Assembly of a new growth cone after axotomy: the precursor to axon regeneration

Frank Bradke[1], James W. Fawcett[2] and Micha E. Spira[3]

Abstract | The assembly of a new growth cone is a prerequisite for axon regeneration after injury. Creation of a new growth cone involves multiple processes, including calcium signalling, restructuring of the cytoskeleton, transport of materials, local translation of messenger RNAs and the insertion of new membrane and cell surface molecules. In axons that have an intrinsic ability to regenerate, these processes are executed in a timely fashion. However, in axons that lack regenerative capacity, such as those of the mammalian CNS, several of the steps that are required for regeneration fail, and these axons do not begin the growth process. Identification of the points of failure can suggest targets for promoting regeneration.

Following injury, axons of the adult mammalian CNS do not regenerate, but those of peripheral nerves can regrow. This difference in regenerative capacity and the hope of finding a treatment for the large number of people with disability resulting from CNS damage have motivated scientists for decades to identify the obstacles that prevent axonal regeneration. Such efforts have led to many important and fundamental discoveries, including the identification of growth inhibitory molecules in CNS myelin and glial scar tissue[1–5] and the elucidation of axonal signalling pathways[6–8].

Before a cut axon can attempt to grow through the inhibitory CNS environment, it first has to assemble a new growth cone at its tip, and it is now becoming clear that many axons in the mammalian CNS fail to complete this crucial step in the regeneration process. Indeed, after injury, CNS axons are often tipped with dystrophic end bulbs, or retraction bulbs[9,10]. These retraction bulbs, which are hallmarks of degenerating axons, are also termed 'frustrated growth cones' because they are considered to be the consequence of the failure to regenerate a growth cone.

In this Review, we will examine the steps that underlie the regenerative reassembly of a growth cone after axotomy and the reasons why this process may fail. In doing so, we will explore the intracellular differences between retraction bulbs and growth cones and ask whether the formation of retraction bulbs can be prevented and whether regeneration can be encouraged. Importantly, we will also consider whether a non-growing retraction bulb can be transformed into a competent growth cone.

[1]*Axonal Growth and Regeneration, German Center for Neurodegenerative Disease, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany.*
[2]*Cambridge University Centre for Brain Repair, Department of Clinical Neurosciences, Robinson Way, Cambridge CB2 0PY, UK.*
[3]*Department of Neurobiology, Life Science Institute, the Hebrew University of Jerusalem, Jerusalem 91904, Israel.*
Correspondence to J.W.F.
e-mail: jf108@cam.ac.uk
doi:10.1038/nrn3176
Published online
15 February 2012

Regenerative capacity after axotomy

In most invertebrate species and primitive vertebrates, axons can regenerate in both the peripheral nervous system (PNS) and the CNS[11–14]. Interestingly, in *Caenorhabditis elegans*, cut axons in young animals almost always regenerate, although as animals age, regeneration of such axons becomes increasingly less probable[14].

Most axons of the mammalian PNS also regenerate after injury and grow towards appropriate targets. Indeed, ultrastructural studies have shown that mammalian PNS axons can start to produce a new growth cone within hours of axotomy[15,16]. The advent of transgenic animals, in which subsets of axons can be labelled with green fluorescent protein (GFP), has made it possible to confirm these anatomical findings and to follow growth cone regeneration or its failure in real time. These studies have shown that following a crush lesion to mammalian peripheral nerves, many axons assemble a new growth cone and start to regrow within 24 hours, and usually within 3 hours[10,17].

Shortly after injury, most mammalian CNS axons retract from the injury site[18]. A few axons sprout for a millimetre or less, but many damaged CNS axons make little regenerative response[9,10]. The axons that have failed to regenerate usually have swollen endings that resemble the dystrophic growth cones that occur when axons are exposed to steep inhibitory substrate gradients[9]. This lack of regeneration must reflect both the intrinsic properties of the axons and their interaction with the inhibitory CNS environment[1,4]. The events that follow

axon damage have been revealed by real-time imaging of the central branches of GFP-labelled sensory axons that have been cut in the dorsal columns of mouse spinal cord[10,19,20]. After axotomy, these sensory axons retract for 200–300 μm and produce a retraction bulb. Only one-third of the cut axons then start to regenerate through the formation of a new terminal growth cone or through sprouting at a node of Ranvier[10,19]. The central branch of a sensory axon is an example of a nerve fibre with a relatively high regenerative potential[21,22]. To learn more about neurons of CNS origin, one study examined axons from the optic nerve, which have a lower intrinsic regenerative response, and showed that after damage, these axons degenerated in a similar fashion to sensory axons but did not sprout[23]. Thus, CNS injury in adult mammals usually leads to a failure to initiate regeneration because of an inability to assemble a new growth cone.

Although mammalian CNS axons do not regenerate *in vivo*, the regeneration of such axons is seen to varying degrees in various primary cell-culture models. Neurons undergo complete axotomy during the dissociation of neural tissue, and when placed in culture, these cells usually either grow an axon or die. Cultured neurons are, however, normally derived from embryos or newborn pups and hence are developing cells, and axon growth occurs from the cell body rather than from the tip of a cut axon[24,25]. As axons only contain a subset of the molecules that are found in the cell body, outgrowth from the soma may have different underlying biology to that of regeneration from the end of a cut axon.

In cultured embryonic day 18 (E18) cortical neurons, 50% of neurites regenerate after damage[26], whereas in cultured hippocampal neurons, axotomy nearly always leads to axon regeneration, with a new growth cone forming within hours of injury[27]. Interestingly, when a hippocampal axon is cut closer than 35 μm from the cell body, a former dendrite can grow to become the new axon; this is an impressive example of the plasticity of neuronal polarity[24,27–30]. Two types of neuron from the adult mammalian nervous system have been shown to survive and grow an axon *in vitro* after dissection and subsequent dissociation: sensory neurons and retinal ganglion cell neurons. After being cut, 65% of adult sensory axons regenerate their growth cone, but only 15% of adult retinal ganglion cell axons do so, indicating a difference in the intrinsic regenerative ability between these two cell types[31,32]. Of note, retinal ganglion cell axons undergo a developmental drop in their regenerative ability a few days after birth[33].

Taken together, these studies show that although adult axons in the PNS and embryonic CNS neurons form growth cones after axotomy, this capability is largely absent in adult mammalian CNS axons. Below, we explore the distinguishing intracellular events that lie behind these differences.

Local protein translation
Mammalian peripheral nervous system axons and axons in many invertebrate species contain ribosomes, messenger RNAs and a Golgi apparatus or equivalent. In such axons, proteins can be synthesized in the axon tip, and if local translation is prevented, the regeneration of a cut axon is inhibited.

Initial events after axotomy

Many of the detailed studies on the events leading to growth cone regeneration have been performed in invertebrate axons, including those of *Aplysia californica*. The great advantages of *A. californica* axons over axons from other model organisms for studies of post-axotomy events are their large size — these axons are ~20 μm in diameter — and their reliable regeneration capacity. Moreover, *A. californica* axons have the same general cytoskeletal organization as mammalian axons; that is, they have a core of microtubules that is surrounded by a submembranous actin–spectrin cortex[34,35]. After axotomy in *A. californica*, a nascent growth cone in the form of a flat lamellipodium forms within 20–30 minutes[36,37]. There are of course differences between axons from different species and parts of the nervous system, but as this Review emphasizes, the similarities are more striking than the differences, and general principles can be drawn (FIG. 1).

Another invertebrate species, *C. elegans,* also offers advantages over other species for studying the molecular events after axotomy because of the ease with which it can be genetically manipulated[8,38]. We have a less complete description of the regenerative events in vertebrate axons, but where observations have been made, the events are strikingly similar to those that occur in invertebrates. In the following paragraphs, we focus mainly on *A. californica* axons, with references to vertebrate axons and other species where appropriate.

Calcium influx

Axotomy disrupts the membrane and exposes an axon's interior to external ionic concentrations. The entrance of calcium into an axon at this point is necessary for the formation of a new growth cone in *A. californica, C. elegans* and mammalian sensory and sympathetic axons[32,39–41]. Indeed, if axotomy is performed in a calcium-free environment, none of these axons will generate a new growth cone and regeneration will fail[32,40–43].

