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Neuronal polarity in *Drosophila*: sorting out axons and dendrites

Melissa M. Rolls

Biochemistry and Molecular Biology, The Pennsylvania State University, 118 Life Sciences, University Park, PA 16802 mur22@psu.edu, (814) 867-1395

Abstract

Drosophila neurons have identifiable axons and dendrites based on cell shape, but it is only just starting to become clear how *Drosophila* neurons are polarized at the molecular level. Dendrite-specific components, including the Golgi complex, GABA receptors, neurotransmitter receptor scaffolding proteins and cell adhesion molecules have been described. And proteins involved in constructing presynaptic specializations are concentrated in axons of some neurons. A very simple model for how these components are distributed to axons and dendrites can be constructed based on the opposite polarity of microtubules in axons and dendrites: dynein carries cargo into dendrites, and kinesins carry cargo into axons. The simple model works well for multipolar neurons, but will likely need refinement for unipolar neurons, which are common in *Drosophila*.

Background

In addition to their ability to allow us to think, walk and talk, one of the most striking features of neurons is that a single cell body, with a single set of instructions in its nucleus, can extend two very different types of processes. Axons are the more outgoing side of the neuron, typically extending long distances to contact other cells, and often bundling into large, readily visible nerves. Dendrites keep a quieter profile, gathering information from their often highly branched, more local processes. Since these two types of processes have different functions, broadly categorized as sending information and receiving information, they also need different sets of proteins, and even organelles. So understanding neuronal polarity involves thinking about the differences between axons and dendrites, and how the cell manages to achieve these differences. Since this could be quite a complicated process, a simple, genetically tractable, model in which it could be studied has considerable appeal. So can we use *Drosophila* to study neuronal polarity?

Invertebrate neurons have not always been considered a good model for neuronal polarity, because it was not clear that they were polarized in quite the same way as mammalian neurons. For example *C. elegans* was thought not to have any neurons with branched dendritic trees until recently (Oren-Suissa et al., 2010). The situation for other invertebrates has not been quite as extreme, but in a 1970 review, “The functional organization of invertebrate ganglia,” Kandel and Kupfermann mention axons many times, but the word dendrite only appears rather uncertainly once (Kandel and Kupfermann, 1970). In their classic review on neuronal polarity, Craig and Banker do not cast doubt on the existence of dendrites in invertebrate neurons, but do wonder whether they use different mechanisms than vertebrates to sort proteins to the two compartments because many of them are unipolar (Craig and Banker, 1994).

In the current review, I aim to bring together recent studies on molecular polarization in *Drosophila* neurons. Just what is sorted to axons and dendrites in *Drosophila*? And which aspects of neuronal polarity could therefore be studied in this powerful genetic system? As background for this, it is important to first take a quick look at the shape of the whole cell.

A view from the outside: what do *Drosophila* neurons look like?

Many *Drosophila* neurons have clearly identifiable axons and dendrites based on the shape of the cell. Axons typically extend far from the cell body before making terminal branches and synapses. Dendrites branch much closer to their point of origin. Shapes of individual neurons have been traced by a variety of methods, including dye injection and generation of single cell GFP-expressing clones, or, on rare occasions, Gal4 drivers with very limited expression (Sink and Whittington, 1991; Lee et al., 1999; Grueber et al., 2002; Roy et al., 2007). In most cases the shape of the cell allows a reasonable assignment of axon or dendrite identity (as in Figure 1A). Where these assignments have later been correlated with marker expression or microtubule polarity (see below), they have proven correct.

