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Review

Actin filament-microtubule interactions in axon initiation and branching

Almudena Pacheco, Gianluca Gallo*

Temple University, Lewis Kats School of Medicine, Shriners Hospitals Pediatric Research Center, Department of Anatomy and Cell Biology, 3500 North Broad Street, Philadelphia, PA 19140, United States

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ABSTRACT

Neurons begin life as spherical cells. A major hallmark of neuronal development is the formation of elongating processes from the cell body which subsequently differentiate into dendrites and the axon. The formation and later development of neuronal processes is achieved through the concerted organization of actin filaments and microtubules. Here, we review the literature regarding recent advances in the understanding of cytoskeletal interactions in neurons focusing on the initiation of processes from neuronal cell bodies and the collateral branching of axons. The complex crosstalk between cytoskeletal elements is mediated by a cohort of proteins that either bind both cytoskeletal systems or allow one to regulate the other. Recent studies have highlighted the importance of microtubule plus-tip proteins in the regulation of the dynamics and organization of actin filaments, while also providing a mechanism for the subcellular capture and guidance of microtubule tips by actin filaments. Although the understanding of cytoskeletal crosstalk and interactions in neuronal morphogenesis has advanced significantly in recent years the appreciation of the neuron as an integrated cytoskeletal system remains a frontier.

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* Corresponding author.
E-mail address: tue86088@temple.edu (G. Gallo).

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1. Introduction

1.1. Overview of the neuronal cytoskeleton

The cytoskeleton is a dynamic and regulated system that shapes the morphology of cells. The importance of cellular morphogenesis is especially evident during development when neurons establish patterns of connectivity and elaborate morphologically complex dendrites and axons. Dendrites tend to be tapered processes that do not extend great distances from the cell body, but can undergo significant amounts of branching. In contrast, neurons generate a single axon of relatively uniform caliber that can be up to meters in length in large animals. Axon branching occurs in the target fields of the main axon and also along the length of the axon through collateral branching (Gibson and Ma, 2011; Kalil and Dent, 2014). Collateral branching involves the generation of a new axon branch from the main axon independent of the growth cone at the tip of the extending axon. This form of branching allows the single axon to establish complex patterns of connectivity in multiple regions of the nervous system and also cover an expanded territory in its synaptic target fields. This review will focus on the initiation of processes from neuronal cell bodies and the collateral branching of axons.

The neural cytoskeleton is composed of three classes of structural elements: actin filaments (often referred to as F-actin for filamentous actin), microtubules and neurofilaments. Neurofilaments will not be considered in this review as there is little to no evidence of their involvement in the initiation or branching of axons. In contrast, the actin filament and microtubule cytoskeleton underlie all stages of neuronal morphogenesis (Dent and Gertler, 2003; Kevenaar and Hoogenraad, 2015). Actin filaments and microtubules are dynamic polymers respectively assembled from ATP-bound actin monomers and GTP-bound α-β-tubulin heterodimers. Actin filaments and microtubules are polarized polymers. In both cases, one end of the polymer exhibits much greater rates of polymerization than the other. These ends are termed the "barbed end" and "plus end" for actin filaments and microtubules, respectively. The opposite ends of the polymers are referred to as the "pointed" and "minus" end for actin filaments and microtubules. The barbed ends of actin filaments are involved in the generation of protrusive structures (e.g., finger-like filopodia and veil-like lamellipodia). At the plasma membrane, a major site for filament polymerization, the barbed ends face the inner leaflet of the membrane. The disassembly of filaments occurs at the pointed end which is usually directed away from the membrane. Filopodia are characterized by a uniform bundle of actin filaments with the barbed ends directed distally toward the tip of the filopodium where their polymerization drives the tip of the filopodium forward. In contrast, lamellipodia exhibit meshworks of actin filaments of varying orientations, but as with filopodia the polymerization of actin filaments near the membrane drives the lamellipodium forward. In both cases the actin filaments also undergo retrograde flow. Retrograde flow refers to the centripetal displacement of the filaments away from the filopodial tip or lamellipodial edge. In axons microtubules have an almost uniform polarity with the plus ends directed toward the terminus of the axon and the minus ends toward the cell body. Within the axon microtubule plus ends undergo bouts of polymerization and depolymerization, collectively referred to as dynamic instability.

