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A Model of Toxic Neuropathy in Drosophila Reveals a Role for MORNN4 in Promoting Axonal Degeneration

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Axonal degeneration is a molecular self-destruction cascade initiated following traumatic, toxic, and metabolic insults. Its mechanism underlies a number of disorders including hereditary and diabetic neuropathies and the neurotoxic side effects of chemotherapy drugs. Molecules that promote axonal degeneration could represent potential targets for therapy. To identify such molecules, we designed a screening platform based on intoxication of Drosophila larvae with paclitaxel (taxol), a chemotherapeutic agent that causes neuropathy in cancer patients. In Drosophila, taxol treatment causes swelling, fragmentation, and loss of axons in larval peripheral nerves. This axonal loss is not due to apoptosis of neurons. Taxol-induced axonal degeneration in Drosophila shares molecular execution mechanisms with vertebrates, including inhibition by both NMNAT (nicotinamide mononucleotide adenylyltransferase) expression and loss of wallenda/DLK (dual leucine zipper kinase). In a pilot RNAi-based screen we found that knockdown of retinophilin (rtp), which encodes a MORNN (membrane occupation and recognition nexus) repeat-containing protein, protects axons from degeneration in the presence of taxol. Loss-of-function mutants of rtp replicate this axonal protection. Knockdown of rtp also delays axonal degeneration in severed olfactory axons. We demonstrate that the mouse ortholog of rtp, MORN4, promotes axonal degeneration in mouse sensory axons following axotomy, illustrating conservation of function. Hence, this new model can identify evolutionarily conserved genes that promote axonal degeneration, and so could identify candidate therapeutic targets for a wide range of axonopathies.

Introduction

Injury to axons occurs in a wide variety of pathological contexts, including diabetic neuropathy, exposure to chemotherapeutic agents during cancer treatment, and hereditary neuropathies such as Charcot-Marie-Tooth disease (Coleman, 2005). In recent years, axonal injury has been appreciated as an early event in CNS degenerative diseases such as Alzheimer’s disease (Stokin et al., 2005) as well as traumatic brain injuries (Xu et al., 2007). These diverse mechanical, toxic, and metabolic insults initiate a stereotypic set of cellular changes beginning with axonal swellings that progress into breaches of membrane integrity and axonal fragmentation (Coleman and Freeman, 2010).

Axon degeneration occurs through a molecular pathway that is evolutionarily conserved. Evidence for this molecular cascade came initially from the remarkable protective effects of expression of nicotinamide mononucleotide adenylyltransferase (NMNAT), the critical component of the Wallerian degeneration slow (WldS) fusion protein (Mack et al., 2001; Araki et al., 2004). Using a mechanism that is still mysterious, NMNAT gain-of-function in the axon blocks axonal degeneration in Drosophila and mouse from a wide range of insults (Mack et al., 2001; Hooper et al., 2006; MacDonald et al., 2006; Press and Milbrandt, 2008; Zhai et al., 2008; Sasaki et al., 2009a). Endogenous pathways also promote axonal degeneration following injury. The dual leucine zipper kinase DLK, or its Drosophila ortholog wallenda, is required for efficient axonal degeneration in response to axotomy or chemotherapy treatment in vitro, and in vivo following sciatic nerve injury (Miller et al., 2009). That the roles of NMNAT and DLK are functionally conserved across species suggests that an ancient cascade executes the removal of irreparably damaged axons. Identification of important players in this cascade may enable strategies to block axon degeneration and could slow or prevent progression of many neurological diseases.

