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FORUM REVIEW ARTICLE

Integrating Opposing Signals Toward Forkhead Box O

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Abstract

Transcription factors are the common convergence points of signal transduction pathways to affect gene transcription. Signal transduction activity results in posttranslational modification (PTM) of transcription factors and the sum of these modifications at any given time point will determine the action of the transcription factor. It has been suggested that these PTMs provide a transcription factor code analogous to the histone code. However, the number and variety of these modifications and the lack of knowledge in general of their dynamics precludes at present a concise view of how combinations of PTMs affect transcription factor function. Also, a single type of PTM such as phosphorylation can have opposing effects on transcription factor activity. Transcription factors of the Forkhead box O (FOXO) class are predominantly regulated through signaling, by phosphoinositide 3-kinase/protein kinase B (also known as AKT) pathway and a reactive oxygen species/c-Jun N-terminal kinase pathway. Both pathways result in increased FOXO phosphorylation yet with opposing result. Whereas PKB-mediated phosphorylation inactivates FOXO, c-Jun N-terminal kinase-mediated phosphorylation results in activation of FOXO. Here we discuss regulation of FOXO transcription factors by phosphorylation as an example for understanding integration of signal transduction at the level of transcription activity. *Antioxid. Redox Signal.* 14, 607–621.

Introduction

THE TRANSCRIPTION FACTOR FAMILY of forkhead proteins participates in numerous cellular processes, such as development, differentiation, metabolism, proliferation, apoptosis, and stress resistance. The family name forkhead is derived from its founding member the *Drosophila melanogaster* Fork head (*fkh*) gene product. A conserved DNA binding domain also known as the forkhead box characterizes Forkhead proteins (43). This domain is a variant of the helix-turn-helix motif and is made up of three alpha helices and two characteristic large loops or butterfly-like wings (20, 106). There is a high degree of sequence homology within the DNA binding domain, but an almost complete lack of similarity in the transactivation domains.

In mammals, the class O of Fox transcription factors (FOXO) contains four members: FOXO1, FOXO3, FOXO4, and FOXO6. The DNA binding domain of FOXO, in particular helix 3, mediates binding to promoters that contain the FOXO consensus motif 5′-TTGTTTAC-3′ (29). In general, it seems that expression of FOXO-regulated genes can be controlled by any of the FOXO transcription factors, and that specificity is obtained either by their specific expression pattern or by isoform-specific regulation. Therefore, throughout this review we will use the general term FOXO, if not specified otherwise.

FOXOs are subject to regulation by many signaling pathways, but initially it was shown that FOXO activity is controlled by insulin/insulin-like growth factor (IGF) signaling, in particular through phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB also known as AKT) signaling resulting in PKB-mediated phosphorylation (Fig. 1) (13, 17, 18, 51, 96, 97). As FOXOs therefore were the first identified downstream transcriptional regulators of this pathway, it became of interest to identify what PI3K/PKB-dependent cellular processes are regulated through FOXO and what gene transcription would therefore be required.

Control of Cell Fate by FOXO

Ectopic expression of FOXO factors in a large variety of mammalian cell types causes a strong inhibition of cell proliferation (21, 50, 64, 69). This antiproliferative effect of FOXO expression is also observed in transformed cell types, such as Ras-transformed cells and cells deficient in phosphatase and tensin homolog (PTEN) (64, 69). Thus, FOXO factors can oppose the growth-stimulating effects of PKB/AKT when expressed at sufficiently high levels. In many cell types, FOXO-induced growth inhibition is associated with a block in cell cycle progression in the G1 phase. FOXOs regulate transcription of the cdk inhibitor $p27^{kip1}$ (64, 69) and FOXO-mediated upregulation of $p27^{kip1}$ controls G1 progression. Next

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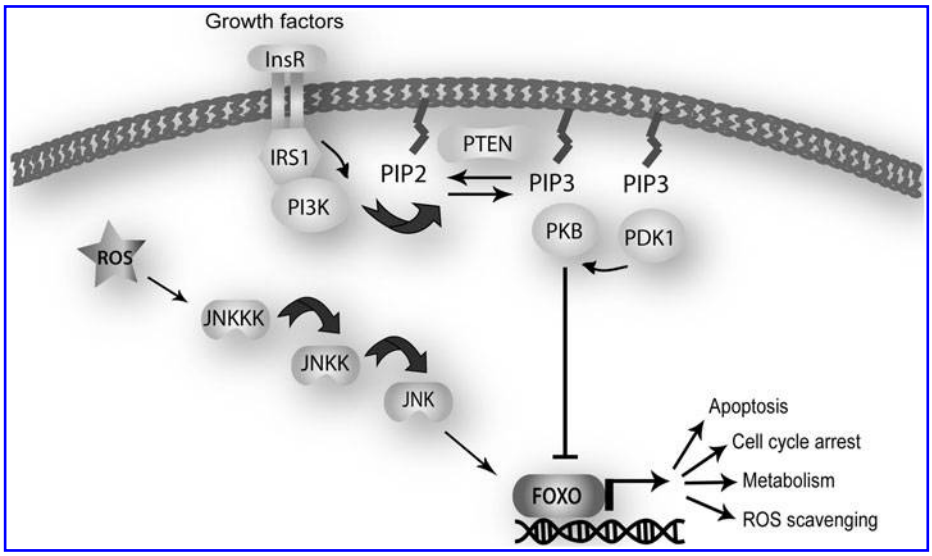


FIG. 1. Opposing signals toward FOXO. A simplified model of the PBK-induced negative and JNK-induced positive signaling pathways toward FOXO. FOXO, Forkhead box O; InsR, insulin receptor; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP, phospho-inositide-phosphate; PKB, protein kinase B; PTEN, phosphatase and tensin homolog.

to $p27^{kip1}$, FOXO-mediated transcriptional repression of D-type cyclins is crucial to the FOXO-induced cell cycle arrest (81, 88).

Under conditions of increased cellular stress, mostly oxidative stress FOXOs may also induce a cell cycle arrest at G2/M (28). However, what target genes mediate G2/M arrest is presently unclear.

Cell cycle arrest in G1/S or G2/M cells oftentimes is a prerequisite to proceed to a specific cellular fate, including quiescence, senescence, differentiation, or cell death. FOXOs are more or less implicated in all these cellular fates. For example, FOXO-induced arrest in DLD-1 cells is timely and reversible, an important criterion for quiescent cells (50). In contrast to quiescence, a number of cell types respond to FOXO activation by inducing apoptosis. Importantly, at least in BaF3 cells, induction of cell death follows a G1 arrest, showing that FOXOs first induce a cell cycle arrest followed by a switch to a desired cell fate (23). A number of FOXO gene targets that may mediate apoptosis have been identified, including Fas-ligand (18), Bim (24, 93), Bcl-6, negatively regulating BCL-XL expression (98), tumor necrosis factor-related apoptosis inducing ligand (65), and tumor necrosis factor receptor-associated death domain (85).

To enable regulation of these complex and diverse cell fates, FOXOs have to regulate other gene programs such as cell metabolism and stress response programs. In mammalian cell systems and in the nematode *Caenorhabditis elegans*, FOXO factors have been shown to mediate protection of cells against oxidative stress. FOXOs control expression of numerous enzymes involved in antioxidant defenses, including manganese superoxide dismutase (49), catalase (70), sestrins (71), and selenoprotein P (104). In addition, FOXO was shown to control expression of growth arrest and DNA damage (GADD)45a protein involved in DNA repair mechanisms (28, 100). Taken together, these data demonstrate that FOXO factors regulate expression of a number of genes that are important in the protection of cells against oxidative stress. As many studies employ H_2O_2 addition to cells, the nature of this oxidative stress is not extensively specified, but at least metabolic stress (*e.g.*, glucose starvation and caloric restriction) is thought to contribute to endogenous regulation of FOXO by cellular stress.

