REVIEW

Neuronal polarity: an evolutionary perspective

Melissa M. Rolls[1,2,*] and Timothy J. Jegla[2,3]

ABSTRACT

Polarized distribution of signaling molecules to axons and dendrites facilitates directional information flow in complex vertebrate nervous systems. The topic we address here is when the key aspects of neuronal polarity evolved. All neurons have a central cell body with thin processes that extend from it to cover long distances, and they also all rely on voltage-gated ion channels to propagate signals along their length. The most familiar neurons, those in vertebrates, have additional cellular features that allow them to send directional signals efficiently. In these neurons, dendrites typically receive signals and axons send signals. It has been suggested that many of the distinct features of axons and dendrites, including the axon initial segment, are found only in vertebrates. However, it is now becoming clear that two key cytoskeletal features that underlie polarized sorting, a specialized region at the base of the axon and polarized microtubules, are found in invertebrate neurons as well. It thus seems likely that all bilaterians generate axons and dendrites in the same way. As a next step, it will be extremely interesting to determine whether the nerve nets of cnidarians and ctenophores also contain polarized neurons with true axons and dendrites, or whether polarity evolved in concert with the more centralized nervous systems found in bilaterians.

KEY WORDS: Axon, Dendrite, Axon initial segment, Microtubule

Introduction: are vertebrates special because of their neurons?

The elaborate behaviors of vertebrates are made possible by the evolution of nervous systems of unparalleled size and complexity. The developmental mechanisms that assemble the vertebrate nervous system and physical mapping of the circuits that underlie behavior are subjects of intense study. But are the neurons of the vertebrate nervous system themselves also unique or special in some way? Do they have vertebrate-only features that facilitate the assembly of large, complex nervous systems? Or is the vertebrate nervous system based on an ancient and highly adaptable neuronal cell type? Understanding the evolutionary history of neurons is critical to understanding how vertebrate nervous systems and behavior came to be. Another very practical reason for asking these questions is that it is helpful to know which features of neurons are shared with and can thus be studied in cheap, efficient invertebrate model systems. Vertebrate-specific neuronal features, in contrast, must be studied in model systems such as zebrafish or mouse, for which time and expense often limit the scientific questions that can be asked.

The polarized functional organization of neurons that facilitates assembly of complex circuits might be a good candidate for being

[1]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA. [2]Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA. [3]Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA.

*Author for correspondence (mur22@psu.edu)

associated specifically with vertebrates. Indeed, in a highly cited review on neuronal polarity from 1994, invertebrate neurons are seen as 'sufficiently different' in terms of organization from vertebrate neurons that the polarized sorting mechanisms are suggested to also differ (Craig and Banker, 1994). However, directional transfer of information between neurons is a key feature of the neuronal circuits that allow all bilaterian animals to move, find food, avoid enemies and perform a myriad of other activities. Thus, at least some aspects of the polar neuron are likely to have ancient origins. In this review, we re-examine the question of whether neuronal polarity is indeed a fundamentally vertebrate innovation, or whether it evolved much earlier in the history of the nervous system.

Overview of neuronal polarity

In vertebrates, directional signaling of neurons is most often accomplished by specialization of neuronal processes into axons and dendrites, with axons as the output side of the cell and dendrites the input side. This specialization is termed neuronal polarity. In assembling the functional circuit maps that underlie behaviors, it is very helpful to know whether a particular neurite is an axon or a dendrite. This is the key to understanding in which direction information flows and how it is processed. So, how do we know which neurites are axons and which are dendrites?

The most familiar layout of a vertebrate neuron is that of a multipolar neuron with a central cell body, which houses the nucleus and most protein synthetic machinery. This cell body gives rise to a single axon and multiple branched dendrites. Axons tend to be longer than dendrites and maintain the same diameter or caliber as they branch, while dendrites are shorter and taper as they branch (Craig and Banker, 1994). The axon is presynaptic and so contains clusters of synaptic vesicles at release sites, and the dendrite is postsynaptic and houses neurotransmitter receptors. The ability of axons to relay the action potential and dendrites to integrate synaptic inputs is in large part dependent on the two compartments having distinct complements of ion channels, the proteins that mediate electrical signaling in the nervous system. At the base of the single axon lies the axon initial segment (AIS), in which ion channels that underlie the action potential are highly concentrated by an underlying spectrin–ankyrin network (Rasband, 2010). Because of the high concentration of channels, the AIS serves as the site of action potential initiation in most polar vertebrate neurons (Bender and Trussell, 2012).

In addition to the axon–dendrite differences that relate closely to the ability to receive or send signals, there are other differences that are less immediately relevant to signaling. For example, dendrites contain some synthetic machinery including ribosomes and Golgi outposts, while axons contain little, if any, of these components (Bartlett and Banker, 1984; Craig and Banker, 1994) (Fig. 1). Similarly, the rough endoplasmic reticulum may extend a little way into the dendrites, but is absent from the axon, although the smooth endoplasmic reticulum is found throughout the neuron (Krijnse-Locker et al., 1995). The cytoskeletal framework of axons and
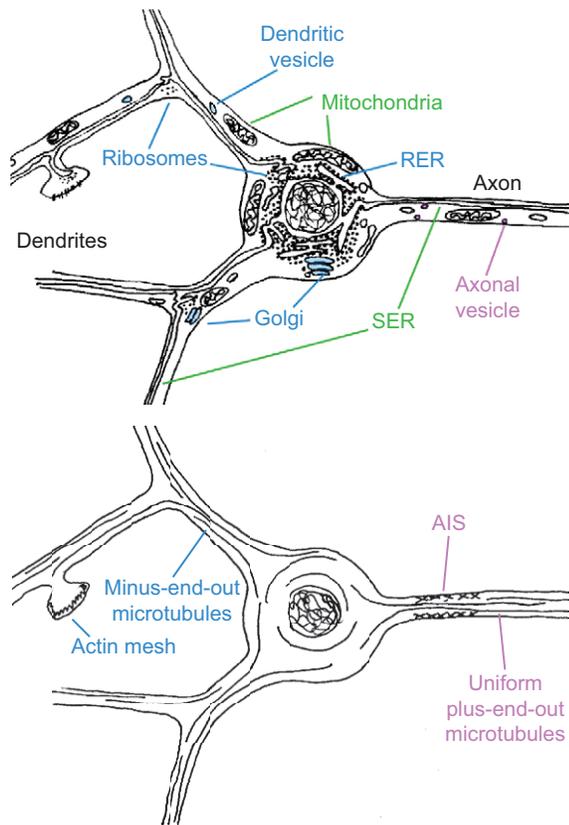
Fig. 1. Key features of a prototypical multipolar neuron. A diagram of the synthetic and secretory machinery localization is shown in the upper panel. The Golgi complex is found in the cell body, with smaller outposts in proximal dendrites, especially at large proximal branch points. Ribosomes are highly concentrated in the cell body, with additional clusters in dendrites, especially at branch points. The rough endoplasmic reticulum (RER) is found in the cell body, and the smooth ER reaches into all regions of the cell. Mitochondria are also found in all compartments. In the lower panel, some of the major cytoskeletal components are shown. The axon initial segment (AIS) is an ankyrin–spectrin meshwork under the plasma membrane. Axonal microtubules are uniformly plus-end-out while dendritic ones are typically 50% or more minus-end-out. Dendritic spines rely on actin for their characteristic shape.

dendrites also differs. Axons contain high levels of neurofilament proteins and dendrites do not, and MAP2 is a dendrite-specific microtubule-binding protein, while dephosphorylated tau, which also associates with microtubules, is found at high levels only in axons (Peng et al., 1986; Craig and Banker, 1994). In addition, in primary cultures of hippocampal pyramidal neurons, which are perhaps the best studied of these prototypic polar neurons, dendritic microtubules have mixed polarity while axonal microtubules have all plus-end-out polarity (Baas et al., 1988).

