(Figure 1B). On the other hand, by forming three-way vertices rather than four-way vertices, COPI polymerization is expected to generate the soccer-ball family of lattices heretofore associated exclusively with clathrin; it will be fascinating to see whether this prediction is borne out by future studies. In the meantime, the results of Lee and Goldberg provide an exciting first glimpse of the COPI cage and set the stage for mechanistic studies of coat assembly and disassembly.

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TGF-β Receptors PAR-ticipate in Axon Formation

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How does a newborn neuron initiate and elaborate an axon? Using cutting-edge approaches, Yi et al. (2010) provide striking evidence that TGF-β signaling via the Par polarity complex is required for axon formation by neocortical pyramidal neurons.

Neurons have a highly polarized morphology with multiple dendrites and a single longer axon, enabling them to receive, process, and transmit information across long distances within the nervous system. The steps and mechanisms for neuronal morphogenesis are only beginning to be understood in the cerebral cortex (Figure 1). An exciting new paper by the Ehlers and Polleux groups (Yi et al., 2010) suggests that transforming growth factor β (TGF-β) signaling via TGF-β receptor TβR2 is required for axon formation in vivo. Further, their findings implicate the Par complex in TGF-β-mediated axon outgrowth.

The model of choice for studying neuronal polarization in the recent decades has been cultured hippocampal pyramidal neurons. This model system, pioneered by Gary Banker, has provided numerous important insights and identified a large number of proteins, including the Par complex, that play roles in axonogenesis in vitro. After dissociation, these neurons must re-establish their polarized structure with one axon and several dendrites. They start by extending immature unspecified neurites and appear multipolar. One of the neurites rapidly outgrows the others, and this longest neurite almost always becomes the axon. Numerous proteins have been shown to selectively accumulate in the nascent axon and some play roles in axon specification or growth.

Although these in vitro studies have provided many hypotheses and candidate molecules for axonogenesis, there are important differences in vivo (Noctor et al., 2004; Polleux and Snider, 2010). In culture, the axon can develop from any of the immature neurites. Axon choice may be influenced by extracellular cues but does not require any. In fact, the notion that neuronal polarity is intrinsic to the cell has dominated the field for many years. By contrast, neurons in vivo are born into an environment that is prepolarized. Neurons polarize with a particular orientation within the tissue and migrate with strict directionality, and the axon always extends from one side of the cell. However, these early steps of axonogenesis are difficult to distinguish in the mammalian brain, and the question of whether (and which) extracellular cues are responsible for polarized axon emergence is an open one. Cortical neurons could receive polarizing cues from their radial glial mother cells either intracellularly by inheritance or extracellularly through contact-mediated signaling, or a polarizing cue may reside elsewhere.

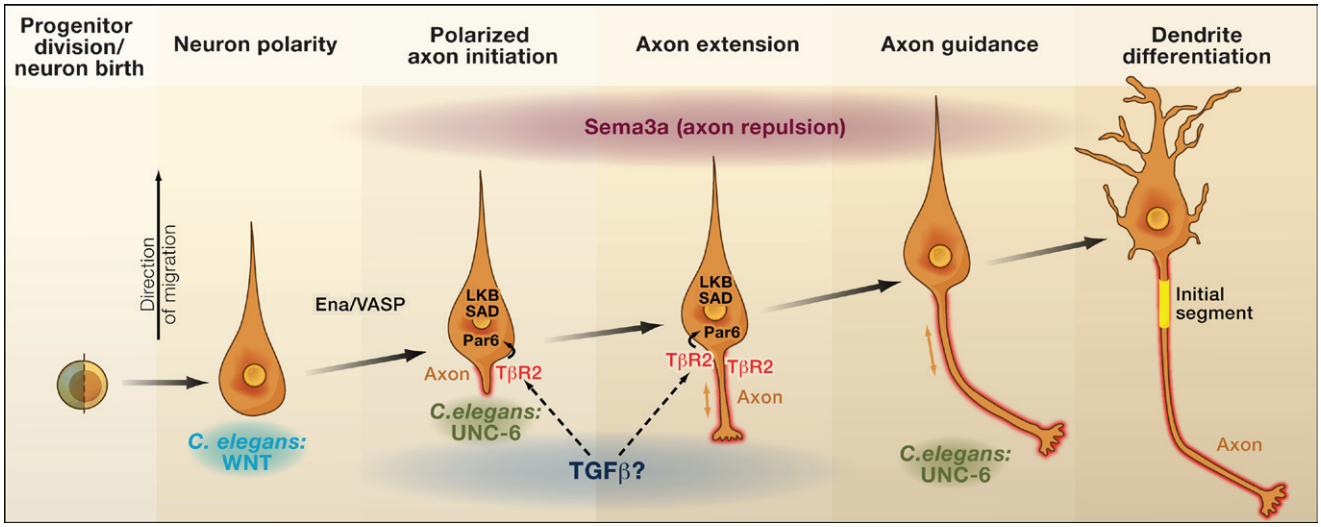

Figure 1. Axonogenesis in Cortical Neurons

A cortical pyramidal neuron born from a radial glial progenitor arises from an asymmetric cell division at the brain ventricle, adopts a neuronal fate, polarizes, and migrates outward along the radial glial fiber into the growing cortical plate. As it migrates, an axon is initiated and extended from the trailing end. A multipolar phase and retrograde migration may precede axon initiation (Noctor et al., 2004). The axon, tipped by a growth cone, elongates toward the ventricle and is guided to its target. After migration is complete, multiple dendrites are elaborated and the axon initial segment forms a diffusion barrier to maintain the distinct molecular composition of the axon.

This simplified model for axonogenesis shows some of the genes with strong in vivo loss-of-function phenotypes for initial axon formation or polarity. The cytoskeletal regulator Ena/VASP is required early for filopodia and neurite initiation (Kwiatkowski et al., 2007). The intracellular kinases LKB and SAD are required for axon initiation or extension but not for migration (Barnes et al., 2007; Kishi et al., 2005). Yi et al. show that transforming growth factor β receptor 2 (TβR2) is required for axon formation (via Par6) and also for neuron migration (not via Par6), presumably by responding to an extracellular TGF ligand. The location of TGF ligand in vivo is not known, but TGF can spatially direct axon specification in vitro. Three other ligand/receptor systems are required for polarized axon growth but not formation. Semaphorin 3a (Sema3A) is required in the cortex for axons to grow ventrally but not for their initiation or extension. In *C. elegans*, Wnt mutants reverse the polarity of axons along the anterior-posterior axis, and UNC-6/Netrin is required to polarize axon initiation and guidance ventrally (Adler et al., 2006; Hilliard and Bargmann, 2006).

Only a few papers have examined axonogenesis in the cortex by genetic loss-of-function studies. For instance, loss of the intracellular kinases SAD-A/B or LKB1, or of the cytoskeletal regulatory Ena/VASP proteins, causes cortical neurons to fail to make an axon (Barnes et al., 2007; Kishi et al., 2005; Kwiatkowski et al., 2007). In these knockout mice, neuron migration is relatively normal despite the lack of an axon. Interestingly, extracellular bone-derived neurotrophic factor (BDNF) can activate the LKB1-SAD pathway in vitro, but evidence for an in vivo role for BDNF in axon specification is lacking (Shelly et al., 2007).

Now, Yi et al. provide dramatic evidence that TGF-β may be an in vivo cue for both axon formation and neuron migration: when the gene encoding TβR2 is deleted from isolated neurons in embryonic day (E) 14.5 cortex by electroporation of cre-recombinase plasmids, the neurons appear bipolar, but most display retarded migration and no axon. If these electroporated brains are dissociated and cultured, the neurons lacking TβR2 remain multipolar and fail to specify an axon. Furthermore, overexpression of the wild-type TβR2 induces extra axons in vitro.

Identification of the TβR2 pathway in axonogenesis begs the questions of what is the ligand, and where is the ligand. Yi et al. begin to address these questions through in vitro experiments. Neurites that contact a bead coated with TGF-β or a stripe of TGF-β more often became the axon. Additionally, cells grown on a uniformly coated TGF-β substrate frequently made supernumerary axons. These experiments suggest that, at least in vitro, TGF-β can play an instructive role for axon formation. Whether this is the case in vivo remains to be determined. The source and location of TGF-β in vivo is uncertain; the three TGF-β proteins have not been examined in E14 mouse brains. What happens to the axons in brains where TGF-β ligands or receptors are knocked out from all cells? Such axons have not been carefully examined, although knockout mice exist and survive late enough into development for this analysis to be done in future work.

Given that TβR2 has been shown to regulate Par6 during epithelial cell plasticity (Ozdamar et al., 2005), Yi et al. tested whether TβR2 might act via Par6 to promote axon formation. They found that a nonphosphorylatable mutant of Par6 blocks axon formation in vitro and prevents the induction of extra axons by overexpressed TβR2. In perhaps the most surprising result, they show that coexpression of a phosphomimetic Par6 mutant in cortical slices rescues axon formation in cells lacking TβR2. Intriguingly, it does not rescue the migration defect, showing that the signaling pathways for neuron migration and axon growth are separable, even though they might both be initiated by the same extracellular cues.

It is interesting in this context to consider two observations by Yi et al. concerning cell and tissue polarity: neurons lacking TβR2 still exhibit a highly oriented bipolar shape, and neurons lacking TβR2 but expressing phosphomimetic Par6 still extend their axons in the correct direction. Hence, might other factors besides

TGF-β provide directional information? In the developing cortex, semaphorin 3A (Sema3a) orients axon outgrowth (Polleux and Snider, 2010), and in the nematode *Caenorhabditis elegans*, netrin and Wnt proteins polarize axons (Adler et al., 2006; Hilliard and Bargmann, 2006).

Other important questions for the future concern how the various pathways required for axonogenesis may interact with each other and coordinate polarization of different cytoskeletal or membrane effectors. The fact that disruption of any one of the TGF-β-Par, LKB1-SAD, or Ena/VASP pathways causes axon loss suggests that they are independently required. What are the intracellular effectors downstream of Par6 that mediate TGF-β signaling for axon formation? A number of good candidates are known from in vitro work and from other systems, but their specific roles and interactions with extracellular factors in the cortex need to be investigated. Given the application of the latest genetic tools and imaging to combine in vitro and in vivo work, as exemplified in Yi et al., the field should be well on its way toward dissecting the steps and molecules involved in axonogenesis.

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