The cellular mechanisms that maintain neuronal polarity

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Abstract | As polarized cells, neurons maintain different sets of resident plasma membrane proteins in their axons and dendrites, which is consistent with the different roles that these neurites have in electrochemical signalling. Axonal and dendritic proteins are synthesized together within the somatodendritic domain; this raises a fundamental question: what is the nature of the intracellular trafficking machinery that ensures that these proteins reach the correct domain? Recent studies have advanced our understanding of the processes underlying the selective sorting and selective transport of axonal and dendritic proteins and have created potential avenues for future progress.

Polarized cells
Cells composed of two distinct domains, which differ in molecular composition. Examples include epithelial cells, oocytes and neurons.

Cargoes
In the context of protein trafficking, cargoes refer to membrane proteins that must be moved from one compartment to another.

Endosomes
Intracellular organelles involved in the trafficking of proteins internalized from the plasma membrane.

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Neurons are polarized cells and typically have multiple dendrites and a single axon that play different parts in electrochemical signalling. Axons and dendrites contain distinct complements of membrane proteins (for example, neurotransmitter receptors, ion channels and guidance receptors) and also differ in the molecular composition of their cytosolic and cytoskeletal elements[1]. Nearly every aspect of neuronal development, neuronal signalling and neuronal plasticity depends on the accurate localization of these proteins to their appropriate domains.

Naturally, neurons are not simply polarized cells. They are composed of many, anatomically and functionally distinct structural elements: for example, postsynaptic specializations and spines, nodes and internodes, and presynaptic terminals. Targeting a protein to either the dendrites or the axon is the crucial step in establishing and maintaining the various distinct subcellular domains that comprise a neuron.

In neurons, the rough endoplasmic reticulum and the Golgi apparatus, where membrane proteins are synthesized and processed, are restricted to the cell body and dendrites, and the bulk of cytosolic protein synthesis also occurs in these regions[2]. This simple observation gives rise to the most fundamental question in neuronal protein trafficking: how is it that axonal and dendritic proteins, which are synthesized together in a common compartment, come to be differentially distributed to distinct destinations within the cell? In this review we focus on this question as it applies to membrane proteins, given that the fundamental cell biological processes that mediate their trafficking are reasonably well understood. We emphasize work in hippocampal and cortical cell cultures, the predominant model systems used to address these problems (BOX 1). The transport and differential distribution of cytosolic proteins and the mechanisms that mediate these processes have only recently begun to receive the attention they merit[3].

Mechanisms of membrane protein trafficking

Vesicles containing newly synthesized membrane proteins bud from the *trans*-Golgi network, undergo microtubule-based transport to reach the axon or dendrites and then deliver their protein cargoes to the plasma membrane by exocytosis (FIG. 1). In addition, many membrane proteins cycle between the cell surface and endosomes, and some cargoes that originate in the Golgi complex pass through endosomes *en route* to the cell surface. Whether vesicles carrying polarized proteins arise from the Golgi complex, the plasma membrane or endosomes, their trafficking must be differentially regulated to ensure that axonal and dendritic cargoes are delivered to the correct domains. The accurate trafficking of proteins between various membrane compartments is essential for all cells, not just for neurons. In fact, our understanding of the protein-trafficking machinery at the molecular level derives largely from studies in other cell types. That said, the size and geometric complexity of neurons put exceptional demands on the trafficking machinery. Thus, it is not surprising that defects in protein trafficking are associated with a great variety of neural diseases, in particular diseases that involve axonal degeneration[4–7].

A large set of proteins is devoted to ensuring the fidelity of membrane protein trafficking (TABLE 1). Vesicle budding is mediated by a set of coat proteins, which are recruited to the membrane from the cytosol. These proteins include cargo adaptors, which bind to and recruit specific transmembrane proteins to the nascent bud, and other proteins that induce membrane curvature and bud fission. Cargo adaptors bind to particular peptide

Box 1 | **Model systems for studying neuronal polarity**

Most of the research on the mechanisms underlying membrane protein trafficking has been performed in dissociated-cell cultures prepared from embryonic rodent brains; in particular, hippocampal cell cultures have been a favoured model system for more than 20 years. There are several reasons for its widespread use: for example, it is relatively easy to express constructs by transfection or electroporation in such cultures, and the cells are well suited for live-cell imaging[121,122]. Cortical cultures are also used (especially for biochemical studies that are limited by the comparatively small number of cells in the hippocampus), but the cell population in these cultures is quite heterogeneous, and not all cells follow the simple developmental programme described for hippocampal neurons[123]. It would be highly desirable to confirm the conclusions from cell-culture models in more intact systems, such as transgenic mice; however, such studies have been rare[124]. This situation may be changing with the advent of the CRISPR method for generating knockout and knock-in mice[120].

Over the past 5–10 years, genetically tractable systems — fruitflies[125,126], nematodes[127–129] and zebrafish[130] — have taken on more prominent roles for the study of vesicle transport and polarity in neurons. For example, genetic studies in *Drosophila* spp. allowed the identification of the proteins that link Kinesin-1 to mitochondria and regulate mitochondrial transport[131–134]. These systems have also provided novel insights into the control of microtubule organization, the regulation of microtubule polarity and the role of microtubule motors in the development of dendritic morphology[135,136]. There are preparations from each of these model organisms that allow for live-cell imaging of vesicular trafficking[56,130,137–140].

Coat proteins
Protein components that bind to the cytosolic face of membranes, serving to concentrate cargoes into new vesicles and to induce bud formation. Some coat proteins form an electron-dense 'coat' that is visible by electron microscopy, hence the name.

Glycoproteins
Proteins that are post-translationally modified by the addition of sugar chains to ectodomain amino acid residues.

Tethering proteins
Also known as tethering factors. Proteins that initiate the first interaction between a vesicle and the target membrane where the vesicle will undergo fusion.

Motor adaptors
Proteins that link kinesin or dynein motors to vesicles or other cargoes.

sequences within the cytoplasmic domains of transmembrane proteins; as a result, only a specific subset of proteins present in the donor compartment is concentrated during vesicle formation, a process referred to as cargo sorting. Other instances of protein sorting may depend on the enrichment of cargo proteins within lipid microdomains or on the concentration of glycoproteins by lectin-like cargo receptors[8].

