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Keeping neurons young and foxy: FoxOs promote neuronal plasticity

Colleen N. McLaughlin and Heather T. Broihier*

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Abstract

Any adult who has tried to take up the piano or learn a new language is faced with the sobering realization that acquiring such skills is more challenging as an adult than as a child. Neuronal plasticity, or the malleability of brain circuits, declines with age. Young neurons tend to be more adaptable and can alter the size and strength of their connections more readily than old neurons. A myriad of circuit- and synapse-level mechanisms that shape plasticity have been identified. Yet molecular mechanisms setting the overall competence of young neurons for distinct forms of plasticity remain largely obscure. Recent studies indicate evolutionarily-conserved roles for FoxO proteins in establishing the capacity for cell-fate, morphological, and synaptic plasticity in neurons.

Keywords

FoxO; Daf-16; neuronal plasticity; synaptic plasticity; microtubule dynamics; neurodegeneration

Welcome to the family (of FoxO proteins)

First identified in *C. elegans* for its role in lifespan regulation and dauer formation [1,2], Daf-16/FoxO is the key transcription factor downstream of Insulin/IGF signaling (IIS) [3,4]. FoxOs are now known to be regulated by other extracellular cues in addition to IIS [5–7]. In general, these pathways control FoxO by regulating its subcellular localization via post-translational modifications, including phosphorylation [8,9]. *C. elegans* and *Drosophila* each have one FoxO homolog, Daf-16 and dFoxO, respectively, while mammals have four: FoxO1, FoxO3, FoxO4, and FoxO6 [10–12].

Over the past twenty years, FoxO proteins have been the focus of studies aimed at revealing the source of their pro-longevity functions. Strikingly, FoxO3 variants are linked to exceptional longevity in seven cohorts of centenarians worldwide [13]. The relationship between FoxOs and youthfulness is likely complex and linked to their central position in

* author for correspondence: heather.broihier@case.edu.

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pathways regulating oxidative stress resistance, proteostasis, cell cycle control and apoptosis [14,15]. Their transcriptional targets include detoxifying enzymes that limit reactive oxygen species (ROS) accumulation as well as pro-autophagy proteins [16–19]. Mechanisms regulating these processes deteriorate in aging cells, suggesting that FoxO's roles in such pathways underlie its functions in healthy aging.

FoxO proteins are expressed in developing and adult neurons [11,20–22], and recent studies indicate that they regulate diverse aspects of neuron development and physiology (Fig. 1; Table 1). How do the functions of FoxO in neurons relate to FoxO functions elsewhere in the body? In some contexts, FoxO's roles in the nervous system are quite analogous to its canonical functions. For example, FoxO proteins regulate neural stem cell homeostasis, similar to their functions in other stem cell populations. They also serve neuroprotective roles in stressed or aging neurons, which are tied, at least in part, to regulation of processes including oxidative stress resistance and autophagy seen in other cell types. Thus, in these settings FoxOs likely promote neuronal health or viability via pathways that are not entirely neuron-specific.

FoxOs also govern neuronal morphogenesis and synapse plasticity, regulating downstream targets central to neuron development and physiology. On the face of it, such neuronal pathways do not have much in common with more generic “healthy-aging” pathways. However, like well-established FoxO-mediated pathways, neuronal pathways largely function to promote youthful cellular attributes—but in this case, FoxOs promote youthful characteristics that are quintessentially neuronal. Specifically, FoxOs act in neurons to permit their flexibility, or plasticity, at the morphological, synaptic, and behavioral levels.

Here we review neuronal functions of FoxO proteins in *Drosophila*, *C. elegans*, and mice—focusing on their roles in cell-type, morphological, and synaptic plasticity. We begin by briefly discussing areas in which neuronal functions of FoxO proteins are somewhat analogous to their functions outside the nervous system. We then turn to FoxO functions in neuronal morphology as well as synapse plasticity and behavior, areas in which FoxO acts in neuron-specific pathways to establish competence for growth and plasticity.