In human patients, the administration of chemotherapeutic agents such as vincristine and paclitaxel (taxol) causes a dose-limiting peripheral neuropathy, such that very aggressive cancers cannot be adequately controlled by these drugs (Malik and Stillman, 2008; Park et al., 2008). Since the timing of chemotherapy treatment is known, strategies to protect axons before and/or during treatment have great potential to minimize this toxic side effect (Windebank, 1999). The identification of genes that promote axon degeneration after injury is a first step toward realizing this therapeutic potential.
To identify molecules underlying axonal degeneration, we devised a novel paradigm for injuring axons in the fruit fly *Drosophila melanogaster* using the chemotherapy drug paclitaxel (taxol). In this model, axons swell, fragment, and are lost, and this loss is not caused by apoptosis. Axons injured in this paradigm can be protected by both NMNAT overexpression and loss of *wallenda/DLK*, demonstrating that the conserved axonal degeneration program mediates this taxol-induced neuropathy. To test the utility of this new model as a gene discovery platform, we performed a pilot RNAi-based screen and identified the MORN (membrane occupation and recognition nexus) family member *retinophilin* as a gene promoting degeneration. Importantly, the mouse ortholog of *retinophilin*, Morn4, also promotes mammalian axonal degeneration.

**Materials and Methods**

*Fly strains*. The following strains were used in this study: Canton S (wild-type), elav-GAL4 (Yao and White, 1994), *pickpocket*-EGFP (referred to as *ppkEGFP*; Grueber et al., 2003), *pickpocket*-CD8:3xGFP (referred to as *ppkCD8GFP*; Shimono et al., 2009), *ppk-GAL4* (Grueber et al., 2007), *wnd2* and *wnd3* mutant alleles (Collins et al., 2006), UAS- *dnNMNAT* (MacDonald et al., 2006), *reten*′ (Venkatachaliam et al., 2010), and *rtp*′ (Mecklenburg et al., 2010). All other strains are from the Bloomington Stock Center. All RNAi lines used are from the Vienna *Drosophila* RNAi Consortium (Retinophilin RNAi; 109000).

The UAS-cytNMNAT1 expression construct was made using mouse *cytNMNAT1*, a mutant version of NMNAT1 with the nuclear localization disrupted (Sasaki et al., 2006). The full-length cytNMNAT1 was inserted into the pUAST expression vector and injected into *w* fly embryos to create transgenic lines. A viable second chromosome insertion of this construct was used for experiments.

*Genetics*. For screening, females with homozygous UAS-Dicer2, elav-GAL4, and *ppkEGFP* were crossed to homozygous UAS-RNAi males to generate progeny of either sex for analysis. For olfactory axotomies, females of a strain containing GAL4 driven by the promotor region of olfactory receptor 82a (*Or82a-GAL4*; Fishilevich and Vosshall, 2005), UAS-CD8GFP, and UAS-Dicer2 were crossed to UAS-RNAi males, and male progeny were analyzed. Other genotypes are as noted in figure legends.

**Taxol treatment**. Embryos from crosses were collected on grape plates, and first instar larvae of both sexes were washed off and resuspended in water with 30 μM paclitaxel (Tocris Bioscience) or an equivalent amount of DMSO (vehicle; Tissue Culture Support Center, Washington University School of Medicine). This liquid was then used to make up instant *Drosophila* media (“blue food;” Carolina Biological Supply). For large-scale screening, 12 well plates were used and plugged with Flugs (Genesee Scientific). Animals on taxol-treated food were incubated at 25°C for 4 d before dissection and analysis.

**Immunocytochemistry**. GFP signal was enhanced with Alexa Fluor 488-rabbit-anti-GFP (1:1000, Invitrogen). Other antibodies used were *Cy3*-HRP (1:1000, Jackson ImmunoResearch) and Rabbit anti-cleaved-Caspase 3 (1:200, Cell Signaling Technology). Secondary antibodies were obtained from Jackson ImmunoResearch and used at 1:1000 dilution.

