Neuronal Morphogenesis: Golgi Outposts, Acentrosomal Microtubule Nucleation, and Dendritic Branching

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http://dx.doi.org/10.1016/j.neuron.2012.11.019

In large cells like neurons, how is microtubule polymerization initiated at large distances from the cell body and the main microtubule-organizing center? In this issue of *Neuron*, Ori-McKenney et al. (2012) demonstrate that Golgi outposts mediate acentrosomal microtubule nucleation and reveal it is crucial for dendrite morphogenesis.

Neurons are some of the most complex and highly polarized cells in our body. Their complex dendritic morphologies underlie their ability to integrate synaptic inputs. For this reason, the mechanisms that drive dendritic morphogenesis have been extensively studied over recent decades (reviewed Whitford et al., 2002). Cytoskeletal dynamics are required for proper formation and maintenance of both dendritic and axonal branches (reviewed Kobayashi and Mundel, 1998; Gallo, 2011). The cytoskeleton is composed of microtubules (MTs), actin filaments, intermediate filaments, and a myriad of regulators that process, order, modify, and remove these structures in a dynamic way. MTs are the largest and longest of these filaments, and are formed by polymerization of α- and β-tubulin dimers. This polymerization gives rise to an inherent polarity along microtubules with a plus-end (where new dimers are polymerized) and a minus-end (where dimers are depolymerized) (reviewed Baas and Lin, 2011).

The site at which microtubules are nucleated in neurons has been an important open question. This has been widely studied in nonneuronal cell types, but because of technical limitations is only recently being addressed in neurons. We have learned from nonneuronal cell types that microtubules are often nucleated at the microtubule-organizing center (MTOC), which is coupled to the centrosome. However, microtubules can also be nucleated from the nuclear envelope, melanosomes, plasma membrane, and the Golgi complex in a process called acentrosomal nucleation (reviewed Vinogradova et al., 2009). MT nucleation at the Golgi has received recent attention as it provides asymmetry in the MT arrays of motile cells and might be important for cell polarization. Four main proteins are thought to be responsible for the MT nucleating ability of the Golgi: γ-tubulin, AKAP450, GM130, and CLASPs, which provide a molecular scaffold for the MT nucleation process (Kollman et al., 2010; Rivero et al., 2009; Efimov et al., 2007).

Neurons present a unique cell biological challenge in this regard, mainly because of the length of their processes, which reach many hundreds of microns to several millimeters in the case of pyramidal cortical neurons in large mammalian brains. It seemed unlikely that most microtubules could be nucleated at the centrosome of a neuron's cell body and still reach the periphery of the dendritic arbor. A few recent studies have shown that, in fact, acentrosomal nucleation occurs in neurons. Stiess et al. (2010) discovered that axon growth can still occur after the centrosome located in the cell body has been ablated, and that very few microtubules emanate from the centrosome in mature neurons. Nguyen et al. (2011) examined microtubule organization in neurons without a functional centrosome and found that microtubules are organized independently of the centrosome. These recent findings have raised three possibilities for new microtubule nucleation in neurons: (1) microtubules are formed at the centrosome, cleaved, and then transported to the proper compartment, (2) microtubules are severed in the periphery, which could provide a scaffold for nucleation/polymerization, and (3) microtubules are nucleated at unknown acentrosomal sites (reviewed by Kuijpers and Hoogenraad, 2011).

In this issue of *Neuron*, Ori-McKenney et al. (2012) provide significant new insights into our understanding of the location of microtubule nucleation in neurons by visualizing acentrosomal MT nucleation in the dendrites of *Drosophila* da neurons. This is a class of large neurons present in the peripheral nervous system of the larva that has become a model system for the study of dendritic morphogenesis (reviewed Jan and Jan, 2010). Their results reveal for the first time that Golgi outpost-associated acentrosomal MT nucleation plays a key role in dendritic morphogenesis.