FoxOs promote cell-fate plasticity by maintaining adult neural stem cell quiescence

Neural stem cells (NSCs) reside largely within two niches in the adult mammalian CNS: the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampus. Adult neurogenesis enables neuronal regeneration in old age or following insult [23]. To maintain stem cell reserves through adulthood, NSCs must balance quiescence with activation. FoxOs are expressed in NSC niches, raising the possibility that they regulate stem cell behavior [24,25]. Indeed, brain-specific knockout of FoxO3 or combinatorial loss of FoxO1/3/4 results in age-dependent depletion of NSCs [24,25]; loss of FoxOs results in precocious NSC proliferation immediately after birth. Following this burst of neurogenesis in FoxO mutants, the NSC pool is depleted, and adult NSC reserves are not maintained. Loss of FoxO is also associated with a rise in ROS levels in NSCs suggesting defects in FoxO-dependent transcription of detoxifying enzymes such as Superoxide Dismutase and Catalase

[17,18]. These findings are in agreement with established roles of FoxO proteins in hematopoietic stem cells, where loss of FoxOs also result in elevated ROS and associated defects in quiescence and self-renewal [26,27].

A subsequent study from the Brunet lab provides key insight into how FoxO3 activity and neuronal specification programs are intertwined [28]. Intriguingly, FoxO binding motifs are frequently found alongside bHLH transcription factor binding motifs, and genome-wide analyses indicate that direct targets of the proneural bHLH protein ASCL1/MASH1 overlap extensively with those of FoxO3. ASCL1 directs proliferation and activation of NSCs [29]. Remarkably, FoxO3 inhibits ASCL1 transcriptional activity thus limiting neuronal specification (Fig. 2A) [28]. This study provides an elegant explanation for how FoxO3 acts to preserve NSC quiescence—by inhibiting ASCL1, a critical activator of neuronal specification.

This study also has implications for the role of FoxO proteins in cellular reprogramming. Specifically, the antagonism between FoxO and ASCL1 suggests that manipulating FoxO activity might alter the efficacy of converting fibroblasts into induced neuronal (iN) cells. Indeed, FoxO3 overexpression prevents iN cell conversion of embryonic fibroblasts, while loss of FoxO3 improves neuronal conversion of fibroblasts from adult mice [30], suggesting that loss of FoxO3 permits full activation of ASCL1 in fibroblasts and activation of downstream neuronal specification programs. Together, these studies argue that FoxOs inhibit neuronal determination in adults and enable cell-fate plasticity into adulthood.

Neuroprotective functions for FoxOs

FoxOs are context-specific regulators of neuronal autophagy

Transcriptional targets of FoxO promote autophagy in many cell types. When Insulin declines during fasting, FoxO translocates to muscle nuclei and promotes the transcription of pro-autophagy genes including Atg12, Bnip3, and LC3b [31]. This protective function is linked to cellular health and organismal longevity. An important and as yet unresolved question is how central FoxO is to autophagy regulation in neurons.

Neuronal FoxOs do stimulate autophagy at least in some circumstances. Cerebellar granule neurons lacking all three Jnk genes have increased levels of autophagy when cultured *in vitro* [32]. FoxO acts via Bnip3 to support heightened autophagy in this background (Table 1). Yet FoxO did not appear to regulate either Bnip3 or autophagy in otherwise wild-type neurons, suggesting that FoxO promotes neuronal autophagy only in some contexts. Notably, Daf-16/FoxO regulates ATG-18, a member of the WIPI family of autophagy proteins, in *C. elegans* chemosensory neurons [33]. However, this requirement for FoxO is not cell-autonomous—Daf-16 functions in the intestine to regulate neuronal ATG-18.

Defining when and where FoxO proteins regulate neuronal autophagy is of central relevance to neurodegenerative disease. These key questions have been addressed in a Parkinson's Disease model. In dopaminergic neurons overexpressing human α -Synuclein, moderate FoxO3 overexpression is protective and drives α -Synuclein into insoluble aggregates [34],

suggesting that FoxO3 stimulates autophagy. Supporting this hypothesis, moderate FoxO3 overexpression increases autophagic flux in human neuroblastoma cells [34].

Several studies have revealed a role for FoxO1 in regulating autophagy induced by ER stress. Loss of XBP1, a transcription factor in the unfolded protein response, results in enhanced autophagy and neuroprotection in models of Huntington's Disease (HD) [35]. FoxO levels are increased in an XBP1 mutant background and ectopic expression of FoxO1 enhances autophagy in transformed Neuro2a cell lines [35]. Additional support for a role for FoxO1 in ER stress-induced autophagy comes from auditory cells [36]. Levels of both autophagy and FoxO1 are increased when ER stress is induced via tunicamycin treatment. Moreover, knockdown of FoxO1 suppresses the increase in autophagy indicating that it is necessary for the response [36].

