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SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

MICROTUBULE POLARITY AND DYNAMICS IN *DROSOHPILA* NEURONAL DENDRITES

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ABSTRACT

The neuron is a polar cell, with axons and dendrites sending and receiving signals, respectively. It has been determined that microtubules play a key role sub-compartmental neuron organization by serving as tracks for motor proteins like kinesin and dynein to transport cargo throughout the cell. As such, microtubule orientation, or polarity, is important to the organization of the cell as a whole. In most cells, the microtubule array is nucleated at the centrosome, with the microtubule minus ends anchored to the centrioles (Alberts et al. 2008). However, in neurons the dendrites have microtubules with minus ends out, nucleated at an unknown source.

My first objective in this study was to screen various candidate proteins to identify those that may be involved in determining microtubule orientation in the dendrite. RNA interference (RNAi) was used to reduce expression of each of the genes that may affect microtubule polarity. Of the proteins screened, only three: pk, esn, and kat-60L1, showed a statistically significant phenotype ($p<0.05$). During the screen, it was observed that reducing levels of Dgrip84, a component of the gamma-tubulin small complex (γ-TuSC) and gamma-tubulin ring complex (γ-TuRC) caused slight changes in the number of growing microtubules in the axon. A more aggressive injury assay was developed to determine more conclusively whether Dgrip84 is involved in microtubule nucleation in the dendrites. The typical injury response involves dramatically increased microtubule dynamics, however I hypothesized that cells lacking γ-TuRC and γ-TuSC proteins will not be able to up-regulate dynamics efficiently. Results showed that all Dgrip RNAi followed a trend of reduced ability to up-regulate dendrite microtubule dynamics in response to axotomy, however the phenotype was only statistically significant ($p<0.05$) for Dgrip91 RNAi and Dgrip128 RNAi. This finding suggests that not only the γ-TuSC, but the γ-TuRC as a whole, is important for non-centrosomal MT nucleation.

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ACKNOWLEDGEMENTS

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Chapter 1

Introduction

What is a neuron?

The neuron is a cell specialized to receive, conduct, and transmit signals (Alberts et al. 2008). These highly-specialized cells are split into three general categories. Sensory neurons are the cells that are responsible for collecting and integrating information from the environment, while motor neurons control movement and coordinate involuntary bodily functions. Interneurons relay and integrate signals from other cells (Alberts et al. 2008). A neuron has an axon for sending signals and dendrites for receiving them. The axon is typically long and thin, while dendrites, especially of peripheral sensory neurons, are short and bushy with many branches to expand the neuron's coverage area (Conde and Caceres 2009, Alberts et al. 2008). Many neurons have extremely elaborate dendritic trees that make connections with hundreds of other neurons.

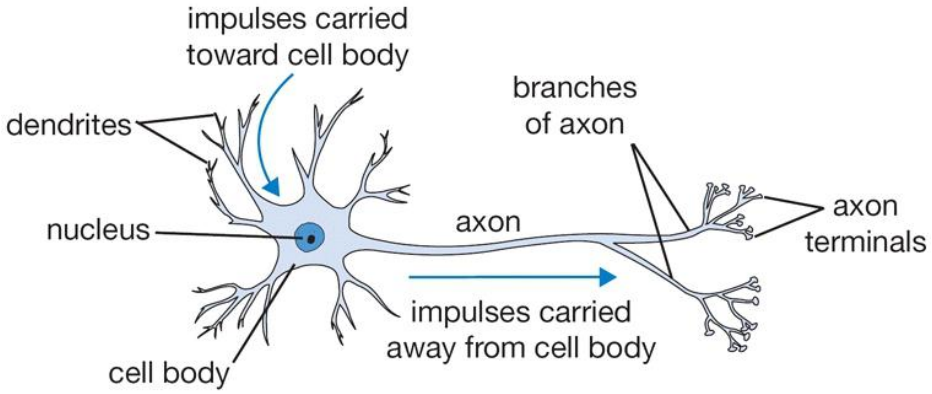

Figure 1-1: Structure of a neuron. The neuron has a long, thin axon for sending signals and shorter, bushy dendrites for receiving signals. (Sherman)

Since the role of the neuron is so fine-tuned, the proper formation of axonal and dendritic arbors is crucially important (Masland 2011). Yet, scientists are far from understanding exactly how neurons and neural networks develop. It is not understood how a neuron specifically develops an axon or dendrite, nor how dendrite growth and branching are regulated. Answering these questions may be important for gaining a greater understanding of common neurological dysfunctions like autism, mental retardation, and hereditary spastic paraplegia.

The neuron is one of the body's most polarized cells, and arguably the one in which polarity is most essential to proper function (Baas 2002, Conde and Caceres 2009). Since the cytoskeleton is crucial to maintaining the cell's shape and structure, much of the research into neuronal polarity has focused on cytoskeletal components like actin and microtubule (MT) fibers (Baas 2002).

Microtubules

Microtubules (MTs) are an important part of the cytoskeleton; they play a role in developing and maintaining cell shape, provide for motility, allow synapse formation and vesicle transport, and are essential to cell division. MTs are composed of thirteen fibrils organized in a long, hollow, cylindrical structure about 25 nm in diameter. Each fibril is composed of α- and β-tubulin dimers and has intrinsic polarity (Alberts et al. 2008). MT growth rate is proportionally dependent on free tubulin concentration and is different at each end of the MT (Zheng et al. 1995). Most growth and catastrophe occurs at the designated dynamic plus-end, which grows approximately three times faster than the minus-end, while the minus end is usually anchored and more stable (Zheng et al. 1995, Alberts et al. 2008, Conde and Caceres 2009).

A third member of the tubulin family, γ-tubulin, is present in much smaller amounts but is essential for MT nucleation (Alberts et al. 2008). The significance of γ-tubulin was realized

when *in vitro* experiments showed that γ-tubulin monomers enhanced the assembly of α/β-heterodimers and stabilized the minus ends of MTs (Li et al.1995). The structure and functionality of γ-tubulin are conserved throughout all eukaryotes and even yeast (Zheng et al. 1995, Oegema et al.1999). γ-Tubulin localizes to MT organizing centers, like the centriole in eukaryotes, and in complex with pericentriolar material serves as a base for MT nucleation. γ-Tubulin has been observed in complexes called the γ-tubulin small complex (γ-TuSC) and γ-tubulin ring complex (γ-TuRC), which have been proven capable of nucleating MTs *in vitro* (Zheng et al. 1995, Alberts et al. 2008). The roles of the γ-TuSC and γ-TuRC are discussed further in chapter 3.

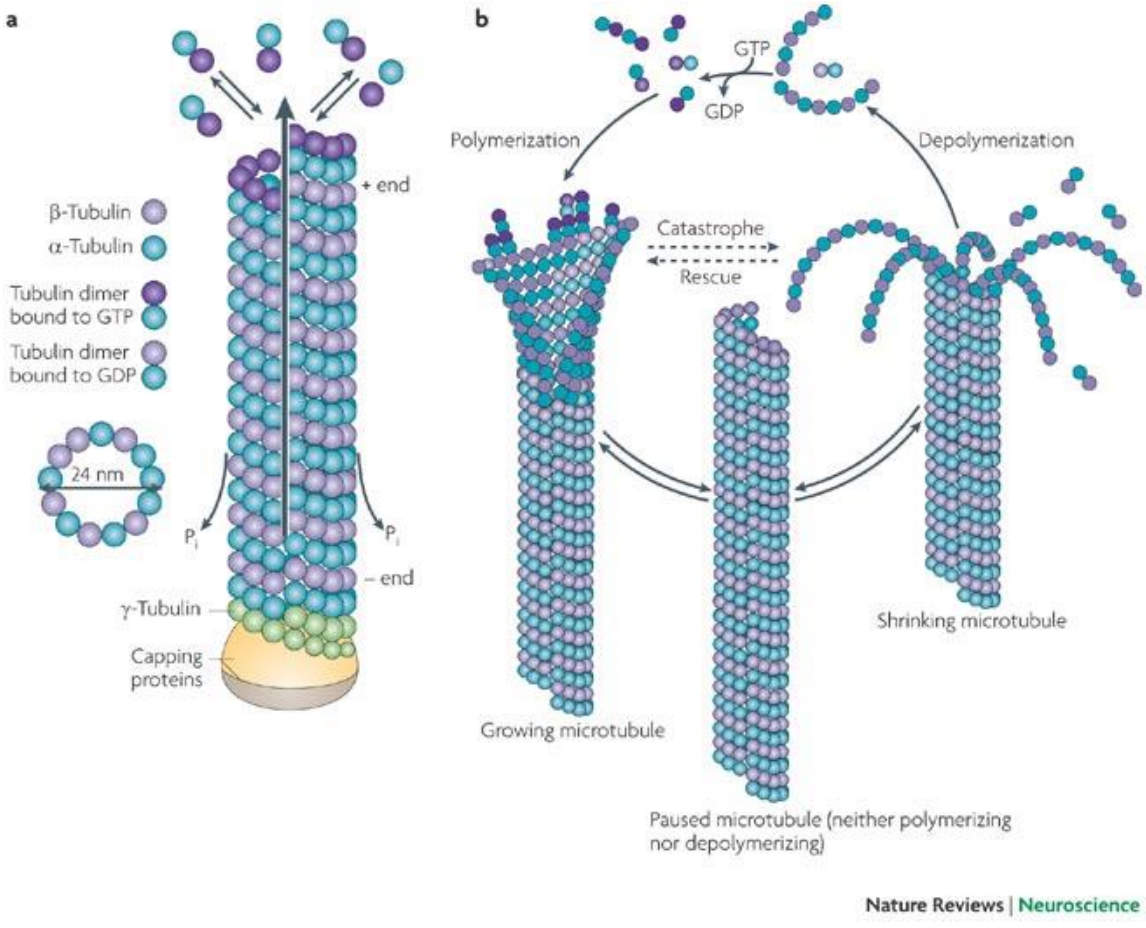

Figure 1-2: Microtubule polymerization. Microtubules are intrinsically polar, composed of α- and β-tubulin dimers. γ-Tubulin and capping proteins like the γ-TuRC in pericentriolar material stabilize and anchor the minus-end. The plus-end is dynamically unstable and goes through rounds of growth, shrinkage, catastrophe, and rescue. (Conde and Caceres 2009).