The elevation of the free intracellular calcium concentration ($[Ca^{2+}]_i$) at both the proximal and distal cut ends of an axon triggers a number of events, including rapid sealing of the membrane, local transformation of the cytoskeleton to form a growth cone or a retraction bulb, activation of long-range retrograde molecular signalling and activation of local protein translation[31,44–46]. In addition to calcium influx down its concentration gradient through the cut axonal ends (FIG. 1a,b), axotomy leads to membrane depolarization that is sufficient to activate voltage-gated calcium channels and that further increases the $[Ca^{2+}]_i$[42,47–51]. In *A. californica, C. elegans* and mammalian neurons, local depolarization may reach the threshold that initiates trains of action potentials in those axons that support them; the action potentials then actively propagate to the cell body and to the axon terminal and further elevate the $[Ca^{2+}]_i$[40,42–51]. Following this initial burst of activity and the ensuing calcium rise, a secondary period of raised $[Ca^{2+}]_i$ may occur as a result of the failure of sodium pumps to cope with the increase in calcium load, leading to reversal of the sodium–calcium exchange pump and release of calcium from intracellular stores[51]. This release, in turn, leads to a more prolonged elevation of axonal and cell body $[Ca^{2+}]_i$.

Although axotomy generates acute trains of action potentials at high frequencies in various neuronal types,

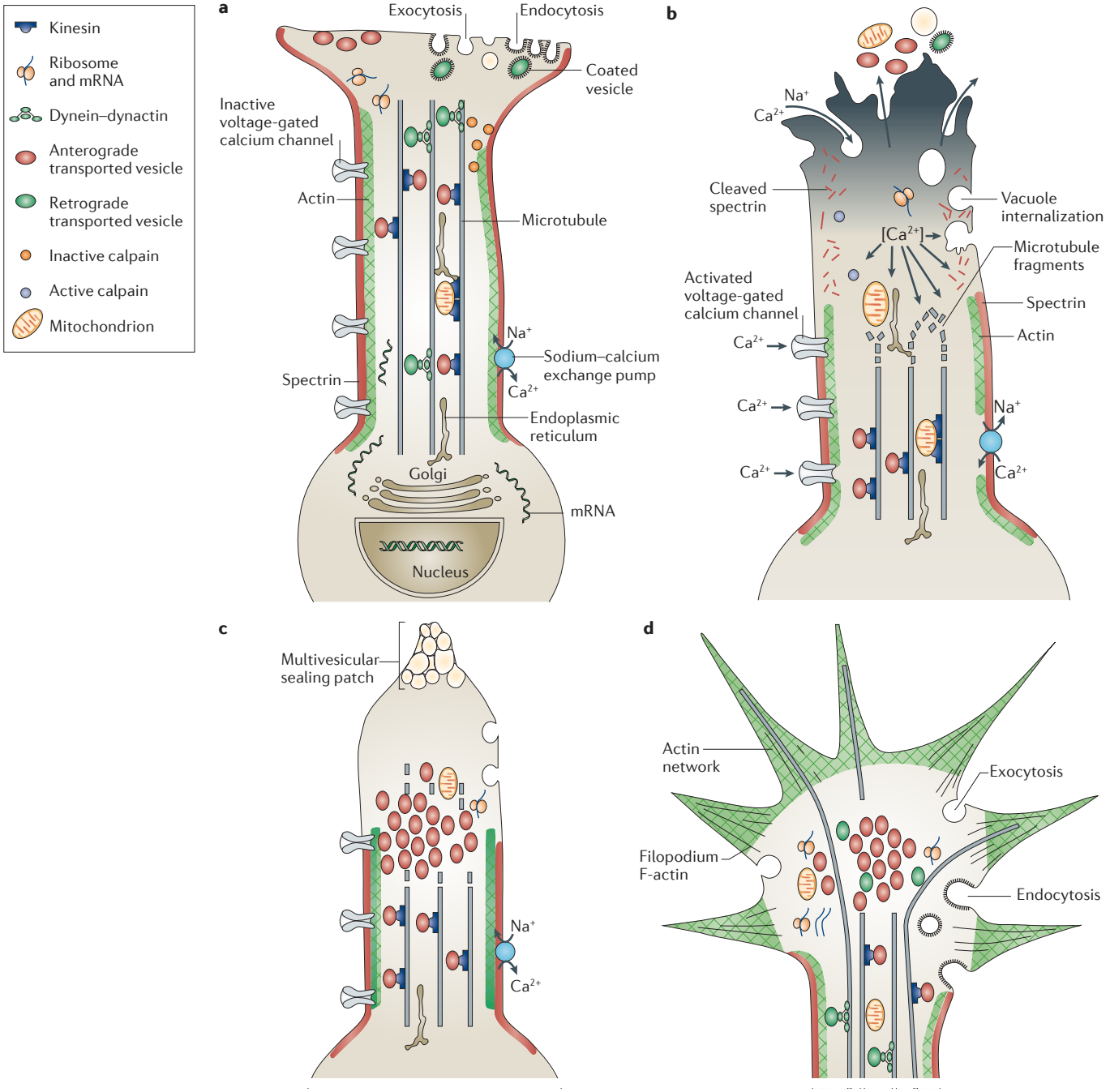

Figure 1 | **Key events in the transformation of the proximal tip of a cut axon into a growth cone. a** | Various components of the intact neuron take part in the transformation of a cut axonal end into a growth cone. **b** | Membrane rupture leads to elevation of the free intra-axonal calcium concentration (shown by the grey shading) by diffusion from the cut end, membrane depolarization, activation of voltage-gated calcium channels and release of calcium from intracellular stores such as the endoplasmic reticulum. Among many other processes, the elevated intracellular calcium concentration ($[Ca^{2+}]_i$) leads to the following: microtubule and actin depolymerization, membrane retrieval, activation of calpains (which proteolytically cleave submembrane spectrin), stimulation of vacuole internalization and activation of numerous other enzymes (not shown). These processes are confined to an axonal segment at the tip of the cut axon and lead to a small volume of the axoplasm being lost before the membrane at the cut end collapses. **c** | In most cases, the membrane of the cut end reseals by membrane collapse and accumulation of vesicles to form a sealing patch. Once a seal is formed, calcium removal mechanisms lower the $[Ca^{2+}]_i$, and this process is followed by microtubule and actin repolymerization. Anterogradely transported vesicles accumulate at the tips of the undamaged microtubule tracks. In *Aplysia californica* neurons, the vesicles fuse with the plasma membrane at regions in which the spectrin skeleton is cleaved. **d** | In the final phase of growth cone reconstruction, actin filaments assemble to generate the mechanical force at the leading edge of the lamellipodium. Microtubules polymerize and point their plus ends towards the plasma membrane. Note that proteins are translated both in the cell body (not shown) and at the growth cone.

these trains only transiently elevate the calcium level in the axons and the cell body. In fact, these $[Ca^{2+}]_i$ transients are not significantly greater than those generated by physiological burst activities. For example, in cultured rat cortical neurons, axonal transection induces a transient $[Ca^{2+}]_i$ elevation to ~1.5 μM in the cell bodies for up to several minutes[51]. This transient elevation of calcium in the cell body is similar in amplitude to that seen during trains of action potentials[52], and therefore it is unlikely to be sufficient by itself to switch the pattern of gene expression into a regenerative mode[41,52–54]. Indeed, the peripheral axons of dorsal root ganglion (DRG) neurons experience a burst of action potentials after axotomy but then show a reduction in electrical activity because their sensory input is reduced[55]. This reduction in activity, in turn, seems to increase the long-term regeneration of DRG axons. Of note, although it is reasonable to assume that axotomy leads to a transient elevation of the $[Ca^{2+}]_i$ in all neurons, axotomy-induced spatiotemporal $[Ca^{2+}]_i$ gradients probably differ between neuronal types. The rate of $[Ca^{2+}]_i$ recovery depends on the time required to generate a membrane seal over the cut end (see below), on the diameter of the axon and on adherence of the axon to the surrounding microenvironment. This rate also depends on the type and distribution of voltage-gated calcium channels that are expressed in the axon, the cell body and the dendrites, the composition and concentration of mobile and immobile calcium buffers, and the density and distribution of calcium pumps and antiporters. Thus, although qualitatively the basic phenomena of axotomy-induced $[Ca^{2+}]_i$ gradients might be similar across neuronal classes, various factors can lead to differences between cell types in such gradients that may affect regeneration potential.

Formation of a membrane seal

If an axon is to survive following axotomy, it needs urgently to regain its ionic homeostasis through the rapid repair of the ruptured membrane (FIG. 1c). Membrane sealing after injury seems to occur in two stages. First, the plasma membrane at the cut end collapses, thereby reducing the diameter of the ruptured membrane and in some cases even leading to fusion of the cut end[47]. Second, vesicles move to the ruptured plasma membrane to form multivesicular structures that fuse with the plasma membrane: the so-called sealing patch. This mechanism is conserved across species that have been examined and allows all cell types to recover from membrane ruptures under various conditions[56,57]. Upon sealing of the ruptured membrane, excess intracellular calcium can be removed.