Elevated FoxO does not stimulate autophagy in all circumstances. Loss of Endophilin-A increases expression of neuronal FoxO3A and its transcriptional target, the E3-ubiquitin ligase FBXO32/Atrogin-1 [37]. In this background, autophagy is markedly reduced, indicating that increased levels of neuronal FoxO3A are insufficient to drive autophagy when Endophilin is lacking. To establish when and where FoxOs regulate neuronal autophagy, it will be key to take both loss- and gain-of-function approaches in multiple *in vivo* contexts. Lastly, cytosolic FoxO1 promotes autophagy and cell death in cancer cells [38,39]. It will be important to determine if neuronal FoxO proteins also act in the cytoplasm to drive autophagy, or whether their pro-autophagy functions in neurons are exclusively at the level of transcription.

FoxOs limit oxidative stress in neurons

FoxO's neuroprotective functions are not exclusively mediated by autophagy. A protective role for dFoxO in the context of Parkinson's Disease has been investigated in *Drosophila* [40]. Loss of the serine/threonine kinase PINK1 causes dopaminergic (DA) neurodegeneration in the *Drosophila* brain [41]. In PINK1 LOF mutants, overexpression of the Sir2 deacetylase in DA neurons rescues degeneration. dFoxO is downstream of Sir2 and required for Sir2-mediated neuroprotection in DA neurons. Overexpression of the detoxifying enzyme Superoxide Dismutase 2 (SOD2), a canonical FoxO target in other cell types, rescues DA neuron degeneration in PINK1 nulls (Table 1) [40], indicating that dFoxO's neuroprotective function here is linked to its role limiting the accumulation of ROS.

Likewise in *C. elegans*, neuroprotective functions of FoxO depend on a transcriptional target implicated in oxidative stress defense. Neurons expressing a polyglutamine (polyQ)-expanded N-term fragment of Huntingtin are dysfunctional [42]. Activation of Sir2 rescues this neuronal dysfunction, and again, the rescue depends on Daf-16. In this context, Daf-16 function requires β -Catenin [43], which binds Daf-16 and enhances its transcriptional activity in cells subjected to oxidative stress (Fig. 2D) [44]. Interestingly, the Wnt receptor Ryk is upregulated in neurons expressing mutant Huntington protein where it promotes neuronal dysfunction by impairing the neuroprotective Daf-16- β -Catenin interaction [6]. What is the relevant Daf-16 target in this context? Daf-16's protective effect requires *ucp-4*,

a mitochondrial uncoupling protein (Table 1). Daf-16 binds the *ucp-4* promoter and *ucp-4* function is required for Sir2-mediated neuroprotection [43].

Suggesting that FoxO pathways have clinical potential in the context of Huntington's Disease, chemical compounds acting in a FoxO-dependent manner sustain neuronal function in the face of polyQ cytotoxicity in *C. elegans* and mammalian neurons [45]. Further more, elevating FoxO-mediated signaling in neurons is broadly neuroprotective. Activating FoxO genetically or pharmacologically confers striking protection in multiple models of motoneuron disease, including overexpression of either mutant SOD1 or mutant p150^{glued} [46]. Defining the upstream regulators and downstream effectors enabling FoxO's protective actions in diverse neurological diseases is essential in order to tap FoxO's therapeutic potential.

FoxOs regulate the neuronal cytoskeleton to enable morphological plasticity

FoxO proteins promote cytoskeletal dynamics during neurodevelopment

Large-scale morphological plasticity is a defining feature of young neurons as they extend axons and dendrites to interconnect and form circuits. Later in development, smaller-scale morphological plasticity predominates as neurons remodel their connectivity in response to genetic and environmental cues.

Drosophila FoxO is expressed in the developing CNS and PNS [20,47]. While dFoxO is cytoplasmic in many neurons, it is predominantly nuclear in motoneurons [20]. Loss of dFoxO does not result in large-scale morphological defects in motoneurons—motor axons reach their target muscles and form neuromuscular junctions (NMJs) of the proper size. However, *dfoxO* mutants fail to undergo activity-dependent plasticity. Wild-type NMJs display activity-dependent structural plasticity; they rapidly bud new boutons following high-frequency stimulation [48,49]. This form of activity-dependent structural plasticity is eliminated in *dfoxO* mutants [5], indicating a requirement for dFoxO in rapid structural plasticity at the NMJ (Fig. 3).