**Mouse DRG cultures**. Embryonic day 12.5 mouse DRG neurons were cultured as described previously (Sasaki et al., 2009b) using embryos of both sexes. Lentinival particles containing *Mor4*-targeting (5′-TGACCTTCTCAAGTGGCTCAA-3′) or control shRNA sequences were packaged as described previously (Araki et al., 2004) and added to DRG cultures at 2 DIV. In *vitro* axotomy was performed at 7–8 DIV. Quantitative reverse transcriptase-PCR analysis of neuronal mRNA was performed using an ABI Prism 7900HT sequence detection instrument (Applied Biosystems) as described previously (Gerits et al., 2011). Following insult, axonal degeneration was quantified from four phase-contrast images per well by ImageJ software that measures fragmentation of axon segments (Sasaki et al., 2009b). Briefly, axon images are bina-
rized, and the ratio of fragmented to total axonal area is computed by using circul arity as an indicator of fragmentation.

**Microscopy.** Images of *Drosophila* larval axons and adult brains were taken on a Nikon Eclipse E600 confocal microscope and are maximal Z-projections. Phase contrast images of DRG axons (20× objective) were taken using an inverted microscope (Eclipse TE 300; Nikon).

To analyze *Drosophila* axons, we took images of nerves between segments A3 and A4 of vehicle-treated third instar larvae or equivalently timed taxol-treated larvae. We counted the number of intact GFP-positive axons per nerve from each image (~100 μm length of nerve per picture). The average number of GFP+axons/nerve in vehicle-treated control (wild-type) animals labeled with ppkEGFP was 1.97 ± 0.06 (SEM) (n = 71 nerves) and was not significantly different in animals labeled with ppkCD8GFP, or between wild-type and rtp mutant strains. For presentation, we normalized data to the average of the wild-type vehicle control of the relevant experiment. All analysis of peripheral sensory axons and olfactory axons was done blind to genotype. One-way ANOVA was used for statistical analysis unless otherwise specified. All error bars are SEM.

For ultrastructural analysis of the *Drosophila* peripheral nerves, samples were fixed in 2% paraformaldehyde/2.5% glutaraldehyde (Polysciences Inc.) in 100 mM phosphate buffer, pH 7.2 overnight at 4°C. Samples were washed in phosphate buffer and postfixed in 0.5% osmium tetroxide (Polysciences Inc./0.08% potassium ferricyanide (Electron Microscopy Sciences)/100 mM phosphate buffer for 1 h, and subsequently in 1% tannic acid (Electron Microscopy Sciences, Fort Washington, PA)/100 mM phosphate buffer for 1 h. Samples were then rinsed extensively in dH2O before en bloc staining with 1% aqueous uranyl acetate (Ted Pella Inc., Redding, CA) for 1 h. Following several rinses in dH2O, samples were dehydrated in a graded series of ethanol and embedded in Eponate 12 resin (Ted Pella Inc.). Sections of 100 nm were cut with a Leica Ultracut UCT ultramicrotome (Leica Microsystems Inc., Bannockburn, IL), stained with uranyl acetate and lead citrate. Electron micrographs were taken on a transmission electron microscope (H-7500; Hitachi). Quantification of axon number was performed on 10 nonconsecutive sections of nerves from at least three independent larvae per treatment condition.

**Results**

Paclitaxel causes axonal injury and loss in *Drosophila*

Because only a few genes have been identified that regulate axonal degeneration, we sought to develop a model of axonal degeneration in *Drosophila* amenable to screens for genes that promote axonal demise. Loss-of-function of a protein participating in the axonal degeneration cascade would be predicted to cause axonal degeneration following taxol treatment is independent of apoptosis. **A–D**, The viral anti-apoptotic protein p35 is unable to rescue sensory axon degeneration. Confocal images of single representative nerves labeled with CD8:GFP (green) and HRP (red). Genotype is ppkGAL4/+;UAS-CD8:GFP/+ (Ctrl, A and C) or ppkGAL4/UAS-p35;UAS-CD8:GFP/+ (p35, B and D). Animals were fed food containing vehicle (DMSO) (A, B) or taxol (C, D) for 4 d. Scale bar (A), 2 μm. **E**, Quantification of GFP-labeled axons per nerve remaining after treatment from the genotypes shown in **A–D**. N = 21, 20, 18, and 16 for the genotypes quantified. NS, Not significant (p > 0.1). **F**, GFP (left column) and activated caspase 3 (right column) in control larvae (ppkGAL4/+;UAS-CD8:GFP/+ that are untreated (top) or treated with 30 μm taxol (second from top). Bottom two sets of images are controls showing caspase 3 activation following ppkGAL4-driven expression of UAS-hid and UAS-reaper (second from bottom), and loss of caspase 3 activation in flies expressing UAS-hid, UAS-reaper, and UAS-p35 (bottom). Scale bar (F), 3 μm.

