THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE ROLE OF CLIP-190, ORBIT/MAST, AND NEB IN MICROTUBULE ORIENTATION, NEURONAL MORPHOLOGY, AND AXONAL REGENRATION IN DROSOPHILA MELANOGASTER

JOANNA M. SKORA

Spring 2010

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Biochemistry and Molecular Biology

Reviewed and approved* by the following:

Melissa Rolls
Assistant Professor of Biochemistry and Molecular Biology
Thesis Supervisor

Craig Cameron
Professor of Biochemistry and Molecular Biology
Honors Advisor

Scott Selleck, Department Head
Department of Biochemistry and
Molecular Biology

* Signatures are on file in the Schreyer Honors College

ABSTRACT

Drosophila melanogaster dendritic arborization (da) neurons offer a model system in which to study microtubule orientation and the effects of disturbing microtubule dynamics. Microtubules serve a crucial function in the cell and are required for diverse processes ranging from cell division, cellular transport and attainment of proper cellular morphology. These cytoskeletal elements have an intrinsic polarity related to the polymerization of tubulin dimers during the dynamic process of their growth and shrinkage. The microtubule orientation in dendrites of *Drosophila* da neurons has been reported as 95% minus-end-out through EB1-GFP dynamics (Rolls et al., 2007). +TIPs are proteins that have been identified as binding or associating with the growing plus-ends of microtubules. Two +TIPs, CLIP-190 and Orbit/MAST, were investigated in this study to determine the effect of their knockdown by RNA interference on microtubule orientation, dendritic morphology, and the cellular response to axon severing. No significant effect was found on microtubule orientation or neuronal morphology in CLIP-190 knockdowns, while there was a significant increase in plus-end-out microtubules in Orbit/MAST knockdowns. These results indicate that Orbit/MAST may play a role in the establishment and maintenance of polarized microtubule arrays in *Drosophila* neurons, but this hypothesis requires further investigation. Both CLIP-190 and Orbit/MAST RNAi knockdown was found to compromise the ability of class I ddaE neurons to respecify one dendrite into an axon-like process following axon severing, indicating that both of these proteins may be required for cellular response to axonal injury. Another important aspect of microtubule function is directional transport of cargo in the cell. Kinesins are molecular motor proteins that use microtubules as tracks to transport cargo, and they are known as plus-end-directed motors. In this study, protein levels of the kinesin-like protein neb were reduced through RNAi and the

effect of neb depletion was studied on microtubule orientation and neuronal morphology in da neurons. Knockdown of neb was found to significantly reduce branching complexity in dendrites of ddaE neurons. These studies shed light on the importance of microtubules and their associated proteins in proper neuronal structure and function.

TABLE OF CONTENTS

INTRODUCTION

Microtubules are crucial for maintaining cell shape and they serve as tracks for intracellular transport of organelles and vesicles. These cytoskeletal elements have an intrinsic polarity, and the arrangement of microtubule arrays in the cell is of great importance to cellular function, especially in polarized cells such as neurons. Microtubule growth occurs at the plus-end by the polymerization of α- and β-tubulin dimers. Microtubules also shrink in a process termed "catastrophe." The dynamic nature of microtubules is based on the hydrolysis of GTP bound to the tubulin dimer, which causes the tubulin to be more prone to depolymerization (Figure 1).

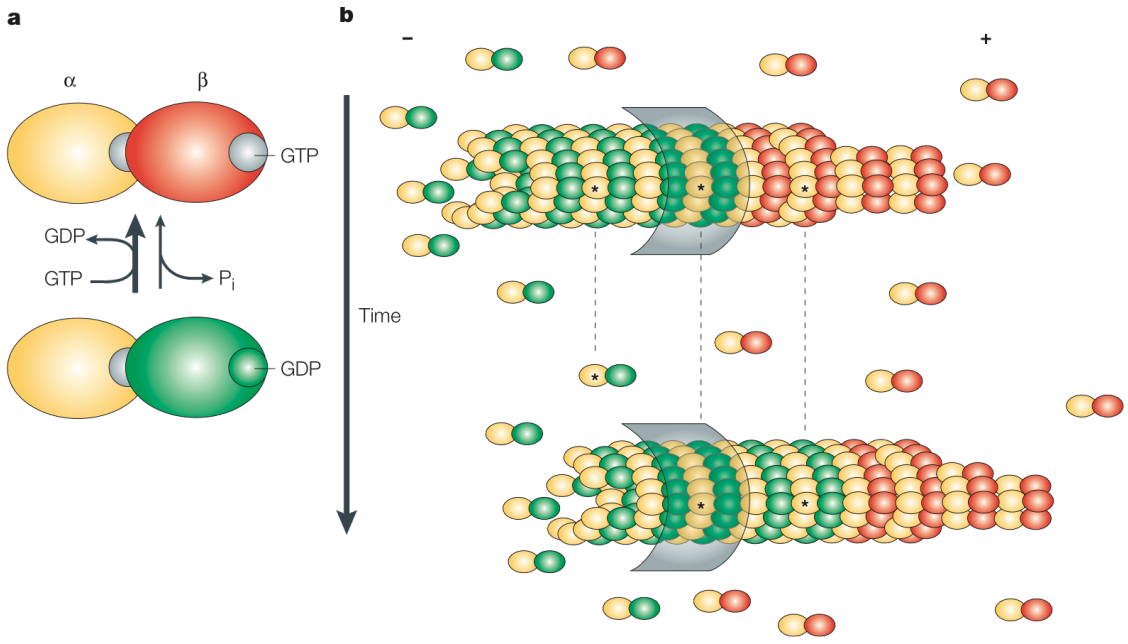

Figure 1. Microtubules form by the polymerization of alpha and beta tubulin dimers into protofilaments. After the dimers are associated with the growing plus end of the microtubule, GTP bound to beta tubulin is hydrolyzed to GDP (Galjart, 2005).

Plus-end-tracking proteins, also known as +TIPs, bind to the growing microtubule plus end with various mechanisms. Two such +TIPs in *Drosophila melanogaster* are CLIP-190 and Orbit/MAST, the proteins investigated in this study. Microtubule organization in neurons may affect dendritic morphology, and may also be involved in the neural phenotypes associated with

various forms of mental retardation (MR) disorders. *Drosophila* is a particularly advantageous model system for the study of microtubule organization and dynamics in neurons, and thus may lead to better understanding of the pathology of MR as manifested in the nervous system.

Drosophila melanogaster **as a model system**

Drosophila is an ideal organism for research purposes due to its short life cycle, easy maintenance, and simple yet powerful genetics. Furthermore, the organism is an excellent model for neural research because *Drosophila* dendrites are more easily accessed than dendrites in other model organisms, allowing for easier *in vivo* study. The study of dendrites has not been as extensive as the study of axons due to such issues with accessibility and recognition of dendrites in organisms such as the rat.

In addition to the ease and convenience accorded by the study of *Drosophila*, many of the proteins in *Drosophila* neurons are conserved in vertebrate neurons, thus allowing for results that are broadly relevant. The neurons of *Drosophila* and vertebrates are similar in polarity, and many features of *Drosophila* dendrites are similar to those in vertebrate dendrites. In one study, several features of vertebrate dendrites, such as an increase in calcium levels upon excitation, the presence of postsynaptic proteins, and the inability to produce synaptic output, were found to be features of *Drosophila* motor neuron dendrites as well (Sánchez-Soriano et al., 2005). Another study found that major polarization and compartmentalization in several different types of neurons in *Drosophila* is similar to that of vertebrate neurons (Rolls et al., 2007). Thus, *Drosophila* is an easily manipulated neural model, and the similarity of *Drosophila* neurons to vertebrate neurons makes the system an ideal one in which to study the effects of neural microtubule organization.

Dendritic arborization (da) neurons in *Drosophila* are a group of sensory neurons found directly under the larval cuticle, making them useful for the study of microtubule organization and dendrite morphology using live imaging with confocal microscopy (Andersen et al., 2005). These neurons have a highly branched dendritic tree which allows for extensive study of the effects of microtubule organization on the shape and branching pattern of dendrites (Figure 2). The neurons are classified based on the complexity of the dendritic tree, with class I having the simplest branching pattern and class IV having the most complex (Grueber et al., 2002).

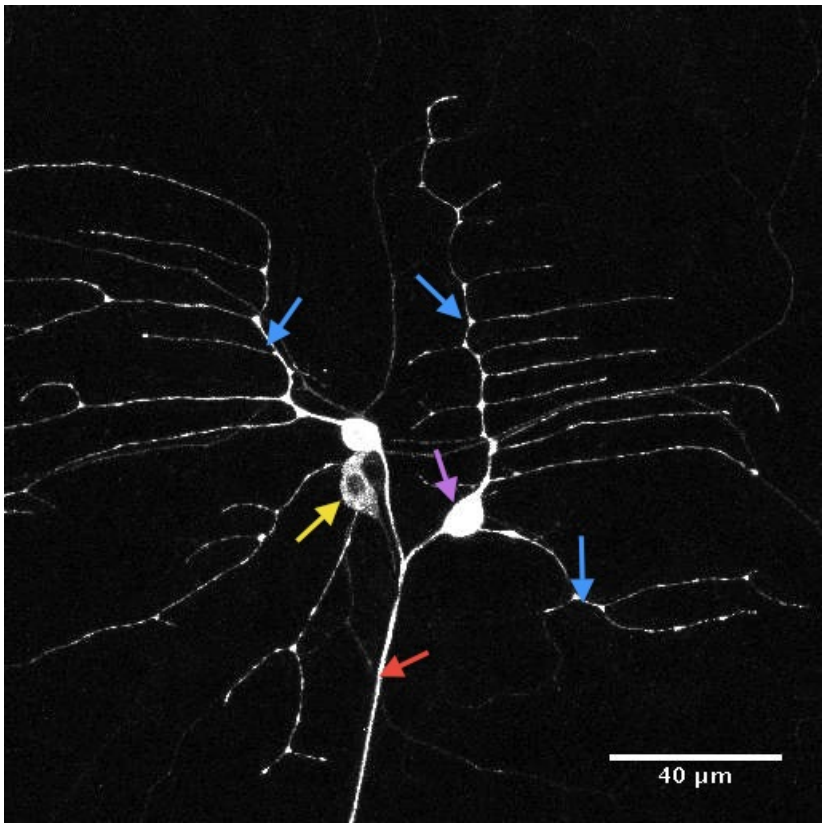

Figure 2. Da neurons labeled with GFP and imaged using confocal microscopy. The red arrow points to the axon bundle, the blue arrows point to dendrites, the purple arrow points to the cell body of the class I da neuron, and the yellow arrow points to the cell body of the class IV da neuron.

