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MINI-REVIEW

Protein trafficking mechanisms associated with neurite outgrowth and polarized sorting in neurons

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Abstract

Neuronal differentiation *in vitro* and *in vivo* involves coordinated changes in the cellular cytoskeleton and protein trafficking processes. I review here recent progress in our understanding of the membrane trafficking aspects of neurite outgrowth of neurons in culture and selective microtubule-based polarized sorting in fully polarized neurons, focusing on the involvement of some key molecules. Early neurite outgrowth appears to involve the protein trafficking machineries that are responsible for constitutive *trans*-Golgi network (TGN) to plasma membrane exocytosis, utilizing transport carrier generation mechanisms, SNARE proteins, Rab proteins and tethering mechanisms that are also found in non-neuronal cells. This vectorial TGN-plasma membrane traffic is directed towards several neurites, but can be switch to concentrate on the growth of a single axon. In a mature neuron, polarized targeting to the specific axonal and dendritic domains appears to involve selective microtubule-based mechanisms, utilizing motor proteins capable of distinguishing microtubule tracks to different destinations. The apparent gaps in our knowledge of these related protein transport processes will be highlighted.

Keywords: exocyst complex, microtubule, motor protein, neurite outgrowth, Rab, SNARE.

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Axonal growth and dendritic tree extension are key morphological features characterizing neuronal development. The process of neurite outgrowth defines neuronal shape, mediates neuronal path finding, and is essential for the eventual establishment of synaptic connections during development. Regulation of neurite outgrowth is also an important aspect of neuronal plasticity as well as neuronal regeneration from injuries or neuropathological conditions. Neurite outgrowth, as studied in cell culture models, can be initiated by the binding of a neurotrophic ligand to its receptor, resulting in cascades of signal transduction events that co-ordinate changes in cellular cytoskeletal components (actin and microtubules) and membrane trafficking events that execute the process. Depending on the model used, neurite outgrowth may (hippocampal neurons) or may not (PC12 cells) lead to a fully polarized neuron with established synaptic connections. A fully polarized neuron exhibits distinct axonal and soma-dendritic domains, with selective microtubule-based targeting of protein components. We are now beginning to get a glimpse of the underlying mechanistic complexity associated with the morphological events of neurite outgrowth and selective microtubule-based transport.

Early signal transduction events in neurite outgrowth

The growth of neurite processes from the cell body involves a massive increase in cell surface area (Futerman and Banker 1996). Membrane expansion at the growth cone requires the addition of new membranes to the growing processes. This is accomplished by the incorporation of *trans*-Golgi network (TGN)-derived exocytic vesicles into the plasma membrane. Exocytic membrane transport from the TGN and the plasma membrane occurs constitutively in all cells. In parallel, a stimuli-regulated exocytic process

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Abbreviations used: EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; KIF, kinesin superfamily; MAP kinase, mitogen-activated protein kinase; NGF, nerve growth factor; SNAP-25, synaptosome-associated protein of 25 kDa; SNARE, soluble *N*-ethylmaleimide sensitive factor attachment protein receptor; TfR, transferin receptor; TGN, *trans*-Golgi network; VAMP, vesicle-associated membrane protein

also exists in neuroendocrine cells. First of all, the initiation of neurite outgrowth would require specifically intensified and oriented exocytosis to a particular small region of the plasma membrane that would become the growing tip of a neurite. How is this achieved?

One important prerequisite may simply be an increase in the supply of materials, a phenomenon invariably tied to the sustained activation of the mitogen-activated protein kinase (MAP) kinase pathway. Nerve growth factor (NGF) treatment can induce differentiation of the rat phaoechromocytoma PC12 line into cells that acquire characteristics of sympathetic neurons (Greene and Tischler 1976). Neurite outgrowth is initiated after 24–48 h of NGF exposure. A morphologically differentiated phenotype characterized by the outgrowth of neurite extending for several cell bodies in length is obvious after 3–4 days. The process of neurite outgrowth in PC12 presumably results from converging signal transduction pathways originating from the binding of NGF to the receptor tyrosine kinase TrkA, culminating in the sustained activation of the MAP kinase pathway (for a review see Kaplan and Miller 2000). Whereas PC12 cells elaborate neurites only after 2 days of exposure to NGF, PC12 lines overexpressing TrkA can begin this process within hours (Hempstead *et al*. 1992; Leoni *et al*. 1999). Specific MAP kinase inhibitors, such as PD98059, absolutely inhibit neurite extension. Further, non-TrkA-binding factors such as epidermal growth factor (EGF) and TrkA-binding ligands such as NGF-mAb complexes that merely result in a transient MAP kinase activation are not neuritogenic. It has also become clear recently that one way NGF (but not EGF) may induce sustained MAP kinase activity is through its induction of persistent Rap1 signaling in endosomes (Wu *et al*. 2001).

