J Mol Med (1999) 77:656–665
Digital Object Identifier (DOI) 10.1007/s001099900050

REVIEW

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Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling

Received: 19 July 1999 / Accepted: 6 September 1999 / Published online: 23 September 1999

Abstract The proto-oncogene protein kinase B (PKB), also known as c-Akt, is a central player in a signaling pathway of which many components have been linked to tumorigenesis. Active forms of PKB as well as of its upstream activator phosphatidylinositol 3-kinase (PI3K) have been found to be responsible for the transforming activities of certain viruses, and the negative regulator of this pathway, PTEN, is a tumor suppressor. The identification of particular downstream targets of PKB has provided us with new insights into the possible mechanism of PI3K/PKB-mediated tumorigenicity. Recently a subfamily of Forkhead transcription factors was identified as additional targets for PI3K/PKB signaling. This review discusses the studies that have led to this conclusion and the possible implications of this finding for our understanding of how PI3K/PKB activity could lead to oncogenesis.

Key words Proto-oncogene · Protein kinase B · Transcription · Forkheads · Tumor suppression

Abbreviations *GSK3* Glycogen synthase kinase 3 · *HNF3* Hepatic nuclear factor 3 · *IGFBP-1* Insulin-like growth factor binding protein-1 · *IGFI* Insulin-like growth factor I · *IRE* Insulin response element · *MLL* Mixed-lineage leukemia · *PDK1* PI(3,4,5)P_3-dependent kinase 1 · *PEPCK* Phosphoenolpyruvate carboxy kinase · *PI(3,4)P_2* Phosphatidylinositol (3,4) diphosphate · *PI(3,4,5)P_3* Phosphatidylinositol (3,4,5) triphosphate · *PI3K* Phosphatidylinositol-3 kinase · *PKB* Protein kinase B · *RAC-PK* Related to A and C protein kinases

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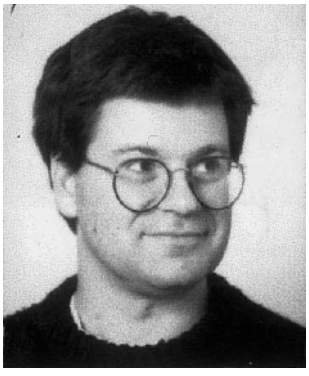

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Introduction

Several years ago two research groups simultaneously cloned a gene that displayed high sequence homology to both the protein kinase C and the protein kinase A family of serine/threonine kinases. Based on this characteristic, it was named protein kinase B (PKB) and RAC-PK (related to A and C protein kinases), respectively [1, 2]. A third research group isolated the same gene in a screen for cDNAs that show similarity to the v-akt gene of the transforming AKT8 virus. Closer sequence comparisons revealed that v-akt cDNA is identical to this new gene fused to the viral gag sequence, and it was named c-akt

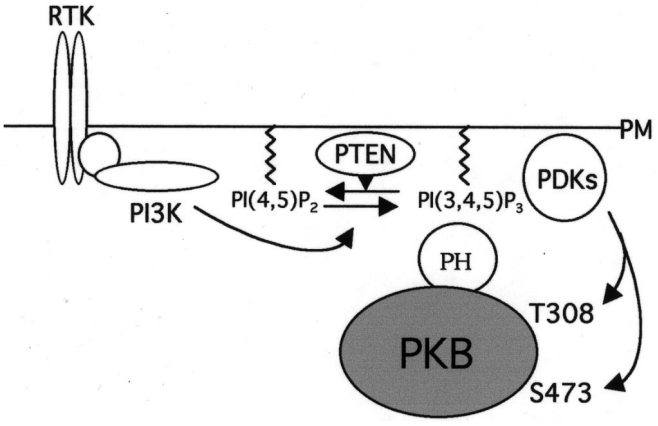

Fig. 1 Schematic representation of growth factor-induced PKB activation. Receptor tyrosine kinases (*RTKs*) activate PI3K that in turn activates PDK1 and 2 (*PDKs*) by the production of $PI(3,4,5)P_3$ lipids. The lipids also recruit PKB to the plasma membrane (*PM*) by means of its pleckstrin homology (*PH*) domain and PKB is subsequently phosphorylated on T308 and S473 by the PDKs, resulting in fully active PKB

[3, 4]. Below we refer to this protein kinase as PKB. PKB-like genes have been found in many types of species, including *Drosophila melanogaster*, *Caenorhabditis elegans*, and humans [1, 5, 6]. One of the three PKB genes, PKBβ, has been shown to be amplified in a number of human ovarian and mammary tumors and in pancreatic cancer cell lines and carcinomas with especially frequent amplifications in undifferentiated tumors [7, 8, 9, 10]. This suggested that this gene has transforming capabilities and contributes to tumor aggressiveness.

PKB is rapidly activated by treatment of cells with growth factors, certain cytokines, and some forms of cellular stress (reviewed in [11]). Activation of the tyrosine kinase activity of the receptors for the various peptide factors and cytokines results in the activation of the heterodimeric phosphatidylinositol-3 kinase (PI3K). PI3K produces 3' phosphorylated phosphoinositide lipids [phosphatidylinositol (3,4) diphosphate, $PI(3,4)P_2$; and phosphatidylinositol (3,4,5) triphosphate, $PI(3,4,5)P_3$] that act as second messengers to recruit PKB to the plasma membrane through its N-terminal lipid-binding pleckstrin homology domain (Fig. 1) ([12] and reviewed in [13]). Once properly localized, PKB becomes phosphorylated on T308 and S473, after which the kinase is fully active [14, 15, 16]. T308 is phosphorylated by the $PI(3,4,5)P_3$-dependent kinase 1 (PDK1), which itself is activated by the lipid products of PI3K activity (Fig. 1) [17, 18]. The kinase that phosphorylates S473, conveniently termed PDK2, has yet to be identified, but a recent study suggests that, by interaction with the PKC-related kinase 2, PDK1 can acquire PDK2-like activity [19]. It has also been proposed that the integrin-linked kinase 4 is PDK2 [20]. The pathway leading to PKB activation can be antagonized by a phosphatase that removes the 3' phosphate from $PI(3,4)P_2$ and $PI(3,4,5)P_3$. Surprisingly, this phosphatase has been identified as the tumor suppressor protein PTEN [21, 22], a gene that for long was known to be deleted or mutated in several types of cancer, including mammary, prostate, and brain carcinomas (Fig. 1) [23, 24, 25]. The fact that the function of PTEN has recently been shown to be the dephosphorylation of $PI(3,4)P_2$ and $PI(3,4,5)P_3$ allows us to speculate that the suppression of tumor growth by PTEN is due to the inhibition of PKB-mediated signaling.