As stated above, the incoming calcium wave is crucial for membrane sealing[47,57,58]. In low extracellular calcium concentrations (<100 μM), the membrane of a severed axon does not seal, leading to axon degeneration. The rate of membrane sealing is affected by adhesion of the membrane (via the extracellular matrix) to surrounding elements that may impede the collapse of the cut ends, and by the dynamics of a number of processes — involving calcium-activated calpain, synaptotagmin, syntaxin and synaptobrevin — that locally control the rate of cytoskeleton restructuring and assembly of the membrane patch[58,59].

Across the various species that have been examined to date, the time required for the axonal membrane to seal following injury has been reported to vary from minutes to hours[42,47,48,56,57–64]. This large variability could reflect differences in the experimental systems or the methods — such as electrophysiology and extracellularly applied fluorescent dyes — that have been used to assess the membrane seal recovery rate. Whereas electrophysiological methods provide an unbiased evaluation of membrane integrity, the uptake of extracellularly applied fluorescent dyes may reflect, to some extent, pinocytotic activity, which may persist after a membrane seal has formed[48]. Reassessment of membrane sealing using the same methods for the different axons could resolve this issue.

Transformation of an axon into a growth cone

After closure of the interrupted plasma membrane, the assembly of a growth cone apparatus can begin. The first steps of growth cone assembly depend on the calcium entry that occurs after axotomy[32,39], as highlighted above. These early regenerative events can be observed in cultured *A. californica* neurons (FIG. 1b) and involve the cytoskeleton[65]. There is a retrograde wave of microtubule depolymerization along an axonal segment of ~100 μm that corresponds in time and space to the incoming calcium wave[35–37,66]. In parallel, actin punctae in adhesion complexes depolymerize[36]. This depolymerization disengages the plasma membrane from its surroundings, creating mechanical freedom for the axonal membrane to collapse, seal and assemble the growth cone structure and machinery. In addition, the submembranous spectrin cortex at the axon tip is cleaved. Calpain proteolytic activity, which is responsible for the cytoskeletal restructuring of the cut end, reaches a peak 10–30 minutes after axotomy and then declines to baseline levels after 45–60 minutes[67]. This brief burst of proteolytic activity is necessary for growth cone formation, as blockage of calpain activation by calpeptin inhibits the transformation of the cut axon into a growth cone[35,67,68] (FIG. 1d).

Under conditions that limit the influx of calcium into the axoplasm but support membrane seal formation, cut *A. californica* axons do not form a growth cone. Rather, these axons form a bulbous structure that bears some similarities to the retraction bulbs that are formed after spinal cord injury[10,40] (FIG. 2a). Under these regeneration-blocking conditions, axotomy is followed by a much reduced level of microtubule depolymerization, and as soon as the $[Ca^{2+}]_i$ recovers, the microtubules repolymerize in such a way that their plus ends reach the plasma membrane at the tip of the cut axon. Furthermore, submembranous spectrin is not cleaved and actin filaments do not polymerize. Under these experimental conditions, anterogradely transported Golgi-derived vesicles accumulate at the tip of the cut axon without fusing with the plasma membrane, presumably because the persistent submembranous spectrin cytoskeleton impedes

fusion (FIG. 2a). However, this failed growth cone regeneration can later be reactivated by local elevation of the $[Ca^{2+}]_i$ by application of ionomycin to the end bulb: this rise in calcium activates calpain, which locally cleaves the submembranous spectrin and initiates growth processes[40] (FIG. 2b).

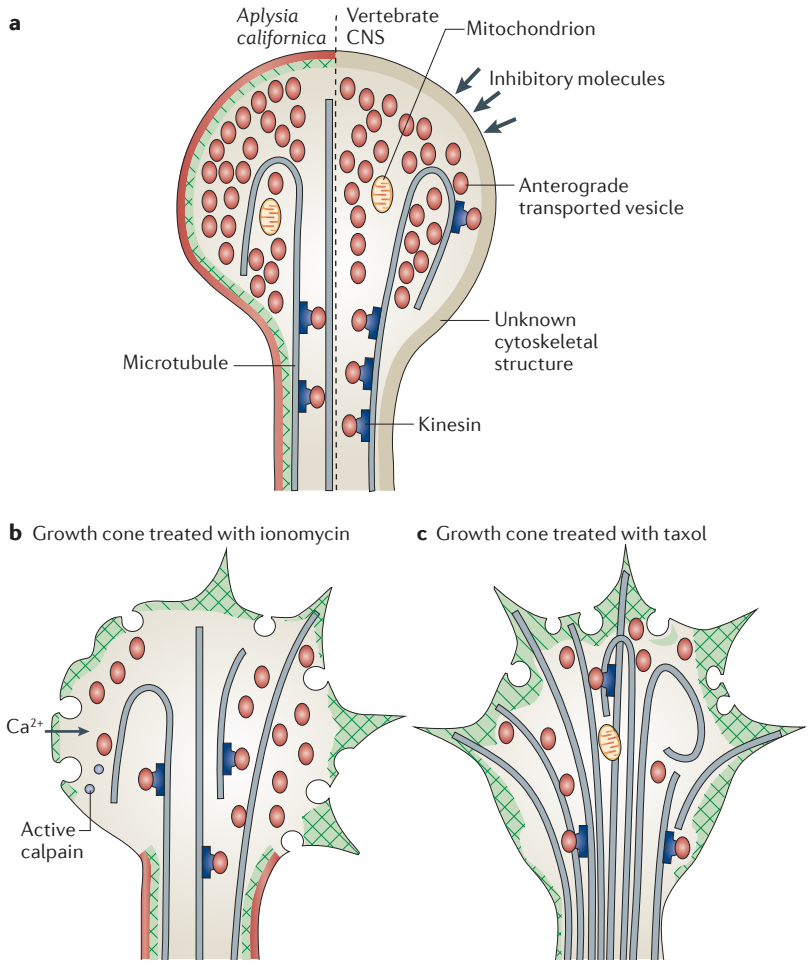

Figure 2 | **Formation of a retraction bulb and its rescue. a** | Retraction bulbs (also known as frustrated growth cones) are typically characterized by an accumulation of anterogradely transported vesicles, mitochondria and microtubules, which may extend in various directions and even turn around to point their plus ends retrogradely. The left-hand side shows a retraction bulb from *Aplysia californica*, whereas the right-hand side shows a retraction bulb from a vertebrate CNS neuron. The cytoskeletal and organelle compositions of these retraction bulbs are probably the same; however, in *A. californica*, experimentally generated bulbs are characterized by the presence of uncleaved submembrane spectrin, which impedes vesicle fusion with the plasma membrane. The ultrastructural organization of the vertebrate retraction bulb submembrane skeleton (represented as the brown submembranous band) is currently unknown. In the mammalian CNS, several growth-inhibitory molecules (shown as arrows) impinge on the end of the cut axon. **b** | Rescue of a retraction bulb is achieved in cultured *A. californica* neurons by transient elevation of the intracellular calcium concentration (achieved experimentally with ionomycin), which activates calpains that remove the submembrane spectrin barrier. Vesicles can then fuse with the plasma membrane and actin filaments can form. **c** | In vertebrate neurons, application of the microtubule-stabilizing reagent taxol leads to regrowth, probably by facilitating the polymerization of microtubules. Such polymerization mechanically stretches the plasma membrane, enhancing vesicle exocytosis. Whether spectrin has a role in these processes in vertebrate CNS neurons and whether taxol exerts similar effects in *A. californica* neurons is unknown.

In normal calcium media, the regeneration of cut *A. californica* axons is successful. Within minutes of calcium recovery, microtubules repolymerize[36,37], and at first, the repolymerized microtubules point their plus ends in random directions (Supplementary information S1 (figure), S2 (movie) and S3 (movie)). Vesicles continue to be transported along microtubules, leading to the formation of two vesicle accumulations or 'traps' at the axon tip: a trap for Golgi-derived vesicles that are driven anterogradely by plus-end-oriented molecular motors (the plus-end trap) and a trap for retrogradely transported cargo driven by minus-end-oriented molecular motors (the minus-end trap; Supplementary information S1 (figure), S4 (movie) and S5 (movie)). The Golgi-derived vesicles accumulated at the plus-end trap later supply the lipid membrane for growth cone construction[37] (FIG. 1d).

The role of and dependence on actin and tubulin restructuring in this first phase of growth cone regeneration has been investigated by using agents that interfere with the polymerization of these cytoskeletal proteins. In the presence of the microtubule-depolymerizing drug nocodazole (at concentrations that do not lead to microtubule depolymerization), the initial calcium-dependent microtubule and actin depolymerization occurs normally. However, microtubule repolymerization and Golgi-derived vesicle accumulation at the axonal tip is prevented. Under these conditions, the actin cytoskeleton still restructures and also forms radial actin filaments. However, interestingly, these actin filaments fail to extend lamellipodia, suggesting that microtubules and/or the accumulation of Golgi-derived vesicles are necessary to establish these projections. Perturbation of actin dynamics by application of cytochalasin D or jasplakinolide prevents growth cone lamellipodial formation but does not inhibit the restructuring of the microtubules and the typical accumulation of Golgi-derived vesicles at the cut axonal end. These observations suggest that the characteristic restructuring of the actin and microtubules at the tip of the cut axon are independent of each other but are both necessary for proper function of growth cones[36,37,40].