The intrinsic polarity of MTs is one of their most important qualities in terms of intracellular transport. Although each is only a few microns long, the MTs can overlap to create long tracks used by motor proteins to carry specific proteins, vesicles, and mRNA to various parts of the cell. Some cargo-carrying motor proteins can only move in one direction on the MT. For example, kinesins move towards the plus-end, while dynein moves towards the minus-end of the MT. The kinesin family, in particular, is very large, suggesting that each kinesin is specific for its cargo and localization (Alberts et al. 2008). Thus, the organization of polar MTs into complex, ordered and dynamic arrays has significant influence on the development and maintenance of the cell as a whole. The structure and polymerization of these arrays is influenced by many factors including regulation of polymerization dynamics, MT-associated proteins, and MT nucleation (Desai 1997).

As mentioned, the polarity of neurons is especially important since axons and dendrites must be organized to perform very different functions. Loss of neuronal polarity has been correlated with changes in MT organization and dynamics (Conde and Caceres 2009). Axons generally have the plus-ends of MTs away from the cell body. It was previously thought that dendrites contained MTs of mixed polarity, however it has been shown in *Drosophila* that the main dendrite branch actually contains almost all

class I da neurons	minus-end-out microtubules
proximal dendrites	94% +/- 3.5%
distal dendrites	55% +/- 15%

Stone, M. 2008.

Figure 1-3: Microtubule orientation in dendrites of a type I da sensory neuron. The axon contains plus-end out microtubules. Contrary to prior belief, the main branch of the dendrite contains almost all minus-end out microtubules. Distal dendrite branches are more mixed. (Stone et al. 2008).

minus-end out MTs (Stone et al. 2008, Figure 1-3). This result is of considerable interest since it is not understood how the minus-ends of MTs, which in most cell types are observed anchored to the centrosome in the cell body, could instead be at the distal end of the main dendrite branch. Several studies have suggested that all neuronal MTs are nucleated from the centrosome in the cell body, released by severing proteins, and transported by dynein or kinesin to the axon or dendrite (Ahmad et al. 1994, 1995, 1998; Conde and Caceres 2009). However, more recent studies have shown that the centrosome is not required for nucleation of MTs in neurons (Nguyen et al. 2011, Colombie et al. 2006). In fact, many *Drosophila* cells do not have functional centrosomes except during mitosis (Rogers et al. 2008).This suggests that there may be a MT nucleation site is present in the dendrites. In the following study, I seek to determine some of the factors that influence the polarity and dynamics of MTs in *Drosophila* ddaE neurons, particularly the dendrite.

Drosophila **as a model system**

Drosophila is used as the model organism in the following study. The organism is well-suited for research due to its short life cycle, easy maintenance, and the fact that many tools have been developed that can be used to manipulate *Drosophila* genetics. Results discovered in *Drosophila* are medically relevant to humans because many of the major developmental pathways are conserved.

Conveniently, the larvae are transparent with neurons just beneath the cuticle, thus the same larva can be imaged over several days without cutting the larva or disrupting normal function. These are peripheral sensory multidendritic arborization neurons are classified by branching complexity (Class I-IV) (Figure 1-4, Greuber et al. 2002). This study makes use of Class I ddaE neurons because the simple structure makes measuring MT polarity and dynamics

easier than in a larger, highly branched, Class IV neuron. In short, the flexibility of genetic tools and ease of maintenance combined with the practicality of microscopy make *Drosophila* an excellent model for *in vivo* studies.

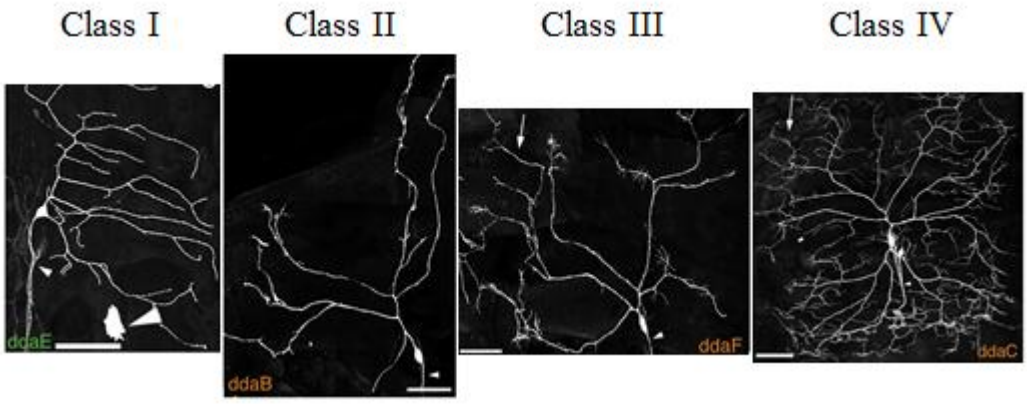

Figure 1-4: Morphologies of dendritic arborization neurons. Examples of Class I-IV dendritic arborization neurons show increasing complexity with each class. Neurons are labeled by mCD8::GFP. Small arrowheads indicate the axon. The large arrowhead in the Class I ddaE image indicates a cell labeled by activity of ELAV-Gal4.The white arrows in the Class III and IV images indicate the position of the segment border, beyond which dendrites mix with those of neurons in the next anterior segment. Images were taken using a confocal microscope. Scale bars are 50 µm. (Greuber 2002, Figures 2-5).

Chapter 2

Microtubule Polarity RNAi Screen

I hypothesize that there is a specific mechanism for nucleating MTs in neuronal dendrites. I base this hypothesis on three established facts. 1) Microtubules in the main dendrite branch are minus-end out (Stone et al. 2008). 2) The centrosome is not required for MT nucleation in *Drosophila* neurons (Nguyen et al. 2011). 3) The centrosome is not even present in most interphase *Drosophila* neurons (Rogers et al. 2008). Given this information, the first goal of my research was to identify proteins that act in a mechanism determining MT polarity in *Drosophila* neurons.

A screen was performed using candidate proteins selected based on potential involvement in controlling MT nucleation. We reasoned that proteins found at centrosomes in other cells, or associated with MT minus ends in some other context may also play a role in localizing MT nucleation sites to dendrites. RNA interference (RNAi) was used to reduce expression of each of the genes that may affect microtubule polarity (Figure 2-1, Table A-1). The polarity of the microtubules in class I dendritic arborization sensory neurons was observed via fluorescent microscopy. End-binding protein 1 (EB1), a protein that binds to the plus tips of growing microtubules, was tagged with green fluorescent protein (GFP). By observing the direction in which the spots of fluorescence move, I deduced the polarity of the microtubules in the cell. Reducing expression of a protein that is essential to this process should present a phenotype that deviates from the wild type polarization.

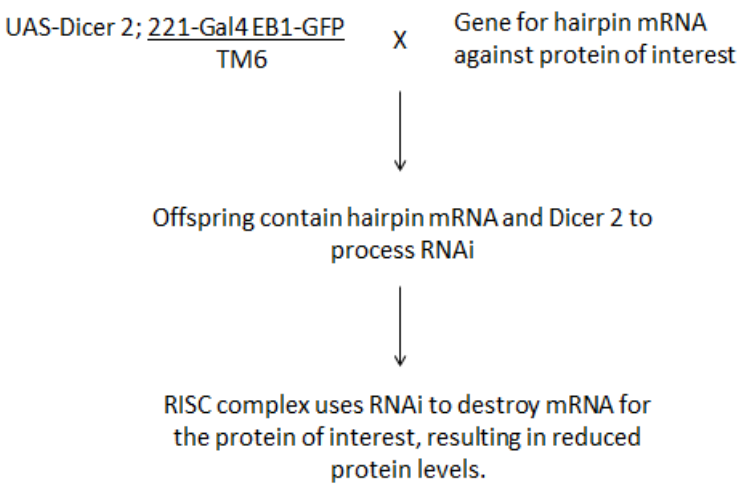

Figure 2-1: Use of RNAi to reduce protein levels in *Drosophila* Virgins containing UAS-Dicer 2 were crossed with males containing an inserted gene for hairpin mRNA against the protein of interest (from Vienna *Drosophila* Resource Center). The offspring use Dicer to process the hairpin mRNA, which is then incorporated as RNAi into the RISC complex and used to reduce levels of complementary mRNA throughout the cell. EB1-GFP is used to visualize the tips of growing microtubules via fluorescent microscopy. TM6 is a balancer to maintain the health of the line.

Candidate proteins were selected based either upon their previously determined interactions with microtubules or roles in other related cell processes. The categories of proteins investigated included microtubule destabilizing proteins, G-proteins, planar cell polarity proteins (PCP), proteins involved with nucleation, and gamma-tubulin ring complex (γ-TuRC) proteins.

Microtubule destabilizing factors (Figure 2-3) include severing proteins like katanin, spastin, and fidgetin. To date, there has been no published account of kat-60L1 specifically exhibiting severing activity, however it is structurally similar enough to katanin that severing activity seems highly likely (Frickey et al. 2004, Stewart et al. 2012). Spastin and fidgetin have been shown to stimulate minus-end depolymerization. Spastin been shown to sever microtubules from the protective γ-tubulin ring complex (γ-TuRC) in mitosis (Zhang et al. 2007). Unlike katanin and spastin, however, purified fidgetin has not yet been shown to sever MT *in vitro* (Roll-Mecak and McNally 2010). For the purposes of this experiment, stathmin, which binds α- and β-tubulin subunits and prevents assembly, is also considered a destabilizing factor (Alberts et al. 2008).

Severing proteins function by forming hexamers that disrupt tubulin-tubulin contacts along MTs and then use ATP hydrolysis to promote end depolymerization or break the long

polymers into smaller pieces (Roll-Mecak and Vale 2008; Roll-Mecak and McNally 2010). Those smaller pieces can then be further disassembled, a process that has been observed *in vitro* (McNally and Vale 1993), or provide a source of more easily transported tubulin or seeds for MT nucleation (Roll-Mecak and McNally 2010).

The severing proteins' interactions with MTs make them candidates for involvement in establishing neuronal polarity. Katanin, kat-60L, and spastin have all specifically been shown to be present in the *Drosophila* nervous system (McNally and Vale 1993). The severing proteins have been shown to play roles in the structure of cells, including neurons. For example, increasing levels of spastin leads to a greater number of dendrite branch points, while decreasing spastin levels moderately inhibits axon growth (Conde and Caceres 2009).

