorylated upon exposure to growth factors and are the most critical for full activation of Akt/PKB are threonine 308 (T308) and serine 473 (S473). T308 resides within the activation loop of the kinase domain and S473 lies in the carboxy-terminal tail. When these two amino acids are mutated to nonphosphorylatable residues activation of the kinase is abolished, whereas mutations to acidic residues render the kinase more active even in the absence of growth factors [62, 36]. The phosphorylation of T308 and S473 induced by IGF-1 or insulin is sensitive to wortmannin, suggesting that this process is dependent on PI 3-kinase [62]. Two possible explanations for this phenomenon are that the binding of phospholipids to the PH domain of Akt/PKB is a prerequisite for its availability to other kinases or that the kinases that phosphorylate T308 and S473 are also dependent on PI 3-kinase. It turns out that both explanations are correct. The enzyme that phosphorylates T308 was purified and cloned [63, 64, 40, 41]. The ability of the enzyme to phosphorylate T308 is dependent on the presence of synthetic PI(3,4,5)P3 in vitro and therefore it was termed 3-phosphoinositide-dependent kinase (PDK1) [64, 41]. PDK1 possesses a PH domain in its carboxy-terminus and binds with high affinity to PI(3,4,5)P3 and more weakly to PI(3,4)P2. Deletion of the PH domain of PDK1 and mutations that decrease binding to PI(3,4,5)P3 strongly decrease its ability to activate Akt1/PKBα [65]. However, the kinase activity of PDK1 is tolerant to low concentrations

of wortmannin. This is likely to be explained by a relatively high affinity of the PH domain of PDK1 to PI(3,4,5)P3 [41, 65]. Although it was reported that PDK1 can be translocated to the plasma membrane upon growth factor stimulation [66], other studies using immunoelectron microscopy, confocal microscopy, and a green fluorescent protein–PDK1 chimera clearly show that it is mostly cytosolic and remains so upon stimulation [65]. Nevertheless a small portion of PDK1 was always found in the plasma membrane even in unstimulated cells and this may be also due to the high affinity of its PH domain to PI(3,4,5)P3. Alternatively another unknown factor is required for the binding of PDK1 to the plasma membrane.

It is possible that the membrane-bound PDK1 may be required for the phosphorylation of Akt/PKB and other membrane-localized substrates, whereas the cytosolic form is required for the phosphorylation of cytosolic proteins such as p70 S6 kinase. Like Akt/PKB, PDK1 is evolutionarily conserved [64, 67] and genetic studies in Caenorhabditis elegans confirm that PDK1 lies upstream of Akt but downstream of PI 3-kinase. PDK1 gain-of-function mutant bypasses the requirement of PI 3-kinase for Akt activation [67].

In addition to T308, the phosphorylation of S473 is also required for maximal activation of Akt/PKB [62, 36]. The findings that PDK1 cannot phosphorylate S473 in vitro or in cotransfection experiments suggested that a distinct kinase activity termed PDK2 is responsible for this function [63, 40, 41] but the identity of this kinase has remained elusive. It has been reported that integrin-linked kinase (ILK-1) is capable of phosphorylating S473 in vitro and in cotransfection experiments [68]. However, others failed to reproduce these results [69]. Recently it was shown that PDK1 interacts specifically in vitro and in vivo with the carboxy-terminus region of protein kinase C-related kinase (PRK2) that was termed PDK1-interacting fragment (PIF) [69]. The interaction of PDK1 with PIF converts it to an enzyme that can phosphorylate both T308 and S473 residues in Akt/PKB [69]. The possibility exists therefore that PDK1 can phosphorylate both residues in vivo depending on postranslational conformational change and/or interaction with another cellular protein. A related observation may be the finding that one point mutation in PDK1 of C. elegans is sufficient to bypass the requirement of PI 3-kinase for Akt/PKB activation [67]. It remains to be determined, however, whether this constitutively active form of PDK1 is capable of phosphorylating both T308 and S473. Interestingly, the minimal functional fragment of PIF contains the putative PDK2 recognition site with serine substituted with the negatively charged aspartate [69]. Thus, PIF may be considered a mimic of PDK2 substrate. While modulation of PDK1 specificity by endogenous PRK2 remains to be demonstrated, it is