Signalling pathways

Cytoskeletal dynamics, local protein translation, transport and trafficking, and other processes that occur within the emerging growth cone are influenced by the signalling pathways that operate in axons, and many of these signalling pathways are, in turn, influenced by the calcium changes described above. The pathways that are necessary for growth cone restoration have been investigated by using pharmacological inhibitors in the simple *in vitro* axotomy models described above and by performing axotomies in *C. elegans* that are deficient in key signalling molecules. In mammalian sensory axons, mitogen-activated protein kinase 1 (MAPK1; also known as ERK2), MAPK3 (also known as ERK1), MAPK11–14 (also known as the p38 MAPKs) and mammalian target of rapamycin (mTOR) inhibitors prevent regeneration of the growth cone, indicating that these kinases are required for growth cone restoration. The

actions of caspase 3 and the proteasome are also required for this process[31,32]. In *C. elegans*, regeneration of axons after laser axotomy can be observed in real time, and this approach has revealed that death-associated protein kinase-like kinase (DLK-1), p38 MAPK 3 (PMK-3), kinase glh-binding protein 1 (KGB-1; a c-Jun amino-terminal kinase) and CCAAT/enhancer-binding protein 1 (CEBP-1) are necessary for initiation of axon regeneration[38,41,46,69]. Of note, DLK-1 overexpression is one of the few manipulations that can increase the probability of regeneration in this model[69].

A large literature exists relating to the manipulation of signalling pathways in the mammalian CNS and enhanced axon regeneration. The studies that make up this body of work focus on axon elongation past the site of damage and therefore do not distinguish between signalling effects in growth cone restitution and those in subsequent axon elongation. However, axons that elongate must first have made a new growth cone. Several signalling molecules and pathways are implicated by these studies, including mTOR, AKT–MAPK signalling, several variants of protein kinase C (PKC), cyclic AMP and downstream signalling from trophic factors[7,70–72].

Providing new membrane

Formation of a new motile growth cone after axotomy requires the recruitment of membrane and its insertion into the neurolemma. The source of this membrane in *A. californica* axons is mostly anterogradely transported vesicles that are derived from the Golgi apparatus[37,40]. In fact, the extension of growth cone lamellipodia after axotomy is prevented by pre-incubation of the neuron with brefeldin A, which disrupts the Golgi apparatus[37,73]. It is conceivable that a fraction of the retrieved plasma membrane from the cut axonal end is reused for the growth process. This possibility is supported by the observation that isolated axonal segments may temporarily and partially create a growth cone[64,74–76]. However, whole-cell patch clamping experiments[48] revealed that axotomy of cultured *A. californica* neurons induced considerable membrane retrieval while a growth cone lamellipodium was extending. This contradiction is reconciled by the results of confocal microscope imaging showing that membrane retrieval occurs mainly at the very tip of the cut axon (the region of the minus-end trap), whereas insertion of Golgi-derived membrane into the neurolemma occurs more proximally, at the so-called growth cone organizing centre[37,48].

Although the mechanisms that localize membrane resources to a newly formed growth cone are partly understood, the mechanisms that facilitate vesicle fusion with the neurolemma are not clear. It is possible that four factors converge to promote fusion: a rise in the concentration of the vesicles within the plus-end vesicle trap; the removal of the submembrane spectrin skeleton that mechanically impedes fusion; an increase in the local membrane stretch (mechanical tension) that is generated by the submembrane microtubules and the actin network[77]; and the spontaneous elevation of the free $[Ca^{2+}]_i$ at this region. It should be noted that although the major supply of materials that is needed to generate membrane may be derived from the cell body and transported along the axon, a fraction of it can be harvested locally. Axons contain the enzymes that allow generation of membrane phosphotidlycholine[78]. Interestingly, after damage, glial cells greatly upregulate the synthesis of apolipoprotein E (APOE) and secrete lipoproteins, cholesterol and phospholipids, which may be taken up by axons, thereby increasing regeneration and growth rates[79]. To conclude, the membrane necessary for plasma membrane growth cone formation is mainly supplied by post-Golgi trafficking and by local stores.

Local translation of proteins

Many of the molecules that are necessary for immediate growth cone assembly are provided by recycling axonal material; however, growth cones also contain proteins that are not part of the structure of mature axon shafts. These new proteins could arrive via axonal transport or through local protein translation. Given these possibilities, it was of great interest to find that some axons — that is, mammalian PNS axons and some invertebrate axons — contain messenger RNAs (mRNAs), ribosomal proteins and Golgi-like structures and can synthesize proteins within the axon[80,81]. In cultured adult rodent sensory neurons, axotomy causes an increase in local protein synthesis, and preventing this synthesis greatly reduces the ability of cut axons to produce a new growth cone[31]. The enhanced synthesis of actin (among other proteins) is necessary for efficient growth regeneration[45]; local translation of actin mRNA has also been reported to regulate axon guidance during development[82]. Interestingly, in both of these situations, recycled actin does not compensate for a lack of locally produced molecules. In *C. elegans*, local translation, which is regulated by several factors including CEBP-1 (which promotes mRNA stabilization), is involved in regeneration[46]. However, not all of the mRNAs for proteins that are necessary for growth cone function have been detected in axons. For example, although mRNAs for many cytoskeletal molecules and molecules that are involved in cytoskeletal dynamics are present in rodent sensory axons, mRNAs for many cell surface adhesion and receptor molecules are absent[45,83] (FIG. 3).

Despite the findings described above, is there evidence for local translation in mammalian adult CNS axons? Some mRNAs are present in axons of immature cortical neurons[84], but ribosomal proteins are undetectable in adult retinal axons[31] and may be absent in other classes of CNS axon. The issue of the availability of machinery for local translation in CNS axons therefore requires further study.

A role for local translation has been described in the conditioning effect — the enhanced regeneration that occurs if a previously cut peripheral axon is re-lesioned. Local synthesis of proteins such as importins and vimentin occurs at sites of peripheral nerve axotomy. Following local synthesis, these proteins are transported back to the neuronal cell body (FIG. 3), where they control the production of various proteins, which participate in the more vigorous regeneration response following subsequent axotomy (2 days or more) after the initial damage[54,81].

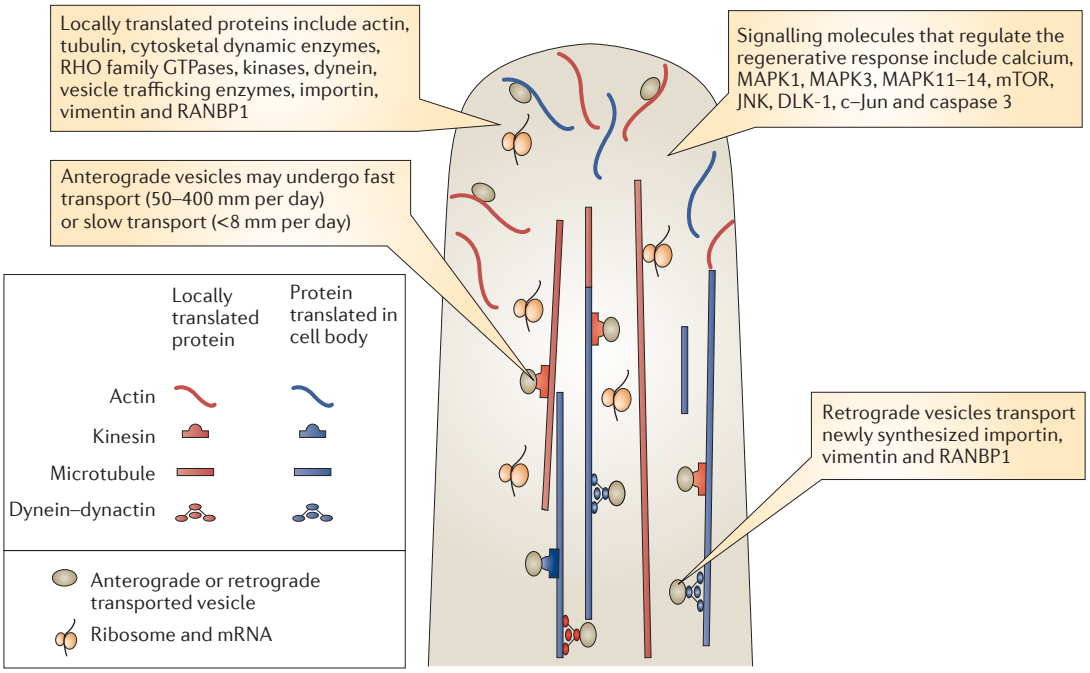

Figure 3 | **Molecular events in growth cone regeneration.** For a new growth cone to form, the appropriate molecules must be deployed in the right places. This is achieved by continual anterograde transport and by local messenger RNA (mRNA) translation. An abbreviated list of some of the key molecules, based on the presence of mRNAs in axons from various species, is shown. Newly synthesized molecules are shown in red. Some of these molecules are transported back to the cell body, where they are involved in the conditioning process. The regenerative events are controlled by several signalling proteins, which are listed on the right. DLK-1, death-associated protein kinase-like kinase 1; JNK, c-Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; RANBP1, Ran-binding protein 1.