Drosophila has served as a successful model system for several disorders affecting the nervous system of vertebrates. For instance, Parkinson's Disease (PD) has been modeled by expression of human proteins associated with PD in *Drosophila*, including wild type and mutant

α-synuclein (Feany and Bender, 2000). In addition, mutations in *parkin* (Pesah et al., 2004), *PINK1* (Park et al., 2006), *DJ-1α* and *DJ-1β* (Meulener et al., 2005) in *Drosophila*, which are homologues of genes associated with PD in humans, produced phenotypes similar to several aspects of human PD and have suggested several therapeutic possibilities for the disease. Thus, using *Drosophila* as an invertebrate model of neurodegenerative disease has been successful and insightful in the past.

Microtubule orientation in neurons

The importance of microtubule organization and orientation in neurons lies in the critical role microtubules have in intracellular transport, shape, and function. Microtubule orientation is important for transport, which is based on two major classes of motors that move in separate directions; kinesins are the primary plus-end-directed motors, while dyneins move towards the minus-end.

The orientation of microtubules in axons had been determined for several different types of neurons in various organisms, ranging from cat sympathetic neurons (Heidemann et al., 1981) to cultured chicken sensory neurons (Baas et al., 1987). The major finding of these studies is that more than 95% of microtubules in axons of vertebrates are oriented away from the cell body, or plus-end-out (Figure 3).

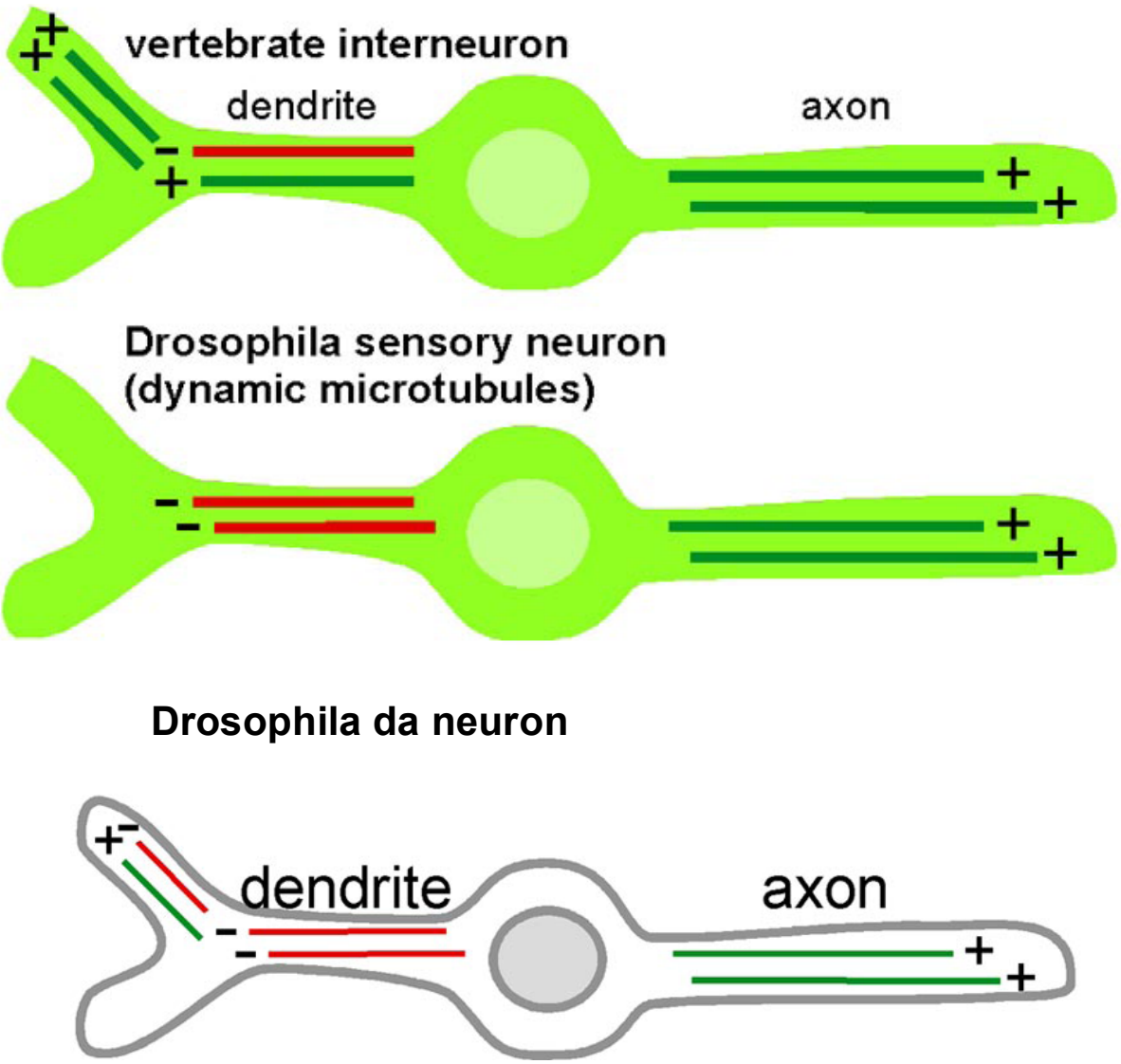

Figure 3. Microtubule orientation in vertebrate and *Drosophila* neurons (Stone et al., 2008).

Studies of axonal microtubule orientation were originally undertaken by introducing tubulin into the cell being studied. The tubulin forms hooks on the sides of existing microtubules, and the handedness of the curvature of the hook (either left or right) is observed by electron microscopy and used to determine the polarity of the microtubule. The hooks are generally right-handed when the microtubule is viewed from plus-end to minus-end (Heidemann and McIntosh, 1980). A new method to determine microtubule orientation in neurons involves

the fusion of green fluorescent protein (GFP) to plus-end-tracking proteins (+TIPs), which associate with the growing plus-end of the microtubule. This method was first used in neurons to determine the microtubule orientation in mouse hippocampal neurons and mouse Purkinje cell neurons, and microtubule orientation in axons was found to be 95% plus-end-out (Stepanova et al., 2003). It is therefore generally accepted that the majority of microtubules in axons are oriented plus-end-out. Thus, axons rely on kinesins for directional intracellular transport of cargo (Hirokawa and Takemura, 2005).

Microtubule organization in dendrites has not been as well documented as that in axons. Studies in frog mitral cells (Burton, 1988) using the hook curvature method *in vivo* and in cultured rodent dendrites (Stepanova et al., 2003) using both the hook method and labeling +TIPs have resulted in dendrite microtubule orientation of approximately 50% plus-end-out in proximal dendrites of vertebrates, and a similar orientation to axonal microtubules in distal dendrites (Figure 3) (Baas et al., 1988). Thus, transport mechanisms into dendrites are still unclear, though current models have suggested that transport relies on plus-end motors as in axons (Hirokawa and Takemura, 2005; Kennedy and Ehlers, 2006; Levy and Holzbaur, 2006; Setou et al., 2004). A more recent study reported that dynein, a minus-end-directed motor, is required for dendritic transport of cargo that is specific to dendrites, such as neurotransmitter receptors (Kapitein et al., 2010).

Studies in da neurons of *Drosophila* found that microtubules in axons are oriented 95% plus-end-out, similar to previous findings in other organisms (Figure 3). However, microtubules in dendrites of da neurons are oriented more than 95% minus-end-out, a striking difference from microtubule orientation in vertebrate dendrites (Rolls et al., 2007; Stone et al., 2008). The opposite orientation of microtubules in da neuron axons and dendrites makes da neurons an ideal

system in which to study the implications of microtubule orientation for different parts of the neuron, as well as the effects of disrupting this system and its effects on neural disorders such as mental retardation.

Mental retardation and Williams syndrome

Microtubule orientation in neurons contributes to cellular shape and function, two aspects that are affected in individuals with various forms of mental retardation (MR). MR comprises a broad spectrum of disorders and affects almost 3% of the population. While the causes for MR vary, abnormalities in dendritic morphology have been observed in all cases where the dendrites have been studied (Kaufmann and Moser, 2000). Dendrites are necessary for receiving and integrating signals at synapses, so it is not surprising that alterations in their morphology and branching patterns are associated with the phenotypes of various forms of MR. The two types of dendritic alterations that have been observed in the dendrites of the cerebral cortex in individuals with MR are a decrease in the complexity and number of dendrite branches and alterations in the number and morphology of dendritic spines (Huttenlocher, 1970, 1975; Purpura, 1974, 1975a, b).

