Annals of Anatomy 193 (2011) 248–258

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Transcribing the path to neurological recovery—From early signals through transcription factors to downstream effectors of successful regeneration

Gennadij Raivich [a,b,*]

[a] Perinatal Brain Repair Group, Department of Obstetrics and Gynaecology, University College London, UK
[b] Department of Anatomy, University College London, UK

ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form 16 January 2011
Accepted 19 January 2011

Keywords:
Nerve regeneration
Transcription factor
Adhesion molecule
Guidance
Peripheral target
Neuropeptide
Cytoskeleton

SUMMARY

The peripheral nervous system is known to regenerate comparatively well and this ability is mirrored in the de novo expression or upregulation of a wide variety of molecules involved in axonal outgrowth starting with transcription factors, but also including growth-stimulating substances, guidance and cell adhesion molecules, intracellular signaling enzymes and proteins involved in regulating cell-surface cytoskeletal interactions. Recent studies using pharmacological agents, and global as well as neuron-selective gene inactivation techniques have shed light on those endogenous molecules that play a non-redundant role in mediating regenerative axonal outgrowth in vivo. The aim of the current review is to sketch the sequence of molecular events from early sensors of injury to transcription factors to downstream effectors that cooperate in successful regeneration and functional recovery.

1. Introduction

That injured peripheral nerves are capable of gradual regeneration and recovery following trauma in humans as well as other mammalian species has been known for several centuries, and sets it apart from the central nervous system, where outright regeneration normally does not occur (Ochs, 1977; West, 1978). In the successfully regenerating peripheral nervous system, the injured neuron elicits a sequence of molecular, ultrastructural and cellular responses that are associated with brisk neurite outgrowth, reinnervation of the target tissue and recovery of function. The key questions from the molecular stand point are what drives these changes, how are they initiated, and how are they translated into a functional regeneration programme.

On the structural level, the neuronal injury response is deducible from striking shifts in the cellular organization of the damaged but surviving, axon-bearing cell. There is a rapid appearance of growth cones at the proximal tip of the lesioned axons, the swelling of the neuronal cell body associated with a strong increase in cellular metabolism and protein synthesis, and the augmentation and regional dispersion of areas of rough endoplasmic reticulum, the so called Nissl bodies (also known as Nissl substance, shoals and granules or the tigroid substance), in neuronal cytoplasm. These latter changes which are easily visible at the light microscope level using basophilic dyes were termed 'chromatolysis' or 'chomatolytic reaction' by Franz Nissl at the end of the 19th century (Nissl, 1894), a term that is still used as a synonym of cellular and molecular changes in lesioned neurons or retrograde reaction following axonal injury (Lieberman, 1971; Kreutzberg and Raivich, 2000).

Over the last 40 years, these morphological changes were gradually complemented by increasing knowledge about the molecular components of the regenerative response. There is a new or at least enhanced synthesis of adhesion molecules, growth associated proteins and structural components needed for axonal elongation. There is also renewed expression of growth factors, cytokines, neuropeptides and other secreted molecules involved in cell-to-cell communication, which may be involved in the activation of neighbouring non-neuronal cells around the cell body of the injured neuron and in the distal nerve fibre tracts.

Interestingly, a large part of this non-neuronal cellular response around the injured neuronal cell body may be associated with the immune surveillance of the injured nervous system that has emerged during the evolution with the need for strategies to prevent the spread of infection (Thomander et al., 1988; Raivich et al., 1999), even when these strategies interfere with the regenerative process. This does not mean that peripheral nerve injuries in human patients, performed in the laboratory or observed in the clinic, are

* Correspondence address: Perinatal Brain Repair Group, Department of Obstetrics and Gynaecology, University College London, 86-96 Chenies Mews, WC1E 6HX London, UK. Tel.: +44 207 679 6068; fax: +44 383 7429.
E-mail address: g.raivich@ucl.ac.uk

0940-9602/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.aanat.2011.01.010

per se not sterile – in almost all cases they are sterile from the beginning, or rapidly become sterile thereafter. However, it has been hypothesized that the neuroimmune surveillance following nerve injury – a finding observed across many different species has emerged, has partly developed to deal with potential neural infections, occurring in the wild.

In many cases, the function of many of the neuronal regeneration-associated components could be partially deduced from their effects and interactions in the cell culture models. The growing availability of pharmacological agents and animal models in which the respective genes are inactivated through global, cell-type specific or conditional mutations has also meant that their effects could also be validated in vivo.

Generally, these studies also reveal that there are a multitude of involved components, some with moderate, some with a more major role that could produce an overlapping network of regenerative response. Thus, the key questions in understanding these changes from the functional and molecular stand point are what drives these changes and how are they brought about to result in successful regeneration. The current article will review the molecules involved, from early sensors, to transcriptional changes in cell body, and finally the appearance of downstream targets mediating axonal growth.