**Figure 2.**

**Figure 3.** Overexpression of mouse NMNAT protects axons and dendrites from taxol-induced degeneration. **A–D**, Confocal images of single representative nerves labeled with ppkEGFP. Genotypes are elavGAL4, ppkEGFP/+ (Ctrl, A and C), or UAS-cyto-NMNAT1/+;elavGAL4, ppkEGFP/+ (NMNAT, B and D). Animals were treated with DMSO (A, B) or taxol (C, D) for 4 d and then analyzed. Scale bar (A), 2 μm. **E**, Quantification of GFP-labeled axons per nerve following taxol treatment in the genotypes shown in **A–D**. N = 10, 17, 17, and 22 for the genotypes quantified. **p < 0.01.** **F, G**, Dendrites and cell bodies of ddaC neurons treated with taxol from control (same genotype as A and C) and UAS-cyto-NMNAT1/+;UAS-CD8:GFP/+ (p35, B and D) animals. Scale bar (F), 10 μm.
of sensory endings is often disrupted and individual axons are
nerve cord following taxol treatment, however the ladder pattern
also observe peripheral nerve defasciculation. In these defascicu-
lar axons using a genetically encoded GFP that labels class IV sensory neurons and their projections (ppkEGFP) (Grueber et al., 2003). Because this marker highlights only a few neurons per hemisegment, it allows single axon resolution within the segmen-
tal nerves leading to the ventral nerve cord.

We find that treatment with taxol causes axonal destruction and loss (Fig. 1). Axonal injury following taxol treatment was apparent after 3 d, with maximal injury after 4 d. In the majority of nerves, GFP is severely fragmented or lost following taxol treat-
ment (Fig. 1C). Interestingly, in nerves where taxol has not caused complete GFP-positive axon loss, we see axonal swellings and debris, hallmarks of mammalian axonal degeneration (Cole-
man, 2005) (Fig. 1B, I). HRP labeling occasionally shows axonal swellings (Fig. 1B) or overall staining intensity decreases (Fig. 1C), likely due to the degeneration of non-GFP-labeled nerves. Examination of nerves using electron microscopy reveals an ap-
proximately twofold decrease in axon number (vehicle, 80.9 ±
3.9 axons per nerve; taxol, 42.7 ± 5.1 axons per nerve; p < 0.001,
Student’s t test) (Fig. 1D, E), suggesting that the GFP loss often corresponds to the frank loss of axons. Nerves in taxol-treated
animals appear unaffected and contain microtubule profiles (Fig. 1F, G). In addition to the phenotypes in GFP-labeled axons, we also observe peripheral nerve defasciculation. In these defascicu-
lated nerves, swellings of single axons that accumulate the microtubule-associated protein Futsch are apparent (Fig. 1H, I). Sensory axons are still present within the neuropil of the ventral nerve cord following taxol treatment, however the ladder pattern of sensory endings is often disrupted and individual axons are fragmented (Fig. 1J, K). Using the same ppkEGFP label, we also examined the effects of taxol on the dendrites of multidendritic sensory neurons in the larval cuticle. Similar to its effects on
axons, taxol causes sensory dendrite swelling and loss (Fig. 1L–
N). To assess whether taxol treatment caused “dying back”–like retraction, we examined larval neuromuscular synapses. Unlike genetic models in Drosophila that disrupt the cytoskeleton (Mas-
aro et al., 2009), we observe normal apposition of presynaptic and postsynaptic markers following taxol treatment, indicating that our model of taxol-induced degeneration does not cause motoneuron synaptic instability (data not shown). Together, these results highlight the range of degenerative phenotypes result-
ing from taxol treatment in flies and reveal morphologi-
cal similarities to degenerating mammalian axons.

