Visualization of microtubule growth in cultured neurons via the use of EB3-GFP

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Several microtubule binding proteins, including CLIP-170 (cytoplasmic linker protein 170), CLIP-115, and EB1 (end-binding protein 1), have been shown to associate specifically with the ends of growing microtubules in non-neuronal cells, thereby regulating microtubule dynamics and the binding of microtubules to protein complexes, organelles, and membranes. When fused to GFP, these proteins, which collectively are called +TIPs (plus end tracking proteins), also serve as powerful markers for visualizing microtubule growth events. We have demonstrated that endogenous +TIPs are present at distal ends of microtubules in fixed neurons. Using EB3-GFP as a marker of microtubule growth in live cells, we have analyzed microtubule dynamics in different neuronal cell types. Our results indicate that microtubules grow slower in neurons than in glia and COS-1 cells. The average speed and length of EB3-GFP movements are comparable in cell bodies, dendrites, axons, and growth cones. In the proximal region of differentiated dendrites 65% of EB3-GFP movements are directed toward the distal end, whereas 35% are directed toward the cell body. In more distal dendritic regions and in axons most EB3-GFP dots move toward the growth cone. This difference in directionality of EB3-GFP movements in dendrites and axons reflects the highly specific microtubule organization in neurons. Together, these results suggest that local microtubule polymerization contributes to the formation of the microtubule network in all neuronal compartments. We propose that similar mechanisms underlie the specific association of CLIPs and EB1-related proteins with the ends of growing microtubules in non-neuronal and neuronal cells.

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