

How to get on the right track

Daniel M Suter & Peter J Hollenbeck

CRMP and ankyrin have been implicated individually in the regulation of neuronal polarity. A study now identifies an interaction between them that controls microtubule organization and thereby protein sorting into axons and dendrites.

Once neurons have completed their last cell cycle, they must migrate to their appropriate location and send out processes to make functional connections with their target cells. A crucial step is determining which process will become the signal-conducting component, the axon, and which processes will become the input components, the dendrites. *In vivo* studies in the mammalian brain have shown that this cellular polarization occurs during the migration of cortical and cerebellar neurons^{1–4}. However, the underlying molecular and cellular mechanisms have been studied mostly using an *in vitro* cell culture system of embryonic rat hippocampal neurons, originally introduced by Banker and colleagues⁵. In this system, many signaling and cytoskeleton-associated proteins that regulate neuronal polarity have been identified over the past 20 years^{6,7}. Still, the precise sequence of molecular events leading to the sorting of distinct proteins to axonal versus dendritic microtubule tracks is not well understood, particularly not *in vivo*. An elegant study by Maniar *et al.* in this issue of *Nature Neuroscience*⁸ has used the power of *Caenorhabditis elegans* genetics to investigate the functional relationship of UNC-33, also known as CRMP (collapsin response mediator protein) and UNC-44, also known as ankyrin, in the sorting of axonal and dendritic proteins in the intact organism. Although previous work had implicated these two proteins, individually, in mediating neuronal polarity, this study suggests a specific molecular cascade for this process. The authors find that UNC-44/ankyrin regulation of UNC-33/CRMP helps organize axonal microtubules, which in turn controls the targeting of presynaptic proteins to the axon.

Previous studies have implicated the microtubule-binding protein CRMP in axon specification in cultured hippocampal neurons⁹, as well as in sensory axon guidance in *C. elegans*¹⁰. Maniar *et al.*⁸ continued the search for a function of CRMP in *C. elegans*, exploiting several advantages of this organism for studying neuronal polarity, including well-described

axonal pathfinding and neuronal morphologies in a stereotypic nervous system, genetic and imaging capabilities and a relative lack of genetic redundancy. They started from the ground up, identifying several *unc-33* alleles in a screen for altered localization of presynaptic proteins. In most of their experiments, the authors focused on the PVD sensory neuron, which produces a characteristic axon that initially grows ventrally then turns anterogradely and also produces many dendritic branches that circle the body (Fig. 1a). In various *unc-33* mutants, fluorescently tagged presynaptic marker proteins RAB-3::mCherry and SAD-1::GFP were mislocalized from axons to dendrites of PVD, as well as other sensory neurons (Fig. 1b). Only expression of the long (but not the medium or short) UNC-33 isoform, UNC-33L, rescued these localization defects, suggesting that the long isoform is sufficient for *unc-33* activity.

Next the authors investigated the localization of UNC-33L using immunofluorescence and expression of UNC-33L::GFP in PVD neurons. They found that the long but not the short isoform of UNC-33 preferential localizes to the axon. Does the *unc-33*-induced mislocalization of presynaptic proteins result from diffusion or from failure of active transport? By assessing RAB-3::mCherry and SAD1::GFP localization in the background of the kinesin-3 (KIF1A) mutant *unc-104*, the authors found that both proper wild-type axonal localization and *unc-33*-induced dendritic mislocalization were completely abolished in the kinesin mutants, indicating that this motor is essential to the sorting process. The normal axonal localization of the kinesin motor UNC-104 was in turn strongly reduced and shifted to dendrites in *unc-33* mutants, suggesting that the mis-sorting of axonal proteins in *unc-33* mutants might be caused by inappropriate localization of the UNC-104 kinesin motor.