Local translation requires ribosomes. Interestingly, these may be retrieved from the environment after peripheral nerve injury, as Schwann cell-derived ribosomal material can be found inside regenerating axons[85].

Trafficking of molecules to the regenerating axon

One feature of injured CNS axons is that their microtubules depolymerize at the axon stump. Thus, these microtubules lose their protrusive activity, which propels axon growth[10]. By contrast, lesioned axons in the PNS retain bundled microtubules and provide support for growing axons. Bundled microtubules distinguish growing growth cones from non-growing retraction bulbs, as microtubule destabilization with nocodazole turns a regenerating PNS growth cone into a non-growing retraction bulb. Conversely, microtubule stabilization with taxol interferes with retraction bulb formation in CNS axons[10,86]. Injury of the CNS axons leads to the formation of retraction bulbs that increase in size over weeks and accumulate vesicles in their interior[10], suggesting that transport continues from the cell body into the injured axon (FIG. 2a). In these injured axons, the tip of the cut axon swells as vesicles continue to accumulate at the plus ends of the microtubule tracks; however, fusion of the vesicles with the plasmalemma might be retarded[10]. It is conceivable that axotomy in CNS neurons does not lead to cleavage of submembranous spectrin, which, as described earlier, is necessary for vesicle fusion and regeneration in *A. californica* axons. This issue requires further study.

The events following axotomy in embryonic hippocampal neurons *in vitro* are very different from those following injury in the adult CNS *in vivo*. In axons of hippocampal neurons, microtubules remain relatively intact after injury[30]. Hence, these cultured CNS axons keep the support structure that is necessary to propel axon regeneration and hence to reactivate their polarization programme. The results of axotomy depend on the distance from the cell body. Severance at a distance of more than 35 µm away from the cell body causes an axon to regrow within hours[24,27–30], whereas severance of an axon at less than 35 µm from the cell body leads to an outgrowth response from one of several neurites: either a growth cone regenerates from the injured axon stump or, after a delay, another neurite or even a dendrite will form a motile growth cone, followed by axon growth[24,27,30]. The production of a new axon is preceded by a change in membrane trafficking into the new axon, suggesting that cargo flow has to be transferred from the old to the new axon[87]. It is possible that the microtubules themselves direct this trafficking into the axon. Microtubules show an increase in acetylation at around 35–40 µm away from the cell body, which could account for the observed polarity change when the axon is cut closer than 35 µm from the soma[30]: the cut would ablate the stable microtubules and hence the landmark that could direct the transport of growth-promoting cargo into the axon. We discuss axon-specific cargoes below.

Taxol
Taxol is a compound that at low concentrations promotes the polymerization of tubulin into microtubules and stabilizes microtubules against depolymerization, and hence may promote axon growth over inhibitory substrates.

Autonomy versus dependency

How autonomous from the cell body is growth cone regeneration? There are four potential sources of molecules for growth cone reconstruction: locally recycled materials from the cut axon; material and organelles that are already in transit in axons; local translation at the injury site; and newly synthesized molecules that are produced through changes in gene expression in the cell body. We have already discussed how molecules are locally reused or translated (in some axons) and how transport vesicles that are already in transit along the axon continue to arrive and deliver molecules to the new growth cone (FIG. 3). The necessity for transported materials in transit from the soma for growth cone assembly is suggested by the observation in *A. californica* that although the distal cut end of an axon (the segment not attached to the cell body) undergoes some of the initial cytoskeletal changes that are seen in a proximal cut end, only the latter is able to produce a competent growth cone[65]. Of note, similar results are seen in mammalian neurons[75]. Moreover, interference of trafficking through local depolymerization of microtubules[40,88] or inhibition of post-Golgi trafficking by pharmacological dispersal of the Golgi apparatus[73,89] stalls axon growth. Thus, clearly, anterogradely transported material is essential for growth cone formation, but where does it come from?

We discussed local translation earlier and showed that efficient regeneration of mammalian PNS axons does not occur without it. But to what extent is growth cone regeneration dependent on new molecules produced in the cell body? Axotomy of peripheral axons changes gene expression in the affected neurons, leading eventually to new proteins entering axons. These changes are involved in the conditioning response, which leads to a more vigorous regenerative response if an axon is cut again[20,90–92]. But are these cell body changes necessary for the initial growth cone regeneration that occurs within hours of injury? Upregulation of rapidly transported molecules such as neuromodulin (also known as GAP43) and cortical-associated protein 23 (CAP23; also known as brain abundant, membrane attached signal protein 1 (BASP1)) occurs 1–3 days after peripheral nerve axotomy. In rodent nerves, experimental axotomies are usually around 1 cm from the cell body. Thus, as the most rapid neuronal transport mechanism delivers molecules at ~20 cm per day (FIG. 3), the fastest a molecule can be transported from the soma to the axon tip is in just over an hour. Growth cone regeneration starts in less than an hour in cultured *A. californica* neurons and within hours in rodent PNS axons[10,17,19], so only in late-regenerating axons could newly synthesized molecules from the cell body arrive in time[93,94]. Moreover, it is notable that only a few proteins are upregulated or transported this rapidly[95]. Axotomy in human PNS neurons may occur up to 1 metre from the cell body. Thus, even if neuronal gene expression changed immediately, it would take 5 days for new proteins to reach the furthest injury sites. These considerations indicate that altered gene expression in the neuronal cell body is unlikely to be essential for initial growth cone assembly — the earliest step in the regenerative process — in mammalian PNS axons, although it is certainly needed for later axon growth.

Taken together, although a continuous membrane and protein supply is necessary for axon elongation, it seems unlikely that new expression of a novel set of genes is necessary to elicit the formation of a new growth cone. However, the changes in gene expression that occur during the conditioning effect may produce proteins that, when they finally arrive in the axon, may enhance the regeneration of the growth cone if the axon is re-lesioned. Later in the regeneration process, as the axon elongates, new proteins coming from the cell body are essential to provide necessary axonal building blocks.

Determinants of a regenerative response

Neurons are an extreme example of cellular compartmentalization, and axons contain only a subset of cellular proteins. This distribution is due to selective transport into axons, to developmental changes in neuronal kinesins and dyneins, and to modifications of tubulin that affect kinesin-based transport[96–101].

It seems possible that selective transport in CNS axons leads to the absence of molecules that are necessary for efficient growth cone regeneration, and may underlie why CNS axons regenerate with greater efficiency if they are cut close to the cell body. It is possible that axons that are cut near to the cell body contain molecules that are not present further down the axon either because of passive diffusion into the axon from the cell body or because the axon initial segment becomes leaky after damage[102]. The first issue for the success or failure of regeneration is therefore whether the necessary molecules (including translational machinery) are actually present within the damaged axon.

One feature of injured CNS axons is that their microtubules depolymerize at the axon stump, whereas axons lesioned in the PNS retain microtubule integrity and bundling[10]. Microtubules are crucially involved in the regenerative response and growth cone formation, as application of the microtubule-destabilizing drug nocodazole turns a regenerating PNS growth cone into a non-growing retraction bulb, and application of taxol to CNS axons diminishes the number of retraction bulbs and increases the number of growth cones[10,86]. Various post-translational modifications, such as detyrosination and acetylation, are linked with stable microtubules that may be relevant to the regenerative response of the axon[100,102,103]. Thus, stable microtubules in the axon may be essential not only for the functioning of the growth cone motor machinery but also to direct transport of cargo that is necessary for axon growth to the axon tip.

The success of some signalling pathway manipulations in promoting axon elongation in the CNS also implies that some signalling events that are necessary for regeneration may not occur in a timely fashion in CNS axons; comparisons of changes after axotomy of CNS and PNS axons are needed to resolve this issue.

Manipulating the regenerative response

In the preceding sections, we have identified the key steps in growth cone regeneration and shown that interference

The conditioning response
A severed peripheral nervous system axon will begin to regenerate after a few hours. If the same axon is cut again 2 or more days later, the speed of axon regeneration increases. This phenomenon is known as the conditioning response.

