Mini-Review

The Secretory Pathway and Neuron Polarization

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The growth of dendrites and axons is accompanied by an enormous increase in cell surface area. A cultured hippocampal neuron that has grown for 7 d *in vitro* can have ~250 μm of dendrites and 3000 μm of axons. Assuming that the diameters of both dendrites and axons are ~1 μm [a reasonable estimate (Fiala and Harris, 1999)], this constitutes ~10,000 μm^2 of surface area, which is ~30 times the surface area of an average soma that is 10 μm in diameter (300 μm^2). Hence, compared with most other cell types, developing neurons face a formidable challenge of adding new membrane to appropriate locations in an efficient manner (for review, see Futerman and Banker, 1996). A number of fascinating questions arise from considering how new membranes are added to the growing axons (Futerman and Banker, 1996; Goldberg, 2003). Where in the neuron are the components of plasma membrane synthesized? How are they transported to the site of membrane addition? Where on a growing axon are new membranes inserted? How does membrane addition coordinate with the cytoskeleton during the extension and retraction of axons? Although the past 10 years have seen significant progresses toward answering these questions, we are still far from having the final answers. We know even less about the differences in the membrane addition processes between axons and dendrites or the molecular machinery involved in these cell biological events.

Accumulating evidence from different types of cells suggests that polarized membrane addition contributes to cell polarization (for review, see Lecuit and Pilot, 2003). Neurons are highly polarized cells, and it is plausible that the control of membrane addition plays an important role in the morphogenesis of distinct subcellular compartments, such as dendrite versus axon, in neurons. Several studies in the past few years and our unpublished results from ongoing studies have provided evidence for such roles of membrane traffic.

The secretory pathway and the differences between dendrite and axon

In the hippocampal neurons described above, the surface area of the axon is ~10 times as much as that of the dendrites, implicating asymmetric transport of membrane to these two subcellular compartments. How such differential transport of membranes to dendrites and axons is achieved is an intriguing but primarily unexplored question.

The secretory pathway provides the membrane supply for cells. The major compartments of the secretory pathway are endoplasmic reticulum (ER), Golgi complex, post-Golgi carriers, and the plasma membrane. Studies on different types of polarized cells have shown that the secretory pathway is a major component in cell polarization. During epithelial polarization of *Drosophila* embryo, new membranes are added to the apical surface at early stage and to the lateral surface at later stage (Lecuit and Wieschaus, 2000). Similarly, in budding yeast, new membranes are added to different parts of the buds at different stages of cell cycle (for review, see Finger and Novick, 1998). In cultured hippocampal neurons, the polarization of the secretory pathway, i.e., preferential delivery of post-Golgi vesicles to the neurite that will later become the axon, precedes morphological polarization (Bradke and Dotti, 1997). In addition to structural polarity, the secretory pathway also contributes to the polarized protein distribution in different intracellular compartments (Lai and Jan, 2006).

Notwithstanding these important findings, whether the secretory pathway plays any role in differentiating dendrite and axon growth is unknown. Recently, we performed a large-scale genetic screen to systematically isolate mutants with defects specific to dendritic growth but not axonal growth (B. Ye, Y. W. Zhang, W. B. Grueber, L. Y. Jan, and Y. N. Jan, unpublished data). We isolated several mutants, termed dendritic arbor reduction (*dar*) mutants, that had reduced dendritic arbors but normal axonal arbors. We found that three of the *dar* genes, *dar2*, *dar3*, and *dar6*, are critical components of the secretory pathway, suggesting that the secretory pathway contributes to the differentiation of dendrites and axons. Similarly, the preferential defects in dendrite growth were observed in cultured hippocampal neurons deficient for a mammalian homolog of the *dar* genes, suggesting that this role of the secretory pathway is evolutionarily conserved. We combined genetic and cell biological approaches to study how the secretory pathway differentiates dendrite and axon growth.

One unique feature of the secretory pathway in neurons is the polarized localization of secretory compartments in the dendrite. In a pioneer work by Steward and Levy (1982), polyribosomes were found in dendrites. Immunogold electron microscopy studies have shown that membranous cisternae in dendritic branches as well as spines contain proteins required for each step of membrane transport through post-ER secretory pathway (Gardiol et al., 1999; Pierce et al., 2001). Furthermore, ER (Gardiol et al., 1999) and functional ER exit sites (Horton and Ehlers, 2003;

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Aridor et al., 2004) are found near Golgi outposts in dendrites. Therefore, in neurons, plasma membrane containing both lipids and membrane proteins can be generated locally in dendrites (Horton et al., 2005). In contrast, Golgi outposts have not been found in axon (Horton and Ehlers, 2003), raising the possibility of a specific regulation of dendrite growth by the function of local secretory pathway.

To test the involvement of Golgi outposts in dendrite growth *in vivo*, we performed laser ablation and time-lapse imaging of dendrite growth in live *Drosophila* larvae (our unpublished data). Our observations from these studies suggest that Golgi outposts are essential for dendritic growth.