tempting to speculate that once S473 or an equivalent residue in other enzymes is phosphorylated it will serve as a catalyst that primes PDK2 activity in PDK1. Another possibility that cannot be completely excluded at present is that T308 phosphorylation permits autophosphorylation of S473 by Akt/PKB itself. This possibility cannot be ruled out by the observation that a kinase-deficient mutant of Akt/PKB can still be phosphorylated on S473 [62], because multimerization with the wild-type Akt/PKB in vivo may enable the phosphorylation of S473 of the mutant protein.

Negative Regulation of Akt/PKB Activity

Similar to other protein kinases, Akt/PKB is subject to negative regulation. The PH domain of Akt/PKB for example may act both as a negative and as a positive regulator of the enzyme. Deletion of the PH domain rendering the enzyme incapable of interaction with 3-phosphoinositides leads to a slightly higher basal kinase activity than that of wild type, but this activity can still be normally elevated in a PI 3-kinase-dependent manner [34, 64]. This suggests that in its inactive form (not bound to 3-phosphoinositides) the PH domain may confer a conformation that is not accessible to PDKs. Another possibility is that the PH domain interacts with a cellular protein that negatively regulates the kinase and binding to 3-phosphoinositides relieves this interaction.

The subcellular localization of Akt/PKB is also tightly regulated and may provide a mechanism for regulating cytoplasmic Akt/PKB activity. After 2 min of stimulation with IGF-1, Akt1/PKBα is translocated to the plasma membrane in a PH domain-dependent manner but following this Akt/PKB is translocated to the nucleus by an unknown mechanism [33]. Nuclear translocation is probably independent of the PH domain or kinase activity because both a mutant that lacks the PH domain and a kinase-deficient mutant of Akt/PKB are also found in the nucleus [33]. The physiological significance of the nuclear localization is not clear. It might be required for phosphorylation of nuclear proteins. Alternatively, the sequestration in the nucleus could be a way to limit the exposure of cytosolic substrates to the kinase and might serve as a mechanism that indirectly negatively controls the kinase. It has to be noted that Akt/PKB targeted to the plasma membrane via a myristoylation signal exhibits constitutively active phenotype in regard to all known functions of activated wild-type enzyme, but fails to translocate to the nucleus [33].

As described above phosphorylation of Akt/PKB is required for its activation. It appears that this phosphorylation is tightly controlled. The facts that the key phosphoserine and phosphothreonine residues in Akt/PKB have a relatively short half-life and that phospha-

tase inhibitors such as vanadate and okadaic acid were shown to augment Akt/PKB kinase activity both indicate that the enzyme is negatively regulated by dephosphorylation [70, 71]. Phosphatase 2A (PP2A) may be the key enzyme associated with dephosphorylation of Akt/PKB in vitro and in vivo [70–72]. Hyperosmotic shock rapidly inactivates Akt/PKB and this is preceded by dephosphorylation of T308 and S473. The dephosphorylation and the decrease in Akt/PKB activity can be prevented by calyculin A, a relatively specific inhibitor of PP2A [72].

It was also shown that the major Src family tyrosine kinase in hematopoeitic cells, Lyn, antagonizes the activation and phosphorylation of Akt/PKB by BCR in B cells [73]. It is possible that Lyn exerts its effect by activation of serine/threonine phosphatases or by activation of a 3-phosphoinositide-preferring phosphatase that antagonizes PI 3-kinase activity.