Orientation of microtubules and the exocyst complex

The signaling step downstream from, or in parallel to, MAP kinase activation that leads to the initiation of neurite extension is not clear. One of the first cellular events that must take place, however, is the reorganization of microtubules towards the future direction of neurite outgrowth. It is apparent that microtubules and microtubule-associated motor proteins mediate delivery of vesicles or cargo containers from the TGN to the plasma membrane, in neuronal and non-neuronal cells alike (Goldstein and Yang 2000). A microtubule-associated factor may therefore be responsible for the precise targeting of vesicles or cargo containers to specific areas of the plasma membrane where outgrowth is to be initiated. One such factor, the exocyst complex, has recently come to light.

The eight-subunit exocyst complex, or the Sec6/8 complex, was first described in yeast but is evolutionally conserved (Hsu *et al*. 1996, 1998). In yeast, the exocyst is found concentrated at bud tips and mutations in the complex subunits result in cytoplasmic accumulation of secretory vesicles and defects in polarized growth (Finger *et al*. 1998). In mammals, the expression of the exocyst complex is ubiquitous, but is specifically enriched in the brain and in growth cones of cultured hippocampal neurons and PC12 cells (Hsu *et al*. 1996, 1998). A role for the exocyst complex in neuronal development was suggested by a delay in neuronal differentiation in mouse embryos with deletion of the *sec8* gene (Friedrich *et al*. 1997).

Recently, Vega and Hsu (2001) provided the first cellular evidence for the involvement of the exocyst complex in directing neurite outgrowth. The authors found that the exocyst is associated with microtubules in PC12 cells. In undifferentiated PC12 cells, it is found largely in the microtubule-organizing center (MTOC). However, in differentiated PC12 cells and cultured hippocampal neurons, the exocyst can be found extending into the growing neurite and colocalizing at the growth cone with markers like synaptotagmin. This redistribution of the exocyst complex upon neuronal differentiation is blocked by inhibition of the MAP kinase pathway. Further, overexpression of a mutant of the exocyst Sec10 subunit abolished neurite outgrowth and promoted cytosolic accumulation of secretory vesicles.

The evidence for the involvement of the exocyst complex in neurite outgrowth is compelling, but what exactly does the complex do? Cellular studies in yeast and non-neuronal mammalian cells are consistent with the role of the exocyst in determining the plasma membrane sites for the fusion of TGN-derived vesicles. It performs the job of a tethering factor, functioning to guide the initial interaction of vesicles with specific regions of the plasma membrane – a step preceding actual vesicle docking and fusion. The exact detail of how this 'tethering' process is performed is still lacking. The large number of subunits in the complex suggests that the exocyst is capable of performing several related jobs. The finding from Hsu's laboratory is important as it suggests that the exocyst complex serves as a link between microtubule organization and vesicle targeting, functioning downstream of the MAP kinase pathway. One could entertain the speculation that the exocyst complex may function, in the first instance, as a remodeling factor for microtubules. Its conformation and localization with respect to the MTOC may be subjected to regulation by the phosphorylation status of one or more of its subunits. The upstream signal from the MAP kinase pathway may modulate this localization, thus initiating the first step in the reorganization of microtubule-dependent vesicular traffic.

There are many questions that remain pertaining to the first step of neurite outgrowth. The most outstanding concerns the selection of the initial site on the plasma membrane for the whole process to begin. During the development of a neuron *in vivo*, this site selection should be oriented by guidance factors in the environment, the

extracellular signals perhaps resulting in the establishment of a focal point for assembly of actin and microtubule-associated factors. In cultured cells *in vitro*, there may well be a similar process of site selection in the form of the first points of focal contact with the growth substratum. With sustained activation of MAP kinase by NGF, the exocyst complex may redistribute to these points, stabilized by possible interaction between subunits of this complex and components of the focal complex. The recent discovery of a direct interaction between Sec3p of the yeast exocyst complex and the GTP-bound form of the actin modulating Rho1 GTPase (Guo *et al*. 2001) as well as the interaction of the mammalian exocyst complex with the brain-enriched RalA GTPase (Brymora *et al*. 2001) lends further support to the notion that the exocyst has multiple interacting partners and may serve to integrate the various processes involved in the initiation of neurite outgrowth.

