Neuro-regeneration: plasticity for repair and adaptation

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Introduction

In order to process information in a manner that is meaningful and useful to the organism, the nervous system is ‘wired’ with remarkable precision. It now seems that the precision of neuronal connections is due largely to the early acquisition of distinct phenotypes by differentiating neurons, which in turn are predictive of neuronal projections and connectivity [1–3]. These neuronal phenotypes are thought to be determined by defined combinations of transcription factors. In olfactory neurons, for example, predetermination extends to the level of groups of neurons expressing single specific receptors, whereas, for spinal cord motoneurons, identity may define groups of neurons with specific neurotransmitter and electrophysiological properties that project to the same muscle, or even to a particular subcompartment of the same muscle. Just as neurons appear to know early on who they are and how they are to connect, they also express sets of genes associated with axon, dendrite and synapse formation at defined times during development. In particular, genes associated with axon formation and elongation are down-regulated either when the target region is reached or when the process of target innervation is completed [4]. The significance of these identity- and intrinsic programme-based properties of the neuronal phenotype for neuro-regeneration is that appropriate programmes of gene expression may have to be re-activated in adult neurons in order to allow regeneration [5,6]. In addition, regeneration and plasticity are likely to be influenced by differences in neuronal phenotype.
Although the major phases of wiring and synapse formation are restricted to specific stages of neural development, the adult nervous system is capable of dramatic activity-regulated plasticity. Ongoing physiological plasticity in the adult is essential for learning and memory. The corresponding long-term changes in circuit activity and synaptic strength probably involve persistent alterations in the molecular composition of affected synapses, but may also involve alterations and remodelling of synaptic structures. In addition, the adult nervous system can carry out extensive re-organization of synaptic circuits in order to adapt to drastic alterations in the pattern of input activity. One well-characterized example is the induction of small experimental scotomas in the eye [7,8]. In these experiments, a circumscribed patch of photoreceptor cells is lesioned, without damaging retina neurons and in particular the retinal ganglion cells that carry visual information from the eye to the brain. Due to the particular synaptic organization of the visual system, such lesions result in the electrical silencing of a corresponding patch of retinal ganglion cells in the neuroretina and of cortical neurons in the primary visual cortex. As a consequence of this inactivation, adjacent projections in the visual cortex expand, the affected cortical patch becomes driven by these neighbouring inputs and the map of the visual world in the primary visual cortex acquires a corresponding distortion. In the visual cortex the blind spot in the visual field is refilled and, depending on the size of the scotoma, the animal can learn to reconstruct and process accurate images of the visual world, i.e. it adapts. Such rearrangements can be reversible; they can also have dramatic non-adaptive consequences, as seen, for example, in phantom limb pain. The mechanisms underlying this plasticity involve the activation of pre-existing but largely silent synaptic connections [7], and possibly also local nerve sprouting and the formation of new synaptic connections [8]. In the context of neuro-regeneration, these findings emphasize the fact that neuronal circuits have a strong capacity for functional rearrangements in the adult. Important issues include the mechanisms that allow, promote and regulate the specificity of such plasticity. Furthermore, are there plastic states in neurons just like there are axon elongation modes, and are there differences in the plasticity competence of neuron types and neural networks?

A critical aspect of neuro-regeneration is the long-distance regrowth of lesioned axons in order to restore functional connections between processing areas that have been disconnected by the lesion. This regenerative process is efficient during development, when gene expression for axonal growth is turned on and the local environment is favourable. In lower vertebrates, efficient regeneration of lesioned axons is also observed in the adult. In adult mammals, however, such regeneration is essentially restricted to the peripheral nervous system (PNS) (Figure 1). Since there may have been little evolutionary pressure to maintain axonal regeneration in the adult central nervous system (CNS), suppression of axonal regeneration may have evolved as a mechanism to confine structural plasticity to appropriate target regions. In addition to axonal regener-
Reactions of adult neurons to axotomy

A lesion of the axon (axotomy) is a traumatic event for neurons that can initiate major changes in gene expression in the cell body [5,6]. Except for a brief period late in development, when for poorly understood reasons lesions induce massive neuronal death, PNS neurons react to axotomy by a switch in their biosynthetic machinery and the re-expression of genes associated with axon formation (see below). This biosynthetic switch or chromatolysis reaction involves a major rearrangement of the endoplasmic reticulum and Golgi apparatus that is presumably related to the transition from a secreting to a growing cell. The signals that initiate this switch in neuronal phenotype are not understood, but may involve a lesion-induced interruption of retrograde inhibitory signalling from the axon periphery to the cell body. In marked con-
Contrast with PNS neurons, axotomy in adult CNS neurons of higher vertebrates can induce substantial cell death [9]. Interestingly, susceptibility to axotomy-induced cell death varies significantly among different CNS neurons, is much more pronounced when axons are lesioned in the vicinity of the cell body, and seems to correlate with the intensity with which the cell body responds to the injury [10]. Unfortunately, reaction intensity also correlates with the potential of CNS neurons to initiate axonal regeneration [10]. As discussed below, this paradoxical relationship between susceptibility to apoptotic cell death and the transition to an axonal growth mode is a major obstacle to axonal regeneration in the adult CNS in higher vertebrates.