Axon initial segment
The axon initial segment is the part of the axon that is closest to the cell body and is the point of initiation for action potentials. It may also act as a selective transport filter for some types of axonal cargo.

in most of these steps can stop this process. However, our ability to inhibit growth cone regeneration is not very useful to injured patients. Are there any points at which it may be possible to intervene to increase the success of growth cone formation? One intervention that has been successful in experimental preparations is microtubule stabilization, which, as described earlier, prevents the rapid depolymerization of microtubules in the axonal tip proximal to the injury site in CNS axons[10]. The possibility that this approach could be the basis of a successful treatment is supported by the demonstration that microtubule stabilization with taxol induces axon growth in cultured neurons that are plated on growth inhibitory substrates, including CNS myelin and chondroitin sulphate proteoglycans[10,86,104]. Importantly, the application of taxol at the lesion site of spinal cord injured rats induces axon regeneration of serotonergic axons, enhances growth cone formation and improves functional outcome[86] (FIG. 2a,c).

A second potential intervention that may be important is the enhancement of the ability of axons to support local translation of mRNAs at the site of axotomy. Whether CNS axons contain ribosomes is not clear; the transport of these organelles may be blocked at the axon initial segment in CNS neurons[31]. If this view is substantiated, temporary and partial deconstruction of the axon initial segment might allow ribosomes and other important molecules to pass into the axon.

The third possible approach concerns the activation of some signalling pathways that promote axon elongation and therefore probably growth cone restitution. For example, the removal of phosphatase and tensin homologue (PTEN) affects various signalling events, and has been successful in promoting axon regeneration in the optic nerve and spinal cord of rodents[6]. Presumably, as a prelude to axon elongation, the changes in signalling that are caused by PTEN removal facilitate the construction of a new growth cone. In *C. elegans*, DLK-1 overexpression can increase the probability of regeneration[69], and it will be interesting to see whether this finding translates to mammalian axons.

It is important to consider at what time an intervention might be successful. Immediate treatment after injury may be impracticable, and if the treatment enhances transport, it may take several days before new molecules arrive at the cut point. Thus, could a delayed treatment work? There are some promising indications that it might. First, in *A. californica* neurons, failed regeneration can be restarted by dissolution of the submembranous spectrin barrier at the axon tip (FIG. 2a,b), and second, the dystrophic club ending in mammalian CNS axons that marks regenerative failure seems to be a highly dynamic structure in terms of anatomy, and might therefore respond to interventions[9,20]. Whether the many molecules that inhibit regeneration in the CNS prevent growth cone assembly or just the later axon elongation phases, and whether the treatments that neutralize inhibition might therefore aid generation of a new growth cone remain unresolved questions.

Conclusions

Successful axon regeneration has to be preceded by successful assembly of a new growth cone. In this Review, we have attempted to show that growth cone assembly is a complex event that depends partly on mechanisms that are shared with axon elongation and partly on mechanisms that are exclusive to the formation of a new growth cone. A cut CNS axon needs to seal its ruptured membrane, regulate intracellular proteolytic events, rearrange its cytoskeleton, regulate transport and direct vesicles to fuse with the plasma membrane. The observation that growth cone regeneration fails in many CNS axons indicates that one or more of these processes is defective. The development of treatments to improve the probability of growth cone regeneration may be fundamental to our attempts to induce axonal regeneration in the injured CNS.

1. Schwab, M. E. Functions of Nogo proteins and their receptors in the nervous system. *Nature Rev. Neurosci.* **11**, 799–811 (2010).
2. Schwab, M. E. How hard is the CNS hardware? *Nature Neurosci.* **13**, 1444–1446 (2010).
3. Fitch, M. T. & Silver, J. CNS injury, glial scars, and inflammation: inhibitory extracellular matrices and regeneration failure. *Exp. Neurol.* **209**, 294–301 (2008).
4. Kwok, J. C., Afshari, F., Garcia-Alias, G. & Fawcett, J. W. Proteoglycans in the central nervous system: plasticity, regeneration and their stimulation with chondroitinase ABC. *Restor. Neurol. Neurosci.* **26**, 131–145 (2008).
5. Pasterkamp, R. J. & Verhaagen, J. Semaphorins in axon regeneration: developmental guidance molecules gone wrong? *Phil. Trans. R. Soc. B* **361**, 1499–1511 (2006).
6. Park, K. K., Liu, K., Hu, Y., Kanter, J. L. & He, Z. PTEN/mTOR and axon regeneration. *Exp. Neurol.* **223**, 45–50 (2010).
7. Hannila, S. S. & Filbin, M. T. The role of cyclic AMP signaling in promoting axonal regeneration after spinal cord injury. *Exp. Neurol.* **209**, 321–332 (2008).
8. Wang, Z. & Jin, Y. Genetic dissection of axon regeneration. *Curr. Opin. Neurobiol.* **21**, 189–196 (2010).
9. Tom, V. J., Steinmetz, M. P., Miller, J. H., Doller, C. M. & Silver, J. Studies on the development and behavior of the dystrophic growth cone, the hallmark of regeneration failure, in an *in vitro* model of the glial scar and after spinal cord injury. *J. Neurosci.* **24**, 6531–6539 (2004).
10. Erturk, A., Hellal, F., Enes, J. & Bradke, F. Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. *J. Neurosci.* **27**, 9169–9180 (2007). **This live imaging study pinpoints microtubule disassembly as a key intracellular event in injured axons that prevents axonal regeneration after spinal cord injury.**
11. Wanner, M. *et al.* Reevaluation of the growth-permissive substrate properties of goldfish optic nerve myelin and myelin proteins. *J. Neurosci.* **15**, 7500–7508 (1995).
12. Gaze, R. M. *The Formation of Nerve Connections* (Academic, London, New York, 1970).
13. Lurie, D. I. & Selzer, M. E. Axonal regeneration in the adult lamprey spinal cord. *J. Comp. Neurol.* **306**, 409–416 (1991).
14. Wu, Z. *et al. Caenorhabditis elegans* neuronal regeneration is influenced by life stage, ephrin signaling, and synaptic branching. *Proc. Natl Acad. Sci. USA* **104**, 15132–15137 (2007).
15. Friede, R. L. & Bischhausen, R. The fine structure of stumps of transected nerve fibers in subserial sections. *J. Neurol. Sci.* **44**, 181–203 (1980).
16. Morris, J. H., Hudson, A. R. & Weddell, G. A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. II. The development of the "regenerating unit". *Z. Zellforsch. Mikrosk. Anat.* **124**, 103–130 (1972).
17. Pan, Y. A., Misgeld, T., Lichtman, J. W. & Sanes, J. R. Effects of neurotoxic and neuroprotective agents on peripheral nerve regeneration assayed by time-lapse imaging *in vivo*. *J. Neurosci.* **23**, 11479–11488 (2003).
18. Windle, W. F. Inhibition of regeneration of severed axons in the spinal cord. *Exp. Neurol.* **69**, 209–211 (1980).
19. Kerschensteiner, M., Schwab, M. E., Lichtman, J. W. & Misgeld, T. *In vivo* imaging of axonal degeneration and regeneration in the injured spinal cord. *Nature Med.* **11**, 572–577 (2005).
20. Ylera, B. *et al.* Chronically CNS-injured adult sensory neurons gain regenerative competence upon a lesion of their peripheral axon. *Curr. Biol.* **19**, 930–936 (2009). **The authors cut single axons (using a two-photon laser) in the spinal cords of living mice and studied growth cone formation and subsequent axon growth.**
21. Richardson, P. M. & Issa, V. M. Peripheral injury enhances central regeneration of primary sensory neurones. *Nature* **309**, 791–793 (1984).
22. Neumann, S. & Woolf, C. J. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* **23**, 83–91 (1999).
23. Knoferle, J. *et al.* Mechanisms of acute axonal degeneration in the optic nerve *in vivo*. *Proc. Natl Acad. Sci. USA* **107**, 6064–6069 (2010).