Next the authors turned their attention to the sorting of dendritic proteins. They observed that a GFP-tagged version of the chemosensory G protein-coupled receptor ODR-10, which is normally found in dendritic cilia of AWB chemosensory neurons, also appears in axons of *unc-33* mutant worms. Interestingly, this mislocalization does not depend on *unc-104* (KIF1A) but on other genes, *odr-4* and *unc-101*, involved in trafficking of vesicles after they emerge from the endoplasmic reticulum.

Altogether, these genetic studies reveal that UNC-33/CRMP controls sorting of both axonal and dendritic proteins.

In the same screen for altered localization of axonal and dendritic proteins, the authors also identified *unc-44* mutants. This gene encodes the lone *C. elegans* homolog of ankyrin, a protein found at the axonal initial segment, where it prevents exchange of axonal and dendritic proteins both *in vitro*¹¹ and *in vivo*¹². In *C. elegans* sensory neurons, *unc-44* mutants mislocalize not only axonal marker proteins but also UNC-33L/CRMP, indicating that ankyrin regulates neuronal polarization by the proper sorting of CRMP (Fig. 1b). UNC-33L/CRMP localization, by contrast, does not depend on UNC-104/kinesin. These genetic interaction experiments defined a molecular pathway in which UNC-44/ankyrin controls the restricted axonal localization of UNC-33/CRMP, which in turn regulates the restricted axonal localization of UNC-104/kinesin and the axonal transport of presynaptic cargo proteins (Fig. 1c).

Finally, the authors found a reduction of microtubule density in axons, along with an increase in density in dendrites, in both *unc-33* and *unc-44* mutants compared to wild-type worms. By expressing GFP-tagged EBP-2, a microtubule plus-end tip-binding protein (the *C. elegans* EB1 homolog), in amphid chemosensory neurons in the head, they were able to determine the polarity of microtubules in axons and dendrites. Live cell imaging of EBP-2::GFP suggested a uniform plus-end-distal microtubule polarity in axons and minus-end-distal polarity in dendrites of these neurons. Both *unc-33* and *unc-44* mutants, however, showed significant bidirectional EBP-2::GFP-movements, suggesting that a mixed microtubule polarity arises in both axons and dendrites when CRMP or ankyrin function is impaired (Fig. 1b).

This study places previously identified polarity proteins into a plausible molecular pathway (Fig. 1c): UNC-44/ankyrin is localized at the axonal initial segment, which restricts the microtubule-binding protein UNC-33/CRMP to the axonal compartment; UNC-33/CRMP in the axon then helps establish and/or maintain preferential plus-end-distal orientation of axonal microtubules, which can in turn be read by microtubule motors such as UNC-104/kinesin to target presynaptic proteins to the axon. This raises several additional

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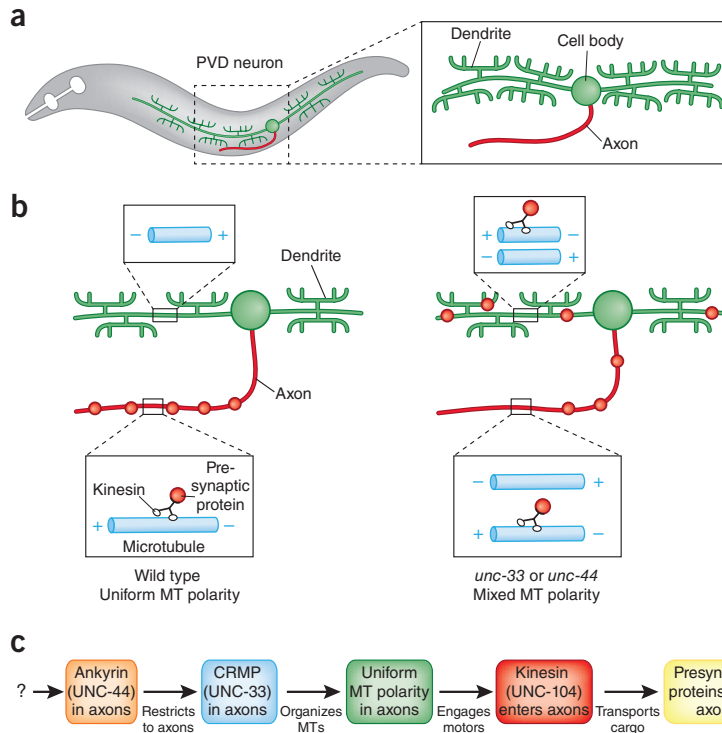