Taxol-Induced Axonal Degeneration is Not Secondary
to Apoptosis

In patients, neuronal cell death is not a major contributor to
taxol-induced neuropathy (Lee and Swain, 2006; Windebank and Grisold, 2008). To test whether this is also true in our Dros-
ophila model, we overexpressed the baculoviral anti-apoptotic protein p35 in sensory neurons (Hay et al., 1994). Expression of p35 with the ppkGAL4 driver (Grueber et al., 2007) effectively blocks activation of Caspase 3 following induction of apoptosis in sensory neurons via the forced expression of the proapoptotic genes hid and reaper (Fig. 2F). However, when p35-expressing
animals are treated with taxol, their peripheral sensory axons are
not preserved (Fig. 2A–E), indicating that axonal loss in this model is not secondary to apoptosis in the sensory cell bodies. In addition, cell bodies of taxol-treated animals fail to label with anti-activated Caspase-3 antibodies (Fig. 2F). These results are consistent with studies that have found a role for caspases in
dendrite pruning but not in injury-induced degeneration (Schoenmann et al., 2010; Tao and Rolls, 2011). These data high-
light the independence of the mechanism of taxol-induced ax-
onal degeneration from that of apoptosis.

NMNAT overexpression can block paclitaxel-induced
axonal degeneration

To determine whether paclitaxel-induced axonal degeneration in flies shares molecular mechanisms with axonal degeneration in mammalian sensory neurons, we asked whether molecules known to block axonal degeneration in mice also protect axons in our Drosophila model. First, we tested whether overexpression of NMNAT could protect fly sensory axons from degeneration. NMNAT is protective for both sensory axons and dendrites in other injury paradigms in the fly (Tao and Rolls, 2011; Wen et al., 2011). We made transgenic flies that allow for the targeted ex-
pression of cytoplasmically targeted mouse NMNAT1 (cyt-
mNMNAT1) (Sasaki et al., 2009a). Following taxol treatment, axons from neurons overexpressing cyt-mNMNAT1 were pre-
served, whereas control axons were lost (Fig. 3). cyt-mNMNAT1 expression also blocked dendrite degeneration following taxol treatment (Fig. 3F, G). These results illustrate conservation of the protective effects of NMNAT for taxol-induced neuropathy be-
tween invertebrates and vertebrates (Wang et al., 2002).
Wallenda/DLK is required for taxol-induced axonal degeneration in *Drosophila*

To examine whether endogenous genes required for axonal degeneration in mammals also participate in taxol-induced degeneration in flies, we examined the effect of loss of the MAP3K Wallenda (DLK in mammals). Wallenda participates in the degeneration cascade following *Drosophila* olfactory axotomy or mouse axonal injury (Miller et al., 2009) and is required for injury signaling following *Drosophila* nerve crush (Xiong et al., 2010). Following taxol treatment, *wallenda* mutants displayed robust protection of peripheral axons (Fig. 4). Similar protection is observed when transgenic RNAi is used to knockdown *wallenda* exclusively in neurons (data not shown). These data demonstrate that known pathways used in mammalian axonal degeneration are conserved members of the taxol-induced degeneration cascade in flies. Furthermore, it illustrates that loss-of-function analysis in taxol-treated animals could be useful to identify novel players in axonal degeneration that may be relevant to mammalian axonal degeneration.

**RNAi interference identifies a role for retinophilin in axonal degeneration**

We undertook a pilot RNAi screen looking for genes that, similar to *wallenda*, would block axonal degeneration when knocked down. We used a collection of inducible, transgenic RNAi strains available from an international consortium (Dietzl et al., 2007). These RNAi lines were expressed under the control of UAS elements using the pan-neuronal driver *elav-GAL4* to express both the RNAi and Dicer2, which enhances efficacy of RNAi knockdown. Under these conditions, ~80% of genes with known phenotypes in the nervous system give the expected phenotype when knocked down (Dietzl et al., 2007).