24. Goslin, K. & Banker, G. Experimental observations on the development of polarity by hippocampal neurons in culture. *J. Cell. Biol.* **108**, 1507–1516 (1989).
25. Dotti, C. G. & Banker, G. A. Experimentally induced alteration in the polarity of developing neurons. *Nature* **330**, 254–256 (1987).
26. Chuckowree, J. A. & Vickers, J. C. Cytoskeletal and morphological alterations underlying axonal sprouting after localized transection of cortical neuron axons *in vitro*. *J. Neurosci.* **23**, 3715–3725 (2003).
27. Bradke, F. & Dotti, C. G. Differentiated neurons retain the capacity to generate axons from dendrites. *Curr. Biol.* **10**, 1467–1470 (2000).
28. Goslin, K. & Banker, G. Experimental observations on the development of polarity by hippocampal neurons in culture. *J. Cell Biol.* **108**, 1507–1516 (1989).
29. Bradke, F. & Dotti, C. G. Establishment of neuronal polarity: lessons from cultured hippocampal neurons. *Curr. Opin. Neurobiol.* **10**, 574–581 (2000).
30. Gomis-Ruth, S., Wierenga, C. J. & Bradke, F. Plasticity of polarization: changing dendrites into axons in neurons integrated in neuronal circuits. *Curr. Biol.* **18**, 992–1000 (2008).
31. Verma, P. *et al.* Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. *J. Neurosci.* **25**, 331–342 (2005).
Study showing that efficient regeneration of sensory axon growth cones requires local protein translation and degradation, but that adult CNS axons lack ribosomes.
32. Chierzi, S., Ratto, G. M., Verma, P. & Fawcett, J. W. The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. *Eur. J. Neurosci.* **21**, 2051–2062 (2005).
33. Goldberg, J. L. *et al.* Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. *Neuron* **33**, 689–702 (2002).
34. Forscher, P., Lin, C. H. & Thompson, C. Novel form of growth cone motility involving site-directed actin filament assembly. *Nature* **357**, 515–518 (1992).
35. Spira, M. E., Oren, R., Dormann, A. & Gitler, D. Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured *Aplysia* neurons. *J. Comp. Neurol.* **457**, 293–312 (2003).
36. Sahly, I., Khoutorsky, A., Erez, H., Prager-Khoutorsky, M. & Spira, M. E. On-line confocal imaging of the events leading to structural dedifferentiation of an axonal segment into a growth cone after axotomy. *J. Comp. Neurol.* **494**, 705–720 (2006).
37. Erez, H. *et al.* Formation of microtubule-based traps controls the sorting and concentration of vesicles to restricted sites of regenerating neurons after axotomy. *J. Cell Biol.* **176**, 497–507 (2007).
Live confocal microscope imaging documentation of the sequence of events leading to the polar reorientation of microtubules after axotomy and the ensuing accumulation of Golgi-derived vesicles and endocytic vesicles in separate traps.
38. Nix, P., Hisamoto, N., Matsumoto, K. & Bastiani, M. Axon regeneration requires coordinate activation of p38 and JNK MAPK pathways. *Proc. Natl Acad. Sci. USA* **108**, 10738–10743 (2011).
39. Kulbatski, I., Cook, D. J. & Tator, C. H. Calcium entry through L-type calcium channels is essential for neurite regeneration in cultured sympathetic neurons. *J. Neurotrauma* **21**, 357–374 (2004).
40. Kamber, D., Erez, H. & Spira, M. E. Local calcium-dependent mechanisms determine whether a cut axonal end assembles a retarded endbulb or competent growth cone. *Exp. Neurol.* **219**, 112–125 (2009).
Live confocal imaging of the subcellular events underlying end bulb formation and its rescue by calpain cleavage of the submembrane spectrin skeleton.
41. Ghosh-Roy, A., Wu, Z., Goncharov, A., Jin, Y. & Chisholm, A. D. Calcium and cyclic AMP promote axonal regeneration in *Caenorhabditis elegans* and require DLK-1 kinase. *J. Neurosci.* **30**, 3175–3183 (2010).
42. Ziv, N. E. & Spira, M. E. Axotomy induces a transient and localized elevation of the free intracellular calcium concentration to the millimolar range. *J. Neurophysiol.* **74**, 2625–2637 (1995).
43. Ziv, N. E. & Spira, M. E. Localized and transient elevations of intracellular Ca^{2+} induce the dedifferentiation of axonal segments into growth cones. *J. Neurosci.* **17**, 3568–3579 (1997).
44. Ambron, R. T. & Walters, E. T. Priming events and retrograde injury signals. A new perspective on the cellular and molecular biology of nerve regeneration. *Mol. Neurobiol.* **13**, 61–79 (1996).
45. Vogelaar, C. F. *et al.* Axonal mRNAs: characterisation and role in the growth and regeneration of dorsal root ganglion axons and growth cones. *Mol. Cell. Neurosci.* **42**, 102–115 (2009).
46. Yan, D., Wu, Z., Chisholm, A. D. & Jin, Y. The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. *Cell* **138**, 1005–1018 (2009).
Real-time imaging study of axon regeneration in *C. elegans* implicating local translation and signalling in successful axon regeneration.
47. Spira, M. E., Benbassat, D. & Dormann, A. Resealing of the proximal and distal cut ends of transected axons: electrophysiological and ultrastructural analysis. *J. Neurobiol.* **24**, 300–316 (1993).
48. Ashery, U., Penner, R. & Spira, M. E. Acceleration of membrane recycling by axotomy of cultured *Aplysia* neurons. *Neuron* **16**, 641–651 (1996).
49. Ziv, N. E. & Spira, M. E. Spatiotemporal distribution of Ca^{2+} following axotomy and throughout the recovery process of cultured Aplysia neurons. *Eur. J. Neurosci.* **5**, 657–668 (1993).
50. Malkinson, G. & Spira, M. E. Imaging and analysis of evoked excitatory-postsynaptic-calcium-transients by individual presynaptic-boutons of cultured *Aplysia* sensorimotor synapse. *Cell Calcium* **47**, 315–325 (2010).
51. Mandolesi, G., Madeddu, F., Bozzi, Y., Maffei, L. & Ratto, G. M. Acute physiological response of mammalian central neurons to axotomy: ionic regulation and electrical activity. *FASEB J.* **18**, 1934–1936 (2004).
Description of the changes in calcium and electrical activity in mammalian CNS axons after axotomy.
52. Friel, D. D. & Chiel, H. J. Calcium dynamics: analyzing the Ca^{2+} regulatory network in intact cells. *Trends Neurosci.* **31**, 8–19 (2008).
53. Navarro, X., Vivo, M. & Valero-Cabre, A. Neural plasticity after peripheral nerve injury and regeneration. *Prog. Neurobiol.* **82**, 163–201 (2007).
54. Rishal, I. & Fainzilber, M. Retrograde signaling in axonal regeneration. *Exp. Neurol.* **223**, 5–10 (2010).
55. Enes, J. *et al.* Electrical activity suppresses axon growth through $Ca_v1.2$ channels in adult primary sensory neurons. *Curr. Biol.* **20**, 1154–1164 (2010).
56. Fishman, H. M. & Bittner, G. D. Vesicle-mediated restoration of a plasmalemmal barrier in severed axons. *News Physiol. Sci.* **18**, 115–118 (2003).
57. McNeil, P. L. & Kirchhausen, T. An emergency response team for membrane repair. *Nature Rev. Mol. Cell Biol.* **6**, 499–505 (2005).
58. Yawo, H. & Kuno, M. How a nerve fiber repairs its cut end: involvement of phospholipase A2. *Science* **222**, 1351–1353 (1983).
59. Bittner, G. D. & Fishman, H. M. in *Axonal Regeneration in the Central Nervous System* (eds Ingoglia, N. A. & Murray, M.) 337–370 (Marcel Dekker, New York, 2000).
60. Krause, T. L., Fishman, H. M., Ballinger, M. L. & Bittner, G. D. Extent and mechanism of sealing in transected giant axons of squid and earthworms. *J. Neurosci.* **14**, 6638–6651 (1994).
61. Regehr, W. G. & Tank, D. W. Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. *J. Neurosci.* **12**, 4202–4223 (1992).
62. Xie, X. Y. & Barrett, J. N. Membrane resealing in cultured rat septal neurons after neurite transection: evidence for enhancement by Ca^{2+}-triggered protease activity and cytoskeletal disassembly. *J. Neurosci.* **11**, 3257–3267 (1991).
63. Yoo, S., Nguyen, M. P., Fukuda, M., Bittner, G. D. & Fishman, H. M. Plasmalemmal sealing of transected mammalian neurites is a gradual process mediated by Ca^{2+}-regulated proteins. *J. Neurosci. Res.* **74**, 541–551 (2003).
64. Benbassat, D. & Spira, M. E. Survival of isolated axonal segments in culture: morphological, ultrastructural, and physiological analysis. *Exp. Neurol.* **122**, 295–310 (1993).
65. Erez, H. & Spira, M. E. Local self-assembly mechanisms underlie the differential transformation of the proximal and distal cut axonal ends into functional and aberrant growth cones. *J. Comp. Neurol.* **507**, 1019–1030 (2008).
An analysis of the structural reorganization of the distal segment of a cut axon and the mechanisms underlying its failure to reassemble a competent growth cone.
66. George, E. B., Glass, J. D. & Griffin, J. W. Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J. Neurosci.* **15**, 6445–6452 (1995).
67. Gitler, D. & Spira, M. E. Real time imaging of calcium-induced localized proteolytic activity after axotomy and its relation to growth cone formation. *Neuron* **20**, 1123–1135 (1998).
68. Gitler, D. & Spira, M. E. Short window of opportunity for calpain induced growth cone formation after axotomy of *Aplysia* neurons. *J. Neurobiol.* **52**, 267–279 (2002).
69. Hammarlund, M., Nix, P., Hauth, L., Jorgensen, E. M. & Bastiani, M. Axon regeneration requires a conserved MAP kinase pathway. *Science* **323**, 802–806 (2009).
A study showing that growth cone regeneration in *C. elegans* requires an MAPK signalling pathway.
70. Liu, H. *et al.* Matrix metalloproteinase inhibition enhances the rate of nerve regeneration *in vivo* by promoting dedifferentiation and mitosis of supporting schwann cells. *J. Neuropathol. Exp. Neurol.* **69**, 386–395 (2010).
71. Park, B. *et al.* Impairment of protein trafficking upon overexpression and mutation of optineurin. *PLoS ONE* **5**, e11547 (2010).
72. Sivasankaran, R. *et al.* PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. *Nature Neurosci.* **7**, 261–268 (2004).
73. Prager-Khoutorsky, M. & Spira, M. E. Neurite retraction and regrowth regulated by membrane retrieval, membrane supply, and actin dynamics. *Brain Res.* **1251**, 65–79 (2009).
74. Schaefer, A. W. *et al.* Coordination of actin filament and microtubule dynamics during neurite outgrowth. *Dev. Cell* **15**, 146–162 (2008).
75. Shaw, G. & Bray, D. Movement and extension of isolated growth cones. *Exp. Cell Res.* **104**, 55–62 (1977).
76. Baas, P. W. & Heidemann, S. R. Microtubule reassembly from nucleating fragments during the regrowth of amputated neurites. *J. Cell Biol.* **103**, 917–927 (1986).
77. Sheetz, M. P., Sable, J. E. & Dobereiner, H. G. Continuous membrane-cytoskeleton adhesion requires continuous accommodation to lipid and cytoskeleton dynamics. *Annu. Rev. Biophys. Biomol. Struct.* **35**, 417–434 (2006).
78. Carter, J. M., Demizieux, L., Campenot, R. B., Vance, D. E. & Vance, J. E. Phosphatidylcholine biosynthesis via CTP:phosphocholine cytidylyltransferase 2 facilitates neurite outgrowth and branching. *J. Biol. Chem.* **283**, 202–212 (2008).
79. Vance, J. E., Karten, B. & Hayashi, H. Lipid dynamics in neurons. *Biochem. Soc. Trans.* **34**, 399–403 (2006).
80. Sotelo-Silveira, J. R., Calliari, A., Kun, A., Koenig, E. & Sotelo, J. R. RNA trafficking in axons. *Traffic* **7**, 508–515 (2006).
81. Yoo, S., van Niekerk, E. A., Merianda, T. T. & Twiss, J. L. Dynamics of axonal mRNA transport and implications for peripheral nerve regeneration. *Exp. Neurol.* **223**, 19–27 (2010).
82. Leung, K. M. *et al.* Asymmetrical β-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nature Neurosci.* **9**, 1247–1256 (2006).
83. Gumy, L. F. *et al.* Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization. *RNA* **17**, 85–98 (2010).
84. Taylor, A. M. *et al.* Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J. Neurosci.* **29**, 4697–4707 (2009).
85. Court, F. A., Hendriks, W. T., MacGillavry, H. D., Alvarez, J. & van Minnen, J. Schwann cell to axon transfer of ribosomes: toward a novel understanding of the role of glia in the nervous system. *J. Neurosci.* **28**, 11024–11029 (2008).
86. Hellal, F. *et al.* Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science* **331**, 928–931 (2011).
This study shows that administration of the clinically approved drug taxol to rats with spinal cord injuries causes axon regeneration and improvement in gait by reducing scarring and inducing axon regeneration.

