A microtubule polymerase is required for microtubule orientation and dendrite pruning in *Drosophila*

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**Abstract**

*Drosophila* class IV ddaC neurons selectively prune all larval dendrites to refine the nervous system during metamorphosis. During dendrite pruning, severing of proximal dendrites is preceded by local microtubule (MT) disassembly. Here, we identify an unexpected role of Mini spindles (Msps), a conserved MT polymerase, in governing dendrite pruning. Msps associates with another MT-associated protein TACC, and both stabilize each other in ddaC neurons. Moreover, Msps and TACC are required to orient minus-end-out MTs in dendrites. We further show that the functions of msps in dendritic MT orientation and dendrite pruning are antagonized by the kinesin-13 MT depolymerase Klp10A. Excessive MT depolymerization, which is induced by pharmacological treatment and katanin overexpression, also perturbs dendritic MT orientation and dendrite pruning, phenocopying msps mutants. Thus, we demonstrate that the MT polymerase Msps is required to form dendritic minus-end-out MTs and thereby promotes dendrite pruning in *Drosophila* sensory neurons.

**Keywords** dendrite; *Drosophila*; microtubule; minus-end-out orientation; pruning

**Subject Categories** Cell Adhesion, Polarity & Cytoskeleton; Neuroscience

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**Introduction**

Neurons often extend their exuberant processes and connections at early developmental stages. Selective removal of their unwanted or redundant dendrites or axons without causing neuronal death, referred to as pruning, is a fundamental strategy to ensure proper wiring in the developing nervous systems (Luo & O’Leary, 2005; Riccomagno & Kolodkin, 2015; Schuldiner & Yaron, 2015). In mammalian brains, some developing neurons remove their long axonal bundles and re-establish functional circuits. Impaired neuronal pruning is associated with autism spectrum disorder (ASD) with increased dendritic spine density in layer V pyramidal neurons (Tang et al, 2014). Neuronal pruning is also essential for the development of invertebrate nervous systems. In *Drosophila*, large-scale remodeling of nervous systems takes place during metamorphosis, a transition stage between larval and adult stages (Yu & Schuldiner, 2014; Kanamori et al, 2015). Of interest, *Drosophila* dendritic arborization (da) neurons, which are part of the peripheral nervous system (PNS), undergo either apoptosis or pruning to generate adult-specific processes. Some dorsal dendrite arborization (dda) neurons, class IV (ddaD and ddaE), selectively prune away their larval dendrites but maintain their larval axons intact (Kuo et al, 2005; Williams & Truman, 2005), whereas others (class III, ddaA and ddaF) are apoptotic during the first day of metamorphosis (Williams & Truman, 2005). Pruning involves blebbing, thinning, and retraction of neuronal branches, reminiscent of neurite degeneration associated with neurodegenerative diseases or spinal cord injury. Thus, developmental pruning might provide an important paradigm to unravel the mechanisms of neurodegeneration in pathological conditions.

*Drosophila* C4da or ddaC neurons have been established as a powerful system to understand the mechanisms of dendrite-specific pruning during early metamorphosis. Induced by a late larval pulse of the steroid hormone 20-hydroxyecdysone (ecdysone), ddaC neurons initially sever their larval dendrites at the proximal regions as early as 4 h after puparium formation (APF) and subsequently undergo rapid fragmentation and phagocyte-mediated debris clearance (Fig 1A; Williams & Truman, 2005; Han et al, 2014). Upon the binding of ecdysone, a heterodimeric ecdysone receptor complex induces many downstream effectors or pathways. Among them are a transcription factor Sox14 (Kirilly et al, 2009, 2011), a cytoskeletal regulator Mical (Kirilly et al, 2009), Headcase (Loncle & Williams, 2014),...
However, the molecular mechanism underlying the formation of dendrite pruning, as knocked down of these factors did not result in dendrite pruning defects in ddaC neurons (Lee et al., 2009; Stone et al., 2014; Tao et al., 2016). Katanin p60-like 1 (Kat-60l), an AAA ATPase related to Katanin-60 (Kat-60) subunit, was reported to play a role in dendrite pruning of ddaC neurons (Lee et al., 2009), although its putative MT-severing function remains to be determined. Par-1 promotes MT breakdown probably via Tau inhibition and thereby dendrite pruning in ddaC neurons (Herzmann et al., 2017). However, despite the view that MT disassembly is a key step in the execution of dendrite pruning, the roles of MT polymerization/depolymerization factors in neuronal pruning remain poorly understood.

MTs are highly dynamic polymers formed by head-to-tail assembly of α- and β-tubulin dimers. These intrinsically polarized structures contain two structurally distinct ends: a fast-growing plus end and a slow-growing minus end that exposes β-tubulins and α-tubulins, respectively (Howard & Hyman, 2003; Akhmanova & Steinmetz, 2015). In contrast to static minus ends, plus ends are highly dynamic and can switch between phases of growth and shrinkage, a process known as “dynamic instability” (Howard & Hyman, 2003; Akhmanova & Steinmetz, 2015). Dynamic plus ends are decorated by MT plus-end tracking proteins (+TIPs) that promote MT dynamics (Akhmanova & Steinmetz, 2008). Highly conserved end-binding proteins (EBs) are the core components of the plus ends that provide a structural platform for recruiting other +TIPs (Honnappa et al., 2009). Neurons are highly polarized cells that extend two types of structurally and functionally distinct processes: a single long axon and multiple short dendrites. MTs are arranged with distinct orientations in axons and dendrites. In mammalian neurons, axons contain uniformly aligned MT arrays with their plus ends distal to the soma (plus-end-out), whereas dendrites are composed of MT bundles with a mixed orientation (Akhmanova & Steinmetz, 2015). In Drosophila and C. elegans, MTs in axons are arranged with a plus-end-out orientation like those in mammalian counterparts (Baas et al., 1988; Stone et al., 2008; del Castillo et al., 2015). However, in dendrites, MTs are arranged with almost exclusive minus-end-out orientation in the major branches, although plus-end-out MTs are present in the terminal branches (Stone et al., 2008; Maniar et al., 2011; Goodwin et al., 2012; Ori-McKenney et al., 2012; Yalgin et al., 2015). Caenorhabditis elegans Unc-33 and Drosophila γ-tubulin were reported to regulate MT polarities in both axons and dendrites (Maniar et al., 2011; Nguyen et al., 2014). Plus-end-directed kinesin motor proteins, kinesin-1/2, mediate MT guidance or sliding to generate minus-end-out orientation of MT arrays in dendrites of C. elegans and Drosophila neurons (Mattie et al., 2010; Yan et al., 2013). Kinesin-1/2 regulate dendrite pruning probably by aligning proper dendritic MT orientation in ddaC neurons (Herzmann et al., 2018). We and others have recently reported that the MT minus-end-binding protein Patronin, which also regulates uniform minus-end-out MT orientation in dendrites (Feng et al., 2019; Wang et al., 2019), is also critical for dendrite pruning (Wang et al., 2019). However, the molecular mechanism underlying the formation of dendritic MT orientation remains largely elusive.

Mini spindles (Msp)/XMAP215/ch-TOG family proteins have been identified as key MT polymerases, which include Stu2/Dis1 in yeasts, Zyg9 in worms, Msps in Drosophila, XMAP215 in Xenopus, and Ch-TOG in mammals (Al-Bassam & Chang, 2011). Msps orthologs consist of multiple tumor-overexpressed gene (TOG) domains at the amino-terminus and a carboxyl-terminal domain (Al-Bassam & Chang, 2011). In vitro studies show that XMAP215 directly binds tubulin dimer via TOG domains to promote multiple rounds of tubulin addition as a MT polymerase (Brouhard et al., 2008). Loss of Drosophila Msps or vertebrate XMAP215/ch-TOG leads to formation of small or aberrant spindle and short astral MTs during mitosis and meiosis (Cullen et al., 1999; Cullen & Ohkura, 2001; Gergely et al., 2003). Msps family proteins can also function at MT-organizing centers (MTOCs) by directly interacting with transforming acidic coiled-coil (TACC) proteins via their carboxyl-terminal domains (Lee et al., 2001; Bellanger & Gonczy, 2003; Kinoshita et al., 2005). TACC proteins recruit Msps/XMAP215 to centrosomes where they act together to assemble centrosomal MTs at the minus ends during mitosis (Cullen & Ohkura, 2001; Lee et al., 2001; Bellanger & Gonczy, 2003; Kinoshita et al., 2005). In postmitotic neurons, XMAP215 and ch-TOG promote MT growth by targeting to the tips of growing MTs in axons (van der Vaart et al., 2012; Lowery et al., 2013). Loss of XMAP215/ch-TOG leads to reduced MT growth rates and thereby impaired axon outgrowth in frog and rodent neurons. Vertebrate TACC proteins, which were initially identified as the centrosome-associated proteins in multiple organisms, colocalize with XMAP215/ch-TOG at MT plus ends and promote axonal outgrowth in cultured neurons (Nwagbara et al., 2014). However, the roles of Msps and TACC in neuronal MT orientation and neuronal pruning have not been documented in invertebrates and vertebrates.