We limited our analysis to *Drosophila* genes with clear mammalian orthologs as determined by Homologene NCBI software.
A subset of 490 RNAi strains was chosen for our pilot screen that was enriched for proteins with enzymatic functions and for genes known to function in the nervous system; this selection was done using Flybase QueryBuilder (www.flybase.org). These strains were screened for degeneration or preservation of protein expression in Drosophila using Flybase QueryBuilder (www.flybase.org). These strains were chosen for our pilot screen that was enriched for proteins with enzymatic functions and for genes known to function in the nervous system; this selection was done using Flybase QueryBuilder (www.flybase.org). These strains were screened for degeneration or preservation of protein expression in Drosophila using Flybase QueryBuilder (www.flybase.org).

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The mammalian rtp ortholog Morn4 is required for axonal degeneration

The closest mammalian ortholog to Drosophila retinophilin is Morn4 (50% identity at the amino acid level) (Mecklenburg, 2007). Morn4 is an uncharacterized protein in mouse and human that also contains four MORN repeats. To test whether Morn4 functions like Retinophilin in promoting axonal degeneration, we knocked down Morn4 expression in cultured mouse embryonic DRG neurons and then cut their axons. Control axons swell and then degenerate within the first 12 h, a process that can be quantified using image analysis software that detects axon fragments as circular particles (Sasaki et al., 2009b; Gerds et al., 2011). Knockdown of Morn4 was very efficient, with only ∼1% of mRNA remaining by quantitative PCR analysis (1.3 ± 0.7%). Knockdown of Morn4 significantly reduced axonal degeneration following axotomy (Fig. 6A,B). Protection was evident for at least 72 h, with knockdown cultures displaying signs of early degeneration (swellings) but no fragmentation. Thus Morn4 may participate in an intermediate step of axonal degeneration, following axonal swelling but preceding fragmentation. The involvement of mouse Morn4 in axonal degeneration highlights the ability of this new Drosophila chemotherapy-induced neuropathy model to identify genes that participate in axonal degeneration in mammals.

Figure 6. Mouse Morn4 participates in axonal degeneration. A, Representative images of axons from cultured mouse DRG neurons infected with shRNAs targeting luciferase (ctrl) or Morn4. Axons are uncut (top images) or 24 h post-axotomy (bottom images). Scale bar, 100 μm. B, Quantification of degeneration index of control shRNA (red trace)– or Morn4 shRNA (blue trace)–infected axons at 0, 9, 24, 48, and 72 h post-axotomy, and uncut control shRNA–infected axons (black trace). Double asterisks indicate p < 0.001 between cut control shRNA and Morn4 shRNA.

Discussion

Here we describe a novel Drosophila model of chemotherapy-induced peripheral neuropathy and demonstrate that the process shares molecular components with the mammalian axonal degeneration program. We then use this platform to identify the Retinophilin/Morn4 protein as a component of an evolutionarily conserved axonal self-destruction cascade.

A Drosophila model of chemotherapy-induced peripheral neuropathy

Chemotherapy drugs such as paclitaxel (taxol) and vincristine cause a dose-limiting peripheral neuropathy in humans. Taxol causes sensory neuropathy which manifests as pain and loss of sensation in extremities, while vincristine causes both sensory and motor dysfunction. These drugs are used widely in both pediatric and adult patients to treat aggressive metastatic cancer (Sparano, 2000; Bradley and Hussian, 2008; Marsh, 2009; Moore and Pinkerton, 2009; Grossi et al., 2010). Because we know so little about the mechanisms causing this severe side effect, we currently have no way to prevent axonal degeneration in these patients.

