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Intrinsic axonal degeneration pathways are critical for glaucomatous damage

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Abstract

Glaucoma is a neurodegenerative disease affecting 70 million people worldwide. For some time, analysis of human glaucoma and animal models suggested that RGC axonal injury in the optic nerve head (where RGC axons exit the eye) is an important early event in glaucomatous neurodegeneration. During the last decade advances in molecular biology and genome manipulation have allowed this hypothesis to be tested more critically, at least in animal models. Data indicate that RGC axon degeneration precedes soma death. Preventing soma death using mouse models that are mutant for BAX, a proapoptotic gene, is not sufficient to prevent the degeneration of RGC axons. This indicates that different degeneration processes occur in different compartments of the RGC during glaucoma. Furthermore, the Wallerian degeneration slow allele (*Wld^Δ*) slows or prevents RGC axon degeneration in rodent models of glaucoma. These experiments and many others, now strongly support the hypothesis that axon degeneration is a critical pathological event in glaucomatous neurodegeneration. However, the events that lead from a glaucomatous insult (e.g. elevated intraocular pressure) to axon damage in glaucoma are not well defined. For developing new therapies, it will be necessary to clearly define and order the molecular events that lead from glaucomatous insults to axon degeneration.

Introduction

Glaucoma is a leading neurodegenerative cause of blindness and is reported to be the second leading cause of blindness worldwide (Quigley & Broman 2006). It is a heterogeneous group of diseases characterized by the dysfunction and death of retinal ganglion cells (RGCs). Approximately 70 million people are affected by glaucoma (Quigley 1996). Major risk factors include increasing age and intraocular pressure (IOP) elevation. However, high IOP by itself is not sufficient to cause glaucoma, as many individuals with high IOP do not develop glaucoma (Ritch et al 1996). Treatments currently center on lowering IOP levels, but do not prevent the development or progression of visual abnormalities in all patients (Gordon et al 2002; Kass et al 2002). To develop better treatments, it is necessary that we

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better understand pressure-dependent and pressure-independent mechanisms that contribute to RGC loss. Given the limitations of working with humans, animal models are an essential component for understanding mechanisms of glaucoma. No single animal model captures all of the nuances of human glaucoma, nor could one since glaucoma differs between patients. However, models used to study glaucoma aim to model the key event in glaucomatous neurodegeneration, early damage to axons in the optic nerve head, and this has been clearly shown for some models. In this review, we do not extensively review animal models of glaucoma as this has been done recently elsewhere (Howell et al 2008; Lasker/IRRF 2010; McKinnon et al 2009; Morrison et al 2005; Pang & Clark 2007; Ruiz-Ederra et al 2005). Instead, we focus on mechanisms contributing to RGC axon degeneration and discuss the early changes that occur in axons prior to axon degeneration. We highlight studies that have directly manipulated biological pathways to test the importance of different pathways/processes that are involved. More extensive discussion on axon degeneration pathways is provided in other reviews in this issue and elsewhere, and many of these pathways need to be tested in glaucoma (reviewed in (Nickells et al submitted)).

Compartmentalized axon degeneration

Neurons have distinct functional compartments and can execute specific self-destruct pathways that are spatially compartmentalized (Whitmore et al 2005). In glaucoma, work using DBA/2J mice (a widely used mouse model of glaucoma) showed that the mechanisms by which RGC somata die differ from those involved in the degeneration of their axons. Genetic ablation of BAX, a proapoptotic molecule, prevented the death of essentially all RGC somata in DBA/2J mice but the axons of these mice still degenerated (Libby et al 2005b). Therefore, for glaucoma, somal death is a BAX-dependant apoptotic process, whereas BAX is not necessary for axonal degeneration. Interestingly, axon degeneration may not be completely independent of BAX as BAX-deficiency did slow axon degeneration in DBA/2J mice (Libby et al 2005b). This possibility is supported by recent work showing that BAX participates in the axon degeneration pathway, independently of somal degeneration (Nikolaev et al 2009).