If all neurons followed the simple segregation rules of our prototype vertebrate multipolar neuron, it would be straightforward to identify universal markers for axons and dendrites. Indeed, MAP2 and dephospho-tau antibodies have been used quite successfully to identify dendrites and axons in studies on mammalian central neurons (Kosik and Finch, 1987). However, if one ventures slightly further afield, these markers are not useful. For example, in *Drosophila*, complex circuits are assembled and neurons appear to have specialized processes, but there is no ortholog of MAP2, and tau is found in both axons and dendrites (Stone et al., 2008). Furthermore, not all vertebrate neurons strictly observe the

functional segregation of axons and dendrites defined in the prototype multipolar neuron. While many axons are exclusively presynaptic and many dendrites are exclusively postsynaptic, abundant exceptions exist. Axo-axonal synapses can directly modulate action potential firing (Inan et al., 2013) or synaptic vesicle release (Ren et al., 2007), and dendro-dendritic synapses can regulate inhibitory circuits (Strowbridge, 2009) in the vertebrate central nervous system (CNS). Are the terms axon and dendrite then simply semantic simplifications, or is there some underlying fundamental difference that distinguishes these processes in all contexts?

We will focus on two aspects of polarity that have the potential to direct the differential distribution of cellular constituents in polar neurons. These two features are (1) the differences in microtubule organization between axons and dendrites, and (2) the specialized sub-membrane skeleton at the AIS. We will first examine the evidence that these cytoskeletal features are functionally important for polarity in vertebrate neurons; then, we will move on to explore whether they might also characterize axons and dendrites in other metazoans.

Microtubule organization in axons and dendrites

Polarized arrays of microtubules can act like signposts to direct traffic of cellular constituents to different places. Microtubules are dynamic polymers that are nucleated by a complex of proteins called the γ-tubulin ring complex, or γ-TuRC. The γ-TuRC most likely acts as a template to organize the 13-protofilament ring structure of the microtubule (Wiese and Zheng, 2006; Raynaud-Messina and Merdes, 2007). Dimers of α-β tubulin can then be added to elongate the microtubule (Alberts et al., 2007). The asymmetry of α-β tubulin dimers differentiates the two ends of the microtubule: the minus end begins with α-tubulin, and β-tubulin is exposed at the growing plus end. α-β tubulin dimers are added at the plus end as the microtubule grows, and are lost from the plus end during catastrophe as the microtubule retracts. Microtubules continuously switch between plus end growth and shrinkage in the process of dynamic instability (Mitchison and Kirschner, 1984). In proliferating cells, most nucleation sites are clustered at the centrosome, so microtubule minus ends are located near the center of the cell and plus ends at the periphery (Bartolini and Gundersen, 2006). This type of organization is not possible in neurons, though, because individual microtubules would need to extend from the centrosome in the cell body through the entire length of the axon or dendrite. In fairly mature axons a single microtubule might be 100 μm long (Bray and Bunge, 1981), but this is still too short to extend to many axonal termini. Microtubules instead form overlapping arrays in axons and dendrites (Baas and Lin, 2011). This arrangement means that minus and plus ends are scattered throughout axons and dendrites. But this scattering of ends is not random. In vertebrate axons all microtubules are oriented with minus ends towards the cell body and plus ends away from the cell body (Baas and Lin, 2011). In two studies in 1988 it was discovered that this was not the case in dendrites. In rat hippocampal neurons *in vitro* (Baas et al., 1988) and frog mitral cells from an adult brain (Burton, 1988), dendritic microtubules were found to be arranged equally plus-end-out and minus-end-out. The authors of both papers noted that this implied that transport in axons and dendrites might work in fundamentally different ways. Could this difference contribute to the development of neuronal polarity?

Cargoes are transported along microtubules by motor proteins that recognize the intrinsic polarity of microtubules and walk to either the plus end or minus end. Most of the several dozen varieties of
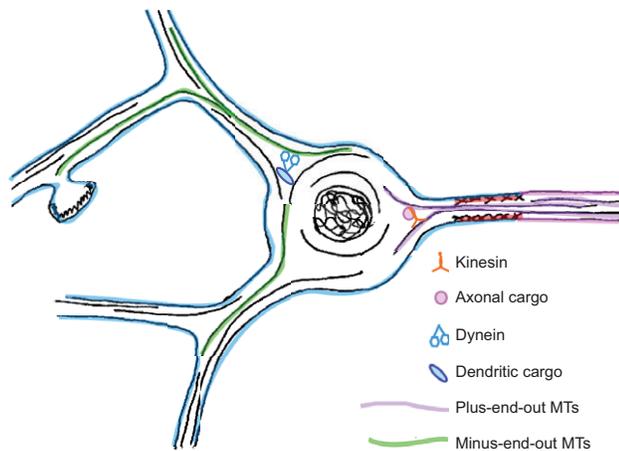
Fig. 2. Microtubule polarity and the AIS can organize polarized distribution of other proteins. The AIS (red mesh) acts as a barrier that keeps axonal plasma membrane proteins (pink) separate from dendritic plasma membrane proteins (blue). In the simplest model for polarized traffic, kinesins bring axonal cargoes into the axon via plus-end-out microtubules and dynein pulls dendritic cargoes into dendrites along minus-end-out microtubules.

kinesin motors walk towards microtubule plus ends, whereas cytoplasmic dynein is the major minus end-directed motor (Alberts et al., 2007). This means that in axons, cargoes are carried outwards from the cell body by kinesins and back again by dynein (Hirokawa et al., 2010; Saxton and Hollenbeck, 2012). In dendrites, one motor could go in both directions, or dynein could take on the role of a specific outbound motor for dendritic cargoes. Such cargoes could include cellular constituents such as Golgi and ribosomes, which are found in dendrites but not axons (Baas and Lin, 2011). Other dendrite-specific cargoes could include postsynaptic proteins and specialized dendritic ion channels. However, the very simple idea that kinesins would carry axon-specific cargoes and dynein would carry dendrite-specific cargoes (Fig. 2) to translate microtubule polarity into more general neuronal polarity fell out of favor for many years. Instead, a variety of kinesin-only models were proposed for polarized transport based on the idea that some kinesins were dendrite specific (Setou et al., 2004; Hirokawa and Takemura, 2005). However, both models of polarized transport rely on fundamental differences in the microtubule cytoskeleton as a basis to direct appropriate cargoes to axons and dendrites. Thus, regardless of the model, microtubules have the potential to underlie many aspects of neuronal polarity.

The AIS is the boundary between the axon and the cell body

The first part of the axon is specialized in many vertebrate neurons to serve as the site of action potential initiation (Bender and Trussell, 2012). The AIS has an especially low excitation threshold because its small surface area favors excitation and, most importantly, it contains a high concentration of voltage-gated Na^+ channels (Grubb and Burrone, 2010; Bender and Trussell, 2012). Thus, graded depolarizations that reach the AIS can initiate an action potential that propagates down the axon. AIS excitation is tightly regulated by synaptic inputs and locally clustered K^+ channels (Grubb and Burrone, 2010; Rasband, 2010; Bender and Trussell, 2012). Shaker (Kv1), Shab (Kv2) and KCNQ2/3 voltage-gated K^+ channels localized to the AIS regulate action potential threshold, duration and frequency (Rasband et al., 1998; Dodson et al., 2002; Pan et al., 2006; Goldberg et al., 2008; Johnston et al., 2008; Lorincz and

Nusser, 2008; Sarmiere et al., 2008; Shah et al., 2008). The AIS ion channel complement is not fixed and can vary across neuronal cell types to facilitate distinct patterns of excitability (Lorincz and Nusser, 2008; Bender and Trussell, 2012).