Altogether, several lines of evidence suggest that PI3K-induced signaling is involved in oncogenic transformation, which might at least in part be due to PKB activity: (a) A p65 form of the p85 regulatory subunit of PI3K has been identified and shown to be oncogenic [26]. (b) A viral oncogene (v-p3k) in the ASV-16 virus is the catalytic subunit of PI3K, which is a potent transforming gene in cultured chicken embryo fibroblasts. These transformed chicken embryo fibroblasts contain high levels of $PI(3,4,5)P_3$ and PKB activity [27]. (c) Full-length PKB fused to the membrane-targeting gag sequence is responsible for the transforming actions of the AKT8 virus and expression of the viral variant of PKB (v-akt or gagPKB) in mammalian cells results in a constitutively active kinase that is able to transform fibroblasts [3, 28]. (d) Glioblastoma and prostate carcinoma cell lines in which the negative regulator of PKB, PTEN, is deleted or mutated show high amounts of PKB activity, and the introduction of wild-type PTEN into these cells results in a reduction in PKB activity and reversion of the transforming phenotype [21, 29, 30].

Biological functions of PKB

Since it was becoming evident that PKB might play a role in oncogenic transformation, substantial effort was made to determine the function of PKB in the cell. The identification of a direct substrate of PKB gave the first clue. Cross et al. [31, 32] showed that PKB is directly responsible for the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3), an enzyme involved in regulating glycogen storage, indicating that PKB is involved in controlling cellular metabolism [31, 32]. Subsequent observations extended such a role for PKB and showed that PKB regulates the uptake of glucose by recruiting the GLUT4 glucose transporters to the plasma membrane, and that PKB directly controls the activity of another glycolysis-regulating enzyme, namely phosphofructo-kinase 2 [33, 34]. A role for PKB in protein translation has also been suggested. PKB mediates the phosphorylation of the translational repressor 4E-binding protein 1, and active alleles of PKB can activate the p70S6-kinase that phosphorylates the S6 ribosomal subunit and stimulates protein synthesis [14, 35].

Although these functions of PKB could account for a permissive role of the kinase in tumorigenesis, a more causative role became apparent with the finding that PKB activity can antagonize apoptotic signals. It was already known that nerve growth factor and insulin-like growth factor I (IGFI) transduce survival signals to the cell by activating PI3K and thereby opposing apoptotic

signals that are elicited by stimuli such as growth factor withdrawal or serum starvation [36, 37]. Shortly thereafter PKB was shown to transduce the PI3K-mediated survival signals by directly phosphorylating and inactivating the pro-apoptotic factors BAD and caspase-9 [38, 39, 41].

Very recently a new target of PKB with potential relations to tumorigenesis has been identified. PKB has been placed directly upstream of endothelial nitric oxide synthase, an enzyme involved in the synthesis of the second messenger nitric oxide (NO) [42, 43]. NO has been implicated in divers physiological processes such as angiogenesis, regulation of blood glucose levels and maintenance of the heart muscle. By regulating the production of endothelial nitric oxide synthase and thereby that of NO, PKB might control processes such as angiogenesis that strongly influence tumorigenicity.

Transcriptional regulation by PKB

Despite these already diverse functions of PKB, yet another role for PKB activity was found, that of regulating gene transcription. Several reports had already indirectly implicated PKB in transcriptional regulation. GSK3 has been shown to be able to negatively regulate the cAMP-response element binding protein and activator protein 1 transcription factors by direct phosphorylation, and inhibition of GSK3 by PKB would therefore result in a positive regulation of these two proteins, although this has never been shown [44, 45]. PKB has more directly been implicated in transcriptional control in two ways. One is the identification of transcription factors that are indirectly controlled by PKB activity. The oncogenic transcription factor c-myc, for instance, has been placed downstream of PKB activity by two different groups. In mesenchymal cells the Ras oncogene is able to inhibit c-myc-induced apoptosis by activating PI3K and PKB, suggesting that PKB can somehow modulate c-myc activity (Table 1) [46]. Furthermore, in BCR/ABL-transformed hematopoietic cells PKB upregulates c-myc activity by increasing c-myc protein levels (Table 1) [47]. Although these two reports claim opposite roles for PKB in c-myc regulation, the contradiction can be explained by the cell type dependent function of c-myc. In mesenchymal cells c-myc induces apoptosis, whereas in hematopoietic cells it induces progression through the cell cycle, and PKB actions on c-myc in both cell types result in a dividing cell population. In addition to c-myc, PKB has been implicated in regulating the transcription factors E2F in T lymphocytes, hypoxia-inducible factor 1 in Ha-Ras transformed NIH3T3 cells, cAMP-response-element-binding protein, and nuclear factor κB (Table 1) [48, 49, 50, 51, 52].

The second way in which PKB has been implicated directly in transcriptional control is the identification of genes that are controlled by PKB activity. Until now three genes that are controlled specifically by PKB have been identified, and all three are insulin-regulated genes involved in maintaining cellular metabolism. First, Liao et al. [53] showed that the insulin-mediated repression of cAMP-induced phosphoenolpyruvate carboxy kinase (PEPCK) gene expression is via the activation of PKB and that PKB is likely to act through a T(G/A)TTTTG sequence, also known as the insulin response element (IRE), in the promoter of the PEPCK gene (Table 1) [53]. Notwithstanding these observations, a previous study claimed that, although the authors report that PI3K is involved, PKB is not downstream of PI3K in the insulin-induced repression of PEPCK [54]. Second, PKB mediates insulin-regulated expression of the fatty acid synthase promoter through an IRE (Table 1) [55]. Third, basal expression of the insulin-like growth factor binding protein-1 (IGFBP-1) gene can be repressed by insulin, and it has been found that PI3K and PKB mediate this effect (Table 1) [56]. Again, the repression by insulin was through two IREs in the promoter. Although the various reports convincingly suggest that a pathway from PKB to a transcription factor that acts on IREs does exist, there was no clue as to what transcription factor(s) may lie downstream of PKB. The identity of such IRE-interacting transcription factors was resolved with the discovery that a subfamily of the Forkhead superfamily of winged-helix transcription factors is directly regulated by PKB activity, and that by regulating this subfamily PKB can control IRE-mediated gene expression.