Axonal degeneration is not only a component of chemotherapy-induced neuropathy but is also a hallmark of many neurological...
diseases including hereditary neuropathies, neurodegenerative diseases such as Alzheimer’s Disease, and trauma including spinal cord injury. Hence, the need is great to identify therapeutic strategies that could slow or block axon loss and thereby prevent progression of neurological symptoms in these and other disorders. To identify such targets, we have developed a model of toxic neuropathy in Drosophila. Treating larval-stage Drosophila with paclitaxel causes axonal degeneration that is anatomically and molecularly similar to that occurring in rodents and, potentially, in human patients undergoing chemotherapy treatment. In Drosophila, as in humans, paclitaxel preferentially damages sensory rather than motor axons (Park et al., 2011). This conservation provides a platform to identify molecules that could serve as future therapeutic targets.

Retinophilin/Morn4 participates in axonal degeneration
We identified Rtp/Morn4 as an evolutionarily conserved component of the axonal degeneration cascade. Furthermore, we observe that Rtp/Morn4 must be present for efficient degeneration following both neurotoxicity (i.e., chemotherapy administration) and traumatic injury (i.e., axotomy). Hence, while the screen uses a neurotoxin as the axonal damaging agent, it can identify components that function downstream of multiple stimuli. Importantly, our screen was able to identify a subtle effect of rtp on the timing of axonal demise. When using RNA interference to implicate a gene in a process, the ability to detect small changes is useful since gene expression is not completely eliminated. Importantly, we were able to confirm this finding using a true loss-of-function mutant in flies. While identification of a Drosophila gene that promotes axonal degeneration is important, we are most interested in genes with an evolutionarily conserved role in the process. Lentiviral-mediated gene knockdown in mouse sensory neurons (Gerdt et al., 2011) is a powerful secondary screen allowing us to identify such genes. Using these methods in combination, we demonstrate that the role of Rtp/Morn4 in the progression of axonal demise is evolutionarily conserved.

While we do not know the mechanism of action of Morn4 in axonal degeneration, Retinophilin has been implicated in both localization of signaling molecules required for termination of phototransduction (Mecklenburg et al., 2010; Venkatachalam et al., 2010) and regulating cytoplasmic calcium levels preceding phagocytosis (Cuttell et al., 2008). Morn4 contains four membrane occupation and recognition nexus (MORN) repeats that can target proteins to lipid membranes (Ma et al., 2006). Potentially, these domains could bring Morn4 to a membrane surface to modulate lipid activity or second messenger generation. Interestingly, Morn4 has recently been implicated in the neuroprotective process of preconditioning following oxygen-glucose deprivation (Dai et al., 2010). Further work to clarify the molecular role of Morn4 in these two seemingly opposite cascades will be necessary.

Axonal degeneration mechanisms and implications for neurological disease
While we know a great deal about the molecular control of the apoptotic process and how it can contribute to disease, we know comparatively little about axonal degeneration despite its importance in a wide variety of pathological states. Many activating insults ultimately use a common pathway (Vohra et al., 2010) that includes the mitochondrial permeability transition pore (Forte et al., 2007; Barrientos et al., 2011) and can be blocked by axonal expression of NMNAT isoforms (Araki et al., 2004; Conforti et al., 2009; Sasaki et al., 2009a; Yahata et al., 2009; Babetto et al., 2010; Sasaki and Milbrandt, 2010). In addition, kinases including DLK, JNK, IKK, and GSK3 promote axonal degeneration (Miller et al., 2009; Gerdt et al., 2011). The relationships among these proteins, as well as the identity of upstream and downstream components of the axonal degeneration program, are still mysterious.

Screens in both Drosophila and mouse (Gerdt et al., 2011) have the potential to reveal molecular targets for therapeutic intervention to block axonal degeneration in the early stages of neurodegenerative diseases, hereditary neuropathies, or before chemotherapeutic treatment. Here we have used a clinically relevant insult in Drosophila to identify a role for Retinophilin/Morn4 in promoting axonal degeneration. Future screening should yield additional candidates whose inhibition could disable this degenerative process.

References