Further evidence of an axonal injury in glaucoma came from testing the effects of the Wallerian degeneration slow (*Wld^s*) allele in two different animal models of glaucoma (Figure 1) (Beirowski et al 2008; Howell et al 2007). The *Wld^s* gene creates a fusion protein containing 70 N-terminal amino acids of ubiquitination factor *Ube4b* linked to full-length nicotinamide mononucleotide adenylyltransferase 1 (*Nmnat1*) (Mack et al 2001). This chimeric protein protects axons from degeneration induced by axonal trauma (Lunn et al 1989; Mack et al 2001; Perry et al 1991; Perry et al 1990; Ribchester et al 1995). *Wld^s* is proposed to directly protect axons but not somata (Adalbert et al 2005; Deckwerth & Johnson 1994; Glass et al 1993; Ikegami & Koike 2003), and so the allele can be used to test the importance of axon degeneration in disease. In DBA/2J mice, the *Wld^s* allele protects from axon degeneration (Howell et al 2007). *Wld^s* more than doubled the number of eyes with no detectable glaucoma compared to standard DBA/2J mice and preserved RGC function (as determined by the pattern electroretinogram). *Wld^s* had a strong protective effect on the survival of optic nerve axons for at least a few months following IOP elevation. In addition, RGC somata survived in DBA/2J. *Wld^s* eyes whose axons were spared. Although the somata were spared, they were not completely protected as on average somal diameter had shrunk by ~10%. RGC somata were largely absent in DBA/2J. *Wld^s* eyes with severe axon loss, indicating that *Wld^s* cannot protect the somata from death if the axon degenerates.

In the second study, the ability of the *Wld^s* protein to protect from RGC loss was assessed in an inducible rat model of glaucoma (Beirowski et al 2008). Transgenic rats were generated

carrying the *Wld^f* gene driven by the α -actin promoter. (This is in contrast to the mouse version of *Wld^f* where the *Ube4b* promoter controls expression of the *Wld^f* protein.) IOP was artificially elevated by translimbal laser photocoagulation of the trabecular meshwork. Due to the different promoter use between mouse and rat, the authors first confirmed that the transgenic rat RGCs expressed the *Wld^f* protein and that RGC axons underwent delayed axon degeneration following optic nerve transection. They then tested whether *Wld^f* affects RGC axon degeneration and somal loss in rats with high IOP. Survival of RGC axons and cell bodies was assessed two and four weeks after induction of ocular hypertension. In wild-type rats, substantial loss of axons was observed and correlated well with cumulative IOP exposure. The *Wld^f* gene delayed axonal degeneration, with a duration similar to that seen in optic nerve transection (approximately 2 weeks). In contrast to the study in DBA/2J, *Wld^f* had little or no effect on somal death.

The two studies report two clear differences. First, axonal protection by *Wld^f* was longer lasting in DBA/2J mice than in the rats. IOP elevation is detected in some DBA/2J eyes by 6 months and in many eyes by 9 months of age (Libby et al 2005a). *Wld^f* protected from optic nerve damage to at least 12 months of age, indicating that eyes were protected for at least a few months. This compares to a two-week protection that was observed in the rat study. Second, *Wld^f* genotype saved somata (when axons were spared) in DBA/2J mice but had little or no effect on somal survival in the rat study. These contrasting results are likely due to the different glaucoma models used. DBA/2J is an inherited, chronic model of glaucoma where IOP elevation and subsequent RGC loss occurs progressively over time. In contrast, the rat model is an inducible, acute model where IOP levels are artificially increased and RGC loss occurs over a shorter window. However, species, genetic background and/or *Wld^f* expression differences could also explain the contrasting results between the two models.

