The Cytoskeleton of the Neuron—An Essay in Celebration of Paul Letourneau's Career

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ABSTRACT: The neuronal cytoskeleton consists of microtubules, actin filaments, neurofilaments, and an array of accessory proteins that regulate and modify these three main filament systems. This essay celebrates the career of Paul Letourneau, a pioneer of the neuronal cytoskeleton, to whom the community owes a debt of gratitude. © 2011 Wiley Periodicals, Inc. Develop Neurobiol 71: 790–794, 2011

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INTRODUCTION

Neurons are arguably the cell type in nature with the greatest dependence upon sophisticated arrays of highly organized cytoskeletal elements for their form and function. The cytoskeleton of eukaryotic cells consists of three major filament systems, namely microtubules, actin filaments, and intermediate filaments, all of which are prominent in neurons. These filament systems contribute to the shape and architecture of the neuron and also fulfill the transport needs of axons and dendrites. There is much interest in understanding how the cytoskeletal arrays of the neuron are organized and established and how they are regulated during development. Rapid progress is being made, using live-cell imaging as well as sophisticated molecular techniques to better understand the neuronal cytoskeleton. During the early days, 2 and 3 decades ago, the most fundamental questions were framed by a small number of investigators using approaches that may seem simple by today's standards but were innovative for their time. It was a time when the imaging technologies of today were still to be realized, and therefore demands were all the greater for creative experimental design and insightful interpretation of indirect data. Among the pioneers was Paul Letourneau, who began his independent research in the mid-1970s. Letourneau's contributions over the decades have been many, but among his most influential were elegant studies characterizing basic features of the neuronal cytoskeleton. The purpose of this article is to review some of these findings and discuss how they opened the door to the contemporary advances we are witnessing today.

NEURONS IN CULTURE

At the time when Letourneau established his laboratory, much of the progress on the axonal cytoskeleton had been made in other laboratories using the radiolabel approach for quantifying the transport of proteins along the axon (see, for example, Hoffman and Lasek, 1975). This work took a biochemical approach

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and was mainly conducted on the adult nervous system. Taking a more visual approach with a more developmental slant, Letourneau worked on cultured neurons. He grew chick sensory neurons in culture, observed them with phase-contrast microscopy and studied them with assays including electron and fluorescence microscopy. In addition, he was a pioneer in the use of drugs that alter microtubules and actin filaments to reveal their functions. These were approaches that would lead to decades of work from a number of laboratories, aimed at understanding the mechanisms that establish the cytoskeletal arrays of neurons, the mechanisms that regulate them and the functions they carry out. While today there is a strong emphasis on *in vivo* models, the early work on primary neuronal cultures opened the door to studies on cellular mechanisms and high-resolution imaging.

PHARMACOLOGICAL ANALYSIS: THE GATEWAY TO FUNCTIONAL ANALYSIS OF THE NEURONAL CYTOSKELETON

As of his 65th year of life, Letourneau has published over 100 scholarly articles and chapters, and hence it would be impossible in this short tribute to do justice to even a fraction of his work. Our own interests in recent years have focused on axonal branching and also on complementary and antagonistic forces that integrate the microtubule and actin cytoskeletons during axonal growth, retraction, and navigation. In this regard, seminal papers come to mind that accentuate Letourneau's contributions to getting us to where we are today. These papers utilized drugs, mainly taxol and cytochalasin, to, respectively, impact microtubules and actin filaments. By conducting experiments aimed at inhibiting the polymerization of actin filaments, Letourneau provided important new insights into the role of this cytoskeletal element in the growth of the axon as well as in the protrusive activity of the growth cone. In other assays that utilized taxol to stabilize microtubules and overstimulate their assembly, Letourneau put forth the first evidence of how regulation of the microtubule array of the axon is essential for its growth and also for its capacity to form branches. Today, sufficient funds enable anyone to purchase antibodies for visualizing many different proteins that participate in such cytoskeletal events as well as RNAi products that will fairly specifically deplete a particular protein of interest. Letourneau is still active today and uses these contemporary approaches. Here, we will focus on a small number of observations from his earlier studies using taxol, and how his results with this microtubule-interacting drug jump-started rapid progress in the field.

TAXOL AND AXONAL GROWTH

Letourneau was first to report on the effects of taxol in the context of axonal growth (Letourneau and Ressler, 1984). When chick sensory neurons were cultured in the presence of high concentrations of taxol, they extended short broad extensions that failed to undergo notable elongation thereafter. Similarly, when axons that had been growing were treated with taxol, they ceased growing. As revealed by electron microscopy, microtubule levels and density were notably increased compared with control neurons, microtubule spacing was notably decreased, and the tips of the axons displayed dramatic abnormal looping of the microtubules. These findings were later confirmed by others showing that taxol caused abnormal microtubule arrays when applied to explant cultures of sensory ganglia (Masurovsky et al., 1985). Other concentrations of taxol permitted axons to grow, but the axons were notably unbranched under these conditions. Experiments using both taxol and cytochalasin were then conducted and permitted conclusions to be drawn on how the microtubule and actin systems of the neuron coordinated with one another during axonal growth. Today, microtubule/actin interactions remain one of the most exciting arenas of study in the field of axonal growth.