2. Axonal injury sensors

The injured nerve appears to provide 3 separate sets of injury signals to the affected neurons, which are schematically summarized in Fig. 1, filling out complementary or potentially synergistic roles (Ambron and Walters, 1996):

(1) Disruption of axonal continuity interferes with the retrograde flow of trophic signals from the normal innervation target, including neurotrophins, their receptors, and their downstream signals (Raivich et al., 1991; Shadiack et al., 2001).
(2) It exposes the tip of the injured axon to the intracellular content of neighbouring axons and Schwann cells, e.g. trophic molecules such as ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), neurotrophin-3 (NT3) and fibroblast growth factors/FGFs (Eckenstein et al., 1991; Funakoshi et al., 1993; Rao et al., 1993; Sendtner et al., 1996, 1997; Kirsch et al., 2003), as well as somewhat later to the extracellular environment of the inflamed neural tissue (Lindholm et al., 1987). Overall, similar signals appear to be used in the autonomic and the somatic – sensory and motor – parts of the peripheral nervous system. For example, LIF is an essential injury signal for sympathetic neurons (Rao et al., 1993), but it's also important for survival and regenerative response in motor (Sendtner et al., 1996) and sensory (Sun and Zigmond, 1996; Ekström et al., 2000; Cafferty et al., 2001) neurons.
(3) It causes a rapid entry of extracellular ions such as calcium and sodium through the transiently opened plasma membrane, before it is resealed (Yoo et al., 2003), which results in depolarization and a train of injury-induced action potentials (Berdan et al., 1993). The influx of calcium and attendant activation of intraaxonal proteases and cytoskeleton remodeling underlie growth cone formation (Spira et al., 2001), and may also be involved in changing the intra-axonal protein synthesis (Zheng et al., 2001).

2.1. How are these signals transmitted to the cell body?

Retrograde flow of trophic signals, for example that of nerve growth factor (NGF) is a rapid process and any interference is easily noted within 12–24 h following injury (Raivich et al., 1991). As to

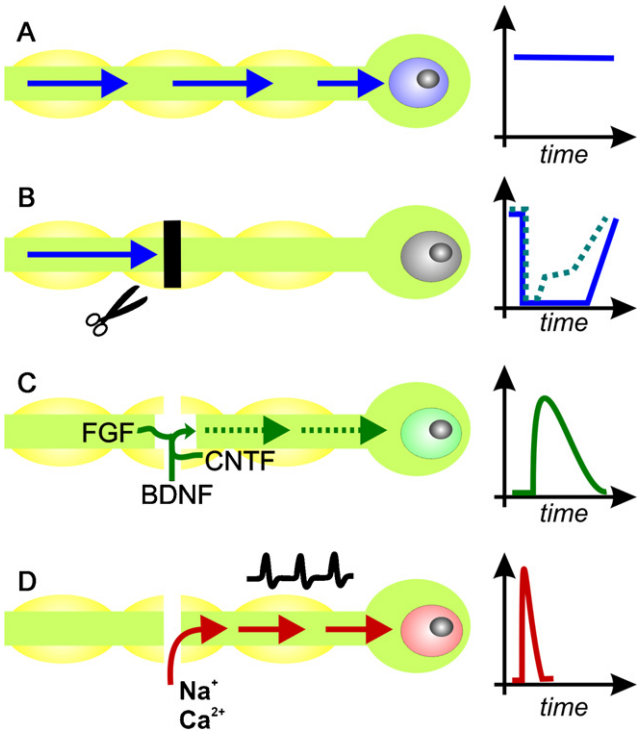

Fig. 1. Hypothetic signals in the initiation of the regenerative programme in the neuronal cell body. The *Y*-axis in the graphs on the right shows the strength of the contact and injury-associated signals arriving in the neuronal cell body and inducing regeneration. (A) In the normal neuron, retrograde signals from the peripheral target, including, but probably not limited to the neurotrophic factors, suppresses the regeneration programme. (B) This suppressive effect is lost after interruption of the axonal transport, disinhibiting the regeneration programme. After the peripheral target is reinnervated, the regeneration programme is stopped. The broken line illustrates the retrograde transport of NGF (Raivich et al., 1991). In fact, the regulation of this classical retrogradely transported neurotrophin is more complex. The complete cessation in the transport of endogenous NGF is limited to just a few days and there is a rapid recovery to intermediate levels. (C) Injury exposes the injured axons to the intracellular content of neighbouring Schwann cells and axons containing growth factors such as FGF and CNTF, immediately leaking out into the extracellular environment, as BDNF (or NGF), synthesized by local cells under inflammatory conditions (Lindholm et al., 1987; Barrette et al., 2008). (D) Rapid entry of extracellular cations (Na^+, Ca^{2+}) through the transiently opened cell membrane, causing depolarization and trains of action potentials. Reproduced from Raivich and Makwana (2007).

the other 2 sets of signals, recent studies have suggested generation and/or activation of proteins with a nuclear localization signal (NLS) sequence (Ambron et al., 1995; Hanz et al., 2003). These NLS proteins in turn link to importins, also known as karyopherins, an intermediary class of proteins that permit NLS-proteins to become attached to the retrogradely transporting dynein motors, and allow them to transfer injury signal(s) to the nucleus of the injured neuron. Interference with this importin/dynein-mediated transport of NLS proteins has been shown to strongly reduce the neurite outgrowth from cultured adult neurons from previously unperturbed sensory ganglia, as well as the much more vigorous outgrowth from cultured neurons that were conditioned by peripheral injury (Hanz et al., 2003).

Electrical signaling: Interestingly, early chromatolytic changes in the cell body of axotomized neurons may precede the arrival of molecules travelling by rapid axonal transport and appear to be mediated by the injury-induced discharges (Huppenbauer et al., 2001). Implicated changes include the rapid elevation of calcium and cAMP (Ambron and Walters, 1996), the transcriptional activation and processing of ribosomal RNA (Kinderman et al., 1998) and the increase of gap junction proteins such as connexin-43 in neighbouring astrocytes (Rohlmann et al., 1994). Inhibition of