Molecular Signaling: How Do Axons Die?

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I. Introduction
II. Axonal Transport and How to Block It
   A. Road closed! General defects in axonal transport
   B. Traffic restrictions: Specific axonal transport defects
   C. One-way street! Unidirectional transport impairment
   D. Banned from driving: Cargo-specific defects
   E. Faulty signals
   F. Traffic congestion: Partial blockages and axonal swellings
   G. Reduced traffic flow: Ageing and decline
   H. No parking! Could increased axonal transport be too much of a “good” thing?
III. The Essentials of Axon Survival
IV. Take It or Leave It: What Axons Do Not Need to Survive
V. Protein Turnover Fast and Slow
VI. Axonal Protein Synthesis
VII. Anterograde Survival Signaling
VIII. Adhesion Molecules
IX. Life After (Cell) Death: The WldS Phenotype
X. Summary: Axonal Transport and Axon Survival Signaling
   Acknowledgments
   References
ABSTRACT

Axons depend critically on axonal transport both for supplying materials and for communicating with cell bodies. This chapter looks at each activity, asking what aspects are essential for axon survival. Axonal transport declines in neurodegenerative disorders, such as Alzheimer’s disease, amyotrophic lateral sclerosis, and multiple sclerosis, and in normal ageing, but whether all cargoes are equally affected and what limits axon survival remains unclear. Cargoes can be differentially blocked in some disorders, either individually or in groups. Each missing protein cargo results in localized loss-of-function that can be partially modeled by disrupting the corresponding gene, sometimes with surprising results. The axonal response to losing specific proteins also depends on the rates of protein turnover and on whether the protein can be locally synthesized. Among cargoes with important axonal roles are components of the PI3 kinase, Mek/Erk, and Jnk signaling pathways, which help to communicate with cell bodies and to regulate axonal transport itself. Bidirectional trafficking of Bdnf, NT-3, and other neurotrophic factors contribute to intra- and intercellular signaling, affecting the axon’s cellular environment and survival. Finally, several adhesion molecules and gangliosides are key determinants of axon survival, probably by mediating axon–glia interactions. Thus, failure of long-distance intracellular transport can deprive axons of one, few, or many cargoes. This can lead to axon degeneration either directly, through the absence of essential axonal proteins, or indirectly, through failures in communication with cell bodies and nonneuronal cells.

I. INTRODUCTION

Axons are more autonomous than we used to think. They can synthesize proteins locally (Giuditta et al., 2002), degrade them (Korhonen and Lindholm, 2004), replicate mitochondria (Amiri and Hollenbeck, 2008), carry out autophagy (Yue et al., 2008), form growth cones and respond to pathfinding cues (Campbell and Holt, 2001; Gumy et al., 2009), and even survive for several weeks without their cell bodies when a single gene is altered (Mack et al., 2001). When they do die, the mechanisms are distinct from those in cell bodies (Coleman, 2005). Ultimately, however, axons depend on two principle sources of support: neuronal cell bodies and glia. This chapter shows how molecular genetic studies are identifying which axonal molecules are most essential for survival.

Axon survival depends on continuous axonal transport, the bidirectional transport of materials from and toward cell bodies. However, some axonal transport cargoes are more essential than others, some have shorter half-lives than others, and in many cases, transport of different cargoes is mediated by
different motor proteins, different scaffold proteins linking to these motors, and different regulatory mechanisms. Some proteins can also be synthesized locally within axons. The combined effect is that some proteins are completely dispensable for long-term axon survival, while a failure to deliver others kills axons in hours.

Molecular genetics gives us some insight into what is most essential. Spontaneous null mutations in man or model organisms and targeted gene disruptions help to identify proteins that axons cannot live without. Among them are many components of the axonal transport machinery itself, some mitochondrial proteins, adhesion molecules, and mediators of the ubiquitin proteasome system and autophagy. However, a surprising array of axonal proteins are not essential for survival. These include highly abundant axonal proteins such as neurofilament subunits, several disease-associated proteins, and key mediators of axon and synapse function. Some axons can even survive days without mitochondria.

Failure of glial support, protein aggregation, inflammatory demyelination, and other mechanisms also cause axon degeneration. These may act partly through impairment of axonal transport, and readers are referred to other recent reviews for more details on what triggers these mechanisms (Muchowski and Wacker, 2005; Nave and Trapp, 2008; Zhao et al., 2005). This chapter aims to move beyond observations that axonal transport is blocked in neurodegenerative disorders by asking which cargoes are delayed, which of the resulting deficiencies kill axons, and what determines how long this takes. Answers to these questions are essential to move toward treating axonopathies. They are also important for understanding the basic biology of this fascinating structure.

II. AXONAL TRANSPORT AND HOW TO BLOCK IT

A. Road closed! General defects in axonal transport

Axonal transport has to deliver proteins, vesicles, organelles, and other components over centimeter-to-meter long distances from their sites of origin in the cell body. This is a feat totally without comparison in any other cell type. Long-range transport is a microtubule-based mechanism mediated in the anterograde direction by a wide range of kinesin superfamily motor proteins and in the retrograde direction by the dynein motor complex. For the details of the emerging molecules and mechanisms, readers are referred to several excellent, recent reviews (Chevalier-Larsen and Holzbaur, 2006; De Vos et al., 2008; Hirokawa and Noda, 2008; Salinas et al., 2008).
What happens when axonal transport fails? After axon injury, the ultimate block of axonal transport, total and permanent isolation of a distal axon from its cell body results in Wallerian degeneration of the distal stump 1–2 days later. Wallerian degeneration involves a poorly understood latent phase, followed by characteristic granular disintegration of the axonal cytoskeleton, glial reaction, and loss of axon continuity. It can be delayed tenfold by the slow Wallerian degeneration protein (WldS) or by related proteins when they are expressed or overexpressed in transgenic animals (Coleman and Freeman, 2010). WldS also delays axon degeneration in some neurodegenerative disorders. Several of these involve disruption of axonal transport, strongly suggesting that the trigger for Wallerian degeneration is the failure to deliver an essential cargo (Coleman and Freeman, 2010). An alternative model, involving an injury signal generated by the lesion, cannot explain how Wallerian-like mechanisms can be triggered without physical injury.