The origin of membranes for neurite outgrowth

Materials needed to elongate the extending neurites are derived from the exocytic pathway. The membranes being added to the growing tips are believed to be derived from exocytosis from the TGN, but in what form and by what mechanism?

There are potentially three pathways of TGN-plasma membrane transport in PC12 cells, a constitutive pathway and two regulated pathways. Of the latter, one generates the large dense-core granules for the storage and release of catecholamines, and the other takes the form of synaptic-like microvesicles responsible for the release of acetylcholine (Shafer and Atchison 1991). Although all three may be involved to varying extends in neurite outgrowth, the regulated pathways appear to be dispensable. Working with TrkA overexpressing PC12 subclones that are totally defective in components of the regulated pathways, Valtorta and co-workers (Leoni *et al*. 1999) showed that these cells contain no known molecular components necessary for regulated exocytosis (including the SNAREs syntaxin 1, VAMP1, VAMP2, SNAP-25 and the regulated pathway specific Rab3A) and are consequentially lacking dense core granules. Neurite outgrowth was, nonetheless, unimpaired in these cells. In addition, their TrkA overexpression enhanced neurite outgrowth in response to NGF, but did not rescue the neurosecretory defect. In these cells at least, neurite outgrowth appears to be exclusively mediated by the constitutive TGN-plasma membrane transport.

If TGN-derived constitutive exocytosis is the source for membrane addition to the growing tip of the neurite, the nature of the transport carrier and the mechanism for their formation may be similar to those described in non-neuronal cells (or undifferentiated PC12 cells). Recent studies using live-cell imaging in non-neuronal cells have revealed that constitutive TGN-plasma membrane transport is mediated by large transport intermediates or cargo carriers (Hirschberg *et al*. 1998; Toomre *et al*. 1999; Polishchuk *et al*. 2000). Rather than the classical picture of transport 'vesicles' averaging between 50 nm-100 nm in diameter, the transport intermediates appear to be pleiomorphic tubular-saccular structures with diameters ranging from 0.3 to 1.7 μm. Differentially tagged apical and basolateral cargo proteins segregate progressively into different domains at the TGN, and exit in separate transport containers, even when expressed in non-polarized PtK_2 cells (Keller *et al*. 2001). The step of transport container generation can be slowed down in HeLa cells expressing a kinase inactive form of protein kinase D (PKD-K618N) at low growth temperatures. Apparently as a result of defective fission of transport carriers from the TGN, these containers take the form of elongated tubules that contain plasma membrane-bound cargo (Liljedahl *et al*. 2001).

Visualization of axonal and dendritic transport using differentiated neurons in culture has also revealed the existence of large carriers of varying shapes and sizes. Dotti and co-workers compared the axonal transport of different cargo molecules tagged with fluorescent proteins of different color (Kaether *et al*. 2000). Yellow fluorescent protein-tagged amyloid precursor protein (APP-YFP) appeared to be transported in elongated tubules (as long as 10 μm) that move very fast. EGFP-tagged synaptophysin transporting structures, on the other hand, are more vesicular in shape and move four times slower. The situation is clearly more complicated in polarized neurons as different cargo carriers are apparently required for the distribution of axonal or dendritic specific proteins.

With respect to neurite outgrowth in PC12 cells, cargo carriers revealed by EGFP-tagged TI-VAMP (a R-SNARE, see below) have been observed in the growing neurites (Martinez-Arca *et al*. 2000). Although there is no absolute demonstration that these structures are derived directly from the TGN, the dynamics of TI-VAMP containing vesicles seem to closely parallel the growth of neurites and the marker itself eventually reaches the plasma membrane. A detailed examination the dynamics of these carriers as they exit the TGN and a thorough analysis of their contents with markers differentially labeled with different fluorescent proteins would be needed to assess if the TGN-plasma membrane transport supporting neurite outgrowth has any unique or distinctive features. The carriers traveling to the outgrowing neurite of PC12 or hippocampal neurons might be different from those going to random destinations on the plasma membrane in an undifferentiated cell. These may, for example, travel at an enhanced velocity due to the acquisition of neuronal specific motor proteins.