Is the somatic Golgi also capable of differentially supplying membranes to dendrites and axons? To address this question, we measured the amount of membrane transport from the soma to dendrites and axons and found that somatic Golgi also contributes to the difference in dendrite and axon growth.

Therefore, our genetic studies and live imaging *in vivo* reveal that the secretory pathway contributes to the differential growth of dendrites and axons through both local and global control of membrane supply.

The secretory pathway and dendritic arbor diversity

One of the most dramatic features of the nervous system is the enormous diversity of dendritic arbor morphology, which is essential for the diverse signal processing ability and the function of different types of neurons. In cat, whereas the total dendrite length of cerebellar granule cells is ~60 μm (Ito, 1984), that of the spinal α-motoneuron is ~52,000 μm (Ulfhake and Kellerth, 1981). The function of the secretory pathway must be dramatically different between different types of neurons.

A recent study supports the idea that the secretory pathway contributes to neuronal diversity. In hippocampal pyramidal neurons, somatic Golgi apparatus is usually oriented toward the apical dendrite and the Golgi outposts are usually localized to the apical dendrite (Lowenstein et al., 1994; Horton et al., 2005). Disruption of this polarized localization of somatic Golgi and dendritic Golgi outposts led to the loss of the apical dendrite in those neurons (Horton et al., 2005). These findings suggest that the Golgi plays an active role in determining which dendrite becomes the apical dendrite and is therefore an important contributor to the morphogenesis of pyramidal neurons.

Furthermore, Golgi outposts are more enriched at dendritic branch points in mammalian neurons (Horton et al., 2005). We found similar enrichment in *Drosophila* neurons (our unpublished data). This evolutionarily conserved distribution of Golgi outposts raise the possibility that dendritic Golgi outposts are involved in branching of dendrites, a process that is crucial for dendritic arbor patterning.

Taking advantages of the morphological diversity of *Drosophila* dendritic arborization neurons, we characterized the distribution of outposts in neurons with different dendritic morphology and found that Golgi outpost distribution is distinctive in each type of dendritic arbors (our unpublished data). These findings point to the strategic distribution of dendritic outposts as a possible mechanism of generating the diversity of dendritic arbors. We are currently performing genetic analysis to examine the distribution of dendritic Golgi outposts in mutants known to have defective dendritic arbors. Such studies will provide insight about how Golgi outposts are involved in generating neuronal diversity.

In summary, recent works have begun to unveil the important roles of the secretory pathway in neuronal polarization. Future studies of mutations that preferentially affect dendrite morphogenesis will further reveal the underlying molecular mechanisms.

References

Aridor M, Guzik AK, Bielli A, Fish KN (2004) Endoplasmic reticulum export site formation and function in dendrites. J Neurosci 24:3770–3776.

Bradke F, Dotti CG (1997) Neuronal polarity: vectorial cytoplasmic flow precedes axon formation. Neuron 19:1175–1186.

Fiala JC, Harris KM (1999) Dendrite structure. In: Dendrites (Stuart G, Spruston N, Hauser M, eds), pp 1–28. New York: Oxford UP.

Finger FP, Novick P (1998) Spatial regulation of exocytosis: lessons from yeast. J Cell Biol 142:609–612.

Futerman AH, Banker GA (1996) The economics of neurite outgrowth: the addition of new membrane to growing axons. Trends Neurosci 19:144–149.

Gardiol A, Racca C, Triller A (1999) Dendritic and postsynaptic protein synthetic machinery. J Neurosci 19:168–179.

Goldberg JL (2003) How does an axon grow? Genes Dev 17:941–958.

Horton AC, Ehlers MD (2003) Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci 23:6188–6199.

Horton AC, Racz B, Monson EE, Lin AL, Weinberg RJ, Ehlers MD (2005) Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. Neuron 48:757–771.

Ito M (1984) The cerebellum and neural control. New York: Raven.

Lai HC, Jan LY (2006) The distribution and targeting of neuronal voltage-gated ion channels. Nat Rev Neurosci 7:548–562.

Lecuit T, Pilot F (2003) Developmental control of cell morphogenesis: a focus on membrane growth. Nat Cell Biol 5:103–108.

Lecuit T, Wieschaus E (2000) Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. J Cell Biol 150:849–860.

Lowenstein PR, Morrison EE, Bain D, Shering AF, Banting G, Douglas P, Castro MG (1994) Polarized distribution of the trans-Golgi network marker TGN38 during the in vitro development of neocortical neurons: effects of nocodazole and brefeldin A. Eur J Neurosci 6:1453–1465.

Pierce JP, Mayer T, McCarthy JB (2001) Evidence for a satellite secretory pathway in neuronal dendritic spines. Curr Biol 11:351–355.

Steward O, Levy WB (1982) Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. J Neurosci 2:284–291.

Ulfhake B, Kellerth JO (1981) A quantitative light microscopic study of the dendrites of cat spinal alpha-motoneurons after intracellular staining with horseradish peroxidase. J Comp Neurol 202:571–583.