Katanin is expressed in developing neurons and is present throughout the neuronal cytoplasm, including at the centrosome (Ahmad et al. 1999). As is the case with microtubule-associated proteins, many of the studies conducted so far investigate severing proteins' interaction with the centrosome and roles in cell division. In *C. elegans,* a loss-of-function mutation in katanin has been shown to cause an inability to form a bipolar meiotic spindle (Mains et al. 1990). Electron microscope tomography of mutant spindles has revealed that fewer, longer MTs are present (Srayko et al. 2006). Katanin has also been shown to stimulate plus-end microtubule depolymerization during mitosis (Zhang et al. 2007), but the fact that katanin is also present elsewhere in the cell suggests that the protein may serve additional roles. In fact, katanin has been shown to also colocalize at the cell edge with cortical actin in an actin-dependent fashion (Zhang et al. 2011, Figure 1-2). Though the severing proteins have been shown to have important roles in cell development a specific role in polarity determination has never been indicated.

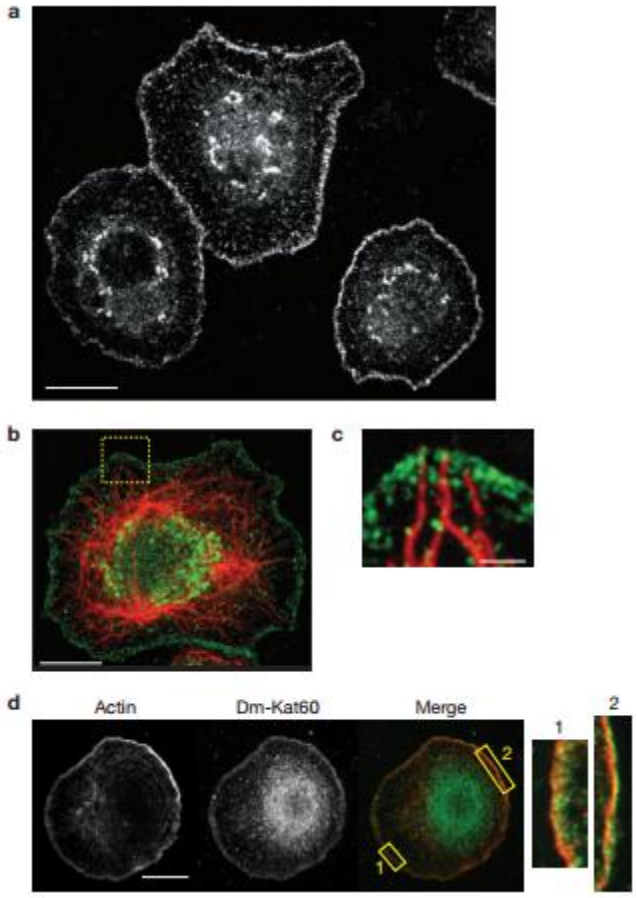

Figure 2-2: Katanin targets the cell cortex of interphase cells. **(a)** Immunofluorescence micrograph showing the localization of Katanin in S2 cells. **(b)** Immunofluorescence micrograph of an interphase S2 cell double labeled for microtubules (anti-α-tubulin; red) and Katanin (green). **(c)** High magnification of the region outlined in **b**. **(d)** Immunofluorescence of an interphase S2 cell double labeled for actin (red) and Katanin (green) and a higher magnification of the two regions outlined in the 'merge' panel. Scale bars 10 μm (**a,b,d**) 2 μm **(c).** (Zhang et al 2011).

The polarity effects of G-protein subunits were also investigated as these subunits are often important in early signaling and development pathways. Along with destabilizing proteins and G-proteins, PCP proteins were tested. Bhanot (1999) describes a set of "core" planar cell polarity (PCP) proteins including frizzled (fz), disheveled (dsh), prickled (pk), and flamingo (fmi, also called starry night, stan) (Gao, 2000). Usui (1999) includes esn and limpet in this group of important PCP proteins. The planar cell polarity (PCP) proteins are involved in organizing cell directionality orthogonal to their apical-basal axes. PCP proteins have been researched most extensively in epithelial cells and *Drosophila* wing cells (Shulman 1998), however, they have also been shown to play critical roles in sensory cells. For example, fmi has been shown to control planar polarity of sensory bristles and sensory organ precursor cells. The related proteins tested in this RNAi screen include espinas (esn), prickled (pk), and limpet (Figure 2-6).

Proteins that played some role at the centrosome or in nucleation of MTs were grouped and screened for effects on MT polarity. These proteins included centrosomin (cnn), shaggy (sgg), and aurora-A. Additionally, Dgrip proteins in the γ-tubulin small complex (γ-TuSC) and γ-tubulin ring complex (γ-TuRC) were also investigated, since they play a role in nucleation and stabilizing the minus ends of microtubules. Several additional protein RNAi trials were also run based on each protein's roles in other related cell processes. These miscellaneous proteins include par-1, src, and ik2.

Results

For each RNAi cross, a series of videos with 200 frames at 2-second intervals was analyzed in order to determine the polarity of the dendrite comb. Figure 1-3 shows a sample of time-lapse frames with arrows indicating the location and progress of comets over time. Parts of the experiment were conducted by two people, each with an internal control for individual differences in microscopy and data analysis techniques. For a control, we used an RNA hairpin (Rtnl2)that has yielded results similar to cells with no hairpins in all assays performed. For the Rtnl 2 RNAi control, 373 comets in a total of 311arvae were observed. Observing the motion of EB1-GFP comets in Rtnl 2 RNAi larvae showed that 85.5% of comets in the main dendrite branch move towards the cell body. In the second control (Caroline's Rtnl 2, Figure 2), a total of 205 comets were observed, showing that 88.3% of comets in the main dendrite branch move towards the cell body.

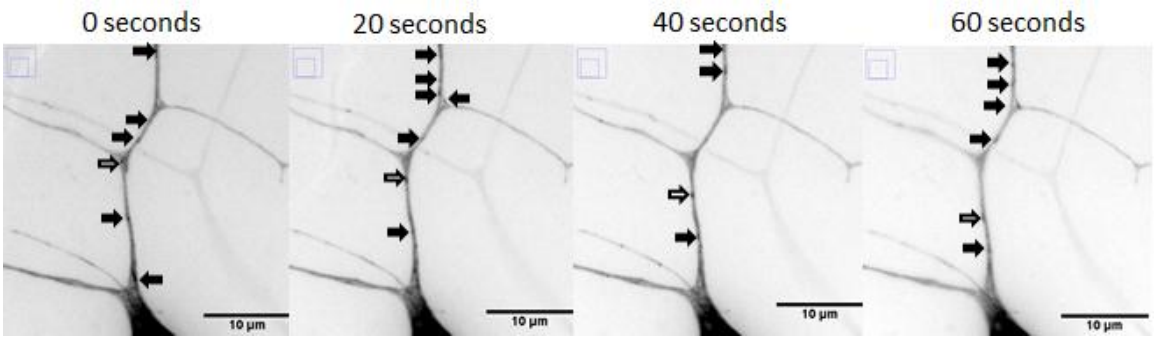

Figure 2-3: Rtnl 2 polarity control Black arrows indicate the location of EB1-GFP comets. White arrows indicate the movement of one comet towards the cell body over one minute. Due to larval movement during live imaging, not all comets are visible in all frames. Image is inverted for ease of viewing.

Kat 60, kat-60L1, and fidgetin RNAi were all compared to the Rtnl 2 RNAi control, while spastin and stathmin RNAi are compared to Caroline's Rtnl 2 RNAi. Fisher's exact test was used to compare the number of comets observed moving towards and away from the cell body. The observed dendrite polarities for Kat 60, fidgetin, spastin, and stathmin RNAi all fell within the expected values. Alternatively, kat-60L1 RNAi displayed slightly mixed polarity with only 70.6% of comets moving towards the cell body ($p < 0.0001$, Figure 2-4).

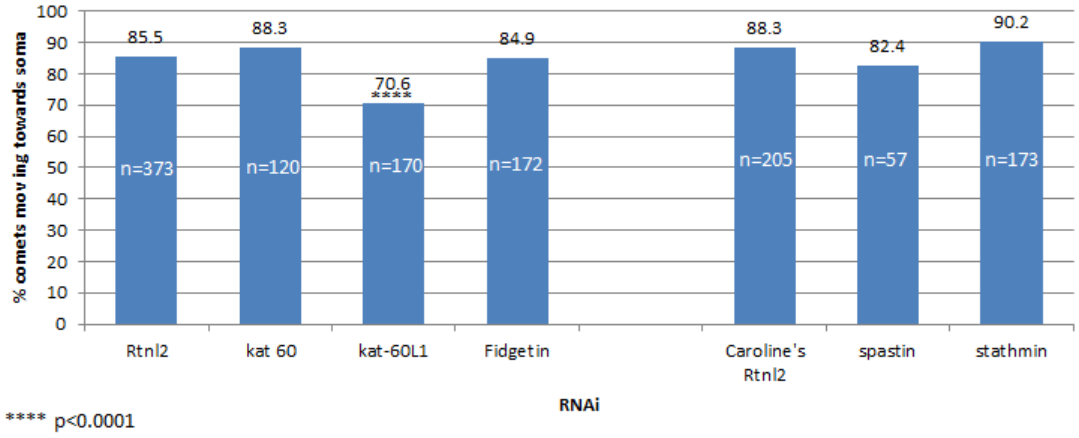

Figure 2-4: Polarity effects of destabilizing proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body. Experiments were conducted by two people working on the same project and internal analysis controls are shown for each.

A selection of G-proteins was tested to see if reduced levels of any specific G-proteins would influence dendrite comb polarity. G-α47A, G-α49B, and G-γ30A RNAi were compared to Rtnl 2 RNAi. G-α60A, G-β76C, and G-α73B RNAi were screened by Caroline and compared to Caroline's Rtnl 2 RNAi. None of the RNAi reductions showed polarity that differed significantly from the control (Figure 2-5).