Similarly to vesicle budding, vesicle fusion also involves a sequence of highly regulated protein–protein interactions. The process is initiated by the formation of molecular links between vesicles and target membranes. These links are mediated by a diverse group of peripherally associated membrane proteins, which are referred to as tethering proteins[9,10]. Then, the formation of a complex between SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) on the vesicle and target membranes drives the fusion of the lipid bilayers, allowing the incorporation of vesicle cargo proteins into the target membrane[11]. The specific tethers and SNAREs present on a vesicle determine where it can fuse.

Vesicle transport, the process that occurs between vesicle budding and fusion, brings a vesicle to its intracellular destination. Owing to their highly extended morphology, neurons are particularly dependent on well-regulated vesicle transport. Long-range vesicle transport is mediated by motor proteins (kinesins and dyneins) that use ATP hydrolysis to translocate along microtubules[12]. Most kinesins move vesicles towards the plus ends of microtubules, whereas cytoplasmic dyneins transport vesicles towards the minus ends of microtubules[13–15]. In most cases, these motors do not bind directly to vesicles but are linked by adaptor proteins, which may also serve to regulate motor activity[16]. Unlike most other trafficking proteins, motor adaptors do not fall into specific protein families but appear to comprise a highly diverse set of proteins.

Membrane budding, transport and fusion events are also regulated by small GTPases, primarily of the RAB and ARF families[17,18]. RABs and ARFs regulate the recruitment or activation of other trafficking proteins, such as cargo adaptors, motors, vesicle coats, tethering factors and SNAREs. RABs and ARFs are crucial for ensuring that different membrane trafficking steps are executed in the proper sequence.

Neuronal membrane protein trafficking
The advent of green fluorescent protein (GFP) technology and advances in live-cell imaging techniques have made it possible to probe the roles of sorting, transport and fusion in the trafficking of polarized proteins in neurons (FIG. 2; see Supplementary information S1 (movie)). Such experiments show that dendritically polarized proteins are sorted into distinct populations of vesicles that do not contain axonal proteins. Vesicles containing dendritic proteins undergo bidirectional microtubule-based transport in dendrites, but their transport is regulated so that these vesicles do not enter the axon[19–22]. Because the carriers that contain dendritically polarized membrane proteins never enter the axon, their fusion does not need to be as tightly regulated as that of vesicles destined for the axon. Thus, selective sorting and selective transport are the key events for maintaining dendritic polarity.

The situation for axonal proteins is more complicated. Vesicles containing axonal proteins cannot be excluded from the dendritic domain because the endoplasmic reticulum and the Golgi apparatus, where axonal proteins are made, extend into the dendritic tree. Vesicles containing axonal proteins undergo microtubule-based transport in dendrites as well as in the axon, but their transport is biased towards the axon; quantitative analyses show that the number of vesicles that enter the axon is between two and four times higher than the number of vesicles entering the average dendrite[19,23]. Although important, this comparatively small transport bias by itself cannot account for the polarity of axonal proteins, given that the surface area of the axon is far larger than that of the average dendrite.

If vesicles containing axonal proteins are not excluded from dendrites, how is the polarity of axonal proteins maintained? In cultured neurons, differential endocytosis is sufficient to account for the polarity of some axonal proteins[24–26]. Such proteins are delivered to both axonal and dendritic surfaces but are rapidly endocytosed in dendrites, so they do not accumulate on the dendritic surface. When endocytosis of these proteins is prevented, they accumulate equally in both the dendritic and axonal membrane and lose their polarity. An alternative pathway selectively delivers other axonal proteins from the Golgi apparatus directly to the surface of the axon without their appearing on the dendritic surface[24,27]. The existence of this pathway implies that vesicles carrying these axonal proteins are unable to fuse with the dendritic membrane. The relative importance of these two different pathways for the polarization of axonal proteins is still unsettled.

Most polarized proteins are not uniformly distributed along axons and dendrites but are instead restricted to
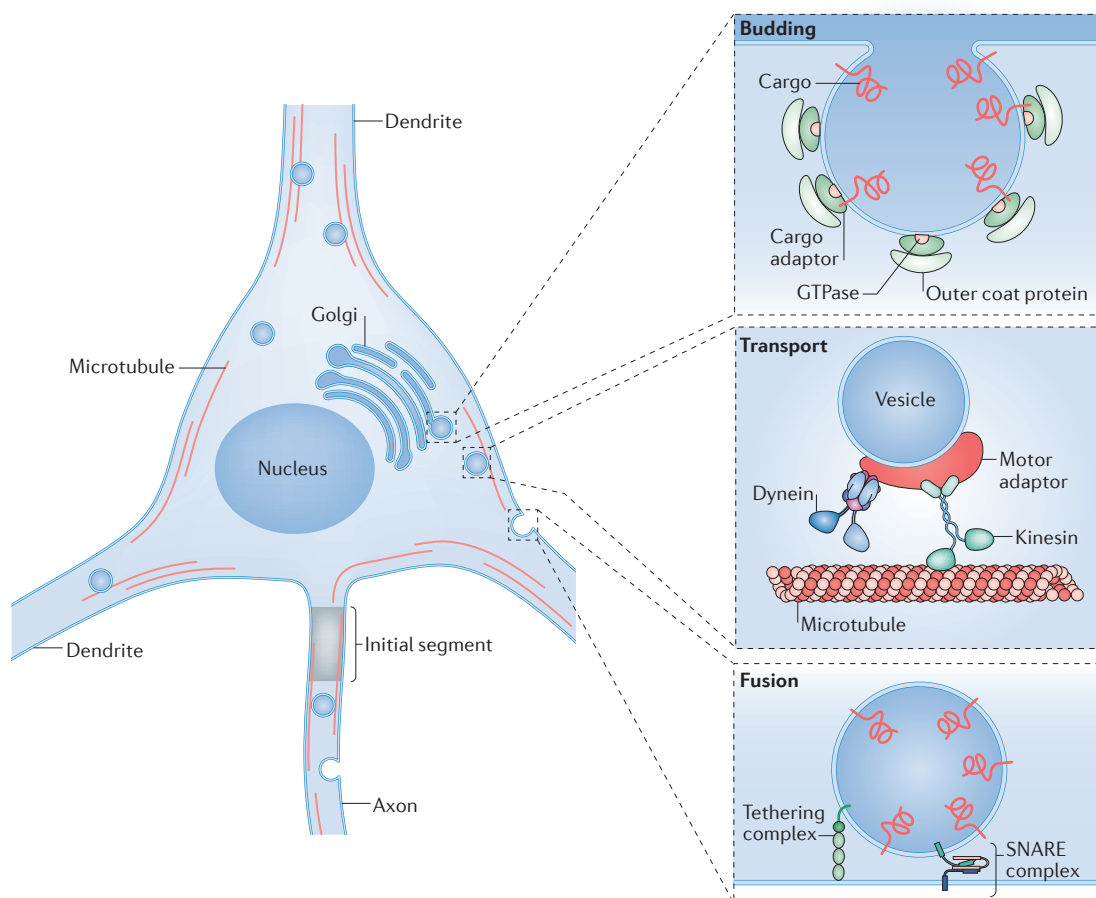
Figure 1 | **Neuronal membrane trafficking.** Neurons are composed of two regions: the somatodendritic domain and the axon. The axon initial segment (grey) marks the boundary between these domains. Axonal and dendritic membrane proteins are synthesized in the rough endoplasmic reticulum (not shown) and undergo post-translational modification in the Golgi complex. These organelles are restricted to the cell body and dendrites. The delivery of proteins from the Golgi complex to the plasma membrane involves budding, transport and fusion events; each of these processes is orchestrated by a complex set of trafficking proteins. Vesicle budding is initiated by the recruitment of a small GTPase from the cytosol to the membrane. In turn, this GTPase binds to and recruits cargo adaptors, which recognize transmembrane 'cargo' proteins containing sorting motifs and concentrate them in the forming bud. Cargo adaptors also recruit additional coat proteins from the cytosol that drive membrane curvature and fission. Long-range vesicle transport is mediated by kinesins and dyneins, which translocate along microtubules. Motors are bound to vesicles by adaptors that may also regulate their activity. Fusion is initiated by tethering factors that form a complex, which links the vesicle to its target membrane. Subsequent formation of a SNARE (*N*-ethylmaleimide-sensitive factor attachment protein receptor) complex drives fusion of the lipid bilayers.

