Mechanisms of Differential Branch Growth Control in the Single Axonal Arbor

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Regulation of branched axonal arbor shape is crucial for proper neuronal wiring as well as for nervous system plasticity. Isolated neurons are capable of extending axons and establishing complicated branched axonal morphology even without cell-extrinsic cues. This occurs by differentially regulating the growth of each terminal in an arbor. However, the mechanisms governing this cell-autonomous process are not fully understood. Here, I present recent findings regarding the intracellular mechanisms mediating terminal-dependent control of growth and retraction in the single axonal arbor.

Key words: Neuron, Axon, Branch, Signaling

1. Introduction

Establishing an accurate axonal branching pattern is crucial for proper information processing. Neurons extend one long axonal process for transmitting signals to distantly located postsynaptic targets. During nervous system development, neurons form axonal branches, sending signals to multiple targets, with some branches simultaneously eliminated by retraction [1–3]. This process, called axonal remodeling, is crucial for generating proper neuronal wiring. For example, during the development of the cerebral cortex, layer five pyramidal neurons send primary axons toward the spinal cord. The original axonal pattern is quite similar between the motor and visual cortices. However, in the later stages of brain development, the neurons in these two different cortical areas establish completely different axonal patterns, through the formation and retraction of axonal branches, and project to different regions [4]. A similar remodeling process is observed in motor neurons, which form axonal branches and send signals to several muscle regions by forming synapses called neuromuscular junctions. In adult animals, each neuromuscular junction is composed of a single neuron. By contrast, in neonatal animals, multiple axons make synapses within each neuromuscular junction. Once animals open their eyes, many branches are eliminated by retraction via activity-dependent mechanisms [5]. In addition, recent studies have found that axonal branch morphology is dynamically changed in several regions of adult animals [6,7]. These observations indicate that reorganization of branched axonal morphology is also crucial for neuronal plasticity.

During axonal remodeling, neurons remove axonal branches that are not required for the network while maintaining branches that are required. It is thought that both extracellular signals and cell-intrinsic systems orchestrate this remarkable process in vivo. However, even neurons isolated in culture, without cell-extrinsic cues, coordinately regulate their axonal branching and establish complicated axonal morphology. Here, I describe recent reports investigating the intracellular signals involved in branch-dependent growth and retraction.

2. Body

2.1 Process-dependent control of growth and retraction in the single axonal arbor

Axonal branches can be generated by a collateral branch forming from a pre-existing axon or from the bifurcation of an extending growth cone [1,8]. In collateral branch formation, a major mode of axonal branching, an F-actin patch is initially formed at the branch site and serves as a precursor for a filopodium or lamellipodium. After the emergence of these F-actin-containing protrusions, microtubules innervate the branch [2,8]. These processes have been extensively studied using young axons of sensory neurons. In this case, the morphological and functional features of a pre-existing primary axonal process and a newly generated collateral branch are clearly different from each other [9]. The primary axonal process is thick and grows continuously, whereas collateral branches are thinner and are often removed by retraction. Many central nervous system neurons extend branches and form complicated axonal arbors. As an axon extends, it becomes thinner, and the primary axonal process and its branches become less distinct from one another. Furthermore, the main axon can disappear by retracting, or the branches can behave as the "main axon," or both [10,11]. Thus, the preexisting axonal process does not persist as a morphologically and functionally distinct process. Hereafter, the term "branch" is used to represent all processes emerging from a branch point, without distinguishing the primary axon from the other branches.

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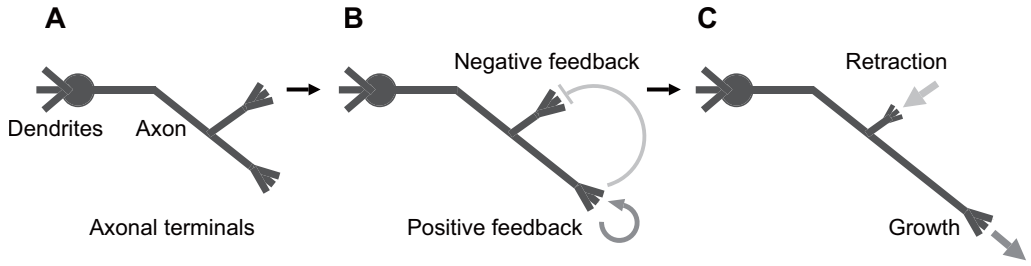

Fig. 1. Differential growth control of axonal branches in a single arbor. (A) A neuron that possesses a branched axon with two terminals. (B) It is hypothesized that signals acting positively on a particular process may negatively affect neighboring processes. (C) These signals may cause differences in process growth and retraction rates and coordinate the axonal arbor shape.

