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Review Article

Regulation of neuronal polarity

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ABSTRACT

The distinctive polarized morphology of neuronal cells is essential for the proper wiring of the nervous system. The rodent hippocampal neuron culture established about three decades ago has provided an amenable *in vitro* system to uncover the molecular mechanisms underlying neuronal polarization, a process relying on highly regulated cytoskeletal dynamics, membrane traffic and localized protein degradation. More recent research *in vivo* has highlighted the importance of the extracellular environment and cell–cell interactions in neuronal polarity. Here, I will review some key signaling pathways regulating neuronal polarization and provide some insights on the complexity of this process gained from *in vivo* studies.

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Introduction

The neuron is a classical example of a polarized cell, usually characterized by a long, thin axon and thick, shorter dendrites. The establishment and maintenance of neuronal polarity are essential for the correct development and function of the nervous system and rely on the exquisite coordination between membrane transport and cytoskeletal dynamics. Disruption of these fundamental processes has been linked to intellectual disability and a variety of neurological diseases [1]. The last four decades have seen great progress in understanding how neuronal polarity is established and maintained, and recent technological advances have enabled scientists to study these events *in vivo* using a range of animal model systems. While the role of the cytoskeleton has been the focus of much effort in the initial studies on neuronal polarity, new concepts such as selective protein degradation, the function of mechanical tension and the interplay with the extracellular environment in an *in vivo* context have been explored in more recent years. After summarizing some key signaling mechanisms underlying neuronal polarization, this mini-review will highlight the latest advances in this fascinating field, with a particular emphasis on the events involved in regulating axon/dendrite establishment *in vivo*.

The cytoskeleton in neuronal polarization

The hippocampal neuron culture established by Dotti et al. in the eighties [2] has been an invaluable tool to dissect signaling pathways regulating neuronal polarization. These neurons, isolated from E18 rat embryos, go through a series of stereotypical stages (Fig. 1A): 1) initial extension/retraction of filopodia; 2) extension of short neurites; 3) extension of the presumptive axon; 4) further growth and branching of the axonal process, dendritic maturation with appearance of spines; 5) formation of functional synaptic contacts. Not surprisingly, dynamic rearrangements of the actin and microtubule cytoskeleton accompany these different stages.

The growth cone of the presumptive axon is characterized by a very dynamic actin cytoskeleton. Indeed, local actin destabilization is sufficient to cause axon initiation [3] by allowing microtubule "invasion" of the central domain of the growth cone, followed by microtubule bundling and consolidation [4]. This process transforms the growth cone of a neurite into the shaft of the nascent axon and continues to be required for axon elongation. The small GTPase Rac acts as a crucial regulator of actin cytoskeletal dynamics in lamellipodia by triggering two major signaling cascades, the P21-activated kinase (PAK)-cofilin pathway and the WAVE-Arp2/3 pathway. While distinct Rac isoforms are likely to act redundantly in different types of neurons [5], the Rac effectors WAVE and PAK1 appear to participate in neuronal polarization, even though with different requirements in distinct types of neurons. For example, overexpression of an active, membrane-tethered WAVE mutant partially rescues axonogenesis in cerebellar granule neurons from *Rac*1 knockout mice [6] while PAK1 is necessary and sufficient for polarization of hippocampal neurons [7]. The small GTPase Cdc42, another modulator of actin dynamics, can also bind PAK1, which in turn activates LIM kinase (LIMK), leading to phosphorylation and inactivation of the actin-depolymerizing factor cofilin (Fig. 1B). Indeed, *Cdc*42 conditional knockout mice show substantial defects in axonogenesis in the cortex and striatum and display abnormally high levels of phosphorylated cofilin [8]. Taken together, these observations strongly support the requirement for a dynamic cycle of actin polymerization/depolymerization in the perspective axon.

Microtubule stabilization is essential for axon initiation, and, surprisingly, is also sufficient to cause extension of supernumerary axons from dendrites of mature neurons [9]. Stabilization of the nascent axon occurs through several microtubule-associated proteins (MAPs), including Collapsin Response Mediator Protein 2 (CRMP2), Map1b and Tau, together with plus-end tip (+TIPS) binding proteins Adenomatous Polyposis Coli (APC), Cytoplasmic Linker Proteins (CLIP)-115 and CLIP-170 [4]. Overexpression of CRMP2 leads to the appearance of multiple axons, and is even sufficient to switch a mature dendrite into an axon [10]. This important molecule contributes to axonogenesis by performing multiple functions: carrying tubulin heterodimers to promote microtubule assembly and stabilization, promoting selective transport of the Sra-1/WAVE actin-regulating complex towards the growing axon, and binding to Numb, a protein involved in clathrin-mediated endocytosis, to regulate endocytosis of the neural cell adhesion molecule L1 in the growth cone of the nascent axon [11].

The function of +TIPs is to allow protrusion of microtubules into the leading edge of the growth cone, facilitating axon initiation and growth. Both CLIP-115 and CLIP-170 are necessary and sufficient for axon formation *in vitro* [12]. APC, CRMP2, Map1b and Tau are substrates of Glycogen Synthase Kinase 3 (GSK3), a key kinase in neuronal polarization [13–16], which phosphorylates them and reduces their microtubule-binding ability. Therefore, local inhibition of GSK3 leads to microtubule stabilization and axon initiation by allowing the binding of these MAPs to microtubules. Notably, MAPs contain multiple phosphorylation sites and are likely to be targets for multiple kinases, some of which are still unknown. Thus, we are still far away from completely understanding how microtubule dynamics is regulated in neuronal polarization.

Centrosome positioning

While centrosome positioning plays a crucial role in many polarization events, its involvement in neuronal polarity may be different depending on the neuronal cell type and the developmental context. For example, in zebrafish retinal ganglion cells axons emerge from the basal side of the cell body, opposite to the apical location of the centrosome [17]. Other reports have instead correlated the axon initiation site to centrosome localization [18,19], suggesting an instructive role for the centrosome in determining axon initiation. However, later live imaging studies in *Drosophila* sensory neurons showed that centrosome positioning *follows* initial polarization, specified by clustering of adherens junction components [20]. In the developing cortex neurons shift from a multipolar to a bipolar morphology during their migration towards the brain surface, with the centrosome aligning towards the extending axon in multipolar-stage neurons, but opposite to the nascent axon in bipolar-stage neurons. In this case the location of the centrosome is associated with the predominant, most protrusive processes rather than with axonal identity, arguing against an instructive role for this organelle in specifying axon initiation [21].