SNARE proteins in neurite outgrowth

Soluble *N*-ethlymaleimide sensitive factor attachment protein receptors (SNARE) mediated docking and fusion is a

common theme in most vesicular transport process, and neurite outgrowth is no exception. However, there is much controversy as to which SNAREs are involved. The involvement of the target (t)-SNARE synaptosome-associated protein of 25 kDa (SNAP-25) was demonstrated as early as 1993 using an antisense oligonucleotide approach (Osen-Sand *et al*. 1993). Contrastingly, inhibition of syntaxin 1A with an antisense approach enhanced neurite sprouting and increased neurite length in dorsal root ganglion and retinal ganglion neurons (Yamaguchi *et al*. 1996). Other early evidence for the involvement of t-SNAREs includes experiments using botulinum neurotoxin type C (which cleaves syntaxins 1, 2 and 3 as well as SNAP-25). Botulinum neurotoxin C1 induces rapid growth cone collapse and impaired neurite outgrowth in chick dorsal root ganglion and retina explants (Igarashi *et al*. 1996). In support of the above, recent reports showed that the overexpression of SNAP-25 in PC12 cells increases the number of neurite per cell (Shirasu *et al*. 2000) or neurite length (Zhou *et al*. 2000), whereas overexpression of syntaxin 1A either has no effect (Shirasu *et al*. 2000) or inhibits neurite extension (Zhou *et al*. 2000).

On the other hand, the demonstration of vesicle (v)-SNARE involvement was not so straightforward in the beginning. Experiments with tetanus neurotoxin [which cleaves vesicle-associated membrane protein 1 (VAMP1) and VAMP2 and blocks neurotransmitter release] had no appreciable effects on axon growth (Ahnert-Hilger *et al*. 1996; Osen-Sand *et al*. 1996). Neurite outgrowth is however, not the only trafficking process that is resistant to tetanus neurotoxin treatment. The resistance of transport to the apical plasma membrane to this treatment actually resulted in the initial interpretation that exocytosis from the apical plasma membrane occurs via a SNARE-independent mechanism (Ikonen *et al.* 1995).

A VAMP1-like v-SNARE, or TI-VAMP, that is insensitive to tetanus neurotoxin and several botulinum toxins was subsequently cloned (Galli *et al.* 1998), and is found in a SNARE complex with SNAP-25 vesicles concentrated in the apical domain of the epithelial CaCo-2 cells. Importantly, TI-VAMP was also found to be concentrated in the leading edge of axonal and dendritic processes in cultured hippocampal neurons (Coco *et al*. 1999). Galli and co-workers recently presented further evidence that TI-VAMP is probably the v-SNARE involved in neurite outgrowth. As mentioned above, the dynamics of EGFP-TI-VAMP vesicles parallel the growth of neurites. Further, expression of the NH_2-domain of TI-VAMP that inhibits the association of the molecule with SNAP-25 also inhibits neurite outgrowth as potently as Botulinum neurotoxin E. On the other hand, overexpression of an NH_2-domain deletion mutant of TI-VAMP enhances SNARE complex formation and stimulates neurite outgrowth.

Experiments from the laboratory of Rothman and colleagues suggest that a typical SNARE complex consists of a four-helix bundle, with the helices contributed by the SNARE domains of three Q-SNAREs and one R-SNARE (Fukuda *et al*. 2000; Parlati *et al*. 2000) (The Q and R in this nomenclature refers to a critically conserved residue of either glutamine or arginine at the center, or the so called ionic 'zero' layer, of the four-helix bundle (Fasshauer *et al*. 1998)). This 3Q + 1R rule seems to hold true as well for new SNARE complexes described for the late endosome (Antonin *et al*. 2000) and ER-Golgi transport (Xu *et al*. 2000). The data summarized above implicates a Q-SNARE SNAP-25 that could contribute two SNARE domains and TI-VAMP, a R-SNARE. SNAP-25, however, appears to not be absolutely required, as neurite outgrowth proceeds in the PC12 mutant lines described above (Leoni *et al*. 1999) that lack detectable SNAP-25. Other candidate Q-SNAREs that may be used include syntaxin 3 and SNAP-23, both of which are found in a SNARE complex with TI-VAMP in epithelial cells (Galli *et al*. 1998). Experimental evidence for their involvement is still lacking.