MICROTUBULES AND AXONAL BRANCHING

Axonal branches can form either through bifurcation of the growth cone or through the formation of interstitial branches along the axonal shaft. In culture, the axons of chick sensory neurons can form either way, although the axons of sympathetic neurons occur predominantly or exclusively via growth cone bifurcation. Letourneau's observations on both types of neurons were limited to growth cone bifurcation; he reported that treatment of either type of neuron with taxol prevented them from developing branches; the neurons were generally unipolar with the single axon displaying no branches (Letourneau et al., 1986). In immunostain analyses of growth cone regions, he noted that control neurons often displayed what appeared to be a quiescent area (with regard to actin-based protrusions), which showed early signs of a splitting of the microtubule array. This was not observed in the taxol-treated neurons. These observa-

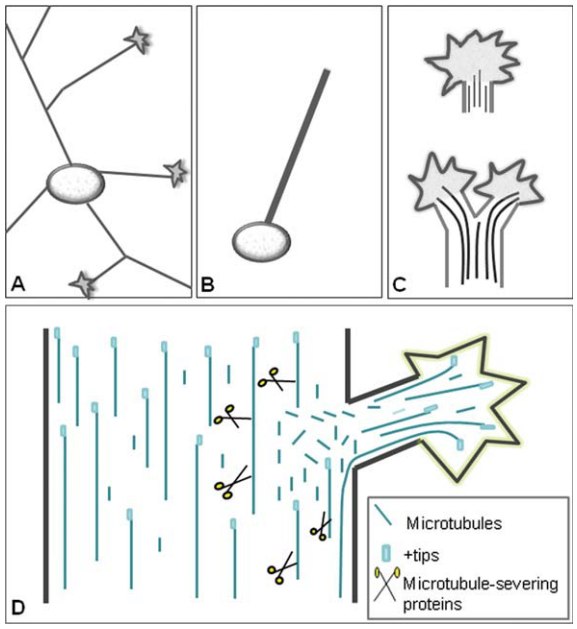

Figure 1 The role of microtubules in axonal growth and branch formation. Schematic diagrams of: A: An actively growing neuron with multiple axons. B: A cultured neuron grown in the presence of taxol. Note the absence of branches and growth cone. C: Letourneau's early model of branch formation. Long microtubules entering the growth cone are pulled laterally and ultimately bifurcate into two branches. D: Contemporary view of how microtubule-severing proteins contribute to branch formation. Long microtubules are cut by severing proteins to generate short ones that are transported into newly forming branches. +tips affiliate with plus ends of microtubules as they assemble within the parent axon and new branches.

tions, shown schematically in Figure 1(A–C), were seminal in that they started the field toward future studies on how interactions between microtubules and the actin cytoskeleton participate in the formation of branches. Sophisticated live-cell imaging as well as further drug studies from other laboratories fortified these early observations and expanded them on interstitial branching (Dent and Kalil, 2001), whereas other studies delved into potential roles for a variety of regulatory proteins, such as MAP1b (Bouquet et al., 2004).

A growing body of evidence suggests that the microtubule array becomes locally "de-stabilized" in order to enable individual microtubules to participate in the formation of interstitial branches (Jeanneteau et al., 2010). Studies are underway to identify the relevant growth factors and signaling pathways in this process. Evidence from our laboratory and others points to a particularly important role for a category of proteins called microtubule-severing proteins; these proteins break the long microtubules in the parent axon into shorter pieces that can then transit into the newly forming branch (Yu et al., 2008; Qiang et al., 2010). Interestingly, taxol would not be expected to prevent the severing proteins from breaking the microtubule (Qiang et al., 2006). However, once a microtubule is severed into a population of shorter ones, the next steps underlying branch formation would presumably include the plus ends of many of the short microtubules undergoing rapid bouts of assembly and their association with a variety of proteins such as +tips that interact with microtubule ends only when they are growing. Such proteins are crucial for the appropriate interaction of the microtubules with the actin-based cortex of the axon, and such interactions are important for axonal branching to occur (Kornack and Giger, 2005). Microtubules stabilized by taxol would lose their highly dynamic properties and therefore would not participate in these sorts of events. Figure 1(D) illustrates these newer ideas about microtubules and axonal branching.

