A model of toxic neuropathy in Drosophila reveals a role for MORN4 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 adenyltransferase) 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 MORN (membrane occupation and recognition nexus) repeat-containing protein, 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.
To identify molecules underlying axonal degeneration, we devised a novel paradigm for injuring axons in the fruit fly Drosophila melanogaster using the chemotherapeutic 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 waldena or dldk, 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. Our work introduces a novel and clinically relevant axonal injury paradigm in Drosophila and has identified a role for MORN4 as a conserved component of the axonal degeneration program.

**Materials and Methods**

*Fly strains.* The following strains were used in this study: Canton S (wild-type), elav-GAL4 (Ya'o and White, 1994), pickpocket-EGFP (referred to as ppKEGFP; Grueber et al., 2003), pickpocket-CD8:3xFP (referred to as ppkCD8GFP; Shimono et al., 2009), ppk-GAL4 (Grueber et al., 2007), wnd<sup>2</sup> and wnd<sup>9</sup> mutant alleles (Collins et al., 2006), UAS-dmNMNAT (MacDonald et al., 2006), retin<sup>1</sup> (Venkatachalam et al., 2010), and rp<sup>1</sup> (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 wild flies to create transgenic lines. A viable second chromosome insertion of this construct was used for experiments.

*Genetics.* For screening, females with homozygous UAS-Dicer2, elavGAL4, 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 Voshall, 2005), UAS-CDEGFP, 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-HP (labels neuronal membranes, red), ppkEGFP labels a subset of sensory neurons, Vehicle (DMSO) treated. B, C, 30 μM taxol treated. Axon degeneration was assessed 4 d after treatment initiation. In B, an intermediate phenotype of swellings and fragmentation is visible with both EGFP (arrowheads) and with HRP (arrows). In C, a more severe phenotype of EGFP and HRP loss is shown. Scale bar: (in A) A–D, 5 μm; D, E, EM cross-section images of nerves from wild-type (Canton S) vehicle- and taxol-treated animals. Scale bars: D, E, 1 μm; F, G, Zoomed-in images of axons from D and E. Scale bar (in F) F, G, 200 nm. H, I, Peripheral nerves stained with antibody to Futsch (22C10). Three nerves are shown in the vehicle; one defasciculated nerve is shown in the taxol-treated animal. Arrowheads point to prominent swellings in individual axons. Scale bar (in H) J, K, K, Class IV sensory axons (ppkEGFP/+; UAS-CDEGFP+/+) in the ventral nerve cord in vehicle (left) and taxol treated (right). Arrows point to intact axons (J) or swollen and fragmenting axons (K). Scale bar: (in J) K, 10 μm, L–N, Dendrites and cell body of ddaC sensory neurons in vehicle-treated (L) or 30 μM taxol-treated (M, N) animals. Scale bar: (in L) L–N, 30 μM. Insets show portions of a dendrite from the larger images. Scale bar, 6 μM.

**Figure 1.** Drosophila peripheral axons are damaged or lost following taxol treatment. A–C, The segmental nerve between segment A3 and A4 is visualized by Cy3-HP (labels neuronal membranes, red). ppkEGFP labels a subset of sensory neurons. A, Vehicle (DMSO) treated. B, C, 30 μM taxol treated. Axon degeneration was assessed 4 d after treatment initiation. In B, an intermediate phenotype of swellings and fragmentation is visible with both EGFP (arrowheads) and with HRP (arrows). In C, a more severe phenotype of EGFP and HRP loss is shown. Scale bar: (in A) A–D, 5 μm; D, E, EM cross-section images of nerves from wild-type (Canton S) vehicle- and taxol-treated animals. Scale bars: D, E, 1 μm; F, G, Zoomed-in images of axons from D and E. Scale bar (in F) F, G, 200 nm. H, I, Peripheral nerves stained with antibody to Futsch (22C10). Three nerves are shown in the vehicle; one defasciculated nerve is shown in the taxol-treated animal. Arrowheads point to prominent swellings in individual axons. Scale bar (in H) J, K, K, Class IV sensory axons (ppkEGFP/+; UAS-CDEGFP+/+) in the ventral nerve cord in vehicle (left) and taxol treated (right). Arrows point to intact axons (J) or swollen and fragmenting axons (K). Scale bar: (in J) K, 10 μm, L–N, Dendrites and cell body of ddaC sensory neurons in vehicle-treated (L) or 30 μM taxol-treated (M, N) animals. Scale bar: (in L) L–N, 30 μM. Insets show portions of a dendrite from the larger images. Scale bar, 6 μM.
rized, and the ratio of fragmented to total axonal area is computed by using circularity 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 were 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, and the ratio of fragmented to total axonal area is computed by using circularity 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).