Physical trauma or axon compression causes a similar, nonspecific block of axonal transport in several human disorders. High intraocular pressure disrupts the flow of materials at one end of the optic nerve (Howell et al., 2007; Martin et al., 2006), resulting in axon degeneration that WldS can delay (Beirowski et al., 2008; Howell et al., 2007). In some models of Alzheimer’s disease pathogenesis, amyloid plaques physically compress nearby structures, including axons (Vickers et al., 2000). Spinal contusion injuries place chronic physical pressure on underlying axons, among other effects. Traumatic brain injury stretches axons, disrupting their cytoskeleton and axonal transport (Stone et al., 2004). Solid tumors, carpal tunnel syndrome, and other pressure palsies similarly restrict axonal transport due to physical pressure.

However, nonspecific impairment of axonal transport does not only result from mechanical disruption. Disruption of the microtubule “rails” along which long-range transport runs is the most obvious way to affect all cargoes. In progressive motor neuronopathy mice (pmn), for example, a loss-of-function mutation in the tubulin-specific chaperone e gene leads to a severe deficiency of microtubules in distal axons (Martin et al., 2002; Schaefer et al., 2007). Spastin, the protein mutated in the hereditary spastic paraplegia SPG4, also has critical roles in microtubule assembly and/or severing (Evans et al., 2005; Riano et al., 2009). Neurotoxic drugs such as Taxol and Vincristine probably alter axonal transport by directly targeting microtubules (Shemesh and Spira, 2009; Silva et al., 2006). Thus, little or nothing can be delivered without microtubules, their building blocks, or the chaperones that help put them together.

B. Traffic restrictions: Specific axonal transport defects

Disruption of axonal transport can be more specific in a number of ways. Ever since the discovery of fast anterograde transport (Weiss and Hiscoe, 1948), the complexity of axonal transport, as we understand it, has been increasing. First,
nerve constituents were found to travel retrogradely as well as anterogradely (Lubinska, 1964). Then, slow anterograde transport was discovered and subdivided (Lasek, 1968). Today, we know a huge array of anterograde motor proteins of the kinesin superfamily carrying different cargoes at different speeds (Hirokawa and Noda, 2008), together with adapter proteins whose roles in regulating attachment of cargo to motor proteins are just beginning to emerge (Horiuchi et al., 2005; Wang and Schwarz, 2009). The range of specific faults that can affect axonal transport is correspondingly wide, resulting in unidirectional impairment, partial impairment, or failure to deliver specific cargoes or groups of cargoes (Fig. 5.1).

C. One-way street! Unidirectional transport impairment

The microtubule-associated protein tau has emerged as an important modulator of axonal transport, but it has differential effects on anterograde and retrograde transport. Tau overexpression slows anterograde transport of mitochondria more than retrograde in cell lines (Stamer et al., 2002), probably reflecting its ability to dissociate the anterograde motor kinesin from microtubules. In contrast, when the retrograde motor dynein encounters tau, it hesitates but does not dissociate (Dixit et al., 2008). In amyotrophic lateral sclerosis studies, primary motoneuronal cultures from the SOD1G93A transgenic mouse model show reduced anterograde transport of mitochondria and enhanced retrograde transport, which may deplete axons of mitochondria (De Vos et al., 2007). One intriguing possibility is
that the paradoxical partial rescue of SOD1<sup>G93A</sup> transgenic mice by a mutation affecting retrograde transport could reflect a rebalancing of anterograde and retrograde transport (Kieran et al., 2005). Molecular switches regulating the direction of axonal transport (Colin et al., 2008; De Vos et al., 2003) provide further scope for directional imbalances when they go wrong, but could also be targets for strategies to restore the balance.

**D. Banned from driving: Cargo-specific defects**

Cargoes travelling in the same direction can be affected to different extents. Viral overexpression of A53T alpha-synuclein in rat substantia nigra to model Parkinson’s disease significantly decreases how much Kif1A, Kif1B, Kif2A, and Kif3A reach the striatum after 8 weeks, while Kif5 is barely changed (Chung et al., 2009). Conversely, mutation of Kif5a alone is sufficient to cause juvenile onset hereditary spastic paraplegia (SPG10) (Reid et al., 2002). Mutation of a Drosophila kinesin 3 family protein specifically blocks transport of synaptic vesicle precursors without altering mitochondria and other cargoes (Pack-Chung et al., 2007). Overexpression of K369I human tau in mice causes a large reduction in the amount of tyrosine hydroxylase, App, Gap43, and some other cargoes in the striatum, but no change in synaptophysin and synaptotagmin (Ittner et al., 2008). The motor proteins, Kif5B, Klc, and Kif1A, are also differentially affected. Finally, a mutant form of Huntingtin carrying an expanded polyglutamine repeat slows Bdnf transport but not that of mitochondria (Gauthier et al., 2004).

Specific axonal transport defects can also result from alterations to scaffold proteins linking motor proteins to their cargoes. Mutation or overexpression of one scaffold protein, the Drosophila Jip-1 homolog Aplip-1, perturbs anterograde transport of synaptobrevin-tagged vesicles but not of mitochondria, and causes axonal swellings and larval paralysis (Horiuchi et al., 2005). Another scaffold protein, milton, is critical for the anterograde axonal transport of mitochondria, but its loss leaves synaptic vesicles unaffected (Glater et al., 2006; Stowers et al., 2002).

Thus, disruption of microtubules affects all traffic by closing the road, while changes to the motors and regulators of axonal transport often stop only some “vehicles.” An intriguing prospect is that different disorders may result from failure to transport different cargoes.