Here, we report the identification of Msps as a novel regulator of dendrite pruning from a large-scale clonal screen. Msps forms a protein complex with TACC in adult neurons, and both stabilize each other in ddaC neurons. Remarkably, we show that Msps and TACC are required for minus-end-out MT orientation in dendrites of ddaC neurons, as impaired msps or tacc function resulted in a drastic decrease of the MT minus-end marker Nod-β-gal and a significant increase of anterograde EB1-GFP comets in the major dendrites. Moreover, attenuation of the kinesin-13 MT depolymerase Klp10A significantly rescued dendrite pruning defects in msps RNAi ddaC neurons, suggesting that excessive MT depolymerization may result in dendritic MT orientation and dendrite pruning defects in mutant neurons. Consistent with this idea, MT depolymerization, which was induced by two MT-destabilizing drugs and Kat-60 overexpression, also led to formation of dendritic MTs with mixed orientations and dendrite pruning defects, resembling msps mutant phenotypes. Thus, our study demonstrates an important and unexpected role of the MT polymerase Msps in regulating minus-end-out MT orientation in dendrites as well as dendrite pruning in Drosophila sensory neurons.

Results

Msps is required for dendrite pruning of sensory neurons

To isolate novel players of dendrite pruning, we carried out a large-scale clonal screen on more than 3,000 mutagenized 3R
Figure 1. Quan Tang et al. The EMBO Journal 39: e103549 | 2020
chromosomes mutagenized by ethyl methanesulphonate (EMS). We induced homozygous mutant clones in a subset of dorsal class IV dda (C4da or ddaC) neurons via the mosaic analysis with a repressible cell marker (MARC-M) system (Lee & Luo, 2001). We isolated one complementation group containing two lethal mutants, l(3)810 and l(3)924. Both alleles exhibited severe dendrite pruning defects in ddaC neurons at 16 h APF (Fig 1C and E). All l(3)810 homozygous ddaC clones failed to sever their dendrites and retained an average of 1416 μm dendrites in the vicinity of their soma (100%, n = 9; Fig 1C, G and H). Likewise, the vast majority of l(3)924 mutant neurons exhibited dendrite severing defects (81%, n = 16; Fig 1E, G and H). By contrast, the wild-type neurons completely pruned their larval dendrites at the same time point (n = 6; Fig 1B, G and H). In addition, simplified dendrite arbors were observed in either l(3)810 or l(3)924 at white prepupal (WP) stage (Fig 1C and E) as well as at larval stage (Appendix Fig S1A).

Like ddaC neurons, wild-type class I ddaD/E sensory neurons also completely pruned away their larval dendrites by 19 h APF (Appendix Fig S1B). l(3)810 mutant ddaD/E neurons failed to prune their larval dendrites; as a result, some processes remained attached to their soma (67%, n = 3; Appendix Fig S1B). Moreover, wild-type class III ddaF neurons were eliminated via apoptosis during early metamorphosis (n = 3; Appendix Fig S1C). Similar to wild-type ones, mutant ddaF neurons homozygous for l(3)810 died by 16 h APF (n = 3; Appendix Fig S1C), suggesting that the l(3)810-associated mutation does not affect ddaF apoptosis.

To identify the molecular lesions of l(3)810 and l(3)924, we performed deficiency mapping and complementation analysis with the existing lethal P-element insertion lines. We narrowed them down to the cytological region 89B1-B2, as both alleles failed to complement with Df(3R)BSC728 and Df(3R)Excel7328 deficiency lines (Fig EV1A). Moreover, both l(3)810 and l(3)924 alleles failed to complement with a lethal P-element line, msps<sup>P</sup> (Fig EV1B), which is inserted to the first intron of msps gene (Cullen et al., 1999). Drosophila Msps belongs to an evolutionarily conserved family of XMAP215/ch-TOG proteins that function as a key MT polymerase in animals, plants, and yeasts (Brouhard et al., 2008; Al-Bassam & Chang, 2011; Li et al., 2012). Subsequent DNA sequencing further revealed that l(3)810 deletes a 47-nt fragment of the msps coding region and presumably generates a truncated Msps protein with the only N-terminal fragment aa1-535 (Fig EV1C). Moreover, immunostaining analyses reveal that Msps protein was undetectable in msps<sup>810</sup> mutant ddaC mutants (n = 4, Fig EV1C), suggesting that l(3)810 is a null or strong hypomorphic allele of msps. Thus, we renamed l(3)810 and l(3)924 as msps<sup>810</sup> and msps<sup>924</sup>, respectively.
terminal TOG domains (Msps-TOGs), is sufficient to co-immunoprecipitate with TACC (Appendix Fig S2A). Reciprocally, Msps-C was specifically pulled down by TACC (Appendix Fig S2A). These experiments indicate that the C-terminal fragment of Msps is essential to mediate its association with TACC, similar to their mammalian counterparts (Thakur et al, 2014). Interestingly, unlike its Xenopus counterpart (Thawani et al, 2018), Msps did not form a protein complex with γ-Tub23C, a major somatic γ-tubulin, in various co-IP experiments using the extracts from S2 cells (Appendix Fig S3A) or adult brains (Appendix Fig S3B).

We next examined whether Msps regulates the stability of TACC in ddaC sensory neurons or vice versa. TACC, which was abundantly expressed in the control RNAi neurons at wL3 stage (n = 33; Fig 2D and H), was strongly reduced in tacc RNAi neurons (n = 16; Fig 2F and H). Interestingly, in msps RNAi (#1) ddaC neurons, TACC levels were significantly reduced in their soma at wL3 stage (n = 13; Fig 2E and H). Likewise, Msps expression, which was enriched in the control RNAi neurons (n = 28; Fig 2I and M), was largely eliminated in the msps RNAi neurons (n = 10; Fig 2J and M). Moreover, Msps levels also exhibited a significant decrease in the soma of ddaC neurons when TACC was knocked down (n = 15; Fig 2K and M). Consistent with those in ddaC neurons, total TACC protein levels were strongly decreased in the larval brain extracts from msps^810/msps^8 heterozygotes (Fig 2N). Total protein levels of Msps were also significantly reduced in the brain extracts from tacc^59/tacc^24 transheterozygotes (Fig 2O). As a control, knockdown of α-Tub84B, which led to a drastic reduction in overall MT density in ddaC neurons (n = 5; Appendix Fig S2B), did not alter the protein levels of Msps and TACC in ddaC neurons (n = 7 and 9, respectively; Fig 2G and L). These results suggest that the decrease in Msps or TACC protein levels is unlikely caused by reduced MT mass upon tacc or msps knockdown, respectively. In addition, the protein levels of Patronin were not affected in msps RNAi or tacc RNAi mutant neurons (n = 17 and 24, respectively; Appendix Fig S2C). Thus, Msps and TACC are required to stabilize each other in postmitotic neurons including ddaC neurons.

**TACC is required for dendrite pruning in ddaC neurons**

Since TACC binds and stabilizes Msps in neurons, we next investigated whether TACC, like Msps, is required for dendrite pruning. We made use of two independent RNAi lines targeting different tacc coding regions (#1, BL65982; #2, v101439). In contrast to no pruning defect in the control RNAi knockdown (n = 15, Fig 3A, D and E), knockdown of TACC, via one or two copies of ppr-Gal4 driver led to similar pruning defects in ddaC neurons at 16 h APF (#1, n = 16, Fig 3B, D and E; #2, n = 25, Fig EV2A). ddaC neurons expressing tacc RNAi #1 or #2 lines failed to prune their larval dendrites (87 and 36%, respectively; Figs 3D and EV2A) and led to the persistence of their larval dendrites (Figs 3E and EV2A). Due to lack of null tacc mutants, we took advantage of the CRISPR/Cas9 technology to generate two large deletions, tacc^59 and tacc^74, which uncover most of the tacc coding region (Fig EV2B). The vast majority of TACC protein was lost in the adult brains transheterozygous for tacc^59 and tacc^24 (Fig 20). Thus, tacc^59 and tacc^24 are two null or strong hypomorphic tacc alleles. Importantly, ddaC neurons derived from tacc^59/tacc^24 transheterozygotes exhibited simplified dendrite arbors at WP stage (n = 3, Fig EV2C) as well as dendrite pruning defects at 16 h APF with full penetrance (n = 24, Fig 3C-E). The dendrite pruning defect is unlikely caused by developmental delay, as the transheterozygous animals exhibited normal head eversion and developed until adulthood. Thus, multiple lines of genetic data demonstrate that TACC, like Msps, plays an important role in regulating dendrite pruning.