specific subdomains, such as nodes of Ranvier or post-synaptic sites. The targeting of proteins to specialized subdomains is discussed in BOX 2.

After reaching the correct destination on the cell surface, to maintain neuronal polarity proteins must be prevented from moving to a different domain by lateral diffusion within the membrane. For most membrane proteins, this is accomplished by anchoring to submembranous scaffolding proteins. For example, postsynaptic receptors bind to various scaffolding proteins[28], and sodium channels are restricted to nodes of Ranvier through their interaction with ankyrin G, βIV spectrin and the underlying actin network[29]. For axonal and dendritic membrane proteins that are not anchored to the sub-membrane scaffold, a diffusion barrier in the initial segment prevents their intermixing (BOX 3).

Submembranous scaffolding proteins
Proteins that form complexes located just beneath the plasma membrane that bind to and concentrate specific sets of plasma membrane and cytosolic proteins.

Mechanisms for selective sorting

To reach the correct intracellular destination, membrane proteins must be packaged into the correct vesicles. This process is mediated by interactions between sorting signals in the cargo proteins and sorting adaptors that recognize these signals and concentrate the proteins in the appropriate vesicles. This observation leads to two questions. What are the sorting signals in axonal and dendritic membrane proteins? What is the machinery that recognizes these sorting signals?

Dendritic protein sorting. Our understanding of dendritic protein sorting began with the identification of sorting sequences in several dendritic proteins[30,31] (TABLE 2). When these sequences are deleted or mutated, the proteins lose their polarity and become uniformly

Table 1 | **A partial list of proteins that regulate polarized trafficking in neurons**

Protein class	Functions	Heterogeneity	Refs
Sorting			
Clathrin-binding adaptors	Recruit transmembrane cargo proteins into vesicle buds; bind to clathrin and other proteins that induce budding and fission	A heterogeneous group of proteins including heterotetrameric adaptors (five members), GGAs (three members) and epsin-related proteins	8,35
Phosphotyrosine-binding adaptors	Recruit transmembrane cargo proteins into vesicle buds	A group of proteins including ARH, Disabled 1, Disabled 2 and Numb	8,35
Retromer	A heteromultimer composed of VPS26, VPS29, VPS35 and two sorting nexins that regulates trafficking between the plasma membrane, endosomes and the Golgi apparatus	Sorting nexins are a single family of 25 members; there are 12 isoforms of VPS26	153
Galectins	Lectin-like proteins that concentrate glycosylated cargoes during vesicle formation	A single protein family with 10 members	17
Transport			
Kinesins	Microtubule motors composed of two kinesin subunits, sometimes also including light chains or accessory subunits, that (with few exceptions) transport cargoes towards the plus ends of microtubules	A single protein family with 45 members	13
Kinesin adaptors	Proteins proposed to mediate the targeting of kinesins to vesicular cargoes	• Proposed Kinesin-1 adaptors include JIP1, JIP3, HAP1, Milton–Miro, NESCA, calsyntenins, GRIP1, CRMP2, ARL8–SKIP and nesprin 4 • Proposed Kinesin-2 adaptors include MINT1 and fodrin • Proposed Kinesin-3 adaptors include liprin α1, DENN/MADD, centaurin α1 and MAGUKs	13
Dyneins	Microtubule motors composed of two heavy chains, two intermediate chains, two light intermediate chains and six light chains that move cargoes to the minus ends of microtubules	Multiple variants of each category of non-catalytic subunit	154
Dynein adaptors	Proteins proposed to regulate dynein activity or dynein recruitment to vesicles	A heterogeneous group of proteins including LIS1, NUDE, NUDEL, Bicaudal D family and RILP	154
Dynactin complex	A heteromultimeric complex that mediates cargo binding to dynein and some kinesins and regulates dynein activity	Components include p150^{GLUED}, p50 dynamitin, ARP1, ARP11, p62, p25, p27, p50, p24 and actin-capping protein	154
Fusion			
Tethering factors	Mediate the initial interaction between a vesicle and its target membrane	A heterogeneous group of proteins and protein complexes, including TRAPPIII, HOPS, CORVET, exocyst, GARP, p230, golgin 97 and EEA1	9
SNAREs	Mediate vesicle fusion by providing the mechanical force that forces membrane mixing and eventual fusion	A single protein family with 40 members	155
Multiple stages			
ARF GTPases	Small GTPases that regulate multiple aspects of membrane trafficking by recruiting specific effector proteins	A single protein family with 29 members	17
RAB GTPases	Small GTPases that regulate multiple aspects of membrane trafficking by recruiting different sets of effector proteins	A single protein family with more than 60 members	17