In addition to its role in action potential initiation, the AIS has a specialized cytoskeletal structure that serves as a barrier for diffusion within the plasma membrane. This diffusion barrier property was discovered in 1999 by using optical tweezers to drag plasma membrane proteins along the axon; they could not be dragged through the AIS (Winckler et al., 1999). Moreover, this barrier localizes to the boundary between axonal plasma membrane proteins like NgCAM and dendritic plasma membrane proteins like the transferrin receptor (Winckler et al., 1999). This diffusion barrier is constructed by a special sub-membrane skeleton that localizes to the AIS. Ankyrin-G (AnkG) is the central player in orchestrating the AIS and acts as a linker protein that bridges transmembrane proteins, including ion channels (Zhou et al., 1998; Pan et al., 2006), and β-IV-spectrin, which in turn binds actin (Grubb and Burrone, 2010; Rasband, 2010; Bennett and Lorenzo, 2013). These proteins work together to set up the electron-dense meshwork under the plasma membrane that has long been known as a distinguishing feature of the AIS (Peters et al., 1991; Jones et al., 2014). This cytoskeletal structure is particularly interesting because, like microtubules, it has the potential to influence the distribution of other proteins.

Functional evidence that microtubules and the AIS direct polarity

The arrangement of microtubules in neurons and the barrier function of the AIS both have the potential to selectively direct traffic to axons and dendrites. But do they? The best way to answer this question is to look at axonal and dendritic components when either microtubule organization or the AIS is genetically (or chemically) disrupted.

For microtubules, reduction of kinesin-6 (or CHO1/MKLP1) by antisense oligonucleotides (Yu et al., 2000) or, more recently, by RNAi (Lin et al., 2012) results in the loss of minus-end-out microtubules from the dendrites of rat sympathetic neurons in culture. If transport along minus-end-out microtubules allows cargo such as ribosomes into dendrites, but not axons, then one would expect these would be lost once minus-end-out microtubules were no longer present. Indeed, ribosomes and membranes that were thought to be Golgi were reduced by antisense oligonucleotides targeting kinesin-6 (Yu et al., 2000). The poorly understood tapering shape of dendrites also became more uniform and axon-like in these experiments. In the more recent RNAi study, the number of minus-end-out microtubules in dendrites was reduced, but not completely eliminated, and dendrites became longer and thinner, but did not entirely lose a dendrite marker, MAP2 (Lin et al., 2012). Overall, these loss-of-function experiments support the idea that minus-end-out microtubules and dendrite identity are closely linked in vertebrate neurons.

For the AIS, acute de-polymerization of actin after polarity is established results in the loss of segregation of axonal and dendritic plasma membrane proteins (Winckler et al., 1999). Long-term (10 day) reduction of AnkG by RNAi results in the appearance of dendritic membrane proteins like KCC2 on the former axon (Hedstrom et al., 2008). Thus, there is very clear experimental support that the cytoskeletal structure at the AIS is required for maintenance of plasma membrane polarity (Szu-Yu Ho and Rasband, 2011). What is less clear is its role in the establishment of the differences between axons and dendrites. Establishment of

polarity requires transporting different cellular components into axons and dendrites. One argument against a role for the AIS in establishing polarity is that the diffusion barrier function of the AIS is established relatively late during polarity development in cultured neurons. A barrier that hinders lipid diffusion is set up after 7–10 days in culture (Nakada et al., 2003), while the axon is first specified around 24 h after culturing (Petersen et al., 2014), and dendrites acquire minus-end-out microtubules around 4 days, with mature microtubule polarity at 7 days (Baas et al., 1989). However, some elements of the AIS cytoskeleton are present by 5 days in culture (Song et al., 2009) so the AIS could play a role in later stages of polarity establishment. Analysis of dextran movement into axons suggests that large dextrans (70 kDa) freely enter axons at 3 days, but have restricted access at 5 days (Song et al., 2009). Analysis of vesicle movement into axons also supports the idea that the AIS cytoskeleton can reach below the surface and restrict entry of a subset of microtubule-based cargoes into axons (Song et al., 2009; Watanabe et al., 2012). It is not completely clear, though, why some cargoes might get stopped by the AIS mesh and others not, or whether some of the evidence for the influence of actin on trafficking might be due to failure to segregate axonal and dendritic cargoes into different vesicles when actin is depolymerized (Petersen et al., 2014). At a minimum, though, the AIS serves as a highly specialized signaling compartment and plays an important role in the maintenance of plasma membrane polarity.

In summary, loss-of-function experiments in vertebrate neurons implicate both polarized microtubules and the AIS in the control of axon and dendrite identity. In order to understand the evolutionary origins of polarity in vertebrate neurons, we must therefore understand the evolutionary origins of differential microtubule polarity and the AIS. Whether these features are shared with various invertebrates is critical to understanding which branches of the metazoan tree implement neuronal polarity in the same way as vertebrates.

Neuronal polarity in model invertebrate bilaterians

Drosophila and *Caenorhabditis elegans* are the most accessible invertebrates for molecular and cellular studies of the nervous system. In the case of *C. elegans*, the entire nervous system, including the vast majority of synapses, has been reconstructed from serial electron micrographs (White et al., 1986) and is accessible at WormAtlas (http://www.wormatlas.org/). The whole animal is optically accessible as it is small and transparent, and various genetic manipulations, including RNAi, work well. For *Drosophila*, genetic manipulation is extremely sophisticated and is built on more than 100 years of study. Decades of detailed genetic analysis of nervous system development are now being supplemented by reconstructions of the nervous system (Chiang et al., 2011) and there is a rapidly growing set of tools to genetically manipulate individual or small subsets of neurons (Jenett et al., 2012). These two models have therefore been ideal for studying polarity in protostome invertebrate neurons.

In *Drosophila*, the majority of neurons are unipolar with a single neurite arising from the cell body (Fig. 3). This single neurite then gives rise to different processes that have been classified as axons or dendrites based on structure and function. For example, unipolar motor neurons have dendrites that branch close to the cell body in the ventral ganglion and then send long axons out to muscles via well-defined nerves (Campos-Ortega and Hartenstein, 1997; Sánchez-Soriano et al., 2005). However, the fact that all processes arise from a single one led to the supposition that polarized traffic might be completely different in vertebrates and invertebrates (Craig
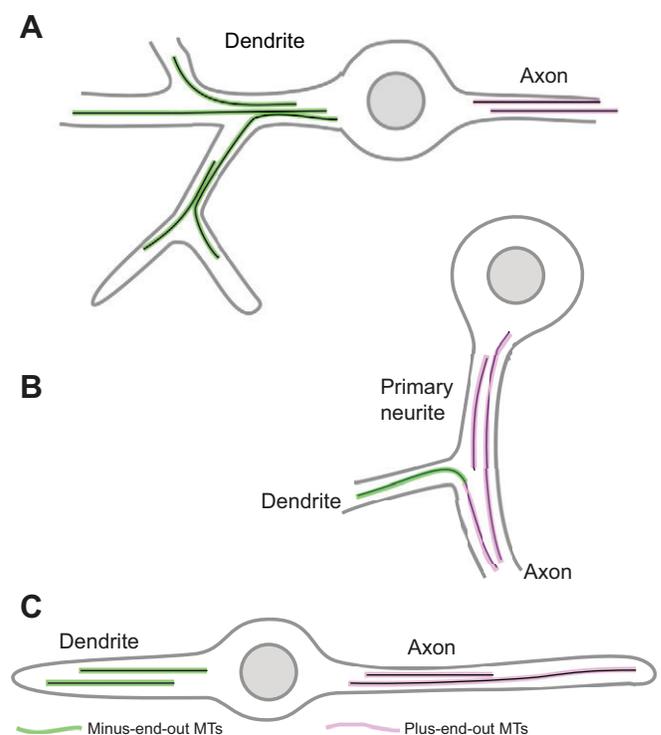

Fig. 3. Microtubules in invertebrate neurons are highly polarized. Schematic diagrams of microtubule organization in *Drosophila* sensory neurons (A) and motor neurons and interneurons (B), and in *Caenorhabditis elegans* motor neurons (C) are shown. MT, microtubule. These diagrams are based on analysis of microtubule growth *in vivo* in *Drosophila* larval neurons (Stone et al., 2008) and an identified motor neuron in *C. elegans* (Goodwin et al., 2012).

and Banker, 1994). Is this difference in where processes arise, directly from the cell body or more distally from a neurite, really a critical determinant of the way polarity is implemented?