A 3-phosphoinositide-specific phosphatase activity was found to reside in the tumor suppressor PTEN (for phosphatase and tensin homologue deleted from chromosome 10), which is mutated or deleted in a wide range of human cancers (reviewed in [74]). PTEN shares homology with dual-specificity phosphatases that can dephosphorylate serine, threonine, and tyrosine residues. However, attempts to confirm PTEN as a protein phosphatase revealed only relatively weak activity [75, 76], suggesting that PTEN is a specialized phosphatase for certain proteins and/or it possesses a different activity. Indeed it was found that PTEN is a potent lipid phosphatase [77, 78]. Overexpression of PTEN significantly reduced PI(3,4,5)P3 production induced by insulin, and PTEN-null cells have higher levels of PI(3,4,5)P3 [79, 77, 80]. A recombinant PTEN dephosphorylates 3-phosphoinositides specifically at position 3 of the inositol ring and has highest specificity for PI(3,4,5)P3 [77]. Since PTEN antagonizes the PI 3-kinase activity its role in tumor suppression may involve Akt/PKB (see discussion below). Experiments in tumor cell lines with inactive PTEN and in PTEN-null fibroblasts showed that these cells exhibit high basal activity of Akt/PKB [78–82]. These results, together with genetic studies in C. elegans demonstrating that PTEN lies in the same pathway with PI 3-kinase/Akt and inhibits Akt [83], established PTEN as a bona fide negative regulator of Akt/PKB.

Another lipid phosphatase that can negatively regulate Akt/PKB activity is SHIP—an inositol 5′ phosphatase that hydrolyzes PI(3,4,5)P3 to PI(3,4)P2. Overexpression of SHIP was shown to inhibit Akt/PKB activity and SHIP-null cells exhibit prolonged activation of Akt/PKB upon stimulation [42, 43]. SHIP1 is mostly found in hematopoeitic cells, while another isoform (SHIP2) is widely expressed in nonhematopoietic cells [84, 85]. The relative roles of PTEN and SHIP in

the regulation of PIP3 levels may well be tissue and cell-type specific.

The Emerging Model for the Regulation of Akt/PKB Activity by Growth Factors

The current model for the activation of Akt/PKB is based on the studies described above and is schematically illustrated in Fig. 2. Upon binding of a growth factor to a tyrosine kinase receptor, the receptor is activated and phosphorylated on tyrosine residues. The phosphotyrosine residues can then recruit Src homology 2 (SH2)-containing proteins (see the review by J. Pessin in this issue). The regulatory subunit p85 of PI 3-kinase is recruited to the active receptor through binding of its SH2 domain to specified phosphotyrosine residues in the receptor, and in the case of insulin receptor this recruitment is largely mediated by the IRS adapter proteins (see the review by J. Pessin in this issue). Recruitment of the catalytic subunit, p110, to the receptor via the regulatory subunit and the close proximity to the plasma membrane lead to its activation. The PI(3,4,5)P3 produced by the activated PI 3-kinase then binds to the PH domain of the dormant cytosolic form of Akt/PKB. Increased production of PI(3,4,5)P3 also activates PDK1 and PDK2. The identity of the latter still remains controversial.

Binding of PI(3,4,5)P3 to the PH domain induces a conformation change and recruitment to the plasma membrane, which in turn expose Akt/PKB to phosphorylation by PDK1 at T308 and subsequent phosphorylation of S473 by PDK2. These events promote full activation of Akt/PKB. Following activation, Akt/PKB is detached from the plasma membrane and translocates to the cytosol and the nucleus. The translocation to the nucleus may have a dual role, one is to have access to target proteins in the nucleus and the other is to regulate its activity in cytoplasm by sequestration in the nucleus. The activity of Akt/PKB is also regulated by PTEN that counteracts PI 3-kinase and by SHIP that converts PI(3,4,5)P3 to PI(3,4)P2.

The model described above is based mostly on experiments performed with Akt1/PKBα. Several reports show that the same paradigm of activation is true for Akt2/PKBβ and Akt3/PKBγ, although the different isoforms can be activated to different extents in different tissues and in response to different stimuli and it is possible that they exhibit different affinities for cellular substrates [71, 28, 86, 87, 30, 21].

Activation of Akt/PKB by PI 3-Kinase-Independent Mechanisms

Several reports have suggested that Akt/PKB could be activated in a PI 3-kinase-independent manner. cAMP-elevating agents such as forskolin, chlorophenylthio-cAMP, prostaglandin E1, and 8-bromo-cAMP

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