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Review

Growth control mechanisms in neuronal regeneration

Ella Doron-Mandel, Mike Fainzilber, Marco Terenzio *

Department of Biological Chemistry, Weizmann Institute of Science, 76100 Rehovot, Israel

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ABSTRACT

Neurons grow during development and extend long axons to make contact with their targets with the help of an intrinsic program of axonal growth as well as a range of extrinsic cues and a permissive *milieu*. Injury events in adulthood induce some neuron types to revert to a regenerative state in the peripheral nervous system (PNS). Neurons from the central nervous system (CNS), however, reveal a much lower capacity for regenerative growth. A number of intrinsic regeneration-promoting mechanisms have been described, including priming by calcium waves, epigenetic modifications, local mRNA translation, and dynein-driven retrograde transport of transcription factors (TFs) or signaling complexes that lead to TF activation and nuclear translocation. Differences in the availability or recruitment of these mechanisms may partially explain the limited response of CNS neurons to injury.

1. Introduction

Neurons extend their axons over great distances during development to make contact with their targets. This is achieved with the help of many signaling pathways and within a growth-favoring *milieu*. However, after the establishment of these contacts, such intrinsic capacity is greatly reduced or lost, especially in the central nervous system (CNS) [1–3]. Often, following a traumatic event, there is the need for neurons to regenerate and revert back to an "elongation mode" that characterizes the developmental stage. While regeneration occurs in the peripheral nervous system (PNS), adult CNS neurons have a vastly reduced regeneration capacity [4]. This disparity underlies the interest in understanding the differences between these two systems in order to discover pathways that facilitate axonal regeneration.

One of the major differences between the CNS and PNS is the surrounding environment in which the injured axons try to regenerate. In the CNS many factors derived from various supporting cells, including myelinating oligodendrocytes, contribute to the creation of a growth-inhibitory environment after injury either by forming physical barriers or, alternatively, by receptor mediated repulsion (reviewed in [5,6]). The most prominent of the latter are the extra-cellular domain of the protein Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte/myelin glycoprotein (OMgp), ephrin B3, ephrin A3, semaphorin 4D, semaphorin 5A, semaphorin 3F, as well as chondroitin sulfate proteoglycans (CSPGs) and the myelin glycolipid sulfatide [6]. In addition, in the CNS a 'glial scar' is formed upon injury by migration of astrocytes, proliferation of reactive astrocytes and accumulation of intermediate filament proteins such as the glial fibrillary acidic protein (GFAP), vimentin and others [6]. A very recent paper showed that systemic administration of a blood–brain barrier permeable microtubule stabilizing drug, epothilone B (epoB), was able to decrease the extent of scarring after spinal cord injury in rodents by interfering with the migration of scar-forming fibroblasts. This drug was also able to induce microtubule polymerization in the axon tip, consequently promoting axonal growth and regeneration, resulting in an improved motor function after the lesion [7].

All of the aforementioned processes are regarded as "extrinsic" cues that prevent CNS regeneration. In accordance with these observations, it was shown that CNS neurons are able to regenerate their axons when given a growth-permissive substrate such as a peripheral nerve graft [8] or a stem-cell derived *milieu* [9], although regeneration beyond these environments is usually very limited. However, a favorable environment is not sufficient for efficient regeneration in CNS neurons [10] and manipulations that successfully enhance axonal regeneration often require combinations of factors affecting both extrinsic and intrinsic mechanisms [11], suggesting that CNS neurons may lack intrinsic mechanisms for promotion of a "regenerative state". The ability of PNS axons to regenerate after injury presents an opportunity to study the

* Corresponding author.
E-mail address: marco.terenzio@weizmann.ac.il (M. Terenzio).

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intrinsic mechanisms underlying axonal regeneration. In this review we will explore the nature of the intrinsic and extrinsic mechanisms that lead to regeneration by comparing the differences between CNS and PNS neurons.

2. The conditioning lesion paradigm

One of the most common models that exemplify the intrinsic differences between the regenerative potential of CNS and PNS neurons is the so-called "conditioning lesion" paradigm. Dorsal root ganglia (DRG) sensory neurons are characterized by a bifurcating axon with two branches, a peripheral and a central branch. While the peripheral branch has the ability to regenerate following injury, the central process does not. Interestingly, if the peripheral branch is injured prior to the central tract lesion, centrally projecting neurites regain their ability to regenerate *in vivo* [12–14]. The "conditioning lesion effect" is observed in DRG neurons grown in culture after injury of their peripheral branch, whereby they shift from their normal highly-branched "arborizing" morphology to an elongating modality of growth [14,15]. DRG neurons cannot, however, be similarly conditioned by an injury in the central branch [15], unless the central lesion was preceded by a peripheral injury [13]. This evidence strongly suggests that, while peripheral branch injury can increase the intrinsic growth capacity of DRG neurons, the same is not true for injury of the central branch.

Findings from the conditioning lesion model raise the question: what are the molecular basis of a regenerative response that can be mounted as a consequence of a peripheral lesion, but not a central one, in the exact same cell? In the following sections of this review we will focus on several different mechanisms that coordinate the regenerative response, including calcium waves, epigenetic modifications, active retrograde macromolecular transport, transcriptional response and local protein synthesis. For each one of these mechanisms we will discuss what is known in terms of differences between PNS and CNS neurons.

