

#57: Fz, Goa, and Axin Recruit Microtubule Nucleation Sites to Dendrite Branch Points  
Alexis Weiner, Dylan Seebold, Nick Michael, Michelle Guignet, Chris Kozlowski, Dylan Barbera, Melissa Rolls

In *Drosophila* neurons, dendrite branch points act as hubs for microtubule organization. We have identified two microtubule control mechanisms that operate at branch points: microtubule steering and regulated nucleation. Apc2 is a component of the steering machinery and g-tubulin is the central nucleation protein. GFP-tagged versions of Apc2 and g-tubulin localize to branch points. To understand how these key regulators are concentrated at their site of action, we performed candidate screens using the very clear localization pattern of Apc2-GFP as a readout. We identified several groups of proteins from the initial screen including proteins that regulate actin polymerization through the Arp2/3 complex, the scaffold ankyrin2 and its membrane protein partner neuroglian, frizzleds, heterotrimeric G proteins and axin. Importantly, several of the proteins identified in the screen themselves localized to dendrite branch points; these included axin and ankyrin2. To determine whether the same suite of proteins is used to position g-tubulin, we knocked down representatives from each group and assayed g-tubulin-GFP at dendrite branch points. We found frizzleds, heterotrimeric G proteins and axin were critical for g-tubulin recruitment. Furthermore, we showed the proteins that were required for g-tubulin were also necessary for proper microtubule polarity in dendrites. We also tested a functional requirement for frizzleds and heterotrimeric G proteins in nucleation by assaying the nucleation-dependent increase in microtubule dynamics after axon injury. These proteins were required for the increase in nucleation in dendrites, confirming their role as critical regulators of g-tubulin localization outside the cell body. The evidence thus far suggests that there exists a partial overlap between pathways responsible for Apc2 and g-tubulin localization. We show here that frizzleds, heterotrimeric G-proteins, and axin act as master regulators through this process to manage microtubule nucleation and steering.