

Minireview

Regulators of kinesin involved in polarized trafficking and axon outgrowth

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Abstract

Proteins such as UNC-76 that associate with kinesin motors are important in directing neurite extension. A small *Caenorhabditis elegans* coiled-coil protein, UNC-69, has now been shown to interact with UNC-76 and to be involved in axonal (but not dendritic) transport and outgrowth, as well as synapse formation.

Vesicle trafficking and neurite outgrowth

Neurons adopt a unique morphology that is different from other cell types in that they extend dendrites and axons from their cell bodies. The dendrites and axons serve two main purposes: to connect distant cells by extending projections between the pre- and postsynaptic targets, and to direct electric signal flow within neurons. Both functions require the proper outgrowth and polarization of neurites, which develop into dendrites and axons.

One of the common mechanisms underlying the outgrowth and polarization of developing neurites is the targeted trafficking of vesicles to the growing neurite tips [1]. One source of materials needed for this growth is thought to be exocytic vesicles derived from the *trans*-Golgi network. These vesicles are transported to sites of growth, where they fuse with the plasma membrane to deliver polarized membrane proteins to the growing neurite. In support of this idea, live imaging of amyloid precursor protein (APP) and synaptophysin, two axonal targeted proteins, tagged with fluorescent proteins in cultured hippocampal neurons,

revealed fluorescence on vesicles moving in an anterograde direction (away from the cell body) in extending axons [2]. Interestingly, whereas synaptophysin was seen more on vesicular structures, APP was found in elongated tubules [2], suggesting that different components are shuttled to the growing neurites on distinct types of transport containers.

How do neurons control all these transport events? If the directional trafficking of vesicular cargos is essential for neurite extension and polarization, what are the molecules regulating the trafficking and fusion processes? Extensive work in the past decade suggests that at least three cellular processes are likely to contribute to neurite polarization and development: microtubule reorientation, motor-dependent cargo transport, and membrane fusion mediated by soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) [1]. The mechanism underlying microtubule reorientation is not yet fully understood, but evidence suggests that the eight-subunit exocyst complex Sec6/8 might have a role in directing microtubule extension and vesicle targeting [3]. The involvement of SNAREs (SNAP-25, syntaxin,

and TI-VAMP) in neurite outgrowth is well documented and nicely reviewed [1,4-10,11]. Here, we focus mainly on the motor-dependent transport in developing neurites and address how this process affects neurite polarization and extension. New work by Su and Tharin *et al.* in this issue of *Journal of Biology* [12] has identified a new potential regulator of the process.

Kinesins in neurite polarization and outgrowth

The kinesins are a large family of microtubule-associated motor proteins that have crucial roles in intracellular trafficking, cell division and signal transduction [13,14]. Like other motors, kinesins contain a conserved 'head' motor domain that hydrolyzes ATP and walks along microtubules, and a divergent 'tail' domain that is thought to bind cargos [15] (Figure 1). Recent work has highlighted the important roles of kinesin in neurite polarization and outgrowth. First, in hippocampal cultures, kinesin-1 specifically accumulates at the tip of neurites that are fated to become axons, and the initiation of an axon extension stabilizes the axonal localization of kinesin-1 [16]. Second, during the extension of dendrites and axons, localization of dendritic proteins (such as the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl D-aspartate (NMDA) glutamate receptor complexes [17,18]) and axonal proteins (such as growth associated protein 43 (GAP-43) and presynaptic

components [18]) is dependent on specific kinesin motors. Knockdown of the kinesin motors using antisense oligonucleotides not only disrupts dendritic or axonal localization of these proteins, but also suppresses neurite outgrowth, presumably by blocking kinesin-dependent vesicle transport [19,20]. This strongly indicates that as molecular motors, kinesins are essential for the trafficking of cargos necessary for both neurite polarization and extension.

One key question that remains unanswered is how kinesins achieve trafficking specificity in developing neurons, as this forms the basis for neurite polarization and differential outgrowth. In principle, two possible mechanisms could be used by the cell to solve the problem. First, kinesins could be selectively trafficked to dendritic and axonal domains and cargos could be transported to these domains by interacting with the motors. Alternatively, motors could have no inherent preference for dendritic or axonal trafficking; instead, the binding of dendritic or axonal cargos to motors would direct them to specific domains. Currently, there is evidence in support of both these possibilities [17,18], indicating that the actual situation *in vivo* could be complicated. Therefore, defining cargo-kinesin motor interactions and establishing their roles in domain-specific trafficking will be critical to elucidating the molecular mechanisms underlying the establishment of mature neuronal structure. The recent work by Su and Tharin *et al.* [12] on the *C. elegans* protein UNC-69 provides new insights into both the molecular mechanisms and the complexity of axonal trafficking.

