UNC-16/JIP3/Sunday Driver: A New Cop on the Organelle Highway

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In this commentary, Qun Zheng and Michael L. Nonet examine Edwards *et al*.'s article in which they provide evidence for a domain in invertebrate neurons similar to the vertebrate axonal initial segment that serves to restrict certain organelles from entering the axonal compartment. In their article published in this month's *GENETICS*, Edwards *et al*. (2013) implicate the UNC-16/JIP3/SYD as a critical regulator of organelle entry into the axonal compartment: "An Organelle Gatekeeper Function for *Caenorhabditis elegans* UNC-16 (JIP3) at the Axon Initial Segment."

NEURONS are highly polarized cells with dendritic and axonal processes that contain distinct cellular machinery to receive and transmit intercellular signals. To create these distinctions, neurons must differentially target both proteins and organelles to these cellular compartments, but how this is achieved remains poorly understood. The vertebrate axon initial segment (AIS) is a specialized subcellular domain in the proximal region of the axon that serves as the boundary between the axon and the somatodendritic compartments in neurons. The AIS was first defined as the specialized axonal domain where electrical inputs from dendrites are integrated and transformed to an all-or-none action potential that instructs signal delivery. Action potentials initiate in the AIS because of the high density of sodium channels and potassium channels clustered in this unique membrane domain (Kole and Stuart 2012). More recently, the AIS has been defined as the site that demarcates the transition between the subcellular identity of the axonal and somatodendritic compartments (Winckler *et al.* 1999). In an article in this month's issue of *GENETICS*, Edwards *et al.* (2013) provide evidence for a similar domain in invertebrate neurons that serves to restrict certain organelles from entering the axonal compartment. Furthermore, they implicate UNC-16/JIP3/SYD as a critical regulator of organelle entry into the axonal compartment.

IN the 1960s, the unique morphological features of the axon initial segment (AIS) were described at the EM level: the plasma membrane is densely undercoated by granular material and the microtubules show special fasciculation (Palay *et al.* 1968). Not until the 1990s was it appreciated that the AIS also functions as a filter for axon asymmetry in addition to its key role in action potential initiation (Winckler *et al.* 1999). In fact, the AIS serves as a boundary restricting both the diffusion of membrane proteins and a filter for restricting the movement of cytosolic proteins from one compartment to another. In vertebrates, a dense ankyrinG–spectrin–actin network forms this AIS cytoskeleton. Detailed measurements showed that, in cultured hippocampal neurons, this filter has a pore size of $<$13 nm, preventing axon entry of 70-kD dextran (Song *et al.* 2009). In the same report, developmental analyses showed that AIS formation is dynamic. The AIS first appears at the end of neuronal differentiation when the axon becomes molecularly distinct from dendrites. Combined with its unique barrier role, this developmental dynamic suggested that AIS is important in developing neuron polarity.

Even though the anatomically simple neurons of worms show similar polarity as vertebrate neurons, morphological evidence for an AIS domain in worm axons has not been described. In this issue of *GENETICS*, Edwards *et al.* (2013) provide some of the first evidence for an AIS-like domain in worm neurons. Furthermore, they extend the concept of an AIS to include not only localizing of proteins, but also regulating the compartment restriction of organelles through characterization of *unc-16* mutants that disrupt somatodendritic *vs*.

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axon targeting of organelles. *sunday driver* (*syd*) encodes a *Drosophila* homolog of the UNC-16/JIP3/SYD family of membrane-bound scaffolding proteins. Prior work in *Drosophila* had defined a defect in the trafficking of synaptic vesicle cargo in *syd* mutants (Bowman *et al.* 2000). However, instead of being lethal like *Drosophila syd* mutants, worm *unc-16* mutants are viable, which allowed Edwards *et al.* to genetically dissect UNC-16 in a null background. In this report they demonstrate that loss of UNC-16 dramatically alters the distribution of multiple axon-restricted organelles, including lysosomes, early endosomes, and Golgi, but not axon-associated components, like ER membranes and mitochondria. Golgi and lysosomal markers were 700 and 800% more abundant, respectively, in distal axonal processes of motor neurons while their counterparts in the cell body were not depleted.

The redistribution of organelles suggested that cargo trafficking was altered by *unc-16* mutations. Edwards *et al.* therefore examined lysosome dynamics in axons of unanesthetized animals using impressive time-lapse imaging. They found that lysosomes in *unc-16* null mutant axons still undergo processive, bidirectional, fast axonal transport. These results challenge the view that UNC-16/JIP3/SYD functions to promote cargo transport in axons, a model that was based primarily on the presence of accumulations of synaptic cargo in axons of *syd* mutant flies (Bowman *et al.* 2000). It was proposed that increased accumulation of membrane cargo in *syd* axons is a result of maternally inherited SYD protein and that gradual depletion of maternal SYD permits cargo entry into axons but is insufficient for extended trafficking to distal axonal regions. The active lysosome trafficking in *Caenorhabditis elegans unc-16* null mutants suggests that SYD regulation of lysosomal trafficking may be more complex.

Based on the *in vivo* dynamics of lysosomes and the presence of UNC-16 in the initial portion of the axon, Edwards *et al.* (2013) propose that UNC-16/JIP3 functions at an invertebrate equivalent of the AIS to restrict the transport of select organelles into the axonal compartment. The exciting discovery that loss of UNC-16 disrupts organelle distribution by altering the behavior of trafficking provides some of the first evidence that *C. elegans* neurons have a specialized structure with functions analogous to the vertebrate AIS. But like most provocative articles, the work highlights numerous questions that remain about the establishment and maintenance of axonal and dendritic polarity.