Using time-lapse microscopy of a genetically-encoded probe for microtubule plus-end (EB1-EGFP), Ori-McKenney et al. (2012) began their study by examining microtubule nucleation events in primary, intermediate and terminal branches of the highly branched class IV da neurons. They confirm previous results showing that in *Drosophila* neurons, primary dendrites contain mostly minus-end distal MTs, while intermediate branches have a mixed orientation of MTs. Interestingly, terminal branches are composed mostly of plus-end distal MTs. After analyzing the dynamics of EB1-EGFP comets in these different branch types, the authors realized that most anterogradely and retrogradely translocating comets initiate within the

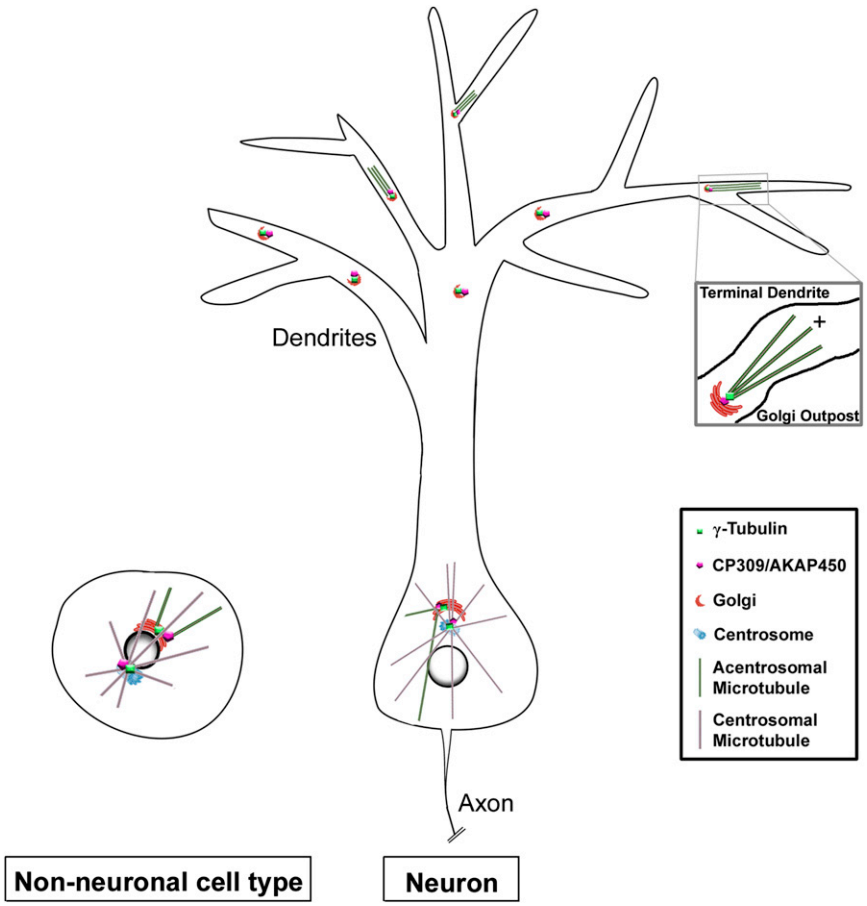

Figure 1. Golgi Outpost-Associated Microtubule Nucleation Sites Regulate Distal Dendritic Branching

In nonneuronal cells (left panel), both centrosome-associated and Golgi-associated acentrosomal microtubule nucleation sites coexist around the nucleus. In large and complex cells such neurons (right panel), in addition to these microtubule nucleation sites present in the cell body, Golgi-outpost-associated acentrosomal MT nucleation sites are distributed throughout the dendritic tree where they regulate microtubule dynamics and dendritic branch stability.

branch, at branch points or at the distal end, but not from the cell body.

This observation reminded the authors of previous work performed in their lab showing that Golgi outposts in *Drosophila* are present along the dendrites, at dendritic branch points, and at the distal tips (Ye et al., 2007), a property also found in mammalian neurons (Horton et al., 2005). To test if Golgi outposts present in dendrites could represent a source for acentrosomal microtubule nucleation, the authors developed transgenic flies expressing two genetically encoded probes to visualize MTs and Golgi outpost dynamics simultaneously. In vivo time-lapse imaging revealed that EB1 comets emerge from approximately 45% of dendritic Golgi outposts (Figure 1). To confirm that these Golgi outposts can nucleate MTs, the authors used an in vitro assay where purified Golgi outposts were collected and incubated with purified α- and β-tubulin dimers and GTP. Indeed, MTs formed on Golgi outposts that contained γ-tubulin and CP309 (the *Drosophila* homolog of AKAP450) but did not form on any Golgi outpost that lacked γ-tubulin. To determine the necessity of γ-tubulin, a function-blocking γ-tubulin antibody was incubated with the purified Golgi outposts prior to addition of γ-tubulin and GTP; as expected, no MTs could be nucleated. To resolve the importance of γ-tubulin and CP309/AKAP450 in vivo, Ori-McKenney et al. (2012) made use of two *Drosophila* mutants where these genes are inactivated. In these mutants, Golgi outposts are still localized to branch points and throughout the dendritic arbor, but mutant neurons show a striking decrease in the number of EB1 comets nucleating from the outposts located in the terminal branches.