Genetic and pharmacological evidence indicate that excessive microtubule (MT) stability inhibits synapse plasticity in *dfoxO* mutants. Loss of dFoxO results in markedly increased levels of stable MTs at NMJs [5,20]. What is the relevant transcriptional target in this context? The mitotic kinesin MKLP1/Pavarotti (Pav-KLP) emerged from a neuronal RT-qPCR screen as a cytoskeletal transcript upregulated in *dfoxO* mutants (Table 1). Validating the approach, synaptic levels of Pav-KLP protein are increased two-fold in *dfoxO* mutants. Demonstrating that Pav-KLP is a key downstream target of dFoxO, Pav-KLP heterozygosity fully suppresses the plasticity phenotype of *dfoxO* nulls (Fig. 3D) [5].

What is the link between Pav-KLP activity and MT network dynamics? Pav-KLP restrains Kinesin-1-mediated sliding of short MT fragments on longer MT tracks (MT sliding) [50]. MT sliding drives initial axon outgrowth, but is limited in older neurons to stabilize neuronal morphology [51]. Pav-KLP is proposed to serve such a stabilizing function and brake MT sliding. In *dfoxO* mutants, elevated levels of Pav-KLP lead to an overly rigid synaptic MT

network that eliminates activity-dependent plasticity. dFoxO then establishes a dynamic MT network by repressing Pav-KLP, setting the stage for morphological plasticity.

dFoxO also promotes MT dynamics in dendrites. Dendritic Arborization (da) neurons, a group of *Drosophila* sensory neurons, extend stereotyped dendrite arbors and are amenable to *in vivo* live imaging [52,53]. Loss of dFoxO results in reduced dendrite branching in all classes of da neurons (Sears and Broihier, 2016). Strikingly, as in motoneurons, loss of FoxO is associated with MT defects. Live analysis of MT growth indicates reduced overall MT dynamics in *dfoxO* nulls. This analysis also uncovered an unexpected function for *dfoxO* in regulating MT polarity. dFoxO is necessary and sufficient for anterograde MT polymerization in dendrites (Fig. 3A). In wild-type da neurons, MTs are predominantly minus-end-out (retrograde polymerizing) at late larval stages [54]. However, earlier in development, during a period of rapid dendrite growth and branching, dendrites contain appreciable numbers of plus-end-out (anterograde polymerizing) MTs [55]. Recent studies establish that anterograde MT polymerization underlies initiation and stabilization of nascent branches [56,57]. Thus, dFoxO regulates a pool of MTs present in young neurons pivotal for dendrite outgrowth and branching. The relevant transcriptional target(s) of dFoxO in this context are not yet known. It is interesting to note that MKLP1 regulates MT polarity in mammalian dendrites [58], raising the possibility that MKLP1/Pav-KLP is a transcriptional target of dFoxO in multiple neuronal populations.

FoxO function in neuronal development is conserved in mammals and *C. elegans*. FoxOs regulate an earlier step of neuronal development in mammals than in either *Drosophila* or *C. elegans*. Simultaneous knockdown of FoxO1/3/6 in hippocampal or cerebellar neurons results in defects in neuronal polarization, with a striking failure of initial axon specification [21]. Via a candidate gene approach, several cytoskeletal genes were identified as potential transcriptional targets, including Pak1 kinase (Table 1). Pak1 levels were significantly decreased in FoxO knockdown neurons relative to controls. Validating the RT-qPCR approach, Pak1 is a direct transcriptional target and a critical downstream mediator of FoxO (Fig. 3B). Pak1 substrates include regulators of cytoskeletal dynamics [59,60], suggesting that FoxOs may promote axon specification in mammals, at least in part, by regulating actin or MTs. FoxO proteins are also involved in later steps of neurodevelopment in mammals. Knockdown of FoxO1/3/6 in cultured cerebellar neurons following initial polarization results in decreased axon outgrowth, and surprisingly, increased dendrite growth [61], indicating that FoxO proteins coordinate axon and dendrite outgrowth. The function for FoxO in axon outgrowth is evolutionarily conserved since axons of *C. elegans* AIY interneurons display markedly stunted growth (Fig. 3C) [61].

As additional neuronal functions for FoxO proteins are identified, it will be important to see if they regulate MT organization at other stages of development. Along these lines, FoxO1 has been shown to bind SnoN1 to repress the MT-stabilizing protein Doublecortin and regulate migration of granule neurons in the cerebellar cortex (Fig. 2C) [62]. While the details of FoxO-mediated signaling pathways vary among systems, in general the preceding studies argue that FoxOs regulate cytoskeletal organization to enable morphological plasticity—an essential feature of developing neurons.

FoxO proteins preserve youthful characteristics of adult neurons

FoxOs are expressed in adult neurons, and recent studies in *C. elegans* provide insight into their functions and downstream targets. A key recent study identified transcripts regulated by Daf-16 downstream of IIS in adult *C. elegans* neurons {Kaletsky:2016dw}. Providing essential support to the hypothesis that FoxOs regulate a distinct transcriptional cohort in neurons, the neuronal IIS/Daf-16 adult transcriptome is dominated not by genes with metabolic functions, but rather is enriched for genes with neuronal functions including transcription factors, kinesins, ion channels, and receptors (Table 1) [63]. This study also argues that FoxOs support neuronal function across lifespan.

What is the role of Daf-16 in adult neurons? Several populations of *C. elegans* neurons exhibit age-dependent deterioration in the form of ectopic neurite sprouting [64–66]. The frequency of excess branches increases with advancing age and is regulated by IIS/Daf-16 signaling. Loss of *daf-2*/Insulin receptor suppresses sprouting, and IIS-mediated suppression requires Daf-16 activity. Loss of Daf-16 on its own results in a modest increase in neurite sprouting, arguing for a neuroprotective role for FoxO in this paradigm [66]. Ectopic neurite sprouting is not the only sign of old age in *C. elegans* neurons. Presynaptic terminals undergo dramatic changes at the ultrastructural level, with pronounced depletion of synaptic vesicles in aged adults [66]. The sheer magnitude of the loss of synapse integrity in old adults suggests that a compensatory response may well exist. It is not clear if ectopic sprouts result from non-specific neuronal deterioration, or if they are a homeostatic response to age-dependent synapse degeneration.

Age-dependent loss of synaptic vesicles is also observed in the DA9 motoneuron [67]. A screen for genetic modifiers of this phenotype led to the identification of Unc-104/KIF1A, a kinesin known to transport synaptic vesicles and active zone proteins. Unc-104 expression declines with age, and remarkably, neuronal overexpression of Unc-104 improves motor function and an age-related decline of learning and memory. Indicating that IIS regulates Unc-104, Unc-104 expression is maintained in old *daf-2*/Insulin receptor mutant animals. Enhanced Unc-104 expression in this background requires *daf-16* [67]. These data argue that Daf-16 maintains synapse integrity via the neuronal kinesin Unc-104 (Table 1) following decreased signaling through the Insulin receptor pathway.

Age-dependent deterioration of *C. elegans* neurons extends to their regenerative ability. Axons in young worms regrow well following laser axotomy [68], while those from older adults do not grow as far [69–71]. Loss of *daf-2*/Insulin receptor improves axon regeneration in old animals indicating that Insulin/IGF1 signaling normally inhibits axon regeneration. Improved axon regeneration in old *daf-2* animals depends on neuronal *daf-16*, raising the exciting possibility that Daf-16 regulates genes acting in cell-intrinsic regeneration programs. Neuron-specific pathways driving axon regeneration are intensively studied, and Dlk-1 kinase is a linchpin in such pathways [70,72,73]. Dlk-1 has been shown to be essential for enhanced regeneration in aged *daf-2* animals, and Daf-16 binds the *dlk-1* promoter in neurons [71]. These findings argue that enhanced regeneration in *daf-2* mutants is likely caused by increased *daf-16*-dependent expression of Dlk-1 (Table 1). Together, the identification of Unc-104/KIF1A and Dlk-1 as transcriptional targets of neuronal Daf-16

underscores the importance of neuron-specific FoxO pathways in promoting key youthful neuronal attributes including synapse integrity and axon regeneration.

FoxOs direct synapse function, plasticity and behavior

To this point, we have reviewed evidence that FoxO proteins regulate cell-fate and morphological plasticity of neurons. But do FoxOs also direct the expression of synaptic proteins to directly control synapse function and plasticity? Genome-wide efforts to identify neuronal targets of FoxOs have turned up synaptic proteins including ion channels and receptors as well as components of the exocytic machinery [63,74], hinting at widespread roles for FoxOs in regulating key synaptic attributes. As discussed below, subsequent functional studies are beginning to illuminate requirements for FoxOs at synapses.

Studies from multiple labs indicate that dFoxO-dependent networks regulate both synaptic function and plasticity in *Drosophila* motoneurons. *dfoxO* mutants display marked defects in baseline neurotransmitter release and synaptic vesicle cycling at the larval NMJ [20,75]. As summarized above, elevated levels of Pav-KLP lead to excessively stable MTs in *dfoxO* mutant motoneurons. Moreover, genetic and cell biological analyses argue that an overly rigid presynaptic MT network underlies defects in baseline synaptic function in *dfoxO* mutants [20]. Thus, the defects in baseline electrophysiological characteristics in *dfoxO* mutants have roots in underlying cytoskeletal defects.

dfoxo mutant motoneurons are also characterized by defects in distinct forms of synaptic plasticity. At the larval NMJ, mutants exhibit a slower rate of onset of long-term facilitation (LTF), indicating decreased excitability [75]. Evidence indicates that this phenotype is caused by disrupting an autocrine signaling pathway that normally limits motoneuron excitability. High levels of glutamate in the synaptic cleft activate the presynaptic metabotropic glutamate receptor (mGluRA), which then signals via Akt to inactivate dFoxO and decrease excitability (Fig. 4A). The decreased excitability observed in *dfoxO* mutants is proposed to reflect this negative regulation of dFoxO by mGluRA. Though the relevant transcriptional targets of dFoxO have not yet been discovered, they may well include proteins that tune neuronal excitability such as ion channel subunits.

In the adult, dFoxO regulates a different type of synaptic plasticity in the CM9 motoneuron [76]. The CM9 NMJ is required for the proboscis extension reflex, which is involved in fly feeding. At this NMJ, the amount of neurotransmitter released in flies fed a low-calorie diet is greater than that in flies fed a high-calorie diet [77], demonstrating diet-induced plasticity. The IIS pathway negatively regulates presynaptic release at the CM9 NMJ in response to increased caloric intake. dFoxO is required for this response; *dfoxO* mutants fed a low-calorie diet do not display enhanced release [76]. A key transcriptional target of dFoxO in CM9 neurons is eukaryotic translation initiator factor 4e-binding protein (4e-BP), an established dFoxO transcriptional target (Table 1) [12,76,78]. The dFoxO-4eBP link begs the question of the identity of the synaptic 4e-BP target. Excitingly, 4e-BP inhibits the translation of Complexin, a component of the synaptic vesicle fusion machinery [79], to increase neurotransmitter release in response to dietary restriction [76]. Thus, FoxO-mediated regulation of 4e-BP is linked directly to Complexin and synaptic vesicle exocytosis

(Fig. 4B). It will be important to establish whether a connection between FoxO and Complexin exists at other synapses and in other forms of plasticity. Suggesting that FoxO does indeed regulate protein translation via 4e-BP at other synapses, dFoxO promotes 4e-BP expression in post-synaptic muscle cells at the larval NMJ to regulate homeostatic plasticity [80].

The roles of FoxO proteins in metabolic and stress response pathways have proven highly conserved, suggesting FoxOs likely regulate synapse function not only in *C. elegans* and *Drosophila* but in mammals as well. Studies investigating FoxO function in synapse plasticity in mammalian systems argue this is likely the case. For example, FoxO6 is prominently expressed in the hippocampus, suggesting roles in learning or memory. Investigation of FoxO6 function in the hippocampus suggests some specificity of FoxO6 function there [74]. FoxO6 mutant mice perform comparably to wild-type controls during learning stages of contextual fear conditioning and novel object recognition, yet exhibit defects in memory formation. Using timed injections of a dominant negative FoxO6 construct, FoxO6 has been shown to function to memory consolidation, not memory retrieval. To provide mechanistic insight, these authors adopted a microarray approach and screened for genes whose expression is induced by object learning in wild-type, but not FoxO6, mutant animals [74]. Via this approach, they identified and validated genes implicated in synapse plasticity including gastrin-releasing polypeptide (Grp) and μ -Crystallin (Crym) [81](Table 1). Intriguingly, genes regulated by FoxO6 were also found to be enriched for activity-dependent MEF2 transcription factor binding sites, suggesting co-regulation of genes by FoxO6 and MEF2 [74].