Figure 1 Model for establishment of neuronal polarity in *C. elegans*. **(a)** Morphology of the PVD sensory neuron in *C. elegans*. **(b)** Wild-type PVD neurons have uniform plus-end-distal microtubule (MT) polarity in axons and minus-end-distal polarity in dendrites. Presynaptic proteins RAB-3 and SAD-1 are delivered to axons and not dendrites through UNC-104/KIF1A-mediated transport. *unc-33/CRMP* and *unc-44/ankyrin* mutants have mixed MT polarity resulting in UNC-104/KIF1A-mediated transport of presynaptic proteins into both axons and dendrites. **(c)** Chain of events for axonal determination and protein sorting suggested by the study of Maniar *et al.*⁸. After UNC-44/ankyrin is localized to the initial segment of the incipient axon, it restricts the location of the MT-binding protein UNC-33/CRMP to these processes. CRMP then organizes the developing MT array into the characteristic plus-end-distal arrangement seen in axons. This allows UNC-104/kinesin-3 motors to engage with the MT array and transport axon-specific cargo, such as the presynaptic proteins RAB-3 and SAD-1.

questions. (i) What mechanism targets UNC-44/ankyrin to the axonal initial segment in the first place? (ii) Does a similar CRMP-dependent mechanism define microtubule organization in axons and dendrites of higher organisms?

In cultured rodent hippocampal neurons, for example, axons show uniform plus-end-distal microtubule polarity, whereas dendrites show mixed polarity¹³. (iii) Do *C. elegans* dendrites generally have their microtubule minus ends

oriented toward the growth cone, as reported for various *Drosophila* neurons¹⁴, or is this property confined to these chemosensory neurons? In this respect, it would also be interesting to know the microtubule polarity of the more complex dendritic structures in PVD neurons. (iv) If UNC-44/ankyrin's main function is to prevent diffusion of UNC-33/CRMP from axonal to dendritic compartment, what directs UNC-33/CRMP to dendrites in the strong *unc-44* mutants? (v) What are the roles of the shorter UNC-33 isoforms that did not rescue *unc-33* polarity defects? (vi) How is preferential minus-end-distal polarity achieved in dendrites of the wild-type sensory neurons in which EB1 tracking was performed? Answers to these questions are likely to bring us closer to a more complete understanding of how axonal and dendritic proteins get on the right track.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Tune in to KCNQ

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A study finds that the voltage-gated K⁺ channel KCNQ4 is expressed in a subset of rapidly adapting, low-threshold mechanoreceptors, where it shapes the response profile to dynamic tactile stimuli.

Our ability to sense and discriminate diverse mechanical stimuli is determined by the expression and function of specialized

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mechanoreceptors in the skin. Although intense mechanical stimuli are detected by the free nerve endings of mechano-nociceptive neurons, nonpainful stimuli are detected by a host of low-threshold mechanoreceptors (LTMRs). The precise stimulus-response characteristics of the diverse array of LTMR types are critical to our ability to perform sensory tasks, such as tactile recognition of complex objects, reading braille, feeling the

buzz of a cellphone or appreciating a caress. However, the molecular basis of LTMR functional diversity remains poorly understood. In this issue of *Nature Neuroscience*, Heidenreich *et al.*¹ demonstrate that the voltage-gated K⁺ channel KCNQ4 is crucial for setting the velocity and frequency preference of a subpopulation of rapidly adapting mechanoreceptors in both mice and humans.