To further determine whether Msps and TACC act in a common pathway or in two parallel pathways during dendrite pruning, we first double knocked down msps and tacc and compared with their single RNAi phenotypes. RNAi knockdown of msps with the control gene (n = 16; Fig 3F, I and J) led to a stronger pruning phenotype than tacc RNAi plus control RNAi knockdown (n = 19; Fig 3G, I and J). Importantly, no significant enhancement was observed in dendrite pruning defects in mutant ddaC neurons expressing both msps and tacc RNAi constructs (n = 16; Fig 3H-J), compared to msps plus control RNAi knockdown (n = 16; Fig 3F, I and J). Likewise, double MARCM ddaC clones of msps^610 and tacc^59 showed pruning defects to a similar extent as msps^810 single-mutant clones (Fig EV2D). These double-mutant analyses indicate no genetic interaction between msps and tacc. Thus, these results, together with the Msps-TACC association, suggest that Msps and TACC act in the same pathway to regulate dendrite pruning. In addition, MARCM ddaC clones derived from either γ-tub23Ca^175:2 or γ-tub23Ca^149:4 mutant did not exhibit any pruning defects (n = 14 and 11, respectively; Appendix Fig S3C). Double knockdown of msps and γ-tub23C did not significantly enhance the pruning defects (n = 16; Appendix Fig S3D), compared to those in msps, control RNAi neurons (n = 24; Appendix Fig S3D). Thus, these data suggest that Msps acts to regulate dendrite pruning with TACC but independently of γ-tubulin in ddaC neurons.

**Msps and TACC are required for proper distribution of dendritic and axonal MT markers**

Msps and TACC were reported to promote MT growth as a MT polymerase in mitotic cells and postmitotic neurons (Brouhard et al, 2008; van der Vaart et al, 2012; Lowery et al, 2013; Nwagbara et al, 2014). We first attempted to investigate whether Msps and TACC regulate overall MT levels in ddaC sensory neurons. Indeed, overall microtubules, which are detected by either the microtubule-associated protein Futsch (22C10) or α-tubulin were significantly reduced in dendrites of msps RNAi ddaC neurons (n = 17 and 11, respectively; Appendix Fig S4A and B), suggesting that Msps is required for overall MT mass in ddaC neurons. We next examined the distribution of two MT markers, namely Nod-β-gal and Karβ-gal, in msps or tacc mutant neurons. The chimeric protein Nod-β-gal, a marker of MT minus ends in Drosophila (Clark et al, 1997), is enriched in dendrites but not in axons in da sensory neurons (Rolls et al, 2007; Zheng et al, 2008). In wild-type ddaC neurons, Nod-β-gal was specifically enriched in the dendrites and absent in the axons (n = 25, Fig 4A). Remarkably, Nod-β-gal was highly concentrated in the soma with a drastic reduction in the dendrites of all msps^810 (n = 6; Fig 4B and G) or msps RNAi (n = 12, Fig 4G) ddaC neurons. To quantify Nod-β-gal alterations in dendrites, we measured its intensity in the major dendrite fragments that are 40 μm away from the soma. In msps^810 (Fig 4H) or msps RNAi (Fig 4H) neurons, dendritic Nod-β-gal levels were drastically reduced to 4 and 23% of that in the control neurons, respectively.

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Figure 2.
Likewise, in all tacc59/tacc74 (n = 14; Fig 4C, G and H) or tacc RNAi (n = 14, Fig 4G and H), Nod-β-gal accumulated to their soma and/ or proximal dendrites with significant reduction in the distal dendrites (36 and 50% of the control levels, respectively). As controls, we also examined the distributions of cellular markers, such as Golgi oustpost marker (ManII-Venus) and mitochondrial marker (Mito-GFP). No aberrant accumulation of these two markers was observed in the soma of msps RNAi neurons (n = 8 and 11, respectively; Appendix Fig S4C). In the distal dendrites of msps or tacc RNAi neurons, mitochondria were reduced in number; however, more Golgi oustposts were present (Appendix Fig S4D). Thus, drastic Nod-β-gal accumulations in the soma with its reduced dendritic signals are unlikely caused by soma-to-dendrite traffic jam.

We next utilized the axon-specific marker Kin-β-gal which previously served as a marker of MT plus ends (Clark et al., 1997). Kin-β-gal localized exclusively in the axons, but was absent in the dendrites of wild-type ddaC neurons (n = 11; Fig 4D and I; Zheng et al., 2008). Remarkably, in msps RNAi or tacc RNAi ddaC neurons, Kin-β-gal was mis-localized to the dendrites (83%, n = 12, Fig 4E and I; 71%, n = 24, Fig 4F and I, respectively). Due to an unknown mechanism, Kin-LacZ often accumulated as several big blobs in msps RNAi dendrites (Fig 4E), which is different from its uniform distribution in the axons.

Thus, the Msps-TACC complex is required for proper distribution of dendrite or axon-specific MT markers in ddaC neurons.

The Msps-TACC complex is required for minus-end-out MT orientation in dendrites

In ddaC neurons, microtubules (MTs) are oriented minus-end-out in dendrites but plus-end-out in axons (Stone et al., 2008). The mis-localization of Nod-β-gal and Kin-β-gal markers in msps-depleted dendrites prompted us to further determine MT orientation in dendrites using the MT plus-end marker EB1-GFP. EB1-GFP labels plus ends of growing MTs, and its comet direction reflects MT orientation in neurons (Stepanova et al., 2003). In the major dendrites of wild-type ddaC neurons, EB1-GFP comets predominantly moved retrogradely toward the soma (97%, n = 18 neurons, 280 comets; Fig 5A and G). EB1-GFP comets were lost in the dendrites of msps810/msps810 transheterozygous mutant neurons (n = 24 neurons; Fig 5B), consistent with its role of Msps/XMAP215 as a MT polymerase (Brouhard et al., 2008; Li et al., 2012). We then took advantage of mspsP18, a weaker msps allele (Chen et al., 2016), to assess EB1-GFP signals. Indeed, msps810/mspsP18 transheterozygous ddaC neurons exhibited detectable EB1-GFP comets. Remarkably, anterograde EB1-GFP comets were significantly increased to 27% in the dendrites of msps810/mspsP18 neurons (n = 20 neurons, 220 comets; Fig 5C and G), compared to approximately 3% in the control neurons (Fig 5A and G). Similarly, anterograde EB1-GFP comets were significantly increased to 50 and 38% in the dendrites of tacc59/tacc74 transheterozygous neurons (n = 24 neurons, 431 comets, Fig 5F and G) neurons. These results, together with Nod-β-gal and Kin-β-gal data, demonstrate that both Msps and TACC are required for MT minus-end-out polarity in the dendrites of ddaC neurons. Of note, the length of EB1-GFP comet track, as measured by the distance of persistent MTs, was significantly reduced in msps810/mspsP18 (Fig 5H), tacc RNAi (Fig 5H), or tacc59/tacc74 transheterozygous (Fig 5I) ddaC neurons, compared to that in the control neurons (Fig 5H). The average number of EB1-GFP comets was reduced in msps810/mspsP18 ddaC neurons (Fig 5I), however, remained the same in tacc RNAi or tacc59/tacc74 transheterozygous neurons (Fig 5I). The average velocity of EB1-GFP comets was reduced in tacc RNAi or tacc59/tacc74 transheterozygous neurons (Fig 5J). The EB1-GFP comets in the dendrites of msps810/mspsP18 (Fig 5C) and tacc59/tacc74 (Fig 5F) mutant neurons appeared to be dimmer than those in the controls (Fig 5A and D). In contrast, we did not observe any MT orientation defect in the dendrites of mutant ddaC neurons transheterozygous for γ- tub23cA14-9 and γ-tub23cA15-2 (n = 6 neurons, 58 comets; Appendix Fig S3E).

Previous studies show that cytoskeletal disassembly or alterations can cause a neuronal stress response that restores MT levels or dynamics via JNK signaling (Massaro et al., 2009; Xiong et al., 2008). Remarkably, in Figure 1D–G, msps RNAi dendrites (Fig 4E), which is different from its uniform distribution in the axons.

Thus, the Msps-TACC complex is required for proper distribution of dendrite or axon-specific MT markers in ddaC neurons.
Figure 3. tacc is required for dendrite pruning in ddaC neurons.

A–C  Live confocal images of ddaC neurons visualized by ppk-Gal4-driven mCD8::GFP expression at WP or 16 h APF. While expression of control RNAi in ddaC neurons (A) did not affect the dendrite morphogenesis nor the pruning processes, knockdown of tacc using RNAi #1 (B) or tacc59/tacc74 transheterozygous mutant (C) severely perturbed the dendrite arborization as well as pruning. Red arrowheads point to the ddaC somas.

D  Percentages of ddaC neurons showing severing defects in (A–C) at 16 h APF.

E  Quantitative analysis of unpruned dendrite lengths in (A–C) at 16 h APF. Error bars represent SEM from three independent experiments. Statistical significances were determined by one-way ANOVA with Bonferroni test. ***P < 0.001, ns indicates statistically not significant. Scale bars represent 50 µm.