ARF, ADP ribosylation factor; ARH, autosomal recessive hypercholesterolaemia protein; ARL8, ADP-ribosylation factor-like protein 8; ARP, actin-related protein; CORVET, class C core vacuole/endosome tethering complex; CRMP2, collapsin response mediator protein 2; EEA1, early endosome antigen 1; GARP, glycoprotein A repetitions predominant; GGAs, Golgi-localized, gamma ear-containing ARF-binding proteins; GRIP1, glutamate receptor-interacting protein 1; HAP1, huntingtin-associated protein 1; HOPS, homotypic fusion and protein sorting complex; JIP, JUN-amino-terminal kinase-interacting protein 1; LIS1, lissencephaly 1; DENN/MADD, differentially expressed in normal versus neoplastic/mitogen-activated protein kinase-activating death domain; MAGUKs, membrane-associated guanylate kinases; MINT1, MUNC18-1-interacting protein 1; NESCA, new molecule containing SH3 at the carboxyl terminus; NUDE, nuclear distribution protein E; RAB, Ras-related in brain; RILP, Rab-interacting lysosomal protein; SKIP, SifA and kinesin interacting protein; SNAREs, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors; TRAPPIII, transport protein particle III complex; VPS, vacuolar protein sorting-associated protein.

distributed throughout the somatodendritic and axonal domains. In many instances, transplanting the relevant sequence to an unpolarized reporter protein is sufficient to cause the reporter to become dendritically polarized. In all cases examined to date, dendritic sorting signals lie within the cytoplasmic domain of single-pass transmembrane proteins or in a cytoplasmic loop of multi-pass transmembrane proteins.

The sorting signals in some dendritic proteins closely resemble the canonical motifs that mediate cargo sorting during clathrin-mediated endocytosis and selective delivery of proteins to the basolateral domain in polarized epithelia[21,31,32]. Canonical sorting motifs are recognized by heterotetrameric adaptor proteins[33–35]. There are five adaptor protein complexes (AP1–AP5), each composed of two large subunits (variously named),
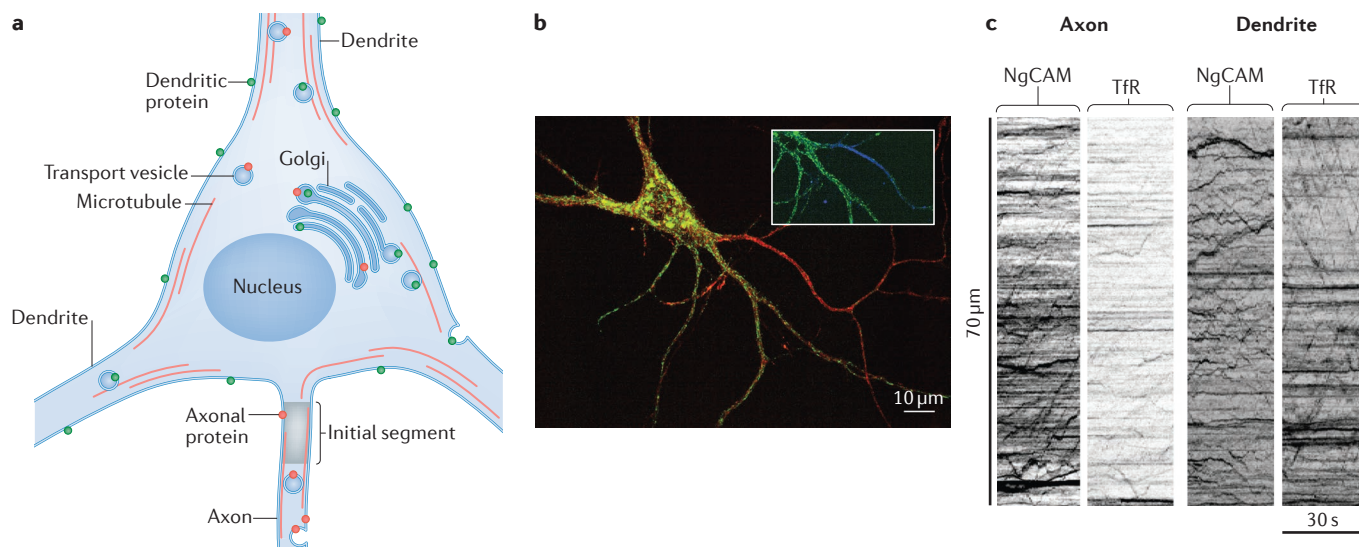
Figure 2 | **The trafficking of polarized proteins in neurons. a** | A drawing of a neuron illustrating the different trafficking patterns of axonal and dendritic proteins. As proteins exit the Golgi complex, dendritic proteins (green) and axonal proteins (red) are sorted into different vesicles, which undergo microtubule-based transport to reach the axon and dendrites. Vesicles containing dendritic proteins are transported bidirectionally in dendrites but do not enter the axon. Vesicles containing axonal proteins are also transported into dendrites, but their transport is biased towards the axon. **b** | Still images from a movie of a living cultured hippocampal neuron expressing an axonal membrane protein, NgCAM (red) and a dendritic membrane protein (green). The inset shows the initial segment, visualized with antibodies against neurofascin (blue). **c** | Kymographs from the cell shown in part **b** compare the movement of vesicles containing the axonal protein NgCAM and vesicles containing the dendritic protein TfR. NgCAM-containing vesicles move in the axon and the dendrite, whereas TfR-containing vesicles move in the dendrite and do not enter the axon. Kymographs plot the maximum intensity at each position along the axon (y axis) as a function of time (x axis). Moving vesicles are represented by the diagonal lines; lines with positive slope represent vesicle movement away from the cell body; lines with negative slope represent movement towards the cell body. Contrast was inverted in kymographs so that bright vesicles appear dark.

one medium-sized μ-subunit (μ1 to μ5) and one small σ-subunit (σ1 to σ5)[35,36]; several subunits exist as multiple isoforms[37,38]. Different family members regulate sorting at different sites in the cell. AP2 binds to the plasma membrane and the remaining adaptors bind to the Golgi complex and endosomal organelles. Heterotetrameric adaptors are recruited to the appropriate membrane by binding to specific phosphoinositides and by interacting with ARF GTPases[39,40].