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protection following an insult and would be a putative therapeutic target. To maximize throughput, we wanted to devise an axonal injury paradigm that did not depend on mechanically injuring each animal individually. In addition, we wanted to use a clinically relevant insult. *Drosophila* has been used as a model of neurodegeneration following cisplatin toxicity (Podratz et al., 2011), and so may respond to chemotherapeutics in a manner analogous to mammals. We therefore attempted to mimic the neuropathy caused in human patients by paclitaxel (taxol) administration. To do this, we fed larval-stage animals food laced with taxol. By beginning treatment after animals have reached the larval stage, we avoid disrupting neurogenesis or axon pathfinding, events that are complete at the time of hatching (for review, see Prokop, 1999). Following treatment, we examined larval peripheral 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 segmental 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 treatment (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 (Coleman, 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 approximately 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 have large holes (Fig. 1E), and free organelles are occasionally seen in these areas. However, remaining axons appear relatively 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 defasciculated 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 (Masaro 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 resulting from paclitaxel treatment in flies and reveal morphological 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 paclitaxel-induced neuropathy (Lee and Swain, 2006; Windebank and Grisold, 2008). To test whether this is also true in our *Drosophila* 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 highlight the independence of the mechanism of taxol-induced axonal 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 expression of cyttoplasmically targeted mouse NMNAT1 (cytmNMNAT1) (Sasaki et al., 2009a). Following taxol treatment, axons from neurons overexpressing cytmNMNAT1 were preserved, whereas control axons were lost (Fig. 3). cytmNMNAT1 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 between 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 gave 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 ppkEGFP-labeled axons following taxol intoxication. From this collection we identified the MORN family member retinophilin [rtp, also called undertaker (uta)]. Retinophilin participates in termination of phototransduction in the fly retina (Mecklenburg et al., 2010; Venkatachalam et al., 2010) and in store-operated calcium release and phagocytosis in fly embryonic macrophages (Cuttell et al., 2008). It contains four MORN repeats, regions that can bind to phospholipid membranes (Ma et al., 2006). Knockdown of retinophilin protected axons from chemotherapy-induced peripheral neuropathy (Fig. 5A). The RNAi protection is quantitatively modest, but consistent (Fig. 5B). To test whether loss of Retinophilin is responsible for this phenotype, we generated a transheterozygote between two previously characterized loss-of-function mutant alleles of rtp (Mecklenburg et al., 2010; Venkatachalam et al., 2010). Axons in this rtp mutant are preserved following taxol treatment (Fig. 5C,D), confirming that removal of retinophilin is indeed axoprotective.

Retinophilin could be specifically involved in chemotherapy-induced neuropathy in the peripheral nervous system, or could play a broader role in injury-induced axonal degeneration. To test whether retinophilin participates in other types of degeneration, we turned to the well established model of olfactory axotomy in Drosophila (MacDonald et al., 2006; Doherty et al., 2009). The cell bodies of olfactory neurons are located in the antenna, and their projections extend into the brain, cross a central commissure, and synapse onto bilateral glomeruli (Fig. 5E, top). By removing the third antennal segment, olfactory axons are severed (MacDonald et al., 2006). We labeled a subset of olfactory axons via expression of GFP driven by the Or82a-GAL4 line (Fishilevich and Vosshall, 2005). Twenty-four hours after axotomy, wild-type axons have degenerated almost completely, with only a few axonal fragments remaining outside the glomeruli (Fig. 5E, middle). In contrast, axons in which retinophilin has been knocked down exclusively within the labeled neurons show a delay in this degeneration, with many axons still extending near the commissure (Fig. 5E, bottom). Using a scoring system to rank the number and length of remaining axons, we find that axon loss is inhibited following rtp knockdown, but much less so than via expression of cyt-mNMNAT1 (NMNAT score = 3.8 ± 0.1) (Fig. 5F,G). Together, these results suggest that Retinophilin promotes the normal axonal degeneration program initiated by both traumatic and toxic axonal injury, and that it functions in a cell-autonomous manner.

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.

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 Hussain, 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
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 (Gerds 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; Gerds 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 (Gerds 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.

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