F–H  Live confocal images of ddaC neurons visualized by ppk-Gal4-driven mCD8::GFP expression at WP or 16 h APF. Simultaneous knockdown of tacc and msp5 via co-expressing tacc RNAi and msp5 RNAi (H) in the ddaC neurons did not enhance the pruning defects, compared to those neurons co-expressing control RNAi and msp5 RNAi (F). Red arrowheads point to the ddaC somas.

I  Percentages of ddaC neurons showing severing defects in (F–H) at 16 h APF.

J  Quantitative analysis of unpruned dendrite lengths in (F–H) at 16 h APF. Error bars represent SEM from three independent experiments. Statistical significances were determined by one-way ANOVA with Bonferroni test. ***P < 0.001, ns indicates statistically not significant. Scale bars represent 50 µm.

Data information: In (D, I), the numbers above the bars represent the sample sizes. Error bars represent SEM from three independent experiments. Statistical significances were determined by one-way ANOVA with Bonferroni test. ***P < 0.001, ns indicates statistically not significant. Scale bars represent 50 µm.

Source data are available online for this figure.
Figure 4.
To investigate whether knockdown of msp5 or tacc causes the induction of JNK signaling, we utilized the puc-LacZ reporter to examine the level of JNK signaling in msp5 or tacc RNAi ddaC neurons. In the control ddaC neurons, puc-lacZ was expressed at a low level in their nuclei \((n = 17, \text{Fig EV3A; } n = 19, \text{Appendix Fig SS5A})\). However, its expression levels were significantly increased when either msp5 \((n = 18, \text{Fig EV3A; } n = 19, \text{Appendix Fig SS5A})\) or tacc \((n = 18, \text{Appendix Fig SS5A})\) was knocked down. The puc-lacZ level in msp5 RNAi neurons was increased more drastically than that in tacc RNAi neurons (Appendix Fig SS5A). We first inhibited the JNK pathway via the treatment with GNE-3511, a potent JNK inhibitor (Feng et al., 2019). GNE-3511 treatment, which completely inhibited the induction of puc-lacZ (Fig EV3A), did not rescue the mixed MT orientation defect in msp5 RNAi \((n = 15, \text{Fig EV3B; } \text{or } \text{tacc RNAi } (n = 14, \text{Fig EV3C}) ddaC neurons, as shown by either Nod-β-gal distribution or EB1-GFP comet direction, respectively. The induction of JNK signaling could also be fully inhibited by the expression of JNKDN, the dominant-negative form of JNK that is encoded by bsk in Drosophila. JNKDN expression did not rescue the defect in Nod-β-gal distribution in msp5 RNAi neurons \((#1, n = 15; #2, n = 17, \text{Appendix Fig SS5B})\). Moreover, both GNE-3511 treatment and JNKDN expression did not rescue the dendrite pruning defects in msp5 RNAi neurons \((n = 12, \text{Fig EV3D; } \#1 \text{ and } #2, n = 16 \text{ and } 16, \text{respectively; Appendix Fig SS5C})\). Thus, Msp5 regulates dendritic MT orientation and dendrite pruning independently of JNK signaling.

Collectively, the Msp5-TACC MT polymerase complex plays an important and JNK-independent role in regulating minus-end-out MT orientation in the dendrites of ddaC neurons.

**Mps6 function is antagonized by the kinesin-13 MT depolymerase Klp10A during dendrite pruning**

Mps6/XMAP215 family proteins localize at MT plus ends to mediate MT growth (Brouhard et al., 2008; Al-Bassam & Chang, 2011), whereas kinesin-related MT depolymerases including Drosophila kinesin-13 Klp10A and its ortholog MCAK are targeted to MT plus ends by EB-1 and induce MT catastrophe at MT ends (Hunter et al., 2003; Mennella et al., 2005). An antagonism between Mps6/XMAP210 and Klp10A/MCAK determines dynamic instability of MTs and spindle size in mitotic cells (Howard & Hyman, 2007). To test potential antagonism between Mps6 and Klp10A during dendrite pruning, we further eliminated Klp10A activity in mps6 RNAi ddaC neurons to examine whether mps6 mutant phenotypes could be rescued. Remarkably, knockdown of klp10A (RNAi #1) in mps6 RNAi neurons significantly restored Nod-β-gal distribution in dendrites \((n = 16, \text{Fig 6B and M})\), compared to mps6, control RNAi neurons \((n = 16, \text{Fig 6A and M})\). Consistently, knockdown of klp10A in mps6 RNAi significantly restored the retrograde movement of dendritic EB1-GFP comets \((n = 29, \text{comets, Fig 7B and G})\), compared to the mps6 RNAi controls \((n = 20 \text{ neurons, } 150 \text{ comets, Fig 7A and G})\). Moreover, knockdown of klp10A largely restored overall MT levels in dendrites of mps6 RNAi neurons \((n = 19, \text{Fig EV4A})\). These data suggest an antagonism between Mps6 and Klp10A in regulating MT orientation and density in dendrites. More strikingly, both dendrite pruning and dendrite arborization defects in mps6 RNAi ddaC neurons were almost fully rescued by knockdown of klp10A \((n = 16, \text{Fig 6H, 6O and P})\), in contrast to those in mps6, control RNAi neurons \((n = 12, \text{Fig 6G, O and P})\). As a control, knockdown of klp10A alone did not disturb dendrite pruning in ddaC neurons \((n = 31, \text{Fig 6O and P})\). Thus, these data suggest that the antagonism between Mps6 and Klp10A is important for both dendritic MT orientation and dendrite pruning.

Given that both impaired MT orientation and reduced MT density were observed in dendrites of mps6 mutant ddaC neurons, we next investigated which one might lead to the dendrite pruning defect in mps6 neurons. To this end, we examined two other MT-related proteins (Prefoldin5/Pfdn5 and Futsch) that regulate tubulin assembly or MT stability (Geissler et al., 1998; Hummel et al., 2000). Although knockdown of pfdn5 or futsch showed a significant reduction in the number of EB1-GFP comets (Fig EV4C), dendritic MT orientation was not impaired (Fig EV4C). Overall MT density was significantly reduced in the dendrites of pfdn5 RNAi ddaC dendrites \((n = 15; \text{Fig EV4B})\). Importantly, dendrite pruning was not affected in either pfdn5 or futsch RNAi ddaC neurons \((n = 19 \text{ and } 15, \text{respectively; Fig EV4D})\). Thus, these data suggest that impaired MT orientation, rather than reduced MT density, is likely responsible for the dendrite pruning defects.

**Excessive MT depolymerization perturbs dendritic MT orientation and dendrite pruning**

Mps6 functions are antagonized by the MT depolymerase Klp10A, raising the possibility that excessive MT depolymerization might lead to dendritic MT orientation and dendrite pruning defects in
Figure 5.
msps mutant neurons. To examine this possibility, we induced MT depolymerization via both pharmacological and genetic approaches. First, we took advantage of two well-characterized MT-destabilizing agents (MDAs), namely colchicine and vinblastine. Colchicine and vinblastine were reported to induce MT catastrophe at the plus ends (Mohan et al., 2013). We first optimized a low concentration of colchicine and fed 72 h AEL larvae for 1 or 2 days. Under such a mild treatment condition, animals completed head eversion at 12 h APF and survived until the pharate adult stage, suggesting normal progression of metamorphosis. Moreover, this mild colchicine treatment did not affect the average number of primary and secondary dendrites in ddaC neurons at WP stage (n = 14; Fig 6J and Appendix Fig S6A). Strikingly, Nod-β-gal exhibited drastic accumulation in the soma with a severe reduction in the dendrites (22% of the control intensity) in all neurons (n = 16; Fig 6D and N), compared to non-treated controls (n = 12; Fig 6C and N). In the larvae treated with colchicine at a lower concentration, anterograde EB1-GFP comets were significantly increased to 15% in the dendrites (n = 31 neurons, 402 comets; Fig 7D and G), indicative of a MT orientation defect. The average track length of EB1-GFP comets drastically decreased (Fig 7H), whereas the average EB1-GFP comet number significantly increased in these neurons (Fig 7I). However, the average velocity of EB1-GFP comets remained unaltered (Fig 7J). Importantly, Kat-60 overexpression resulted in ectopic branches at the proximal dendrites of all ddaC neurons (n = 18, Fig 6L), which were also observed in msps or tacc mutant neurons (Appendix Fig S1A and Fig EV2C). Dendrite severing was inhibited in most of Kat-60-overexpressing ddaC neurons at 16 h APF (63%, n = 30; Fig 6L, O and P), compared to no severing defect in the control neurons (0%, n = 29; Fig 6L, O and P). In contrast, Kat-60L1 overexpression did not affect dendrite pruning (n = 15; Fig EV5B). Finally, similar to that in msps RNAi neurons, overall microtubule levels were significantly reduced in dendrites of colchicine/vinblastine-treated and Kat-60-overexpressing ddaC neurons (Fig EV5C), as detected by the anti-α-Tubulin antibody. Thus, MDA treatment and katanin overexpression phenotype msps mutants.