**E. Faulty signals**

It is not enough for axonal transport to deliver cargoes to axons. They must also be deposited in the correct location or risk ending up centimeters away from where they are needed. The signaling pathways controlling this are starting to become clear and provide further scope for axonal transport to go wrong. For
example, the Rho GTPase miro confers calcium sensitivity on mitochondrial transport (Guo et al., 2005; Wang and Schwarz, 2009). Under normal circumstances, this causes mitochondria to accumulate in regions of high calcium influx such as synapses where ATP synthesis and calcium sequestration are important (Macaskill et al., 2009). Another consequence, however, is that mitochondrial movements stall with excessive calcium influx, as in excitotoxicity (Rintoul et al., 2003). Continued movement of other cargoes in high calcium conditions shows the specificity of this effect (Brady et al., 1984). NGF and Tnf also cause mitochondria to pause, probably acting through PI3 kinase and Jnk signaling pathways, respectively (Chada and Hollenbeck, 2004; Stagi et al., 2006). NGF seems to preferentially affect anterograde transport and not stall other cargoes, whereas TNF disrupts mitochondrial transport in both directions along with synaptophysin transport. Finally, some proteins such as Gap43 are anchored to vesicle membranes by palmitoylation (El-Husseini et al., 2001). This process is essential for their axonal targeting but likely has little effect on proteins that use other mechanisms.

**F. Traffic congestion: Partial blockages and axonal swellings**

Limited disruption of transport can take an entirely different form. Instead of a few specific cargoes being severely disrupted, the flow of many cargoes can be partially restricted. Organelle-filled swellings in kinesin or dynein-deficient *Drosophila* appear as “traffic jams,” but mitochondria continue to move into and out just as a queue on the motorway has cars leaving at one end and joining at the other (Pilling et al., 2006). Overexpression of tau in *Aplysia* causes similar axonal swellings, but live imaging shows that microtubule whorls within them continue to support transport (Shemesh et al., 2008; Fig. 5.2). Only a fraction of cargoes become trapped. The same may be true in mammals. Axonal swellings near amyloid plaques in a mouse Alzheimer’s disease model grow to the size of neuronal cell bodies and are packed with short, rounded mitochondria and markers of poor axonal transport (Fig. 5.2). Remarkably, however, more distal axon regions survive many months without degenerating, indicating that enough material flows through the swellings to support distal axons (Adalbert et al., 2009).

**G. Reduced traffic flow: Ageing and decline**

Axonal transport falls dramatically with ageing, affecting fast and slow transport of many cargoes both in retrograde and anterograde directions (Castel et al., 1994; Fernandez and Hodges-Savola, 1994; Frolikis et al., 1997; Li et al., 2004; McQuarrie et al., 1989; Tashiro and Komiya, 1994; Viancour and Kreiter, 1993). In rats aged two years, the average velocity of axonal transport is less than half
that in young rats and may decline by as much as 71% (Frolkis et al., 1997; Minoshima and Cross, 2008). Similar events occur in older primates (Kimura et al., 2007). Until noninvasive methods can be applied in man, we can only guess how much more transport slows over the course of 80 years or more. As axonal transport impairment is clearly established as a cause of some neurodegenerative conditions (Martin et al., 2002; Reid et al., 2002), this age-related decline could predispose to a range of age-related disorders or even cause some.

Thus, young axons appear to transport far more material than they need for survival, because otherwise, an age-related decline would cause massive axon death. This could explain the survival of swollen axons in young amyloid mouse models (Adalbert et al., 2009; Spires et al., 2005; Fig. 5.2). If the defect were imposed on a lower basal level of transport, the consequences might be far worse.

**Figure 5.2.** Partial blockage of axonal transport in amyloid or tau pathology. (A) Axons (green) swell next to amyloid plaques (red), and rounded mitochondria (blue) accumulate in these swellings. (B) Microtubule whorls (arrows) are evident in some swellings. (C) Similar microtubule diversions (green EB3 staining) form in Aplysia when tau is overexpressed (see also supplementary movie in Shemesh et al., 2008). (D) Despite the extensive swelling, axons in the mouse amyloid model shown in A and B remain continuous and morphologically normal at more distal sites (white arrows) for several months, indicating continued flow of at least some transport. A, B, and D reproduced from Adalbert et al. (2009) with permission from Oxford Journals. C reproduced from Shemesh et al. (2008) with permission from John Wiley & Sons, Inc.
(Fig. 5.3), just as amyloid pathology worsens when axonal transport is deliberately impaired (Stokin et al., 2005). Similarly, the decline in transport with age could underlie the age-associated axonal swelling found in YFP-H transgenic mice (Bridge et al., 2007).

**H. No parking! Could increased axonal transport be too much of a “good” thing?**

Finally, axonal disorders are so frequently associated with impaired transport that it is tempting to assume that enhancing transport can only be good for axonal health. However, when mitochondrial movement in axons is increased by deleting the mitochondrial docking protein syntaphilin, this reduces the density of mitochondria in axons and presynaptic terminals, thus impairing calcium buffering at nerve terminals (Kang et al., 2008). There are also subtle but significant motor defects.

Figure 5.3. Age-related decline in axonal transport and the threshold for disease. The decline in axonal transport with age could predispose or even trigger neurodegenerative disease below a certain threshold (dashed line). The reasons for falling below this threshold may include acute events such as toxins, viruses, or demyelination that lead to a sudden decline in transport (red line), cumulative events such as metabolic disorders causing accelerated declines over a long period (green line), or a lower starting point, for example, due to polymorphisms in transport regulating proteins (orange line).
Thus, axonal transport may have an optimum level such that too much could be as damaging as too little. Axonal components need to be distributed along the axon length not just delivered to the end, so failure to dissociate cargoes from the transport machinery would prevent cargoes reaching the correct destination just like failure to attach to transport machinery in the first place. A unidirectional increase also risks a gradual buildup of excess components at one end of the axon and a progressive deficiency at the other. Increased traffic may wear out the “road” or saturate motor complexes needed for other cargoes. Our understanding of how altered axonal transport leads to axon degeneration has come a very long way in recent years, but these and many other intriguing questions remain to be answered.