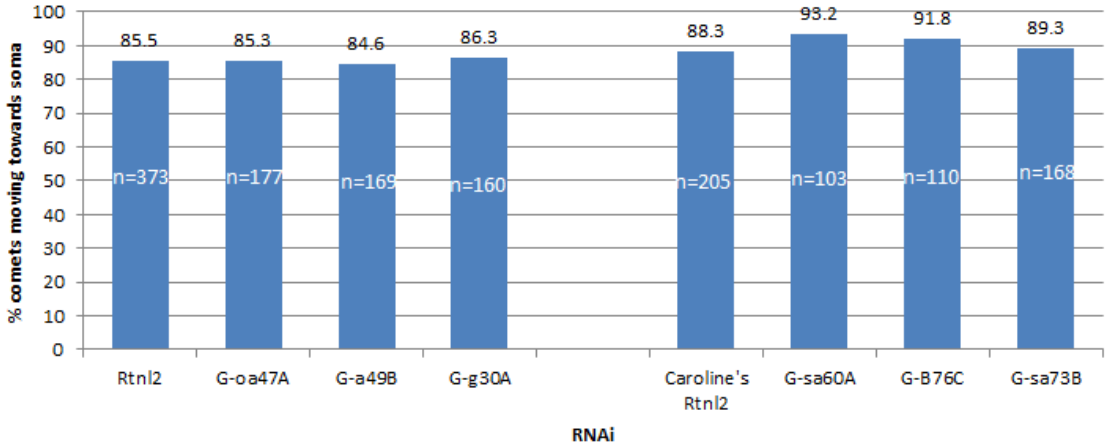

Figure 2-5: Polarity effects of G-proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body. Experiments were conducted by two people working on the same project and internal analysis controls are shown for each.

Proteins associated with planar polarity cells were also tested to see whether reducing their levels by RNAi would affect dendrite comb polarity. Espinas (esn), prickled (pk), and limpet were all tested. All planar polarity proteins tested showed moderately higher proportions of comets moving towards the soma as compared to the control. However, only esn and pk showed a statistically significant increase ($p < 0.05$) (Figure 2-6).

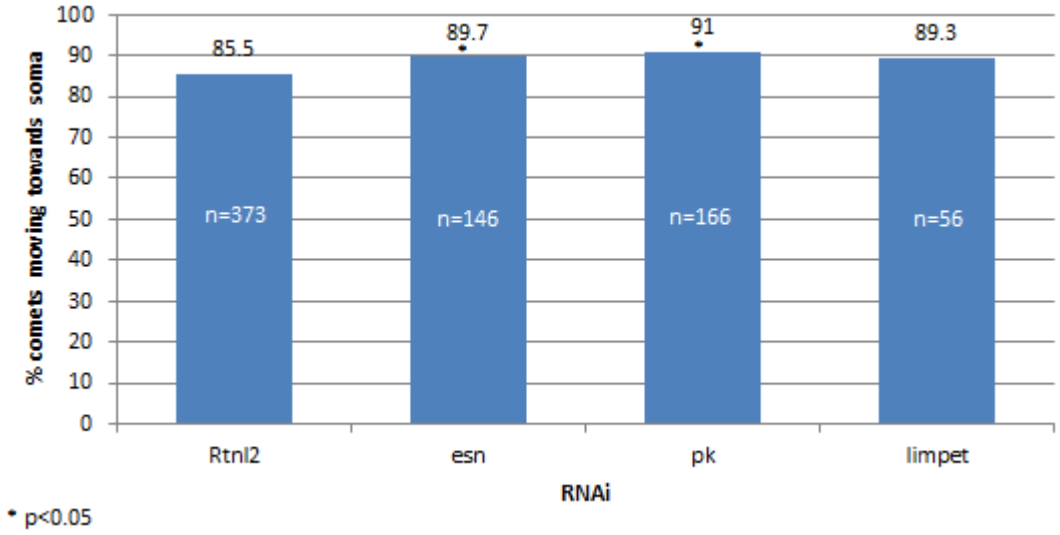

Figure 2-6: Polarity effects of planar cell proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body.

Three proteins associated with nucleation, centrosomin (cnn), shaggy (sgg), and aurora-A were reduced with RNAi to see if they affected dendrite comb polarity. Sgg and aurora-A both showed increases in the proportion of EB1-GFP comets moving towards the cell body, however, only the result of aurora-A RNAi proved statistically significant ($p<0.05$). Cnn actually showed the opposite trend, however this result was not statistically different from the control (Figure 2-7).

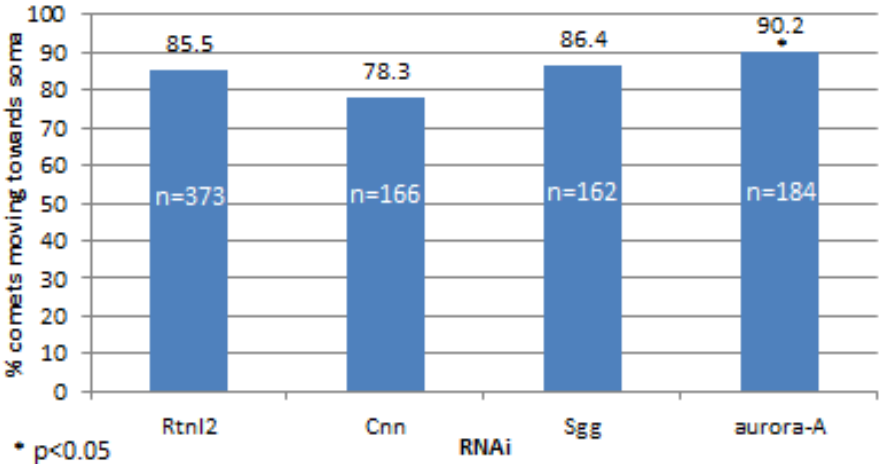

Figure 2-7: Polarity effects of nucleation proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body.

Dgrip proteins, components of the γ-tubulin small complex (γ-TuSC) and γ-tubulin ring complex (γ-TuRC) were reduced individually with RNAi. Dgrip71 and Dgrip163 showed slightly mixed polarity, with 76.8% and 79.1% of EB1-GFP comets moving towards the soma, respectivley. However, Fisher's exact contingency test showed neither result to be statistically significant (Figure 2-8). Although not shown here, a slight decrease in microtubule dynamics was noticed in the axons of Dgrip84 RNAi neurons. This observation is discussed further in Chapter 3.

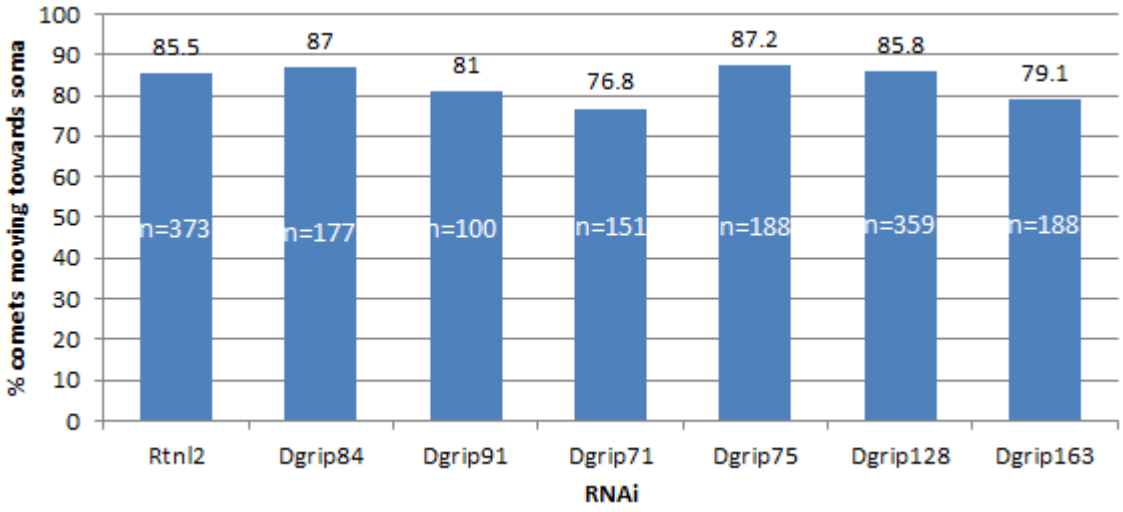

Figure 2-8: Polarity effects of Dgrip proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body.

Other uncategorized proteins were chosen as candidates based on their roles in other cellular functions. Par-1, and src RNAi were compared to Rtnl 2 RNAi. Ik2 RNAi was compared to Caroline's Rtnl 2 RNAi. Only aurora-α showed a statistically significant difference in dendrite comb polarity with 90.2% of EB1-GFP comets moving towards the soma ($p < 0.05$, Figure 2-9).

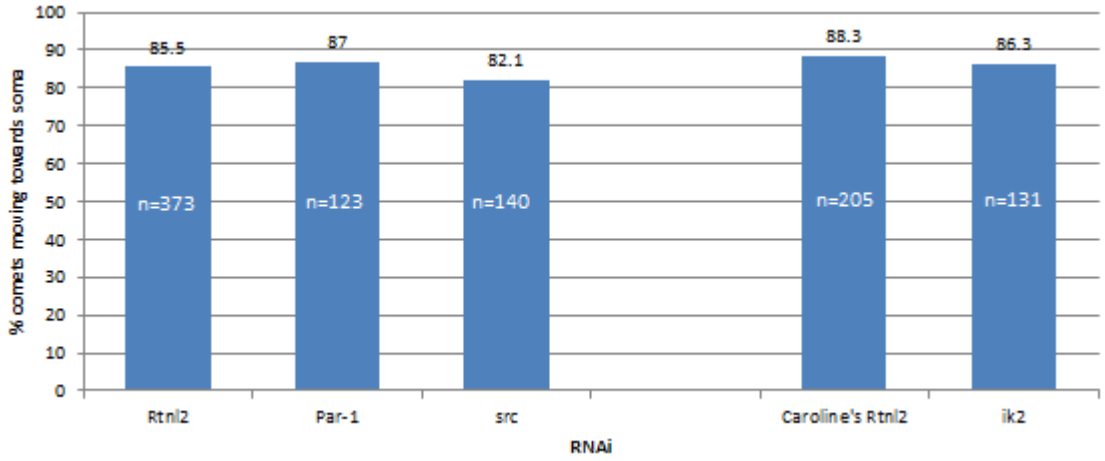

Figure 2-9: Polarity effects of miscellaneous proteins. Protein levels were reduced by RNAi. Polarity was deduced by observing the number of EB1-GFP comets moving towards and away from the cell body.