In summary, we provide multiple lines of genetic, cell biological, and pharmacological evidence demonstrating that the conserved MT polymerase MspS is required to form minus-end-out MTs in dendrites and thereby promotes dendrite pruning in ddaC sensory neurons (Fig 8).

**Discussion**

Despite that MT disassembly precedes neuronal pruning, major MT-severing factors appear to be dispensable for dendrite pruning in...
ddaC neurons (Lee et al., 2009; Stone et al., 2014; Tao et al., 2016). Here, we have identified an unexpected role of a key MT poly-
merase MspS in dendrite pruning of ddaC neurons. First, genetic
analyses with *msps* mutants, the rescue experiments, and additional
RNAi lines highlight an important role of *msps* in regulating
dendrite pruning in ddaC neurons. Second, we show that MspS
forms an *in vivo* protein complex with TACC in adult neurons; both
proteins stabilize each other in ddaC neurons independently of MT

Figure 6.
mass. Third, TACC, like Msp, is required for dendrite pruning. Fourth, the dendrite pruning defects in msp knockdown neurons are likely caused by excessive Klp10A, a MT-depolymerizing kinesin. Consistently, excessive MT depolymerization, which is induced by both MDA treatment and katanin overexpression, resembles all msp loss-of-function phenotypes with regard to both dendritic MT orientation and pruning. Thus, Msp plays an important role in dendrite pruning in ddaC sensory neurons (Fig 8).

The vertebrate orthologs of Msp were reported to regulate MT dynamics and axon outgrowth in neurons from frogs and mammals (van der Vaart et al., 2012; Lowery et al., 2013), but their roles in neuronal MT orientation remain unknown in both invertebrates and vertebrates. In this study, we demonstrate, for the first time, that Mps and its binding partner TACC play an important role in governing minus-end-out orientation of dendritic MTs, which is very likely required for dendrite pruning in ddaC sensory neurons. TACC is able to recruit Msp to centrosomal MTs during mitosis, and both proteins move toward the plus ends in mitotic cells of early Drosophila embryos (Lee et al., 2001). Thus, TACC might also target Msp to the MT plus ends to polymerize minus-end-out MTs in the dendrites of ddaC neurons.

How does Msp regulate MT minus-end-out orientation in ddaC dendrites? Msp might associate with the MT plus ends and promote persistent MT growth against MT depolymerization events in ddaC dendrites, which leads to the formation of long and stable MT filaments. Plus-end motors kinesins, via MT guidance or sliding, may orient growing MTs in a minus-end-out orientation in the dendrites (Mattie et al., 2010; Yan et al., 2013). These minus-end-out MTs may form stable bundles that anchor within the dendrites. In the absence of Msp, dendritic MTs are depolymerized from their plus ends or along the lattices mediated by excessive depolymerization activity of kinesin-13 and/or other MT-severing factors. It has been shown that microtubule-severing factors are able to sever MTs into short pieces (McNally & Vale, 1993), which may serve as MT seeds. Short MT seeds might be re-oriented in either plus-end-out or minus-end-out direction with equal probability, resulting in a mixed MT polarity in the dendrites of msp mutant neurons. In line with this idea, knockdown of the MT-depolymerizing factor Klp10A in msp mutant neurons significantly restored minus-end-out MT filaments in the dendrites. Moreover, MDA treatments and Kat-60 overexpression that depolymerize MTs from the MT ends also led to mixed MT orientation in dendrites, identical to msp loss-of-function mutants. The Msp vertebrate ortholog XMAP215 interacts with γ-tubulin via its carboxyl-terminal portion to synergistically stimulate MT nucleation in vitro (Thawani et al., 2018). Interestingly, γ-tubulin was reported to regulate the minus-end-out MT orientation in dendrites of class I da sensory neurons (Nguyen et al., 2014). It is conceivable that Mps and γ-tubulin might be part of the asymmetric MT nucleation machinery which is responsible for the generation of non-centrosomal MTs with a minus-end-out orientation in dendrites of ddaC neurons. Unexpectedly, we found no notable biochemical/genetic interaction between Mps and γ-tubulin in Drosophila. Future studies will be required to identify such an asymmetric MT nucleation/polymerization machinery in neurons.

Our study supports a link between dendritic MT orientation and dendrite pruning defect. Both msp and TACC mutant ddaC neurons, which showed impaired MT orientation in dendrites, had dendrite pruning defects. Moreover, knockdown of klp10A restored the minus-end-out MT orientation in dendrites and rescued the dendrite pruning defect in msp RNAi or mutant neurons. MDA treatment and katanin overexpression led to impaired MT orientation as well as dendrite pruning defects, phenocopying msp mutant neurons. Growing evidence supports a likely causal link between minus-end-out MT orientation and dendrite pruning. First, Cnn and APC1/2, two known regulators of dendritic MT orientation (Mattie et al., 2010; Yalgin et al., 2015), are required for dendrite pruning (Wang et al., 2019). Second, the Rumpf laboratory has recently reported that both kinesin-1 and kinesin-2 mutant ddaC neurons that exhibited mixed dendritic MT orientations (Mattie et al., 2010) had dendrite pruning defects (Herzmann et al., 2018). Third, we and others have recently reported that the MT minus-end-binding protein Patronin, which also regulates uniform minus-end-out MT orientation in dendrites (Feng et al., 2019; Wang et al., 2019), is also critical for dendrite pruning (Wang et al., 2019). In contrast, we further show here that knockdown of other MT-related proteins, Pδn5 and Futsch, which did not affect minus-end-out MT orientation in dendrites, did not cause any dendrite pruning defect in ddaC neurons. Thus, all these data strongly support a likely causal link between minus-end-out MT orientation and dendrite pruning.
Figure 7.
How mis-oriented MT arrays impact on dendrite pruning is currently unknown. It is conceivable that dendrites with a minus-end-out MT orientation might be more susceptible to dendrite pruning. Severing of dendrites usually takes place at proximal region of dendrite arbors, where the plus ends of MTs are enriched, raising the possibility that the dynamic plus ends are more prone to severing and disassembly. Alternatively, organelles, for example, endosomes and lysosomes, move along the minus-end-out dendritic MTs in ddaC neurons (Satoh et al., 2008), which might facilitate the efficient endo-lysosomal degradation of Nrg during dendrite pruning (Zhang et al., 2014).

In summary, this study reveals a new paradigm that a conserved MT polymerase Msps plays an important role in dendrite pruning in Drosophila sensory neurons. Furthermore, we show that Msps is required to regulate dendritic MT orientation and thereby promotes dendrite pruning.

**Materials and Methods**

**Fly strains**

ppk-Gal4 on II and III chromosome (Grueber et al., 2003), SOP-flp (#42) (Matsubara et al., 2011), msps\(^8\) (Cullen et al., 1999), g-msps (HN267) (Cullen et al., 1999), UAS-Mical\(^N\)-co (Terman et al., 2002), UAS-EB1-GFP (Stone et al., 2008), UAS-Kin-\(\beta\)-gal (Clark et al., 1997), UAS-Venus-Kat-60L1 (Stewart et al., 2012), UAS-Man\(\beta\)-Ven (Wang et al., 2017), UAS-ManII-GFP (Ye et al., 2007), UAS-\(\gamma\)Tub23C-GFP (Nguyen et al., 2014), msps\(^18\)\(^\alpha\) (Chen et al., 2016), puc-lacZ (Martin-Blanco et al., 1998), tacc\(^{59}\), tacc\(^{74}\), msps\(^810\), and msps\(^924\) (this study).

The following stocks were obtained from Bloomington Stock Centre (BSC): UAS-mCD8::GFP, FRT82B, UAS-Dicer2, tacc RNAi #1 (BL#65982), ppk-CD4-tdGFP (BL#35842), Gal4\(^{47-77}\) (BL#8737), UAS-
CD4-tdtom (BL#35841), UAS-Nod-β-gal (BL#9912), nanos-Cas9 (BL#54591), klp10A RNAi #1 (BL#33963), tubP-Gal80, Gal4109/L80, elav-Gal4C155 (BL#458), UAS-Kat-60 (BL#64115), UAS-Mito-GFP (BL#8442), γ-tub23C115-2 (BL#7042), γ-tub23C114-7 (BL#7041), UAS-JNKDN(BskDN) #1 (BL#6409), and UAS-JNKDN (BskDN) #2 (BL#9311).

The following stocks were obtained from the Vienna Drosophila RNAi Centre (VDRC): mpsps RNAi #1 (v21982), control RNAi (v36355, v25271), tacc RNAi #2 (v101439), γ-tub23C RNAi (v19130), fustch RNAi (v6972), pfdn5 RNAi (v29812), and γ-tub84B RNAi (v33427).

The following stocks were obtained from National Institute of Genetics, Japan: msps RNAi #2 (5000R-3).

**EMS mutagenesis**

Isogenized w*;FRT82B male flies were fed with 25 mM EMS. Mutant chromosomes were balanced over TM6B prior to isolation of lethal or semi-lethal lines. These lines were then used for the following MARCM analysis.