FOXO activity has also an important impact on cell metabolism. For instance, in situations where there is lack of or impaired insulin signaling, such as during fasting or diabetes, FOXOs play an important role in regulating gene expression important for hepatic gluconeogenesis, like glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (67, 89). In addition, FOXO1 plays an important role in lipid metabolism where it regulates microsomal triglyceride transfer protein (MTP) production (45), a membrane-associated chaperone protein that catalyzes transfer of lipids to nascent apolipoprotein B. MTP is necessary for very low density lipoprotein (VLDL) formation; as the liver-specific $MTP^{-/-}$ mice are unable to produce VLDL (79) and genetic mutations in the gene encoding MTP (*MTTP*), they cause abetalipoproteinemia (eliminated VLDL formation) in humans (10). Thus, in situations of impaired insulin signaling, FOXO1 is active and induces MTP production and thereby increased VLDL levels, resulting in hypertriglyceridemia (45). Next to the regulation of MTP, FOXO1 also promotes expression of apolipoprotein CIII (3). ApoCIII inhibits the clearance of triclycerides, so increased ApoCIII expression by FOXO1 can result in hypertriglyceridemia as well.

Besides the liver, FOXOs also play an important metabolic function in muscle cells, where they are involved in muscle atrophy and myoblast differentiation. Muscle atrophy is a process in which rapid loss of muscle proteins takes place. This process occurs systemically with diseases like diabetes and cancer and during fasting, circumstances in which FOXOs are activated. So not unexpectedly, during catabolic states, FOXOs translocate to the nucleus and induces antrogin-1 and MuRF-1 gene expression, atrophy-specific ubiquitin ligases, important in the proteosomal-mediated degradation of muscle proteins (86, 94). Recently, Zhao *et al.* and Mammucari *et al.* discovered an additional role for FOXO3 in the regulation of muscle atrophy, by regulation of autophagy through transcriptional regulation of a set of autophagy genes (61, 120). Thus, muscle atrophy is regulated through two different pathways: ubiquitin/proteosomal pathway and autophagic/lysosomal pathway, in which FOXO plays an essential role by transcriptional regulation of genes involved in both pathways.

Next to the above-described metabolic functions of FOXO, they are important in regulating metabolic processes in a subset of other tissues/cell types as well; pancreatic β-cells, adipose tissue, hypothalamus, and endothelial cells [for more detailed information, see reviews (9, 68)].

FOXOs Through Evolution

All of the above studies are in fact guided by the initial observation that FOXO transcription factors are orthologous to the nematode *C. elegans* abnormal dauer formation-16 (DAF-16) protein. DAF-16, like FOXO, is also negatively regulated by the PI(3)K (*age-1*)/PKB (*akt-1/2*) pathway (72, 76, 99). In *C. elegans*, DAF-16 promotes entry into the Dauer stage. The Dauer stage represents an alternative larval stage that is induced by starvation, a dauer pheromone, or high temperature. This developmental arrest is associated with reduced metabolic activity and increased resistance to oxidative stress. Besides controlling dauer diapause, DAF-16 has also been shown to be a key component in controlling *C. elegans* lifespan. Thus, increased DAF-16 activity is responsible for enhanced *C. elegans* lifespan and further studies have shown that at least to some extend this DAF-16/FOXO function is preserved through evolution. This observation has turned out to be key of current thinking with respect to aging. Consequently, all effects of FOXO on cell fate as described above are held against the light of its impact on organismal lifespan, and are used to further our understanding of lifespan and aging.

Biological aging can be defined as a timely decline of functional ability of cells, tissue, or organism, and changes in lifespan observed in model organisms are thought to reflect at least in part the process of aging. Many theories have been put forward to explain the phenomenon of aging, among which the so-called free-radical theory of aging likely best explains the function of DAF-16/FOXO in lifespan. In essence, this theory postulates that because organisms use molecular oxygen (O_2) for efficient generation of ATP, they are also continuously exposed to the damaging effects of cellular reactive oxygen species (ROS) that is produced because of O_2 consumption. The accumulating damaging effects of ROS are then believed to underlie the natural process of aging. Importantly, whereas aging itself is not a disease, ROS-induced cellular damage is generally considered important in the etiology of almost all age-related disease, including cancer and diabetes. In agreement with the above, DAF-16/FOXO has been shown to regulate a number of genes (*e.g.*, manganese superoxide dismutase, sestrin3, and catalase) that are involved in conferring resistance against increased cellular ROS. The apparent importance of DAF-16/FOXO in mediating resistance against increased ROS led to the suggestion that DAF-16/FOXO in return is also subject to regulation by ROS. Indeed, numerous studies by others and us have shown that in contrast to insulin signaling, increased ROS result in activation of DAF-16/FOXO. Most importantly, at least within the context of this review it has been shown in mammalian cells, as well as in *C. elegans* and *D. melanogaster*, that direct c-Jun N-terminal kinase (JNK)-mediated phosphorylation of DAF-16/FOXO mediates activation of DAF-16/FOXO after increased ROS (Fig. 1) (25, 73, 105). In agreement, enhanced JNK activity increases lifespan in *C. elegans* and *D. melanogaster* and this requires DAF-16 and dFOXO, respectively (73, 105). Although JNK-mediated phosphorylation is important to regulate FOXO after increased ROS, the activity of FOXOs is further tightly regulated by other PTMs such as acetylation, methylation, and ubiquitination, all of which are regulated after changes in cellular ROS. All these PTMs affect FOXO function and this can occur in various ways. FOXOs shuttle between nucleus and cytoplasm and PTMs can shift the equilibrium of this shuttling to either site. Also, when bound to DNA, PTMs are required to unfold transcriptional activity through recruitment of coactivators/repressors (*e.g.*, p300/CBP; SIRT). Finally, FOXO protein stability is regulated by PTMs, Thus, FOXO activity can be regulated through multiple means and multiple PTMs and this provides a challenge in understanding the interplay between the various PTMs. Here, we will discuss the opposing signals, of activation and inactivation, mediated by phosphorylation on FOXOs.

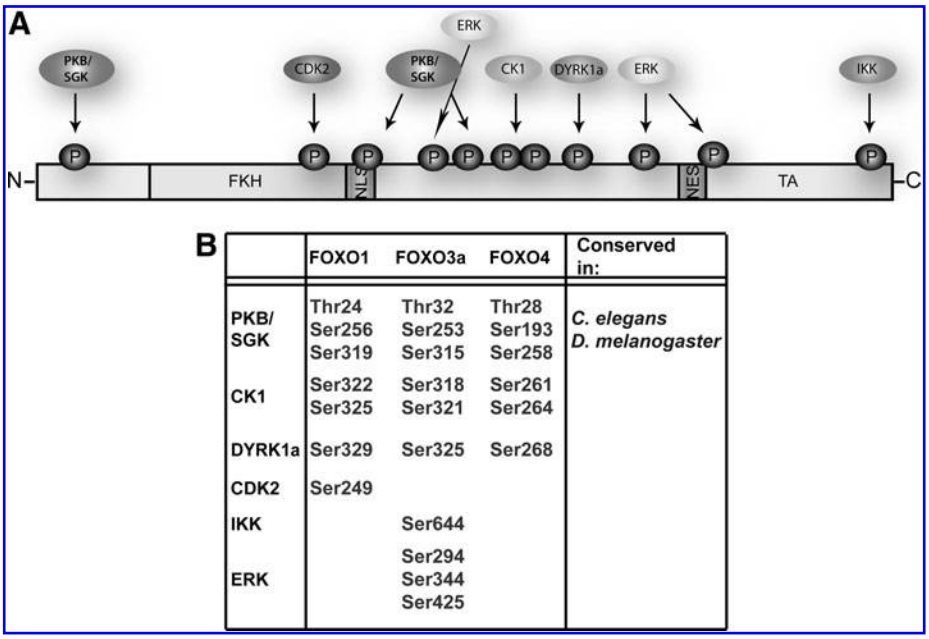
Signal Transduction Pathways Regulating FOXO Phosphorylation Status

Growth factor signaling

FOXO transcription factors are regulated by the insulin signaling pathway through PI3K and PKB (Fig. 1). All FOXO members as well as DAF-16 contain three conserved PKB phosphorylation sites (Fig. 2). *In vivo* all three sites are phosphorylated after growth factor, in particular insulin and IGF-1, treatment (18, 34, 51, 82). PKB-induced phosphorylation of FOXO on these three sites results in the binding to 14-3-3 and this correlates with export of FOXO out of the nucleus (17, 34, 83, 119). Shuttling of proteins between nucleus and cytoplasm is a highly regulated process and requires accessory proteins such as importins and exportins (59). At present there is still little detailed knowledge as to how FOXO shuttling is regulated. Domains within FOXO that could function as nuclear export signal (NES) or nuclear localization signal (NLS) have been assigned but are supported by limited experimental evidence. For FOXO4 a nonclassical NLS that surrounds the PKB phosphorylation site Ser-193 has been defined (17). Shuttling of FOXO4 was shown to be a Ran- and Crm1-dependent mechanism and PKB-mediated phosphorylation of FOXO4 on Ser-193 functionally inactivates the NLS, causing the continuously shuttling transcription factor to be detained in the cytoplasm (17). The primary sequence of the FOXO4 NLS shows that it is not a classical NLS and indeed none of the classical importins were found to bind FOXO4 and at present the importin responsible for nuclear import is unknown.