Discussion

Interestingly, of the severing proteins tested, only kat-60L1 showed a statistically significant polarity change. Based on the functions of kat-60L1 and related katanin determined in previous studies, it is reasonable to think that that kat-60L1 would play a role in establishing neuronal polarity. Work in *Drosophila* has shown that kat-60L1 is required in late larval development for normal neuron structure and has also been implicated indendritic pruning (Sherwood et al. 2012, Lee et al . 2009). Without kat-60L1, the neurons have fewer, shorter dendrite branches, reducing the density of the dendritic arbor and the perceptive area of the overlying epidermis. This aberrant structure is reflected in a loss of sensation of noxious or painful stimulus (Stewart et al. 2012). Increased MT polarity-mixing was not the only result of reduced kat-60L1 activity; a reduced number of growing MT was also observered, though not quantitated (data not shown). This finding is supported by Stewart's similar observation, revealing reduced numbers of growing MT in kat-60L1 mutant dendrite branches proximal to the terminal branches. Although loss of kat-60L1 is detrimental, exogenous overexpression of kat-

60L1 in *Drosophila* eye or muscle caused no detectable effects on MT distribution or the tissue as a whole (Stewart et al. 2012).

Besides studies in *Drosohpila,* studies in plant cells have shown that katanin could be required for functions beyond dissolution of MT structures (Burk et al. 2001, Murata et al. 2005). Scientists have been able to develop a more complete udnerstanding of katanin's cellular mechaanism in plants over other systsems in part because loss-of-function mutants are still viable and easy to visulaize, while dendrites contain comparatively dense MT bundles, making it challenging to directly observe MT severing (Roll-Mecak 2010). In vivo imaging of GFP-tubulin has revealed that many cortical MTs nucleate off the wall of pre-existing MTs, always at 40 degree angles. In one plant katanin mutant, AT1G80350, loss of katanin function blocked the release of newly formed MTs from the prior MT template, which explaiend the lack of parallel, polar MT organization and abnormal cell shape (Murata et al. 2005). Similar studies in nematode embryos and cultured neurons (Ahmad et al. 1999) also propose additional roles for katanin and related proteins like kat-60L1. It seems likely that katanin and related proteins like kat-60L1 could be required for organization, postnucleation processing, and polarity determination (Srayko 2006).

Along with kat-601L, two proteins involved in planar cell polarity, esn and pk, also had an effect on MT polarity. Limpet, the third protein in the planar cell polarity set, also had a similar but not statistically significant effect on MT polarity. My original hypothesis was that reducing the levels of a protein required for organizing MT polarity would cause any deviation from the wild-type, however I truly expected the result to be a decreased number of minus-end out MTs in the dendrite. Oddly enough, and unlike kat-60L1, reduced levels of proteins involved in planar cell polarity actually caused the proportion of EB1-GFP comets moving towards the cell body to increase.

In contrast with the severing and destabilizing proteins, there is comparatively little literature on planar cell polarity proteins interacting with MTs. As mentioned, PCP proteins play significant roles in determinig polarity of other cells. The polarization of wing epithelial cells likely reflects internal reorganization of the cytoskeleton inresponse to the cell's external environment, sometimes responding to a very small gradient. These proteins are also present in neurons, however, their documented role is somewhat different. In the development of dendritic arbors, several of these proteins also play a role in ensuring that there is minimal crossover of dendrites, as it would inefficient for ddaE neurons to have dendrites with a significant amount of crossover.

Prickled (pk), one of the proteins shown to have a slight effect on MT polarity in ddaE neurons (Figure 1-6) has been implicated as a negative feedback loop in the PCP pathway in *Drosohpila* epithelial cells. It is thought that pk allows the cell to respond to slight differences in dsh localization in the early cell (Tree et al. 2002). Espinas (esn), another one of the proteins shown to have a slight effect on MT polarity in ddaE neurons, belongs to the *Drosophila* PET-LIM domain family, is related to testin, and is a close cognate with pk (Figure 1-6). Esn has been shown to bind to the C-tail of flamingo (fmi), a a seven-pass transmembrane protein downstream of frizzled (fz) in the PCP pathway (Matsubara et al. 2011, Gao et al. 2000, Chae et al. 1999). Fmi and related proteins have been shown to be involved in a myriad of developmental processes, many of which suggest interaction with MTs (Jan and Jan 2010). These processes include, but are not necessarily limited to growth control of dendrites in *Drosophila* and mammals (Sweeney et al. 2002, Shima et al. 2007), target selection of *Drosophila* photoreceptor axons (Lee et al. 2003), and axonal navigation and tract formation in mouse brains and *C. elegans* (Stiemel et al . 2010). Mutations in fmi alter the polarity of the cuticular structures in all regions of the *Drosophila* adult body and result in overextension of multidendritic neuronal dorsal dendrites (Lu et al. 1999, Gao et al. 2000). Most convincingly, the fact that fmi mutants affect spindle orientation in sensory

organ precursor cells indicates that this pathway somehow acts on MT organization. After early development, the level of fmi mRNA expression decreases, suggesting a crucial role for both esn and fmi in early organization of neural systems (Chae et al. 1999).

The available literature makes it clear that PCP proteins and related proteins like esn, pk, and limpet may be involved in determining neuronal polarity, however the exact mechanism by which these proteins interact with MTs and why decreasing their levels results in a greater proportion of EB1-GFP comets moving towards the cell body rather than increased polarity mixing remains to be uncovered. Future studies should continue to investigate the functions of PCP proteins specifically in neurons, perhaps using null mutants or double RNAi to induce a possible synergistic effect. Such studies could be of great interest because these proteins are highly conserved from flies to humans, and may hold the secret to morphologically based diseases including mental retardation and autism.

Oddly, one of the nucleation proteins showed the same effects as the PCP proteins. Aurora-A, a mitosis-specific serine/threonine kinase exhibited an unexpected increase in the proportion of EB1-GFP comets moving towards the cell body (Roghi et al. 1988, Figure 1-7). Aurora-A has been implicated in MT organization and spindle assembly. Aurora-A functions to recruit pericentriolar material including centrosomin (cnn) and γ-tubulin. Aurora-A specifically binds the carboxy-terminal domain of *Drosophila* cnn. The NH_2-terminal half of cnn then recruits gamma tubulin to the centrosome (Terada et al. 2003).

The effects of aurora-A depletion have been observed in diverse organisms, with significant focus on the *C. elegans* homolog air-1. Air-1 has been shown to localize to centrosomes where it is required for normal spindle assembly. When air-1 levels were reduced by RNAi, normal mitotic spindles were never observed, and although the presence of a-tubulin near the mitotic spindles increased, it only reached 40% of the normal concentration in wild-type cells, indicating that mitotic centrosomes in air-1 RNAi cells organize fewer MTs. It was also shown in

C. elegans that air-1 is required to recruit centrosomal γ-tubulin during centrosomal maturation (Hannak et al. 2001).

Given this background, it would be expected that aurora-A might have an impact on MT nucleation and dynamic levels, but not on polarity. Terada et al. (2003) have shown that aurora-A and cnn are mutually dependent for localization at the spindle poles, so it is odd that aurora-A RNAi neurons exhibit a slight but significant change in polarity but cnn RNAi neurons show a trend in the opposite direction, however statistically insignificant (Figure 1-7). This seems even more unusual in light of findings that embryos from cnn mutant mothers undergo mitosis on highly abnormal spindles lacking γ-tubulin and without astral MTs, which Barbosa et al. suggest indicates that cnn is most important in holding the structure of the centrosome together (2000). Since aurora-A and cnn bind each other directly and are in the same pathway for recruiting γ-tubulin to the centrosome, it is odd that the two RNAi would have different effects. These disparate results may indicate that aurora-A serves an additional role in the neuron besides just at the centrosome.

Oddly, none of the Dgrip proteins appeared to have an effect on polarity, which would have been expected given observations in previous studies. An analysis of Dgrip84 mutants produced cells with highly unusual monopolar or unpolarized spindles (Colombie et al. 2006). Then again, Colombie et al. observed primarily mitotic or male meiotic cells; Dgrip84 null mutants only showed slight differences from the wild-type in interphase. However, this outcome may also be attributable to the pitfalls of using RNAi, as discussed below.

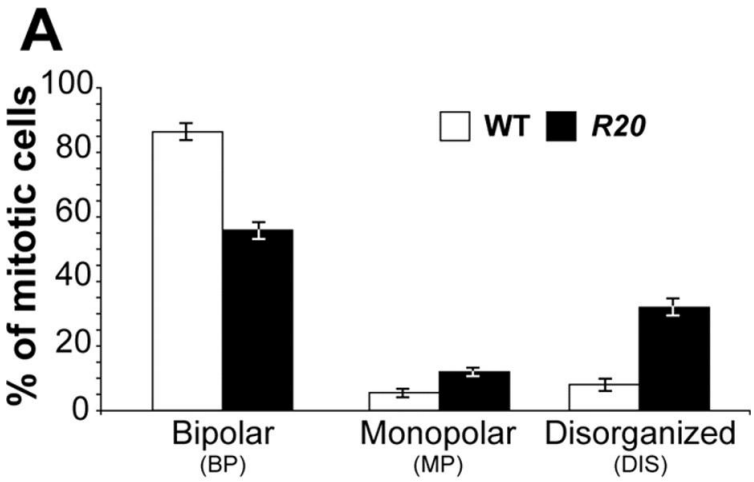

Figure 2-10: Abnormal mitotic organization in Dgrip84 null neuroblasts. Percentage of bipolar spindles is decreased, whereas the percentage of monopolar and disorganized figures is increased. The percentage of bipolar (BP), monopolar (MP), and disorganized (DIS) mitotic figures was calculated using spindle labelling (α-tubulin antibodies) of nine L3 larval brains for each genotype (WT, n = 910; and R20, n = 1622). (Colombie et al.. 2006).