**MARCM analysis of da neurons**

Embryos were collected every 1–2 days and cultivated on cornmeal food at 25°C. For analyzing dendrite phenotype of ddaC, ddaD/E, or ddaF neurons, animals at WP stage were first collected onto moistened tissue paper at 25°C overnight. Pupal cases were removed for confocal imaging of da neurons at 16 or 19 h APF correspondingly. MARCM clones labeled by GFP were analyzed using confocal Microscopy.

**Brain protein extraction for Western blot and co-immunoprecipitation (co-IP)**

Larval brains or adult fly heads were dissected and ground in 1 to 1 mixture of lysis buffer (Pierce, Cat#87788) and loading dye. Standard Western blots were conducted immediately to analyze protein contents. For co-IP, flies were collected and decapitated by vigorous shaking after frozen. The lysis buffer (Pierce, Cat#87788) with freshly added protease inhibitor (Roche, Cat#11697498001) was used to extract proteins from the fly heads. GFP or Venus-tagged proteins were immunoprecipitated with anti-GFP beads (Chromotek, GFP-Trap A) and subjected to standard Western blot analyses. Each Western blot or co-IP assay was repeated for 3–6 times.

**Cell culture and co-IP**

S2 cells were maintained in Express Five SFM (Life Tech) supplemented with 1% l-glutamine (Life Tech) at 25°C. Transfections of destination vectors were conducted following Qiagen Effectene transfection procedure (Qiagen, Cat#301427). Transfected S2 cells were homogenized with lysis buffer (25 mM Tris pH 8, 27.5 mM NaCl, 20 mM KCl, 25 mM sucrose, 1 mM DTT, 10% (v/v) glycerol, 0.5% NP40, and protease inhibitors), as previously described (Wong et al., 2013). Standard Western blot was then conducted to analyze co-immunoprecipitated protein components. Each co-IP assay was repeated for three times.

**Immunohistochemistry**

w1118 larvae and WP samples for each set of experiments were dissected in cold PBS and processed simultaneously. For Futsch (22C10) and α-tubulin staining, the following fixation procedures were performed in order to assess MT mass without unpolymerized tubulin subunits. Larvae were dissected in cold Ca²⁺+-free HL3.1 saline, and their muscles were removed (Yalgin et al., 2011; Tenenbaum & Gavis, 2016). The dissected fillets were fixed in freshly prepared PHEM fixing buffer with 0.25% glutaraldehyde, 3.7% paraformaldehyde, 3.7% sucrose, and 0.1% Triton X-100. The samples were then quenched with 50 mM ammonium chloride for 5 min (Witte et al., 2008). The samples within the same group of experiments were stained in the same tube and mounted in VECTASHIELD mounting medium. The samples were directly visualized by Leica SPE-II confocal microscope and processed in parallel. Data analysis and statistics were performed via Excel (Microsoft) and GraphPad Prism software.

**Antibodies**

The following antibodies and dilutions were used in this study: Guinea pig anti-Msps (IF 1:500, WB 1:3,000, a gift from H. Wang), rabbit anti-TACC (IF 1:500; WB 1:3,000, a gift from J.W. Raff and H. Wang), mouse anti-β-Galactosidase (IF 1:1,000, Promega, Cat#Z3781), rabbit anti-GFP (WB 1:5,000, Invitrogen, Cat#A11212), mouse anti-Futsch (IF 1:50, 22C10, DSHB), rabbit anti-beta actin (WB 1:3000, Abcam, Cat#ab8227), mouse anti-alpha tubulin (WB 1:3,000, IF 1:500, Sigma, Cat#T9026), mouse anti-γ-tubulin (1:500, Sigma, Cat#T5326), rabbit anti-Patronin (a gift from M. Gonzalez-Gaitan, 1:500), mouse anti-Myc (WB 1:3,000, Sigma, Cat#M4439), mouse anti-Myc-HRP (WB 1:10,000, Invitrogen, Cat#R951-25), rabbit anti-Flag (WB 1:3,000, Sigma, Cat#F7425), mouse anti-Flag-HRP (WB 1:10,000, Sigma, Cat#ab8592), Cy3-conjugated goat anti-mouse antibody, Cy3-conjugated goat anti-Guinea pig antibody, 488-conjugated goat anti-rabbit antibody, Cy3-conjugated goat anti-rabbit antibody, and 649-conjugated goat anti-HRP antibody (IF 1:500, Jackson Laboratories, Cat#115-165-003, Cat#106-165-003, Cat#101-165-003, Cat#ab545-003, Cat#123-495-021).

**MDA treatments**

Embryos were collected at 12-h intervals and cultivated on standard food. The larvae were then transferred to the food containing 20 μg/ml colchicine (Sigma Aldrich Cat#C9754), 15 μM vinblastine (Tocris Cat#1256), or 50 μM GNE-3511 (Millipore CAS#5.33168.0001). WPs were collected after 1–2 days of drug feeding and used for confocal imaging at 16 h APF. Wandering 3rd instar larvae were collected after 1–2 days of drug feeding and used for anti-Msps staining. For EB1-GFP movies, a lower concentration of 1 μM GNE-3511 was used for the treatment, and 96 h AEL larvae were collected after 1 day of drug feeding and used for live imaging of EB1-GFP.

**Plasmid construction**

The coding sequences of msps, tacc, kat-60, kat-60L1, and kat-80 were amplified from EST clones (DGRC, Bloomington). The
respective fragments were cloned into Gateway entry vectors pENTR/D-TOPO or pDONR/Zeo (Invitrogen), followed by cloning into Gateway destination vectors (pAMW, pAFW) (DGRC) via LR reaction.

**Generation of tacc mutants via CRISPR/Cas9**

Two different guide RNAs (gRNAs) targeting tacc exons were cloned into the pCFD4 vector following the standard procedures published previously (Port et al., 2014). The following primer set was used: 5’-TATATAGAAAGATATCCGCTGAACTTCGCAACGTCAGCTATGAAGCCAGTTTTAGAGCTAGAAATTAGCAAG-3’, 5’-ATTCTAATCTGCTATTTCTTATATAGGAAAGATATCCGGGTGAACTTCGCAAGCCTTTTAAAACATCTCTAATGACGTCTCAGCTCACGCTTAAATTTGAAATAGGTC-3’. Transgenic flies were generated by BestGene Inc and crossed with nanos-Cas9 flies to generate mutant tacc lines. Mutants with large genomic deletions were isolated by PCR and confirmed by DNA sequencing. Embryo microinjection services were provided by BestGene Inc.

**Live imaging of EB1-GFP comet**

Larvae at desired developmental stages were immersed with halocarbon oil (Santa Cruz, Cat#sc-250077) and mounted to slides for confocal imaging. Time-lapse imaging of EB1-GFP comet was performed with Olympus FV3000 using 60x oil lens with 3x zoom. Eighty-three frames were acquired at 2.25-s intervals with 6 Z-steps. Kymographs were generated for Z-projected time-lapse images using KymographBuilder plugin in ImageJ.

**Analysis of ddaC dendrites**

Dendrite images for larvae and WP were taken using Leica SPE-II with 40x oil lens. To image full arbor of ddaC neurons at different stages, multiple images were acquired and stitched using ImageJ plugin MosaicJ. Dendritic termini number was calculated using plugin Simple Neurite Tracer. The severing defect was defined by the presence of dendrites that remain attached to the soma at 16 h APF. The total length of unpruned dendrites was measured in a 275 × 275 μm region of the dorsal dendritic field using ImageJ. Sholl analyses of dendrite morphogenesis were conducted using ImageJ. Plots of average length, number of intersections, and SEM were generated using GraphPad Prism software.

**Quantification of immunostaining**

Images were acquired from projected z-stacks (at 1.5 μm intervals) to cover the entire volume of ddaC/D/E sensory neurons using the confocal microscopy Leica TSC SP2. To quantify the fluorescence intensities, cell nuclei (puc-lacZ) or whole soma (Patronin/Msp1/TACC immunostaining) contours were drawn on the appropriate fluorescent channel based on the GFP channel or relative cellular position in ImageJ software. After subtracting the background (Rolling Ball Radius = 30) on the entire image of that channel, we measured the mean gray value in the marked area in ddaC and/or ddaE on the same images and calculated their ratios. The ratios were normalized to the corresponding average control values and subjected to statistical analysis for comparison between different conditions. Graphs display the average values of ddaC soma/nucleus or ddaC/ddaE ratios and the standard error of the mean (SEM) normalized to controls. The number of ddaC neurons (n) examined in each group is shown on the bars. Inset shows the ddaC neurons labeled by ppk-Gal4-driven UAS-mCD8-GFP expression.

To quantify the alterations of dendritic Nod-β-gal distribution, we measured its intensity in the 20 μm of major dorsal dendrites which were 40 μm away from the soma. The number of ddaC neurons (n) examined in each group is shown on the bars. Dorsal is up in all images.