One complicating factor in thinking about polarity in *Drosophila* neurons is where dendrites arise. We are used to thinking about dendrites emerging directly from the cell body, and this is the cellular outline into which models of polarized sorting have been drawn, but, in many *Drosophila* neurons, dendrites arise from a primary neurite, not directly from the cell body (Grueber et al., 2005; Sanchez-Soriano et al., 2005). For example, most motor neurons in the embryonic ventral nerve cord are unipolar; a single primary neurite arises from the cell body. Within 10–20 microns from the cell body dendrites arise. The neurite then continues out of the nerve cord and becomes an axon that innervates muscles of the body wall (Sink and Whittington, 1991). However, not all embryonic motor neurons are unipolar; some are multipolar and have dendrites that emerge directly from the cell body. Other types of neurons are very clearly multipolar. Probably the best-studied example is the multidendritic sensory neurons just under the larval and adult cuticle (Grueber et al., 2002; Shimon et al., 2009). These neurons are picture-perfect examples of textbook neuronal polarity. They have a single axon that emerges from the cell body and fasciculates with other axons in a nerve as it heads towards the central nervous system (Figure 1A). Multiple dendrites emerge from other regions of the cell body and form elaborate branched structures that tile the body wall (Grueber et al., 2002).

Is there really a difference between unipolar and multipolar cells; clearly flies can generate both? One study has tried to tackle this issue directly and has found flexibility of the position of dendrite emergence of motor neurons in vitro and in vivo (Sanchez-Soriano et al., 2005). Flexibility of position of dendrite emergence can also be seen in multipolar sensory neurons. When the cell adhesion molecule neuroglian is lost, multidendritic sensory neurons that normally have dendrites emerging only from the cell body also sprout dendrites from the proximal axon (Yamamoto et al., 2006). This flexibility suggests there is not an intrinsic difference in how polarity works in unipolar and multipolar neurons.

Aside from shape, another argument that the processes that look like dendrites are dendrites, and that they are analogous structures to vertebrate dendrites, is the way they develop. Many

vertebrate neurons in vivo, including retinal ganglion cells in zebrafish (Zolessi et al., 2006), initially grow an axon from the cell body, followed later in development by emergence of dendrites. Most *Drosophila* neurons whose development has been studied in vivo start life as small round balls. Next, a lamellipodium arises from one side of this ball, and gradually extends to become a nascent axon with a growth cone at the tip. Later on, dendrites grow out, just as in vertebrates. This same course of events is seen in unipolar motor neurons (Sink and Whittington, 1991) and multipolar sensory neurons (Gao et al., 1999).

One point to note about *Drosophila* dendrites is that they do not have clearly defined spines that house postsynaptic sites. Some sensory dendrites do have actin-rich spikes that protrude from dendrites, and not axons (Grueber et al., 2002), so there is at least some capacity to generate this type of structure in a polarized manner, and, as in mammals, it is polarized to dendrites.

Drosophila neurons thus have morphologically and developmentally identifiable axon and dendrites. Now, we will move on to the major focus of the review: molecular differences between axons and dendrites, and how these might be generated.

Who is where?

A standard polarized neuron has dendrites that are post-synaptic and axons that are pre-synaptic. Of course there are variations on this theme, for example dendrites of sensory neurons are set up to sense information about their surroundings rather than to receive signals from other neurons, but it is helpful to think about a flow of information from dendrites to axons. Thus receptors are expected to be concentrated in dendrites, and synaptic vesicles in axons. As well as this polarity of content that is clearly related to overall functional polarity of neurons, other types of proteins and organelles have also been shown to be polarized to axons or dendrites in vertebrate neurons. We will make a quick survey of what is known about how each type of contents is localized in *Drosophila* to get a better picture of a standard *Drosophila* neuron (Figure 1B).

synaptic components

Presynaptic proteins have been examined most thoroughly in motor neurons as the neuromuscular junction is large and beautiful and thus used extensively for studying synapse development and organization (Prokop and Meinertzhagen, 2006; Ruiz-Canada and Budnik, 2006). Very specific localization of proteins to motor neuron axon terminals has been observed with antibody staining and tagged proteins. Some nice examples are the active zone components cacophony (Kawasaki et al., 2004) and bruchpilot (Wagh et al., 2006), and synaptic vesicle proteins synaptobrevin (Estes et al., 2000) and synaptotagmin (Zhang et al., 2002; Sanchez-Soriano et al., 2005).