### III. The Essentials of Axon Survival

Having established that axonal transport can be impaired in a cargo-specific manner, it makes sense to ask which cargoes are most essential for axon survival. Specific failure to deliver these would endanger axon survival more than when less essential cargoes are blocked. Alternatively, when transport declines generally, as in ageing or with mechanical pressures, these are the cargoes whose absence is most likely to precipitate axon degeneration. For individual proteins, molecular genetic studies can help to identify which are the most critical, as genetic disruption also stops their delivery to axons. Table 5.1 lists some proteins in this category.

Interestingly, these proteins can be clustered into a relatively small number of common themes that begin to tell us what functions are most critical for axon survival. Around half are connected with axonal transport or vesicle trafficking. Interestingly, not all motor proteins, scaffold proteins, or regulators of axonal transport are essential for axon survival (see Table 5.2). Probably, the motor proteins in Table 5.1 are those that carry essential cargoes. Microtubule integrity clearly needs to be closely regulated by chaperones and severing proteins as it probably influences all cargoes. Vesicle trafficking also clearly links to axonal transport, and the likely involvement of lipid rafts in axonal delivery of some membrane proteins illustrates how cholesterol and lipid metabolism could also fit this theme (el-Husseini Ael and Bredt, 2002). Both protein synthesis and degradation will influence the axonal levels of specific proteins, and the requirement for efficient protein degradation is also consistent with other reports that proteasome inhibition is toxic to axons (Kane et al., 2003; Laser et al., 2003). An important role for adhesion molecules also emerges, probably mediating essential axon–glia interactions (below).
<table>
<thead>
<tr>
<th>Protein</th>
<th>Context</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Axonal transport/vesicle trafficking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alsin</td>
<td>Human disorder, null mouse</td>
<td>ALS/HSP</td>
<td>Deng et al. (2007), Yamanaka et al. (2006)</td>
</tr>
<tr>
<td>Atlastin</td>
<td>Human disorder</td>
<td>Hereditary spastic paraplegia</td>
<td>Meijer et al. (2007)</td>
</tr>
<tr>
<td>Bpag1</td>
<td>Null mouse</td>
<td>Rapid axon degeneration</td>
<td>Guo et al. (1995)</td>
</tr>
<tr>
<td>Dynein</td>
<td>Mouse missense mutations</td>
<td>Motor/sensory axon loss</td>
<td>Hafezparast et al. (2003), Ilieva et al. (2008)</td>
</tr>
<tr>
<td>Huntingtin</td>
<td>Conditional null mouse, null fly</td>
<td>Progressive axon/synapse loss</td>
<td>Dragatsis et al. (2000)</td>
</tr>
<tr>
<td>Jnk1</td>
<td>Null mouse</td>
<td>Spinal cord axon degeneration</td>
<td>Chang et al. (2003)</td>
</tr>
<tr>
<td>Kif1b beta</td>
<td>Heterozygous null mouse</td>
<td>Peripheral neuropathy</td>
<td>Zhao et al. (2001)</td>
</tr>
<tr>
<td>Kif5a</td>
<td>Human disorder, null mouse</td>
<td>Hereditary spastic paraplegia</td>
<td>Reid et al. (2002), Xia et al. (2003)</td>
</tr>
<tr>
<td>Masparadin</td>
<td>Human disorder</td>
<td>Hereditary spastic paraplegia</td>
<td>Simpson et al. (2003)</td>
</tr>
<tr>
<td>p150Glued</td>
<td>Human disorder, Tg mouse</td>
<td>Motor neuron disease</td>
<td>Laird et al. (2008), Puls et al. (2003)</td>
</tr>
<tr>
<td>Spastin</td>
<td>Human disorder</td>
<td>Hereditary spastic paraplegia</td>
<td>Patel et al. (2002)</td>
</tr>
<tr>
<td>Spastizin</td>
<td>Human disorder</td>
<td>CNS axonopathy</td>
<td>Tarrade et al. (2006)</td>
</tr>
<tr>
<td>Tbce</td>
<td>Mouse missense mutation</td>
<td>Early motor neuron disease</td>
<td>Martin et al. (2002)</td>
</tr>
<tr>
<td>Vps54</td>
<td>Mouse missense and gene trap</td>
<td>Motor neuron disease</td>
<td>Schmitt-John et al. (2005)</td>
</tr>
<tr>
<td><strong>Cholesterol and other lipid metabolisms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyp7b1</td>
<td>Human disorder</td>
<td>Hereditary spastic paraplegia</td>
<td>Tsaousidou et al. (2008)</td>
</tr>
<tr>
<td>iPla2β</td>
<td>Human disorder, null mouse</td>
<td>Neuroaxonal dystrophy</td>
<td>Shinzawa et al. (2008)</td>
</tr>
<tr>
<td>Npc1</td>
<td>Human disorder, null mouse</td>
<td>Axon swelling, lysosome defect</td>
<td>Pacheco et al. (2009)</td>
</tr>
<tr>
<td>Nte</td>
<td>Human toxicity, null mouse</td>
<td>Dying back axonopathy</td>
<td>Read et al. (2009)</td>
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<tr>
<td>Sap</td>
<td>Null mouse</td>
<td>Axonal spheroids</td>
<td>Oya et al. (1998)</td>
</tr>
<tr>
<td><strong>Protein synthesis</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GARS</td>
<td>Human disorder</td>
<td>Peripheral neuropathy</td>
<td>Antonelli et al. (2006)</td>
</tr>
<tr>
<td>Smn</td>
<td>Human, null mouse, zebrafish MO</td>
<td>Motor neuron disease</td>
<td>Carrel et al. (2006), Cifuentes-Diaz et al. (2002)</td>
</tr>
</tbody>
</table>

(Continues)
The table lists proteins with a likely axonal or axonal delivery role, whose genomic loss-of-function or dominant negative mutation results in axon degeneration. Defects that are clearly developmental have been excluded to focus on the maintenance of mature axons. Functional categories should be viewed only as a guide, as proteins may have multiple functions or their functions may not yet be fully characterized.
Table 5.2. Proteins Whose Functional Loss Does Not Cause Axon Degeneration

<table>
<thead>
<tr>
<th>Protein</th>
<th>Context</th>
<th>Related deficiency in nulls/roles of protein</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>App</td>
<td>Null mouse</td>
<td>Reduced synaptic vesicle density</td>
<td>Wang et al. (2005)</td>
</tr>
<tr>
<td>Ataxin 3</td>
<td>Null mouse</td>
<td>Increased ubiquitination in the brain</td>
<td>Schmitt et al. (2007)</td>
</tr>
<tr>
<td>Bace</td>
<td>Null mouse</td>
<td>Hypomyelination</td>
<td>Willem et al. (2006)</td>
</tr>
<tr>
<td>Calpain 1</td>
<td>Null mouse</td>
<td>Calpain activated in Wallerian degeneration</td>
<td>Grammer et al. (2005)</td>
</tr>
<tr>
<td>Caspase 6</td>
<td>Null mouse</td>
<td>Necessary for some forms of axon degeneration</td>
<td>Zheng et al. (1999)</td>
</tr>
<tr>
<td>Cd38</td>
<td>Null mouse</td>
<td>Enhanced neuronal NAD$^+$ levels</td>
<td>Sasaki et al. (2009)</td>
</tr>
<tr>
<td>EphA4</td>
<td>Null mouse</td>
<td>Effects on axon regeneration</td>
<td>Goldshmit et al. (2004)</td>
</tr>
<tr>
<td>Imac/kinesin 3</td>
<td>Mutant Drosophila embryo</td>
<td>Synaptogenesis defects</td>
<td>Pack-Chung et al. (2007)</td>
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<tr>
<td>Jip1</td>
<td>Null mouse</td>
<td>Protects from excitotoxic stress</td>
<td>Whitmarsh et al. (2001)</td>
</tr>
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<td>Jnk2</td>
<td>Null mouse</td>
<td>Protects from apoptosis</td>
<td>Ries et al. (2008)</td>
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<td>Jnk3</td>
<td>Null mouse</td>
<td>Protected from excitotoxicity</td>
<td>Yang et al. (1997)</td>
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<tr>
<td>K,1.1</td>
<td>Null mouse</td>
<td>Increased excitability, epilepsy</td>
<td>Smart et al. (1998)</td>
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<tr>
<td>Milton</td>
<td>Drosophila second instar</td>
<td>Absence of axonal mitochondria</td>
<td>Stowers et al. (2002)</td>
</tr>
<tr>
<td>Miro</td>
<td>Drosophila third instar</td>
<td>Very few axonal mitochondria</td>
<td>Guo et al. (2005)</td>
</tr>
<tr>
<td>Nefh</td>
<td>Null mouse</td>
<td>Increased microtubule density</td>
<td>Zhu et al. (1998)</td>
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<td>Nefl</td>
<td>Null mouse</td>
<td>Reduced caliber</td>
<td>Zhu et al. (1997)</td>
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<td>Nefm</td>
<td>Null mouse</td>
<td>Reduced caliber</td>
<td>Elder et al. (1998)</td>
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<td>Nefm</td>
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<td>Reduced caliber</td>
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<td>Nefm</td>
<td>Null mouse</td>
<td>Reduced caliber</td>
<td>Elder et al. (1998)</td>
</tr>
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<td>Nnos</td>
<td>Null mouse</td>
<td>Delayed axon regeneration</td>
<td>Keilhoff et al. (2002)</td>
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<td>p110β</td>
<td>Kinase dead knockin mouse</td>
<td>Poor axon regeneration</td>
<td>Eickholt et al. (2007)</td>
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<td>Presenilin 1</td>
<td>Conditional null mouse</td>
<td>Inhibits amyloid pathology</td>
<td>Dewachter et al. (2002)</td>
</tr>
<tr>
<td>Presenilin 2</td>
<td>Null mouse</td>
<td>Inhibits amyloid pathology</td>
<td>Dewachter et al. (2002)</td>
</tr>
<tr>
<td>Rab3</td>
<td>Quadruple null mouse</td>
<td>Evoked synaptic release deficiency</td>
<td>Schluter et al. (2004)</td>
</tr>
<tr>
<td>Scn2b</td>
<td>Null mouse</td>
<td>Reduced severity in EAE</td>
<td>O'Malley et al. (2009)</td>
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(Continues)
The table lists proteins with prominent roles in axonal or neuronal function that are dispensable for axon survival, at least in the experimental system indicated.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Context</th>
<th>Related deficiency in nulls/roles of protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scn8a</td>
<td>Conditional null mouse</td>
<td>Impaired motor function</td>
<td>Levin et al. (2006)</td>
</tr>
<tr>
<td>Scn9a</td>
<td>Human null mutations</td>
<td>Insensitivity to pain</td>
<td>Cox et al. (2006)</td>
</tr>
<tr>
<td>Sir2</td>
<td>Adult fly comp. heterozygote</td>
<td></td>
<td>Avery et al. (2009)</td>
</tr>
<tr>
<td>Sod2</td>
<td>Mouse conditional null</td>
<td></td>
<td>Misawa et al. (2006)</td>
</tr>
<tr>
<td>Syntaphilin</td>
<td>Null mouse</td>
<td>Low mitochondrial density, short-term facilitation</td>
<td>Kang et al. (2008)</td>
</tr>
<tr>
<td>α-Synuclein</td>
<td>Spontaneous mouse null</td>
<td></td>
<td>Specht and Schoepfer (2004)</td>
</tr>
<tr>
<td>Synaptotagmin</td>
<td>Null neonatal mouse and null early adult</td>
<td>Synaptic vesicle depletion, defective calcium-dependent release</td>
<td>Geppert et al. (1994), Loewen et al. (2001)</td>
</tr>
<tr>
<td>Tau</td>
<td>Null mouse</td>
<td>Exacerbates Npc1 and Map1b phenotypes</td>
<td>Dawson et al. (2001)</td>
</tr>
</tbody>
</table>
IV. TAKE IT OR LEAVE IT: WHAT AXONS DO NOT NEED TO SURVIVE

Conversely, there are many proteins whose functional absence has little effect on axon survival (Table 5.2). Null mouse or Drosophila studies, especially when limited to early developmental stages, cannot of course rule out degeneration over the longer term in larger human axons, but failure to deliver these proteins to axons does not precipitate axon degeneration within the confines of the experimental system listed. Some have other functional consequences, but axons survive.