One point that is important to note is that while this RNAi screen can identify proteins that may be invovled in establishing neuron polarity, it cannot conclusively rule out proteins that show no deviation from the wild-type. Using RNAi may not entirely eliminate the protein from the cell. If the protein is maternally transcribed before transcription of the embryonic genome begins, it will not be removed by RNAi. The embryo itself also contains the wild-type gene for the protein of interest. It can still be transcribed, and possibly translated if the RNAi is not 100% efficient. Particularly stable proteins may still be present in large enough amounts to maintain a wild-type phenotype, especially if only low levels of the protein are requried. With this experimental setup, it would also be impractical to determine the levels of the protein in question by a Western blot because this would require surgically extracting ddaE neruons, of which there are only 16 in each larva. Despite its fallbacks, RNAi also has many advantages as an experimental tool; with an established library of RNAi lines, it is a cheap and time-efficient technique that does not require multiple steps to clone, breed, and rebalance heterozygous or null mutant lines. Given the advantages and disadvantages, RNAi was a suitable tool for this screen, and identified several factors important to establishing wild-type MT polarity in dendrites including esn, pk, aurora-A, and most significatnly, kat-60L1.

Chapter 3

The γ-tubulin Complexes

γ -Tubulin is required for microtubule nucleation and is present in both interphase and mitotic spindle bodies (Oakley et al. 1990). *In vivo,* γ-tubulin acts in a variety of complexes rather than as a monomer (Li and Joshi 1995). Two such complexes, the γ-TuSC and γ-TuRC have been characterized in *Drosophila* (Oegema et al. 1999). γ -Tubulin and these complexes nucleate MT assembly and may play a significant role in establishing MT polarity *in vivo* (Oakley et al. 1990). The complexes and γ-tubulin are conserved from yeast to human homologs (Zheng et al. 1995, Geissler et al. 1996, Murphy et al. 1998). The γ-TuSC appears to be especially important; mutations in any of the three genes, Dgrip84, Dgrip91, or γ-tubulin is lethal and recessive (Oakley et al. 1990), although some conditional mutants survive and can be studied (Colombie et al. 2006). The γ-TuRC is larger, consisting of Dgrip71, 75, 128, and 163 in addition to γ-TuSC proteins, and has an appearance of an open ring with approximately the same diameter as a MT (Zheng et al. 1995).

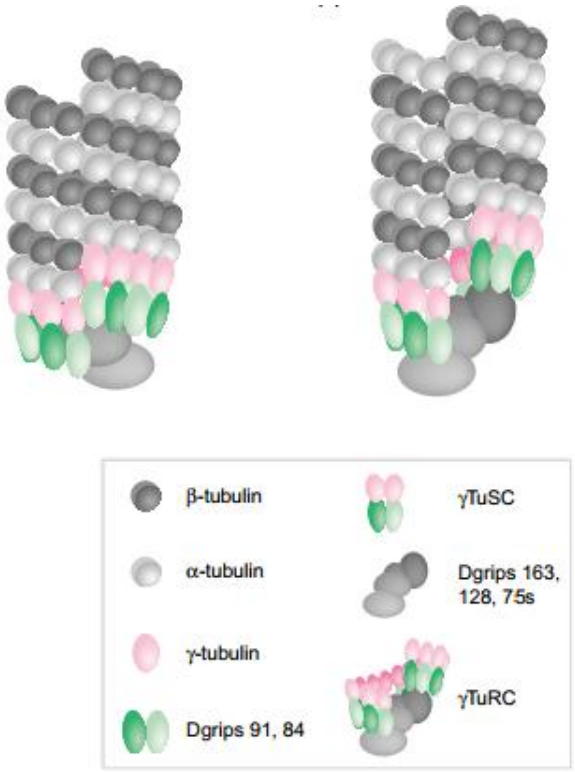

Figure 3-1: Model structure of γ-TuSC and γ-TuRC. The γ-TuSC and γ-TuRC function as bases for MT polymerization. Several models have been proposed describing their specific organization. The model on the left shows a proposed structure containing 14 γ-tubulin molecules, while the model on the right diagrams a proposed structure containing 12 γ-tubulin molecules.
(Moritz and Agard 2001)

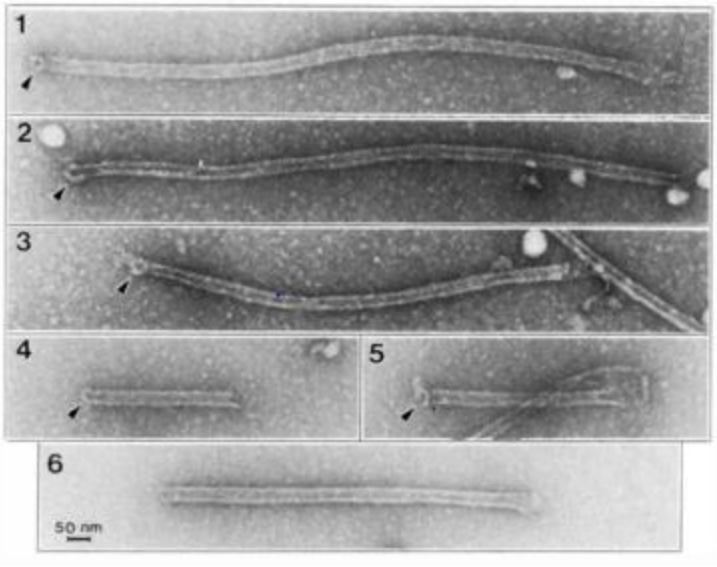

Figure 3-2: Electron microscopy of microtubules nucleated in the presence or absence of purified γ-TuRC. Panels 1-5, selected microtubules nucleated in the presence of the γ-TuRC; arrowheads point to a ring structure at one end of each microtubule. Panel 6, a typical microtubule nucleated in the absence of the γ-TuRC.
(Zheng et al. 1995)

Although the initial polarity screen for Dgrip proteins did not show any significant change in polarity, there seemed to be fewer growing MTs in the axon (data not shown). Since the γ-TuRC plays a role in MT nucleation, it was thought that other proteins in the complex might play similar roles. The number of growing microtubules in the axons in neurons with RNAi

against specific components of the γ-TuRC was observed, however, no significant effect was seen consistently. In order to obtain more conclusive results, an injury assay was developed to determine whether a phenotype was actually present.

The typical injury response involves dramatically increased microtubule dynamics and reversal of dendrite polarity to create a new axon (Stone et al. 2010, Figure 3-1). However, I hypothesized that cells lacking γ-TuSC or γ-TuRC components would not be able to up-regulate MT dynamics efficiently. I also predicted that reducing expression of γ-TuSC proteins will have a greater effect on the ability of cells to up-regulate MT dynamics than reduced γ-TuRC. If a phenotype is present, the injury assay should make it more evident than using RNAi alone.

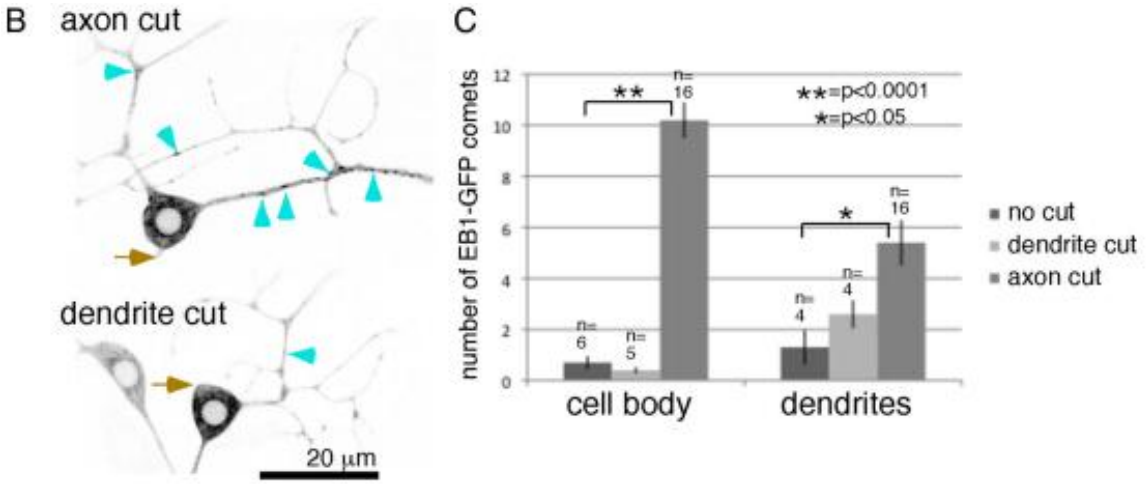

Figure 3-3: Injury response (B) Panels from movies acquired 24h after axon or dendrite severing are shown. Images were inverted for ease of showing EB1-GFP comets in dendrites. (C) The number of EB1-GFP comets in the cell body or a region of the dendrite was counted in single frames from uninjured neurons from neurons 24h after axon or dendrite severing. Three frames were averaged for each animal, error bars, SD of the average from all animals. N= number of neurons scored. Unpaired t-tests were used to determine whether the number of comets was significantly increased after dendrite or axon cutting. No significant difference between number of comets in the cell body after dendrite cutting was found. Significant differences were found for cell bodies and dendrites after axon cutting. (Stone et al. 2010)

Results

γ -tubulin 37C RNAi was used as a control showing normal injury response because γ-tubulin 37C is found only in very early *Drosophila* embryos as a result of maternal deposition. The larval neurons used in the assay have a normal dose of γ-tubulin 23C, and thus a normal phenotype. In normal, uninjured neurons, there are a few comets, mostly moving towards the cell body (Figure 3-4a). The control injury response (Figure 3-4b) shows dramatic MT regulation and reversal of polarity as was expected (Stone et al. 2010). Uninjured Dgrip91 RNAi looks very similar to the uninjured control (Figure 3-4c). However, the injury response in Dgrip91 neurons is greatly diminished (Figure 3-4d). The results of the same experiment with RNAi depleting the other γ-TuSC and γ-TuRC components are shown in Figure 3-5. It appears that the γ-TuSC component Dgrip91 plays a more significant role in MT up-regulation because its depletion has a more severe effect. However, this cannot be said for certain because RNAi only decreases protein level; the proteins are not entirely eliminated from the cell. The ability of the neurons tested to up-regulate MT may differ based on the effectiveness of the RNAi rather than the importance of the protein.