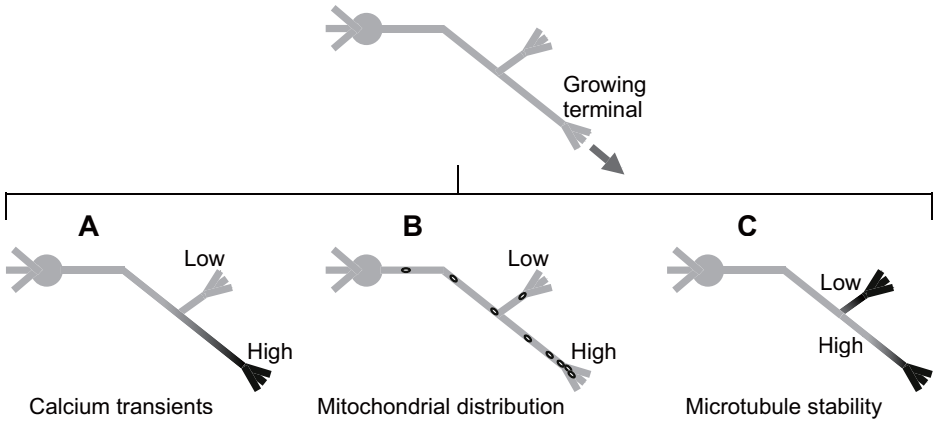

Fig. 2. Process-dependent differences that contribute to differential growth. (A) A growing terminal process exhibits higher Ca^{2+} transients than a neighboring branch that extends less. (B) Mitochondria are preferentially sorted into a growing process. (C) Microtubule stability is higher in longer processes than in shorter neighboring processes, which causes posttranslational modification differences.

The axonal branches of isolated hippocampal neurons alternate periods of growth; when one side of the branch actively grows, the other remains quiescent. The growth and quiescent states of a branch switch after a few hours [10]. These observations suggest that within a single axonal arbor, growth and retraction can be regulated in branch-dependent manner. Intriguingly, studies using isolated cortical neurons have shown that activation of growth on one side branch increases the retraction rate of another branch [12]. Thus, it can be hypothesized that there are mechanisms that maintain the growth of particular axonal branch during a period of time and that simultaneously send negative signals to neighboring branches to enhance their retraction (Fig. 1). As described below, recent studies have demonstrated that certain intracellular events (i.e., calcium signaling, mitochondrial sorting, and microtubule regulation) participate in this cellular function and contribute to the differential growth or retraction of an axonal branch.

2.2 Calcium transients

Calcium (Ca^{2+}) signals play pivotal roles in controlling dendritic and axonal morphology [13,14]. Entry of Ca^{2+} from voltage-dependent Ca^{2+} channels locally enhances branch growth [15,16]. Using Ca^{2+} imaging in branched axons of cortical neurons, Hutchins and Kalil [12,15] found that these neurons exhibit spontaneous localized calcium transients. In their system, primary axons exhibit more rapid outgrowth than collateral branches do. Intriguingly, local Ca^{2+} transients frequently occur in a primary axon but not in its collateral branches, and the frequency of Ca^{2+} transients positively correlates with the growth rate of axonal terminals [12] (Fig. 2A). Different frequencies of Ca^{2+} transients are thought to be involved in the differential control of terminal growth and retraction. When Bay K8644, an agonist for the L-type voltage-dependent Ca^{2+} channel, is applied in culture, it enhances localized Ca^{2+} transients and the differential growth of processes. By contrast, application of nifedipine, an antagonist at this same Ca^{2+} channel, blocks both localized Ca^{2+} transients and differential growth. Furthermore, induction of local Ca^{2+} transients by using caged Ca^{2+} increases growth of UV-stimulated axonal processes, whereas it causes retraction of unstimulated processes [12]. Downstream signaling molecules, such as calcium/calmodulin-dependent protein kinase II is thought to be involved [15]. However, the mechanisms whereby the outgrowth of one side of a branch inhibits the extension of a neighboring process remain undetermined.