There are a number of surprises in this list. Axons can survive in the absence of proteins essential for synaptic function, without some of their most abundant proteins, or without proteins closely associated with neurodegenerative diseases.

Redundancy is one obvious explanation why axons survive without some proteins. For example, mice lacking tau show no obvious axonal defects (Dawson et al., 2001; Yuan et al., 2008), but when Map1b is also removed, the axonal phenotype is far worse than for either individual deletion (Takei et al., 2000). In this particular example, the question whether this is a degenerative phenotype as well as developmental still needs to be resolved, but it illustrates the point that the function of some axonal proteins can be filled by others.

Synaptic vesicle trafficking and function can be impaired without compromising axon survival. Rab3 proteins, a family of GTP-binding proteins with seemingly ubiquitous roles in vesicular transport, have essential roles in evoked release of synaptic vesicles. Structurally, however, there is no apparent change in brain or synapse structure even when all four isoforms are deleted simultaneously in mice (Schluter et al., 2004). Drosophila embryos deficient in Imac, a kinesin 3 protein essential for synaptic vesicle transport, develop axons that contact their muscles, even if they lack extended branches and mature synapses (Pack-Chung et al., 2007), and both Drosophila and mouse axons can develop without any signs of degeneration in the absence of synaptotagmin (Geppert et al., 1994; Loewen et al., 2001). Thus, while some aspects of vesicle trafficking are essential for axon survival (Table 5.1), profound defects in synaptic vesicle trafficking or function do not necessarily cause axon degeneration.

Axons are also remarkably tolerant of disruptions to neurofilaments, the major class of intermediate filaments in neurons, in contrast to the devastating effects of microtubule disruption (above). All three neurofilament subunits can be deleted without loss of axonal viability, although axonal caliber decreases without NF-M or NF-L (Elder et al., 1998; Zhu et al., 1997, 1998). Deletion of the peripheral nerve intermediate filament protein peripherin also affects only a small subset of the axons that express it (Lariviere et al., 2002).

Several proteins not required for axon survival are mutated in neurodegenerative diseases. App, tau, α-synuclein, and ataxin 3 are mutated in some hereditary forms of Alzheimer’s disease, frontotemporal dementia, Parkinson’s
disease, and spinal cerebellar ataxia, respectively. Furthermore, Bace and PS1 are responsible for cleaving the pathogenic Aβ1-42 species from its precursor App as a key step in Alzheimer’s disease pathogenesis. Considering the major role of synapse loss played in many of these diseases, the ability of axons and synapses to survive without these proteins suggests that gain-of-toxic-function mechanisms (e.g., protein aggregation) are most likely to underlie their roles in disease. Alternatively, mice could be too short-lived, or their axons too short, to model the degenerative stages of the human disease in some cases.

Perhaps most remarkably of all, second instar larvae with the Drosophila mutation milton have axons that survive up to 5 days without mitochondria. Milton is a scaffold protein essential for anterograde axonal transport of mitochondria, but without it, axons develop and retain a normal ultrastructure and continue to transport other components, including synaptic vesicles (Glater et al., 2006; Stowers et al., 2002). Another mutant, miro, also has a severe depletion of axonal mitochondria but retains basal neurotransmitter release (Guo et al., 2005). The larvae reach the third instar, extending axons as long as 1.5 mm (Tom Schwarz, personal communication). A possible explanation for how these axons generate enough ATP to survive comes from SCG primary culture studies, which show that glycolysis makes a significant contribution to axonal ATP synthesis (Tolkovsky and Suidan, 1987; Wakade et al., 1985).

Together, these observations support the concept that axon survival depends much more on some cargoes than others, and Tables 5.1 and 5.2 begin to highlight which proteins are fundamentally important for axons to survive and which are dispensable.

V. PROTEIN TURNOVER FAST AND SLOW

Of course, the response of axons to long-term absence of proteins only partially models the deficiency caused by blocking axonal transport. For example, an acute block of axonal transport (e.g., by injury, ischemia, or toxins) impairs delivery of new cargo, but molecules or organelles already present in axons may be unaffected. Thus, even if their function is essential for axon survival (Table 5.1), the effect may not be felt until the axonal pool turns over. The effects of chronic transport impairment (e.g., in ageing, amyloid or other axonal swelling pathology (Fig. 5.2), or faulty axon–glia interactions) may also depend on half-life. This is because short-lived cargoes are less likely to reach distal axons than long-lived ones if the journey takes too long. Thus, a possible basis for “dying back” disorders (Cavanagh, 1979) is a delay in axonal transport of proteins that are both short-lived and essential for axons, depriving distal axons of the replenishment they constantly need.
Like any mixture of proteins, axonal proteins have a large range of half-lives. At one extreme, the light neurofilament protein has a half-life estimated at 3 weeks to 2.5 months, depending on the experimental system (Millecamps et al., 2007; Yuan et al., 2009). In contrast, App and Aplp2 have half-lives of around 4 h after being transported through the hamster optic nerve to the superior colliculus (Lyckman et al., 1998). Some axonal proteins are even less stable. Ornithine decarboxylase has a half-life of only 5–30 min in many mammalian tissues, although its axonal half-life has not been reported (Iwami et al., 1990).

It is hard to reconcile some of these half-lives with the time taken for proteins to reach axon termini. In the longest human axons, the most rapidly transported cargoes take around 2 days to reach the distal ends. Thus, a half-life of 4 h may suffice for App to reach the terminals of 1-cm hamster optic nerve axons, but little or none should reach neuromuscular junctions in our toes, and the viability of long blue whale axons starts to appear really questionable. For ornithine decarboxylase, a notoriously unstable protein, the problem is even greater, and yet it is abundant in motor axons and their terminals (Junttila et al., 1993). Axonal protein synthesis could be one explanation for a subset of axonal proteins (see below). Another intriguing prospect is that cargoes are somehow “privileged” while they are being transported, so that the ubiquitin proteasome system does not “see” them. Once released from the transport machinery, their half-life could suddenly shorten.