**Statistics**

For pairwise comparison, two-tailed Student’s t-test was applied to determine statistical significance. One-way ANOVA with Bonferroni test was applied to determine significance when multiple groups were present. Error bars in all graphs represent standard error of the mean (SEM). Statistical significance was defined as ***P < 0.001, **P < 0.01, *P < 0.05, n.s., not significant. The number of samples (n) in each group is shown on the bars.

**Expanded View for this article is available online.**

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**Author contributions**

FY, QT, and YW conceived and designed the study. QT performed most of the experiments. MR conducted some Western blot and JNK experiments. SB performed some EB1-GFP movies, JNK, and drug treatment experiments. YW conducted some EB1-GFP and Nod-β-gal experiments. LYC carried out some JNK experiments. QT, YW, and FY analyzed the data. FY and QT wrote the paper.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**

Akhmanova A, Steinmetz MO (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol 9: 309–322

Akhmanova A, Steinmetz MO (2015) Control of microtubule organization and dynamics: two ends in the limelight. Nat Rev Mol Cell Biol 16: 711–726

Ali-Bassam J, Chang F (2011) Regulation of microtubule dynamics by TOS-domain proteins XMAP215/Dis1 and CLASP. Trends Cell Biol 21: 604–614

Baas PW, Detch JS, Black MM, Banker GA (1998) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci USA 85: 8335–8339

Bellanger JM, Gonczy P (2003) TAC-1 and ZYG-9 form a complex that promotes microtubule assembly in C. elegans embryos. Curr Biol 13: 1488–1498
Brouhard GJ, Stear JH, Noetzel TL, Al-Bassam J, Kinoshita K, Harrison SC, Howard J, Hyman AA (2008) XMAP215 is a processive microtubule polymerase. Cell 132: 79 – 88

del Castillo U, Winding M, Lu W, Gelfand VI (2015) Interplay between kinesin-1 and cortical dynein during axonal outgrowth and microtubule organization in Drosophila neurons. Elife 4: e10140

Chen K, Koe CT, Xing ZB, Tian X, Rossi F, Wang C, Tang Q, Zong W, Hong WJ, Taneja R et al (2016) An2- and Msps-dependent microtubule growth governs asymmetric division. J Cell Biol 212: 661 – 676

Clark IE, Jan LY, Jan YN (1997) Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the Drosophila oocyte, epithelium, neuron and muscle. Development 124: 461 – 470

Cullen CF, Deak P, Glover DM, Ohkura H (1999) mini spindles: a gene encoding a conserved microtubule-associated protein required for the integrity of the mitotic spindle in Drosophila. J Cell Biol 146: 1005 – 1018

Cullen CF, Ohkura H (2001) Msps protein is localized to acentrosomal poles to ensure bipolarity of Drosophila meiotic spindles. Nat Cell Biol 3: 637 – 642

Diaz-Velazco JD, Morelli MM, Bailey M, Zhang D, Sharp DJ, Ross JL (2011) Drosophila katanin-60 depolymerizes and sever at microtubule defects. Biophys J 100: 2440 – 2449

Feng C, Thayagarajan P, Shorey M, Seebold DY, Weiner AT, Albertson RM, Rao KS, Sagasti A, Goetschius DJ, Rolls MM (2019) Patronin-mediated minus end growth is required for dendritic microtubule polarity. J Cell Biol 218: 2309 – 2328

Geissler S, Siegers K, Schiebel E (1998) A novel protein complex promoting formation of functional alpha- and gamma-tubulin. EMBO J 17: 952 – 966

Gergely F, Draviam VM, Raff JW (2003) The ch-TOG/XMAP215 protein is essential for spindle pole organization in human somatic cells. Genes Dev 17: 336 – 341

Goodwin PR, Sasaki JM, Juo P (2012) Cyclin-dependent kinase 5 regulates the polarized trafficking of neuropeptide-containing dense-core vesicles in Caenorhabditis elegans motor neurons. J Neurosci 32: 8158 – 8172

Grueber WB, Ye B, Moore AW, Jan LY, Jan YN (2003) Dendrites of distinct classes of Drosophila sensory neurons show different capacities for homotypic repulsion. Curr Biol 13: 618 – 626

Gutierrez-Caballero C, Burgess SG, Bayliss R, Royle SJ (2012) A genetic pathway composed of Sox14 and Mical governs severing of dendrites during pruning. Neuron 73: 1005 – 1015

Hummel T, Krukke K, Roos J, Davis G, Klampt C (2000) Drosophila Futsch/22C10 is a MAP18-like protein required for dendritic and axonal development. Neuron 26: 357 – 370

Hunter AW, Caplow M, Coy DL, Hancock WO, Dize S, Wordeman L, Howard J (2003) The kinesin-related protein MCAK is a microtubule depolymerase that forms an ATP-hydrolysing complex at microtubule ends. Mol Cell 11: 445 – 457

Kanamori T, Kanai M, Dairyo Y, Yasunaga K, Morikawa RK, Emoto K (2013) Compartmentalized calcium transients trigger dendrite pruning in Drosophila sensory neurons. Science 340: 1475 – 1478

Kanamori T, Togashi K, Koizumi H, Emoto K (2015) Dendritic remodeling: lessons from invertebrate model systems. Int Rev Cell Mol Biol 318: 1 – 25

Kanistina K, Noetzel TL, Pelletier L, Mechtler K, Drechsl E, Schwager A, Lee M, Raff JW, Hyman AA (2005) Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis. J Cell Biol 170: 1047 – 1055

Koizumi T, Togashi K, Koizumi H, Emoto K (2015) Dendritic remodeling: lessons from invertebrate model systems. Int Rev Cell Mol Biol 318: 1 – 25

Kirillo D, Yu C, Huang Y, Wu Z, Bashirullah A, Low BC, Kolodkin AL, Wang H, Yu F (2009) A genetic pathway composed of Sox14 and Mical governs severing of dendrites during pruning. Nat Neurosci 12: 1497 – 1505

Kirillo D, Wong J, Lim EK, Wang Y, Zhang H, Wang C, Liao Q, Wang H, Liou YC, Yu F (2011) Intrinsic epigenetic factors cooperate with the steroid hormone ec dysone to govern dendrite pruning in Drosophila. Neuron 72: 86 – 100

Kuo CT, Jan LY, Jan YN (2005) Dendrite-specific remodeling of Drosophila sensory neurons requires matrix metalloproteinases, ubiquitin-proteasome, and ec dysone signaling. Proc Natl Acad Sci USA 102: 15230 – 15235

Lee T, Luo L (2001) Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neuronal development. Trends Neurosci 24: 251 – 254

Lee MJ, Gergely F, Jeffers K, Peak-Chew SY, Raff JW (2001) Msps/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour. Nat Cell Biol 3: 643 – 649

Lee HH, Jan LY, Jan YN (2009) Drosophila Ikk-related kinase ik2 and Katanin p60-like 1 regulate dendrite pruning of sensory neuron during metamorphosis. Proc Natl Acad Sci USA 106: 6363 – 6368

Li W, Moriwaki T, Tani T, Watanabe T, Kaibuchi K, Goshima G (2012) Reconstitution of dynamic microtubules with Drosophila XMAP150, EB1, and Sentin. J Cell Biol 199: 849 – 862

Loncle N, Williams DW (2012) An interaction screen identifies headcase as a regulator of large-scale pruning. J Neurosci 32: 17086 – 17096

Lowery LA, Stout A, Faris AE, Ding L, Baird MA, Davidson MW, Danuser G, Van Vactor D (2013) Growth cone-specific functions of XMAP215 in restricting microtubule dynamics and promoting axonal outgrowth. Neural Dev 8: 22

Luo L, O’Leary DD (2005) Axon retraction and degeneration in development and disease. Annu Rev Neurosci 28: 127 – 156

Maniak TA, Kaplan M, Wang GJ, Shen K, Wei L, Shaw JE, Koushika SP, Bargmann CI (2011) UNC-33 (CRMP) and ankyrin organize microtubules and localize kinesin to polarize axon-dendrite sorting. Nat Neurosci 15: 48 – 56

Mao CX, Xiong Y, Xiong Z, Wang Q, Zhang YQ, Jin S (2014) Microtubule-severing protein Katanin regulates neuromuscular junction development and dendritic elaboration in Drosophila. Development 141: 1064 – 1074

Martin-Blanco E, Gampel A, Ring J, Virdee K, Kirov N, Tolkovsky AM, Martinez-Arias A (1998) Puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev 12: 557 – 570

Massaro CM, Pielage J, Davis GW (2009) Molecular mechanisms that enhance synapse stability despite persistent disruption of the spectrin/ankyrin/ microtubule cytoskeleton. J Cell Biol 187: 101 – 117