Axonal growth during development and regeneration correlates with the expression of a distinct group of genes in neurons. The term ‘growth-associated proteins’ (GAPs) initially referred to proteins that are greatly induced and rapidly transported in regenerating nerves, thus possibly playing a direct role in axon elongation [5]. The neural protein GAP-43 [4] is possibly the best known example of such a GAP. GAP-43 is a major protein kinase C substrate and calmodulin-binding protein in the brain. It accumulates selectively at the plasma membrane of axons, where it affects growth cone activity and vesicle fusion through mechanisms that have not yet been elucidated. The current list of known GAPs includes a variety of vesicle- and/or membrane-associated proteins, including cytoskeleton-regulating proteins (e.g. SCG-10), cell adhesion molecules [e.g. N-CAM (neural cell adhesion molecule)], receptors for extracellular-matrix proteins (e.g. \( \beta_1 \)-integrin), the low-affinity nerve growth factor receptor, and non-receptor tyrosine kinases (e.g. c-Src). Further genes and proteins associated with axon growth include cytoskeletal proteins such as actin and tubulin isoforms, and transcription factors such as c-Jun [5,10].

A combination of PNS-graft regeneration experiments (see also next section) and the application of molecular markers for axonal growth has provided valuable insights into the possible relationships between the expression of GAP genes and the actual growth process. These experiments have revealed a strong correlation between the expression of GAP-43 and competence for axonal growth. In one set of experiments, lesioned optic nerve axons only grew into peripheral nerve grafts when a lesion was applied within about 1 mm of the eye [11]. Unfortunately, such lesions induce massive death of retinal ganglion cells, i.e. the neurons that convey all information from the eye to the brain. Lesions further into the CNS had far less devastating consequences for retinal ganglion cell survival, but resulted in the complete absence of axonal regeneration into grafts [12]. Apparently, an intense cell body reaction is required for regeneration, but this can also lead to apoptosis. GAP-43 expression was only detected in a subpopulation of lesioned ganglion cells, and only with proximal lesions [12]. Labelling of regenerating axons with lipophilic dyes that diffuse back into the cell body (retrograde labelling) revealed that the few ganglion cells that extended axons into the grafts all expressed elevated levels of GAP-43, suggesting that a cell body reaction visualized by the expression of this GAP may be
causally associated with successful axon elongation. However, with such lesions GAP-43 induction was also detected in the absence of the graft [13], and thus of effective regeneration. Many neurons that expressed GAP-43 did not regenerate, indicating that the expression of GAP-43, and presumably also of further growth-related genes, is not sufficient for nerve regeneration.

Strong evidence in support of a link between a cell body reaction (defined by the induction of GAP-43) and the vigour of axon elongation into a peripheral nerve graft was provided by experiments with dorsal root ganglion (DRG) neurons. The axon of these PNS neurons bifurcates close to the cell body, giving rise to branches growing to the periphery and into the CNS. Peripheral root lesions lead to robust induction of GAP-43 and nerve regeneration, whereas dorsal root lesions lead to very poor regeneration, no growth of axons into the spinal cord and no detectable induction of GAP-43. Application of a peripheral nerve graft next to a lesioned dorsal root results in the slow growth of processes into the graft, and in a delayed but significant induction of GAP-43 [14,15]. Crushing of the peripheral nerve branch of the same DRG neurons at the time of nerve grafting to their dorsal root led to greatly accelerated process growth into the graft and to rapid induction of GAP-43. Therefore a vigorous cell body reaction induced by a peripheral root lesion and reflected by the induction of GAP-43 mRNA significantly accelerates the elongation of dorsal root axons in the nerve graft [15]. While the peripheral nerve lesion induced rapid and transient GAP-43 expression in a very large number of DRG neurons, it did not lead to a comparable elevation in the numbers of dorsal root axons growing into the peripheral nerve graft [15]. As with the retinal ganglion cell experiments mentioned above, the peripheral nerve lesion apparently affected growth vigour, but not the absolute numbers of dorsal root axons able to regenerate into the graft. It will be important to determine whether the absence of regeneration in lesioned and GAP-re-expressing CNS neurons is due to some particular intrinsic properties of these neurons or, alternatively, to lesion-related unfavourable properties of their local environment.

