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FoxO regulates microtubule dynamics and polarity to promote dendrite branching in Drosophila sensory neurons

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ABSTRACT

The size and shape of dendrite arbors are defining features of neurons and critical determinants of neuronal function. The molecular mechanisms establishing arborization patterns during development are not well understood, though properly regulated microtubule (MT) dynamics and polarity are essential. We previously found that FoxO regulates axonal MTs, raising the question of whether it also regulates dendritic MTs and morphology. Here we demonstrate that FoxO promotes dendrite branching in all classes of Drosophila dendritic arborization (da) neurons. FoxO is required both for initiating growth of new branches and for maintaining existing branches. To elucidate FoxO function, we characterized MT organization in both *foxO* null and overexpressing neurons. We find that FoxO directs MT organization and dynamics in dendrites. Moreover, it is both necessary and sufficient for anterograde MT polymerization, which is known to promote dendrite branching. Lastly, FoxO promotes proper larval nociception, indicating a functional consequence of impaired da neuron morphology in *foxO* mutants. Together, our results indicate that FoxO regulates dendrite structure and function and suggest that FoxO-mediated pathways control MT dynamics and polarity.

1. Introduction

Dendrite architecture is established during development and lays the groundwork for neuronal connectivity and function. Dendrites acquire simple or complex morphologies depending on the degree of branching and growth of their arbors. Regulation of dendrite branching and growth requires accurate integration of cell-intrinsic and cell-extrinsic factors. On the cell-intrinsic side, cohorts of transcription factors direct the expression of downstream effector molecules that together impart cell-type specific morphologies. While a number of transcription factors have been implicated in dendrite morphogenesis, the remarkable morphological diversity of dendrite arbors suggests that others remain to be identified.

Dendritic arborization (da) neurons are sensory neurons that innervate the larval epidermis and are grouped into four classes (classes I-IV) based on the size and shape of their dendrite arbors (Corty et al., 2009; Grueber et al., 2002). Work in this system has detailed cytoskeletal characteristics that distinguish dendrite morphologies of classes of da neurons (Grueber et al., 2003; Jinushi-Nakao et al., 2007). For instance, simple class I arbors and complex class IV arbors differ in the extent to which their dendrite branches are populated by stable microtubules (MTs). The MT-associated protein (MAP) Futsch/MAP1B, binds and stabilizes MTs (Halpain and Dehmelt, 2006; Hummel et al., 2000; Roos et al., 2000). In class I neurons, many branches contain Futsch, while in class IV neurons, Futsch is confined primarily to main branches (Grueber et al., 2002; Jinushi-Nakao et al., 2007). Moreover, loss of Futsch increases branching of class I neurons (Yalgin et al., 2015). Together these data suggest that dynamic MTs are particularly critical in generating the highly branched dendrite arbors in class IV neurons.

The stereotyped and superficial positions of da neurons, as well as the two-dimensional shapes of their dendrite arbors, have greatly facilitated *in vivo* live imaging of dendrite growth and cytoskeletal dynamics in this system (Rolls et al., 2007; Stone et al., 2008). Such studies have established that da neuron dendrites have mixed MT polarity during developmental stages characterized by rapid dendrite growth and branching (Hill et al., 2012). In other words, MT polymers are a mixture of plus-end-out (anterograde polymerizing) and minus-end-out (retrograde polymerizing) filaments. MT polarity matures over the course of larval development to an almost entirely minus-end-out orientation (Hill et al., 2012). The presence of plus-end-out MTs during stages of extensive branching suggests that anterograde MT polymerization may play a role in generating dendrite arbors. This hypothesis is supported by recent studies demonstrating a function for anterograde MT polymerization in facilitating nascent branch formation and stabilization (Ori-McKenney et al., 2012; Yalgin et al., 2015).

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Transcription factor-mediated pathways play leading roles in regulating cytoskeletal assembly and organization in da neurons (Lefebvre et al., 2015; Santiago and Bashaw, 2014), suggesting that developmental competence for dendrite growth and branching is established by cell-intrinsic factors. Interestingly, a number of transcription factors selectively regulate either the MT or actin cytoskeleton in da dendrites. For example, Cut and Lola control actin organization while Abrupt, Dar1, and Knot regulate MTs (Ferreira et al., 2014; Jinushi-Nakao et al., 2007; Yalgin et al., 2015; Ye et al., 2011). Identifying the suite of transcription factors regulating da neuron dendritogenesis and defining the cytoskeletal features they regulate is key to deciphering how these factors collaborate to control neuronal morphology.

We set out to test whether the transcription factor FoxO regulates development of da neuron dendrites. FoxO proteins regulate neural stem cell homeostasis, neuronal polarity, neurite outgrowth, synaptic function, and memory consolidation (Christensen et al., 2011; la Torre-Ubieta et al., 2010; Paik et al., 2009; Renault et al., 2009; Salih et al., 2012). In addition, we previously found that the sole FoxO ortholog in Drosophila regulates MT organization in presynaptic terminals of motor neurons (Nechipurenko and Broihier, 2012). Together, these studies demonstrate that FoxO proteins are evolutionarily conserved regulators of neuronal structure and function. However, a role for FoxO proteins in dendrite arborization during neurodevelopment has not been investigated.