There is also good evidence that postsynaptic proteins are targeted to motor neuron dendrites. For example, the postsynaptic scaffolding protein, homer, localizes to embryonic motor neuron dendrites, but not axons (Diagana et al., 2002), and a tagged GABA receptor (Resistance-to-Dieldrin, Rdl) also localized to dendrites, but not axons, of embryonic motor

neurons (Sanchez-Soriano et al., 2005). Thus motor neurons seem to be classically polarized with respect to pre- and postsynaptic markers.

The synaptic polarity of other neurons is not quite as well documented, but there are certainly examples of synaptic polarity in a variety of neurons. For example, the synaptic vesicle marker syb-GFP and neurotransmitter receptor Rdl-HA localize to different compartments in CCAP peptidergic neurons (Santos et al., 2007). Although sensory neuron dendrites are not postsynaptic, they are the input side of the cell, and this is reflected by receptor localization. The DEG-EnaC family member, pickpocket (ppk), localizes to dendrites, but not axons, of sensory neurons with complex branching patterns (Zheng et al., 2008); ppk is most likely a channel that is required for nociception by these neurons (Zhong et al., 2010).

Of course, as in vertebrates, there are exceptions to the simple rule that dendrites receive signals and axons send them. In particular, central neurons that process information tend to have processes that both send and receive signals. For example, the serotonergic interneuron, CSD, has projections that may be both pre- and post-synaptic (Roy et al., 2007). Similarly, mushroom body neuron dendrites most likely contain synaptic vesicles (Rolls et al., 2007). Thus overall, *Drosophila* and vertebrate neurons look as though they follow similar rules, and bending of the rules, for localization of synaptic components.

While synaptic proteins can be targeted specifically to either axons or dendrites, they are not generally found throughout the membrane of this compartment. Rather, they are most often localized just to synaptic sites. This type of localization tends to involve scaffolding proteins that concentrate and anchor proteins at their site of function (Bruneau et al., 2009; Oswald and Sigrist, 2009). Thus enrichment of synaptic proteins either to axons or dendrites does not necessarily reflect overall differences in the axonal or dendritic plasma membrane, or overall polarized transport to one compartment or the other, so it is important to look at other types of proteins as well.

cell adhesion proteins

In vertebrate neurons, plasma membrane proteins that are not directly involved in neurotransmission can also localize specifically to axons or dendrites of mature neurons. These probably better represent overall plasma membrane polarity than synaptic components. Two good examples are transferrin receptor, which is localized to dendrites, and NgCAM (neuron-glia cell adhesion molecule), which is localized to axons in cultured rat hippocampal neurons (Burack et al., 2000). Do these proteins also polarize in *Drosophila*? The NgCAM-YFP fusion protein that localizes to axons in hippocampal neurons, localizes to the primary neurite in unipolar mushroom body and olfactory projection neurons in the *Drosophila* larval brain (Rolls et al., 2007). The localization of this marker is thus restricted to a subregion of the neuron, but is not the same as its localization in mammalian cells. Perhaps this reflects the fact that NgCAM has multiple targeting signals (Yap et al., 2008), only a subset of which are active in *Drosophila* neurons. It may be that its localization to the primary neurite in *Drosophila* neurons reflects an ankyrin-dependent anchorage, as this mechanism targets NgCAM to the initial region of axons in mammalian neurons (Boiko et al., 2007). So, if expression of NgCAM does not give a clear answer

about plasma membrane polarity in *Drosophila*, are there endogenous proteins that support its existence?

The clearest example of a polarized cell adhesion molecule in *Drosophila* neurons is Dscam. The *Dscam* gene can generate thousands of splice forms, and the variety of proteins produced is important for self-avoidance of neurites during development (Hughes et al., 2007; Matthews et al., 2007; Hattori et al., 2009). This variation includes two different transmembrane domains that can encode proteins targeted to different regions of the neuron. GFP-tagged constructs containing transmembrane domain 1 (TM1) are present in the cell body and dendrites, but not axons, when expressed in mushroom body neurons (Wang et al., 2004). Thus, there is at least one example of a highly polarized endogenous cell adhesion molecule in *Drosophila*.