The formation of new actin filaments and microtubules requires an initial nucleation event forming the seed of the polymer that will be subsequently elongated through polymerization. Actin filaments are nucleated through a variety of molecular systems that can generate individual filaments or give rise to a new filament from the side of an existing filament (Skau and Waterman, 2015). The nucleation of actin filaments can occur anywhere in the cell where the relevant nucleation systems are targeted and activated. Nucleation mechanisms are usually localized at the inner leaflet of the plasma membrane or other intracellular membranes. In contrast, the majority of axonal microtubules are considered to be nucleated at the somatic centrosome. However, recent evidence indicates that neuronal microtubules may also be nucleated independent of the centrosome (Kuijpers and Hoogenraad, 2011). Within the axon microtubules are considered to undergo active transport. The transport delivers microtubules to the distal axon, thereby contributing to axon extension. The polymerization of microtubules in axons, particularly at the terminus of the axon, is also of fundamental importance to axon extension (Dent and Gertler, 2003).

The axon is supported by a parallel array of microtubules. Depolymerization of microtubules results in the fragmentation of the axon. Other than to serve as the major structural support for the axon, an additional role of axonal microtubules is to provide the substrate for the axonal transport of a variety of cargoes ranging from organelles, protein complexes and mRNAs (in protein particles) (Maeder et al., 2014). The dynamic instability of axonal microtubules is greatest at the terminus of the growing axon. The microtubules along the axon shaft have decreased dynamics and increased structural stability. In contrast, in a developing neuron the concentration of actin filaments is greatest at the terminus of the axon comprising the growth cone. The growth cone is a highly motile structure characterized by actin filament dependent filopodia and/or lamellipodia. The concentration of actin filaments drops precipitously along the axon behind the growth cone (usually comprising the distal 10–15 μm of the axon). Within the axon proper actin filaments have been described to occur in a variety of super-structures including small localized meshworks termed actin filament patches, circumferential small bundles of actin, and also filament populations arranged longitudinally (Arnold and Gallo, 2014; Ganguly et al., 2015)

In summary, both microtubules and actin filaments have fundamental roles in the development of axons. The two cytoskeletal systems have mostly distinct roles in axonal biology, but cooperate in order to generate a fully functional axon. This review will focus on how actin filaments and microtubules can coordinate one another through indirect physical interactions and the regulation of cellular mechanisms that are controlled by one cytoskeletal element but converge on the other.

1.2. The cytoskeletal basis of axon initiation and branching

Because the details of the cytoskeletal involvement in neuronal morphogenesis are usually investigated using *in vitro* model system, which provide a high degree of spatio-temporal detail, this section begins with an overview of the relevant aspects of neuronal morphogenesis from *in vitro* models. The majority of neuronal cell types exhibit a single axon and multiple dendrites. *In vitro* both axons and dendrites differentiate from an initial set of undifferentiated short processes generated by the cell body termed "minor processes" (Neukirchen and Bradke, 2011a; Sainath and Gallo, 2015). One of these processes then becomes the axon and the rest develop into dendrites. The formation of minor processes is mediated by the extension of actin filament dependent filopodia or lamellipodia from the cell body. Microtubules subsequently enter the filopodium and allow the filopodium to mature into a minor process. An increase in the levels of actin filaments and the generation of frequent dynamic protrusions by the growth cone of one of the minor processes is an early hallmark of the differentiation into an axon. The differentiated axon then begins to elongate at a much greater rate than the remaining processes. The formation of an axon collateral branch from the main axon shaft follows a relatively similar sequence as that of the formation of minor processes from the cell body. As noted previously, the axon exhibits