FORCES THAT PUSH ON THE MICROTUBULE ARRAY DURING AXON GROWTH

A "push and pull" relationship between microtubules and the actin cytoskeleton was a fresh idea when proposed by Letourneau and others in the 1980s. As framed by Letourneau, the theory was that the axon is pulled out by actin-based protrusive activity while at the same time pushed out by the microtubule array undergoing anterograde transport (Letourneau et al., 1987). When neurons were treated with a low dose of cytochalasin, the axons ceased observable actin-based protrusive activity but continued to grow, and this level of growth was maintained even at higher doses of the drug. Letourneau concluded that "pull" assisted axonal growth but that "push" was sufficient for axons to grow [Fig. 2(A)]. This begs the question of what exactly is the push. In the early 1990s, it was reported by Letourneau and other laboratories (using photobleach analysis) that the microtubule mass in the axon does not, in fact, undergo a unified concerted march forward at the rate of what had been termed the slow component of axonal transport (Edson et al., 1993). More recent studies have documented that only very short microtubules in the axon undergo rapid intermittent transport in the axon, whereas the longer microtubules are essentially stationary (Wang and Brown, 2002; He et al., 2005). Nevertheless, Letourneau's idea of the entire microtubule array existing under forces was prescient, in that

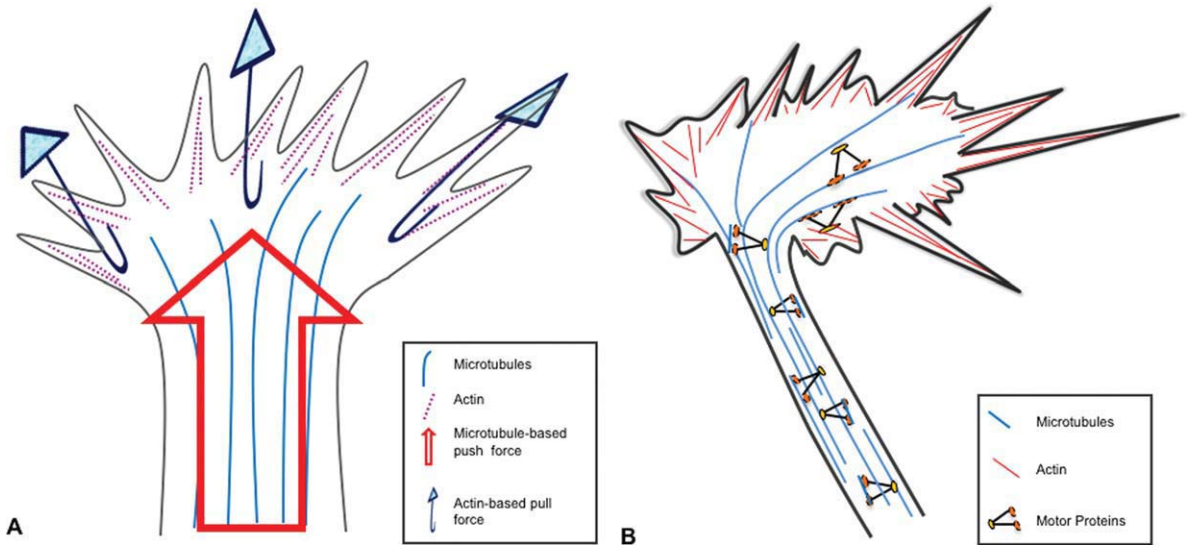

Figure 2 Interaction of cytoskeletal elements via forces in the axon. A: According to Letourneau's early model, the axon is pushed forward by the microtubule array while being pulled out by actin-based protrusive structures. B: According to contemporary models, forces generated by molecular motor proteins transport short microtubules down the axon, and also impinge upon long stationary microtubules to regulate axonal growth, retraction, and turning.

contemporary studies on molecular motor proteins suggest that the same motor proteins that transport the short microtubules impose forces on the longer ones (Myers et al., 2006; Myers and Baas, 2007; Liu et al., 2010). In this view [as shown in Fig. 2(B)], the longer immobile microtubules are under a complex balance of forces generated by motors that are critical for determining whether the axon grows or retracts and also for regulating the distribution of microtubules in the growth cone, underlying its turning behavior. For example, cytoplasmic dynein is essential for microtubules to resist the myosin-II-powered retrograde flow of actin filaments in the growth cone and also the myosin-II-powered contractility of the cortical actin of the axonal shaft, whereas kinesin 5 plays a critical role in regulating the selective distribution of microtubules in the growth cone (Myers et al., 2006; Grabham et al., 2007; Nadar et al., 2008).

CONCLUDING REMARKS

When taxol was first used for cell biological studies, there was no hint of the existence of +tips, a powerful class of proteins that interact with the plus ends of microtubules only when they undergo bouts of assembly. Microtubule-severing proteins had not yet been discovered, and the molecular motors that fuel microtubule transport were vaguely known as "the transport machinery." There was also little or no information on the various post-translational modifications of tubulin that accumulate within microtubules after they are stabilized (for review, see Janke and Kneussel, 2010). Thus, the black box was far more opaque back when the early experiments with taxol were reported and interpreted. Today, the neuronal cytoskeletal field is moving forward in these and other arenas, building on a solid foundation established by the pioneers. We are pleased with the opportunity to express gratitude and congratulations to Dr. Paul Letourneau for his decades of pioneering work. Through elegant experimental design and available tools, he established a body of literature that forms a foundation for present and future breakthroughs on a topic critically important to the development of the nervous system.

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