Another Q-SNARE that has been reported to be involved in neurite outgowth is the endosomal syntaxin 12/13 (Advani *et al*. 1998; Tang *et al*. 1998), previously reported to function in recycling from the early endosome to the plasma membrane (Prekeris *et al*. 1998). Syntaxin 12/13 transcripts and protein are particularly enriched in brain tissues (Tang *et al*. 1998; Tang *et al.*, unpublished). Catsicas and colleagues have recently shown that overexpression of syntaxin 13 enhances neurite outgrowth in NGF-stimulated PC12 cells (Hirling *et al*. 2000). It is not clear how an endosomal SNARE may affect neurite outgrowth, although it points towards the possibility that recycling from endosomal compartments may also contribute to membrane addition to the growth tip.

Rab proteins in neurite outgrowth

Early evidence implicating the involvement of a Rab protein in neurite outgrowth came from analysis in cultured hippocampal neurons using an antisense approach. Antisense oligonucleotide against Rab8 inhibit neurite outgrowth, while antisense oligonucleotides of Rab3A have no effect (Huber *et al*. 1995). The Rab8 antisense oligonucleotide treated cells also exhibit a dramatic reduction in the number of vesicles undergoing anterograde transport (Huber *et al*. 1995). In fact, antisense oligonucleotides of the Rab guanine nucleotide dissociation inhibitor (GDI) also inhibit axonal outgrowth of hippocampal neurons (D'Adamo *et al*. 1998) and neurite outgrowth in the regulated secretory pathway defective, Rab3A-negative PC12 cells (Leoni *et al*. 1999). Unimpaired neurite outgrowth in Rab3A-negative cells is in good agreement with earlier data, but the effect of RabGDI antisense oligonucleotides does point towards an absolute requirement for a Rab in neurite outgrowth.

The accumulated data so far therefore implicate only Rab8 in the early stages of neurite outgrowth. There are of

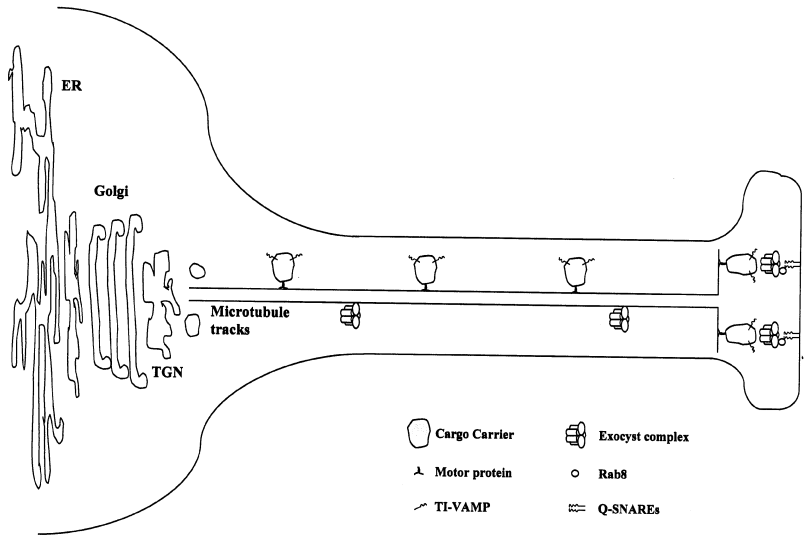

Fig. 1 Components of the cellular membrane transport machinery that had been shown to be involved in neurite outgrowth.

course several other SNAREs (including Rab10, Rab12 and Rab13) localized to the vicinity of the TGN whose role in neurite outgrowth is not yet known. Figure 1 is a schematic representation of the major players involve in the early process of neurite outgrowth. Two further points are noteworthy. First, Rab8, together with Rab10, are the mammalian proteins with the highest homology to yeast Sec4p (Chen *et al*. 1993; the master controller of yeast post-Golgi traffic. Sec4p has been shown to interact directly with the yeast exocyst complex (Guo *et al*. 1999), providing a hint of the characters involved in the regulation of directional transport to the growth tip. However, the mammalian exocyst complex has yet to be shown to be interacting with any Rab protein. Another interesting point to note here is that Rab8 is eventually excluded from axons and is only found in dendrites when neurons becomes fully polarized.