Several studies have shown a link between MR and microtubule organization. A disruption in microtubule organization is associated with the dendritic abnormalities that accompany MR. Studies labeling the Golgi and observations through electron microscopy identified microtubule disarray in cortical neurons of children with severe MR (Bodick et al., 1982; Purpura et al., 1982). In addition to these observations, several MR disorders are also associated with genes that are linked to microtubule organization. This study focuses on Williams syndrome, a rare genetic MR disorder that is caused by the deletion of approximately 28 genes from one copy of chromosome 7 (Figure 4).

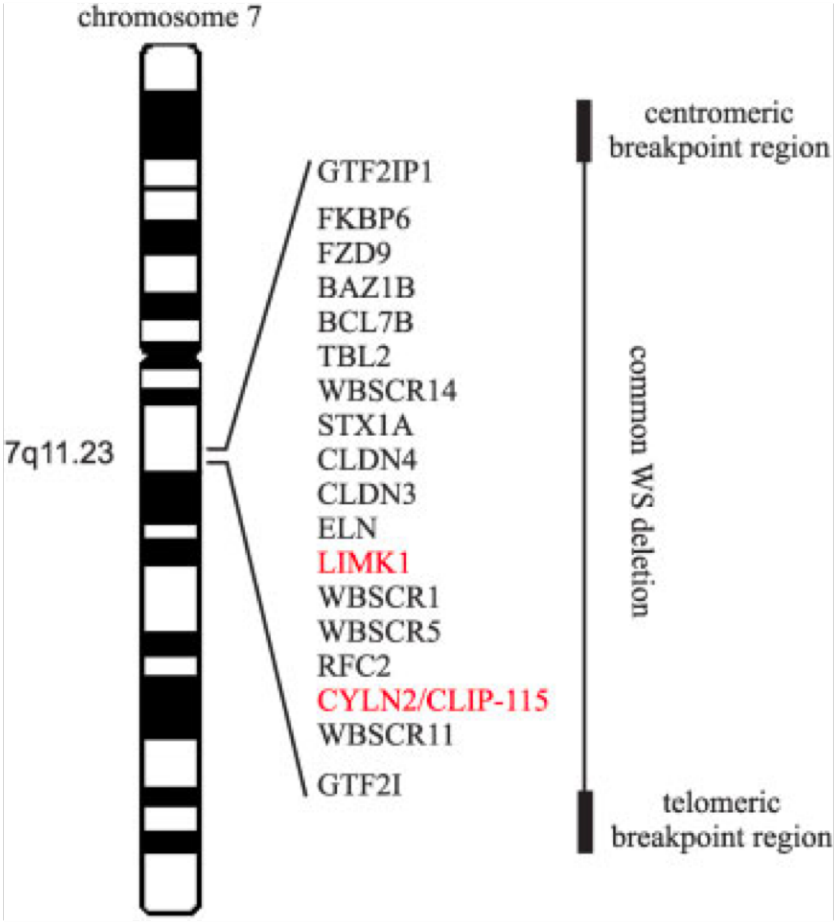

Figure 4. A map of the region of chromosome 7 deleted in Williams syndrome (Hoogenraad et al., 2004).

Williams syndrome is estimated to affect as many as 1 in every 7,500 live births (Strϕmme et al., 2002). The disorder manifests with distinct facial features, slowed growth, cardiovascular abnormalities (Beuren et al., 1962; Williams et al., 1961); endocrine, gastrointestinal, and orthopedic abnormalities (Morris, 1998); and various neurological and learning impairments. Two genes associated with the cytoskeleton, *LIMK1* and *CYLN2*, are part of the deletion on chromosome 7 in Williams syndrome. *LIMK1* encodes a protein kinase that regulates actin dynamics (Arber et al., 1998). *LIMK1* knockout mice display increased locomotion, decreased spatial abilities, and learning abnormalities, which are phenotypic traits characteristic of Williams syndrome (Meng et al., 2002). *CYLN2* encodes the cytoplasmic linker

protein of 115 kD (CLIP-115), which regulates microtubule dynamics (Komarova et al., 2002). *CYLN2* knockout mice manifest phenotypic abnormalities in growth, motor coordination, and ventricular volume (Hoogenraad et al., 2002). Thus, both genes link cytoskeletal defects to phenotypic abnormalities observed in Williams syndrome patients.

CLIPs and CLASPs in *Drosophila melanogaster*

While there are two CLIP proteins found in humans (CLIP-115 and CLIP-170), only one CLIP homologue, CLIP-190, is found in *Drosophila* (Galjart, 2005). CLIP-190 is a +TIP that is more similar to CLIP-170 than to CLIP-115, but because it is the only CLIP homologue in flies, it must perform all the functions of both CLIP proteins found in humans. CLIP proteins were so named due to their observed function of regulating interactions between microtubules and other intracellular organelles (Pierre et al., 1992). There has been no reported effect of the loss of CLIP-190 on *Drosophila* neurons.

CLASPs are CLIP associating proteins, and the only homologue of CLASPs in *Drosophila* is known as Orbit/MAST (Galjart, 2005). Orbit/MAST is a +TIP required for oogenesis and cell division in *Drosophila* and associates with the mitotic spindle (Máthé et al., 2003). Orbit/MAST has also been shown to have a role in axon guidance at growth cones (Lee et al., 2004). Further investigation of Orbit/MAST is necessary to determine if it has a role in microtubule dynamics or dendritic function in da neurons of *Drosophila*.

The role of microtubule polarity in directional transport

One crucial function of microtubules is to serve as tracks for directional transport inside the cell. Due to their intrinsic polarity, microtubules participate in directional transport of cellular cargo such as vesicles, organelles, mRNAs, and proteins (Hirokawa, 1998). This is especially important in neurons due to their long projections away from the cell body. It is

known that axonal directional transport utilizes plus-end kinesin motor proteins (Hirokawa and Takemura, 2005), but the mechanism of dendritic transport of cargo is still unclear. In this study, the kinesin like protein neb is investigated through RNAi knockdown. neb is required for mitosis, meiosis, and abdominal segmentation in *Drosophila* (Ruden et al., 1997).

Overview of experimental approach

The functions of CLIP-190, which have never been phenotypically analyzed, Orbit/MAST, and neb in *Drosophila* were investigated through RNA interference and confocal microscopy. Introduction of RNA hairpins resulted in the reduction of protein levels. *Drosophila* larvae were imaged *in vivo* and the following were assayed in da neuron dendrites: 1) microtubule orientation by counting comets of EB1-GFP for knockdowns of CLIP-190, Orbit/MAST, and neb, 2) dendrite morphology for knockdowns of CLIP-190, Orbit/MAST, and neb and 3) axon regeneration in response to axon severing for knockdowns of CLIP-190 and Orbit/MAST. I hypothesize that CLIP-190 and Orbit/MAST play a role in the regulation of microtubule dynamics in dendrites, and that these proteins are important for attainment of proper dendritic morphology. I also hypothesize that neb is involved in the attainment of proper dendritic morphology due to the importance of kinesins in cargo transport. In addition, I hypothesize that the normal axon regeneration response may be affected with reduced protein levels of CLIP-190 and Orbit/MAST. Following the severing of an axon, a dendrite is elicited to change its identity and regenerate into an axon. This requires a dramatic reversal of microtubule orientation and is usually accompanied by one of the dendritic processes undergoing extensive tip growth (Stone et al., 2010). The +TIPs CLIP-190 and Orbit/MAST may be required for the microtubule reversal events that occur due to the association of these proteins with growing microtubules.

MATERIALS AND METHODS

Maintenance of *Drosophila melanogaster* lines

Drosophila lines were grown on standard *Drosophila* media and stored at 25°C. During preparation for crossing lines, one of the lines to be crossed was stored alternately at 18°C for 16h and room temperature for 8h and virgins were acquired from this line twice a day following the end of each time period at a given temperature.

RNAi line generation

RNAi lines were obtained from the Vienna *Drosophila* RNAi Center (VDRC, Vienna, Austria) for CLIP-190, Orbit/MAST, neb, and the control Rtnl2 (Dietzl et al., 2007). The following VDRC stock numbers correspond to the above lines, respectively: 107176, 26051, 31329, and 33318. These lines express hairpin RNAs under UAS control. The Rtnl2 RNAi line was used as a control in all experiments because there have been no reported abnormalities resulting from the absence of Rtnl2 in *Drosophila*. For microtubule orientation assays, da neuron dendritic morphology assays, and axon-severing assays, RNAi lines were crossed to UAS-dicer2; 221-Gal4, UAS-EB1-GFP. Larval progeny were collected on food caps every 24h at 25°C for microtubule orientation and dendritic morphology assays and at room temperature for axon-severing assays. Larvae for axon-severing assays were aged at 25 °C for 24h and at room temperature following axon severing. For all other assays, larvae were aged at 25°C prior to imaging.

Microtubule orientation assay of RNAi knockdowns

Microtubule orientation assays were done for RNAi knockdowns of CLIP-190, Orbit/MAST, neb, and Rtnl2 (control). Live larvae were collected 48-72 hours after egg laying, washed in Schneider's insect medium, and mounted dorsal side up on a glass microscope slide

containing a dry agarose pad. The agarose pad helped to prevent movement of the larvae during live imaging. The larvae were covered with a glass coverslip, which was taped down on both sides to further prevent movement of the animals. Live images of the larvae were obtained on an Olympus FLUOVIEW (FV1000) confocal microscope by collecting single frames every 2 s in order to track microtubule dynamics. The larvae were imaged under 63x oil magnification. Time series movies consisted of 200 frames. EB1-GFP dynamics were tracked in the main trunk of the comb dendrite in class I da neurons of *Drosophila* larvae (Figure 5). Specifically, the ddaE neuron was analyzed due to its simple, easily recognized, and reproducible branching pattern (Grueber et al., 2002). Images were analyzed using ImageJ software (http://rsb.info.nih.gov/ij/; NIH). In each main trunk, EB1-GFP comets were scored as either going towards the cell body (CB, minus-end-out) or away from the CB (plus-end-out). Comets were only counted if seen moving in a specific direction for three or more consecutive frames. Statistical analyses were done using Fisher's exact test.