In *Drosophila*, there are also neurons that resemble canonical vertebrate multipolar neurons. The best-studied example is dendritic arborization neurons in the larval body wall. These cells respond to sensory stimuli including harsh touch (Hwang et al., 2007) and gentle touch (Tsubouchi et al., 2012). They have extensive branched dendrite arbors and long axons that bundle together in nerves to take signals from the periphery to the central nervous system (Bodmer and Jan, 1987; Gao et al., 1999; Grueber et al., 2002). Although the dendrites of these cells are sensory, they share cellular features with mammalian dendrites. For example, ribosomes (Hill et al., 2012) and Golgi outposts (Ye et al., 2007) are present in dendrites, but not axons of these cells. Although these cells are normally classically multipolar, with axons and dendrites arising directly from the cell body, there are some genetic backgrounds in which dendrites can arise from the axon in these cells as they do in unipolar neurons (Yamamoto et al., 2006). Is there then perhaps not quite so rigid a distinction between unipolar and multipolar neurons? Several other examples support the idea that the lines that separate unipolar and multipolar neurons are somewhat blurred. For example, when *Drosophila* unipolar neurons are removed from the animal and grown in culture, they sprout dendrites directly from the cell body (Sánchez-Soriano et al., 2005). Similar flexibility also exists in some cultured rodent neurons, which can grow axons from dendrites [for a nice example see fig. 4 in Burack et al. (Burack et al., 2000)]. Likewise, hippocampal oriens–alveus interneurons have an axon

that sprouts from the dendritic tree rather than the soma (Martina et al., 2000).

Although sending axonal and dendritic traffic out of the cell body together in a single process must cause some trafficking challenges, neurons in *Drosophila* and mammals seem able to accomplish this when necessary. Unipolar shape alone therefore does not necessarily mean that *Drosophila* neurons have a fundamentally different mechanism of polarization with respect to vertebrate multipolar neurons. But how does the microtubule arrangement in neuronal processes compare between vertebrates and *Drosophila*?

Microtubule polarity in *Drosophila* neurons has been analyzed in multipolar larval sensory neurons, unipolar motor neurons and unipolar interneurons in intact animals (Stone et al., 2008). In all cases, axonal microtubules are exclusively plus-end-out as in vertebrates. The dendrites, however, held a surprise. Like vertebrate neurons, they are distinguished from axons by the presence of minus-end-out microtubules. But in *Drosophila*, almost all microtubules are minus-end-out (Stone et al., 2008). This is true in unipolar and multipolar neurons. Polarity was also examined in the primary neurite of the motor neuron in this study. This result was another surprise: microtubules were plus-end-out (Fig. 3), not mixed (Stone et al., 2008), meaning cargoes traveling from the cell body to the minus-end-out dendrite would need to travel first on plus-end-out microtubules, then switch motors and move onto minus-end-out microtubules. It is not known how this switch is orchestrated, but studying trafficking in these unipolar neurons might provide important clues to the sorting of axonal and dendritic cargoes.

As *Drosophila* dendrites are minus-end-out rather than mixed, does this mean that mechanisms that underlie axon and dendrite differences are not the same in flies and vertebrates? One piece of evidence that suggests overall similarity derives from a developmental study. Early in development, dendritic microtubules in multipolar *Drosophila* neurons are mixed, as in vertebrates, but over time they gradually mature to more than 90% minus-end-out (Hill et al., 2012). The fact that dendrites have mixed polarity during development in *Drosophila* suggests that dendrites in *Drosophila* and vertebrates are fundamentally similar and may share a common evolutionary history.

However, the maturation of *Drosophila* dendrites to minus-end-out emphasizes the importance of minus-end-out microtubules for dendritic trafficking. Indeed, *Drosophila* neurons deficient in subunits of dynein, the major minus end-directed motor, have strong dendrite growth and transport defects (Liu et al., 2000; Satoh et al., 2008; Zheng et al., 2008). While kinesins were the focus of dendrite transport studies in mixed polarity mammals for many years (Setou et al., 2000; Setou et al., 2002; Hirokawa and Takemura, 2005), dynein has now also been shown to have a role in the classic polarity model of primary cultures of rodent hippocampal neurons (Kapitein et al., 2010). Thus, in both *Drosophila* and vertebrate neurons, axons have plus-end-out microtubules, and anterograde transport is mediated by kinesins, while dendrites have a significant number of minus-end-out microtubules, and dynein is at minimum an important contributor to anterograde transport.

Caenorhabditis elegans neurons typically have a much simpler shape than either vertebrate or insect neurons. Many *C. elegans* neurons have one or two unbranched processes extending from the cell body (White et al., 1986). The only neurites that are highly branched are newly discovered sensory dendrites that cover the body wall (Halevi et al., 2002; Tsalik et al., 2003). For the majority of neurons that have unbranched processes, morphology is of little help for classification of axons or dendrites. Instead, synaptic architecture has been used. In many cases the neurites are both presynaptic and

postsynaptic (White et al., 1986). But there are some neurons that have a process that is entirely postsynaptic, and this process is typically called the dendrite (White et al., 1986). Until recently it was not clear that these processes shared any features other than their postsynaptic nature with dendrites in vertebrates. However, in 2012 microtubule polarity was analyzed in a motor neuron that had one of these dendrite-like processes. In this cell the dendritic microtubules were minus-end-out and the axonal microtubules were plus-end-out as in a *Drosophila* neuron (Goodwin et al., 2012). Thus, although *C. elegans* neurons may not have elaborately branched dendrites, this compartment is still distinguished from the axon by minus-end-out microtubules.

If the microtubule layout is generally quite similar in vertebrate and model invertebrate neurons, is the AIS also similar? Less information is available to answer this question. One view is that the AIS is a vertebrate-specific innovation. This view is based in large part on analysis of ankyrins and ankyrin-binding motifs in voltage-gated ion channels. Specifically, AnkG is a vertebrate-specific member of the ankyrin gene family. The AnkG binding motif in voltage-gated Na^+ channels that is required for clustering at the AIS is present in chordates that predate vertebrates (Hill et al., 2008). Thus, the Na^+ channel ankyrin-binding motif predates AnkG, suggesting that an ancestral ankyrin with the ability to bind channels may have already been present at the AIS in the first chordates. A similar AnkG-binding motif in the AIS-clustered K^+ channel KCNQ2/3 evolved independently with jawed fishes (Hill et al., 2008). These observations have led to a model in which the 'excitozone' of the AIS evolved within the chordate/vertebrate lineage to reliably and specifically initiate action potentials at the AIS, and this channel clustering at the AIS and nodes of Ranvier was a critical innovation that allowed for the evolution of rapid saltatory conduction in myelinated axons (Hill et al., 2008).

This chordate/vertebrate AIS model predicts that there is no ankyrin-dependent clustering of voltage-gated Na^+ or K^+ channels in the initial segment of invertebrate axons and implies that the AIS of invertebrate neurons may not be an efficient zone for action potential initiation. However, many studies have shown that action potentials initiate in the proximal axon in diverse invertebrate neurons (including unipolar neurons) from lobster, leech, *Aplysia* and insects (Edwards and Ottoson, 1958; Tauc, 1962; Goodman and Heitler, 1979; Gu et al., 1991; Melinek and Muller, 1996). Therefore, the ability of the proximal axon to serve as the site of action potential initiation appears to be an ancient shared feature of vertebrate and invertebrate neurons rather than a recent vertebrate innovation.

