Quick guide

FOXO transcription factors

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What are they? FOXO proteins are a subgroup of the Forkhead family of transcription factors. This family is characterized by a conserved DNA-binding domain (the 'Forkhead box', or FOX) and comprises more than 100 members in humans, classified from FOXA to FOXR on the basis of sequence similarity. These proteins participate in very diverse functions: for example, FOXE3 is necessary for proper eye development, while FOXP2 plays a role in language acquisition. Members of class 'O' share the characteristic of being regulated by the insulin/PI3K/Akt signaling pathway.

How did this family get named 'Forkhead'? Forkhead, the founding member of the entire family (now classified as FOXA), was originally identified in *Drosophila* as a gene whose mutation resulted in ectopic head structures that looked like a fork. Forkhead proteins are also sometimes referred to as 'winged helix' proteins because X-ray crystallography revealed that the DNA-binding domain features a 3D structure with three α-helices flanked by two characteristic loops that resemble butterfly wings.

How many FOXOs are there? In invertebrates, there is only one FOXO gene, termed *daf-16* in the worm and *dFOXO* in the fly. In mammals, there are four FOXO genes, *FOXO1*, *3*, *4*, and *6*.

Hey, what about FOXO2 and FOXO5? *FOXO2* is identical to *FOXO3* (a.k.a. *FOXO3a*, as opposed to *FOXO3b*, a pseudogene). *FOXO5* is the fish ortholog of *FOXO3*.

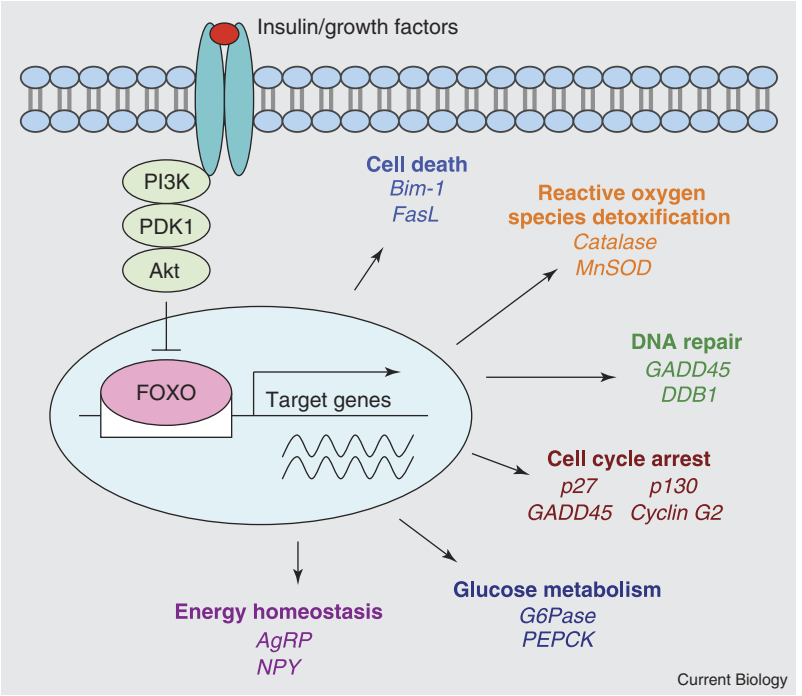

Figure 1. In the absence of insulin or growth factors, FOXO transcription factors are located in the nucleus, where they specify target gene expression (see text for details).

FOX hunting... *FOXO* genes were first identified in humans because three family members (1, 3, and 4) were found at chromosomal translocations in rhabdomyosarcomas and acute myeloid leukemias. Just after FOXO factors were identified in human tumor cells, the crucial role of DAF-16 in organismal longevity was discovered in worms. DAF-16 activity was shown to be negatively regulated by the insulin/PI3K/Akt signaling pathway. Subsequent experiments in mammalian cells showed that mammalian FOXO proteins were directly phosphorylated and inhibited by Akt in response to insulin/growth factor stimulation. Thus, FOXO factors are evolutionarily conserved mediators of insulin and growth factor signaling.

Why are they important? FOXO transcription factors are at the interface of crucial cellular processes, orchestrating programs of gene expression that regulate apoptosis, cell-cycle progression, and oxidative-stress resistance (Figure 1). For example, FOXO factors can initiate apoptosis by activating transcription of FasL, the ligand for the Fas-dependent cell-death pathway, and by activating the pro-apoptotic Bcl-2 family member Bim. Alternatively, FOXO factors can promote cell-cycle arrest; for example, FOXO factors upregulate the cell-cycle inhibitor p27kip1 to induce G_1 arrest or GADD45 to induce G_2 arrest. FOXO factors are also involved in stress resistance via upregulation of catalase and MnSOD, two enzymes involved in the detoxification of reactive oxygen species. Additionally, FOXO factors facilitate the repair of damaged DNA by upregulating genes, such as GADD45 and DDB1. Other FOXO target genes have been shown to play a role in glucose metabolism, cellular differentiation, muscle atrophy, and even energy homeostasis.