Although all FOXO members harbor a putative NES sequence, its function remains unclear. Nuclear export of Forkheads is sensitive to leptomycinB treatment, indicating a Crm1-dependent export mechanism (17). Deletion of the putative NES clearly affects nuclear export of FOXO4, yet surprisingly Crm-1 binding appears unaffected by phosphorylation and the NES deletion does not prevent binding between FOXO4 and Crm1. This leaves the possibility that an additional protein in complex with Forkhead/Crm1 might provide the required NES.

Serum- and glucocorticoid-inducible kinase (SGK) is highly homologous to PKB and like the family of PKB kinases (PKBα, β, and γ), the SGK family also consists of three members (SGK1, 2, and 3). SKGs phosphorylate the same consensus phosphorylation site in target proteins (RXRXXS/T) as PKB, and therefore SGK can phosphorylate FOXO3 at the same sites as PKB (*e.g.*, Thr-32, Ser-253, and Ser-315 in FOXO3a) (Fig. 2) (19). However, SGK preferentially phosphorylates



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	FOXO1	FOXO3a	FOXO4	Conserved in:
PKB/ SGK	Thr24 Ser256 Ser319	Thr32 Ser253 Ser315	Thr28 Ser193 Ser258	*C. elegans* *D. melanogaster*
CK1	Ser322 Ser325	Ser318 Ser321	Ser261 Ser264	
DYRK1a	Ser329	Ser325	Ser268	
CDK2	Ser249			
IKK		Ser644		
ERK		Ser294 Ser344 Ser425		

FIG. 2. Phosphorylation-dependent inactivation of FOXO. (A) Schematic representation of FOXO domains and phosphorylated residues. **(B)** Kinases involved in phosphorylation and subsequent inactivation of FOXO. Specific phosphorylation sites of the different FOXO members are indicated. Numbering of all phosphorylation sites is for human FOXO. CDK2, cyclin dependent kinase 2; ERK, extracellular signal-regulated kinase; FKH, Forkhead domain; IKK, IκB kinase; NES, nuclear export signal; NLS, nuclear localization signal; TA, transactivation domain.

Thr-32 and Ser-315, whereas PKB preferentially phosphorylates Thr-32 and Ser-253 in FOXO3a (19).

Not only does SGK/PKB-mediated phosphorylation provide a binding interface for 14-3-3 proteins, but also PKB-mediated phosphorylation of Ser-319 of FOXO1 has also been shown to act as a docking site for subsequent phosphorylation by casein kinase 1 (CK-1). CK-1 recruitment in this way results in phosphorylation of two additional sites, Ser-322 and Ser-325, in FOXO1 (Fig. 2) (84).

Initial *in vitro* kinase experiments using purified his-tagged PKB indicated phosphorylation of an apparent non-PKB site (51). Further analysis revealed that a kinase(s) harboring a natural his-tag apparently copurifies with his-tagged PKB. Dual-specificity tyrosine-phosphorylated and regulated kinase 1a was identified in these purified PKB fractions as a natural his-tag expressing contaminant and responsible for phosphorylation of Ser-329 in FOXO1 and Ser-268 in FOXO4 (Fig. 2) (107). Interestingly, Ser-329 phosphorylation appears constitutive as this is at least not regulated by insulin like growth factor signaling, or by overexpression of PI(3)K. Taken together, combined PKB and CK-1-mediated phosphorylation and constitutive dual-specificity tyrosine-phosphorylated and regulated kinase 1a phosphorylation results in a consecutive stretch of phosphorylated residues (Ser-319, 322, 325, and 329 in FOXO1), and it is suggested that this negatively charged patch in FOXO greatly enhances nuclear export of FOXO1 in a Ran-Crm1-specific manner (84).

Ikk-dependent signaling

Immunohistochemical analysis of certain tumors revealed apparent FOXO3a localization in the cytoplasm, while no hyperactive PKB/AKT could be detected. This led to the suggestion that in these tumors, other kinases, besides PKB, are responsible for relocalizing FOXO3a to the cytosol. This resulted in the identification of IκB kinase (IKK) as being responsible for Ser-644 phosphorylation of FOXO3a (Fig. 2). The phosphorylation of FOXO3a on Ser-644 seems to be specific for the human and mouse FOXO3a, as this site is not conserved among the other members of the FOXO family. The classical role of IKKs is to regulate NFκB during cytokine-induced and inflammatory responses and in agreement phosphorylation of FOXO3a on Ser-644 is induced after TNFα stimulation. Ser-644 phosphorylation is suggested to inhibit, like PKB through nuclear exclusion of FOXO, followed by poly-ubiquitination and consequent degradation. Thus IKK-mediated Ser-644 phosphorylation results in inhibition of FOXO3a transcriptional activity and this may occur in a PKB-independent manner, as a mutant of FOXO3a, mutated in all three PKB sites, is still suppressed by IKK (37). However, PKB has been shown to positively regulate IKK (74), and thus may act upstream rather than downstream of IKK, and Ser-644 phosphorylation would thereby provide an additional mechanism, whereby PKB is able to inhibit FOXO3a function.

DNA damage signaling

ROS-induced DNA damage is considered a main driving force of aging. Thus, a linkage between DNA damage and FOXO function is to be expected. Initially, it was shown that FOXO activation results in increased GADD45a gene expression (28, 100). GADD45a has been implicated in DNA repair, and in agreement it was shown that FOXO activation results in GADD45a-dependent repair of an *ex-vivo* UV-damaged plasmid (100). It was shown recently that GADD45a has a key role in active DNA demethylation (8). This may explain how GADD45a facilitates DNA repair, but also indicates that FOXO through GADD45a may contribute to reactivation of genes silenced by DNA methylation.

A high-throughput mass-spectrometry analysis of proteins phosphorylated on serine followed by glutamine (SpQ), the consensus for ataxia telangiectasia mutated (ATM) and Rad3-related-mediated phosphorylation, revealed FOXO1 to be a potential ATM substrate (62). This would be in accordance with aforementioned possibility that FOXO-induced gene expression may facilitate DNA repair. Subsequently, it was

shown that FOXO and ATM can interact, but rather than serving as a substrate, FOXO was suggested to be required for ATM to become activated upon DNA damage. Cells in which FOXO3a expression was reduced by siRNA showed complete lack of ATM activation after DNA damage (101). How FOXO senses DNA damage and how this then is transmitted to ATM remains yet unresolved. Alternatively, a recent report shows that in hematopoietic stem cells, FOXO3a deficiency compromises ATM expression, suggesting that loss of ATM activation is primarily due to loss of ATM expression (110).