Table 1 Transcription factors and target genes suggested to be controlled by PKB activity *(CREB* cAMP response element binding protein, *FAS* fatty acid synthase, *HIF* hypoxia-inducible factor, *IL2* interleukin-2, *NFκB* nuclear factor κB, *VEGF* vascular endothelial growth factor)

PKB stimulus	Transcription factor	Target gene	Reference
v-Ras/hypoxia	c-myc	?	46
v-Ras	HIF-1	VEGF	49
IL2	E2F	?	48
Insulin	?	PEPCK	53
Insulin	?	IGFBP-1	56
Insulin	?	FAS	55
Serum	CREB	?	50
myrPKB	NFκB	IL2	51

Forkhead transcription factors

The superfamily of Forkhead transcription factors consists of approximately 90 members, with orthologues expressed in an array of species ranging from yeast to man (reviewed in [57]). All members of the family show high sequence homology within their DNA-binding "winged-helix" domain to the fork head protein of *D. melanogaster*, which is involved in the formation of terminal structures in the early fly embryo [58]. The prototype of Forkheads in mammals are the α, β, and γ isoforms of the hepatic nuclear factor 3 (HNF3). HNF3 is a liver-enriched transcription factor that has been identified as an activator of liver-specific gene expres-

sion in rat [59]. In vivo, HNF3 proteins have been reported to bind to highly related IREs in both the IGFBP-1 and PEPCK promoters [60, 61]. Whether insulin is able to modify this interaction of HNF3 with the IRE still remains elusive.

A subset of Forkhead transcription factors has been associated with tumorigenesis. The Forkhead gene qin of the ASV-31 virus is responsible for the transforming activity of the virus and is closely related to the teleencephalon-specific brain factor-1, a Forkhead transcription factor of which knock-out mice show severe cerebral hemisphere abnormalities [62, 63]. Furthermore, in acute lymphoid leukemias the vast majority of chromosomal translocations that cause the disorder disrupt the mixed-lineage leukemia (MLL) transcription factor gene [64]. The proteins resulting from such chromosomal breaks are fusion proteins consisting of the DNA binding domain of MLL fused to the transactivation domain of another transcription factor. Two of those other transcription factors are the Forkhead genes AFX and AF6q21 [65, 66]. A similar event occurs in rhabdomyosarcomas. Here a chromosomal translocation results in a fusion between the PAX3 DNA-binding domain and the transactivation domain of the Forkhead protein FKHR [67].

A connection between PKB and Forkhead transcription factors?

The initial clues as to what lies downstream of PKB activity in terms of transcription regulation came from studies performed in the nematode *C. elegans*. Two groups independently reported a pathway in the worm that controls dauer formation, a developmental stage of the animal which ensures survival in adverse conditions by lowering its metabolism and closing its mouth and anus. In this way it can live up to ten times longer than a normal adult. When conditions improve, the worm returns to the development program and lives a normal 15-week adult life (reviewed in [68]). Genes that regulate this dauer formation are called *daf* genes. It was found by Ogg et al. [69] and Lin et al. [70] that the pathway controlling this dauer formation is regulated by pheromones that activate the DAF-16 protein. DAF-16 in turn is negatively regulated by DAF-2 via AGE-1 (also known as DAF-23). Surprisingly, DAF-2 is a an insulin-receptor-like protein, AGE-1 is PI3K-like protein, and DAF-16 proved to be a transcription factor of the Forkhead family with highest homology to mammalian AFX, FKHR, and FKHRL1 (Fig. 2) [65, 69, 70, 71, 72, 73, 74]. Later, also a PKB-like (AKT1, AKT2), a PDK1-like (PDK1), and a PTEN-like (DAF-18) protein were placed in this dauer formation pathway, suggesting a fully conserved signaling route towards PKB activation between worm and man (Fig. 2) [6, 75, 76]. Intriguingly, DAF-16 As well as AFX, FKHR, and FKHRL1 contain multiple putative PKB phosphorylation sites that can be recognized by the amino acid sequence RXRXXS/T, as determined by Alessi et al. [65, 71, 74, 77] (Table 2). This could indicate that these transcription factors are direct targets of insulin-activated PKB.

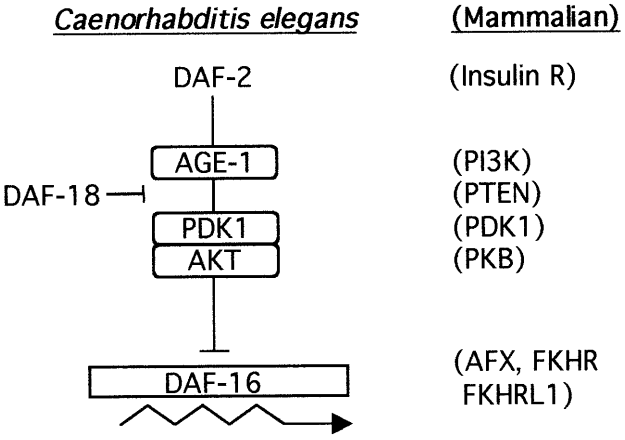

Fig. 2 Regulation of DAF-16 in *C. elegans*. Genetic analysis of dauer formation in *C. elegans* has revealed that DAF-16 is negatively regulated by AKT, which in turn is activated by AGE-1 and DAF-2. *Brackets* mammalian orthologues of the various nematode genes

Table 2 PKB consensus phosphorylation sites of known or putative substrates of PKB (*eNOS* endothelial nitric oxide synthase, *PFK2* phosphofructo-kinase 2)