87. Bradke, F. & Dotti, C. G. Neuronal polarity: vectorial cytoplasmic flow precedes axon formation. *Neuron* **19**, 1175–1186 (1997).
88. Zakharenko, S. & Popov, S. Dynamics of axonal microtubules regulate the topology of new membrane insertion into the growing neurites. *J. Cell Biol.* **143**, 1077–1086 (1998).
89. Futerman, A. H. & Banker, G. A. The economics of neurite outgrowth — the addition of new membrane to growing axons. *Trends Neurosci.* **19**, 144–149 (1996).
90. Richardson, P. M. & Verge, V. M. Axonal regeneration in dorsal spinal roots is accelerated by peripheral axonal transection. *Brain Res.* **411**, 406–408 (1987).
91. Shoemaker, S. E., Sachs, H. H., Vaccariello, S. A. & Zigmond, R. E. A conditioning lesion enhances sympathetic neurite outgrowth. *Exp. Neurol.* **194**, 432–443 (2005).
92. Cafferty, W. B. *et al.* Conditioning injury-induced spinal axon regeneration fails in interleukin-6 knock-out mice. *J. Neurosci.* **24**, 4432–4443 (2004).
93. Chong, M. S. *et al.* The downregulation of GAP-43 is not responsible for the failure of regeneration in freeze-killed nerve grafts in the rat. *Exp. Neurol.* **129**, 311–320 (1994).
94. Mason, M. R., Lieberman, A. R., Grenningloh, G. & Anderson, P. N. Transcriptional upregulation of SCG10 and CAP-23 is correlated with regeneration of the axons of peripheral and central neurons *in vivo*. *Mol. Cell. Neurosci.* **20**, 595–615 (2002).
95. Nilsson, A., Moller, K., Dahlin, L., Lundborg, G. & Kanje, M. Early changes in gene expression in the dorsal root ganglia after transection of the sciatic nerve; effects of amphiregulin and PAI-1 on regeneration. *Mol. Brain Res.* **136**, 65–74 (2005).
96. Rasband, M. N. The axon initial segment and the maintenance of neuronal polarity. *Nature Rev. Neurosci.* **11**, 552–562 (2010).
97. Nishimura, K., Akiyama, H., Komada, M. & Kamiguchi, H. βIV-spectrin forms a diffusion barrier against L1CAM at the axon initial segment. *Mol. Cell. Neurosci.* **34**, 422–430 (2007).
98. Nishimura, T. & Goll, D. E. Binding of calpain fragments to calpastatin. *J. Biol. Chem.* **266**, 11842–11850 (1991).
99. Hirokawa, N., Noda, Y., Tanaka, Y. & Niwa, S. Kinesin superfamily motor proteins and intracellular transport. *Nature Rev. Mol. Cell Biol.* **10**, 682–696 (2009).
100. Hammond, J. W. *et al.* Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. *Mol. Biol. Cell* **21**, 572–583 (2010).
101. Kapitein, L. C. *et al.* Mixed microtubules steer dynein-driven cargo transport into dendrites. *Curr. Biol.* **20**, 290–299 (2010).
102. Schafer, D. P. *et al.* Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. *J. Neurosci.* **29**, 13242–13254 (2009).
103. Konishi, Y. & Setou, M. Tubulin tyrosination navigates the kinesin-1 motor domain to axons. *Nature Neurosci.* **12**, 559–567 (2009).
104. Sengottuvel, V., Leibinger, M., Pfreimer, M., Andreadaki, A. & Fischer, D. Taxol facilitates axon regeneration in the mature CNS. *J. Neurosci.* **31**, 2688–2699 (2011).

Acknowledgements
This Review was written as a result of a meeting sponsored by the UK Academic Study Group and the Institute of Advanced Studies of the Hebrew University of Jerusalem, Israel. The laboratory of M.E.S is supported by grants from The Israel Science Foundation, The Israel Ministry of Health, The United States–Israel Binational Science Foundation, the European Commission and the Charles E. Smith Family and Professor Joel Elkes Laboratory for Collaborative Research in Psychobiology. The laboratory of F.B. is supported by the Deutsches Zentrum für Neurodegenerative Erkrankungen, the Deutsche Forschungsgemeinschaft, the International Foundation for Research in Paraplegia and the Human Frontier Science Program. J.W.F. is supported by the UK Medical Research Council, Engineering and Physical Sciences Research Council, European Union Framework 7 Programme Spinal Cord Repair, Plasticise and Angioscaff, Henry Smith Charity, Christopher and Dana Reeve Foundation, and the UK National Institute of Health Research Cambridge Biomedical Research Centre.

Competing interests statement
J.F. declares competing financial interests; see the Web version for details.

FURTHER INFORMATION

Frank Bradke's homepage: http://www.dzne.de/en/centers/bonn-cologne-juelich/research-groups/bradke.html

SUPPLEMENTARY INFORMATION

See online article: S1 (figure) | S2 (movie) | S3 (movie) | S4 (movie) | S5 (movie)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF