Axon selection
From a polarized cytoplastm to a migrating neuron

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The shape of a neuron supplies valuable clues as to its function. Neurons typically extend a single long, thin axon, which will transmit signals and several shorter and thicker dendrites, which will receive signals. The understanding of the means by which neurons acquire a polarized morphology is a fundamental issue in developmental neurobiology. The current view suggests that axon selection involves a stochastic mechanism. However, new data suggest that a polarized cytoplasm not only determines the position of neurite emergence, but also sets the conditions for morphological polarization. In vertebrates, neurons migrate before establishing their final morphology. Recent work shows that the polarized cytoplasm also determines how neurons migrate. Thus, neuronal migration might influence the processes by which neurons form an axon.

Neurons come in many shapes and sizes; in general, however, they maintain two domains: the axon and the somatodendritic compartment. It has become clear that the formation of these domains is the consequence of polarized differences in the subcellular mechanics of membrane delivery, actin dynamics and microtubule stability.1-4 It is not well understood, however, how the final positioning of these two domains is determined.

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Axon Selection

The prevailing idea of the mechanisms underlying axon selection involves the stochastic selection of one of several, presumably equally competent, minor neurites by a competitive mechanism based on a contest between neurites that favors the establishment of the axonal fate ("tug of war" model5,6). This concept is supported by the fact that axon formation can be directed by external factors when neurons are plated on to patterned substrates.7

This hypothesis triggered a search for molecules that could act locally in a selected growth cone to initiate axonal outgrowth. Recently, several proteins that impose cellular asymmetry were identified to participate in axon growth, in part via signaling to the actin and microtubule cytoskeleton.1,2,8 The question remains how these polarized molecules are concentrated in one neurite before, or during, the axon specification. Axon selection has been suggested to depend upon the translocation of a plus end motor protein (KIF5C), which belongs to the kinesin-1 superfamily.9 This motor protein concentrates in one or two growth cones before axon formation, as well as in the established axon itself. KIF5C accumulation prior to axon formation is dynamic, and this protein constantly moves along neurites to reach the different neurite tips. Recent work supports the idea that there is a "radial movement" from the cell body to the neurite tip underlying the accumulation of KIF5C in the growth cone and that probably this and other motor proteins follow this principle to transport polarized signaling molecules to the axonal growth cone.1,2 In this regard, it was shown that molecules involved in axonal outgrowth, such as mPar3 and...
phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), are polarized in developing neurons as a result of kinesin-mediated transport to the nascent axon tip.\textsuperscript{10,11} If it is the case that the kinesin family proteins are following this radial movement, it may be that the origin of this movement determines the position of axon outgrowth.

**Centrosome Position Determines Neuronal Polarity**

Recent data have shown that the site of axon formation is determined by cytoplasmic polarization;\textsuperscript{12} the centrosome, Golgi apparatus and endosomes are clustered proximal to the site of the first neurite extension, and the axon has the highest probability of emerging from this location (Fig. 1A–C).\textsuperscript{12} Moreover, polarized microtubule polymerization and membrane transport precede the formation of the primary neurite.\textsuperscript{12} This is in accordance with observations that the centrosome and the Golgi are oriented towards the growing axon in cultured cerebellar granule neurons.\textsuperscript{13} These findings challenge the concept that axonal fate is random, and suggest a cell-autonomous mechanism for the regulation of axon initiation.

The concept that axonal fate depends on centrosome position is consistent with established roles for the centrosome in neuronal differentiation and other biological processes, in which the centrosome is involved in defining cell polarity.\textsuperscript{14} For instance, it was shown that, after fertilization, zygotes of *C. elegans* achieve polarization when the centrosome, provided by the sperm, is proximal to the embryo cortex and triggers the accumulation and segregation of the PAR proteins.\textsuperscript{15} In this system, the centrosome is required to initiate, but not to maintain, polarity. Similarly, in cultured hippocampal neurons, it was shown that, when the axon is truncated to the length of the remaining neurites, another neurite could then become the axon\textsuperscript{16} without the rotation of the centrosome to this new, alternative, axon.\textsuperscript{17} This indicates that the centrosome is involved in initiating polarization and suggests that it confers to the first neurite a quantitative, rather than a qualitative, signal that provides this neurite with a growth advantage over the others.

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**Figure 1.** Axon selection can be predicted. (A) Time-lapse analysis of a freshly isolated rat hippocampal neuron. The cell has a dynamic sprout (asterisk). (B) The same cell as in (A), cultured for two days, formed an axon (arrows) from the place of the initial sprout (asterisk). (C) Tau-1 immunostaining confirmed the axonal identity of the neurite highlighted in (B). Time: minutes; scale bar: 10 mm.
Neurites appear in a sequential fashion, with the second neurite emerging opposite to the first. This bipolar pattern of neurite differentiation underlies a bipolar organization of the cell that consists of more microtubules and membrane activity initially near the centrosome and, later, in the opposite pole. This intrinsic bipolarity is maintained during development since, upon axotomy, the new axon formed with higher probability at the opposite pole of the original axon. These data suggest that the polarity information represents the quantity of material that every neurite needs to receive in order to support a determined growth rate. When all neurites are forced to grow at the same rate, multiple axons are produced.

In vitro versus in vivo

The concept that intrinsic polarity predetermines the site of axon outgrowth is supported by earlier in situ observations of differentiating grasshopper neurons in which Lefort and Bentley (1989) found that the centrosome and Golgi apparatus are found at the site of the future axon. In apparent contrast, however, in retinal ganglion cells and neurons from the cerebellum of the developing zebrafish embryo, centrosome position does not predict the site of axon emergence. Interestingly, in the retinal ganglion cells the centrosome and the Par3 protein are localized opposite to the site of axon elongation even when the external environment is disrupted. The behavior of those cells resembles the bipolar radial neuronal progenitors from the developing cortex with the centrosome in the apical process or endfeet radiating microtubules towards the basal process to control the nuclear movement throughout the cell cycle. These findings are in agreement with the concept of an intrinsic mechanism underlying axon selection. However, fruit flies lacking centrioles develop a largely normal nervous system.

It remains to be elucidated whether, in neurons without centrioles, a polarized cytoplasm still underlies morphological differentiation. Along these lines, it was shown that the Golgi apparatus is a source of a large number of non-centrosomal microtubules that may compensate for a lack of centrioles. Moreover, some animal cells have centrosomes composed only of pericentriolar material with centrioles being undetectable. Despite a lack of centrioles, those cells are able to maintain an elaborate microtubule cytoskeleton.

Importantly, it was shown that the secreted UNC-6/netrin protein, which can attract or repel migrating cells, induces neuronal asymmetry and defines the site of axon formation early in the development of neurons of C. elegans. These findings indicate that axon formation may have both conceptual and mechanistic similarities to the polarization that occurs during cell migration. Changes in migration direction, following exposure to external stimuli, are associated with a re-orientation of the centrosome towards the leading edge. Therefore, it is conceivable that an initial step of axon formation may occur when extracellular cues influence the orientation of the centrosome and polarized cytoplasm towards the future site of axon elongation.

Centrosome Motility is Essential for Proper Axon Formation in the Neocortex

In the developing cortex, the first signs of axon outgrowth in neurons that will end up in upper layers is evident in neurons located in the subventricular zone (SVZ) and intermediate zone (IZ) that display a multipolar morphology. The multipolar neuron represents a transitional stage between the newborn bipolar neurons that ascend from the ventricular zone (VZ) to the SVZ/IZ, and the bipolar neurons that migrate out of the IZ and into the cortical plate (CP), elongating axons apically towards the VZ.

An interesting question that arises from these observations concerns the mechanisms by which the VZ-targeted apical axon is selected. Throughout migration, the centrosome is actively producing microtubules in a bipolar fashion, which propels the nucleus and exerts a pulling force toward the leading process. Thus, it is conceivable that, to preferentially send material necessary for axon specification, whereas neuronal migration sustains axon elongation. In future studies, it will be important to determine how external cues regulate the centrosome motility prior to axon formation, as environmental influences are known to pattern the cortical efferent projections.
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