PKB substrate	PKB phosphorylation site
Known	
GSK3	RARTSS[9]
PFK2	RMRRN**S**[466]/RPRNY**S**[483]
BAD	RGRSR**S**[136]
Caspase-9	RRRFS**S**[196]
eNOS	RIRTQ**S**[1177]
Putative	
AFX	RPRSC**T**[28]/RRRAA**S**[193]/RPRSS**S**[258]
FKHR	RPRSC**T**[24]/RRRAA**S**[256]/RPRTS**S**[319]
FKHRL1	RPRSC**T**[32]/RRRAV**S**[253]/RSRTN**S**[315]
DAF-16	RDRCN**T**[54]/RTRER**S**[24°0]/ RERSN**T**[242]/RPRTQ**S**[314]

Standard amino acid single-letter codes are used. Boldface is the serine (*S*) or threonine (*T*) residue that is phosphorylated (known) by PKB or that lies within a possible PKB phosphorylation site (putative). Numbers indicate the position of the serine or threonine residue in the protein

Regulation of Forkhead transcription factors by PKB

We and others hypothesized that in mammalian cells growth factors regulate the activity of AFX, FKHR, and/or FKHRL1 via a PI3K/PKB pathway, and that PKB does so by direct phosphorylation of the transcription factors. To this end, epitope-tagged versions of the transcription factors were transiently expressed in a variety of mammalian cell lines, and the details of growth factor induced phosphorylation of the Forkheads were investigated. Total phosphorylation of AFX, as measured by in-

corporation of [32P]orthophosphate, was increased upon insulin treatment of A14 cells [78]. This phosphorylation is not exclusive for insulin since we have observed phosphorylation of AFX upon treatment of A14 and Cos-7 cells with epidermal growth factor and of Rat1 and porcine aortic endothelial cells expressing the platelet-derived growth factor receptor with platelet-derived growth factor (G. Kops and B. Burgering, unpublished observations). The same approach was taken to show that FKHR is phosphorylated by insulin in hepatocytes [79]. Two other studies concerning FKHRL1 and FKHR show that IGFI increases the phosphorylation of specific sites within the proteins, namely the PKB consensus sites, but it was not shown whether other sites are phosphorylated upon growth factor treatment [80, 81]. The involvement of PI3K in insulin-induced phosphorylation became apparent when the insulin effect on total phosphorylation of AFX and FKHR was shown to be decreased by pretreatment of the cells with the PI3K inhibitor wortmannin. For AFX it was shown that insulin-activated PI3K exerts its effect on phosphorylation through activation of PKB, since the insulin-induced phosphorylation is decreased by expression of a dominant-negative form of PKB [78]. In the case of FKHRL1 and FKHR the involvement of PKB in PI3K-mediated phosphorylation of the transcription factors is very likely, although not formally confirmed. Several reports have shown that wortmannin or the functionally similar compound LY294002 is able to inhibit insulin- or IGFI-induced phosphorylation of either of the three putative PKB sites [80, 81, 82]. Yet this leaves the possibility that in FKHR and FKHRL1 these sites are phosphorylated by PI3K-dependent kinases other than PKB. p70S6-kinase, for instance, is regulated by PI3K and has a recognition sequence (RXXS/T) that inherently lies within each PKB consensus site [83], and moreover the insulin-induced phosphorylation of FKHR is decreased when hepatocytes are treated with the p70S6-kinase inhibitor rapamycin [79].

PKB can directly phosphorylate all three Forkhead transcription factors. Various active forms of PKB are able to phosphorylate bacterially expressed [78, 80, 81, 82, 84] or immunoprecipitated ectopically expressed Forkhead proteins in vitro [78, 84]. Differences between the three transcription factors with regard to the specifics of the sites that are phosphorylated by PKB do exist, however. As noted above, all three Forkheads contain three PKB consensus phosphorylation sites (Table 2, Fig. 3), one N-terminal threonine, and two C-terminal serines. In AFX, PKB phosphorylates both C-terminal serines but not the N-terminal threonine, whereas in FKHRL1 all three sites are phosphorylated [78, 80]. For FKHR the details of PKB-mediated phosphorylation are the subject of debate. Whereas Rena et al. [81] and Tang et al. [84] suggest a triple phosphorylation of FKHR, Nakae et al. [79] claim phosphorylation of only the first of the C-terminal serines. Whether these differences in phosphorylation by PKB represent differences in the function and/or regulation of the Forkheads remains to be investigated. It is clear, however, that phosphorylation by PKB on two (AFX) or three (FKHRL1, FKHR) sites is essential for insulin-induced inhibition of the transcription factors. Bandshift analyses have shown that all three Forkheads are able to bind IREs, and it was furthermore demonstrated that they are able, via the IREs, to transactivate a variety of reporter constructs, including those containing the IGFBP-1 and Fas ligand promoters [78, 80, 82, 84, 85]. Insulin or IGF1 treatment of cells or expression of active forms of PKB inhibits this Forkhead-induced transactivation, and this is due to direct phosphorylation of the transcription factor by PKB, since mutants that can no longer be phosphorylated by PKB are no longer inhibited by insulin, IGFI, or active PKB.

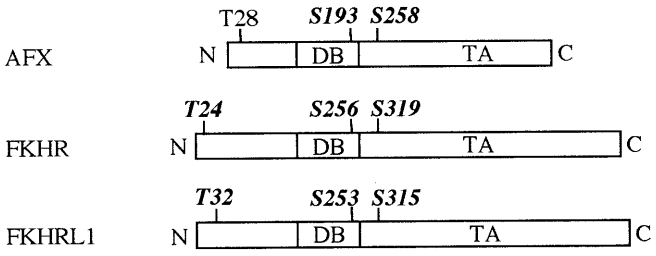

Fig. 3 Schematic representation of AFX, FKHR, and FKHRL1. The positions of the PKB consensus sites are indicated by the serine (*S*) and threonine (*T*) residues. *Boldface*, *italic* sites that have been shown to be phosphorylated by PKB. *DB* DNA-binding domain; *TA* transactivation domain; *N* amino-terminus; *C* carboxy-terminus