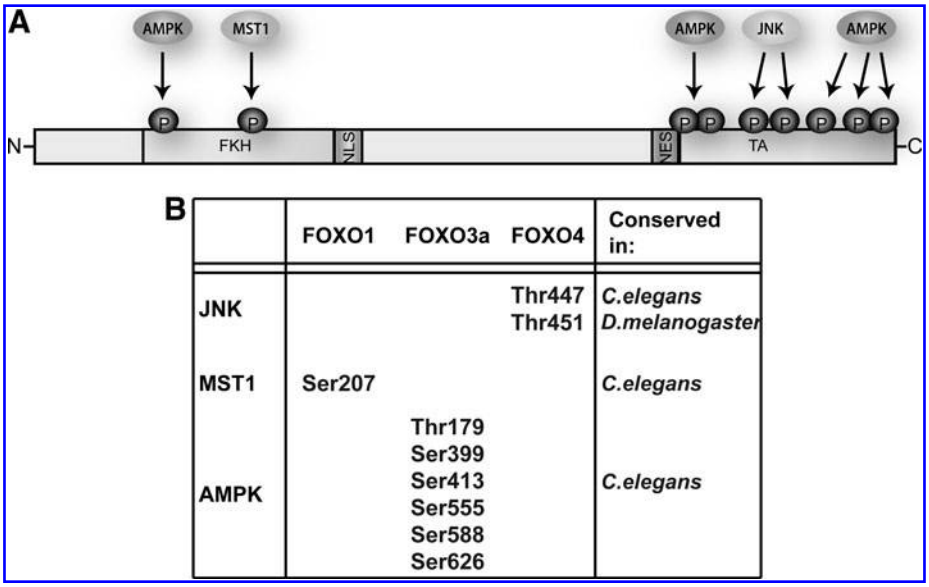
Further, cyclin-dependent kinase 2 (CDK2) is reported to directly phosphorylate FOXO1 on Ser-249 (Fig. 2) (38). Phosphorylation on this site results in cytoplasmic localization and consequent inhibition of FOXO1 transcriptional activity. Thus, DNA damage through inhibition of CDK2 would activate FOXO to either induce a cell cycle arrest and concomitant repair, or to induce cell death. In addition, the same group reported CDK1 to also phosphorylate Ser-249 in FOXO1 (57). This would suggest that FOXO activation after DNA damage occurs at both G1/S and G2/M and that FOXO activation does not link to a specific mode of DNA damage repair. Thus, the involvement of FOXO in DNA repair appears general rather then specific.

However, and in contrast to the above, Yuan *et al.* reported CDK1-mediated FOXO1 phosphorylation on Ser-249 to activate FOXO1 (116). Here it was shown that upon CDK1-mediated phosphorylation, FOXO1, at least partially, is released from 14-3-3 and that this mediates cell death in neurons (116). At least *in vivo* neurons are mostly postmitotic and this may explain a different mode of action for FOXO in cycling cells. Yet, in addition, the same authors describe a role for FOXO1 in proliferating cells, where upon phosphorylation by CDK1, active FOXO1 regulates expression of the mitotic regulator polo-like kinase (116). Thus, how these data, of Ser-249 phosphorylation activating or inactivating FOXO1, can be reconciled at the biochemical level is at present not clear.

Stress-dependent signaling

Next to the many ways identified for the negative regulation of FOXO, more recently, several pathways have been identified to be important in activation of FOXO. One of the kinases described to activate FOXO is JNK (Fig. 1). Upon cellular stress, JNK can directly phosphorylate FOXO (on different sites compared to PKB) (Fig. 3) and thereby overrules the negative phosphorylation by PKB, resulting in nuclear translocation and transcriptional activation of, in particular, FOXO4 (25). The consensus sites for JNK phosphorylation in FOXO4 are not conserved among the other FOXOs, indicating more specified functions for the different FOXO proteins. However, next to the direct phosphorylation of FOXO4 by JNK, JNK is also reported to phosphorylate 14-3-3 proteins and thereby release 14-3-3 from its interaction partners. Indeed, a similar role for JNK is reported for the binding of FOXO to 14-3-3, which is abrogated upon phosphorylation of 14-3-3 by JNK, resulting in translocation of FOXO to the nucleus (95). Since 14-3-3 binding regulates all FOXO family members, this might provide a more general function of JNK toward activation of FOXO. In addition, JNK-mediated activation of FOXO is conserved in both *C. elegans* (73) and *D. melanogaster* (105), where JNK-mediated activation of DAF-16 or dFOXO results in stress resistance and lifespan extension.

Another kinase reported to be important for activation of FOXO3a is mammalian STE20-like protein kinase 1 (MST1). Under conditions of oxidative stress MST1 phosphorylates FOXO3a on Ser-207 (Fig. 3). Upon phosphorylation, FOXO3a is released from 14-3-3 proteins and translocates to the nucleus to promote transcription of its target genes (55). However, structural analysis has shown that Ser-207 phosphorylation is incompatible with DNA binding (16) and this led to the suggestion that upon nuclear entry Ser-207 needs to be specifically dephosphorylated before FOXO becomes active. The MST1-mediated activation of FOXO is conserved in *C. elegans*, where the MST1 ortholog, CST1, is important in regulation of DAF-16-dependent lifespan extension (55). Recently, another group has shown that MST1 requires JNK-mediated phosphorylation on Ser-82 for its full activation and activity toward FOXO3a regulation. Mutation of Ser-82 to alanine abrogates the MST1 mediated phosphorylation of Ser-207 in FOXO3a (12). However, MST1 is also a well-known



	FOXO1	FOXO3a	FOXO4	Conserved in:
JNK			Thr447 Thr451	*C.elegans* *D.melanogaster*
MST1	Ser207			*C.elegans*
AMPK		Thr179 Ser399 Ser413 Ser555 Ser588 Ser626		*C.elegans*

FIG. 3. Phosphorylation dependent activation of FOXO. (A) Schematic representation of FOXO domains and phosphorylated residues. **(B)** Kinases involved in phosphorylation and subsequent activation of FOXO. Specific phosphorylation sites of the different FOXO members are indicated. Numbering of all phosphorylation sites is for human FOXO. AMPK, AMP-dependent protein kinase.

upstream regulator of stress kinases, including JNK and p38 (31). Thus, it could be equally well possible that a Ser-82 mutant of MST1 is impaired in JNK activation and therefore displays reduced ability to regulate FOXO. Clearly, further studies should shed light on the complicated interplay between MST1 and JNK signaling.

Nutrient signaling

In situations of low energy levels, the AMP-dependent protein kinase (AMPK) plays an important role in activation of energy-producing pathways and inactivation of energy-consuming pathways. AMPK exerts its function through numerous substrates, including FOXO3a (33). AMPK phosphorylates FOXO3a at six different sites (Fig. 3) and thereby activates its transcriptional program. Unlike JNK- and MST1-mediated regulation of FOXO, AMPK does not regulate FOXO3a subcellular localization, meaning that FOXO3a needs to be nuclear to become activated by AMPK. This can either be a result of lack of growth factors and thereby no PKB/GSK signaling, or activation of the JNK/MST1 signaling pathway to localize FOXO to the nucleus, where AMPK can phosphorylate and further accelerate FOXO mediated transcription. Again this pathway is conserved in *C. elegans*, where upon some forms of dietary restriction, AMPK is activated and mediates DAF-16-dependent stress resistance and lifespan extension (32).

Regulation of FOXO activity by protein phosphatases

Because of the tight control of FOXO proteins by phosphorylation, a role for phosphatases in FOXO regulation can be expected. Protein phosphatase 2A (PP2A) has been shown to directly dephosphorylate FOXOs although the various studies differ within the details. Yan *et al.* reported that after knockdown of PP2A, but not PP1, there is increased phosphorylation on the PKB sites Thr-24 and Ser-256 of FOXO1 (112). In contrast, Singh *et al.* showed similar results for FOXO3a, yet provided additional evidence that compared to FOXO3a, PP2A did not dephosphorylate FOXO1 and only slightly FOXO4 (90). A major confounding factor is obviously the ability of PP2A to also dephosphorylate a number of the upstream FOXO regulators, including PKB (4, 44). However, Singh *et al.* excluded to some extend such an indirect role of PP2A on FOXO *in vivo*. In addition, dephosphorylation by PP2A apparently affected FOXO function as in the presence of PP2A inhibitors, FOXO1 is no longer able to induce apoptosis (112).