Molecular localization studies in *Drosophila* have confirmed that voltage-gated Na^+ and K^+ channels can be concentrated either just at an AIS-like domain or from the putative AIS along the rest of the axon in invertebrate neurons. An immunohistological study of an identified unipolar *Drosophila* motor neuron showed that voltage-gated Na^+ channels were highly concentrated along the axonal plasma membrane starting about 100 μm from the cell body (Kuehn and Duch, 2013), consistent with the previous electrophysiological studies on the site of action potential initiation in unipolar neurons. In Kenyon cells, which are central unipolar neurons responsible for higher order processing, GFP-tagged elk and shal K^+ channels concentrate in an AIS-like domain (Trunova et al., 2011). Elk channels regulate action potential threshold in vertebrate neurons (Zhang et al., 2010), while shal channels encode transient A-currents, which influence both threshold and frequency (Jerng et al., 2004). These studies at a minimum suggest that the initial segment of *Drosophila* axons can serve as a boundary for ion channel

distribution, and suggest that there may be a mechanism for clustering ion channels there. While these findings are intriguing, more work is required to determine whether this axonal channel localization is ankyrin dependent in the same manner as in vertebrates, or relies on alternative mechanisms.

In addition to organizing plasma membrane proteins, the first part of the axon seems to influence intracellular trafficking in both *C. elegans* and *Drosophila*. In *C. elegans*, the microtubule-binding protein CRMP is localized to a specific region of the proximal axon in an ankyrin-dependent manner (Maniar et al., 2012). Moreover, loss of CRMP or ankyrin disrupts polarized trafficking and allows presynaptic proteins to enter dendrites (Maniar et al., 2012). Intriguingly, this same study showed that loss of ankyrin or CRMP resulted in mixed polarity microtubules in axons and ciliated sensory dendrites (Maniar et al., 2012), although a link between the AIS and microtubule polarity has not been made in other systems. In another study, live imaging of membrane traffic in *C. elegans* neurons indicated that the proximal axon acts as a filter that keeps somatic components like the Golgi out of axons (Edwards et al., 2013). In at least one *Drosophila* neuron, the lateral td cell, there is also a specialized region of the axon that influences retrograde transport of neuropeptide vesicles and seems to keep them out of the cell body (Wong et al., 2012). Again, these studies hint that basic features of the AIS, including action potential initiation and influencing traffic into and out of the axon, are present in invertebrate neurons.

So when did neuronal polarity evolve?

Experiments in *Drosophila* and *C. elegans* suggest that neuronal polarity and its cytoskeletal underpinnings predate the evolutionary divergence of vertebrates, insects and nematodes (Fig. 4). All three have polarized microtubules that are plus-end-out in axons and at least 50% minus-end-out in dendrites, and appear to share at least some aspects of AIS function. We therefore suggest that axons and dendrites in two of the three major recognized bilaterian lineages (Halanych et al., 1995; Philippe et al., 2005) – deuterostomes (including chordates) and ecdysozoans (including insects and nematodes) – are likely to have a common evolutionary origin. If so, their axons and dendrites are orthologous structures, and *C. elegans* and *Drosophila* are valuable models for studying the mechanisms underlying neuronal polarity. But what about the third major branch

of the bilaterian tree, the lophotrochozoans, which includes annelids and mollusks? While molecular studies of neuronal polarity have not been carried out in lophotrochozoans, *Aplysia* (mollusk) and leech (annelid) have unipolar neurons of similar anatomy to insect unipolar neurons, and an axon initial segment that is used as the site of action potential initiation (Tauc, 1962; Gu et al., 1991; Melinek and Muller, 1996). Thus, all three major groups of bilaterians have the ability to concentrate ion channels in axons relative to the somatodendritic compartment. While additional molecular studies are needed to determine mechanisms of polarization in invertebrates, including lophotrochozoans, it is already clear that some of the major mechanisms for polarization are at least as old as the ecdysozoan/chordate ancestor, and we speculate that they may have been present in a common ancestor of all extant bilaterians (Fig. 4).

If mechanisms for generating neuronal polarity were established before the radiation of the major bilaterian lineages, when might they have first evolved? Two additional extant groups of metazoans harbor nervous systems – the cnidarians and the ctenophores. Fig. 4 depicts the modern view of metazoan phylogeny, which places cnidarians as a sister group to bilaterians, and suggests that ctenophores branched from the very base of the metazoan tree (Hejnol et al., 2009; Ryan et al., 2013; Moroz et al., 2014). Cnidarians are the more extensively studied of these two groups, and have viable genetic models such as the starlet sea anemone *Nematostella vectensis* (Anthozoa). The backbone of the cnidarian nervous system is a distributed nerve net with little centralization in the Anthozoa (Marlow et al., 2009; Nakanishi et al., 2012), the basal extant cnidarian lineage. While some centralization does occur in various jellyfish lineages (Mackie, 2004; Satterlie, 2011), it is likely that ancestral cnidarians had minimal nervous system centralization. Evidence against the presence of polarized neurons in cnidarians includes the presence of bidirectional chemical synapses in scyphozoans and a hydrozoan (*Hydra*) (Kinnamon and Westfall, 1982; Anderson and Grünert, 1988). However, other hydrozoans do have polarized chemical synapses (Anderson and Spencer, 1989) and many cnidarians display a variety of highly complex motor behaviors that require directional neuronal signaling (Anderson and Spencer, 1989; Mackie, 2004). On a molecular level, cnidarians share almost all the major gene families of neuronal signaling proteins with bilaterians (Putnam et al., 2007), including all gene
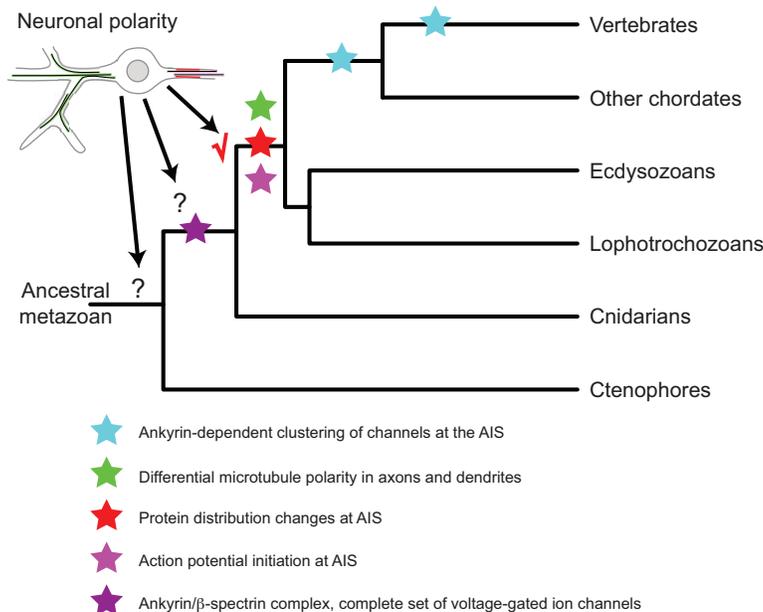

Fig. 4. Schematic phylogeny of the evolution of neuronal polarity. The evolutionary relationships of key groups of animals with nervous systems are shown. These relationships are based on recent sequence analysis (Ryan et al., 2013; Moroz et al., 2014). We argue that at least a minimal AIS-like domain is likely to be present in all bilaterians including ecdysozoans like *Drosophila* and *C. elegans* and lophotrocozoans like leech and *Aplysia*. Polarized microtubules in axons and dendrites are also found in different bilaterian branches. It is currently an open question whether these key features of polarized neurons are found in cnidarians and ctenophores.

families of voltage-gated ion channels (Nayak et al., 2009). The biophysical properties of the cnidarian channels in these families are highly conserved (Jegla et al., 1995; Jegla and Salkoff, 1997; Sand et al., 2011; Gur Barzilai et al., 2012; Jegla et al., 2012; Martinson et al., 2014), suggesting they play similar physiological roles. Furthermore, *Nematostella* has key components of the AIS barrier including β-spectrin (Putnam et al., 2007; Bennett and Lorenzo, 2013) and an ankyrin ortholog with a full β-spectrin binding domain (Putnam et al., 2007). Investigation of synapse arrangement, microtubule polarity and diffusion barriers in *Nematostella* neurons could shed light on whether common mechanisms for generating neuronal polarity predate the cnidarian/bilaterian split, or whether they arose in concert with nervous system centralization in the bilaterians.