What is the mechanistic explanation for the transcriptional repression of the Forkheads by PKB? Considering the position of the two serine phospho-acceptors (Fig. 3), one might argue that it is either control of DNA-binding activity or regulation of the function of the transactivating domain. At present the most important mechanism of inhibition of the Forkhead transcription factors that are controlled by PKB seems to be regulation of subcellular localization. Transiently expressed AFX in A14 cells resides in the nucleus and treatment of the cells with insulin or coexpression of active PKB results in the redistribution of AFX to the cytoplasm in a phosphorylation-dependent manner (G. Kops and B. Burgering, unpublished observations). The same type of regulation has been observed for FKHRL1 and a mouse orthologue of FKHR, FKHR1 [80, 82]. As argued for FKHRL1, this redistribution could be due to phospho-serine dependent interactions with cytoplasmic 14-3-3 proteins causing retention of the protein in the cytoplasm, but it is also possible that the phosphorylation of the AFX, FKHRL1, and FKHR1 proteins is required for nuclear export, possibly by binding nuclear 14-3-3 proteins that contain an nuclear export signal, such as Rad24 [80, 86]. On the other hand, all three Forkheads contain a very good consensus nuclear export signal within their primary amino acid sequence, and for mouse FKHR1 it has been shown that this sequence is required for nuclear exclusion of a truncated form of FKHR1. It is still possible, however, that

inhibition of Forkhead activity by PKB occurs at more levels than redistribution of the protein. None of the reports on PKB-mediated regulation of Forkhead transcription factors has tested whether DNA binding is affected by phosphorylation, although Nakea et al. [79] claim to have observed a decrease in DNA binding when the PKB site in the DNA-binding domain (the first of the C-terminal serines) of FKHR was phosphorylated.

The roles of PI3K and PKB have been well established in the regulation of AFX, FKHR, and FKHRL1. In the case of AFX, however, the PI3K/PKB pathway is not the only route mediating the phosphorylation induced by insulin. Treatment of cells with wortmannin or expression of dominant-negative PKB decreases but does not abolish insulin-induced total phosphorylation of AFX [78]. Although not stated explicitly, a similar partial effect by PI3K inhibition was observed for insulin-induced phosphorylation of FKHR and IGFI-induced phosphorylation of FKHRL1. Insulin-induced ^{32}P-orthophosphate incorporation into FKHR was only partly inhibited by wortmannin [79], and an IGFI-induced mobility shift of FKHRL1 was only partly inhibited by LY294002 [80]. Furthermore, peptide maps of mouse FKHR1 show phosphorylated residues apart from the PKB-mediated phosphorylation sites although it is not shown of which ones the phosphorylation is growth factor induced [82]. Altogether, this indicates the existence of multiple routes that mediate the growth factor induced phosphorylation of the Forkhead factors. In our study of insulin-mediated phosphorylation of AFX, the pathway in addition to PI3K/PKB from the insulin-receptor was identified and shown to consist of Ras signaling to the Ral GTPase. Activation of this pathway, as with the PI3K/PKB pathway, resulted in phosphorylation and inactivation of AFX [78]. It will be interesting to determine whether activation of this pathway also results in the phosphorylation and inactivation of the other Forkheads, as for DAF-16 it has already been suggested that an AGE-1/AKT-independent pathway originates from the DAF-2 receptor [6].

New perspectives on PKB function

Until now the effect of PKB on diverse processes such as metabolism, protein synthesis, and apoptosis has been thought to be by directly affecting proteins involved in these processes, such as GSK3, 4E-binding protein 1, and BAD, respectively (Fig. 4). Identification of the Forkhead transcription factors as direct downstream targets of PKB further refines this view of how PKB regulates cellular events. In addition to direct regulation by phosphorylation, PKB apparently regulates these processes by a seemingly indirect mechanism, namely transcription. The Forkheads have already been functionally implicated in the regulation of metabolism and apoptosis (Fig. 4). In the insulin-responsive A14 and HepG2 cell lines, AFX and FKHR can regulate the IGFBP-1 promoter [78, 85]. We and others have furthermore observed that AFX and FKHR can bind to IREs from the PEPCK and tyrosine amino-transferase genes (G. Kops and B. Burgering, unpublished observations and [87]).

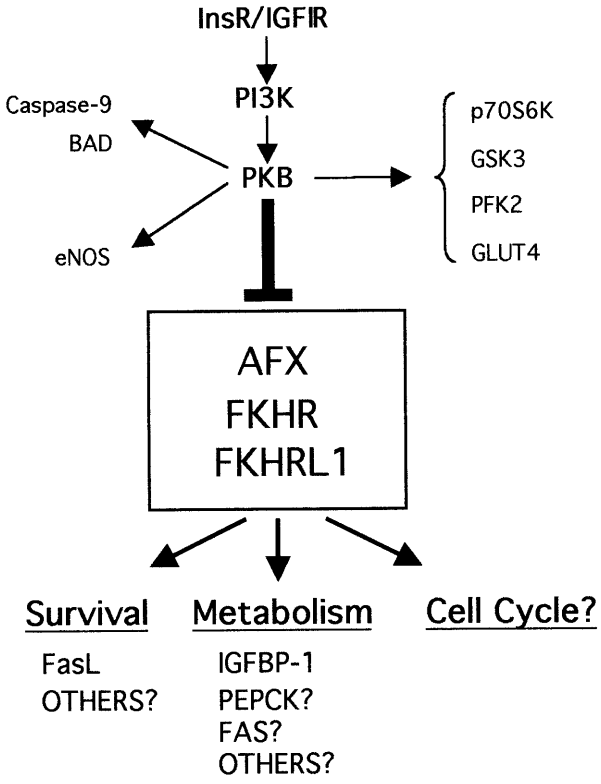

Fig. 4 Pathway leading to inactivation of AFX, FKHR, and FKHRL1. Insulin - or IGFI - receptor-activated PI3K leads to the activation of PKB which directly inactivates the Forkhead transcription factors. By inactivating the Forkheads PKB might affect cellular survival, metabolism and/or cell cycle progression

Overexpression of FKHRL1 and FKHR results in apoptotic cell death [80] [84]. In the event of FKHRL1-induced apoptosis, a target gene suggested to be able to mediate this effect is the Fas ligand gene (*FasL*), but other genes involved in Forkhead-induced apoptosis may have yet to be identified (Fig. 4 and [80]). In the same cell type as used for the FKHRL1 study, however, AFX does not seem as potent as FKHR and FKHRL1 in inducing apoptosis (R. Medema, G. Kops, and B. Burgering, unpublished observations). This may represent a functional difference between the Forkheads with respect to target genes, but it could also indicate a cell type specific expression difference. AFX expression especially is quite restricted, with the clearest expression of mRNA in muscle cells and hematopoietic cells [65, 88], whereas FKHR and FKHRL1 seem to be expressed more ubiquitously [71, 74]. This variance in expression between AFX, on the one hand, and FKHR and FKHRL1, on the other, may possibly explain potential functional differences.