First, it remains unclear whether the worm AIS-like compartment shares any structural components with the vertebrate AIS. No ultrastructural evidence for a specialized cytoskeletal structure in the proximal region of axons has been described in the literature, although much of the published work describing *C. elegans* ultrastructure was performed under unusual fixation conditions (White *et al.* 1986). Ion channels are clustered at the AIS in vertebrates via specific domains, yet equivalent domains are not present in the analogous worm channels (Hill *et al.* 2008). Thus, it is unknown whether *C. elegans* neurons even have a membrane diffusion barrier in the proximal axon. No cellular markers that specifically delineate an AIS-like subcellular domain have been described either. The critical core cytoskeletal component that underpins the vertebrate AIS is ankyrinG, which is localized selectively to the AIS and is essential for AIS formation (Zhou *et al.* 1998). However, worms do not encode a specialized ankyrin gene homologous to ankyrinG. Rather, the worm genome encodes a single ankyrin gene (*unc-44*) that expresses at least seven distinct splice forms of diverse structure (Boontrakulpoontawee and Otsuka 2002), one of which could subserve an equivalent function to ankyrinG. However, the role of *unc-44* in lysosome trafficking has not been examined, although recent work has provided evidence that it is involved in establishment of neuronal polarity (Maniar *et al.* 2012). Thus, a key step forward will be to define the molecular composition of the worm proximal axonal domain to determine if its subcellular cytoskeletal architecture shares any similarity with the vertebrate AIS.

Second, the mechanism by which UNC-16/JIP3 regulates organelle trafficking remains elusive. Edwards and colleagues propose that the protein functions as an organelle traffic cop in the proximal axonal domain. While this model is attractive, there is currently no direct evidence in support of such a localized site of action for UNC-16/JIP3. The protein is expressed broadly in the soma and proximal axon and also localized sparsely in the distal axon. The major defined interaction partners of UNC-16/JIP3 are kinases and motors. Specifically, UNC-16/JIP3 interacts physically with kinesin-1 (UNC-116/KIF5) and dynein (Bowman *et al.* 2000; Cavalli *et al.* 2005; Arimoto *et al.* 2011). Although the motor that transports lysosomes in *C. elegans* has not been defined, kinesin-1 transports lysosomes in vertebrate cells (Rosa-Ferreira and Munro 2011). Unexpectedly, Edwards and colleagues found UNC-116 was not required for axonal lysosome accumulations induced by loss of UNC-16. Furthermore, in prior studies loss of UNC-16 was shown to partially suppress the defect in axonal transport of synaptic vesicles caused by the disruption of the KIF1A UNC-104 motor (Byrd *et al.* 2001). These two findings suggest that loss of UNC-16 leads to changes in cargo–motor interactions resulting in other motors transporting lysosomal and synaptic vesicle cargo in absence of UNC-16/JIP3. In vertebrates, distinct motor–cargo combinations have been documented to have differing abilities to transport through the AIS and the main feature that correlates with the ability to enter the axon is the transport efficiency (speed and run length) of the cargo–motor complex (Song *et al.* 2009). Thus, an alternative model to UNC-16/JIP3 functioning as a gatekeeper is that UNC-16 specifies the cargo–motor interactions for native transport and that disrupting UNC-16/JIP3 results in altered organelle transport. Characterization of the interactions of UNC-16/JIP3 with components of the axonal filter will play a critical role in defining the mechanism underlying the UNC-16/JIP3 gatekeeper function.

Third, what cellular events engage UNC-16/JIP3 to modulate organelle trafficking and for what biological purposes is organelle trafficking altered? In addition to interacting directly with several motor proteins, UNC-16/JIP3 is also a scaffolding protein for the c-jun N-terminal kinase (JNK)

pathway (Matsuura *et al.* 2002). Edwards and colleagues demonstrate that disruption of the JNK-1 kinase yields a lysosomal accumulation phenotype similar to, but less severe than, that seen with loss of UNC-16. This implies that organelle trafficking and subcellular targeting is regulated at least in part by JNK signaling pathways. In mammalian systems, axonal damage has been shown to activate JNK and stimulate retrograde transport of a JNK-SYD labeled vesicle complex (Cavalli *et al.* 2005). The possibility exists that JNK–SYD interactions modulate AIS permeability for protein/organelle axon entry, reverting the cell to a state similar to earlier in neuronal differentiation before the AIS has formed. This "de-differentiation" step could play a role in post-axon injury repair processes.

In summary, Edwards *et al.* examined organelle distribution and trafficking behavior in the absence of UNC-16/JIP3/SYD and elegantly demonstrate that organelle transport in mutant axons is not stalled, but rather is differentially directed in the cell. Mechanistically, they propose that UNC-16 regulates the behavior of the organelle transport machinery in the proximal axon. These observations provide strong evidence for the existence of a cytoplasmic gate in the proximal axon of invertebrate neurons analogous to the cytoplasmic filter of the vertebrate AIS. The unusual lysosomal accumulation phenotype of these mutants should provide a powerful visual assay to apply to worm genetics to dissect the pathways regulating trafficking and restriction of organelle entry into the axonal compartment as well as an inroad to elucidating the structural components of the invertebrate proximal axon filter.

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