Three 'canonical' sorting motifs have been identified, and their interaction with heterotetrameric adaptors is understood at the structural level[34,35] (TABLE 2). Two of these motifs are recognized directly by adaptor subunits: tyrosine-based Yxxφ motifs, which bind the μ-subunit of adaptor proteins, and acidic dileucine motifs ([DE]xxxL[LI]), which bind at the interface between the σ-subunit and one of the large subunits. A third canonical motif, [YF]xNPx[YF], is recognized by phosphotyrosine-binding (PTB) domains on a different set of adaptors, including the autosomal recessive hypercholesterolaemia protein (ARH; also known as LDLRAP1), Disabled homologue 1 and Disabled homologue 2. In this context, the PTB domains specifically recognize non-phosphorylated tyrosines. The PTB-containing adaptors in turn bind to the large subunit of heterotetrameric adaptors so that the two sets of adaptors work together to concentrate cargoes containing the [YF]xNPx[YF] motif and incorporate them into forming vesicles.

Subsequent work demonstrated a key role for heterotetrameric receptors in the sorting of dendritic proteins[21,41]. One study focused on two proteins, transferrin receptor (TfR) and Coxsackievirus and adenovirus receptor (CAR), both of which contain canonical Yxxφ motifs[21]. The authors identified a single tryptophan residue in the μ1A subunit of AP1 that, when mutated, abolishes binding to the Yxxφ motifs in these proteins. When the mutant μ1A subunit was overexpressed in cultured hippocampal neurons, it resulted in a dominant negative phenotype, causing a complete loss of polarity for both TfR and CAR[21]. Presumably, AP1 complexes containing the mutant μ1A subunit are unable to sort TfR and CAR into dendritically targeted vesicles, allowing them to leak into vesicles destined for the axon. Consistent with this hypothesis, in cells expressing the mutant μ1A subunit, TfR was found in vesicles that were transported into the axon, a situation that is never observed in normal cells[19,21].

It is likely that AP1 or other members of this family are also involved in the dendritic sorting of proteins containing other canonical motifs, although this has not yet been investigated. Low-density lipoprotein receptor (LDLR) and several other members of the LDLR family contain [YF]xNPx[YF] motifs and are dendritically polarized in cultured neurons[31,42]. Mutating the [YF]xNPx[YF] motif in LDLR causes it to lose its polarity[31]. In epithelial cells, AP1 and the PTB-binding protein ARH cooperate to mediate the basolateral sorting of LDLR[43], and neurons express ARH and other proteins that also recognize

Canonical sorting motifs
Sorting sequences that share a common three-dimensional structure that allows them to interact with identified binding pockets in heterotetrameric adaptor proteins.

REVIEWS

Box 2 | **Targeting proteins to subdomains of axons and dendrites**

Many polarized proteins are not uniformly distributed throughout the axonal or somatodendritic domains but are clustered at specific sites, such as nodes, paranodes, internodes and presynaptic terminals in the axon and postsynaptic sites on somata, shafts and spines in the somatodendritic domain. The mechanisms that underlie the maintenance of such microdomains are likely to be different for dendrites and axons. Molecular heterogeneity in the dendritic membrane could be generated by 'diffusion trapping', whereby dendritic proteins may not need to be delivered directly to specific subdomains but could simply diffuse within the plasma membrane and then bind to membrane scaffolds at postsynaptic sites[141]. The sequences that mediate postsynaptic localization are different from those that mediate sorting. For example, mutating residues in metabotropic glutamate receptors that bind to scaffolding proteins disrupts their synaptic localization but leaves their polarity unaffected[142]. Conversely, disrupting the dendritic sorting signal in excitatory amino acid transporter 3 (EAAT3) causes it to appear on both the axonal and dendritic surface; however, in dendrites, EAAT3 retains its postsynaptic localization[143]. In the diffusion trap model, proteins destined for different sites do not need to be packaged into separate vesicles.

By contrast, diffusion trapping is an inefficient method for generating membrane heterogeneity in axons. In large myelinated axons, where nodes can be up to 1 millimetre apart, nodes and paranodes account for as little as 0.1% and 1% of the axonal surface, respectively. Presynaptic sites likewise account for only a small fraction of the axonal surface and can be far from the cell body. Given this geometry and the presence of diffusion barriers created by the glia–axon junctions at paranodes, proteins destined for axonal subdomains must be delivered in the vicinity of their target sites. Thus, nodal proteins, intermodal proteins and presynaptic proteins are likely to be packaged into distinct sets of vesicles.

the LDLR sorting motif[44,45]. The situation for proteins containing the [DE]xxxL[LI] motif is less clear. The dendritic polarity of Shal potassium channels depends on a conserved sequence containing an acidic dileucine motif, which differs to some extent from the canonical sequence[46]; however, it is not clear whether the sorting of these channels is mediated by AP1. Neurons express several additional proteins that bind to the acidic dileucine motifs in Shal potassium channels and that could be involved in their sorting[47]. Some dileucine-containing proteins that are basolaterally sorted in epithelia, presumably by AP1, are not dendritically polarized in hippocampal neurons[32]. Expression of different σ1 isoforms in neurons and epithelial cells might be the reason why acidic dileucine motifs are differently interpreted in the two types of cells.

Although many dendritic proteins do not contain canonical sorting motifs, heterotetrameric adaptors may nonetheless participate in their sorting. Metabotropic glutamate receptor 1 (mGluR1) and the GluN2A and GluN2B subunits of NMDA-type glutamate receptors do not contain canonical sequences but bind to the μ1A subunit[21]. Expressing a mutant μ1A subunit disrupts the dendritic polarization of these glutamate receptors. AMPA receptors, which are also dendritically polarized, do not bind to AP1 but do bind to AP4 (REF. 41). The interaction of AP4 with AMPA receptors is mediated by transmembrane AMPA receptor regulatory proteins, which bind to the μ4 subunit. Knocking out the μ4 subunit causes AMPA receptors to lose their polarization and enter the axon, but has no effect on the distribution of metabotropic glutamate receptors and NMDA receptors[41]. Taken together, these results suggest that at least two different vesicle populations convey polarized

proteins to the dendritic membrane and that the budding of these vesicle populations is regulated by either AP1 or AP4. The other dendritic cargoes carried by these two vesicle populations and the trafficking proteins that regulate their transport and fusion remain to be determined.

Despite the exciting recent progress towards elucidating the dendritic sorting machinery, many important questions remain unanswered. It seems unlikely that sorting signals recognized by heterotetrameric proteins can fully account for the complexities of dendritic sorting. Of the approximately 4,800 predicted single-pass transmembrane proteins in the human genome, more than 1,000 have Yxxφ motifs[48]. A priori, it seems unlikely that all of these proteins are dendritically polarized. Indeed, several well-known proteins that contain such sequences, including amyloid precursor protein (APP), neural L1 cell adhesion molecule (L1CAM) and the neurotrophin receptors TRKA, TRKB and TRKC, are not dendritically polarized. Thus, additional factors must determine the circumstances that allow such motifs to be recognized by dendritic sorting adaptors. In addition, many of the sorting adaptors that have important roles in other cell types have yet to be investigated in the context of neuronal polarity[8] (TABLE 1).