The catalytic subunit of PP2A requires targeting subunits to exert its proper function (42). The PP2A targeting subunit B65epsilon is identified as a binding partner of FOXO3a, yet B65epsilon did not appear to mediate PP2A-directed dephosphorylation (90). The protease calpain has been shown to regulate FOXO3a in part through PP2A (11). The B56 alpha and gamma targeting subunits of PP2A are *in vitro* substrates of calpain, and calpain regulates B56 alpha stability *in vivo*, suggesting a direct role of calpain in the regulation of PP2A function. However, this was linked to regulation of PKB activity rather than dephosphorylation of FOXO3a directly. Indeed, in this respect, a genetic screen in *C. elegans* revealed pptr-1, a B56 regulatory subunit of the PP2A holoenzyme to regulate PKB activity in the worm (75). In agreement, mammalian B56beta regulates PKB phosphorylation at Thr 308 in 3T3-L1 adipocytes (75). Thus, PP2A regulates both PKB and FOXO and the concerted action on both is to increase FOXO activity. Likely, the relative contribution of PP2A on both determines within the experimental setup the conclusion as to what player mediates the effect of modulating PP2A activity. In addition, resolving what targeting subunit of PP2A is required for its effect on FOXO may help to further understand the details of dephosphorylating the different FOXO members.

Regulation of phosphorylation by methylation

The PKB phosphorylation sites of FOXOs adhere to the consensus sequence for PKB phosphorylation, that is, RXRXXS/T. Recently, it was shown that Arg-248 and -250 within the FOXO1 consensus encompassing the second PKB site [R(248)XXR(250)XS(253)] are subject to methylation by the methyltransferase protein arginine *N*-methyltransferase 1 (PRMT1) (111). Oxidative stress induces PRMT1 binding to FOXO1 and mediates methylation on Arg-248 and Arg250 in mouse FoxO1. Methylation on these sites abrogates PKB-mediated phosphorylation on Ser-253. Therefore, PRMT1-mediated methylation of FOXO1 prevents its nuclear exclusion, polyubiquitination, and proteosomal degradation (111). Thus, besides dephosphorylation, PRMT1-mediated FOXO methylation presents another mean to reduce phosphorylation on the PKB sites. This involvement of methylation again nicely illustrates the importance of crosstalk between different PTMs to fine-tune FOXO regulation. FOXO3a has been described to directly regulate B cell translocation gene 1, which is a positive regulator of PRMT1, suggesting a positive feedback loop where FOXO potentiates itself *via* induction of a B cell translocation gene 1/PRMT1 complex and subsequently abrogates PBK mediated phosphorylation (7).

Mechanisms of Phosphorylation Dependent FOXO Regulation

Phosphorylation-dependent control of FOXO ubiquitination and degradation

As illustrated above, crosstalk between PTMs is an important mode of FOXO regulation, and phosphorylation of FOXO thus indirectly regulates other PTMs of FOXO. Phosphorylated residues often serve as docking sites for additional regulatory proteins, including kinases, as illustrated above for CK-1. Phosphorylation of serine/threonine residues followed by a proline is also oftentimes a docking site for the peptidylisomerase peptidyl prolyl isomerase 1 (PIN1) and indeed also FOXOs bind PIN1 (15). The consequence of PIN1 binding to proteins is not uniform as its major action is to induce a conformational change in the target protein by inducing a *cis* to *trans* isomerization of the peptide bond between the phosphorylated residue and the proline at +1. This conformational change was initially shown to increase dephosphorylation of target proteins [*e.g.*, c-myc (114)] and as such PIN1 functions in a manner similar to the targeting subunits of phosphates like PP2A. However, in case of FOXO no apparent effect was seen on the phosphorylation status of both PKB and JNK sites. In contrast PIN1 binding appeared to affect deubiquitination of FOXO through increasing the activity of the deubiquitinating enzyme ubiquitin-specific peptidase 7 toward FOXO (15).

At present there is no clear evidence that acetylation of FOXOs is dependent on FOXO phosphorylation. Recently, the acetyltransferase p300/CBP-associated factor (PCAF) was reported to repress FOXO1 in a PKB-dependent manner, since phosphorylation of the PKB site Ser-253 of FOXO1 is required for the interaction between PCAF and FOXO1 (115). However, inhibition by PCAF occurred independent of its acetyltransferase activity (115).

In contrast to acetylation, ubiquitination of FOXOs is tightly coregulated with phosphorylation. Originally, it was noted that several important substrates of PKB display decreased protein half-life after PKB-mediated phosphorylation (78). This suggested that PKB phosphorylation in general regulates the recruitment of an E3 ligase to induce polyubiquitination and degradation of its target proteins. The F-box protein S-phase kinase-associated protein 2 (Skp2) has been identified as a putative E3 ubiquitin ligase responsible for polyubiquitination of several PKB substrates, including FOXOs (Fig. 4A) (39).

Skp2 is a component of SCF^{Skp2} (Skp1, Cul1, and F-box protein Skp2) ubiquitin E3 ligase complex that targets the CDK inhibitor $p27^{Kip1}$ and other substrates for ubiquitin-dependent proteolysis (60). Given the role of FOXO in $p27^{Kip1}$ regulation, this already suggested a mode of concerted regulation. Indeed, Skp2 has been shown to respond to PI3K/PKB signaling in multiple ways. Initially, it was shown that PI3K activation through loss of PTEN results in increased Skp2 expression (60); however, this lacked a mechanism. Subsequent studies showed that Skp2 binds to PKB and that Skp2 is phosphorylated by PKB at Ser-72. Phosphorylation of Ser-72 results in Skp2 translocation to the cytoplasm, but the consequence of this remains unclear. Gao *et al.* show that in addition to nuclear exit, Ser-72 impairs Cdh1 binding and consequently APC-Cdh1-mediated Skp2 destruction, thereby providing rationale for the observed increase of Skp2 in PTEN-negative cells (30). Lin *et al.* show that Ser-72 phosphorylation results in increased SCF^{skp2} complex formation. Clearly, Skp2 when stably in complex with Skp1 and Cul1 will be indirectly protected from interacting with Cdh1 and degradation (56). Thus, in contrast to PKB substrates such as $p27^{kip1}$, $p21^{cip1}$, Tsc2, and FOXO, the consensus is that Skp2 is protected from destruction by PKB-mediated phosphorylation and this results in enhanced Skp2-dependent E3 ligase activity.

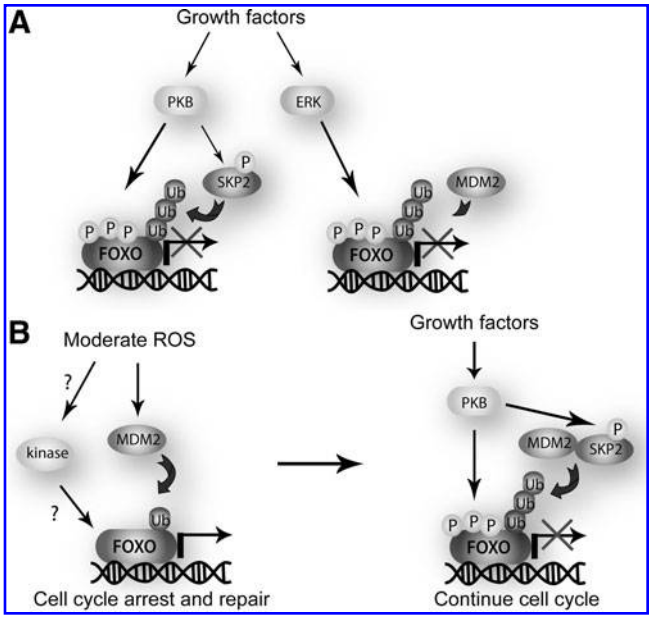


FIG. 4. FOXO and ubiquitination. (A) Upon growth factor signals, FOXOs are phosphorylated by either PKB or ERK, resulting in recruitment of the E3 ligases SKP2 and MDM2, which polyubiquitinate FOXO resulting in its degradation. PKB can also activate SKP2 directly by phosphorylation. **(B)** Model of a switch between MDM2-mediated monoubiquitination *versus* polyubiquitination, where moderate stress can induce monoubiquitination and thereby activation of FOXO, resulting in cell cycle arrest and potential damage repair. If the damage is repaired and cell cycle can progress, FOXO can again be inactivated by growth factor-induced polyubiquitination and degradation. MDM2, murine double minute 2; SKP2, S-phase kinase-associated protein 2.