The advent of polarized sorting

The mature and fully differentiated neuron epitomizes membrane polarity, with the axon and the somatodendritic domains maintaining separate, unique structures and protein compositions that are absolutely necessary for the function of the neuron to both receive and transmit signals. Using cultured hippocampal neurons as a model to study neuronal development, Dotti and co-workers recognized five distinct phases in neuronal growth upon plating of hippocampal cells. (for a review see Bradke and Dotti 2000a,b). Neurite outgrowth occurs shortly after plating. Within 24 h, one of the neurites starts to elongate very rapidly while the growth of other neurites appears to be temporarily halted. This fast-growing neurite becomes the future axon, and the others will constitute the dendrites when the neuron is fully mature (with the formation of synaptic junctions in about 10 days). Morphological polarization therefore has a rather early onset.

What are the events that lead towards this morphological polarization? A general picture linking regulated changes in the microtubules and the actin cytoskeleton has begun to appear (discussed in Bradke and Dotti 2000a,b). In brief, the event begins with changes in actin dynamics, mediated by the Rho family proteins (the small GTPases, Rho, Rac, Cdc42 and their associated regulators) (Ridley 2001). These changes lead to increased actin filament turnover and destabilize the actin cytoskeleton. The resultant reduction of steric hindrance allows increased vectorial cytoplasmic flow into the neurite growth cone (Bradke and Dotti 1997). Furthermore, it may facilitate polymerization and distal extension of microtubules, thus providing the trafficking railways for enhanced membrane traffic flow into this particular neurite.

Membrane traffic flow at this stage may still be non-polar, at least in terms of cargo content. This is evidenced by the fact that proteins restricted only to dendrites in mature neurons such as the R1 subunit of glutamate receptor, are also delivered to the growing axon. Extending their studies into cultured hippocampal neurons, Galli's group showed that the expression of the N-terminal domain of TI-VAMP inhibits the outgrowth of both dendrites and axons, while expression of the N-terminal deletion mutant has the opposite effect (Martinez-Arca *et al*. 2001). Therefore, the advent of apparent morphological polarization is definitely associated with reorganization of the TGN-plasma membrane trafficking process – from several different directions for multiple neurites to a single direction of the growing axon. However, the non-selective nature of the TGN-plasma membrane traffic may not have change yet. If this is true, polarization of membrane transport, in this case, may lag behind morphological polarization. The implication would be that the former is not a direct cause of the latter.

Selective transport in polarized sorting

There are two major ways whereby the distinctive identity of

axons and dendrites can be maintained in a mature, polarized neuron. The first would involve some kind of mechanism for the physical partitioning of cytoplasmic contents, akin to the role played by tight junctions in polarized epithelial cells. Evidence for such a mechanism is accumulating. In the case of the initial segment of the axon, for example, there exists a general diffusion barrier resulting from dense packaging of membrane proteins via their interactions with specialized cytoskeletal components (known as the initial segment fence Winckler *et al*. 1999; Winckler and Mellman 1999).

More relevant to our discussion would be the mechanisms for selective sorting and separate transport or targeting of axonal and dendritic cargo. As mentioned above, it has been recently demonstrated that different cargo proteins can segregate into different domains at the TGN and be subsequently incorporated into different transport carriers, even in a non-polarized cell (Keller *et al*. 2001). There are known mechanistic bases for this type of segregation in both polarized and non-polarized cells, such as selective interaction of a sorting signal with a coat protein or recruitment into lipid rafts. Determinants for separate axonal versus dendritic transport or targeting must occur downstream to this more general segregation process. There are two possibilities here: selective microtubule-based transport with 'smart' motor proteins that can distinguish different microtubules that lead to different destinations, or selective fusion of carriers with specific plasma membrane domains. Both mechanisms may be utilized for any given carrier.

Although selective microtubule-based transport has long been speculated to transport cargo to specific domains of a neuron, a direct demonstration of such a process is possible only with live cell imaging techniques. Bankers and colleagues expressed EGFP-tagged transferin receptor (TfR), a dendritic protein and neuron-glia cell adhesion molecule (NgCAM) and found that TfR-EGFP containing carriers are preferentially transported into dendrites and excluded from axons. NgCAM-EGFP containing carriers, on the other hand, were transported into both dendrite and neurons. The transport of both TfR and NgCAM carriers was inhibited by the microtubule-disrupting agent, nocodazole, indicating that they were microtubule-based (Burack *et al*. 2000). Although the generality of the process remains to be established by the examination of more markers, it appears that selective dendritic transport of TfR is sufficient to account for its polarized distribution. For the case of NgCAM, specific downstream fusion events of its carriers with the axonal but not the dendritic plasma membrane or its selective retention upon entry into the axon would be required to achieve its polarized distribution.