While cnidarians have the genetic trappings of polar neurons and sufficiently complex neural circuits to suggest some benefit from neuronal polarity, the situation is even less clear for the ctenophores, which represent the most ancient extant group of metazoans with nervous systems. Analysis of ctenophore genomes suggests that their nervous systems could be very different. Many classes of neuronal proteins, including many families of voltage-gated ion channels, shared by cnidarians and bilaterians appear to be missing (Ryan et al., 2013; Moroz et al., 2014). Interestingly, we found no evidence for ankyrin or β-spectrin genes in the genomes of two distinct ctenophores (Ryan et al., 2013; Moroz et al., 2014), suggesting that the backbone of the vertebrate AIS cytoskeleton is not present in ctenophores. It has even been suggested that the nervous systems of ctenophores and cnidarians/bilaterians are so different that they might have evolved independently (Moroz et al., 2014; Ryan, 2014). No information is available on the cellular structure of ctenophore neurons, but ctenophores have both diffuse nerve nets and some centralized neuronal structures (Moroz et al., 2014). Molecular investigation of neuronal polarity in ctenophores could help shed light on the question of whether ctenophore and cnidarian/bilaterian nervous systems have common evolutionary origins. If mechanisms of polarization are shared, then it would strongly support a single, common origin of the major aspects of neuronal structure. However, distinct mechanisms for generating polarity or a lack of polarity in ctenophore neurons would support a model in which at least our axons and dendrites, if not our entire neurons, evolved after the divergence from ctenophores.

Conclusions

We suggest that current evidence points to a common evolutionary origin for neuronal polarity in bilaterians. Vertebrates, flies and nematodes share distinct microtubule arrangements in axons and dendrites and can concentrate proteins at an AIS-like domain. Furthermore, the proximal axon is the favored site for action potential initiation in all major bilaterian lineages. Assuming neuronal polarity is present in all bilaterian lineages, cnidarians and ctenophores hold the key to determining when and how many times neuronal polarity evolved. If these animals lack neuronal polarity, or have different polarization mechanisms, then typical axonal/dendritic neuronal polarity might be a bilaterian innovation. However, the presence of common polarization mechanisms in cnidarians and/or ctenophores would suggest a single origin for neuronal polarity at the time of the evolution of the first complex neural networks in early metazoans.

References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2007). *Molecular Biology of the Cell*. New York, NY: Garland Science.

Anderson, P. A. and Grünert, U. (1988). Three-dimensional structure of bidirectional, excitatory chemical synapses in the jellyfish *Cyanea capillata*. *Synapse* **2**, 606-613.

Anderson, P. A. and Spencer, A. N. (1989). The importance of cnidarian synapses for neurobiology. *J. Neurobiol.* **20**, 435-457.

Baas, P. W. and Lin, S. (2011). Hooks and comets: the story of microtubule polarity orientation in the neuron. *Dev. Neurobiol.* **71**, 403-418.

Baas, P. W., Deitch, J. S., Black, M. M. and Banker, G. A. (1988). Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc. Natl. Acad. Sci. USA* **85**, 8335-8339.

Baas, P. W., Black, M. M. and Banker, G. A. (1989). Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. *J. Cell Biol.* **109**, 3085-3094.

Bartlett, W. P. and Banker, G. A. (1984). An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture. I. Cells which develop without intercellular contacts. *J. Neurosci.* **4**, 1944-1953.

Bartolini, F. and Gundersen, G. G. (2006). Generation of noncentrosomal microtubule arrays. *J. Cell Sci.* **119**, 4155-4163.

Bender, K. J. and Trussell, L. O. (2012). The physiology of the axon initial segment. *Annu. Rev. Neurosci.* **35**, 249-265.

Bennett, V. and Lorenzo, D. N. (2013). Spectrin- and ankyrin-based membrane domains and the evolution of vertebrates. *Curr Top Membr* **72**, 1-37.

Bodmer, R. and Jan, Y. N. (1987). Morphological differentiation of the embryonic peripheral neurons in *Drosophila*. *Roux's Arch. Dev. Biol.* **196**, 69-77.

Bray, D. and Bunge, M. B. (1981). Serial analysis of microtubules in cultured rat sensory axons. *J. Neurocytol.* **10**, 589-605.

Burack, M. A., Silverman, M. A. and Banker, G. (2000). The role of selective transport in neuronal protein sorting. *Neuron* **26**, 465-472.

Burton, P. R. (1988). Dendrites of mitral cell neurons contain microtubules of opposite polarity. *Brain Res.* **473**, 107-115.

Campos-Ortega, J. A. and Hartenstein, V. (1997). *The Embryonic Development of Drosophila Melanogaster*. Berlin: Springer.

Chiang, A. S., Lin, C. Y., Chuang, C. C., Chang, H. M., Hsieh, C. H., Yeh, C. W., Shih, C. T., Wu, J. J., Wang, G. T., Chen, Y. C. et al. (2011). Three-dimensional reconstruction of brain-wide wiring networks in *Drosophila* at single-cell resolution. *Curr. Biol.* **21**, 1-11.

Craig, A. M. and Banker, G. (1994). Neuronal polarity. *Annu. Rev. Neurosci.* **17**, 267-310.

Dodson, P. D., Barker, M. C. and Forsythe, I. D. (2002). Two heteromeric Kv1 potassium channels differentially regulate action potential firing. *J. Neurosci.* **22**, 6953-6961.

Edwards, C. and Ottoson, D. (1958). The site of impulse initiation in a nerve cell of a crustacean stretch receptor. *J. Physiol.* **143**, 138-148.

Edwards, S. L., Yu, S. C., Hoover, C. M., Phillips, B. C., Richmond, J. E. and Miller, K. G. (2013). An organelle gatekeeper function for *Caenorhabditis elegans* UNC-16 (JIP3) at the axon initial segment. *Genetics* **194**, 143-161.

Gao, F. B., Brenman, J. E., Jan, L. Y. and Jan, Y. N. (1999). Genes regulating dendritic outgrowth, branching, and routing in *Drosophila*. *Genes Dev.* **13**, 2549-2561.

Goldberg, E. M., Clark, B. D., Zagha, E., Nahmani, M., Erisir, A. and Rudy, B. (2008). K⁺ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. *Neuron* **58**, 387-400.

Goodman, C. S. and Heitler, W. J. (1979). Electrical properties of insect neurones with spiking and non-spiking somata: normal, axotomized, and colchicine-treated neurones. *J. Exp. Biol.* **83**, 95-121.

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

Grubb, M. S. and Burrone, J. (2010). Building and maintaining the axon initial segment. *Curr. Opin. Neurobiol.* **20**, 481-488.

Grueber, W. B., Jan, L. Y. and Jan, Y. N. (2002). Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. *Development* **129**, 2867-2878.

Gu, X. N., Muller, K. J. and Young, S. R. (1991). Synaptic integration at a sensory-motor reflex in the leech. *J. Physiol.* **441**, 733-754.

Gur Barzilai, M., Reitzel, A. M., Kraus, J. E., Gordon, D., Technau, U., Gurevitz, M. and Moran, Y. (2012). Convergent evolution of sodium ion selectivity in metazoan neuronal signaling. *Cell Reports* **2**, 242-248.

Halanych, K. M., Bacheller, J. D., Aguinaldo, A. M., Liva, S. M., Hillis, D. M. and Lake, J. A. (1995). Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* **267**, 1641-1643.

Halevi, S., McKay, J., Palfreyman, M., Yassin, L., Eshel, M., Jorgensen, E. and Treinin, M. (2002). The C. elegans ric-3 gene is required for maturation of nicotinic acetylcholine receptors. *EMBO J.* **21**, 1012-1020.