The way in which PKB activity contributes to cellular transformation is generally thought to be by the inhibiting apoptosis. In agreement with this, expression of PTEN restores the susceptibility of PTEN-deficient cells

from PTEN knock-out mice to agonist-induced apoptosis [22]. Nonetheless, in PTEN-negative human glioma cells the reintroduction of a wild-type PTEN allele suppresses the growth of the cells, however, not by an increase in apoptosis but rather by inhibiting their progression through the cell cycle at the G_1 phase [89]. This observation revealed a surprising new role for PKB in cell growth regulation, but no targets of PKB have yet been identified that can transduce the effect of PKB activity on the cell cycle. The Forkhead transription factors may be mediators of this effect (Fig. 4).

Tumor suppression by Forkhead transcription factors?

AFX and FKHR contribute to chromosomal translocations that lead to leukemias and rhabdomyosarcomas, respectively. Interestingly, the PAX3-FKHR fusion product resulting from the t(2;13) translocation upregulates the receptor tyrosine kinases platelet-derived growth factor receptor and MET, although neither gene is a target for FKHR or PAX3 separately [90, 91]. Both growth factor receptors are very potent activators of PI3K (reviewed in [92]), which in turn is able to inactivate FKHR. This process thereby would have effects on cellular survival. This suggests that the t(2;13) translocation that already knocks out one allele of FKHR can inactivate the second allele by upregulating PI3K-activating receptor tyrosine kinases. The transforming capability of the PAX3-FKHR fusion product could therefore at least in part be due to its ability to inactivate FKHR or even any of the other two Forkheads.

In a similar manner, the way in which the t(X;11) translocation that creates the MLL-AFX fusion protein contributes to the onset of leukemia can be viewed in a new light. In this particular case, the translocation may also have a double function, yet one slightly different from that which creates PAX3-FKHR. The AFX gene lies on the X chromosome, indicating that males have only one allele [65]. A translocation of the AFX gene to chromosome 11 would therefore result not only in the potent transcriptional activator MLL-AFX, but also in the functional knock-out of AFX itself in males. This latter effect could then conceivably contribute to the leukemia, providing that AFX is indeed involved in cell growth inhibiting processes such as apoptosis and cell cycle arrest.

Conclusions

Over the past year many groups have shown the control of a subfamily of Forkhead transcription factors by PKB. These studies have completed a pathway to the nucleus that is initiated by the insulin or IGFI receptor and is mediated by PI3K and PKB. Genetic complementation studies had earlier suggested this route to exist in the nematode *C. elegans*. The biochemical evidence for the same route in mammalian cells provides a unique example of a complex regulatory cascade that is totally conserved between worm and man. In the future it will be of great interest to determine the target genes of the Forkheads, which can give insight into the functions and specificity of the transcription factors. For DAF-16 one target gene has already been identified, namely the cytosolic catalase [93]. This protein is likely to be involved in reducing the amount of free radicals in the dauer larvae, thereby contributing to the extended life span that results from the dauer phenotype. Although no cytosolic catalase has been found in mammals, it will be interesting to see what genes are regulated by AFX, FKHRL1, and FKHR, and whether these genes can contribute to an extended life span, for instance, by regulating metabolic processes or by regulating the cell cycle. Come what may, the identification of the subfamily of Forkhead transcription factors as downstream targets of PKB signaling sheds new light on the mechanism by which the proto-oncogenic kinase induces may induce cellular transformation.

References

1. Coffer PJ, Woodgett JR (1991) Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. Eur J Biochem 201:475–481 (published erratum appears in Eur J Biochem 205:1217)
2. Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proc Natl Acad Sci U S A 88:4171–4175
3. Bellacosa A, Testa JR, Staal SP, Tsichlis PN (1991) A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science 254:274–277
4. Staal SP (1987) Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci U S A 84:5034–5037
5. Franke TF, Tartof KD, Tsichlis PN (1994) The SH2-like Akt homology (AH) domain of c-akt is present in multiple copies in the genome of vertebrate and invertebrate eucaryotes. Cloning and characterization of the Drosophila melanogaster c-akt homolog Dakt1. Oncogene 9:141–148
6. Paradis S, Ruvkun G (1998) Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev 12:2488–2498
7. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR (1992) AKT2, a putative oncogene encoding a member of a subfamily of protein- serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci U S A 89:9267–9271
8. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, et al (1995) Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int J Cancer 64:280–285
9. Miwa W, Yasuda J, Murakami Y, Yashima K, Sugano K, Sekine T, Kono A, Egawa S, Yamaguchi K, Hayashizaki Y, Sekiya T (1996) Isolation of DNA sequences amplified at chromosome 19q13.1-q13.2 including the AKT2 locus in human pancreatic cancer. Biochem Biophys Res Commun 225:968–974
10. Cheng JQ, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR (1996) Amplification of AKT2 in human

pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. Proc Natl Acad Sci USA 93: 3636–3641
11. Coffer PJ, Jin J, Woodgett JR (1998) Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335:1–13
12. Franke TF, Kaplan DR, Cantley LC, Toker A (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science 275:665–668
13. Toker A, Cantley LC (1997) Signalling through the lipid products of phosphoinositide-3-OH kinase. Nature 387:673–676
14. Burgering BM, Coffer PJ (1995) Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 376:599–602
15. Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR, Tsichlis PN (1995) The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell 81:727–736
16. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15:6541–6551
17. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Curr Biol 7:261–269
18. Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick F, Tempst P, Coadwell J, Hawkins PT (1998) Protein kinase B kinases that mediate phosphatidylinositol 3,4,5- trisphosphate-dependent activation of protein kinase B. Science 279:710–714
19. Balendran A, Casamayor A, Deak M, Paterson A, Gaffney P, Currie R, Downes CP, Alessi DR (1999) PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2. Curr Biol 9:393–404
20. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S (1998) Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc Natl Acad Sci USA 95:11211–11216
21. Haas-Kogan D, Shalev N, Wong M, Mills G, Yount G, Stokoe D (1998) Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. Curr Biol 8:1195–1198
22. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95:29–39
23. Pennisi E (1997) New tumor suppressor found-twice. Science 275:1876–1878
24. Maehama T, Dixon JE (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:13375–13378
25. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK (1998) The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA 95:13513–13518
26. Jimenez C, Jones DR, Rodriguez-Viciana P, Gonzalez-Garcia A, Leonardo E, Wennstrom S, von Kobbe C, Toran JL, R-Borlado L, Calvo V, Copin SG, Albar JP, Gaspar ML, Diez E, Marcos MA, Downward J, Martinez AC, Merida I, Carrera AC (1998) Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. EMBO J 17:743–753
27. Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK (1997) Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. Science 276:1848–1850
28. Marte BM, Rodriguez-Viciana P, Wennstrom S, Warne PH, Downward J (1997) R-Ras can activate the phosphoinositide 3-kinase but not the MAP kinase arm of the Ras effector pathways. Curr Biol 7:63–70
29. Wu X, Senechal K, Neshat MS, Whang YE, Sawyers CL (1998) The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 95:15587–15591
30. Davies MA, Lu Y, Sano T, Fang X, Tang P, LaPushin R, Koul D, Bookstein R, Stokoe D, Yung WK, Mills GB, Steck PA (1998) Adenoviral transgene expression of MMAC/PTEN in human glioma cells inhibits Akt activation and induces anoikis. Cancer Res 58:5285–5290
31. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785–789
32. van Weeren PC, de Bruyn KM, de Vries-Smits AM, van Lint J, Burgering BM (1998) Essential role for protein kinase B (PKB) in insulin-induced glycogen synthase kinase 3 inactivation. Characterization of dominant-negative mutant of PKB. J Biol Chem 273:13150–13156
33. Deprez J, Vertommen D, Alessi DR, Hue L, Rider MH (1997) Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. J Biol Chem 272:17269–17275
34. Kohn AD, Barthel A, Kovacina KS, Boge A, Wallach B, Summers SA, Birnbaum MJ, Scott PH, Lawrence JC Jr, Roth RA (1998) Construction and characterization of a conditionally active version of the serine/threonine kinase Akt. J Biol Chem 273:11937–11943
35. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N (1998) 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev 12:502–513
36. Yao R, Cooper GM (1995) Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. Science 267:2003–2006
37. Yao R, Cooper GM (1996) Growth factor-dependent survival of rodent fibroblasts requires phosphatidylinositol 3-kinase but is independent of pp70S6 K activity. Oncogene 13:343–351
38. Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME (1997) Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 275:661–665
39. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G (1997) Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 278:687–689
40. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell- intrinsic death machinery. Cell 91:231–241
41. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC (1998) Regulation of cell death protease caspase-9 by phosphorylation. Science 282: 1318–1321
42. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999) Activation of nitric oxide synthase in endothelial cells by Akt- dependent phosphorylation. Nature 399: 601–605
43. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC (1999) Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 399:597–601
44. de Groot RP, Auwerx J, Bourouis M, Sassone-Corsi P (1993) Negative regulation of Jun/AP-1: conserved function of glycogen synthase kinase 3 and the Drosophila kinase shaggy. Oncogene 8:841–847
45. Fiol CJ, Williams JS, Chou CH, Wang QM, Roach PJ, Andrisani OM (1994) A secondary phosphorylation of CREB341 at Ser129 is required for the cAMP-mediated control of gene expression. A role for glycogen synthase kinase-3 in the control of gene expression. J Biol Chem 269:32187–32193
46. Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J, Evan G (1997) Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. Nature 385:544–548

47. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, Choi JK, Trotta R, Wlodarski P, Perrotti D, Chan TO, Wasik MA, Tsichlis PN, Calabretta B (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3 k/Akt-dependent pathway. EMBO J 16:6151–6161
48. Brennan P, Babbage JW, Burgering BM, Groner B, Reif K, Cantrell DA (1997) Phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F. Immunity 7:679–689
49. Mazure NM, Chen EY, Laderoute KR, Giaccia AJ (1997) Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras- transformed cells through a hypoxia inducible factor-1 transcriptional element. Blood 90:3322–3331
50. Du K, Montminy M (1998) CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 273:32377–32379
51. Kane LP, Shapiro VS, Stokoe D, Weiss A (1999) Induction of NF-kappaB by the Akt/PKB kinase. Curr Biol 9:601–604
52. Sizemore N, Leung S, Stark GR (1999) Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF-kappaB p65/RelA subunit. Mol Cell Biol 19:4798–4805
53. Liao J, Barthel A, Nakatani K, Roth RA (1998) Activation of protein kinase B/Akt is sufficient to repress the glucocorticoid and cAMP induction of phosphoenolpyruvate carboxykinase gene. J Biol Chem 273:27320–27324
54. Agati JM, Yeagley D, Quinn PG (1998) Assessment of the roles of mitogen-activated protein kinase, phosphatidylinositol 3-kinase, protein kinase B, and protein kinase C in insulin inhibition of cAMP-induced phosphoenolpyruvate carboxykinase gene transcription. J Biol Chem 273:18751–18759
55. Wang D, Sul HS (1998) Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway. Involvement of protein kinase B/Akt. J Biol Chem 273:25420–25426
56. Cichy SB, Uddin S, Danilkovich A, Guo S, Klippel A, Unterman TG (1998) Protein kinase B/Akt mediates effects of insulin on hepatic insulin- like growth factor-binding protein-1 gene expression through a conserved insulin response sequence. J Biol Chem 273:6482–6487
57. Kaufmann E, Knochel W (1996) Five years on the wings of fork head. Mech Dev 57:3–20
58. Weigel D, Jurgens G, Kuttner F, Seifert E, Jackle H (1989) The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the Drosophila embryo. Cell 57:645–658
59. Costa RH, Grayson DR, Darnell JE Jr (1989) Multiple hepatocyte-enriched nuclear factors function in the regulation of transthyretin and alpha 1-antitrypsin genes. Mol Cell Biol 9:1415–1425
60. Unterman TG, Fareeduddin A, Harris MA, Goswami RG, Porcella A, Costa RH, Lacson RG (1994) Hepatocyte nuclear factor-3 (HNF-3) binds to the insulin response sequence in the IGF binding protein-1 (IGFBP-1) promoter and enhances promoter function. Biochem Biophys Res Commun 203:1835–1841
61. O'Brien RM, Noisin EL, Suwanichkul A, Yamasaki T, Lucas PC, Wang JC, Powell DR, Granner DK (1995) Hepatic nuclear factor 3- and hormone-regulated expression of the phosphoenolpyruvate carboxykinase and insulin-like growth factor-binding protein 1 genes. Mol Cell Biol 15:1747–1758
62. Xuan S, Baptista CA, Balas G, Tao W, Soares VC, Lai E (1995) Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. Neuron 14: 1141–1152
63. Li J, Thurm H, Chang HW, Iacovoni JS, Vogt PK (1997) Oncogenic transformation induced by the Qin protein is correlated with transcriptional repression. Proc Natl Acad Sci USA 94:10885–10888
64. McCabe NR, Burnett RC, Gill HJ, Thirman MJ, Mbangkollo D, Kipiniak M, van Melle E, Ziemin-van der Poel S, Rowley JD, Diaz MO (1992) Cloning of cDNAs of the MLL gene that detect DNA rearrangements and altered RNA transcripts in human leukemic cells with 11q23 translocations. Proc Natl Acad Sci U S A 89:11794–11798
65. Borkhardt A, Repp R, Haas OA, Leis T, Harbott J, Kreuder J, Hammermann J, Henn T, Lampert F (1997) Cloning and characterization of AFX, the gene that fuses to MLL in acute leukemias with a t(X;11)(q13;q23). Oncogene 14:195–202
66. Hillion J, Le Coniat M, Jonveaux P, Berger R, Bernard OA (1997) AF6q21, a novel partner of the MLL gene in t(6;11)(q21;q23), defines a forkhead transcriptional factor subfamily. Blood 90:3714–3719
67. Sublett JE, Jeon IS, Shapiro DN (1995) The alveolar rhabdomyosarcoma PAX3/FKHR fusion protein is a transcriptional activator. Oncogene 11:545–552
68. Thomas JH (1993) Chemosensory regulation of development in C. elegans. Bioessays 15:791–797
69. Lin K, Dorman JB, Rodan A, Kenyon C (1997) daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. Science 278:1319–1322
70. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature 389:994–999
71. Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher FJd, Emanuel BS, Rovera G, Barr FG (1993) Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. Nat Genet 5:230–235 (published erratum appears in Nat Genet 6:214)
72. Morris JZ, Tissenbaum HA, Ruvkun G (1996) A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature 382:536–539
73. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science 277:942–946
74. Anderson MJ, Viars CS, Czekay S, Cavenee WK, Arden KC (1998) Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics 47:187–199
75. Ogg S, Ruvkun G (1998) The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell 2:887–893
76. Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G (1999) A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Genes Dev 13:1438–1452
77. Alessi DR, Caudwell FB, Andjelkovic M, Hemmings BA, Cohen P (1996) Molecular basis for the substrate specificity of protein kinase B; comparison with MAPKAP kinase-1 and p70 S6 kinase. FEBS Lett 399:333–338
78. Kops GJ, de Ruiter ND, De Vries-Smits AM, Powell DR, Bos JL, Burgering BM (1999) Direct control of the Forkhead transcription factor AFX by protein kinase B. Nature 398:630–634
79. Nakae J, Park BC, Accili D (1999) Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a wortmannin-sensitive pathway. J Biol Chem 274:15982–15985
80. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–868
81. Rena G, Guo S, Cichy SC, Unterman TG, Cohen P (1999) Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. J Biol Chem 274:17179–17183
82. Biggs WH, 3rd, Meisenhelder J, Hunter T, Cavenee WK, Arden KC (1999) Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. Proc Natl Acad Sci U S A 96:7421–7426
83. Erikson E, Maller JL (1988) Substrate specificity of ribosomal protein S6 kinase II from Xenopus eggs. Second Messengers Phosphoproteins 12:135–143

84. Tang ED, Nunez G, Barr FG, Guan KL (1999) Negative regulation of the Forkhead transcription factor FKHR by Akt. J Biol Chem 274:16741–16746
85. Guo S, Rena G, Cichy S, He X, Cohen P, Unterman T (1999) Phosphorylation of serine 256 by protein kinase B disrupts transactivation by FKHR and mediates effects of insulin on insulin-like growth factor-binding protein-1 promoter activity through a conserved insulin response sequence. J Biol Chem 274:17184–17192
86. Lopez-Girona A, Furnari B, Mondesert O, Russell P (1999) Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein. Nature 397:172–175
87. Durham SK, Suwanichkul A, Scheimann AO, Yee D, Jackson JG, Barr FG, Powell DR (1999) FKHR binds the insulin response element in the insulin-like growth factor binding protein-1 promoter. Endocrinology 140:3140–3146
88. Parry P, Wei Y, Evans G (1994) Cloning and characterization of the t(X;11) breakpoint from a leukemic cell line identify a new member of the forkhead gene family. Genes Chromosomes Cancer 11:79–84
89. Li DM, Sun H (1998) PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. Proc Natl Acad Sci U S A 95:15406–15411
90. Ginsberg JP, Davis RJ, Bennicelli JL, Nauta LE, Barr FG (1998) Up-regulation of MET but not neural cell adhesion molecule expression by the PAX3-FKHR fusion protein in alveolar rhabdomyosarcoma. Cancer Res 58:3542–3546
91. Epstein JA, Song B, Lakkis M, Wang C (1998) Tumor-specific PAX3-FKHR transcription factor, but not PAX3, activates the platelet-derived growth factor alpha receptor. Mol Cell Biol 18:4118–4130
92. Porter AC, Vaillancourt RR (1998) Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. Oncogene 17:1343–1352
93. Taub J, Lau JF, Ma C, Hahn JH, Hoque R, Rothblatt J, Chalfie M (1999) A cytosolic catalase is needed to extend adult lifespan in C. elegans daf-C and clk-1 mutants. Nature 399:162–166