In agreement with the above, platelet-derived growth factor treatment of chicken embryo fibroblasts (CEFs) transformed through overexpression of activated forms of PI3K and/or PKB induced a strong reduction in FOXO1 half-life (<1 h) (5). Importantly, this also suggests that inhibition of FOXO function through increased degradation is a gain of function for cells transformed through increased PI-3K signaling. Huang *et al.* found that, in contrast to FOXO1, FOXO3 and FOXO4 do not interact with Skp2 (39). However, growth factor signaling affects FOXO3 stability (78), indicating that a Skp2-dependent degradation of FOXO3, and possibly also FOXO4, cannot be ruled out.

Clearly, in growth factor signaling through PKB, Skp2 is an important regulator of FOXO stability, but in addition murine double minute 2 (MDM2) has been suggested as E3 ligase mediating polyubiquitination and degradation of FOXO in growth factor signaling through MAPkinase/extracellular signal-regulated kinase (ERK) (Fig. 4A). FOXOs can be phosphorylated *in vitro* by several proline-directed kinases, including MAPK/ERK (6). Subsequently, it was shown that MAPK/ERK-mediated phosphorylation of FOXO3a on Ser-294, Ser-344, and Ser-425 (Fig. 2) results in increased interaction of FOXO3a with the E3 ligase MDM2, thereby enhancing MDM2-dependent degradation of FOXO3a (113). In addition, Fu *et al.* describe PKB-dependent polyubiquitination of FOXO1 and FOXO3a mediated by MDM2 (27).

Phosphorylation-dependent control of FOXO monoubiquitination and activation

Whereas MDM2 may mediate polyubiquitination and degradation of FOXO3a during growth factor signaling, MDM2-mediated monoubiquitination has been suggested to mediate FOXO activation during increased cellular oxidative stress (Fig. 4B). After increased ROS, FOXO4 becomes monoubiquitinated, and this results in nuclear localization and increased transcriptional activity (103). Recently, Brenkman *et al.* found MDM2 to be the E3 ligase responsible for this stress-induced FOXO4 monoubiquitination (14).

Thus, upon growth factor signaling, FOXO factors become phosphorylated and subsequently polyubiquitinated resulting in degradation. However, upon oxidative stress, where activation of FOXO factors is favored, FOXO4 becomes monoubiquitinated by MDM2, which results in its activation. Whether MDM2 is able to monoubiquitinate the other FOXO transcription factors remains to be established, as well as the

possible involvement of a kinase, before the MDM2-mediated FOXO monoubiquitination. Nevertheless, in terms of regulation, MDM2 may critically determine the activity status of FOXO. At present the model would be that under conditions of moderate cellular ROS increase, FOXOs become active through MDM2-dependent monoubiquitination and that this facilitates repair. If repair has been completed, cell cycle progression may resume and growth factors may override the FOXO-imposed cell cycle block by inducing MDM2-dependent FOXO degradation (illustrated in Fig. 4B).

Integrating Opposing Signals

Considering that PKB and JNK likely represent the major negative and positive regulatory input on FOXO, respectively (Fig. 1), it is of importance to understand the general crosstalk between, and regulation of PKB and JNK activity. Several studies have described various means of crosstalk between PI3K/PKB and JNK. Here we will describe in brief several observations in this respect possibly relevant in understanding FOXO regulation.

Control of JNK signaling occurs through the so-called scaffold proteins (see Fig. 5). These scaffold proteins are responsible of orchestrating the proper assembly of the JNK kinase cascade, which typically consists of a JNKK and a JNKKK [reviewed in (22)]. Second, due to their specific cellular locations these scaffold proteins are likely responsible for ensuring that JNK signaling is tailored toward the proper substrates given the stimulus context in which JNK activation occurs.

MST1 belongs to the STE20 group of MAPKKKs and is the mammalian homolog of *Drosopohila* HIPPO. In *D. melanogaster* as well as in mammals MST1/hippo is shown to restrict cell growth and survival and genetics in *Drosophila* has outlined a pathway [reviewed in (36)]. Whereas in *Drosophila* Mst1 signals through transcriptional control by Yorkie of cyclinE and DIAPs (Drosophola inhibitor of apoptosis), studies in mammalian cell lines indicate other mediators, including JNK and p38 pathways through MKK4/7 and MKK3/6, respectively (31), but also H2B and FOXO. PKB can phosphorylate MST1 on Thr-120 and thereby inhibits MST1 function toward JNK as well as its role in apoptosis (Fig. 6a) (41, 117). In addition, PBK has been reported to phosphorylate MST1 on Thr-387 and thereby inhibits MST1-mediated FOXO3 nuclear translocation and activation (41). Besides MST1, PKB also phosphorylates other JNKKKs. For example, apoptosis signal-regulating kinase 1 (ASK1) is a redox-sensitive JNKKK that mediates in part ROS-dependent JNK activation. PKB phosphorylates ASK1 at Ser-83 and thereby inhibits ASK1 (Fig. 6b) (46). In addition to the inhibition of JNKKKs, PKB is also described to inhibit the JNKK, MKK4 by direct phosphorylation on Ser-78 (Fig. 6c) (77).

Further, PKB has been described to regulate the function of JNK scaffolds. PKB interacts with JNK interacting protein 1 (JIP1), but here the precise functional consequence of this interaction remains unclear. PKB binding to JIP correlates with inhibition of JNK activation (Fig. 6d) (48). In addition, binding of PKB to JIP1 is also acclaimed to affect PKB activity itself, but both inhibition and activation of PKB have been reported (47, 92). In this respect at least activation of PKB through binding to JIP1 is most extensively documented. JIP1 binds to the pleckstrin homology domain of PKB and as such may substitute for PI3P lipids, which otherwise bind the pleckstrin homology domain of PKB and this is essential for PKB activation. Further, it has been shown that 3-phosphoinositide-dependent protein kinase 1 (PDK1), the kinase responsible for phosphorylation of PKB within its activation loop, is also recruited to JIP1. However, PDK1 does not bind JIP1 directly, but through RalGDS an exchange factor for the small GTPase Ral (35). The existence of a quaternary complex consisting of PKB/RalGDS/JIP1/PDK1 has not yet been demonstrated directly, but if present would indeed suggest that this will result in PKB activation. An interesting consequence of such a mechanism of PKB activation would be that this represents, first, a mechanism of PKB activation that can occur independent of PI3P lipids and, second, therefore a possibility to activate PKB in cellular domains devoid of membranes. PI3K-

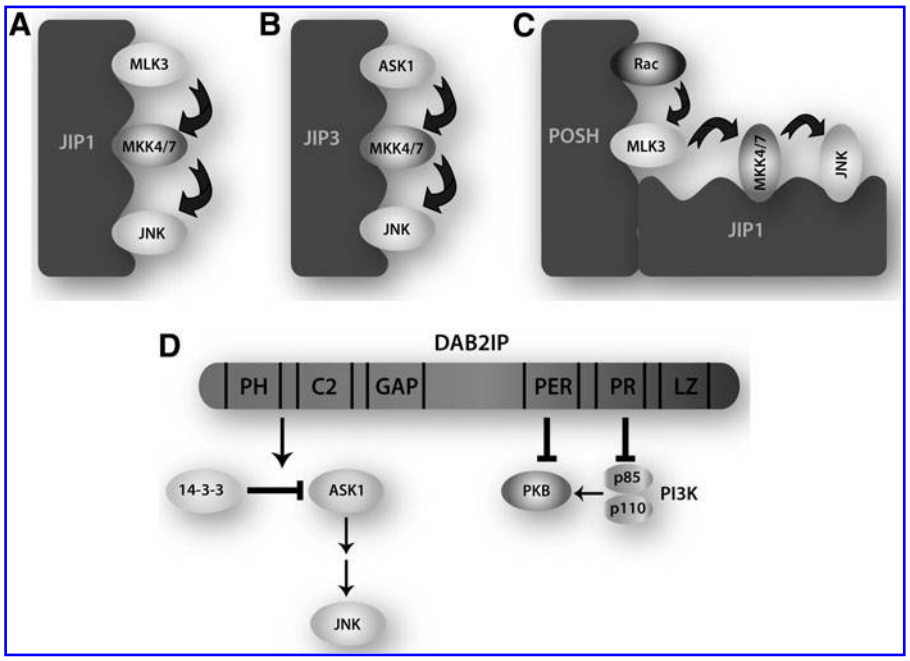


FIG. 5. Overview of stress-regulated JNK scaffold proteins. (A, B) JNK interacting proteins 1 and 3 (JIP1/JIP3) both regulate JNK activation by assembling MAPKKKs and MAPKKs important for JNK activation. **(C)** POSH together with JIP1 provides another scaffold platform for stress-dependent JNK signaling. **(D)** DAB2IP regulates JNK signaling by stress-dependent activation of ASK1. ASK1, apoptosis signal-regulating kinase 1; C2, protein kinase C-conserved domain; DAB2IP, DOC2/DAB2 interactive protein; GAP, GTPase activating protein; JIP1, JNK interacting protein 1; LZ, leucine zipper; PER, period-like domain; PH, pleckstrin homology; POSH, plenty of SH3 domains; PR, proline-rich region.