protein synthesis and processing machinery

Ribosomes, rough endoplasmic reticulum (RER) and the Golgi complex can be identified in electron micrographs, and so have long been known to be highly concentrated in the cell body and proximal dendrites of mature vertebrate neurons, with lesser localization in distal dendrites (Craig and Banker, 1994). In *Drosophila* neurons, ribosomes and RER are also highly concentrated in the cell body (Rolls et al., 2007). It is less clear how much of either extends into dendrites, if any. Polarized dendritic localization of the Golgi complex has, however, been convincingly described. Golgi outposts are found in the large dendritic arbors of class IV multidendritic sensory neurons, where they often localize to dendrite branch points (Ye et al., 2007), and are rarely found in axons of these cells (Zheng et al., 2008). Polarized dendritic localization of mRNA has not been demonstrated thus far in *Drosophila* neurons. In one study, fluorescently tagged nanos RNA localized outside the cell body of multidendritic neurons in a 3' UTR-dependent manner, but the RNA localized to axons as well as dendrites (Brechtel and Gavis, 2008). A GFP-tagged RNA-binding protein, Imp, also has been shown to localize to axons (Boylan et al., 2008). Thus, whether there is polarized distribution of mRNA remains an open question, but it does seem likely that when the Golgi complex is found outside the cell body, it is localized only to dendrites.

cytoskeletal proteins

The other major class of proteins that has polarized distribution in vertebrate neurons is cytoskeletal proteins. In fact, if one would like to use antibody staining to prove that a process is an axon or dendrite, most likely this will involve antibodies to a cytoskeletal protein. Antibodies to MAP2 exclusively stain dendrites, and those that recognize dephosphorylated tau are classic axonal markers (Craig and Banker, 1994). In addition to these microtubule-associated proteins (MAPs), phosphorylated intermediate filament proteins (neurofilaments) are enriched in axons (Benson et al., 1996), and ankyrin G is specifically localized to the axon initial segment (Jenkins and Bennett, 2001).

MAPs that are exclusively localized to axons or dendrites have not yet been identified in *Drosophila*. The GFP-tagged microtubule-binding domain of bovine tau appears enriched in axons over dendrites when expressed in central neurons, but it is present in dendrites (Rolls et al., 2007). Likewise endogenous *Drosophila* tau tagged with GFP can be seen in dendrites

as well as axons (Stone et al., 2008). There are several potential reasons for this lack of polarized localization. One is that *Drosophila* tau is the protein most similar to human tau, but it is also the most similar protein to human MAP2. Mammalian tau and MAP2 have a conserved microtubule-binding domain, and there is only one protein in *Drosophila* with this region, and it is the *Drosophila* tau protein (Goldstein and Gunawardena, 2000). So the relative simplicity of the *Drosophila* cytoskeletal proteome could explain the absence of specialized axonal and dendritic MAPs. Alternately, differently modified forms that cannot be detected with available tools could be localized to axons and dendrites. Indeed mammalian tau is only a good axonal marker if you use an antibody that specifically recognizes dephospho-tau (Papasozomenos and Binder, 1987).

Axon-specific neurofilaments have also not been identified in *Drosophila*, but in this case it is unlikely that time will change this state of affairs. No proteins, aside from nuclear lamins, that could be intermediate filament subunits have been identified in *Drosophila*, even in this era of sequenced genomes (Goldstein and Gunawardena, 2000).

Ankyrins present a more complex story. Ankyrins link the spectrin submembrane skeleton with plasma membrane proteins, including NgCAM/L1 and voltage gated sodium channels at the axon initial segment (AIS) in vertebrates. This axon initial segment localization is mediated by a special ankyrin, ankyrin G, which is highly enriched in this part of the axon. It is thought that *Drosophila* do not have true AIS because the short sequences that target sodium and potassium channels to the AIS are present only in chordates (Hill et al., 2008; Rasband, 2010). But there is some evidence that ankyrin isoforms localize to specific regions of neurons in *Drosophila*.