In summary, diverse axonal proteins have half-lives ranging from months down to hours or even minutes. Mechanisms to understand how the more labile proteins reach the distal ends of longer axons require further investigation. However, most labile proteins are likely to be depleted quickly when transport fails, and distal regions are likely to suffer the greatest decline.

VI. AXONAL PROTEIN SYNTHESIS

After many years of controversy, it is now generally accepted that some axonal proteins can be translated locally using axonally targeted mRNAs (Giuditta et al., 2002; Gumy et al., 2009). This seems to concern only a subset of axonal proteins, and how much of these proteins is synthesized in axons remains unclear. However, there is a clear capacity to synthesize some proteins locally using a system that can respond to local events such as axonal damage (Gumy et al., 2009; Perlson et al., 2005) and extracellular stimuli (Willis et al., 2007).

Axonal protein synthesis may partially answer how labile proteins are supplied to distal axons. For example, ornithine decarboxylase mRNA is present in axons, at least after a conditioning lesion (Willis et al., 2007). Thus, the paradox that a large amount of this very labile protein exists in distal axons (above) could be explained if most of the protein derives from local synthesis.
Other mRNAs in both mammalian and Aplysia axons cluster around certain types of protein, in particular, those encoding protein translation machinery, cytoskeletal proteins, proteasome subunits, mitochondrial proteins, and heat shock proteins (Gumy et al., 2009; Moccia et al., 2003; Willis et al., 2007), together with some membrane and secreted proteins (Merianda et al., 2009). However, mRNAs encoding many of the essential proteins listed in Table 5.1 are conspicuously absent from the list of axonal mRNAs reported so far (Willis et al., 2007). Thus, axonal protein synthesis does not in general appear to be designed to supply essential proteins that axonal transport fails to deliver. Instead, its main function may be to facilitate a rapid axonal response to local events such as trauma, neurotrophic signaling, or pathfinding cues (Campbell and Holt, 2001; Gumy et al., 2009).

Nevertheless, there are indications that local protein synthesis does influence axon survival. First, spinal muscular atrophy, a lower motor neuron disease with early axon loss (Cifuentes-Diaz et al., 2002), is caused by loss-of-function mutations in the survival motor neuron (smn) protein. Although there is some controversy, the essential function of smn does seem to be axonal, where it helps deliver mRNA for β-actin (Carrel et al., 2006; Conforti et al., 2007). Second, glycyl tRNA synthetase, a tRNA-charging enzyme whose loss-of-function causes Charcot-Marie-Tooth Disease Type 2D, is abundant in peripheral axons where it seems likely to function in local synthesis.

VII. ANTEROGRADE SURVIVAL SIGNALING

Together, the preceding sections tell us that (a) a subset of axonal proteins are critically important for survival, (b) some protein half-lives appear barely compatible with delivery to distal axons before these proteins degrade, and (c) a largely nonoverlapping subset of axonal proteins can be locally translated in axons. When delivery of many cargoes is impaired, those that limit axon survival are likely to be essential, short-lived, and unable to be locally synthesized (Fig. 5.4). Essential proteins with longer half-lives will remain abundant in axons until the dwindling supply of short-lived, essential proteins has already killed the axon. Locally synthesized proteins could be maintained for as long as the translation machinery remains active, which could be a long time if the protein synthesis machinery can be supplied by associated glia, as recently proposed (Court et al., 2008). Thus, when the supply of specific cargoes fails, the life or death of an axon, and whether it dies quickly or slowly, will depend on whether the affected cargo is in this central category.

One example of such a protein has recently been reported. The NAD⁺ biosynthesis enzyme nicotinamide mononucleotide adenylyltransferase 2 (Nmnat2) is a highly labile molecule whose presence in axons, at least in
primary culture, is essential for their survival (Gilley and Coleman, 2010). Its rapid turnover in axons is normally replaced by fast anterograde axonal transport of newly synthesized protein, but if transport or synthesis of Nmnat2 fails, this triggers Wallerian-like degeneration of the axon. An important future direction will be to identify more such proteins, particularly by knowing more about protein half-lives in axons. Manipulation of the expression, transport, or degradation of such proteins could open new therapeutic opportunities in axonal disorders. It would also be interesting to know whether neurons use similar mechanisms to regulate axonal survival in disease, after injury or during development.

Another approach to understanding anterograde survival signals is to study the axonal roles of proteins that mediate survival signaling in many cells. Although mechanisms of axon degeneration and cell death differ (Deckwerth...
and Johnson, 1994; Finn et al., 2000), some steps could still be shared. Exposure of phosphatidyl serine on the external surface of degenerating axons directly mirrors one event during apoptosis (Ivins et al., 1998; Sievers et al., 2003), and the involvement of the apoptotic effector caspase 6 in axon death after trophic factor deprivation also suggests similarities (Nikolaev et al., 2009). Thus, it is interesting that survival and stress signaling pathways involving PI3 kinases, Erk1/2, Erk5, and Jnk all have important axonal roles, including retrograde signaling of axonal damage, neurotrophin signaling, and regulating axonal transport (Cavalli et al., 2005; Chada and Hollenbeck, 2004; Horiuchi et al., 2005; Perlson et al., 2005; Watson et al., 2001). Mek/Erk signaling and Jnk signaling have also been reported to influence axon survival (Macinnis and Campenot, 2005; Miller et al., 2009). An important future direction will be to understand the anterograde transport of these molecules and their regulators, and what influence this has on axon survival.