While FoxO6 and MEF2 may cooperate to regulate activity-dependent gene transcription, a recent paper provides strong evidence that FoxO3 cooperates with MeCP2 and HDAC3 to regulate synapse plasticity in the hippocampus [82]. Mutations in MeCP2 cause the neurodevelopmental disorder Rett Syndrome and a specific disease-causing allele, MeCP2^{R306C}, interferes with the ability of MeCP2 to bind histone deacetylase HDAC3, suggesting that HDAC3 is a key MeCP2 interactor [83]. Transcriptional and chromatin profiling analyses indicate that MeCP2 and HDAC3 both activate expression of a gene battery required for proper cognition and social behavior. Hinting that FoxO may act in parallel with MeCP2-HDAC3, promoters of these genes are enriched for FoxO binding sites. While HDAC3 is best known as a histone deacetylase, it deacetylates FoxO1 and FoxO3 in the liver [84]. Excitingly, in the context of MeCP2, HDAC3 also deacetylates FoxO3, promoting its localization to promoters of genes regulated by MeCP2-HDAC3 including BDNF and NGF1B (Fig. 2B). Lastly, neural progenitor cells derived from an individual with the MeCP2^{R306C} allele display elevated levels of acetylated FoxO3 and decreased recruitment of FoxO3 and HDAC3 to active gene regulatory regions, arguing for disease relevance of this interaction. It will be essential to determine whether loss of FoxO3 in the hippocampus has consequences for cognition and social behavior similar to that of MeCP2 and HDAC3. Together, these studies suggest that FoxO proteins act in parallel with other key neuronal transcription factors to drive downstream gene expression required to modulate memory and behavior.

Concluding Remarks

It is well established FoxO protein functions outside of the nervous system are evolutionarily conserved; FoxOs direct metabolic functions and the oxidative stress response to regulate organismal health and longevity. Recently it has become clear that neuronal functions of the FoxO family are likewise highly conserved among *C. elegans*, *Drosophila*, and mammals. FoxOs are critical for promoting cell-type, morphological, and synaptic plasticity in neurons. Perhaps not surprisingly, FoxOs act to neuron-specific pathways to execute these functions—acting in concert with other neuronal transcriptional factors to regulate the expression of genes involved in cytoskeletal dynamics, vesicle cycling, and synapse plasticity. Defining the ensemble of genes regulated by FoxO proteins and dissecting their regulatory mechanisms are crucial first steps toward a comprehensive understanding of FoxO protein function in morphological and synaptic plasticity in the developing and adult nervous system.

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Trends Box

FoxO proteins maintain youthful neuronal characteristics by performing functions that are both analogous to their roles in peripheral tissues and also unique to neurons.

Similar to their roles in other stem cell populations, FoxOs promote adult neural stem cell multipotency by inhibiting fate specification.

FoxOs promote neuronal health and viability by regulating oxidative stress and autophagy in multiple neurodegenerative disease contexts, resembling their functions in the periphery.

In developing and mature neurons, FoxO proteins preserve neuronal vitality by enabling morphological plasticity through regulation of cytoskeletal dynamics and organization.

FoxOs regulate genes required at the synapse for neuron-specific processes such as learning and memory.

The ability of FoxOs to execute a wide array of neuron-specific functions is explained by that fact that many of their neuronal targets are distinct from targets in non-neuronal cells.

Outstanding Questions

Can neuronal activity regulate FoxO nucleo-cytoplasmic shuttling, DNA binding ability, or transcriptional activity?

In what neuronal contexts is FoxO downstream of Insulin/IGF signaling? What are other the upstream regulators of FoxOs that guide their distinct functions in neurons? Are the upstream regulators of FoxO TFs context-specific, i.e. do the upstream pathways change depending on developmental or disease state?

What are the downstream targets of FoxOs independent of Insulin/IGF signaling? How do the developmental targets compare with the transcriptional targets of FoxO in adulthood?

Are the neuronal mammalian FoxO isoforms (FoxO1, FoxO3, and FoxO6) redundant in any context? To what extent are the target genes of the mammalian FoxOs similar? Or do they have mostly non-overlapping targets?

What factors or signaling pathways inhibit FoxO-dependent NSC quiescence? Are these pathways different in development versus adulthood?

Do FoxO isoforms promote neuronal autophagy outside of a disease context? Are the roles of FoxOs in autophagy purely at the level of transcriptional regulation or does cytoplasmic FoxO have non-canonical functions in autophagy?

Separate from their roles in establishing neuronal polarity, do FoxOs promote morphological plasticity of mammalian axons or dendrites? If so, do FoxOs accomplish this through regulation of cytoskeletal dynamics?

The central nervous system is composed of two main cell types: neurons and glia. Are FoxO transcription factors expressed in CNS glia? Do FoxOs regulate glial cell biology or development?

Neuronal functions of FoxO transcription factors

Figure 1. FoxO transcription factors are evolutionarily-conserved regulators of neuronal form and function

Overview of neuronal FoxO functions in commonly studied model organisms.