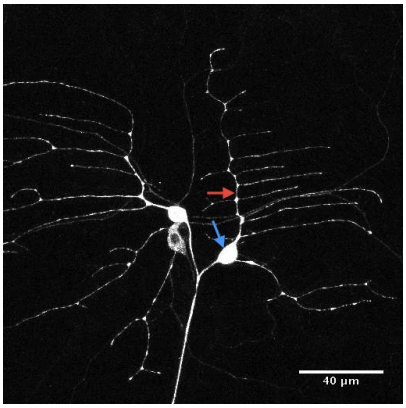

Figure 5. Dendritic arborization neurons in *Drosophila* larva. The blue arrow points to the class I da neuron, and the red arrow points to the main trunk of the comb dendrite, where microtubule orientation was analyzed by tracking EB1-GFP dynamics.

da neuron dendritic morphology assay of RNAi knockdowns

Dendritic morphology assays of da neurons were done for RNAi knockdowns of CLIP-190, Orbit/MAST, neb, and Rtnl2 (control). Following the mounting of each larva on a

microscope slide as described above for the microtubule orientation assay, the da neurons were imaged by taking 0.5 μm focus steps above and below the plane, resulting in a Z-plane projection. These images were also analyzed using ImageJ software.

Dendritic morphology was scored qualitatively by assessing the general appearance of the da neurons as compared to the Rtnl2 da neurons. Quantitative scoring was also done by counting the number of branches/projections from the main trunk of the comb dendrite of the class I da neuron for each image. In addition, an analysis measuring branching complexity was done in which the number of projections that branched further after branching off of the main trunk was compared to the number of total branches off of the main trunk. Statistical analyses were done using a two-tailed Student's *t*-test.

Axon-severing assays of RNAi knockdowns

RNAi embryos of CLIP-190, Orbit/MAST, and Rtnl2 were collected at room temperature 0-24h after egg-laying and cultured in standard *Drosophila* media at 25°C for an additional 48 hr before axon severing. Axons of class I da neurons were cut using a Micropoint UV laser (Photonic Instruments, St. Charles, IL), and larvae were imaged by collecting frames every 2 s using a LSM510 confocal microscope (Carl Zeiss, Thornwood, NY). Following axon severing, the larvae were removed from the microscope slide by applying a drop of Schneider's insect medium in order facilitate removal from the dry agarose pad. The larvae were then placed in standard *Drosophila* media and cultured at room temperature until further imaging. Microtubule orientation in the main trunk of all dendrites of the class I da neuron and dendrite tip growth in the same neuron were assayed in larvae 24, 48, 72, and 96h after axon severing by observing EB1-GFP dynamics and taking a Z-stack, respectively.

RESULTS

RNAi knockdown of Orbit/MAST, but not CLIP-190, has an effect on microtubule orientation in class I da neurons

In order to investigate the role of CLIP-190 and Orbit/MAST in the maintenance of microtubule polarity in da neurons, EB1-GFP comets were counted in the main trunk of the class I neuron comb dendrite for RNAi knockdowns of these proteins. Comets moving towards the cell body indicated minus-end-out microtubules, while comets traveling in the opposite direction were plus-end-out (Figure 6).

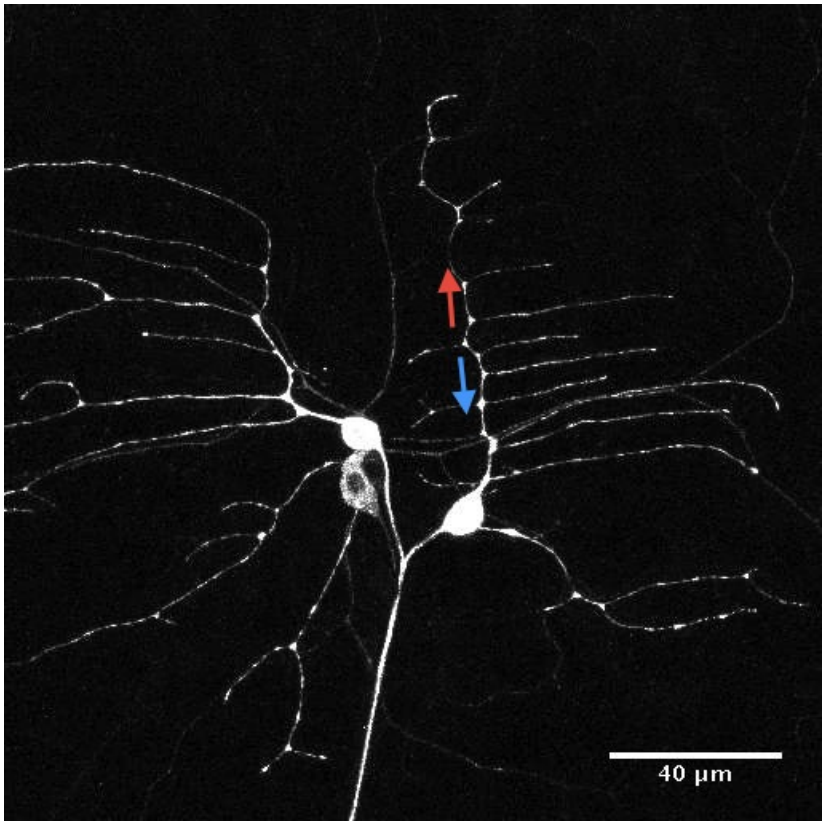

Figure 6. Microtubule orientation in the main trunk of class I da neuron comb dendrites. EB1-GFP comets moving towards the cell body (blue arrow) were classified as minus-end-out microtubules, while comets moving away from the cell body (red arrow) were classified as plus-end-out.

CLIP-190 RNAi knockdowns had 90% (n=175 in 12 neurons) of microtubules minus-end-out, a result that was not significantly different from the control (Rtnl2 RNAi). RNAi knockdown of Orbit/MAST had a statistically significant effect on microtubule orientation

(Table 1). While the RNAi knockdowns of Rtnl2 had 92% (n=224 in 14 neurons) minus-end-out microtubules in the main trunk, Orbit/MAST RNAi knockdowns showed 85% (n=280 in 13 neurons) of microtubules minus-end-out. This effect requires further investigation due to its subtle nature. Future studies on microtubule orientation in Orbit/MAST knockdowns should use a different Gal-4 driver to drive expression of UAS-EB1-GFP in order to validate the reliability of the findings.

Table 1. Effects of RNAi knockdown of CLIP-190 and orbit/MAST on microtubule orientation in class I da neurons of *Drosophila.*

RNAi Line	Number of Neurons Analyzed	Total Number of Comets Counted	Total Number Minus-End-Out	Percentage Minus-End-Out
Rtnl2 (control)	14	224	206	92%
CLIP-190	12	175	158	90%
Orbit/MAST	13	280	237	85%

RNAi knockdown of neb does not have an effect on microtubule orientation in class I da neurons

RNAi knockdowns of neb were also studied in order to determine if neb is involved in the maintenance of microtubule orientation in da neurons. As compared to Rtnl2, RNAi knockdowns of neb did not significantly differ in microtubule orientation in main trunks of class I da neuron dendrites. neb RNAi knockdowns had 95% (n=147 in 11 neurons) minus-end-out microtubules (Table 2).

Table 2. Effect of RNAi knockdown of neb on microtubule orientation in class I da neurons of *Drosophila.*

RNAi Line	Number of Neurons Analyzed	Total Number of Comets Counted	Total Number Minus-End-Out	Percentage Minus-End-Out
Rtnl2 (control)	14	224	206	92%
neb	11	147	140	95%

Dendritic morphology is not affected by RNAi knockdown of CLIP-190 but may be affected by RNAi knockdown of Orbit/MAST

Dendritic morphology was first measured by qualitative comparison of the da neurons in Orbit/MAST and CLIP-190 RNAi knockdowns to neurons in Rtnl2 RNAi knockdowns. CLIP-190 RNAi knockdowns did not have any apparent morphological features that caused them to differ from the Rtnl2 controls (Figure 7). Orbit/MAST RNAi knockdowns, however, did show several features not seen in the control, notably deformed cell bodies in class IV da neurons and elongated cell bodies with thickened initial dendritic segments in class I da neurons (Figure 7). These features were not observed in all of the Orbit/MAST images, however, and require further investigation using a class IV neuron specific driver.