Hedstrom, K. L., Ogawa, Y. and Rasband, M. N. (2008). AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. *J. Cell Biol.* **183**, 635-640.

Hejnol, A., Obst, M., Stamatakis, A., Ott, M., Rouse, G. W., Edgecombe, G. D., Martinez, P., Baguñà, J., Bailly, X., Jondelius, U. et al. (2009). Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proc. Biol. Sci.* **276**, 4261-4270.

Hill, A. S., Nishino, A., Nakajo, K., Zhang, G., Fineman, J. R., Selzer, M. E., Okamura, Y. and Cooper, E. C. (2008). Ion channel clustering at the axon initial segment and node of Ranvier evolved sequentially in early chordates. *PLoS Genet.* **4**, e1000317.

Hill, S. E., Parmar, M., Gheres, K. W., Guignet, M. A., Huang, Y., Jackson, F. R. and Rolls, M. M. (2012). Development of dendrite polarity in *Drosophila* neurons. *Neural Dev.* **7**, 34.

Hirokawa, N. and Takemura, R. (2005). Molecular motors and mechanisms of directional transport in neurons. *Nat. Rev. Neurosci.* **6**, 201-214.

Hirokawa, N., Niwa, S. and Tanaka, Y. (2010). Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron* **68**, 610-638.

Hwang, R. Y., Zhong, L., Xu, Y., Johnson, T., Zhang, F., Deisseroth, K. and Tracey, W. D. (2007). Nociceptive neurons protect *Drosophila* larvae from parasitoid wasps. *Curr. Biol.* **17**, 2105-2116.

Inan, M., Blázquez-Llorca, L., Merchán-Pérez, A., Anderson, S. A., DeFelipe, J. and Yuste, R. (2013). Dense and overlapping innervation of pyramidal neurons by chandelier cells. *J. Neurosci.* **33**, 1907-1914.

Jegla, T. and Salkoff, L. (1997). A novel subunit for shal K+ channels radically alters activation and inactivation. *J. Neurosci.* **17**, 32-44.

Jegla, T., Grigoriev, N., Gallin, W. J., Salkoff, L. and Spencer, A. N. (1995). Multiple Shaker potassium channels in a primitive metazoan. *J. Neurosci.* **15**, 7989-7999.

Jegla, T., Marlow, H. Q., Chen, B., Simmons, D. K., Jacobo, S. M. and Martindale, M. Q. (2012). Expanded functional diversity of shaker K+ channels in cnidarians is driven by gene expansion. *PLoS ONE* **7**, e51366.

Jenett, A., Rubin, G. M., Ngo, T. T., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B. D., Cavallaro, A., Hall, D., Jeter, J. et al. (2012). A GAL4-driver line resource for Drosophila neurobiology. *Cell Reports* **2**, 991-1001.

Jerng, H. H., Pfaffinger, P. J. and Covarrubias, M. (2004). Molecular physiology and modulation of somatodendritic A-type potassium channels. *Mol. Cell. Neurosci.* **27**, 343-369.

Johnston, J., Griffin, S. J., Baker, C., Skrzypiec, A., Chernova, T. and Forsythe, I. D. (2008). Initial segment Kv2.2 channels mediate a slow delayed rectifier and maintain high frequency action potential firing in medial nucleus of the trapezoid body neurons. *J. Physiol.* **586**, 3493-3509.

Jones, S. L., Korobova, F. and Svitkina, T. (2014). Axon initial segment cytoskeleton comprises a multiprotein submembranous coat containing sparse actin filaments. *J. Cell Biol.* **205**, 67-81.

Kapitein, L. C., Schlager, M. A., Kuijpers, M., Wulf, P. S., van Spronsen, M., MacKintosh, F. C. and Hoogenraad, C. C. (2010). Mixed microtubules steer dynein-driven cargo transport into dendrites. *Curr. Biol.* **20**, 290-299.

Kinnamon, J. C. and Westfall, J. A. (1982). Types of neurons and synaptic connections at hypostome-tentacle junctions in *Hydra*. *J. Morphol.* **173**, 119-128.

Kosik, K. S. and Finch, E. A. (1987). MAP2 and tau segregate into dendritic and axonal domains after the elaboration of morphologically distinct neurites: an immunocytochemical study of cultured rat cerebrum. *J. Neurosci.* **7**, 3142-3153.

Krijnse-Locker, J., Parton, R. G., Fuller, S. D., Griffiths, G. and Dotti, C. G. (1995). The organization of the endoplasmic reticulum and the intermediate compartment in cultured rat hippocampal neurons. *Mol. Biol. Cell* **6**, 1315-1332.

Kuehn, C. and Duch, C. (2013). Putative excitatory and putative inhibitory inputs are localised in different dendritic domains in a *Drosophila* flight motoneuron. *Eur. J. Neurosci.* **37**, 860-875.

Lin, S., Liu, M., Mozgova, O. I., Yu, W. and Baas, P. W. (2012). Mitotic motors coregulate microtubule patterns in axons and dendrites. *J. Neurosci.* **32**, 14033-14049.

Liu, Z., Steward, R. and Luo, L. (2000). *Drosophila* Lis1 is required for neuroblast proliferation, dendritic elaboration and axonal transport. *Nat. Cell Biol.* **2**, 776-783.

Lorincz, A. and Nusser, Z. (2008). Cell-type-dependent molecular composition of the axon initial segment. *J. Neurosci.* **28**, 14329-14340.

Mackie, G. O. (2004). Central neural circuitry in the jellyfish *Aglantha*: a model 'simple nervous system'. *Neurosignals* **13**, 5-19.

Maniar, T. A., Kaplan, M., Wang, G. J., Shen, K., Wei, L., Shaw, J. E., Koushika, S. P. and Bargmann, C. I. (2012). UNC-33 (CRMP) and ankyrin organize microtubules and localize kinesin to polarize axon-dendrite sorting. *Nat. Neurosci.* **15**, 48-56.

Marlow, H. Q., Srivastava, M., Matus, D. Q., Rokhsar, D. and Martindale, M. Q. (2009). Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev. Neurobiol.* **69**, 235-254.

Martina, M., Vida, I. and Jonas, P. (2000). Distal initiation and active propagation of action potentials in interneuron dendrites. *Science* **287**, 295-300.

Martinson, A. S., van Rossum, D. B., Diatta, F. H., Layden, M. J., Rhodes, S. A., Martindale, M. Q. and Jegla, T. (2014). Functional evolution of Erg potassium channel gating reveals an ancient origin for IKr. *Proc. Natl. Acad. Sci. USA* **111**, 5712-5717.

Melinek, R. and Muller, K. J. (1996). Action potential initiation site depends on neuronal excitation. *J. Neurosci.* **16**, 2585-2591.

Mitchison, T. and Kirschner, M. (1984). Dynamic instability of microtubule growth. *Nature* **312**, 237-242.

Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., Grigorenko, A. P., Dailey, C., Berezikov, E., Buckley, K. M. et al. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature* **510**, 109-114.

Nakada, C., Ritchie, K., Oba, Y., Nakamura, M., Hotta, Y., Iino, R., Kasai, R. S., Yamaguchi, K., Fujiwara, T. and Kusumi, A. (2003). Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. *Nat. Cell Biol.* **5**, 626-632.

Nakanishi, N., Renfer, E., Technau, U. and Rentzsch, F. (2012). Nervous systems of the sea anemone *Nematostella vectensis* are generated by ectoderm and endoderm and shaped by distinct mechanisms. *Development* **139**, 347-357.

Nayak, S. K., Batalov, S., Jegla, T. J. and Zmasek, C. M. (2009). Evolution of the human ion channel set. *Comb. Chem. High Throughput Screen.* **12**, 2-23.