How are they regulated? FOXO proteins are tightly regulated to ensure that transcription of specific target genes is responsive to environmental conditions. A major form of regulation is Akt-mediated phosphorylation of FOXO in response to insulin or growth factors (Figure 1).

Phosphorylation at three conserved residues results in the export of FOXO factors from the nucleus to the cytoplasm, thereby inhibiting FOXO-dependent transcription. FOXO proteins are also phosphorylated by other protein kinases, including JNK or Mst1, which phosphorylate FOXO under conditions of oxidative stress. This phosphorylation causes the translocation of FOXO from the cytoplasm to the nucleus, thus opposing Akt's action. In addition to being post-translationally modified by phosphorylation, FOXO proteins also bind to co-activator or co-repressor complexes and become acetylated or deacetylated. For example, the deacetylase SIRT1 increases FOXO DNA-binding ability by deacetylating FOXO in response to oxidative stress. FOXO proteins are also monoubiquitinated under conditions of oxidative stress and this increases transcriptional activity. Finally, FOXO proteins can also be polyubiquitinated and targeted for protein degradation. The unique phosphorylation, acetylation, and ubiquitination status of FOXO under specific environmental conditions may provide specificity in the regulation of subsets of FOXO target genes.

What is the role of FOXO in longevity? FOXO factors have been shown to prolong lifespan in invertebrates. The worm orthologue, DAF-16, activates a program of genes that extend longevity by promoting resistance to oxidative stress, pathogens, and damage to protein structure. Mutations in the insulin receptor or PI3K extend longevity up to threefold, and this extension is reverted when *daf-16* is mutated. In flies, overexpression of *dFOXO* is sufficient to increase longevity. The role of FOXO factors in mammalian longevity is currently being explored. Mice that are deficient for either the insulin receptor or the insulin-like growth factor receptor-1 can live up to 30% longer than wild-type mice, suggesting that FOXO factors could be involved in mammalian longevity. Furthermore, FOXO target genes involved in stress resistance are conserved between invertebrates and mammals, suggesting that the function of FOXO in organismal stress resistance and longevity is evolutionarily conserved.

Isn't it strange that FOXO could induce both stress resistance* and *cell death? The regulation of stress-resistance genes and pro-apoptotic genes by FOXO is not necessarily a paradox. FOXO factors may orchestrate different patterns of gene expression based on the intensity of the stimulus, perhaps activating stress-resistance genes under mild conditions but pro-apoptotic genes when the intensity of stress stimuli increases beyond a certain threshold. It is also possible that FOXO factors regulate different genes in different cell types, causing apoptosis in some cells (e.g. neurons, lymphocytes) while promoting survival in others. Importantly, the induction of apoptosis by FOXO may cause the death of damaged or abnormal cells, therefore benefiting the longevity of the entire organism.

Is there a connection between FOXO and cancer? Because FOXO proteins were originally identified in human tumors, and because they play an important role in cell-cycle arrest, DNA repair, and apoptosis — cell functions that go awry in cancer — the FOXO family is thought to coordinate the balance between longevity and tumor suppression. Consistent with this idea, in certain breast cancers, FOXO3 is sequestered in the cytoplasm and inactivated. Expression of active forms of FOXO in tumor cells prevents tumor growth *in vivo.* Additionally, protein partners of FOXO, such as p53 and SMAD transcription factors, are tumor suppressors. Investigating the ensemble of FOXO protein partners will provide insight into the connection between aging and cancer.

Can you live without FOXO? It depends if you are a worm, a fly, or a mammal. Worms lacking *daf-16* or flies lacking *dFOXO* are viable but do not show an increase in lifespan following mutations in the insulin/PI3K/Akt pathway. *FoxO1*-null mice die at embryonic day 10.5 from defects in angiogenesis. *FoxO3*- and *FoxO4*-null mice have also been produced and are viable: *FoxO3*-null mice exhibit an age-dependent infertility in females, while *FoxO4*-null mice have no apparent phenotype. *FoxO6*-null mice are currently being generated. The four mammalian isoforms may have both distinct and overlapping functions, and compensation of one member by another may mask the function of individual FOXOs. Investigating the role of FOXO factors in longevity and tumor suppression will require more complex mouse models in which multiple *FoxO* genes are deleted.

What remains to be explored? More FOXO target genes remain to be discovered, as do regulators of FOXO function. An exciting area of future exploration will be to determine how FOXO factors mediate cell non-autonomous processes in the entire organism. The recent discovery that FOXO can upregulate neuropeptides in the hypothalamus suggests that FOXO can regulate animal behavior, and future studies will elucidate how hormones and neuronal signaling cause FOXO-dependent transcription of target genes that affect the entire organism.

Where can I find out more?

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