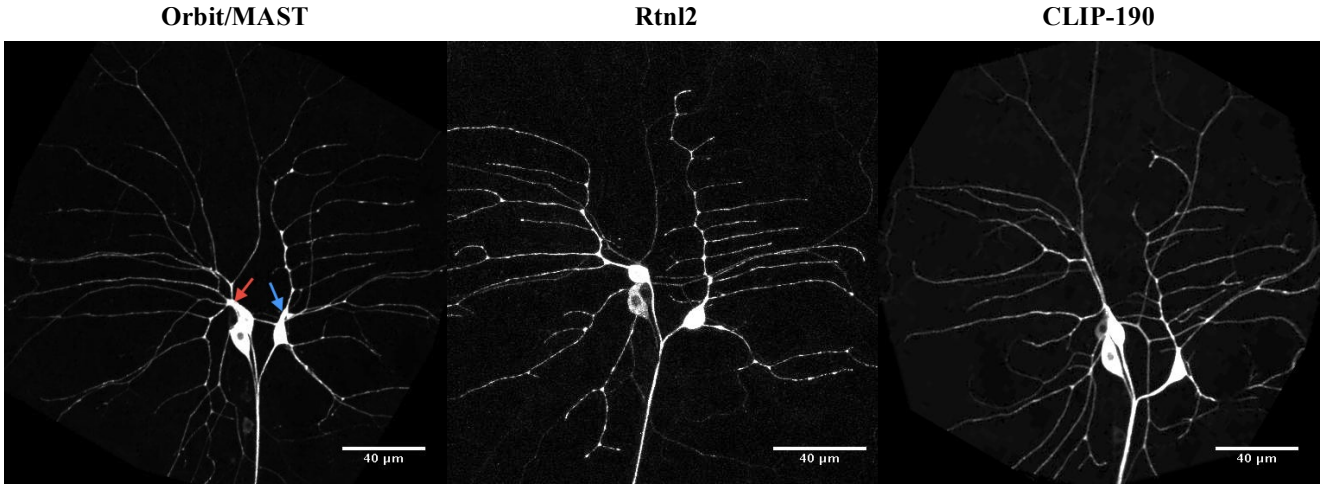

Figure 7. Neuron overview images of RNAi knockdowns showing neuronal and dendritic morphology of da neurons. The blue arrow points to the elongated class I neuron cell body observed in some Orbit/MAST RNAi knockdowns, and the red arrow points to the deformed class IV cell body seen in some Orbit/MAST RNAi knockdowns.

Quantitative analysis was next undertaken to determine if da neuron morphology was affected by RNAi knockdown of CLIP-190 or Orbit/MAST. First, the number of dendritic

branches off of the main trunk of each class I comb dendrite was counted (Table 3). The average number of branches did not significantly differ from the control (8.1, n=7) for neither CLIP-190 knockdowns (7.9, n=8) nor Orbit/MAST knockdowns (8.3, n=7).

Table 3. Effects of RNAi knockdown of CLIP-190 and Orbit/MAST on the number of branches off of the main trunk of class I da neuron comb dendrites.

Orbit/MAST Neurons	CLIP-190 Neurons	Rtnl2 Neurons (control)
9	8	8
10	8	10
7	8	8
10	9	10
6	6	4
7	9	9
9	5	8
—	10	—
8.3	**7.9**	**8.1**

A measure of branching complexity was also obtained for CLIP-190 and Orbit/MAST knockdowns (Table 4). The number of branches that experienced further branching after the initial branch point on the main trunk of the comb dendrite was compared to the total number of branches off of the main trunk. No significant difference from the control (0.41, n=7) was found for CLIP-190 knockdowns (0.42, n=8) and Orbit/MAST knockdowns (0.39, n=7).

Table 4. Effects of RNAi knockdown of CLIP-190 and Orbit/MAST on branch complexity in class I da neurons.

Orbit/MAST Neurons	CLIP-190 Neurons	Rtnl2 Neurons (control)
4/9	4/8	2/8
3/10	4/8	3/10
2/7	3/8	3/8
1/10	3/9	5/10
4/6	3/6	2/4
4/7	5/9	5/9
3/9	2/5	3/8
—	2/10	—
0.39	**0.42**	**0.41**

RNAi knockdown of neb reduces branching complexity but does not have an effect on branch number

Neuronal and dendritic morphology in RNAi knockdowns of neb was first observed qualitatively and then quantitatively analyzed. Knockdowns of neb appeared to have less complex branching patterns compared to the Rtnl2 control knockdowns (Figure 8).

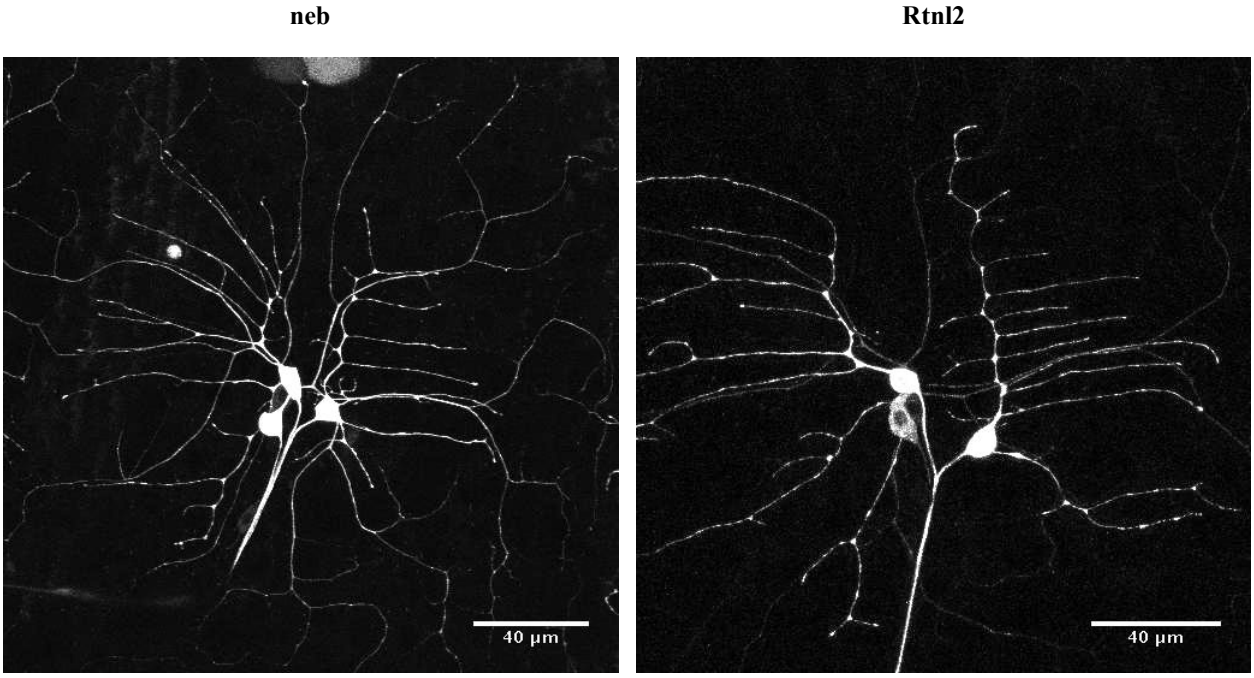

Figure 8. Neuron overview images showing neuronal and dendritic morphology of da neurons.

The number of branches off of the main trunk of the comb dendrite in the class I neuron was counted for knockdowns of neb, and branching complexity was analyzed in the same manner as for CLIP-190 and Orbit/MAST knockdowns. The mean number of branches in neb knockdowns (7.1, n=19) did not differ significantly from Rtnl2 (8.1, n=7), as shown in Table 5.

Table 5. Effect of RNAi knockdown of neb on the number of branches off of the main trunk of class I da neuron comb dendrites.

neb Neurons	Rtnl2 Neurons (control)
6	8
7	10
5	8
9	10
6	4
8	9
8	8
9	—
4	—
5	—
6	—
9	—
5	—
9	—
7	—
9	—
10	—
8	—
5	—
7.1	**8.1**

RNAi knockdowns of neb differed significantly from Rtnl2 on the measure of branching complexity (Table 6). While Rtnl2 knockdowns had a mean branching ratio of 0.41 (n=7), neb knockdowns had a ratio of 0.26 (n=19), significantly lower than the controls (p=0.04767).

Table 6. Effect of RNAi knockdown of neb on branch complexity in class I da neurons.

neb Neurons	Rtnl2 Neurons (control)
4/6	2/8
3/7	3/10
3/5	3/8
1/9	5/10
1/6	2/4
0/8	5/9
1/8	3/8
2/9	—
1/4	—
1/5	—
1/6	—
1/9	—
2/5	—
3/9	—
3/7	—
2/9	—
2/10	—
1/8	—
1/5	—
0.26*	**0.41**

CLIP-190 and Orbit/MAST are required for the normal axon regeneration response following axonal severing in class I da neurons

In order to determine if RNAi knockdown of CLIP-190 or Orbit/MAST have a subtle effect on the establishment or maintenance of microtubule polarity, axons in class I ddaE neurons were severed in RNAi knockdowns of CLIP-190, Orbit/MAST, and Rtnl2 using a pulsed UV laser, and these larvae were observed 24, 48, 72, and 96h after the initial cut. This assay was a more sensitive test of whether these proteins are required when microtubule dynamics are changing quickly in all remaining neuronal processes. Microtubule dynamics were assayed in the main trunk before the second branch point of each dendrite of the class I ddaE neuron at each time point. Dendrites with more than 75% of microtubules moving away from the cell body were classified as plus-end-out (axonal orientation) and dendrites with more than

75% of microtubules moving towards the cell body were classified as minus-end-out (dendritic orientation). Dendrites with intermediate microtubule populations (greater than 25% and less than 75% in a particular direction) were classified as mixed orientation. Dendrites were numbered in each ddaE neuron in order to facilitate comparison among groups. The comb dendrite was classified as dendrite 1, and the dendrite nearest the axons of the neuron cluster was classified as dendrite 2. Some cells contained a third dendrite (dendrite 3) in the center of the first two dendrites (Figure 9). One cell analyzed had five dendrites, and the dendrites in between dendrite 1 and dendrite 2 were numbered from dendrite 1 down toward dendrite 2 in increasing order. In addition, an overview was also obtained at each time point to observe the neuronal processes for morphological changes after axon severing.

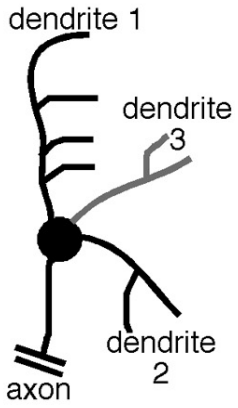

Figure 9. Dendritic numbering scheme used in axon severing assay (Stone et al., 2010).