The experiments of Banker and colleagues also provide some clues to the problem discussed above – that of the temporal relationship between morphological polarization and polarization of membrane transport. Their results clearly show both dendritic and axonal marker proteins are significantly polarized by developmental stage 3, and selective dendritic transport of EGFP-TfR is also evident at this stage (Silverman *et al*. 2001). With these timelines, it may be safe to assume that the onset of polarization of membrane transport does not really lag behind the morphological appearance of the axon.

Finally, how is selective microtubule-based transport achieved? In other words, how does a carrier acquire the specific 'smart' motor protein that would mediate trafficking along selected microtubule tracks? A putative answer to this question is provided by the recent demonstration that the dendrite-specific motor protein, KIF17, mediates dendritic transport of NMDA receptor containing carriers via specific and direct interaction with a PDZ-domain of mLin-10 (Mint1/X11) (Setou *et al*. 2000). mLin-10 is a component of the complex enriched in NMDA receptor containing carriers that contain mLin-2 (CASK), Mlin-7 (MALS/Velis) and the NR2B subunit of the NMDA receptor. The *C. elegans* LIN-2, LIN-7 and LIN-10 complex functions in the polarized targeting of Let-23, the worm equivalent of mammalian epidermal growth factor (EGF) receptor (Kaech *et al*. 1998). The mammalian homologues of these proteins are enriched in brain, and can be found at the Golgi region (Borg *et al*. 1999; Whitfield *et al*. 1999). The interaction between KIF17 and mLin10 is specific, as other kinesin family proteins do not appear to interact with mLin-10. An *in vitro* microtubule gliding assay also revealed that NR2B containing membrane vesicles purified using anti-KIF17 antibody can be mobilized in a plus end-directed manner by recombinant KIF17, but not by a mutant KIF17 lacking the mLin-10 binding tail. Thus, it appears that the cargo itself (NR2B) can select the 'smart' motor protein (KIF17) via the mLin-2, − 7 and − 10 complex. It would be interesting to see if other dendritically targeted glutamate receptors exhibit such a motor protein selection mechanism.

Future perspectives

The results and discoveries described above provide us with a very rough picture of the molecular events pertaining to the protein trafficking processes associated with neurite outgrowth and neuronal polarization. Much remains to be learned. First of all, precise knowledge of the players involved in the TGN-plasma membrane transport responsible for the initial sprouting of is still lacking, and there is still no good explanation as to how the sprout site is determined. Although it is clear now that components of the regulated secretory pathway are not involved, much remains unknown about the generation of membrane carriers from the TGN, especially how the composition of these carriers changes with time. The precise identity of the Q-SNAREs responsible for plasma membrane fusion is still not known. Although the involvement of Rab GTPase (Rab8) and a tethering complex (the exocyst complex) has been implicated,

their mode of function is not at all clear (unlike other transport events where Rab proteins have been shown to co-ordinate membrane attachment by interactions with tethers and SNAREs).

Furthermore, the identity of cellular components that could serve to link the changes in actin and microtubule cytoskeleton to that of membrane transport is also lacking. Without knowing what these molecules are, it would be difficult to come to a mechanistic understanding of, for example, the reorganization of traffic flow that results in the onset and rapid growth of the young axon.

Although microtubule-based polarized carrier transport has been demonstrated, understanding of the mechanistic details will take some doing. Each population of carriers may associate with more than one type of motor proteins. The analysis can be further complicated by the progressive changes in the relative abundance of all the above factors with time as differentiation progresses. One should also bear in mind that current neuronal culture models are far from being able to fully recapitulate events *in vivo*. Neurons within the developing brain are subjected to a myriad of extracellular factors and cues from glial cells that would influence its growth both positively and negatively. The task confronting the field is daunting, but there is cause for optimism. Constantly improving new technologies such as live cell imaging would refine analysis. Importantly, genomic and proteomic tools that has became available with the sequencing of the mammalian genomes will definitely aid future investigations.

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