To establish the role of Golgi-associated acentrosomal MT nucleation during dendritic arborization, the authors used the two mutants mentioned above and performed Scholl analysis which allows a quantitative assessment of the effect on dendritic branching as a function of distance from the cell body. Remarkably, the primary and secondary branches formed properly but a drastic reduction in the number of terminal branches occurred in γ-tubulin and CP309 mutant neurons, leading to a significant simplification of dendritic arborization. To understand why terminal branches were more specifically affected in these mutants, the authors compared distal branches with or without EB1 comet formation in vivo and determined that EB1 comet formation correlated with branch growth or stability, whereas the lack of comet formation correlated with a high probability of branch retraction. This suggested that Golgi outpost-associated acentrosomal MT nucleation is critical for terminal branch stabilization. The authors confirmed that in the γ-tubulin and CP309 mutant neurons, significantly fewer EB1 comets entered terminal branches and the majority of terminal branches retracted.

These results provide compelling evidence defining the critical role of Golgi outpost-associated acentrosomal MT nucleation during dendritic morphogenesis. The authors propose a likely scenario where all three modes of acentrosomal MT nucleation are involved in proper formation and maintenance of dendritic morphology. This work highlights potential differences between axonal and dendritic morphogenesis because Golgi outposts are not present from the axon (Horton et al., 2005). These results also beg the question of whether or not acentrosomal MT nucleation plays an important role in dendritic morphogenesis of vertebrate neurons. This is likely to be the case because (1) the main molecular components of Golgi-associated acenstrosomal MT nucleation are

conserved in vertebrates and (2) Golgi outposts are present in vertebrate neuron dendrites (Horton et al., 2005). Many other exciting questions remain to be addressed. Is the extent of Golgi-associated acentrosomal MT nucleation different in neuronal subtypes characterized by significantly different dendritic complexity, such as hippocampal neurons versus Purkinje cells? Is this process of acentrosomal MT nucleation used in other large, highly polarized cell types in the developing brain, such as dividing radial glial progenitors? What are the molecular mechanisms regulating the position, number and activity of Golgi-outpost acentrosomal MT nucleation sites in dendrites? Without any doubt, future studies will tackle the questions raised by these exciting new results.

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Dystroglycan Adds More Sugars to the Midline Cocktail

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http://dx.doi.org/10.1016/j.neuron.2012.11.017

In this issue of *Neuron*, Wright et al. (2012) identify two novel mediators of α-dystroglycan glycosylation in mouse and unravel a novel function of glycosylated dystroglycan in axon guidance by providing evidence for direct binding of α-DG to the midline chemorepellent Slit2.

The addition of glycan chains is a key step during the biosynthesis of many extracellular proteins, membrane bound receptors, and lipids. The structural diversity of these sugar polymers, further expanded by addition of sulfate, phosphate, and acetyl groups, is tremendous, possibly exceeding that of proteins (Ohtsubo and Marth, 2006). An increasing number of human diseases have been found to be caused by mutations in genes encoding glycosyltransferases and glycosidases (so-called congenital disorders of glycosylation or CDG; Freeze et al., 2012). In most cases, the development of the nervous system is affected (Freeze et al., 2012), such as in dystroglycanopathies, which are all linked to abnormal glycosylation of α-dystroglycan (α-DG).

Dystroglycan is a transmembrane protein expressed in various cell types that binds to laminin, a key component of the extracellular matrix (Hohenester and Yurchenco, 2012). The dystroglycan complex has thus been established as a crucial mediator of communication between factors of the extracellular matrix. The biosynthesis pathway of dystroglycan entails intracellular posttranslational proteolytic processing of a propeptide derived from a single mRNA, creating the α and β subunit of the mature dystroglycan (Hohenester and Yurchenco, 2012). Interestingly, following this initial cleavage, the two subunits reassemble noncovalently upon reaching the plasma membrane. The β-dystroglycan spans the plasma membrane, thus mediating intracellular signaling processes, while the α-dystroglycan is responsible for extracellular binding of ligands.