**Role of extrinsic factors in axonal regeneration**

Pioneering experiments by Albert Aguayo’s group have established that the local environment in the adult CNS of higher vertebrates plays a major role in preventing axonal regeneration after a lesion [16] (Figure 2). Thus not only do central neurons regenerate axons into a peripheral nerve graft, but peripheral axons fail to regenerate axons into a central nerve graft. Several factors are responsible for these differences. The main growth-promoting activity in peripheral nerve explants is due to living Schwann cells activated by the absence of axon contact. In contrast, CNS nerves prevent axon growth due to inhibitory activities associated with oligodendrocytes and their product, CNS myelin [17]. In addition, lesioned peripheral nerves contain higher levels of growth-promoting neurotropic factors than do lesioned central nerves. The experiments
with peripheral nerve grafts also revealed the existence of intrinsic differences in the regenerating ability and vigour of lesioned axons. Thus certain types of neurons, e.g. cerebellar Purkinje cells [18], consistently failed to regenerate axons into peripheral nerve grafts.

In addition to differences in growth-promoting and -inhibitory activities in peripheral compared with central nerves, the formation of a glial scar, mainly associated with reactive astrocytes, is a major factor that prevents regeneration in the CNS [19]. Recent studies have provided evidence that it is not the astrocytes themselves, but the appearance of inhibitory proteoglycans in the extracellular matrix associated with scar material, that prevents axon growth. Thus in two models of axonal growth in the adult CNS, i.e. growth upon microlesions and extension of axons by adult DRG neurons implanted into the corpus callosum of an adult rat, failures coincided spatially with the presence of proteoglycans [19]. Remarkably, implanted adult DRG neurons extended long axons into the host CNS white matter, suggesting that inhibitory factors from oligodendrocytes may inhibit but do not suppress axonal elongation (see next section). A recent study suggests that deposition of inhibitory proteoglycans may be triggered by blood-derived factors that would invade the CNS as a consequence of a local breakdown of the blood–brain barrier. Clearly, the identification of signalling pathways that regulate the production of such inhibitory molecules could have major clinical implications for promoting regeneration in the CNS. In addition to the neutralization of oligodendrocyte inhibitors and the prevention of inhibitory proteoglycan production, grafting of glial cells that promote and guide axon growth also promises to provide substantial benefits for regenerating axons. In particular, a recent study pro-

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**Figure 2.** Intrinsic (blue) and extrinsic (black) factors that promote regeneration (top) and local nerve sprouting (bottom) in the adult

Abbreviations: PSA, polysialic acid; CAP-23, cortical cytoskeleton-associated protein of 23 kDa.
vided evidence that glial cells derived from the olfactory system not only greatly promote axon elongation but also help regenerating axons to manage the difficult transition into the CNS environment [20].

**Role of intrinsic neuronal components in axonal regeneration**

Experimental evidence supporting the view that neurons express axonal growth programmes during development, which are greatly attenuated or absent in the adult, derives from co-culture and transplant experiments that used combinations of neurons and tissues from different developmental stages. In one dramatic type of experiment, dissociated embryonic neurons were transplanted into lesioned adult rat brains [21]. When very young neurons were transplanted, they grew long processes in the pre-lesioned adult host brain. Clearly, this is in marked contrast with the lesioned host neurons. In these experiments, extensive growth was detected along myelinated tracts in the CNS. These experiments also provided evidence that grafts of dissociated embryonic neurons may integrate functionally into the host brain, thus providing a potential strategy to counteract functional deficits in certain neurodegenerative diseases, e.g. Parkinson’s disease. The application range of such graft strategies is, however, presently limited by the poor survival of transplanted neurons. The actual intrinsic neuronal mechanisms that allow young neurons to grow in an adult CNS environment are not clear. Neurons may differ in general axonal growth programmes that define an elongation mode or elongation vigour. An alternative view is that neurons differ in their expression profiles for the machinery that detects and amplifies guidance signals from the environment. *In vitro* experiments with dissociated neurons and CNS white matter sections, for example, suggest that young neurons may be less sensitive to inhibitory proteins on the surface of oligodendrocytes [17].