Rtnl2 RNAi knockdowns did not show the anticipated results as the control for this study, as compared to the results obtained by Stone et al. (2010). Only one neuron out of four analyzed showed tip growth in dendrite 3 by 96h, although the microtubule orientation of this dendrite was mixed rather than plus-end-out, as would be expected for a dendritic process that was converted into an axon (Figure 10). Thus, the results obtained by Stone et al. (2010) were used as the control in this study.

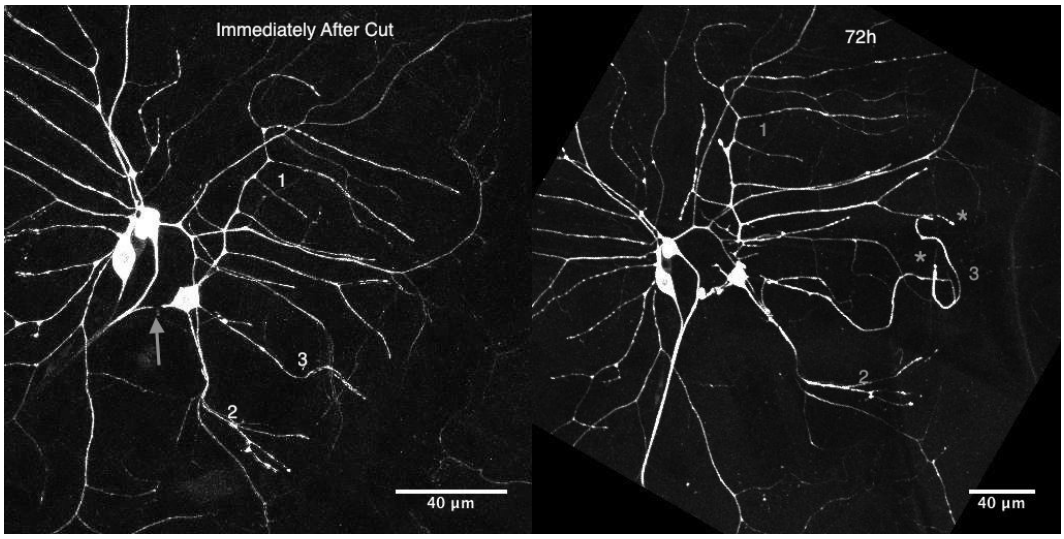

Figure 10. An example of the tip growth that occurred 72h following axon severing in Rtnl2 RNAi knockdown. This growth only occurred in one larva. The arrow points to the severed axon, and asterisks label the growing dendrite tips.

Stone et al. (2010) were the first to report the cellular response of ddaE neurons in *Drosophila* to axon severing, and their methods were identical to those used in this study. Their analysis was conducted on larvae heterozygous for 221-Gal4 and UAS-EB1-GFP. According to Stone et al. (2010), the majority of ddaE neurons (66%) initiate dendritic tip growth by 96h after axon severing, and this tip growth is usually observed for dendrite 2 (closest to the axon bundle). The period between 0h and 48h following axon severing is marked by a 10-fold upregulation in microtubule dynamics and the specification of one dendrite into an axon-like process. While at 24h many dendrites observed have plus-end-out microtubule orientation, by 48h one dendrite usually remains as the only dendrite to still have plus-end-out orientation, with the remaining dendrites reverting back to minus-end-out orientation. It is this plus-end-out dendrite that is observed to initiate extensive tip growth by 96h (Stone et al., 2010) (Figure 11).

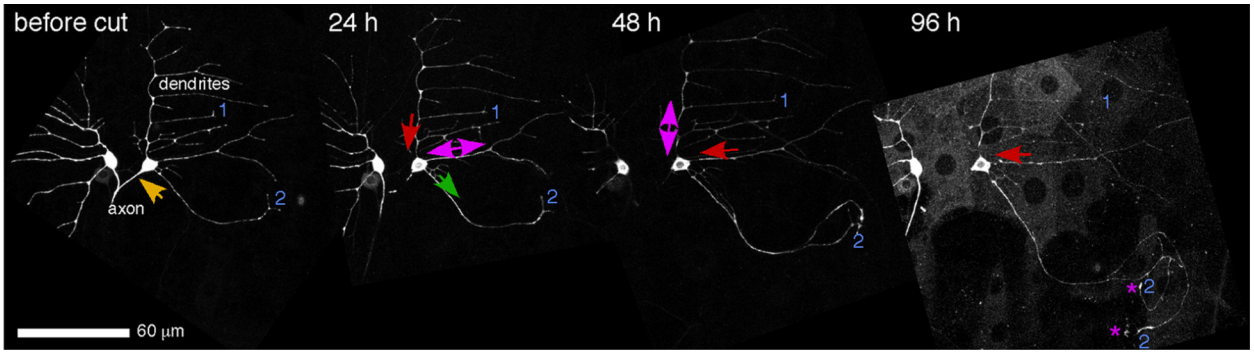

Figure 11. Axon severing in class I ddaE neurons elicits a dendrite to change microtubule polarity within 48h after severing, followed by dendrite tip growth (purple asterisk). The axon is denoted with a yellow arrow. Plus-end-out microtubules are shown with a green arrow, minus-end-out microtubules are shown with a red arrow, and mixed polarity is shown with a double-arrow (Stone et al., 2010).

Extensive tip growth like that seen by Stone et al. (2010) was not observed for any of the neurons analyzed in CLIP-190 (n=5) and Orbit/MAST (n=6) RNAi knockdowns, although a minority of the knockdowns did display abnormal growth patterns in dendritic processes (Figure 12). RNAi knockdowns of Orbit/MAST, but not of CLIP-190, had small projections growing out of the dendrites between branch points, and these projections became more noticeable over time (Figure 12). In addition, a majority of the dendrites for both RNAi knockdowns appeared to switch between minus-end-out, plus-end-out, and mixed microtubule polarity at almost every time point, and this pattern of switching polarity appeared random (Figure 12).

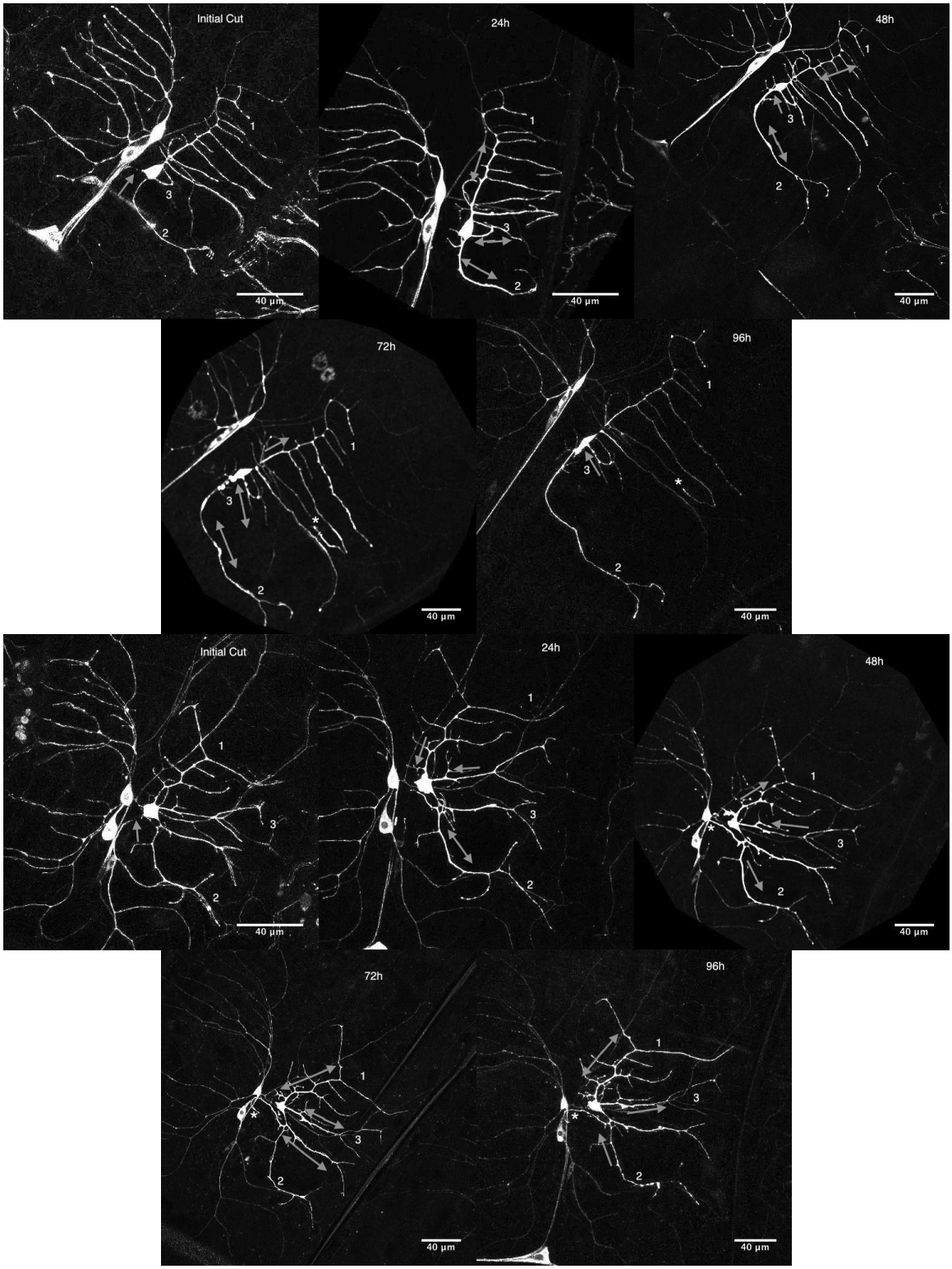

Figure 12. Axon severing assays of CLIP-190 (top 5) and Orbit/MAST (bottom 5) RNAi knockdowns. Arrows denote microtubule polarity 24h-96h following axon cutting, and point towards the plus ends of microtubules in each dendrite. Double-headed arrows signify mixed orientation. In the initial cut images, the arrow denotes the severed axon. Asterisks label abnormal growth in dendritic processes. Note the small dendritic projections sprouting from dendrites of Orbit/MAST RNAi starting at 48h after axon severing.