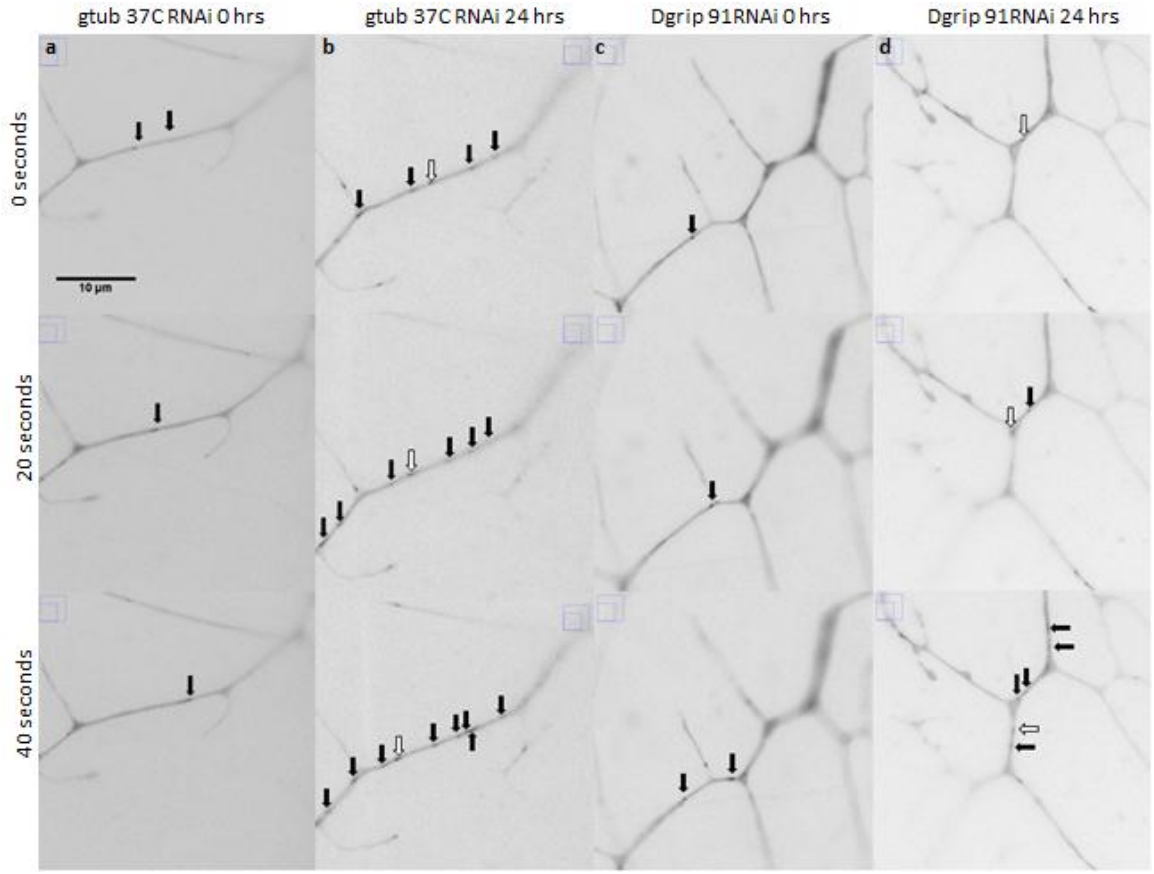

Figure 3-4: Change in microtubule dynamics in response to axotomy. For each genotype, gtub37C RNAi **(a, b)** or Dgrip 91 RNAi **(c, d)**, a series of 200 frames at 2-second intervals was taken immediately after axotomy (0hrs) and 24 hours later. Three images ten frames (20 seconds) apart were selected to show the injury response. Images are inverted for ease of showing EB1-GFP comets. Arrows indicate the location of EB1-GFP comets showing the plus-tips of microtubules. In images where multiple comets are seen, white arrows track the position of a single comet to indicate the direction in which the comets are moving. In all images, the cell body is towards the top-right of the image. The scale bar in the top left image applies to all images in the set. Images were taken on a Zeiss fluorescence microscope.

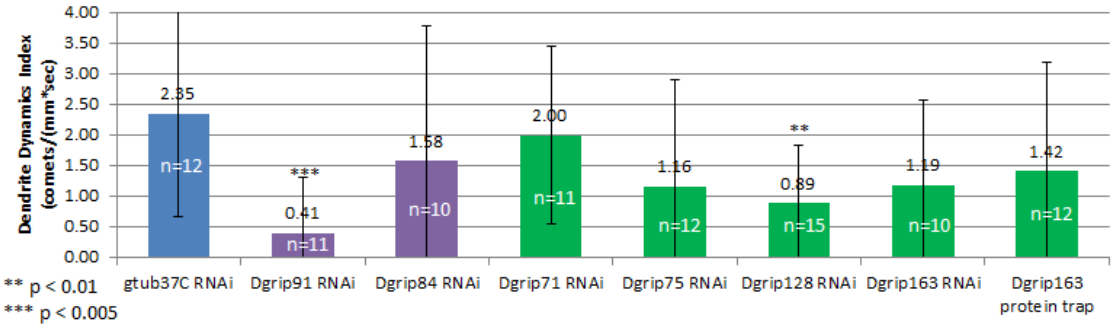

Figure 3-5: Microtubule up-regulation in response to axotomy. Neurons from larvae with RNAi against each γ-TuSC and γ-TuRC component were observed to determine the extent of microtubule up-regulation. For each larva, a video of 200 frames at 2 second intervals was taken immediately after axotomy. A second video was taken 24 hours later. The number of EB1-GFP comets passing through a 10-micron length was determined for each video, and the difference between the 24-hour and initial videos was calculated. This number was divided by the number of frames and seconds per frame, ultimately giving a dynamics index in units of comets/(sec*mm). γ-TuSC components are colored purple, while γ-TuRC components are colored green. The control gtub37C is colored blue. Error bars indicate standard deviation of each sample.

Discussion

The results of the injury assay show that neurons with Dgrip91, a key γ-TuSC component, and Dgrip128, a γ-TuRC component, RNAi have a significantly reduced ability to up-regulate MT dynamics after axotomy ($p < 0.005$ and $p < 0.01$, respectively). Although other RNAi samples were not statistically significant, all followed the trend of diminished capacity to up-regulate MT dynamics. Each sample had considerable variability, with some larvae showing more dramatic responses to injury while others showed no response at all. Thus, each sample had a large standard deviation. It would be beneficial to repeat trials to gain a larger sample size for each cross, especially since less than half of the larvae initially cut are still alive and in the larval stage after 24 hours.

In comparison to findings from similar studies, it makes sense that Dgrip91 would produce the most dramatic effect, as it is a component of the γ-TuSC. Colombie et al. (2006)

showed that Dgrip84, Dgrip91, and γ-tubulin are all required for mitosis and male meiosis, and none is fully redundant, so a strong effect would be expected for any of these three RNAi crosses. Similar results were expected for Dgrip84 RNAi, however the decrease observed was not statistically significant ($p>0.05$) (Figure 3-5). This may indicate either that Dgrip84, while a part of the γ-TuSC, is not as important to its function as Dgrip91. However, this result may also be due to variable efficiency of RNAi. I think the latter explanation is more likely since other studies show Dgrip84 to be essential (Colombie 2006). Perhaps a form of selection bias occurred in that the larvae that survived to three days had comparatively high levels of Dgrip84 protein, thus displaying a phenotype that resembles the wild type.

It would be beneficial to confirm the RNAi results with Dgrip84 null mutants. In fact, such an experiment was attempted, but failed due to complications. Two Dgrip84 heterozygous-null lines, $Dgrip84^{PG36}$ and $Dgrip84^{PG66}$ were provided by Brigitte Raynaud-Messina of the Center of Research in Health and Pharmacology in Toulouse, France. PG66 mutants exhibited very early lethality, but PG36 was only semilethal, allowing 50% of hemizygous males to reach the adult stage (Colombie et al. 2006, Figure 3-6).

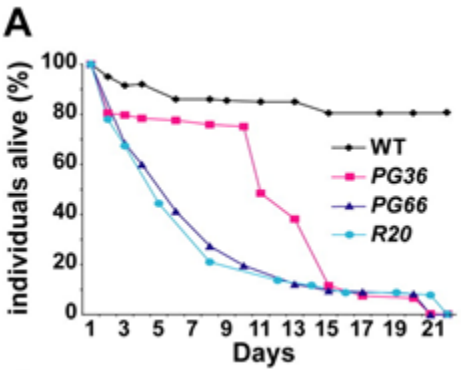

Figure 3-6: Characterization of Dgrip84 mutants. The three Dgrip84 mutations induce different patterns of lethality. One hundred first instars of each mutant genotype and wild-type (WT) were followed until adult stage. The percentage of live individuals— independently of the developmental stage— was determined. The PG66 and R20 alleles induce an early lethality, whereas the PG36 allele induces a belated lethality (Colombie et al. 2006).

Preliminary polarity studies were conducted using the offspring of PG36 female virgins and males from the line UAS-Dicer 2; 221-Gal 4, EB1-GFP/TM6. The plan was to select male hemizygous null larvae (identified by gonad size); however, the loss of Dgrip84 affected male meiosis and prevented normal gonad development, making it impossible to identify male larvae (Colombie et al. 2006). I initially expected that 50% of the larval males would survive even though they could not be phenotypically identified as male

larvae, so each imaged larvae was saved individually, and its sex was determined when the adult eclosed. However, the male survivability observed was much lower than expected. Of the 46 larvae imaged, only 1 male survived to adulthood (data not shown).

I am unsure why such high lethality was observed for males, but hypothesize that while phenotypically normal larvae can withstand the stress of the experiment, it was too much for the unhealthy hemizygous PG36 male larvae to handle. It may also be due to association with a different genetic balancer. The stocks provided were balanced over FM7c, but I rebalanced them over FM7i-actin GFP so that I would be able to select for larvae carrying the PG36 gene of interest during imaging. I suspect that this might be a partial cause of the effect because I also noticed other phenotypic differences from those described. Colombie reports that although 50% of PG36 males survived to adulthood, all were sterile. In contrast, I observed a handful of surviving fertile males in a population of a few thousand. This observation leads me to believe that when the line is balanced over FM7i-actin GPF it has a slightly different phenotype than when it is balanced over FM7. Ultimately, since the line with the highest reported male viability failed to produce a sufficient number of surviving hemizygous males, it unfortunately seems that it may be impractical study the effects on microtubule polarity of a Dgrip84 null mutant.