As well as a very generally expressed ankyrin, *Drosophila* expresses an additional ankyrin, ankyrin2/Dank2, predominantly in neurons. There are short, medium (Ank2-M) and giant (Ank2-L and Ank2-XL) isoforms of Dank2. The short isoform localizes to the cell body, and not axons (Hortsch et al., 2002), while the M and L isoforms are enriched in axons (Hortsch et al., 2002; Koch et al., 2008; Pielage et al., 2008), and the XL isoform is found in cell bodies and axons (Koch et al., 2008). The only isoform whose localization with respect to dendrites has been determined is Ank2-M (also known as long): in multidendritic sensory neurons it is present in dendrites as well as the axon (Yamamoto et al., 2006). Thus while there are differently localized ankyrin isoforms, how their localization relates to overall neuronal polarity is not clear. Nor is it clear whether there will be an initial segment-specific ankyrin analogous to vertebrate ankyrin G. However, Dank2 and neuroglian, the *Drosophila* equivalent of NgCAM, do at least work together in the proximal axon to control excess dendrite sprouting (Yamamoto et al., 2006). The only additional hint that there may be a special organization of the ankyrin-spectrin submembrane skeleton in proximal axons or primary neurites is the observation that vertebrate NgCAM is enriched in the primary neurite of *Drosophila* central neurons (Rolls et al., 2007). If there is a specialized cytoskeleton somewhere in the primary neurite or proximal axon, perhaps it could be responsible for organizing the spike initiation zone, which is also predicted to be somewhere in this region (Gouwens and Wilson, 2009).

At least one other cytoskeleton-binding protein has a restricted distribution in *Drosophila* neurons. When tagged with GFP, the adenomatous polyposis coli (APC) family member Apc2 is localized to puncta in the cell body and dendrites of central and peripheral neurons, as well as to a short region of the primary neurite or axon, but not at all to distal axons (Rolls et al., 2007; Stone et al., 2010). As APC family members can associate with both the microtubule and actin cytoskeleton, and a host of other proteins (Dikovskaya et al., 2001), it is not clear what is targeting Apc2-GFP to these regions of the cells. Thus while there is clearly enrichment of Apc2 and other cytoskeleton-binding proteins in specific regions of *Drosophila* neurons, it is so far unclear how these enrichment domains relate to overall axon-dendrite polarity.

In fact, these localization patterns suggest that neurons are divided into subdomains within axon and dendrite compartments at the level of the cytoskeleton. A recent study on developing axons suggests that this is true even early in neuron life. Diffusion boundaries, which are presumably mediated by specialized cytoskeletal organizations, were found between regions of growing axons in primary cultures of *Drosophila* neurons. Specifically, a distal axonal compartment enriched for ROBO2 and ROBO3, as well as a proximal compartment enriched for DRL, are separated by a plasma membrane diffusion barrier (Katsuki et al., 2009).

While our understanding of who is where in *Drosophila* neurons is still not as sophisticated as it is in vertebrate neurons, even this invertebrate clearly has the capacity to generate highly polarized neurons with axon-dendrite differences as well as additional regional specialization. It should therefore be possible to use this system to understand how a single cell can send different sets of proteins to different compartments. So, the next question is: what has *Drosophila* already told us about how a neuron, with a single protein synthesis factory in the cell body, generates axons and dendrites that are different from one another?

How do proteins and organelles get where they need to be?

Mechanisms of polarized transport can be quite complex. For example, one of the best-studied axonal proteins in mammalian neurons has dendritic and axonal targeting signals, and gets to axons via transcytosis from dendrites (Yap et al., 2008). One strategy for dealing with this potential complexity is to start with the simplest possible model and work up from that. In *Drosophila* neurons, the simplest possible model is actually very simple, and involves thinking about the tracks for polarized transport: microtubules.

Perhaps the most striking difference between *Drosophila* axons and dendrites is the polarity of their microtubules. Microtubules are intrinsically polarized filaments, with a minus end that is the site of nucleation and a plus end at which subunit addition and loss constantly occurs in a process of dynamic instability. Microtubule motors recognize this polarity, and move in one direction, either to the plus or minus end depending on the motor. For a long time it was assumed that *Drosophila* microtubule polarity would be the same as that of mammalian neurons in culture: plus-end-out microtubules in axons and mixed microtubule polarity in dendrites. And circumstantial data was consistent with this model. For example, a fusion protein with a plus end-directed motor accumulated in axons, and one to a minus end-

directed motor accumulated in dendrites (Clark et al., 1997). When microtubule polarity was finally directly examined, axonal microtubules were found to be plus-end-out as predicted, but a surprise was in store in dendrites (Rolls et al., 2007). Rather than mixed polarity, greater than 90% of microtubules had minus-end-out polarity in dendrites of multidendritic peripheral neurons and central neurons (Rolls et al., 2007; Stone et al., 2008). Thus microtubules have essentially opposite polarity in *Drosophila* axons and dendrites.

The simple model of polarized transport is thus that plus end-directed motors (most kinesins) are responsible for transport into axons and minus end-directed motors (primarily cytoplasmic dynein with potential help from minus end-directed kinesin *ncd*) are responsible for transport into dendrites. Thus any cargo synthesized in the cell body would simply have to bind to a specific motor to be transported in one direction or the other.

So how consistent is the simple model with the available experimental data? In *Drosophila*, most of the relevant data involves analysis of mutant phenotypes. And, although this is a very simple model, the data actually fits it very well. Phenotypes of neurons lacking dynein subunits have been described in several studies. Clones of interneurons in the larval mushroom body that harbored mutations in either dynein heavy chain (*Dhc64C*) or the dynein complex component *Lis1*, had severely reduced dendrites; axons had abnormal swellings, but were not as strongly affected as dendrites (Liu et al., 2000). In retrospect, this result is consistent with the bulk of dendritic, but not axonal, components requiring dynein for transport from the cell body (and axonal retrograde transport also being dependent on dynein). Since then, additional mutant and RNAi studies have focused on the role of dynein in transport of specific proteins or organelles into dendrites. One example is mitochondria: mitochondria localize to both axons and dendrites, but when levels of a component of dynein, *dlic*, are reduced in mutant clones, mitochondria are still found in axons, but not dendrites (Sato et al., 2008). Similarly, endosome localization to dendrites is dependent on dynein (Sato et al., 2008; Zheng et al., 2008). Moreover, when dynein is disrupted, proteins or organelles that are normally restricted to dendrites, including *ppk*, *Dscam*[TM1], and the Golgi, lose their dendritic localization and escape into axons (Yang et al., 2008; Zheng et al., 2008). Recent work also suggests that dynein may be a major motor for dendritic transport in mammalian neurons (Kapitein et al., 2010). Although the kinesin, *ncd*, is minus end-directed, and could thus potentially help dynein in dendritic transport, there is no evidence so far that it does.

The flip side of the role of dynein in dendritic transport is the role of kinesin in axonal transport. However, while there is just one cytoplasmic dynein, there are many kinesin motors, so the situation is a little more complicated. The kinesin that has been studied the most is conventional kinesin (*Khc*, kinesin-1). When mitochondrial localization was analyzed in mutant *Khc* clones in parallel with the *dlic* clones described above, mitochondria were still seen in dendrites, but were dramatically reduced in axons (Sato et al., 2008) consistent with complementary roles of these two motors in axonal and dendritic transport. One complication of the studies on *Khc* is that mutations in either *Khc* or dynein often cause gross axonal transport defects that are similar, perhaps because the motors work together (Martin et al., 1999). However, detailed studies are generally consistent with a role for *Khc* in anterograde axonal transport of mitochondria (Pilling et al., 2006). Another

kinesin, Unc-104/Imac (a kinesin-3), has also been shown to have a role in anterograde axonal transport; its cargoes are synaptic vesicles (Pack-Chung et al., 2007; Barkus et al., 2008). Many additional cargoes await experiments to pair them with specific kinesins, but overall the simple model of dynein for dendritic transport and kinesins for axonal transport seems to fit with motor loss-of function studies.

Of course the simple model is just that, and leaves many issues to be resolved, including how a cargo lets go of the motor when it gets where it needs to be. There is some progress being made on the motor regulation front in both mammals and *Drosophila*. Both phosphorylation and binding of cargo can regulate motors (Horiuchi et al., 2007; Verhey and Hammond, 2009).

The simple model also runs into trouble when unipolar neurons are considered. If the key to efficient polarized transport is for a cargo to bind to the appropriate motor in the cell body after synthesis and then be carried out towards microtubule minus ends in dendrites or plus ends in axons, what happens when there is a single exit route from the cell body? Knowing the polarity of microtubules in this single exit route, the primary neurite, is key. Microtubule polarity in primary neurites of unipolar neurons has so far been described in only a single cell, the CSD interneuron in the brain (Stone et al., 2008), so there is definitely room for more information on this topic. But, in this neuron, the arrangement of microtubules is very hard to reconcile with our simple, and so far useful, model of polarized transport. Microtubule polarity in the primary neurite was plus-end-out as in the axon and the dendrites had minus-end-out polarity (Stone et al., 2008). If one extrapolates from this to draw a map of microtubules in this cell (Figure 2), it is unclear how dendrites receive any cargo at all, as there are no (or few) microtubules that travel from the primary neurite into dendrites. Indeed, analysis of endosome movement confirmed the rarity of this pathway (Stone et al., 2008). Is there then a transport pathway from axons to dendrites, or a concerted switching of motors at the primary neurite-dendrite junction? The answer to these questions awaits live imaging of dendritic cargo transport in unipolar neurons.

The other scenario that is problematic for our simple model of polarized transport is interneurons with pre- and post-synaptic sites on a single process. This type of mixed polarity process is common in vertebrate and invertebrate interneurons which integrate signals (Craig and Banker, 1994). One likely example is the CSD interneuron mentioned above. The process with minus-end-out polarity has presynaptic, as well as most likely postsynaptic, sites (Roy et al., 2007). How do synaptic vesicles, which normally accumulate in axons, get into, and stay in, these dendrites, but not other ones? How this polarity rule-bending works has not been addressed in any system, but wrestling with it may help us to develop a more complete model of neuronal polarity.

Do fly neurons consider themselves polarized?

It is very difficult to think of a way to ask neurons just how they feel about their axons and dendrites: are they really fundamentally different places, and if so, what is important for making them different? But they may have already given us an answer. When their axon is severed, multidendritic sensory neurons mount a full-scale response: they globally rearrange

their microtubules and initiate axon regeneration (Stone et al., 2010). However, when a dendrite is severed in exactly the same way microtubules do not rearrange (Stone et al., 2010), and there is no major regeneration response (Sugimura et al., 2003; Stone et al., 2010). So, when probed in this manner, *Drosophila* neurons do seem to be telling us that axons and dendrites are fundamentally different!

In these injury experiments, axons and dendrites were severed close to the cell body, completely removing them. This type of proximal axotomy experiment has been performed in many different vertebrate systems, from sea lamprey (Hall and Cohen, 1983; Hall et al., 1989) to hippocampal neurons in culture (Gomis-Ruth et al., 2008), to cats and rats (Cho and So, 1992; Rose et al., 2001; MacDermid et al., 2002; Hoang et al., 2005; Fenrich et al., 2007). In each of these cases, growth of a new process that looked like an axon was initiated from a dendrite, rather than any remaining axon stump or the cell body. Exactly the same thing was observed in *Drosophila* (Stone et al., 2010), suggesting that vertebrate and fly neurons deal with a polarity challenge, complete loss of the single axon, with the same strategy: polarity reversal.

This type of experiment allows the neurons to tell us how they feel about their own polarity, and perhaps also what is important for distinguishing axons and dendrites. If a dendrite must become an axon during this type of regeneration, then we can determine what key features of the dendrite must change for this conversion to be successful. Microtubule polarity definitely changes. Microtubules reverse from minus-end-out to plus-end-out polarity, and regrowth of the axon initiates only after this happens (Stone et al., 2010). So microtubule polarity is at least one key feature of axon or dendrite identity.

Odd and ends

One and Only

Many neurons in both vertebrates and flies maintain tight control over axon number, and the number is one. The one and only one axon rule has been known and puzzled over for a long time (Dotti and Banker, 1987; Peters et al., 1991), but remains unexplained. Perhaps the power of *Drosophila* genetics may some day contribute to understanding this aspect of polarity.

Growing up

Almost all of the protein localization and mutant studies mentioned in the previous sections relied on larval neurons. The larva is a functional, mature body form: it eats, moves, and behaves. But is the adult even more mature? Perhaps some of the markers that are not clearly polarized in larval neurons could become polarized in adult cells that have a longer lifetime and also often a more complex shape? One intriguing hint that this might be the case is the localization of the microtubule-binding protein futsch, a MAP1 homolog. In larval neurons, for example multidendritic sensory neurons, futsch is clearly present in dendrites as well as axons (Hummel et al., 2000). But it is described (although not shown) as being absent from the dendrites of these same multidendritic cells in adults (Shimono et al., 2009). So perhaps this is the first example of a polarized MAP in *Drosophila* neurons, but only in the adult.

Polarity in culture?

Polarized cultures of mammalian neurons, especially rat hippocampal neurons, have been extremely useful for studying polarity (Kaeck and Banker, 2006), but there are very few culture systems in which dendrites polarize. In *Drosophila* the situation is similar. Primary cultures of neurons are relatively straightforward to generate, and can form obvious presynaptic specializations. However, it is not so clear whether specialized postsynaptic processes develop (Kuppers-Munther et al., 2004; Oh et al., 2008). Dendrite-like processes have been described in several studies (Sanchez-Soriano et al., 2005; Kraft et al., 2006), but not fully polarized dendrites.

It's a wrap!

As well as beautiful, highly polarized neurons, *Drosophila* have glia that stand up well to scrutiny under the microscope (Freeman and Doherty, 2006; Awasaki et al., 2008; Banerjee and Bhat, 2008; Doherty et al., 2009). These cells form close relationships with their neuronal partners, and these contacts can influence neuron shape (Yamamoto et al., 2006). Moreover, glia seem to be able to recognize axons, bundle them into clearly visible nerves, and take more pains to wrap axons than dendrites. The easiest place to see this is in the periphery where neurons are spread out (Figure 1). Axons of multidendritic sensory neurons are ensheathed in glia before the cell body, and the wrapping only ever reaches the first segment of the dendrite in larvae (Yamamoto et al., 2006). Maybe the glia also have a message for us about the importance of neuronal polarity, and *Drosophila* as a model system in which to study it.

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Figure 1. Overview of *Drosophila* neuronal polarity

A portion of the *Drosophila* peripheral nervous system is shown on the left in A. This is the dorsal cluster of larval dendritic arborization neurons, which are mechanosensory. The ddaE cell, which has a relatively simple dendritic arbor, is on the right of the cluster; its axon and dendrites are indicated with arrows. Its axon joins with axons from other neurons to form a nerve that is wrapped in glial membranes. These membranes also wrap the cell bodies and proximal dendrites, but not distal dendrites. To take this image, whole living larvae were mounted on a microscope slide. The larvae had one copy each of a tau protein trap (GFP inserted into the *Drosophila* tau gene such that the endogenous tau is fused with GFP, described in (Stone et al., 2008), repo-Gal4, which drives expression in glia, and UAS-mCD8-RFP, which labels membranes in response to Gal4 in glia. B.

Localization of some of the markers discussed in the text is shown in the context of a multipolar neuron.

multipolar neuron

unipolar neuron

Figure 2. Map of microtubule polarity in *Drosophila* multipolar and unipolar neurons

These maps are extrapolated from the behavior of growing microtubule plus ends labeled with EB1-GFP, and also the movement of endosomes through dendrite branches and through the primary neurite-axon-dendrite junction (Stone et al., 2010). Dendritic microtubules are almost all minus-end-out, except at dendrite tips, and axonal microtubules are plus-end-out. Based on analysis of one cell type, the primary neurite also has plus-end-out polarity, which means that there are no direct microtubule tracks from the primary neurite into the dendrite.