Axonal protein sorting. Compared with the sorting of dendritic proteins, axonal protein sorting is not well understood. Sorting signals have been identified in only a few axonally polarized proteins, and so far no coherent picture has emerged. The targeting of L1CAM and its chick homologue NgCAM has received particular attention[24,27]. Signals in the ectodomain of NgCAM are sufficient to mediate its targeting to the axon, and the fibronectin repeats were identified as the most important factors for this process. In addition, sequences in the cytoplasmic domain of NgCAM were found that may be responsible for its targeting to the axon through a transcytotic pathway[49], and neuron-enriched endosomal protein 21 kDa (NEEP21) and EH domain-containing protein 1 (EHD1) were identified as proteins that are important for regulating this trafficking[50,51].

The targeting of members of the voltage-gated potassium channel K_V1 family has also been investigated in cultured hippocampal neurons[52]. *In situ*, these channels are concentrated at the axon initial segment and at juxtaparanodes within the axon; in neuronal cultures, where the neurons lack myelin, these channels are axonally polarized, although the degree of their polarization is modest. The T1 domain, a cytoplasmic region near the amino terminus that mediates tetramerization, is essential for axonal polarity[53,54]. An accessory channel subunit, $K_V\beta$, which binds to the T1 domain, also plays an important part in channel targeting[55]. It is unclear whether the axonal localization signals identified in potassium channels regulate sorting during vesicle budding or control retention within the axon after non-selective delivery. To date, no sorting receptors for axonal membrane proteins have been identified in vertebrate neurons, but recent work shows a role for AP-3 in axonal sorting in *Caenorhabditis elegans*[56].

Microtubule motor proteins in neurons

Once axonal and dendritic membrane proteins are sorted into distinct vesicles, selective transport delivers them to their proper targets. Before considering the mechanisms for selective transport, we first consider the properties of neuronal motor proteins and microtubules pertinent to their roles in vesicle transport.

Neuronal microtubule organization. In mature neurons, the organization of axonal and dendritic microtubules is quite different. In axons, 90% of microtubules are oriented with their plus ends away from the cell body[57–59]. Dendritic microtubules have a mixed polarity that varies with the distance from the cell body[59–61]. In proximal dendrites, about half of the microtubules are oriented in each direction; near dendritic tips, 90% of the microtubules are oriented with their plus end out (away from the cell body), as they are in axons. In axons, kinesins mediate anterograde transport and dynein mediates retrograde transport; in dendrites, both motors could mediate bidirectional transport. In developing neurons, microtubules in axons and dendrites have a similar orientation, with 90% of microtubules oriented with their plus end out[62,63].

In addition to these differences in microtubule organization, the microtubules in axons and dendrites contain a different population of microtubule-associated proteins and differ in the post-translational modifications of their tubulin subunits[64]. The microtubules in the initial segment also have unique biochemical properties, including a high affinity for certain microtubule tip-binding proteins; however, the significance of this remains unclear[23,65,66].

Exploring the behaviour of motor proteins in axons and dendrites. Individual neurons express 15–20 different kinesins that are thought to participate in plus-end-directed vesicle transport[67]. Like all other eukaryotic cells, neurons express only one cytoplasmic dynein heavy chain but express multiple isoforms of the non-catalytic subunits[68]. Two types of assays have been developed to analyse the translocation of motor proteins in living neurons. To date, these assays have been largely used to examine the properties of different kinesins, but in principle they might be adapted to analyse dynein movements as well.

One assay (the truncated motor assay) involves the expression of constitutively active kinesins (generated by deleting the autoinhibitory domain that normally prevents translocation until kinesins bind to their cargo)[23,69,70]. Such kinesins 'walk' spontaneously in the absence of cargo. Although individual kinesin dimers only move a few micrometres before dissociating from the microtubule[71–74], the density of microtubules in neurons is so high that constitutively active kinesins rapidly reattach and resume their movement. Thus, motor domains that move efficiently on axonal microtubules accumulate at axon tips[23,69,75]. Although proximal dendrites contain microtubules of mixed polarity, the preponderance of plus-end-out microtubules in distal dendrites enables kinesins to accumulate at dendritic tips as well. By expressing a series of constitutively active kinesin motor domains, it has been possible to systematically evaluate the accumulation patterns of most of the neuronal kinesins that are thought to participate in vesicle transport[75]. Interestingly, only two basic patterns were observed: some kinesins accumulate only in the axon, others accumulate in both dendrites and the axon. Conspicuously, kinesin motor domains that accumulate only at dendrite tips have not been identified.

A second strategy for investigating motor selectivity in intact neurons uses an inducible dimerization method to link constitutively active motors to 'artificial' cargoes[76,77]. Given that peroxisomes seldom undergo long-range transport, are roughly the size of transport vesicles and are easy to label with GFP constructs expressing peroxisomal targeting sequences, these organelles are convenient for this purpose[78]. This approach was used to compare the ability of 23 different kinesins to translocate peroxisomes in axons and dendrites[79]. All were able to move peroxisomes into axons, but only a subset mediated significant transport in dendrites. Consistent with the results of the truncated motor assay, no kinesins were found to preferentially move peroxisomes in dendrites. When KIF5B and KIF17 motor domains were linked to peroxisomes by drug-induced heterodimerization, the peroxisomes began to undergo highly processive, long-range anterograde axonal transport within minutes; only short movements were observed in dendrites[77]. These results are consistent with the results from the truncated motor assay[75], which showed that KIF5B and KIF17 were axon-preferring kinesins. After linking the dynein complex to peroxisomes via the dynein regulator Bicaudal D2, peroxisomes underwent long-range, bidirectional movements in dendrites but did not enter the axon[70,77]. This pattern of dynein-mediated transport is

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Table 2 | **Dendritic sorting signals**