Graft regeneration experiments have provided molecular evidence for at least two classes of genes associated with axonal elongation [13]. Thus, while GAP-43 expression was correlated with elongation competence, levels of β-tubulin transcripts were only elevated when actual elongation into the peripheral nerve graft took place. This is reminiscent of the regulation of these transcripts during development. In this case, tubulin and actin transcript levels correlate with axon elongation, whereas GAP-43 expression can persist at high levels during a protracted period of target innervation, when axons arborize extensively within the target region, where they form increasing numbers of synaptic connections. These findings provide valuable molecular correlates for at least two intrinsic growth states in developing and regenerating neurons.

Explant co-culture experiments using hamster retina (as the source of axons) and midbrain tectum (the target region) have provided an *in vitro* experimental system with which to dissect the contributions of intrinsic neuronal properties and the local environment in axonal regeneration [22]. The main finding of these studies was that, while embryonic (day 15) and day 0
postnatal retinal axons regenerated into tectum of any age, including adult, most axons from day 2 postnatal and older retinae failed to grow into tectal explants. Significantly, even embryonic tectal explants failed to attract axon growth from day 2 postnatal and older retinae. These findings provide evidence for a dramatic developmental decline in the intrinsic ability of retinal axons to elongate in tectal explants. Neurons from older retinae did, however, grow processes inside the retina explants, and it was mainly growth into the target region (i.e. tectal) explants that was absent. Therefore a remaining question is the extent to which these findings reflect differences in axon growth per se, as opposed to growth into CNS explants. The molecular mechanisms that control an intrinsic elongation programme in neurons may be linked to the anti-apoptotic protein Bcl-2 [23]. Thus the expression of Bcl-2 was apparently necessary and sufficient to promote the elongation of retinal ganglion cell axons in mice. This effect of Bcl-2 was apparently not simply due to its anti-apoptotic properties, suggesting that a molecular switch to an axonal elongation mode in neurons requires Bcl-2. Overexpression of Bcl-2 failed, however, to completely rejuvenate neurons, as late embryonic and early postnatal neurons failed to grow into adult tectum even in the presence of excess Bcl-2. Elucidation of the relative contributions of further intrinsic growth-regulating mechanisms and inhibitory signals in the adult environment may provide crucial leads towards promoting nerve regeneration in the adult CNS.

Factors that control nerve sprouting and synaptogenesis in the adult

Nerve sprouting is involved in target innervation during development, de-afferentation-induced repair, and possibly also use-related plasticity in the adult [6]. During development, when incoming axons reach their target region they branch extensively to establish a large number of synapses. This process of terminal arborization can be preceded by a waiting period, when no axonal growth is detected. At least in some systems, synaptogenesis proceeds for a protracted period of time, as more target sites are added through the generation of additional postsynaptic neurons and the growth of dendrites. During this process, innervation involves sprouting of collaterals, at a time when axonal elongation and the expression of several GAP genes have subsided. Towards the end of this period excess collaterals are eliminated through an activity-dependent process that produces a quantitative refinement of synaptic connections. Synapse elimination can proceed as new target sites are innervated by sprouting collaterals, i.e. there is some overlap in time between the two processes. This suggests that nerve sprouting and synapse retention are not governed by the same factors.

In the adult PNS and CNS, reactive nerve sprouting is a major adaptational mechanism to compensate for lesion-induced de-afferentation. Possibly due to specific contact-mediated inhibition, and like collateral sprouting during
development, sprouting in the adult is confined to specific target areas [24]. In neurodegenerative diseases, re-innervation through sprouts from neighbouring neurons can effectively delay the appearance of detectable deficits. It is presently not clear whether, and to what extent, local nerve sprouting in the adult also contributes to activity-dependent network plasticity in the absence of local de-afferentation. Studies initiated in *Aplysia* and recently extended to rodents have provided evidence that activity-regulated alterations in synaptic structure may be a substrate for persistent modifications of synaptic function [25]. Whether presynaptic expansion at synaptic structures may be related to nerve sprouting, and whether new synaptic connections are formed in the adult in the absence of physical de-afferentation, remain to be determined.

All forms of neurite outgrowth, including terminal arborization and nerve sprouting, are induced and guided by extrinsic signals from the local environment [26]. For example, nerve sprouting *in vivo* can be induced by the local application of neurotropins or other neurotropic factors. Although the actual functional roles of such mechanisms in nerve sprouting and terminal arborization are not yet clear, diffusible factors could trigger sprouting, or even attract sprouts by chemotropic mechanisms. Such a mechanism is likely to induce the sprouting of collaterals that is involved in the innervation of several types of targets during development [26]. In addition to diffusible activities, contact-mediated mechanisms play a critical role in nerve sprouting. This is particularly well documented for the adult neuromuscular junction, where sprouts are induced and guided by the processes of activated Schwann cells [27]. The neuromuscular junction must be viewed as a functional unit involving three cellular elements: the presynaptic motor nerve terminal, the postsynaptic skeletal muscle fibre, and a cluster of synapse-associated glia, the terminal Schwann cells. The involvement of the terminal Schwann cell is based on the facts that it senses and reacts to transmitter release and that it interacts selectively with the synaptic extracellular matrix and nerve processes. Similar cellular arrangements are found at certain types of central synapses, but it is not clear whether astrocytic processes can regulate nerve sprouting in the CNS.