Irrespective of the differences, both studies indicate that protecting RGCs from axonal degeneration should be explored further as a treatment for human glaucoma (possibly as part of a combinatorial treatment regimen). The mechanisms by which *Wld^f* protects neurons are not fully understood (Araki et al 2004; Laser et al 2006) and are discussed in more detail elsewhere in this special issue. However, these studies suggest that neuroprotective strategies that involve NAD biosynthesis or additional functions of *Nmnat*, possibly in combination with BAX inhibitors, should be tested for glaucoma.

Mechanisms of distal axon degeneration in glaucoma

Both dying back and Wallerian degeneration have been proposed as mechanisms of distal axon degeneration in glaucoma. Dying back is the process that involves the slow and progressive (weeks or months) degeneration of a stressed neuron from its terminals and distal axon towards the cell body (Whitmore et al 2005). Wallerian degeneration occurs in response to severe focal damage such as transection (Whitmore et al 2005). The distal axon, separated from the cell body by the lesion, rapidly disassembles (days) in a characteristic way with the accumulation of dense bodies and neuroaxonal spheroids. The exact molecular processes are not clear but Wallerian degeneration and dying back likely share similar mechanisms, as the *Wld^f* allele slows both forms of degeneration. Some studies support a role for dying back as the major mechanism of distal axon degeneration in glaucoma (Figure 2). Disruption of axonal transport, as observed in many glaucoma models, may limit transport and communication between distal axons and cell bodies leading to the degeneration of the axons by dying back. In DBA/2J mice, axon degeneration is evident at distal locations of the optic pathway and coincides with a lack of axonal transport at the level of the superior colliculus (Figure 2 and (Crish et al 2010; Schlamp et al 2006)).

However, data also support a more Wallerian degeneration-like mechanism for distal axon degeneration in glaucoma. In DBA/2J mice, swollen axon regions with disorganization of axonal contents and accumulation of organelles and neurofilaments, reminiscent of the neuroaxonal swellings of Wallerian degeneration, are observed from the optic nerve head all the way back to the superior colliculus (Howell et al 2007). However, in the same eyes, milder and highly focal swelling of individual axons was the far more common form of damage and was restricted to axon segments in the ONH. We speculate that these focally swollen and damaged axon segments represent regions of slowed or disrupted axonal transport, which may lead to the degeneration of the damaged axon by dying back. The presence of two types of damage in DBA/2J mice suggests that distal degeneration of axons occurs by both Wallerian degeneration and dying back, depending on the severity of damage to individual axons. Both of these degeneration processes are likely to occur in human glaucoma. It is possible that the relative contribution of each process to axon degeneration varies between different glaucoma patients (e.g. a greater contribution of Wallerian degeneration in severe angle-closure glaucoma patients) and even between different axons in the same eye.

Axon degeneration is caused by an insult in the ONH

Early studies in humans and primates suggested that the ONH was an important site in glaucoma (Anderson & Hendrickson 1974; Quigley & Addicks 1980a; b; 1981; Quigley et al 1981; Quigley & Anderson 1977; Quigley et al 1979; Quigley et al 1983). RGC axons exit the eye through a specialized region of the ONH known either as the lamina cribrosa (LC) or the glial lamina. In humans and species with larger optic nerves the LC is comprised of plates of extracellular matrix (ECM) that provide support for the bundles of axons as they pass through the posterior wall of the eye. The ECM plates are covered by astrocytes that provide support to the neurons (Anderson DR & HA 1992). The laminar region in the rodent ONH has been termed the glial lamina (Howell et al 2007). It has striking similarities to the LC in primates, primarily because of the presence of a network of astrocytes but the ECM plates are absent (Howell et al 2007; May & Lütjen-Drecoll 2002; Schlamp et al 2006). Despite these differences, the earliest RGC axon damage appears to occur in the laminar region in species with either an LC or a glial lamina. Also, the characteristic pattern of RGC dysfunction/loss observed in both human glaucoma (Shields 1992) and animal models (Howell et al 2007; Jakobs et al 2005; Schlamp et al 2006) is best explained by crucial damage to axon bundles in the laminar region (reviewed in (Nickells et al submitted)).