3. Calcium waves

Calcium influx in the axoplasm represents a fast signaling avenue in response to injury that is able to trigger several mechanisms connected to axonal growth. The consequent inversion of the normal calcium/sodium flux creates a depolarization that is propagated along the axon all the way to the cell body [16]. In *Caenorhabditis elegans* the amplitude of such depolarization waves may correlate positively with the extent of sensory neuron regeneration, while the opposite is also true, in that inhibition of calcium signaling reduces the regenerative potential of injured axons [17]. Interestingly, the underlying mechanism inducing a calcium wave in response to injury between central and peripheral neurons might differ greatly. For instance, while it was shown that in cortical neurons the generation of a calcium wave requires both calcium and sodium voltage-dependent channels [18], in sympathetic neurons there is no such dependency on sodium channels [19].

Changes in intracellular calcium levels activate downstream effectors that in turn regulate regeneration. One of the most prominent calcium responsive mechanisms is the activation of adenylate cyclase (AC) and the subsequent raise in cyclic AMP (cAMP) levels. Indeed, local increase of cAMP influences the establishment of a functional growth cone after axotomy. In *C. elegans* sensory neurons calcium-dependent enzymes lead to an increase of cAMP, which promotes the rearrangement of the cytoskeleton needed for the growth cone assembly [17]. In rodents, activation of extracellular-signal regulated kinase 1,2 (ERK) is required for the formation of a competent growth cone after axotomy in dorsal root ganglia axons, while depletion of extracellular calcium or the inhibition of cAMP – protein kinase A (PKA) significantly impair this process [20]. Similar results have also been reported in *Aplysia*, where the assembly of an effective growth cone machinery, which is able to initiate axonal regeneration, is dependent on calcium influx [21]. Indeed, structural organization of the cytoskeleton at an axonal lesion site is altered by conditions that limit calcium influx [21]. Such alterations cause delays in the fusion of anterogradely transported vesicles to the plasma membrane of the cut axonal end, affecting the ability of the growth cone to regenerate [21]. Along the same lines, cAMP promotes regeneration in peripheral sensory neurons, where its levels are elevated after injury [22].

It is not clear, however, whether cAMP can contribute to CNS neurons regeneration. The effect of cAMP on the regeneration of sensory neurons after spinal cord lesion was modest in comparison to its effects after peripheral injury in the same cell type [23]. In retinal ganglia cells (RGCs) cAMP was suggested to facilitate cell survival rather than axonal regeneration [24], and to play a role in modulating inflammation-induced regeneration, through its effects on oncomodulin binding in the retina [25].

A recent study has determined that the release of calcium from internal stores is essential to the generation of a calcium wave after nerve injury of mouse sensory neurons (Fig. 1A) [26]. Furthermore, the back-propagating calcium wave following axonal injury in DRG neurons arrives all the way to the soma and causes nuclear export of the histone deacetylase 5 (HDAC5) in a protein kinase Cμ (PKCμ) – dependent manner (Fig. 1A). This event facilitates axon regeneration *in vitro* and *in vivo* by leading to an increased acetylation of histone H3, thus inducing the up-regulation of regeneration-associated genes (RAGs) such as Jun, KLF4, KLF5, Fos, and Gadd45a [26] (Fig. 1A). An interesting suggestion is that this early calcium-dependent mechanism of HDAC5 nuclear export primes the neuronal cell body for a second slower signaling dependent on retrograde transport along microtubules in the axon [26] (see below). In addition, following its injury-induced nuclear export, HDAC5 is transported to axon tips where it accumulates, ultimately resulting in local deacetylation of tubulin, which in turn promotes growth-cone dynamics and axon regeneration [27]. Interestingly, the aforementioned PKCμ activation and increased histone acetylation was not observed in RGCs; and HDAC5 accumulation and consequent tubulin deacetylation was not detected in the axon tips of RGCs [26,27]. These observations support the notion that one or more of the steps in the cascade of events stemming from the back-propagating calcium wave to the activation of PKCμ and nuclear export of HDAC5 differ between CNS and PNS neurons. The exact steps underlying the disparity between the two systems remain, however, to be determined.

A key difference between the central and the peripheral response to axonal injury is the tendency of central axons to form a retraction bulb, while peripheral axons form a growth cone that enables regeneration a short time after injury [28]. This differential response seems to be mediated by the dynamics of microtubules leading to stabilization (growth cone) in the case of the PNS and de-stabilization (retraction bulb) in the case of the CNS [29]. In *Aplysia* neurons, axonal lesion was shown to cause a re-orientation of microtubule polarity at the cut end, which supports the sorting and concentrating of different membrane resources to specific sites on the injured axon, thereby transforming the axonal stump into a motile growth cone [30]. While a detailed overview of this topic is outside the scope of the review, we will mention that the major identified effectors of this differential response are the histone deacetylases HDAC6 [31], HDAC5 [27] and the kinesin family member KIF3C [32]. Specifically, axonal injury triggers microtubule deacetylation in PNS but not in CNS neurons [27]. Histone deacetylation has been suggested to be connected with microtubule dynamics, which are essential for the

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