In addition to factors in the environment, intrinsic neuronal components also affect the competence of nerves to sprout in the adult. Thus transgenic mice overexpressing GAP-43 selectively in adult neurons exhibit dramatic spontaneous nerve sprouting [28]. To determine whether GAP-43 produced a true gain-of-function phenotype, toxin- and lesion-induced nerve sprouting was compared in control and transgenic mice. To allow for an assessment of the role of GAP-43 in induced sprouting, the experiments were carried out under conditions that also lead to nerve sprouting in the absence of GAP-43. These experiments revealed a dramatic potentiation of induced sprouting in the presence of GAP-43, indicating that this GAP is an intrinsic determinant of nerve sprouting [28]. Whether neurons that maintain substantial levels of GAP-43 expression in the adult also have a greater potential for nerve sprouting remains to be determined. A similar study provided evidence that CAP-23
(cortical cytoskeleton-associated protein of 23 kDa) is a growth-associated protein with sprout-promoting properties similar, but not identical, to those of GAP-43 [29]. GAP-43 and CAP-23 are locally abundant and highly regulated proteins that share a number of biochemical and cell biological properties, and are expressed in partially overlapping subsets of neurons in the adult. One further neuronal protein that may be viewed as an intrinsic determinant of nerve sprouting is the polysialylated form of the cell adhesion protein N-CAM. The differential expression of such intrinsic determinants of nerve sprouting may affect both the pattern and the regulation of nerve sprouting during development and in the adult.

**Perspectives**

The recent advances in identifying and counteracting factors that prevent the regeneration of lesioned axons in the adult CNS of higher vertebrates have uncovered an astonishing capacity for local use-dependent plasticity of neuronal circuits in the adult. Such plasticity is apparently responsible for the observation that regeneration of only 1–5% of the axons from a completely lesioned fibre tract, e.g. the cortico-spinal tract essential for voluntary motor control, can be sufficient to induce remarkable restitution of function. Interestingly, functional restitution is detected mainly for tasks that involve simple synaptic circuitry, whereas more complex functions are not or only poorly recovered. Observations made on the restitution of Parkinson-type deficits upon implantation of dopaminergic neurons into the striatum led to similar conclusions. Clearly, therefore, very significant progress towards promoting effective neuro-regeneration in the adult CNS has been made in the last few years. While cautious optimism is no longer a matter of faith, one major challenge still remaining is to develop and refine procedures that would effectively promote axonal regeneration in patients. In addition, however, the recent findings have emphasized the need to understand the factors that control the effectiveness and specificity of structural and functional plasticity in the adult nervous system. The establishment of genetic models in mice and flies promises to provide definitive information about the mechanisms that control axon elongation and use-dependent plasticity. Such information should lead to the identification of promising targets for intervention in patients. Given the dramatic pace at which molecular and systems neuroscience are establishing common areas of research, this promises to be an exciting research field over the next few years.
Summary

- Specificity of connectivity is essential to nervous system function. It is determined by intrinsic programmes of gene expression that define neuronal phenotypes, and by activity-dependent mechanisms. Neuroregeneration in the adult may involve re-activation of growth programmes within the constraints of neuron-type specific phenotypes.
- Lesion-induced re-induction of an axonal growth mode in adult neurons correlates with a vigorous cell body reaction that can also lead to apoptotic cell death. Directing the cell body reaction towards regeneration is a major goal towards improving regeneration.
- Extrinsic factors that prevent axonal regeneration in the adult CNS of higher vertebrates include inhibitory components on the surface of oligodendrocytes and CNS myelin, and proteoglycans associated with scar material; grafts of certain glial cells can promote regeneration.
- Local nerve sprouting and synaptic plasticity can produce dramatic functional adaptation to lesions in the adult and greatly enhance the impact of the partial regeneration of lesioned axons; nerve sprouting is promoted by diffusible and contact-mediated extrinsic mechanisms, and by intrinsic neuronal components.
- As a result of recent discoveries, significant progress in promoting axonal regeneration and recovery of function in the adult can be anticipated.

References