Various studies have shown that the earliest damage to axons is observed in the ONH in glaucoma (Anderson & Hendrickson 1974; Anderson & Hendrickson 1977; Howell et al 2007; Quigley & Anderson 1976; Quigley & Addicks 1980c; Quigley et al 1981; Quigley et al 1983; Schlamp et al 2006). However, demonstrating that the first signs of axon damage occur within the lamina is not proof that axons are insulted within the lamina. In the general case, it is well established that the first site of neuronal degeneration may be remote from the site of insult (reviewed in (Conforti et al 2007)). For example, in transected motor axons, neuromuscular junctions that are many centimeters from the lesion degenerate first. The axons immediately adjacent to the lesion remain intact for two to three times longer than the distant terminals (Beirowski et al 2005). To test if an axon injury occurs in the glial lamina in glaucoma, a study was performed that was based on the well-established pattern of axon degeneration and survival that occurs in response to axon injury in the peripheral nervous system (reviewed in (Whitmore et al 2005)). Direct and focal axon injury in the peripheral nervous system results in degeneration of the distal portion of the axon that is isolated from the cell body by the lesion. However the proximal portion of the axon attached to the surviving cell body can survive up to the proximity of the axon insult. It was reasoned that in BAX-deficient DBA/2J mice, because all the RGC somata survive after insult, the

proximal axon should survive up to the site of injury. This reasoning was first tested and confirmed using optic nerve crush in BAX-deficient DBA/2J mice. Following crush, the proximal axon segment survived up to the crush site for at least 30 days. Next, survival of the proximal axon was used to locate a site of insult in glaucoma. Importantly, in eyes of DBA/2J mice with severe glaucomatous damage (e.g. distal axons degenerating), RGC axons survived from the RGC bodies to the anterior edge of the glial lamina. No axons survived to the middle or posterior of the lamina. This provided experimental evidence for a major axon insult occurring within the lamina of the optic nerve or very close to its anterior edge.

Early axonal changes that occur prior to axon degeneration

The processes that damage RGC axons in the ONH are not clear. However, observations from several different animal models of glaucoma show that disruption of axonal transport at the ONH is consistently seen early in the disease process. In monkeys with experimental glaucoma, anterograde and retrograde transport is affected in the ONH (Anderson & Hendrickson 1974; Dandona et al 1991; Radius & Anderson 1981). Electron microscopy studies showed that mitochondria and dense bodies accumulate anterior and posterior to the lamina cribrosa after IOP elevation (Gaasterland et al 1978) (Quigley & Anderson 1976). This accumulation of organelles is observed as early as one hour after induction of high IOP (Quigley & Anderson 1976). Under conditions of transient IOP elevation, this axonal blockade is reversed when IOP is normalized (Levy 1974; Minckler et al 1977; Quigley & Anderson 1976). Similarly, acute elevation of IOP in the rat causes disruption of axonal transport and cytoskeletal changes that presumably sustain axonal transport blockade (Chidlow et al 2011; Pease et al 2000; Quigley et al 2000; Salinas-Navarro et al 2010). Finally, in DBA/2J mice, anterograde axonal transport is disrupted from the retina to the superior colliculus and in regional patterns that are similar to the regional degeneration of axons in glaucoma (Crish et al 2010). Compromised axonal transport likely results in a reduction in the flow of neurotrophic factors in RGCs (reviewed in (Baltmr et al 2010)). It has not yet been shown that a loss of neurotrophic factors directly leads to axon degeneration. However, delivery of neurotrophic factors improves RGC survival in animal models of glaucoma (Martin et al 2003; Pease et al 2009) and may be a therapeutic target for human glaucoma (Baltmr et al 2010; Saragovi et al 2009). Overall though, many early changes occur to RGC axons at the optic nerve head that could trigger axon degeneration, and all these early changes may be considered as avenues for neuroprotective treatments.

While it is clear that damage to axons in the ONH is an important early event in glaucoma, the molecular signaling pathways that trigger axon degeneration are not well defined. Elevation of intra-axonal calcium levels, and its downstream effects on mitochondrial dysfunction, are often proposed to be important in axonal self destruction in glaucoma (reviewed in (Kong et al 2009; Whitmore et al 2005)). Increased release of calcium from the ER causes the uptake and accumulation of calcium in the mitochondrial matrix. This accumulation of calcium promotes the opening of the permeability transition pore (mPTP) that results in the swelling, membrane rupture, and depolarization of mitochondria leading to an energetic failure (Whitmore et al 2005). Recent data have demonstrated that opening of the mPTP induces axonal degeneration, and that inhibition of this complex protects axons (Barrientos et al 2011). Opening of the mPTP, mitochondrial swelling and mitochondrial dysfunction were all downstream of important axonal changes that follow injury, such as intra-axonal calcium elevation and activation of signaling pathways that are known to be important in axonal degeneration (Barrientos et al 2011).

Recently, the importance of axonal mitochondria in RGC death in glaucoma has gained traction (Osborne 2008; 2010). The role of mitochondrial dysfunction in axonal

degeneration is highly relevant in glaucoma, since higher concentrations of mitochondria are found in the prelaminar and laminar regions of RGC axons (Barron et al 2004; Minckler et al 1977; Morgan 2004). Axons in the prelamina and lamina regions are unmyelinated resulting in higher energy demands and the need for more mitochondria. Consequently, disturbances in mitochondrial function can lead to metabolic stress and dysfunction of RGC axons with possible degeneration (Yu-Wai-Man et al 2011). In fact, disruption of axonal transport at the lamina region in glaucoma could be indicative of mitochondrial dysfunction and energy failure (Anderson 1999; Anderson & Hendrickson 1974). Changes in mitochondrial dynamics/morphology in mice (Abe & Cavalli 2008), and mitochondrial dysfunction in glaucoma patients (Abu-Amro et al 2006) have been reported. In addition to lower energy production, mitochondrial abnormalities could result in increased oxidative stress. Mice with a mutation that impairs mitochondrial polymerase proofreading function, accumulate mitochondrial mutations, have accelerated, age-related loss of retinal function, and their RGCs are more vulnerable to high IOP (Kong et al 2011). Thus, increases in mitochondrial dysfunction with increasing age, as well as loss of function, are likely to be important components of the age-related increase in glaucoma risk.

Regardless of the cause of axonal injury in glaucoma, an active molecular process is likely to occur both proximally (axon injury signaling to cell body) and distally (axon degeneration) to the site of injury. To optimally prevent RGC death in glaucoma, it will be important to understand the signaling pathways that control these distinct molecular processes. c-Jun N terminal kinase (JNK) signaling could potentially be a key mediator of both processes. JNK signaling is known to contribute to both axon injury signaling and axon degeneration in other systems (Abe & Cavalli 2008; Cavalli et al 2005; Lindwall & Kanje 2005; Miller et al 2009; Sengupta Ghosh et al 2011). JNK signaling appears to be involved in RGC death in various animal models of glaucoma (Kwong & Caprioli 2006; Tezel et al 2004; Yang et al 2008) and was shown to be active in human glaucoma (Tezel et al 2004). In a recent study, we found that activated JNK was present at the site of injury soon after mechanical injury to RGC axons (Libby, unpublished observations). Genetic ablation of JNK 2 and 3 protected the RGC somas from degeneration following the axonal injury, supporting the hypothesis that a JNK pathway signals axonal injury to the soma (Libby, unpublished observations). In contrast, a recent study proposed that targeting JNK3 alone did not prevent RGC loss or axon degeneration in an inducible model of glaucoma (Quigley et al 2011). This may agree with our study where JNK2 disruption alone was not protective, and/or may reflect difficulties of identifying a protection given the little damage that occurred in the inducible model. Further experiments are needed to fully determine the effectiveness of inhibiting JNK signaling as a potential treatment for glaucoma.

The role of glial cells in axon injury and degeneration

Glaucoma is very complex and axon degeneration may be initiated, modulated or propagated by different cell types including glia. Axon injury not only has a profound effect on the neuron, but also triggers changes in glial cell types such as astrocytes, microglia and Muller glia (reviewed in (Bringmann et al 2006; Perry et al 2010; Sofroniew 2009)). In addition to responding to injury, it is possible that glial cells may contribute to the initiation or propagation of axon injury. This is true for retinal and optic nerve glia, which may independently affect RGC health. These other cell types may also mediate protective responses. The protective and damaging responses of different cell types will need to be distinguished when prioritizing potential neuroprotective therapies for glaucoma.

Astrocytes are a major component of the neurovascular unit providing nutrients and support for neurons (Kimelberg & Nedergaard 2010). Microglia are the resident macrophages of the central nervous system sensing and responding to changes in the environment (Perry et al

2010). For glaucoma, a number of studies have used gene expression profiling to identify glial responses using a variety of animal models and human tissue (e.g. (Howell et al 2011b; Johnson et al 2011; Johnson et al 2007; Kompass et al 2008; Nikolskaya et al 2009; Panagis et al 2009; Steele et al 2006; Yang et al 2007). Two profiling studies propose that glial changes occur at the ONH prior to any significant RGC axon damage. In DBA/2J mice, upregulation of extracellular matrix (ECM) proteins by astrocytes and activation of both the endothelin and complement systems by non-neuronal cells occurred very early in the ONH and retina (Howell et al 2011a). In a rat model of glaucoma, astrocyte proliferation was reported to be a very early process (Johnson et al 2011).

For glaucoma, the changes described in glia are likely to consist of both protective and damaging responses (Johnson & Morrison 2009; Morgan 2000). In addition, these changes may be primary (in response to intraocular pressure changes) and/or secondary (in response to early RGC stress and/or damage). Early changes in extracellular matrix proteins may be a direct result of increased mechanical stress and strain due to IOP elevation. These changes occur in primates and rodents and may reflect an astrocyte-mediated response to protect axons from strain-induced injury (Burgoyne 2010; Del Zoppo et al 2006; Nickells et al submitted; Roberts et al 2009). Recently, fortified astrocytes (with dense, cytoskeletal filaments) in the ONH have been described in a rat model of glaucoma. In response to pressure, dorsal processes of the fortified astrocytes are torn away from the sheath and it is proposed that the damage to axons is not mechanical but as a consequence of regional loss of metabolic support from the astrocytes (Dai et al 2011).

Astrocytes in the ONH are phagocytic (Nguyen et al 2011), a process that may be increased as a result of injured or stressed axons expressing 'eat me' signals (Grimsley & Ravichandran 2003). Phagocytosis of cellular debris can be beneficial (to minimize inflammatory damage), but if the astrocytes directly phagocytose parts of the RGC axon in glaucoma it may turn out to be detrimental.

Non-neuronal cells have also been shown to express potentially damaging molecules including complement components and endothelins (Chauhan 2008; Rosen & Stevens 2010). The complement cascade has important roles in innate immunity and in the pruning of neuronal synapses. Microglia synthesize and secrete key molecules of the complement cascade and may phagocytose synapses (reviewed in (Perry et al 2010; Rosen & Stevens 2010)). Increased complement expression is reported in the optic nerve and retina for various glaucoma models and human glaucoma (reviewed in (Nickells et al submitted)). DBA/2J mice mutant for complement component *C1qa* mice have a significant reduction in RGC loss and axon degeneration compared to wild-type DBA/2J mice (Howell et al 2011a). Endothelins are reported to be expressed in multiple cell types in glaucoma including microglia (Howell et al 2011a) and astrocytes (Murphy et al 2010; Prasanna et al 2005). Endothelins can cause vasoconstriction, glial cell activation, inhibition of axonal transport, and induce RGC death (Howell et al 2011a; Sasaoka et al 2006; Taniguchi et al 2006). Inhibition of the endothelin system by administration of the endothelin receptor antagonist Bosentan reduced RGC loss in DBA/2J mice (Howell et al 2011a).