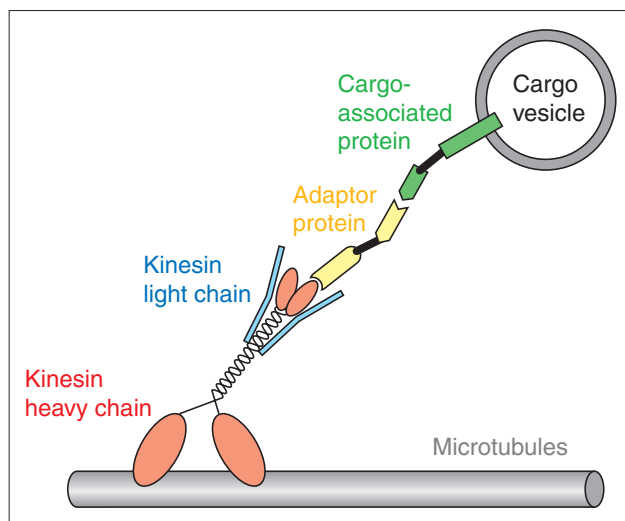


Figure 1
Schematic diagram of a kinesin-cargo complex. The cargo vesicles are thought to be associated with kinesins through the interactions between cargo-associated proteins (green) and adaptor proteins (yellow) as well as those between adaptors and kinesins (red). The heads of kinesin motors, which contact the microtubule, hydrolyze ATP and perform microtubule walking.

UNC-69, a coiled-coil protein important for polarized trafficking and axonal outgrowth

Using a genetic approach in *C. elegans*, Su and Tharin *et al.* [12] have not only identified a novel component of a protein complex that has crucial roles in axonal targeting, but have also uncovered potential roles for the complex in synapse assembly. UNC-69 is a small, evolutionarily conserved coiled-coil protein [12]. In *unc-69* mutants several outgrowth defects are observed, including premature termination of axonal processes, ectopic extension of branches, and de-fasciculation of axon bundles [12]. This spectrum of phenotypes resembles the disruption of UNC-76 [21,22], a protein whose *Drosophila* homolog binds to the carboxyl terminus of the kinesin heavy chain [23]. Loss of *unc-76* function phenocopies the defects of *Drosophila* kinesin mutants in axonal transport, suggesting that UNC-76 coordinates with kinesin to regulate cargo trafficking in axons [23].

The similarity of the *unc-69* and *unc-76* mutant phenotypes suggests that UNC-69 and UNC-76 could function together to regulate normal axon development. Indeed, UNC-69 and UNC-76 interact through a conserved coiled-coil domain,

which is at least partly required for the function of UNC-76 *in vivo* [12]. Through extensive analysis of double mutant combinations among *unc-69*, *unc-76* and other classes of outgrowth mutants (such as *unc-6*, which is defective in a netrin, *unc-119*, which lacks human retina gene 4 (*HRG4*), and so on), Su and Tharin *et al.* [12] showed that UNC-69 and UNC-76 act in a single pathway to regulate axon development. Interestingly, UNC-69 and UNC-76 proteins colocalize to puncta distributed in both the axon and the soma, consistent with the model that they form a protein complex *in vivo* [12]. Taking these results together, Su and Tharin *et al.* [12] have provided strong evidence that UNC-69 is involved in normal axon development. By interacting with UNC-76, UNC-69 is likely to be tethered to kinesin complexes, and it may coordinate with UNC-76 and kinesins to guide the vesicle trafficking in the growing axon that is necessary for axon extension and development.

In addition, the authors [12] also found that the UNC-69-UNC-76 complex might be involved in regulating synaptogenesis. Deciphering whether an axonal-outgrowth protein participates in synaptogenesis is complicated by the fact that axonal outgrowth precedes synapse assembly and thus has an effect on synapse formation. Using hypomorphic alleles of *unc-69*, however, Su and Tharin *et al.* [12] found that these mild alleles exhibited defects only in synaptogenesis (specifically, in clustering of puncta marked by the synaptic-vesicle marker synaptobrevin) but not defects in axonal outgrowth, suggesting a direct involvement of UNC-69 in synapse formation. One possibility is that UNC-69 directs the axonal trafficking of a type of cargo vesicle used in the assembly of synapses. Further characterization of the synaptic defects in *unc-69* mutants and the identification of interactions between UNC-69 and synaptic proteins should refine our understanding of the role of UNC-69 in synaptogenesis.

Several interesting questions remain. Firstly, what is the exact role of UNC-69 in the UNC-76 protein complex? Unlike motor mutants that cause general transport defects, loss of *unc-69* function leads to defects only in growth and synaptic-protein localization in axons, but not in dendrites, suggesting that its function is axon-specific [12]. One possibility is that UNC-69 is a cargo-associated protein and that its binding to a kinesin complex directs the cargo to axons in a similar manner to that of other known cargo-associated proteins [14]. Alternatively, UNC-69 might act as an adaptor and recruit other proteins to the UNC-76 complex, which then specify the destination of cargos. Consistent with a role for UNC-69 in cargo selection, SCOCO, its vertebrate homolog, interacts in yeast two-hybrid assays with ADP-ribosylation factor-like protein 1 (ARL1), a membrane-associated small GTPase involved in post-Golgi transport [24].

Secondly, how is formation of the UNC-69-UNC-76 complex regulated *in vivo*? Under normal conditions, UNC-69 and UNC-76 are seen on the same cargo-like, axonal and perinuclear puncta, whereas in *unc-116* (kinesin) mutants they mislocalize to non-overlapping regions [12]. This indicates that the interaction between the two proteins is probably dynamic, and other proteins whose localization is dependent on UNC-116 kinesin might help to regulate the interaction and localization of the two proteins.

In summary, Su and Tharin *et al.*'s work [12] has provided evidence for an evolutionarily conserved function of UNC-69 in axon development in both the nematode and vertebrates. Further characterization of the proteins and/or vesicular cargos associated with UNC-69 and UNC-76 is likely to unravel the complexities of protein trafficking and targeting in developing axons.

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