In this study, we demonstrate that Drosophila FoxO regulates dendrite development of da neurons. We find that FoxO is expressed in da neurons, and loss of FoxO results in decreased dendrite branching in all da neuron classes. To understand how FoxO promotes dendrite branching, we undertook a time-lapse analysis and demonstrate that FoxO stimulates initiation of new branch growth and also stabilizes existing branches. We hypothesized that these morphological defects result from aberrant MT organization. In line with this hypothesis, analyses of *foxO* loss-of-function (LOF) and overexpressing neurons demonstrate that FoxO regulates MT dynamics. Specifically, we find that FoxO promotes overall MT dynamics as well as anterograde MT growth. Taken with our previous study of FoxO function in motoneurons, these findings indicate that FoxO regulates MT organization in both motor axons and sensory dendrites. Lastly, we examined whether FoxO is required for da neuron function. Class IV da neurons are nociceptive, sensing noxious heat and mechanical stimuli (Hwang et al., 2007; Tracey et al., 2003). We find that nociceptive responses are attenuated in *foxO* mutant larvae, indicating that FoxO is required for both structure and function of da neurons. Together, these findings extend *in vivo* functions of neuronal FoxO proteins to include dendrite arborization and suggest that regulating MT dynamics is a core neuronal function of FoxO family members.

2. Results

2.1. FoxO acts cell-autonomously to regulate class IV dendrite morphology

Our previous work established that FoxO organizes presynaptic MTs at the neuromuscular junction (NMJ) (Nechipurenko and Broihier, 2012). Because MT organization and dynamics are central to dendrite growth and branching, we hypothesized that FoxO regulates dendrite morphology. Class IV da cells are the largest and most elaborate of the da neurons, providing an ideal cell type in which to explore a possible function for FoxO in dendrite morphology. Dendrite outgrowth of class IV cells begins late in embryogenesis and continues through early larval stages, when it is characterized by a rapid growth as the arbor covers its receptive field. Following this phase, dendrite growth transitions to a phase of scaling growth in third instar larvae where growth of dendrite arbors and overall animal growth are synchronized (Parrish et al., 2009).

We examined dendrite growth and branching in early (72 h AEL; After Egg Laying) and late (120 h AEL) third instar larvae in ddaC, a well-characterized Class IV cell (Grueber et al., 2002). We labeled membranes of *foxO* nulls ($foxO^{\Delta 94}$) (Slack et al., 2011) and controls with membrane-targeted GFP via a class IV Gal4 driver to permit morphological analyses. Consistent with previous reports (Colombani et al., 2005), we do not detect a difference in overall body size between *foxO* mutants and controls. We find that at 72 h AEL, $foxO^{\Delta 94}$ animals are 2.0 ± 0.14 mm long ($n=12$) and control animals are 2.0 ± 0.15 mm long ($n=20$). At 120 h AEL, $foxO^{\Delta 94}$ animals are 3.2 ± 0.07 mm long ($n=38$) and control animals are 3.2 ± 0.06 mm long ($n=39$). We first assessed ddaC branching at 72 h AEL. We find that loss of FoxO results in a 46.4% reduction in branch number, and a 27.7% reduction in overall dendrite length (Fig. 1A–D). We utilized Sholl analysis to quantify branching as a function of distance from the soma (Sholl, 1953). We find that relative to controls, *foxO* nulls display decreased branching at both proximal and medial regions of the arbor (Fig. S1A). Decreased dendrite branching in *foxO* mutants leads to large regions of non-innervated epidermis within the area covered by individual class IV cells. We developed an ImageJ macro to first overlay a grid of 250 μm^2 squares on dendrite arbors, and then analyze internal coverage as reflected by squares with/without a dendrite branch (Jinushi-Nakao et al., 2007; Stewart et al., 2012). We find that *foxO* nulls display a 2.2-fold increase in the proportion of empty squares relative to controls (Fig. 1E–G), consistent with decreased dendrite branching. Together, these analyses argue that FoxO regulates the early, rapid phase of dendrite outgrowth and branching.

We next examined if the decrease in dendrite branching observed at 72 h AEL persists until 120 h AEL, the late third instar stage. At 120 h AEL, we find a 33.2% reduction in branch number (Fig. 1H–J) and a 28.7% reduction in overall dendrite length in *foxO* nulls (Fig. 1K). We again utilized Sholl analysis to quantify branching as a function of distance from the soma, and find reductions in branching throughout the arbor in *foxO* nulls relative to controls (Fig. S1B). We next quantified internal coverage and find that *foxO* nulls display a 1.7-fold increase in the proportion of empty squares relative to controls (Fig. 1L–N). Together, these findings indicate that loss of FoxO results in a sustained decrease in dendrite branching and a corresponding increase in epidermal area lacking innervation.

To assess cell autonomy, we undertook a clonal analysis of class IV ddaC using MARCM (Lee and Luo, 1999; Grueber et al., 2002). At 120 h AEL, *foxO* null ddaC clones display a 29.7% reduction in branch number relative to control cells (Fig. 2A–C), consistent with the phenotype observed in *foxO* nulls. *foxO* null clones also display a 24.4% reduction in total dendrite length at this stage (Fig. 2D). Sholl analysis reveals a similarly shaped arbor as observed in *foxO* null animals (Fig. S1C). We again tested internal coverage using an overlaid grid and find that *foxO* null ddaC clones display a 1.6-fold increase in the proportion of empty squares relative to controls at 120 h AEL (Fig. 2E–G). Because neither dendrite length nor branching of ddaC are more severely disrupted in *foxO* null animals than in *foxO* mutant clones ($p > 0.05$ for both), we conclude that FoxO acts cell-autonomously in class IV ddaC neurons to promote dendrite branching and growth.

2.2. FoxO is expressed in da neurons and regulates class I-III dendrite morphology

Our MARCM analysis implies that FoxO protein is expressed in class IV da neurons. In line with a cell-autonomous function, FoxO is expressed in ddaC neurons (Fig. 3A), as assessed with an anti-