Figure 2. Important cofactors that regulate neuron-specific FoxO functions

A) During neurogenesis, FoxO3 and ASCL1 share multiple target genes. In this context, FoxO3 inhibits ASCL1 transcriptional activity to promote neuronal stem cell quiescence. B) FoxO3 functions in the hippocampus where it is deacetylated by HDAC3. Deacetylation promotes FoxO3 localization to genes that are regulated by MeCP2 to control aspects of plasticity and behavior. C) In the cerebellar cortex, FoxO1 binds the SnoN1 transcription factor to regulate granule neuron migration. D) Daf-16 serves neuroprotective functions through a direct interaction with β -catenin in oxidatively-stressed *C. elegans* neurons.

Figure 3. Neuronal FoxOs enable morphological plasticity

A) dFoxO modulates axonal microtubule dynamics necessary for activity-dependent structural plasticity via transcriptional repression of the mitotic kinesin Pav-KLP. B) dFoxO regulates dendrite plasticity by promoting anterograde microtubule polymerization and overall microtubule network dynamics. C) FoxO1/3/6 regulate neuronal polarization and axon growth through the Pak1 kinase. D) Daf-16 promotes axonal outgrowth of developing AIY interneurons in *C. elegans*.

Figure 4. dFoxO acts in distinct molecular pathways in developing and adult motoneurons to promote synaptic function

A) In developing motoneurons, homeostatic regulation of neuronal activity occurs when high levels of synaptic glutamate feeds back onto presynaptic mGluRA receptors to downregulate neuronal excitability. Here, mGluRA signals via PI3K to promote Akt-dependent inhibition of dFoxO resulting in decreased motoneuron activity. B) Adult CM9 motoneurons exhibit a type of diet-induced synaptic plasticity whereby a high-calorie diet leads to decreased neurotransmitter release. Increased caloric intake activates Insulin signaling which functions to inhibit dFoxO's transcriptional regulatory ability. Inhibited dFoxO function causes decreased expression of its transcriptional target, 4e-BP. As a result, 4e-BP is unable to inhibit translation of Complexin, a protein that acts to diminish synaptic vesicle release.

Table 1

Important FoxO targets in the nervous system

Targets in black have been experimentally validated and targets in blue have not been fully validated.

Selected Neuronal FoxO target genes					
Protein Name	Target	Target Function	Directness of Regulation	Direction of regulation	Reference
Daf-16	Ucp-4	Mitochondrial uncoupling protein	Likely direct	Positive	43
	Fkh-9	Forkhead transcription factor	Likely direct	Positive	63
	CREB	Transcription factor; functions in long-term memory	Unknown	Positive	
	Irk-3	Inward Rectifying K channel		Negative	
	Str-131	seven-transmembrane receptor		Negative	
	Srd-28	Serpentine Receptor		Negative	
	Nhr-2	Nuclear Hormone Receptor family		Negative	
dFoxO	UNC-104/KIF1A	Kinesin motor; transports synaptic vesicles	Unknown	Positive	67
	Dlk-1	Dual-leucine zipper kinase; intrinsic regulator of axon regeneration	Likely direct	Positive	71
	4e-BP/Thor	inhibitor of translational initiation	Likely direct	Positive	76
	SOD2	superoxide dismutase; ROS clearance	Unknown	Positive	40
	Shortstop	Actin-MT crosslinking	Unknown	Positive	5
FoxO1	Pav-KLP/MKLP1	Kinesin-6 family member; inhibits neuronal MT network dynamics	Unknown	Negative	62
	Dex	Doublecortin; MT-associated protein; promotes MT stability	Likely direct	Negative	
	Bnip3	Bcl2 family member; induces autophagy	Unknown	Positive	32
FoxO3	BDNF	Brain-derived neurotrophic factor; regulates neuronal survival & plasticity	Likely direct	Positive	82
	NR4A1/NGF1B	Nerve growth factor; regulates neuronal survival & plasticity	Likely direct	Positive	
	Crym	u-crystallin; may regulate neuronal specification	Unknown	Negative	31
FoxO6	Grip	Gastrin releasing polypeptide; modulates plasticity and memory formation	Unknown	Negative	
	Par6	PDZ domain containing protein	Unknown	Positive	21
FoxO1, FoxO3, FoxO6	Kif5A	Kinesin family member		Positive	
	Disc1	Scaffolding protein		Positive	
	CRMP-2	Collapsin response mediator protein; MT regulator		Positive	
	Pak1	Protein kinase; can regulate MT dynamics		Positive	