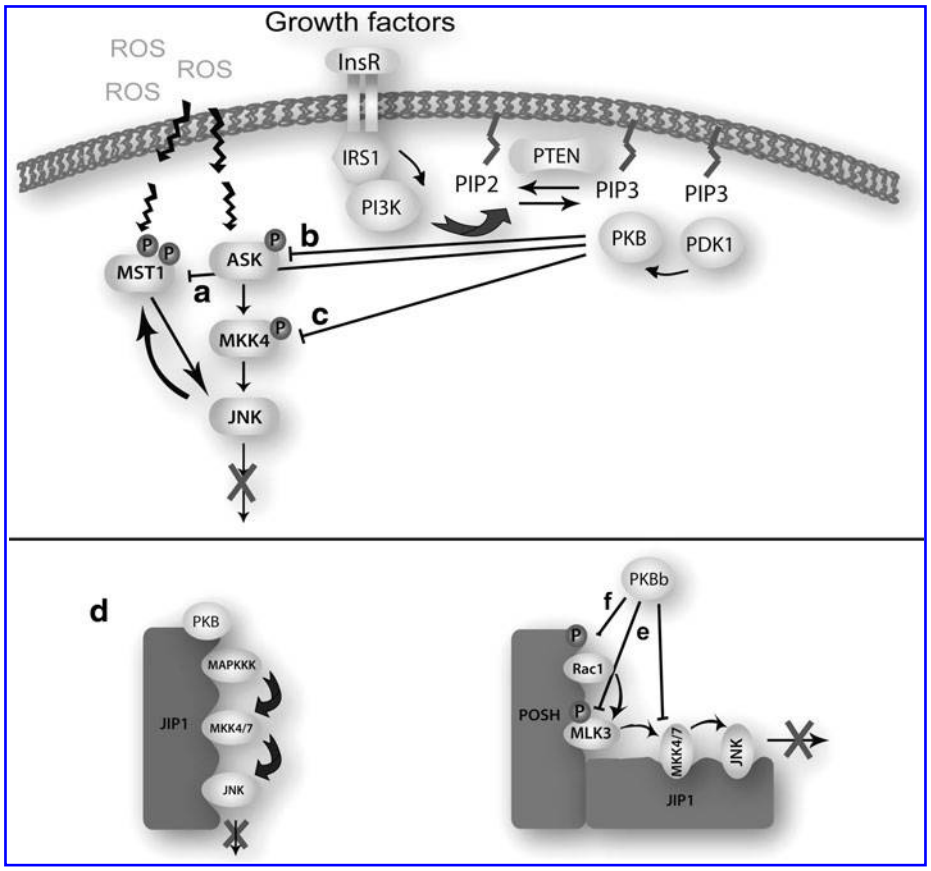


FIG. 6. Inactivation of JNK signaling by PKB. Schematic overview of PKB-mediated inhibition of JNK signaling pathway. PKB phosphorylates several MAPKKK, like MST1 **(a)**, ASK1 **(b)**, and MLK3 **(e)** and the MAPKK, MKK4 **(c)**, subsequently inhibiting their function. Additionally, PKB can regulate several JNK scaffold proteins, like JIP1 **(d)** and POSH **(f)**, thereby preventing proper complex assembly and subsequent JNK activation. MLK3, mixed lineage kinase 3; MST1, mammalian STE20-like protein kinase 1.

independent activation of PKB has been reported but with little detail as to the mechanism involved.

As summarized in Figure 5, JNK activation can occur through multiple different scaffold platforms and JIP1 is not the only JNK pathway scaffold regulated by PKB. Plenty of SH3 domains (POSH) is another scaffold for JNK signaling to which PKB can bind (26, 58). POSH appears to bind specifically to PKBb and not PKBa, whereas such isozyme preference has not been documented for JIP1. The precise action of PKB in this complex is unclear. Figueroa *et al.* provide evidence that PKB phosphorylates mixed lineage kinase 3 (MLK3), a JNKKK, and as such regulates MLK3 binding to POSH and hence JNK activation (Fig. 6e) (26). Lyons *et al.* provide evidence that PKB phosphorylates Ser-304 of POSH, which lies within the binding domain of POSH for the small GTPase RAC. Consequently, this phosphorylation impairs binding of RAC and subsequent JNK activation (Fig. 6f) (58). To complicate matters further, interaction between POSH and JIP1 (52) as well as JIP1 and JIP3 (91) has been described; thus, under conditions of heterodimerization, PKB can also affect JNK scaffolds indirectly. Overexpression of POSH, similar to JNK activation, results in extended lifespan of *D. melanogaster* (2).

As described above and summarized in Figure 6, PBK has many ways to inhibit stress-induced activation signals toward FOXOs; however, in situations where survival and proliferation is not favorable, the PKB signaling pathway needs to be turned off. Indeed, there are several examples in which oxidative stress signaling can inhibit the insulin pathway.

One example is the direct effect of ROS on PKB kinase activity. Inside its kinase domain, PKB has two cysteins that can form a disulphide bond upon oxidation, which results in increased binding of PP2A to PKB and subsequent dephosphorylation of PKB on Thr308 and Ser-493 resulting in inhibition of PKB activity (Fig. 7a) (40, 66). In contrast, PTEN, a lipid PI3-phosphatase that metabolizes PtIns(3,4,5)P3 and inhibits PI3-kinase signaling, is highly sensitive to inactivation *in vitro* by hydrogen peroxide (53). Thus, on one hand, ROS can activate PKB by inhibition of PTEN; on the other hand, it inhibits PKB activity by recruiting PP2A. Possibly, there is a difference in short-term and long-term ROS, where short-term ROS can still activate PKB, as this is still favorable for proliferation, whereas long-term ROS will result in inactivation of PKB.

Inhibition of PKB also occurs through activation of JNK, as JNK is able to directly phosphorylate insulin receptor substrate (IRS) (Fig. 7b) (1, 54), thereby inhibiting the insulin pathway. In obese conditions, there is excessive activation of JNK, resulting in phosphorylation and inactivation of IRS and subsequent attenuated insulin signaling and thereby the development of insulin resistance.

Besides inhibition of the insulin pathway, JNK mediates a positive feedback loop by phosphorylation of the JNKKK, MLK3, thereby enhancing the MLK3/JNK signaling (Fig. 7c) (87). In addition, as already described above, JNK can phosphorylate and activate MST1 (Fig. 7d) (12). Further, JNK has been implicated in regulation of stability of several of its upstream signaling members, like POSH, JIP1, and MLK3, providing another way of amplifying its own signaling (109).