Motif	Protein	Sorting sequence	Location	Refs
Yxxφ	TfR	YTRF	Cytoplasmic N-terminal tail	21,30, 31
	CAR	YNQV	Cytoplasmic C-terminal tail	21
	NiV-F	YSRL	Cytoplasmic C-terminal tail	156
	DNER	YEEF	Cytoplasmic C-terminal tail	157
	α7 nAChR	YIGF	Cytoplasmic loop M3–M4	158
[DE]XXXL[LI]	$K_V4.2$	FETQHHHLLCLEKTT	Cytoplasmic C-terminal tail	46
FxNPxY	LDLR	FDNPVY	Cytoplasmic C-terminal tail	31
Non-canonical	Neuroligin 1	VVLRTACPPDYTLAMRRSPDDVPLMTPNTITM	Cytoplasmic C-terminal tail	159
	Telencephalin	GEVFAIQLTS	Cytoplasmic C-terminal tail	124
	PIgR	RARHRRNVDRVSIGSYR	Cytoplasmic C-terminal tail	31
	EAAT3	KSYVNGGFAVDK	Cytoplasmic C-terminal tail	143

α7 nAChR, α7 nicotinic acetylcholine receptor; CAR, Coxsackievirus and adenovirus receptor; DNER, Delta/Notch-like epidermal growth factor-related receptor; EAAT3, excitatory amino acid transporter 3; $K_V4.2$, potassium channel 4.2; LDLR, low-density lipoprotein receptor; NiV-F, Nipah virus fusion glycoprotein; PIgR, polyimmunoglobulin receptor; TfR, transferrin receptor.

exactly what one would predict, based on the polarity orientation of neuronal microtubules. The absence of long-range kinesin-mediated dendritic transport in the peroxisome assay is somewhat unexpected, given that Kinesin-1 family members (KIF5A, KIF5B, KIF5C) are the principal motors mediating mitochondrial transport and that Kinesin-1 and KIF17 have been implicated in mediating the transport of dendritic channels and receptors[80–83]. When motors are tethered to vesicles by direct links to transmembrane proteins rather than through their normal adaptors, it may be that some regulatory elements are lost. Myosins that associate with vesicles, such as myosin Va, do not generate long-range movements when evaluated in the peroxisome assay[84,85].

Biochemical differences between axonal and dendritic microtubules. The results described above show that some kinesins translocate preferentially on axonal microtubules, which raises the following question: what are the differences between axonal and dendritic microtubules that could account for these translocation preferences? Although axonal and dendritic microtubules differ in many respects, recent attention has focused on the role of post-translational modifications of tubulin in the regulation of kinesin translocation[86–89]. Tubulin is subject to several post-translational modifications, including acetylation of a luminal lysine residue, glutamylation of several glutamate residues near the carboxyl terminus and removal of the C-terminal tyrosine (detyrosination) and the penultimate glutamate residue[90–94]. These modifications occur after tubulin subunits are assembled into microtubules. Several lines of evidence, although indirect, suggest that post-translational modifications of tubulin contribute to the selectivity of kinesin translocation. First, glutamylation and detyrosination occur near the C terminus of the protein (where motor proteins bind), and in cells some kinesins walk preferentially on microtubules that are enriched in these modifications[73,89,95,96]. Second, axonal microtubules are enriched in glutamylated, acetylated and detyrosinated tubulin, and several

kinesins accumulate preferentially at axonal tips in the truncated motor assay[88,97]. Finally, after paclitaxel treatment, which stabilizes microtubules and globally increases all three of these modifications, axon-selective kinesins lose their selectivity and accumulate at both dendritic and axonal tips[88]. In addition to tubulin modifications, pockets of GTP–tubulin that escape the hydrolysis that normally follows polymerization may also be involved in driving axon-selective transport[97,98].

Although these results suggest that post-translational modifications of tubulin can influence motor translocation, until recently it has been difficult to tease out how specific post-translational modifications influence kinesin motility. Using a strategy for chemically modifying microtubules following expression of mammalian tubulins in yeast, the Vale laboratory showed that glutamylation significantly enhances the motility of Kinesin-1 in *in vitro* assays[96]. Detyrosination also increases Kinesin-1 motility, although the effect is modest[89]. With these new strategies, it should soon be possible to determine how particular post-translational modifications of tubulin influence the motility of different kinesins and of dynein as well.

Selective microtubule-based transport
Axon-selective transport. Carriers containing axonally polarized membrane proteins are not excluded from dendrites, but their transport is biased towards the axon. Two factors could contribute to this bias. First, because of the mixed polarity of dendritic microtubules, more plus-end-out microtubules enter the axon than the average dendrite. Thus, one would expect some degree of axonal bias for transport mediated by any kinesin. Second, microtubules leading from the cell body into the axon may be biochemically modified so that axon-preferring kinesins walk on them preferentially[23,97]. If the latter idea is correct, then expressing kinesins with motor domain mutations that alter their selectivity in the truncated motor assay or the peroxisome assays should interfere with axon-selective vesicle transport.

Dendrite-selective transport. Vesicles carrying dendritically polarized proteins are largely excluded from the axon. When these vesicles approach the base of the axon, their transport is arrested[20,99]. After pausing, some vesicles are transported back to the cell body, whereas others fuse with the plasma membrane; few, if any, pass into the axon itself. Several different hypotheses have been put forward to explain this behaviour[100–102]. Although all of these hypotheses are provocative, none of these models appears to be consistent with all key observations.

One class of models proposes that the transport of vesicles containing dendritically polarized proteins is largely mediated by dynein[62,77,103]. Dynein could mediate bidirectional transport within dendrites (where microtubules have a mixed polarity), but vesicles transported by dyneins would not enter the axon (where microtubules are oriented with their plus end out). Based on simulations, it was shown that dynein-mediated transport would be sufficient to carry vesicles far out into the dendrites[77], even though the population of minus-end microtubules declines with the distance from the cell body[62]. Several lines of evidence, however, suggest that dendritic vesicles are not moved exclusively by dynein. First, interfering with transport mediated by Kinesin-1 or Kinesin-2 family members, using RNAi or dominant-negative strategies, reduces steady-state levels of dendritic receptors and channels[81,82,104], consistent with the idea that these kinesins mediate transport to the dendritic membrane. In addition, certain Kinesin-3 family members associate specifically with the vesicles that transport polarized proteins to dendrites, and increasing the activity of these motors causes dendritic vesicles to enter the axon[105]. Finally, selective dendritic transport is observed early in development[99,106], when the microtubules in both dendrites and axons are predominantly oriented plus end out[62,63]. Although dynein alone is unlikely to mediate dendritic transport, it clearly has a role in dendritic transport because expressing p50 dynamitin (also known as DCTN2), which inhibits dynein, causes dendritic proteins to enter the axon[77]. Knockdown of the dynein regulators NUDEL (also known as NDEL1) and lissencephaly 1 (LIS1) also causes dendritic vesicles to enter the axon; however, the explanation for this result is unclear[107].