Conclusion

It is clear that an early site of injury to RGCs in glaucoma is the axon segment in the ONH, but fundamental questions remain. While it is now understood that many different cell types respond in glaucoma, it is unclear if the earliest pathological events are intrinsic or extrinsic to RGC axons. Furthermore, it is not known which pathways control distal axon degeneration and proximal axon signaling after injury. Although early changes are known to occur in glial cells, the contribution of these early changes to glaucoma needs further

investigation. Some of these changes will be protective while others will be damaging. Targeting early, damaging events has the potential to provide robust neuroprotection for glaucoma patients and perhaps for patients suffering from other axonopathies.

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Figure 1. Wallerian degeneration slow (*Wld^S*) allele protects or delays axon degeneration in two models of glaucoma

(A–F) In DBA/2J mice, the *Wld^S* allele protected both RGC axons (A–C) and soma (D–F) at two key time points (Libby et al 2005a). A and B show representative images of optic nerve cross sections stained with paraphenylenediamine from a wild type DBA/2J eye with severe glaucoma (A) and a DBA/2J *Wld^S* eye with no glaucoma (B). (C) Assessment of over 100 eyes showed that *Wld^S* more than doubled the number of eyes with no glaucoma (green, hashed bars) compared to wild type DBA/2J mice. (D–F) *Wld^S* also protected RGC somata. In eyes with no optic nerve damage, RGC somata were also preserved. (G–O) In a model where IOP elevation was induced experimentally in rats, *Wld^S* delayed axon degeneration (G–H) but had no effect on somal survival (J–O). (G–H) At 2 weeks following IOP elevation, proximal axons from *Wld^S* rats (H) had significantly less axon damage compared to wild type rats (G). Interestingly, in the wild type rats, greater axon damage was observed in proximal axons (I, squares) compared to distal axons (I, triangles) two weeks after IOP

elevation. This data suggests dying back is not the major mechanism of axon degeneration in this model. (J–O) *Wld^s* does not protect the soma. Higher magnification confocal stacks showing loss of dendritic arborization and cell body shrinkage in glaucomatous RGCs from both wild-type (K, M) and *Wld^s* rats (L, N) as compared to control RGCs from untreated retinas (J). The majority of control RGCs showed more than one immunopositive process (arrow in J depicts example RGC with six processes) while, in particular, 4 weeks of glaucoma triggered massive loss of arborization that resulted in RGCs with only one (K and L, arrows) or no processes (M and N, arrows).

A–F reproduced from Howell et. al., 2007 (J. Cell Biology). G–O reproduced from Beirowski et. al., 2008 (Eur. J. Neuro.). Permission requested.

Figure 2. Dying back as a mechanism for axon degeneration in glaucoma

(A–C) Schlamp et al used the diI labeling technique in DBA/2J mice to show that axon degeneration followed a die-back pattern from the distal end of the optic nerve to the proximal end. The images shown are dorsal views of a healthy young mouse (A), and two old mice with degenerating nerves that typically exhibit reduced label distally (in relation to cell body) (B, C). Ch = Chiasm, RON = right optic nerve, LON = left optic nerve. (D–F) Crish et al stained optic nerve cross sections stained with Toluidine blue to identify and quantify the number of degenerating axons (or profiles, arrowed). Sections were assessed from the proximal (D) and the distal (E) portions of the optic nerve. (F) A greater number of degenerating profiles were observed in distal optic nerve sections compared to proximal optic nerve sections. This supports dying back as the major mechanism of axon degeneration in DBA/2J glaucoma.

A–C reproduced from Schlamp *et al.*, 2006 (BMC Neuro). D–F reproduced from Crish *et al.*, 2010 (PNAS).