As already mentioned, PKB is able to interfere with the JNK signaling pathway at the level of scaffold proteins. Another,

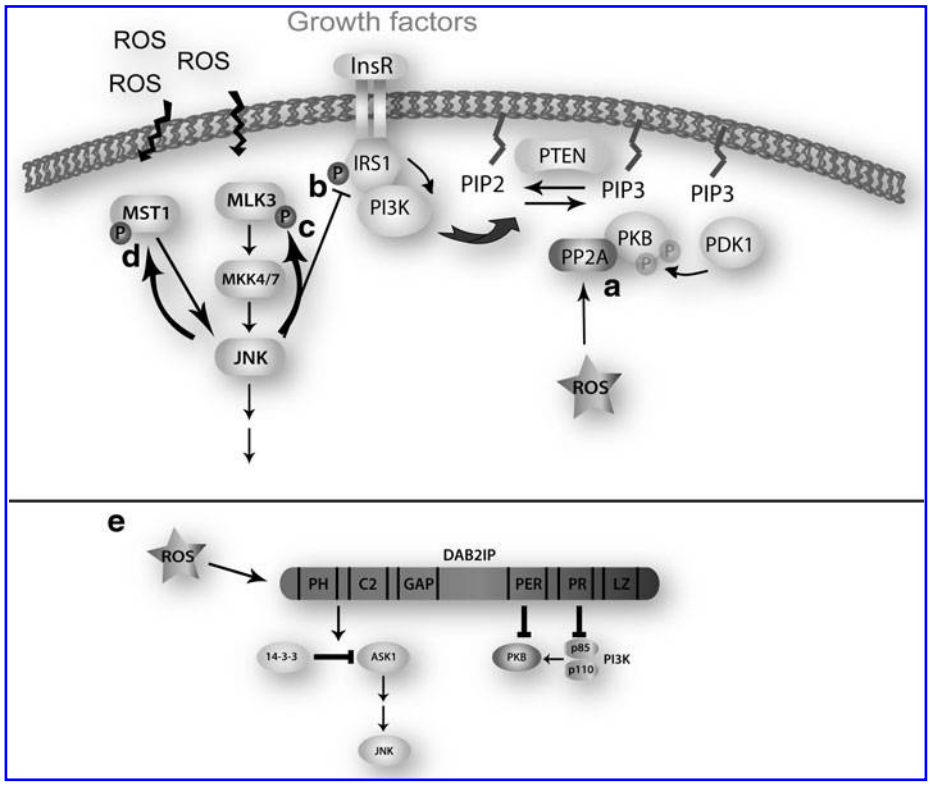


FIG. 7. Inactivation of PKB signaling by stress signals. Schematic overview of ROS-mediated PKB inactivation. Inactivation of PKB can occur directly by ROS, thereby attracting PP2A, resulting in dephosphorylation and inactivation of PKB **(a)** or indirectly *via* activation of JNK, which subsequently can phosphorylate and inactivate IRS1 **(b)**. Activation of JNK results in several positive feedback loops, by direct phosphorylation and thereby activation of MLK3 **(c)** and MST1 **(d)**. Additionally, stress signals result in assembly of a scaffolding complex consisting of DAB2IP/ASK1 interacting proteins **(e)**, which can directly activate ASK1, by releasing ASK1 from 14-3-3, thereby enhancing JNK signaling. PP2A, protein phosphatase 2A; ROS, reactive oxygen species.

somewhat unique scaffold protein, called DOC2/DAB2 interactive protein (DAB2IP) or ASK1 interacting protein, has also been implicated in regulation of JNK, but in addition coordinates inactivation of PKB (Fig. 7e). DAB2IP is originally identified as a member of the RasGAP family; in addition to its GAP activity, it has been identified to be involved in apoptosis by mediating dissociation of ASK1 from its inhibitor 14-3-3, thereby activating JNK signaling (118). Recently, Xie *et al.* show the ability of DAB2IP to regulate both the inhibition of PI3K/PKB and activation of ASK1 in response to stress signals (Fig. 7e) (108), so thereby providing another way of inhibiting PKB signaling, while enhancing JNK signaling.

Taken together, it is clear that PKB can interfere with many of the signal transduction pathways leading to JNK activation, and the other way around, JNK can interfere with many players in the insulin pathway. Further detailed knowledge is needed to understand when, where, and how PKB interferes with JNK and JNK interferes with PKB. In addition, detailed knowledge about the JNK signaling cascade(s) that impinge on FOXO is also required to understand how PKB and JNK interfere in FOXO regulation.

Future Directions

Even more kinases?

Several high-throughput screens, including siRNA screens, have been performed to discover novel regulators of FOXO function. Of relevance to this review is a recent siRNA screen in *Drosophila* Schneider S2 cells dedicated to find kinases/phosphatases regulating dFOXO (63). This screen yielded numerous hits that provide novel insight into the issues described above. For example knockdown of inositol-requiring protein 1 (IRE-1) represses dFOXO transcriptional activity. IRE-1 is a stress-activated endonuclease resident in the ER that is conserved in all known eukaryotes. IRE-1 is an essential mediator of all aspects of the unfolded protein response (UPR). UPR gene expression in mammals relies largely on pancreatic ERK and ATF6. Further, mammalian IRE-1 proteins activate JNK by recruiting the TRAF2 protein to the ER membrane independently of their endonucleolytic activity (102). Thus, IRE-1 may couple UPR to FOXO activation by regulating JNK.

Next to IRE-1, this screen identified the TAO1 kinase as a potential FOXO regulator. Initially, TAO1 kinase was suggested to be a checkpoint kinase. However, this turned out erroneously, as the employed siRNA against TAO1 displayed Mad2 downregulation as an off-target effect, thereby explaining its effect on the M-phase checkpoint. More plausible within the context of FOXO regulation is the observation that TAO1 can impinge on p38 after DNA damage (80).

Also interesting hits of this screen are a number of regulators of inositol lipid metabolism, for example, inositol(myo)-1(or 4)-monophosphatase 1 (IMPA)NP, Phosphatidylinositol 4-kinase (PI4K), phosphatidylinositol-4-phosphate 5-kinase type I alpha, and DAG kinase delta II. As PKB activity is dependent on PI3P lipids, these kinases/phosphatases may all indirectly affect PKB activity. Although not a surprising conclusion, it does reveal apparent critical steps in inositol metabolism toward PKB and thus potential targets for small molecules to inhibit PKB and to reactivate FOXO, for example, in tumors.

Clearly, our understanding of FOXO regulation in general and regulation through phosphorylation is far from complete. As both kinases and phosphatases are considered being druggable, further research may yield compounds that can (re)activate FOXO and provide benefits that may come at least somewhat close to the beneficiary effect of activating DAF-16 in *C. elegans*.

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Abbreviations Used

AMPK = AMP-dependent protein kinase
ASK1 = apoptosis signal-regulating kinase 1
ATM = ataxia telangiectasia mutated
C2 = protein kinase C-conserved domain
CDK1/2 = cyclin dependent kinase 1/2
CK-1 = casein kinase 1
DAB2IP = DOC2/DAB2 interactive protein
DAF-16 = abnormal dauer formation 16
ERK = extracellular signal-regulated kinase
FKH = Forkhead domain
FOXO = Forkhead box O
GADD = growth arrest and DNA damage
GAP = GTPase activating protein
IKK = IκB kinase
IRE-1 = inositol-requiring protein 1
IRS = insulin receptor substrate
JIP1 = JNK interacting protein 1
JNK = c-Jun N-terminal kinase
LZ = leucine zipper
MDM2 = murine double minute 2
MLK3 = mixed lineage kinase 3
MST1 = Mammalian STE20-like protein kinase 1
MTP = microsomal triglyceride transfer protein
NES = nuclear export signal
NLS = nuclear localization signal
PCAF = p300/CBP-associated factor
PDK1 = 3-phosphoinositide-dependent protein kinase 1
PER = period-like domain
PH = pleckstrin homology
PI3K = phosphoinositide 3-kinase
PIN1 = peptidyl prolyl isomerase 1
PKB = protein kinase B
POSH = plenty of SH3 domains
PP2A = protein phosphatase 2A
PR = proline-rich region
PRMT1 = protein arginine *N*-methyltransferase 1
PTEN = phosphatase and tensin homolog
PTM = posttranslational modification
ROS = reactive oxygen species
SGK = serum- and glucocorticoid-inducible kinase
Skp2 = S-phase kinase-associated protein 2
TA = transactivation domain
UPR = unfolded protein response

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