Neurotrophins are also now known to be transported anterogradely in axons, including Bdnf, NT-3, and Gdnf (von Bartheld et al., 2001). This process influences the functions and survival of postsynaptic cells (Caleo et al., 2003; Fawcett et al., 1998), associated glia (Ng et al., 2007), and the axon itself (Menna et al., 2003), at least during development. Neurotrophin receptors involved in retrograde signaling clearly also have to be delivered by anterograde axonal transport. Thus, anterograde cargoes that may regulate axon survival include well-studied intracellular signaling proteins and intercellular ligands and their receptors whose delivery to axons influences cross-talk with supporting glia and other cells.

VIII. ADHESION MOLECULES

Adhesion molecules also have roles in survival signaling in many cell types and feature prominently in the list of proteins essential for axon survival (Table 5.1). Without efficient anterograde transport, these will also be unable to fulfill their important axonal roles such as contributing to axon–glial interactions. Mechanisms of glial support for axons, an absolute requirement for axon survival in vivo, have been extensively reviewed elsewhere (Nave and Trapp, 2008), so this chapter focuses on events on the axonal side.

Axonal L1 CAM is needed to maintain, but not to establish, Schwann cell ensheathment of unmyelinated sensory axons (Haney et al., 1999), probably through interaction with Schwann cell β1-integrins (Itoh et al., 2005). Without it, this axon subset is progressively lost. Interestingly, unmyelinated sympathetic fibers are unaffected, suggesting that different mechanisms mediate this interaction in different peripheral nerve axons. L1 also appears to be essential in the optic nerve prior to myelination, with axonal ankyrinB probably acting
downstream. Despite normal development of L1 positive axons in ankyrinB null mice, L1 is lost unusually early in postnatal life. This is followed by catastrophic axon swelling and degeneration of the entire optic nerve within the space of 2 weeks (Scotland et al., 1998). AnkyrinB seems to have an essential role in stabilizing L1 and linking the membrane to the actin cytoskeleton to provide mechanical strength.

In myelinated axons, gangliosides appear to play a similar role. Gangliosides are glycosphingolipids in the axolemma that function as membrane-anchored ligands for myelin-associated glycoprotein (Mag), a glycoprotein located on adaxonal membranes of myelin-producing cells in CNS and PNS that is also required for axon survival (Nguyen et al., 2009; Yin et al., 1998). Mice lacking the complex ganglioside synthetic protein Galgt1 develop progressive axon degeneration in peripheral nerves and dorsal column of the spinal cord (Chiavegatto et al., 2000; Pan et al., 2005). A more severe phenotype develops in mice also lacking Siat9, which are unable to synthesize simple gangliosides too (Yamashita et al., 2005).

Thus, another function of anterograde axonal transport important for axon survival is to deliver these enzymes or their products into axons, in order to maintain axon–glia interactions.

**IX. LIFE AFTER (CELL) DEATH: THE WLD<sup>S</sup> PHENOTYPE**

To return to a topic introduced at the beginning, it is interesting to reexamine the delayed Wallerian degeneration in Wld<sup>S</sup> mice in the light of the discussions above. Recent advances in understanding the Wld<sup>S</sup> mechanism are summarized elsewhere (Coleman and Freeman, 2010), but an important new point emerges here. Axon injury or cell death (Deckwerth and Johnson, 1994) interrupts the supply of all cargoes from the cell body. Some will be essential, some nonessential. Some will have shorter half-lives than others, and some will be locally synthesized in the distal axon stump. Among the essential missing factors, Nmnat2 seems to have a key role in triggering Wallerian degeneration when its level falls below a threshold needed for survival (Gilley and Coleman, 2010; see above). This may explain why injured axon stumps degenerate after a latent phase of around 36 h (Beirowski et al., 2005; Lubinska, 1977). Delaying this degenerative mechanism by substituting Nmnat2 with Wld<sup>S</sup> allows axons to survive for 2–3 weeks, indicating that axons do not need replenishment of most cargoes on this timescale. Thus, any protein whose loss triggers degeneration in 36 h should have a half-life that is a clear outlier from the population of essential axonal proteins. Moreover, to preserve injured or transport-impaired axons even longer, it would be useful to identify the next most labile, essential cargoes.
Further understanding of the mechanism by which WldS prolongs the survival of severed distal axon stumps should be an excellent way to shed light on the nature of anterograde axon survival signaling.

X. SUMMARY: AXONAL TRANSPORT AND AXON SURVIVAL SIGNALING

This chapter emphasizes that axonal proteins are not all equal in their essential nature, their transport mechanisms, their half-lives, and their capacity for local synthesis. Essential axonal proteins fall into a surprisingly narrow list of categories, while those that are dispensable for axon survival are not necessarily those we might have expected. There is an enormous range of protein half-lives, and some proteins will have difficulty making it to the ends of long axons unless there are mechanisms to stabilize them during transport or to synthesize them locally. Functionally, we can find clues to which proteins limit axon survival from molecular genetic studies. However, an equally promising area to explore is the axonal roles of signaling pathways that control survival in other cell types, as these same pathways have axonal functions but may be subject to different control mechanisms. Intracellular transport over such long distances is unique to axons and while this poses problems for delivering essential cargoes, it also provides a unique mechanism for regulating death programs. Long-range survival signaling and axonal transport may be two ways of looking at the same thing.

Acknowledgments

This chapter reflects memorable presentations and discussions with many colleagues in addition to the cited publications. These include but are not limited to Christine Beattie, Scott Brady, Anthony Brown, Felipe Court, James Fawcett, Marc Freeman, Paul Glynn, Larry Goldstein, Georg Haase, Peter Hollenbeck, Christine Holt, Erika Holzbaur, Keith Martin, Chris Miller, Thomas Misgeld, Hugh Perry, Evan Reid, Richard Ribchester, Tom Schwarz, Zu-Hang Sheng, Aviva Tolkovsky, Jeff Twiss, Xinnan Wang, and Dianna Willis. I am equally grateful to present former members of the Coleman laboratory for open and constructive discussions on many topics related to this chapter. I am also grateful to Hilda Tsang for provision of a literature summary of hereditary spastic paraplegia that contributed to Tables 5.1 and 5.2. The author is funded by the Biotechnology and Biological Sciences Research Council (BBSRC).

References


5. Axon death