Pan, Z., Kao, T., Horvath, Z., Lemos, J., Sul, J. Y., Cranstoun, S. D., Bennett, V., Scherer, S. S. and Cooper, E. C. (2006). A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. *J. Neurosci.* **26**, 2599-2613.

Peng, I., Binder, L. I. and Black, M. M. (1986). Biochemical and immunological analyses of cytoskeletal domains of neurons. *J. Cell Biol.* **102**, 252-262.

Peters, A., Palay, S. L. and Webster, H. D. (1991). *The Fine Structure of the Nervous System: Neurons and their Supporting Cells*. New York, NY: Oxford University Press.

Petersen, J. D., Kaech, S. and Banker, G. (2014). Selective microtubule-based transport of dendritic membrane proteins arises in concert with axon specification. *J. Neurosci.* **34**, 4135-4147.

Philippe, H., Lartillot, N. and Brinkmann, H. (2005). Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. *Mol. Biol. Evol.* **22**, 1246-1253.

Putnam, N. H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V. V. et al. (2007). Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86-94.

Rasband, M. N. (2010). The axon initial segment and the maintenance of neuronal polarity. *Nat. Rev. Neurosci.* **11**, 552-562.

Rasband, M. N., Trimmer, J. S., Schwarz, T. L., Levinson, S. R., Ellisman, M. H., Schachner, M. and Shrager, P. (1998). Potassium channel distribution, clustering, and function in remyelinating rat axons. *J. Neurosci.* **18**, 36-47.

Raynaud-Messina, B. and Merdes, A. (2007). Gamma-tubulin complexes and microtubule organization. *Curr. Opin. Cell Biol.* **19**, 24-30.

Ren, M., Yoshimura, Y., Takada, N., Horibe, S. and Komatsu, Y. (2007). Specialized inhibitory synaptic actions between nearby neocortical pyramidal neurons. *Science* **316**, 758-761.

Ryan, J. F. (2014). Did the ctenophore nervous system evolve independently? *Zoology (Jena)* **117**, 225-226.

Ryan, J. F., Pang, K., Schnitzler, C. E., Nguyen, A. D., Moreland, R. T., Simmons, D. K., Koch, B. J., Francis, W. R., Havlak, P., Smith, S. A. et al.; NISC Comparative Sequencing Program (2013). The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* **342**, 1242592.

Sánchez-Soriano, N., Bottenberg, W., Fiala, A., Haessler, U., Kerassoviti, A., Knust, E., Löhr, R. and Prokop, A. (2005). Are dendrites in *Drosophila* homologous to vertebrate dendrites? *Dev. Biol.* **288**, 126-138.

Sand, R. M., Atherton, D. M., Spencer, A. N. and Gallin, W. J. (2011). jShaw1, a low-threshold, fast-activating K(v)3 from the hydrozoan jellyfish *Polyorchis penicillatus*. *J. Exp. Biol.* **214**, 3124-3137.

Sarmiere, P. D., Weigle, C. M. and Tamkun, M. M. (2008). The Kv2.1 K+ channel targets to the axon initial segment of hippocampal and cortical neurons in culture and in situ. *BMC Neurosci.* **9**, 112.

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

Satterlie, R. A. (2011). Do jellyfish have central nervous systems? *J. Exp. Biol.* **214**, 1215-1223.

Saxton, W. M. and Hollenbeck, P. J. (2012). The axonal transport of mitochondria. *J. Cell Sci.* **125**, 2095-2104.

Setou, M., Nakagawa, T., Seog, D. H. and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* **288**, 1796-1802.

Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M. and Hirokawa, N. (2002). Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83-87.

Setou, M., Hayasaka, T. and Yao, I. (2004). Axonal transport versus dendritic transport. *J. Neurobiol.* **58**, 201-206.

Shah, M. M., Migliore, M., Valencia, I., Cooper, E. C. and Brown, D. A. (2008). Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. USA* **105**, 7869-7874.

Song, A. H., Wang, D., Chen, G., Li, Y., Luo, J., Duan, S. and Poo, M. M. (2009). A selective filter for cytoplasmic transport at the axon initial segment. *Cell* **136**, 1148-1160.

Stone, M. C., Roegiers, F. and Rolls, M. M. (2008). Microtubules have opposite orientation in axons and dendrites of *Drosophila* neurons. *Mol. Biol. Cell* **19**, 4122-4129.

Strowbridge, B. W. (2009). Role of cortical feedback in regulating inhibitory microcircuits. *Ann. N. Y. Acad. Sci.* **1170**, 270-274.

Szu-Yu Ho, T. and Rasband, M. N. (2011). Maintenance of neuronal polarity. *Dev. Neurobiol.* **71**, 474-482.

Tauc, L. (1962). Site of origin and propagation in spike in the giant neuron of *Aplysia*. *J. Gen. Physiol.* **45**, 1077-1097.

Trunova, S., Baek, B. and Giniger, E. (2011). Cdk5 regulates the size of an axon initial segment-like compartment in mushroom body neurons of the *Drosophila* central brain. *J. Neurosci.* **31**, 10451-10462.

Tsalik, E. L., Niacaris, T., Wenick, A. S., Pau, K., Avery, L. and Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the *C. elegans* nervous system. *Dev. Biol.* **263**, 81-102.

Tsubouchi, A., Caldwell, J. C. and Tracey, W. D. (2012). Dendritic filopodia, Ripped Pocket, NOMPC, and NMDARs contribute to the sense of touch in *Drosophila* larvae. *Curr. Biol.* **22**, 2124-2134.

Watanabe, K., Al-Bassam, S., Miyazaki, Y., Wandless, T. J., Webster, P. and Arnold, D. B. (2012). Networks of polarized actin filaments in the axon initial segment provide a mechanism for sorting axonal and dendritic proteins. *Cell Reports* **2**, 1546-1553.

White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. B* **314**, 1-340.

Wiese, C. and Zheng, Y. (2006). Microtubule nucleation: gamma-tubulin and beyond. *J. Cell Sci.* **119**, 4143-4153.

Winckler, B., Forscher, P. and Mellman, I. (1999). A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature* **397**, 698-701.

Wong, M. Y., Zhou, C., Shakiryanova, D., Lloyd, T. E., Deitcher, D. L. and Levitan, E. S. (2012). Neuropeptide delivery to synapses by long-range vesicle circulation and sporadic capture. *Cell* **148**, 1029-1038.

Yamamoto, M., Ueda, R., Takahashi, K., Saigo, K. and Uemura, T. (2006). Control of axonal sprouting and dendrite branching by the Nrg-Ank complex at the neuron-glia interface. *Curr. Biol.* **16**, 1678-1683.

Ye, B., Zhang, Y., Song, W., Younger, S. H., Jan, L. Y. and Jan, Y. N. (2007). Growing dendrites and axons differ in their reliance on the secretory pathway. *Cell* **130**, 717-729.

Yu, W., Cook, C., Sauter, C., Kuriyama, R., Kaplan, P. L. and Baas, P. W. (2000). Depletion of a microtubule-associated motor protein induces the loss of dendritic identity. *J. Neurosci.* **20**, 5782-5791.

Zhang, X., Bertaso, F., Yoo, J. W., Baumgärtel, K., Clancy, S. M., Lee, V., Cienfuegos, C., Wilmot, C., Avis, J., Hunyh, T. et al. (2010). Deletion of the potassium channel Kv12.2 causes hippocampal hyperexcitability and epilepsy. *Nat. Neurosci.* **13**, 1056-1058.

Zheng, Y., Wildonger, J., Ye, B., Zhang, Y., Kita, A., Younger, S. H., Zimmerman, S., Jan, L. Y. and Jan, Y. N. (2008). Dynein is required for polarized dendritic transport and uniform microtubule orientation in axons. *Nat. Cell Biol.* **10**, 1172-1180.

Zhou, D., Lambert, S., Malen, P. L., Carpenter, S., Boland, L. M. and Bennett, V. (1998). AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J. Cell Biol.* **143**, 1295-1304.