DISCUSSION

This study sought to investigate the roles of CLIP-190, Orbit/MAST, and neb in microtubule dynamics, neuronal morphology, and the axonal regeneration process using RNAi knockdowns. The first goal of this study was to determine the role of CLIP-190, a plus-end microtubule tracking protein, and Orbit/MAST, a CLIP-associating protein (CLASP), in the maintenance of microtubule polarity in class I da neurons of *Drosophila*. Next, neuronal and dendritic morphology were analyzed in da neurons in these knockdowns. In addition, axons of class I ddaE neurons were severed in CLIP-190 and Orbit/MAST RNAi knockdowns and the neurons were observed for 4d following axon cutting. The analysis focused on microtubule polarity and tip growth in the dendrites of ddaE neurons that had severed axons. Finally, microtubule orientation and neuronal morphology were investigated in knockdowns of the kinesin neb.

CLIP-190 has no published phenotype, but it is known to associate with plus-ends of growing microtubules (Galjart, 2005). I sought a neuronal phenotype for CLIP-190 based on the fact that its homologue in humans, CLIP-115, is encoded by a gene (*CYLN2*) that is deleted in Williams syndrome, a rare form of mental retardation (Komarova et al., 2002). Orbit/MAST was investigated due to its association with CLIP-190. The microtubule orientation assay did not produce statistically significant results for CLIP-190 RNAi knockdowns, indicating that CLIP-190 does not play a role in establishing and maintaining microtubule orientation in class I da neurons under normal physiological conditions. Microtubule orientation in the control, Rtnl2, was comparable to findings by others using the 221-Gal4 driver (our unpublished data).

While no effect was found in CLIP-190 knockdowns on microtubule polarity, other aspects of microtubule dynamics that were not investigated in the present study may rely on

CLIP-190. Future studies should include an analysis of EB1-GFP comet velocity, an analysis of the average number of comets per neuron in a fixed time period, and an investigation of microtubule dynamics in dendritic tips rather than the main trunks of the dendrite. In addition, phenotypic analysis of CLIP-190 could be investigated using lines that have a P-element transposon insertion in a portion of the CLIP-190 gene. The next step in the phenotypic investigation of CLIP-190 should involve the generation of a null allele of CLIP-190 to determine the effects of lacking a functional gene encoding CLIP-190 on a full scale, rather than only in specific cells where expression is driven and RNAi knockdown occurs.

Orbit/MAST was found to have a statistically significant effect on microtubule orientation, with 85% microtubules plus-end-out as compared to 92% in Rtnl2. However, the subtlety of this effect calls for further investigation using a different Gal-4 driver. Orbit/MAST has been shown to have several crucial functions in *Drosophila*, such as its involvement in oogenesis and cell division (Máthé et al., 2003) and its role in axon guidance (Lee et al., 2004). Orbit/MAST is required for proper oogenesis, and it organizes a polarized microtubule network that allows the oocyte to differentiate (Máthé et al., 2003). Thus, the involvement of Orbit/MAST in microtubule dynamics and the establishment of the proper microtubule polarity in neurons is consistent with the findings regarding its role in oogenesis.

In addition to studying microtubule orientation, I also investigated the role of CLIP-190 and Orbit/MAST in establishing normal neuronal and dendritic morphology in da neurons. Microtubules serve a major structural function in the cell and are required as tracks for transport of important cargo. Thus, I hypothesized that the knockdown of proteins associated with growing microtubule plus ends may have an ultimate phenotypic manifestation as a structural deformity. My quantitative results for measures of branch number and branching complexity in

class I da neuron comb dendrites indicated no significant difference between knockdowns of both proteins investigated and controls. However, my results for Orbit/MAST RNAi knockdowns remain inconclusive due to my qualitative observation of several features that were not present in all Orbit/MAST images but seemed to be more prevalent than in control images. These features included deformed cell bodies in class IV cells and thickened dendrite initial segments in class I cells of several Orbit/MAST knockdowns. A larger sample size is required to generate more conclusive results.

Analysis of the axon regeneration response in CLIP-190 and Orbit/MAST RNAi knockdowns revealed that this response does not occur in a normal fashion, as compared to the results reported by Stone et al. (2010). RNAi knockdown of Rtnl2 was also observed to produce an abnormal response to axon severing. Because Rtnl2 RNAi has not been used as a control in axon severing studies, there was no data available for comparison. In addition, loss of Rtnl2 in *Drosophila* has not been shown to affect any cellular processes, but this has not been confirmed in an assay of this nature. Thus, the results reported by Stone et al. (2010) were used as a control in the axon severing assay.

While Stone et al. (2010) observed a majority of class I ddaE neurons to initiate the conversion of a dendrite to an axon within 96h following axon severing, RNAi knockdowns of CLIP-190 and Orbit/MAST did not show this axon regeneration response. Two specific axon regeneration features were absent in these knockdowns: 1) the reversal of microtubule polarity from minus-end-out to plus-end-out in one dendrite of the injured neuron within 48h following axon severing and 2) the initiation of extensive dendrite tip growth in the dendrite that reversed microtubule orientation by 96h after injury. Rather than specifying one dendritic process to become the new axon, both RNAi knockdowns were observed to switch microtubule polarity in

all dendrites at all time points, and microtubule orientation in these process was not constant after 48h as observed by Stone et al. (2010). This lack of a normal response may indicate that both CLIP-190 and Orbit/MAST are required for the cell to "decide" which dendrite will take on axonal properties following the loss of its axon.

While the microtubule orientation and neuronal morphology assays in this study focused on analyzing cellular response to the loss of either CLIP-190 or Orbit/MAST under normal physiological conditions, the axon severing assay tested the cell's need for these proteins in a situation not usually experienced by neurons: the loss of an axon. The results indicate that these proteins may be part of a larger signaling pathway that allows microtubules in wild type cells to reverse polarity in a specific dendrite and thus initiate that dendrite to respecify into an axon. Maintained reversal of microtubule polarity is required for a dendrite to initiate the extensive tip growth associated with axonal respecification (Stone et al., 2010). This maintained microtubule polarity reversal was observed to occur prior to extensive tip growth in the control cells analyzed by Stone et al. (2010). Thus, the inability of RNAi knockdowns of CLIP-190 and Orbit/MAST to sustain one dendritic process with a reversed microtubule orientation may be the reason that extensive tip growth was not observed in these neurons.

RNAi knockdowns of the kinesin-like protein neb were analyzed in the same manner as those of CLIP-190 and Orbit/MAST for microtubule orientation and neuronal morphology. Kinesins travel along microtubules and transport cargo towards the plus-end, and these are the motors that transport cargo into axons, which have mostly plus-end-out microtubules (Hirokawa and Takemura, 2005). However, transport mechanisms into dendrites are still undetermined and may also involve kinesins (Hirokawa and Takemura, 2005; Kennedy and Ehlers, 2006; Levy and Holzbaur, 2006; Setou et al., 2004). Kinesin transport may be especially important in dendrite

tips, where microtubule orientation is 55% minus-end-out, which is much more mixed than in the main trunk (Stone et al., 2008). While RNAi knockdown of neb did not significantly affect microtubule orientation in the main trunk of class I da neuron dendrites or branching number, I did find a statistically significant effect of neb knockdown on branching complexity. Branching complexity showed a significant decrease, which may be due to the reliance of dendritic tips on kinesin motor transport of important cargo required for growth and development of the tips.

The use of RNA interference as the primary means for removal of protein from the cell is the main limitation of this study. Many of the findings in this study were negative, and one reason for this may be that the RNAi did not work effectively. Unlike genetic techniques that block gene transcription into mRNA or remove the gene from the cell altogether, RNAi depends on the efficient action of argonaute to identify and destroy mRNA that is transcribed in the cell, based on its complementary sequence to short interfering RNAs (siRNAs) contained in the RNA-induced silencing complex (RISC) (Macrae et al., 2006). Thus, the mRNA that escapes detection may still be transcribed, causing low levels of protein to exist in the cell. Therefore, further experiments should focus on techniques other than RNAi to determine the neuronal functions of CLIP-190, Orbit/MAST, and neb.

Microtubule orientation and dynamics have crucial roles in proper neuronal functioning, morphology, and signaling. A dramatic example of the pathology seen when cellular microtubule arrays are disturbed can be observed in various forms of mental retardation (Bodick et al., 1982; Purpura et al., 1982). This study sought to better understand the effects of reducing protein levels of several proteins associated with microtubule functioning on orientation of microtubules and morphology of the affected neurons. Elucidation of the particular role played by CLIP-190, Orbit/MAST, and neb is an important step in understanding the function of +TIPs

in regulating microtubule dynamics and the effects of reducing kinesin levels on dendritic morphology.

REFERENCES

Andersen, R. *et al.* Calcium/calmodulin-dependent protein kinase II alters structural plasticity and cytoskeletal dynamics in Drosophila. *J. Neurosci.* **25**, 8878-88 (2005).

Arber, S. *et al.* Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **393**, 805-9 (1998).

Bass, P. W., Sinclair, G. I. & Heidemann, S. R. Role of microtubules in the cytoplasmic compartmentation of neurons. *Brain Res.* **420**, 73-81 (1987).

Baas, P. W. *et al.* Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc. Natl. Acad. Sci. USA* **85**, 8335-9 (1988).

Beuren, A. J., Apitz, J. & Harmjanz, D. Supravalvular aortic stenosis in association with mental retardation and a certain facial appearance. *Circulation* **26**, 1235-40 (1962).

Bodick, N. *et al.* Microtubular disarray in cortical dendrites and neurobehavioral failure. II. Computer reconstruction of perturbed microtubular arrays. *Brain Res.* **281**, 299-309 (1982).

Burton, P. R. Dendrites of mitral cell neurons contain microtubules of opposite polarity. *Brain Res.* **473**, 107-15 (1988).

Dietzl, G. *et al.* A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* **448**, 151-6 (2007).

Feany, M. B. & Bender, W. W. A Drosophila model of Parkinson's Disease. *Nature* **404**, 394-8 (2000).

Galjart, N. CLIPs and CLASPs and cellular dynamics. *Nat. Rev. Mol. Cell Biol.* **6**, 487-498 (2005).

Grueber, W. B., Jan, L. Y. & Jan, Y. N. Tiling of the Drosophila epidermis by multidendritic sensory neurons. *Development* **129**, 2867-78 (2002).

Heidemann, S. R. & McIntosh, J. R. Visualization of the structural polarity of microtubules. *Nature* **286**, 517-9 (1980).

Heidemann, S. R., Landers, J. M. & Hamborg, M. A. Polarity orientation of axonal microtubules. *J. Cell. Biol.* **91**, 661-5 (1981).

Hirokawa, N. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* **279**, 519-26 (1998).

Hirokawa, N. & Takemura, R. Molecular motors and mechanisms of directional transport in neurons. *Net. Rev. Neurosci.* **6**, 201-14 (2005).

Hoogenraad, C. C. *et al.* Targeted mutation of Cyln2 in the Williams syndrome critical region links CLIP-115 haploinsufficiency to neurodevelopmental abnormalities in mice. *Nat. Genet.* **32**, 116-27 (2002).

Hoogenraad, C. C. *et al.* LIMK1 and CLIP-115: linking cytoskeletal defects to Williams syndrome. *Bioessays* **26**, 141-50 (2004).

Huttenlocher, P. R. Dendritic development and mental defect. *Neurology* **20**, 381 (1970).

Huttenlocher, P. R. Synaptic and dendritic development and mental defect. *UCLA Forum Med. Sci.* **18**, 123-40 (1975).

Kapitein, L. C. *et al.* Mixed microtubules steer dynein-driven cargo transport into dendrites. *Curr. Biol.* **20**, 290-9 (2010).

Kaufmann, W. E. & Moser, H. W. Dendritic anomalies in disorders associated with mental retardation. *Cereb. Cortex* **10**, 981-91 (2000).

Kennedy, M. J. & Ehlers, M. D. Organelles and trafficking machinery for postsynaptic plasticity. *Annu. Rev. Neurosci.* **29**, 325-62 (2006).

Komarova, Y. A. *et al.* Cytoplasmic linker proteins promote microtubule rescue in vivo. *J. Cell Biol.* **159**, 589-99 (2002).

Lee, H. *et al.* The microtubule plus end tracking protein Orbit/MAST/CLASP acts downstream of the tyrosine kinase AbI in mediating axon guidance. *Neuron* **42**, 913-26 (2004).

Levy, J. R. & Holzbaur, E. L. Cytoplasmic dynein/dynactin function and dysfunction in motor neurons. *Int. J. Dev. Neurosci.* **24**, 103-11 (2006).

Macrae, I. J. *et al*. Structural basis for double-stranded RNA processing by Dicer. *Science* **311**, 195-8 (2006).

Máthé, E. *et al.* Orbit/Mast, the CLASP orthologue of Drosophila, is required for asymmetric ste cell and cystocyte divisions and development of the polarised microtubule network that interconnects oocyte and nurse cells during oogenesis. *Development* **130**, 901-15 (2003).

Meng, Y. *et al.* Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Nature* **35**, 121-33 (2002).

Meulener, M. *et al.* Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Curr. Biol.* **15**, 1572-7 (2005).

Morris, C. A. Genetic aspects of supravalvular aortic stenosis. *Curr. Opin. Cardiol.* **13**, 214-9 (1998).

Park, J. *et al.* Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature* **441**, 1157-61 (2006).

Pesah, Y. *et al.* Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131**, 2183-94 (2004).

Pierre, P. *et al.* CLIP-170 links endocytic vesicles to microtubules. *Cell* **70**, 887-900 (1992).

Purpura, D. P. Dendritic spine "dygenesis" and mental retardation. *Science* **186**, 1126-8 (1974).

Purpura, D. P. Dendritic differentiation in human cerebral cortex: normal and aberrant developmental patterns. *Adv. Neurol.* **12**, 91-134 (1975).

Purpura, D. P. Normal and aberrant neuronal development in the cerebral cortex of human fetus and young infant. *UCLA Forum Med. Sci.* **18**, 141-69 (1975).

Purpura, D. P. *et al.* Microtubule disarray in cortical dendrites and neurobehavioral failure. I. Golgi and electron microscope studies. *Brain Res.* **281**, 287-97 (1982).

Rolls, M. M. *et al.* Polarity and intracellular compartmentalization of Drosophila neurons. *Neural Dev.* **2**, 7 (2007).

Sánchez-Soriano, N. *et al.* Are dendrites in Drosophila homologous to vertebrate dendrites? *Dev. Biol.* **288**, 126-38 (2005).

Setou, M., Hayasaka, T. & Yao, I. Axonal transport versus dendritic transport. *J. Neurobiol.* **58**, 201-6 (2004).

Stepanova, T. *et al.* Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). *J. Neurosci.* **23**, 2655-64 (2003).

Stone, M. C., Roegiers, F. & Rolls, M. M. Microtubules have opposite orientation in axons and dendrites of Drosophila neurons. *Mol. Biol. Cell* **19**, 4122-9 (2008).

Stone, M. C. *et al.* Global up-regulation of microtubule dynamics and polarity reversal during regeneration of an axon from a dendrite. *Mol. Biol. Cell* **21**, 767-77 (2010).

Strømme, P., Bjørnstad, P. G. & Ramstad, K. Prevalance estimation of Williams syndrome. *J. Child Neurol.* **17**, 269-71 (2002).

Williams, J. C., Barratt-Boyes, B. G. & Lowe, J. B. Supravalvular aortic stenosis. *Circulation* **24**, 1311-8 (1961).

Academic Vita of Joanna M. Skora

424 Waupelani Drive Apt. I-34, State College, PA 16801
(732) 600-3782
jms5456@psu.edu

Education

The Pennsylvania State University, University Park, PA, May 2010
Bachelor of Science, Biology (Neuroscience Option), Psychology Minor
Schreyer Honors College

Honors in Biochemistry and Molecular Biology
Thesis: The role of CLIP-190, Orbit/MAST, and neb in microtubule orientation, neuronal morphology, and axonal regeneration in *Drosophila melanogaster*
Thesis Supervisor: Melissa Rolls

Experience

Undergraduate Research — May 2008-Present
Biochemistry and Molecular Biology Department at Penn State, Dr. Melissa Rolls

- Research microtubule dynamics and the cell biology of neurons in *Drosophila melanogaster*
- Generate and analyze data for honors thesis

Patient Floor and Emergency Department Volunteer — January 2008-Present
Mount Nittany Medical Center

- Transport patients for discharge and medical testing
- Assist nurses with floor maintenance and equipment transport
- Refer patients and families to correct locations
- Train incoming volunteers

Student Red Cross Club Active Member and Volunteer — September 2006-Present
Pen State Student Red Cross Club

- Volunteer at student-run blood drives
- Trained as an on-site coordinator for blood drives in Spring 2010
- Responsible for ensuring blood drives reach the goal amount of donations
- Help with fundraising for the Four Diamond Fund and THON

Extern — May 2008
Hackensack University Medical Center

- Observed laparoscopic cholecystectomy (removal of the gallbladder)
- Performed patient rounds and office hours with Dr. Hans Schmidt of Advanced Laparoscopic Associates

Research Assistant for the Family Life Project — September 2007-May 2008
Human Development and Family Studies Department at Penn State

- Observed and coded for specific child behaviors

- Generated and analyzed data for the Family Life Project, a large collaborative research project conducted by researchers at Penn State and the University of North Carolina at Chapel Hill, with the goal of better understanding young child development in rural counties

Box Office Attendant June-August 2007
Marquee Cinemas
- Managed the box office and concessions area
- Trained new employees

Sales Associate June 2007
New York & Company
- Supervised areas of the sales floor and fitting rooms

Awards and Honors

A & A Unger Memorial Scholarship (2009-2010)
George H. Deike Scholarship (2009-2010)
Penn State Bookstore Scholarship (2007-2010)
Bunton Waller Scholarship (2006-2010)
Academic Excellence Scholarship (2006-2009)
Schreyer Honors College Summer Research Grant (2008, 2009)
Anna Belle Beier Scholarship (2006, 2008-2009)
Pre-Med Scholarship (2008)
Mathues Scholarship in Science (2007-2008)
President's Freshman Award (2007)
Ed Beier, Arthur and Barnes A Scholarship (2006-2007)
Academic Competitiveness Grant (2006-2007)
The University Trustee Scholarship (2006-2007)
Internal Scholarship, Agriculture Department (2006)
Oswald Scholarship (2006)