Other reports suggest that myosin motors play a key part in dendrite-selective transport[20,84,101,108]. According to this model, dendritic vesicles, but not axonal vesicles, bind myosin Va. When dendritic vesicles approach the initial segment, they encounter actin filaments; myosin Va engages with these filaments, pulls the dendritic vesicles away from microtubules and moves them for a short distance back towards the soma, thereby blocking their entry into the axon proper. In support of this model, expression of dominant-negative myosin Va allows dendritic proteins, including mGluR1 and $K_V4.2$, to enter the axon, disrupting their polarization[101]. Furthermore, adding a myosin Va-binding domain to an unpolarized reporter protein results in its dendritic polarization. Further evaluation of this model will require a clearer picture of the organization of actin at the base of the axon and of its proximity to the microtubules that mediate vesicle transport. Furthermore, it is not known whether all of the elements required by this model arise early enough in development to account for dendrite-selective transport.

Another possibility is that selective dendritic transport depends not on a unique set of motors associated with dendritic vesicles but on differences in the regulation of motors when they bind to different cargo vesicles. Setou *et al.* present evidence that Kinesin-1 family members, the principal motors that mediate axonal vesicle transport, are also responsible for the dendrite-selective transport of AMPA receptors[109]. They propose that dendrite-selective transport depends on interactions with specific cargoes or cargo adaptors that 'steer' kinesins to the appropriate domain. Transport mediated by KIF17, which appears to be dedicated primarily to dendritic transport[81,82], may also represent an example of cargo steering, given that KIF17 behaves as an axon-selective motor in the peroxisome and truncated motor assays[75,77]. Although the idea of cargo steering is consistent with a substantial part of the available data, no structural mechanism has been proposed to explain how cargo interactions, which are mediated by the tail domain, might influence interactions with microtubules, which are mediated by the motor domain.

Finally, local signalling events near the base of the axon could increase dynein-based transport or inhibit kinesin-based transport, thereby preventing dendritic vesicles from entering the axon. In some instances, it has been shown that kinesins and dyneins bind to vesicles through a common adaptor and that post-translational modifications of this adaptor determine which of the two motors is active[110]. Transport can also be regulated by phosphorylation of kinesins themselves, either at sites within the motor domain that regulate the efficiency of translocation or at sites on auxiliary subunits that regulate cargo binding[111]. At present, it is not known whether these mechanisms for motor regulation play a part in dendrite-selective transport.

Selective vesicle fusion

The final step in the delivery of membrane is vesicle fusion with the plasma membrane. Little is known about where the vesicles that carry neuronal membrane protein fuse with the plasma membrane or about the tethers and SNAREs that regulate this fusion. Although it has been shown that vesicles carrying $Na_V1.6$ sodium channels fuse at a higher frequency in the initial segment than in the cell body[112], in general the importance of selective fusion for maintaining neuronal polarity has not been investigated. In polarized epithelia, mistargeting apical SNAREs to the basolateral domain causes apical proteins to be delivered there, indicating that the regulation of fusion is crucial for maintaining polarity[113,114]. All in all, remarkably little is known about the SNAREs and tethers found on the different populations of vesicles that transport polarized proteins or about the proteins that interact with them to deliver the cargoes to the somatodendritic and axonal membranes.

REVIEWS

Outlook

Understanding the trafficking of any vesicle of interest requires knowledge of the specific proteins that control its budding, transport and fusion. From this perspective, our understanding of the trafficking of polarized proteins in neurons is fragmentary, at best. We do not even know how many different vesicle populations mediate the transport of axonal and dendritic proteins. For each distinct vesicle population, there must be a different set of coat proteins and cargo adaptors that recognize different sorting signals in the cargo proteins, and each population may bind to different molecular motors, motor adaptors, RABs, tethers and SNAREs.

Although a systematic identification of the relevant vesicle populations and their trafficking proteins is an essential step, many other key questions need to be addressed. One question concerns the trafficking pathways used by polarized proteins. Dendritic proteins are directly delivered to the dendritic membrane; vesicles containing dendritic proteins never enter the axonal domain. By contrast, axonally polarized proteins use several delivery pathways, and their relative importance is unknown. Are most axonal proteins delivered directly to the axon, or do they reach the axon by transcytosis from the dendritic membrane?

A second series of questions concern the mechanisms underlying the selective transport of vesicles containing dendritic and axonal proteins. How are dendritic vesicles prevented from entering the axon? Does this process involve the coordinated action of dynein and kinesins? Are myosins involved? How are vesicles with axonal proteins directed preferentially towards the axon? Is the difference in the number of plus-end-out microtubules that enter axons and dendrites sufficient to account for axon-selective transport? Do kinesins recognize a subset of microtubules that lead from the cell body into the axon?

Additional questions concern the role of selective vesicle fusion in polarized trafficking. Are vesicles containing axonal proteins unable to fuse with the dendritic plasma membrane? Are proteins destined for specific microdomains, such as nodes of Ranvier and postsynaptic sites, transported in dedicated vesicles that fuse only at these locations?

The trafficking pathways for unpolarized proteins also merit attention. Do these proteins simply leak into the various vesicle populations that carry polarized cargoes, or is there a dedicated pathway for them? Given the vast difference in the size of axonal and dendritic arbors, what mechanism ensures that unpolarized proteins attain identical concentrations in both domains?

Finally, and perhaps most importantly, too little attention has been given to the links that must occur between the process of protein sorting, which determines the cargoes present in a vesicle and hence the identity of a vesicle, and the subsequent steps in the trafficking process that determine the fate of a given vesicle. To ensure that newly formed vesicles are transported to the appropriate domain and can deliver their cargoes to the specific region of plasma membrane that comprises the domain, these vesicles must also contain the correct SNAREs and tethers and must bind to the right motor adaptors and motor proteins. How are these other components recruited to each vesicle population? Do cargo adaptors have a role in the recruitment process? Are some trafficking proteins lost or recruited during the lifetime of a vesicle? How is this sequence of changes orchestrated and what role does it have in polarized trafficking?

Over the past decade, the question of how neuronal polarity develops has been the subject of intensive investigation[115–117], but the mechanisms that underlie the maintenance of polarity have received less attention. With the advent of modern imaging techniques[118], convenient methods for editing the mammalian genome[119,120] and better models for neuronal polarity in genetically tractable organisms (BOX 1), the time may be ripe for a concerted effort to address this fundamental problem.

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