Along with characterization of Dgrip84 mutants, Colombie et al. (2006) also provide important evidence that the centrosomes alone cannot organize MT in interphase S2 cells. This was shown after when cold MT disassembly caused a decrease in the number of regrowth points in Dgrip84 mutants relative to the control. Furthermore, the MTs that were produced were abnormally long. Similar observations have been made in studies involving yeast γ-TuRC homologs and probably result from an increase in the concentration of free tubulin in consequence of low MT nucleation sites (Jung et al. 2001). Similar results were also obtained when katanin was mutated, suggesting that the two may as part of the same mechanism (Srayko et al. 2006). Colombie's (2006) results are especially interesting in the context of a somewhat

related study in plants (Burk et al. 2001, Murata et al. 2005). The complexity of the *Drosophila* interphase cytoskeleton makes study difficult, however research in plants may provide clues about the combine roles of γ-TuRC and severing proteins. In a review focused on MT-severing enzymes, Roll-Mecak and McNally (2010) diagram how the γ-TuRC is thought to bind to the walls of stable MTs at 40° angles and serve as new nucleation sites (Figure 3-7). Severing proteins then cleave the nascent MT from the γ-TuRC, allowing the branched array to rearrange into a polar parallel array.

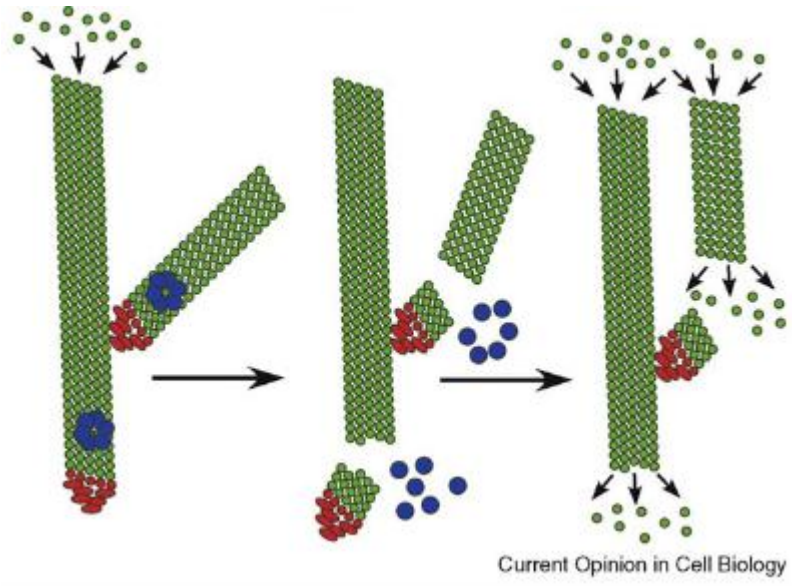

Figure 3-7: Schematic representation of branched nucleation and severing patterns to generate parallel MT arrays in plant cells. γ-tubulin ring complex, shown in red, binds to the wall of a pre-existing MT and nucleates polymerization of a new MT at a 40° angle. Assembly of katanin rings, shown in blue, results in severing of these branched structures. Severing by katanin allows branched arrays to rearrange into parallel arrays. (Roll-Mecak and McNally 2010).

If this model holds true in *Drosophila*, it could explain why Colombie et al. observed MT growth without a focal point as well as why decreasing severing proteins like katanin and kat-60L1 contribute to the altered polarity observed in dendrites (Figure 1-4). However, the fact that RNAi against Dgrip proteins did not produce significant changes in polarity does not fit as well in this model (Figure 2-8). Still, this may be a result of the cell requiring only low levels of these proteins to maintain a normal phenotype. The fact that all Dgrip proteins, not only those in the γ-TuSC, inhibit the ability of the cell to up-regulate MT dynamics in response to injury indicates that the γ-TuRC as a whole plays an important role in MT dynamics and possibly polarity.

Furthermore, reduced levels of pk and esn, proteins not known to interact with centrosomal proteins, cause deviations from wild-type MT polarity, suggesting that the centrosome alone is not responsible for organizing MTs in the dendrites. In conclusion, these findings provide further evidence for the idea that neuronal dendrites contain non-centrosomal MT nucleation sites responsible for establishing and maintaining the MT array that is so important to the neuron's structure and function as a whole.

Appendix A

Materials and Methods

RNAi screen for Microtubule Polarity Candidates

Transgenic flies containing short gene fragments (300-400 bp) as inverted repeats under a GAL4/UAS promoter system were obtained from the library at the Vienna Drosophila RNAi Center (VDRC, Table A-1). Male flies were selected from these lines and mated to virgin females of the genotype UAS-Dicer2; UAS-221Gal4, EB1-GFP/TM6. Virginity was ensured by removing all adult flies from a bottle and then collecting newly-eclosed females before the age at which they will begin to mate (8 hours at room temperature or 16 hours at 18°C). Embryos were laid in media comprised of cornmeal, brewer's yeast, agarose, glucose, and sucrose, and collected at 24-hour intervals. The embryos were matured for three days at 25°C.

Three-day larvae were cleaned in PBS or Schneider's media and then placed on agarose slides dorsal side up and secured with a cover-slip. A Zeiss fluorescence microscope was used to visualize sensory dendritic arborization ddaE dorsal sensory neurons just under larval cuticle in the third through fifth segments. The motion of fluorescent comets produced by GFP-tagged EB1 attached at the plus-ends of growing microtubules was observed and used to deduce the direction of microtubule growth. Each larva was imaged for 200 frames at 2-second intervals. Image J software was used to analyze the obtained videos.

Table A-1. RNAi VDRC stock numbers. Stocks for the RNAi screen discussed in chapter 2 were provided by VDRC. The stock number for each gene is given below in the order results were presented in chapter 2.

Protein	VDRC#	Protein	VDRC#	Protein	VDRC#
Rtnl2	33320	G-sa60A	24958	Dgrip84	34730
Kat 60	24764	G-B76C	109528	Dgrip91	104667
Kat-60L1	31598	G-sa73B	17054	Dgrip71	100830
Fidgetin	24746	Espinas	32038	Dgrip75	30131
Spastin	33110	Prickle	101480	Dgrip128	29073
Stathmin	32370	Limpet	100716	Dgrip163	108686
G-oa47A	19124	Centrosomin	100415	Par-1	52556
G-a94B	105300	Shaggy	7005	Src	100708
G-g30A	102706	Aurora-A	108446	Ik2	103748

Injury Assay

The axon of a ddaE dorsal sensory neuron was severed using a pulsed laser. A Zeiss fluorescence microscope was used to visualize sensory dendritic arborization ddaE dorsal sensory neurons just under larval cuticle in the third through fifth segments. The larvae were then saved in individual media dishes for 24 hours at 25°C. After 24 hours, the larvae were imaged again and the change in microtubule dynamics in the trunk of the dendrite comb was observed as a function of axon injury. Microtubule dynamics were measured as the difference in the number of fluorescent comets observed to be passing through a 10- micron segment in either direction over the course of 200 frames taken at 2-second intervals. Microtubule dynamics are measured in units of EB1-GFP comets per millimeter per second. Image J software was used to analyze the obtained videos.

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EDUCATION:

The Pennsylvania State University, Schreyer Honors College, University Park, PA
B.S. Biochemistry and Molecular Biology; Summer 2013
Minor in Global Health; Summer 2013

EXPERIENCE:

Penn State Biochemistry and Molecular Biology Department — May 2010 - Present
Research Assistant

- Mastered research techniques including:

Confocal microscopy	Spectrophotometry
PCR	Gel extraction and purification
Restriction digests	*Drosophila genetics*
Plasmid transformation	Fly stock maintenance

- Developed new methods of data interpretation for specific experiments
- Trained new lab members in lab procedures and experiment protocol

PUBLICATIONS:

Stone MC, Rao K, Gheres KW, Kim S, Tao J, La Rochelle C, Folker CT, Sherwood NT, Rolls MM. 2012. "Normal spastin gene dosage is specifically required for axon regeneration" *Cell Reports*, *2*(5):1340-1350.

PRESENTATIONS:

"The role of APC-2 and related proteins in dendrite polarity and dynamics." Poster presented at Pennsylvania State University Eberly College of Science Alumni Poster Session

COMMUNITY SERVICE:

Mount Nittany Medical Center, State College
Emergency Department Volunteer — 2012 - present

- Aided in transition from old to new emergency department
- Directed, escorted, and transported hospital patients and visitors
- Comforted patients, family, and visitors
- Handled confidential health documents and ensured patient privacy
- Maintained well-stocked, sanitary, comfortable environment for patients and hospital staff

Physical Therapy Volunteer — Summer 2012

- Aided in patient treatment and exercises
- Practiced patient-physician and family-physician interaction

Penn State University Health Services — 2012 - present
Physical Therapy Volunteer

- Aided in patient treatment and exercises
- Maintained exercise and treatment equipment
- Handled confidential health documents and ensured patient privacy

Penn State Dance Marathon, THON — 2009 - present

- Raised approximately $85,000 for pediatric cancer research and treatment with a team of dedicated students
- Represented Penn State Blue Band in Dance Marathon by dancing for 46 hours without sitting or sleeping

Mission Mexico Service Trip to Casa Hogar de los Niños in Tijuana, Mexico — 2010 - present

- Raised funds to pay for food, clothes, utilities needed at the orphanage
- Contributed to community improvement projects including cleaning up garbage, constructing a fence, painting, and planting a garden
- Improved basic Spanish skills

LEADERSHIP:

Schreyer Honors College: The GLOBE, Executive Board Chair — 2011 - present

- Organized book club, speaker events, student panels, and service opportunities
- Set rules and precedents for the organization in future years
- Planned and coordinated social events

HONORS:

- Academic Excellence Scholarship
  Schreyer Honors College 2009-2012
- Dean's List (all 7 semesters), 2009-2012
- Morrow Family Endowed Prize
  Penn State Biochemistry and Molecular Biology department, 2012
- Phi Beta Kappa, 2012
- Jacqueline Hemming Whitfield Research Endowment
  Penn State Biochemistry and Molecular Biology department, 2011
- Summer Undergraduate Research Fellowship
  Penn State Biochemistry and Molecular Biology department, 2011
- Hutchings Scholarship
  Penn State Biochemistry and Molecular Biology department, 2011
- Morrow Family Scholarship
  Penn State Biochemistry and Molecular Biology department, 2011
- National Residence Hall Honorary, 2011
- National Society of Leadership and Success, 2010
- Gold Key Honors Society, 2010
- Pennsylvania Governor's School of Science, 2008