Matsubara D, Horisuchi SY, Shimono K, Usui T, Uemura T (2011) The seven-pass transmembrane cadherin Famingo controls dendritic self-avoidance via its binding to a LIM domain protein, Espinas, in Drosophila sensory neurons. Genes Dev 25: 1982 – 1996
Nwagbara BU, Faris AE, Bearce EA, Erdogan B, Ebbert PT, Evans MF, Port F, Chen HM, Lee T, Bullock SL (Stone MC, Albertson RM, Chen L, Rolls MM (Stepanova T, Slemmer J, Hoogenraad CC, Lansbergen G, Dortland B, De Stewart A, Tsubouchi A, Rolls MM, Tracey WD, Sherwood NT (Schuldiner O, Yaron A (2020 of Mattie FJ, Stackpole MM, Stone MC, Clippard JR, Rudnick DA, Qiu Y, Tao J, Allender DL, Parmar M, Rolls MM (2010) Directed microtubule growth, +TIPs, and kinesin-2 are required for uniform microtubule polarity in dendrites. Curr Biol 20: 2169 – 2177

McNally FJ, Vale RD (1993) Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell 75: 419 – 429

Mennella V, Rogers GC, Rogers SL, Buster DW, Vale RD, Sharp DJ (2005) Functionally distinct kinesin-13 family members cooperate to regulate microtubule dynamics during interphase. Nat Cell Biol 7: 235 – 245

Mohan R, Katrukha EA, Doodhi H, Smal I, Meijering E, Kapitein LC, Steinmetz MO, Akhmanova A (2013) End-binding proteins sensitize microtubules to the action of microtubule-targeting agents. Proc Natl Acad Sci USA 110: 8900 – 8905

Nguyen MM, McCracken CJ, Milner ES, Goetschius DJ, Weiner AT, Long MK, Michael NL, Munro S, Rolls MM (2014) Gamma-tubulin controls neuronal microtubule polarity independently of Golgi outposts. Mol Biol Cell 25: 2039 – 2050

Nwagbara B, Faris AE, Bearce EA, Erdogan B, Ebbert PT, Evans MF, Rutherford EL, Enzenbacher TB, Lowery LA (2014) TACC3 is a microtubule plus end-tracking protein that promotes axon elongation and also regulates microtubule plus end dynamics in multiple embryonic cell types. Mol Biol Cell 25: 3350 – 3362

Ori-Mckenney KM, Jan LY, Jan YN (2012) Golgi outposts shape dendrite morphology by functioning as sites of acentrosomal microtubule nucleation in neurons. Neuron 76: 921 – 930

Port F, Chen HM, Lee T, Bullock SL (2014) Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in Drosophila. Proc Natl Acad Sci USA 111: E2967 – E2976

Riccomagno MM, Kolodkin AL (2013) Sculpting neural circuits by axon and dendrite pruning. Annu Rev Cell Dev Biol 31: 779 – 805

Rolls MM, Satoh D, Clyne PJ, Henner AL, Uemura T, Doe CQ (2007) Polarity and intracellular compartmentalization of Drosophila neurons. Neural Dev 2: 7

Satoh D, Sato D, Tsuya T, Saito M, Ohkura H, Rolls MM, Ishikawa F, Uemura T (2008) Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. Nat Cell Biol 10: 1164 – 1171

Schuldiner O, Yaron A (2015) Mechanisms of developmental neurite pruning. Cell Mol Life Sci 72: 101 – 119

Stepanova T, Slemmer J, Hoogenraad CC, Lansbergen G, Dortland B, De Zeeuw CI, Grosfeld F, van Cappellen G, Akhmanova A, Galjart N (2003) Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). J Neurosci 23: 2655 – 2664

Stewart A, Tsubouchi A, Rolls MM, Tracey WD, Sherwood NT (2012) Katanin p60-like1 promotes microtubule growth and terminal dendrite stability in the larval class IV sensory neurons of Drosophila. J Neurosci 32: 11631 – 11642

Stone MC, Roegiers F, Rolls MM (2008) Microtubules have opposite orientation in axons and dendrites of Drosophila neurons. Mol Biol Cell 19: 4122 – 4129

Stone MC, Albertson RM, Chen L, Rolls MM (2014) Dendrite injury triggers DLK-independent regeneration. Cell Rep 6: 247 – 253

Tang G, Gudsnuk K, Kuo SH, Cotrina ML, Rosoklija G, Sosunov A, Sonders MS, Kaner E, Castagna C, Yamamoto A et al (2014) Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron 83: 1131 – 1143

Tao J, Feng C, Rolls MM (2016) The microtubule-severing protein fidgetin acts after dendrite injury to promote their degeneration. J Cell Sci 129: 3274 – 3281

Tenenbaum CM, Cavis ER (2016) Removal of Drosophila muscle tissue from larval fillets for immunofluorescence analysis of sensory neurons and epidermal cells. J Vis Exp 117: 54670

Terman JR, Mao T, Pasterkamp RJ, Yu HH, Kolodkin AL (2002) MICALs, a family of conserved flavoprotein oxidoreductases, function in plexin-mediated axonal repulsion. Cell 109: 887 – 900

Thakur HC, Singh M, Nagel-Steger L, Kremer J, Prummba D, Fansa EK, Ezzaohini H, Nouri K, Gremer L, Abts A et al (2014) The centrosomal adaptor TACCC and the microtubule polymerase ch-TOG interact via defined C-terminal subdomains in an Aurora-A kinase-independent manner. J Biol Chem 289: 74 – 88

Thawani A, Kadzik RS, Petry S (2018) XMAP215 is a microtubule nucleation factor that functions synergistically with the gamma-tubulin ring complex. Nat Cell Biol 20: 575 – 585

van der Vaart B, Franken MA, Kuijpers M, Hua S, Bouchet BP, Jiang K, Grigoriev I, Hoogenraad CC, Akhmanova A (2012) Microtubule plus-end tracking proteins STAIN1/2 and ch-TOG promote axonal development. J Neurosci 32: 14722 – 14728

Wang Y, Zhang H, Shi M, Liu YC, Lu L, Yu F (2017) Sec71 functions as a GEF for the small GTPase Arf1 to govern dendrite pruning of Drosophila sensory neurons. Development 144: 1851 – 1862

Wang Y, Rui M, Tang Q, Bu S, Yu F (2019) Patronin governs minus-end-out orientation of dendritic microtubules to promote dendrite pruning in Drosophila. Elife 8: e39964

Williams DW, Truman JW (2005) Cellular mechanisms of dendrite pruning in Drosophila: insights from in vivo time-lapse of remodeling dendritic arborizing sensory neurons. Development 132: 3631 – 3642

Witte H, Neukirchen D, Bradke F (2008) Microtubule stabilization specifies initial neuronal polarization. J Cell Biol 180: 619 – 632

Wong JJ, Li S, Lim EK, Wang Y, Wang C, Zhang H, Kirilly D, Wu C, Liou YC, Wang H et al (2013) A Cullin1-based SFC3 ubiquitin ligase targets the InR/PI3K/TOR pathway to regulate neuronal pruning. PLoS Biol 11: e1001657

Xiong X, Wang X, Ewanek R, Bhat P, Diantonio A, Collins CA (2010) Protein turnover of the Wallenda/DLK kinase regulates a retrograde response to axonal injury. J Cell Biol 191: 211 – 223

Yalgin C, Karim MR, Moore AW (2011) Immunohistochemical labeling of microtubules in sensory neuron dendrites, tracheae, and muscles in the Drosophila larva body wall. J Vis Exp 57: 3662

Yalgin C, Ebrahimi S, Delandre C, Yoong LF, Akimoto S, Tran H, Amikura R, Spokony R, Torben-Nielsen B, White KP et al (2015) Centrosomin represses dendrite branching by orienting microtubule nucleation. Nat Neurosci 18: 1437 – 1445

Yan J, Chao DL, Toba S, Koyasu K, Yasunaga T, Hirotsune S, Shen K (2013) Kinesin-1 regulates dendrite microtubule polarity in Caenorhabditis elegans. Elife 2: e00133

Ye B, Zhang Y, Song W, Younger SH, Jan LY, Jan YN (2007) Growing dendrites and axons differ in their reliance on the secretory pathway. Cell 130: 717 – 729

Yu W, Solowska JM, Qiang L, Karabay A, Baird D, Baas PW (2005) Regulation of microtubule severing by katanin subunits during neuronal development. J Neurosci 25: 5573 – 5583

Yu F, Schuldiner O (2014) Axon and dendrite pruning in Drosophila. Curr Opin Neurobiol 27: 192 – 198

Zhang H, Wang Y, Wong JJ, Lim KL, Liu YC, Wang H, Yu F (2014) Endocytic pathways downregulate the L1-type cell adhesion molecule neuregian to promote dendrite pruning in Drosophila. Dev Cell 30: 463 – 478

Zheng Y, Wildonger J, Ye B, Zhang Y, Kita A, Younger SH, Zimmerman S, Jan LY, Jan YN (2008) Dynin is required for polarized dendritic transport and uniform microtubule orientation in axons. Nat Cell Biol 10: 1172 – 1180