2.3 Mitochondria distribution

In animal cells, mitochondria play a major role in ATP synthesis. In addition, these organelles are involved in calcium signaling and apoptotic signals [17,18]. Within axons, mitochondria are sparsely distributed throughout the arbor, but they are enriched in areas in which energy consumption is high as compared with other regions, such as synapses and nodes of Ranvier [19–21]. The microtubule motors ki-

nesins and cytoplasmic dyneins mediate anterograde and retrograde transport, respectively, of mitochondria in the axon [22]. Adaptor proteins, such as milton and syntabulin, mediate interactions between mitochondria and kinesin [22]. A previous study by Ruthel [23] and Hollenbeck using isolated cultures of hippocampal neurons has revealed a mechanism for the active sorting of mitochondria into growing axonal branches. At a given axonal branch point, mitochondria more frequently enter the actively growing branch than the non-growing branch, resulting in different mitochondrial densities between the two branches (Fig. 2B). This mitochondrial sorting at an axonal branch point does not precede the terminal growth of branches. Rather, it follows the elongation of the axon. Application of mechanical stimulation to the growth cone induces terminal growth, which is not accompanied by the activation of mitochondrial sorting into the target branch. In addition, disruption of the growth cone structure by cytochalasin treatment does not block the accumulation of mitochondria in the growing branch [23]. These observations suggest that there is a selective sorting of mitochondria into growing axonal branches that is not directly coupled with growth cone function. Although it has not yet been demonstrated, accumulation of mitochondria in a particular branch likely depletes mitochondria from the neighboring branch to negatively regulate branch growth.

2.4 Microtubule regulation

Kinesin-mediated axonal transport plays critical roles in the formation and maintenance of the axon. During axonal formation, various differences in the state of the microtubules (e.g., posttranslational modifications, GDP/GDP binding, and microtubule-associated protein-mediated decoration) are observed between axons and other minor processes that give rise to dendrites [24–26]. Among the various posttranslational modifications of tubulins, tyrosination and acetylation are affected by microtubule stability, that is, stable microtubules contain more detyrosinated and acetylated tubulins [27]. In axonal arbors, microtubules near growth cones are unstable and abundant with tyrosinated and deacetylated tubulins, whereas microtubules in longer processes are more stable than those in shorter processes at the region near the branch point [11,28,29]. Consistent with these observations, longer processes are more enriched with detyrosinated and acetylated microtubules than their neighboring shorter processes when compared at the same distance from the axonal branch point (Fig. 2C).

Detyrosination and acetylation of microtubules are suggested to enhance kinesin-dependent transport [24,30,31]. In a branched axonal arbor, the motor domain of kinesin (constitutively active kinesin) preferentially accumulates in the terminals of longer processes that are enriched in detyrosinated and acetylated tubulins. Axonal processes with the motor domain of kinesin enriched at the terminals exhibit lower retraction rates. Furthermore, local inhibition of kinesin function by chromophore-assisted light inactivation increases the retraction rate of the target process but not that of the neighboring process [11]. These results suggest that a process-dependent difference in microtubule stability within a single arbor contributes to differential terminal retraction by regulating kinesin-mediated axonal transport. Similar to a statement in the section above discussing mitochondrial sorting, kinesin transport into one side of a branch may result in the depletion of this molecule from the neighboring branch. Thus, this system may explain the mechanism by which process growth on one side of an axonal branch negatively affects growth of the spatially separated branch.

3. Conclusion

Although regulating axonal branch shape is a critical function of neurons during developmental neuronal wiring well as in neural plasticity, the intracellular systems involved in this function have not been fully elucidated. Three different intracellular systems contributing to differential branch growth in a neuronal arbor are discussed herein. It is known that the anterograde transport of mitochondria is mediated by kinesin and that Ca^{2+} signaling regulates kinesin functions, including mitochondrial trafficking. Thus, these systems may communicate with one another. To fully understand the intracellular mechanisms by which neurons elaborate their axonal arbors, it will be important to demonstrate the precise molecular links among these machineries as well as to determine the additional molecules involved in this process.

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