Axon injury and stress trigger a microtubule-based neuroprotective pathway

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Axon injury elicits profound cellular changes, including axon regeneration. However, the full range of neuronal injury responses remains to be elucidated. Surprisingly, after axons of *Drosophila* dendritic arborization neurons were severed, dendrites were more resistant to injury-induced degeneration. Concomitant with stabilization, microtubule dynamics in dendrites increased. Moreover, dendrite stabilization was suppressed when microtubule dynamics was dampened, which was achieved by lowering levels of the microtubule nucleation protein γ-tubulin. Increased microtubule dynamics and global neuronal stabilization were also activated by expression of expanded polyglutamine (poly-Q) proteins SCA1, SCA3, and huntingtin. In all cases, dynamics were increased through microtubule nucleation and depended on JNK signaling, indicating that acute axon injury and long-term neuronal stress activate a common cytoskeleton-based stabilization program. Reducing levels of γ-tubulin exacerbated long-term degeneration induced by SCA3 in branched sensory neurons and in a well established *Drosophila* eye model of poly-Q–induced neurodegeneration. Thus, increased microtubule dynamics can delay short-term injury-induced degeneration, and, in the case of poly-Q proteins, can counteract progressive longer-term degeneration. We conclude that axon injury or stress triggers a microtubule-based neuroprotective pathway that stabilizes neurons against degeneration.

Many animals generate a single set of neurons that must function for the entire life of the individual. Each neuron typically has a single axon that transmits signals to other neurons or output cells such as muscle. As axons can extend long distances, they are at risk for injury, and, if the single axon is damaged, the cell can no longer function. Many neurons thus mount major responses to axon injury. The best characterized of these responses is axon regeneration, the process in which a neuron extends the stump of the existing axon or grows a new axon from a dendrite (1–3).

In addition to the regenerative response, axon injury can cause other less well-understood changes. For example, in mammalian dorsal root ganglion cells, injury of the peripheral axon causes a transcriptional response that increases the capacity of the central axon to regenerate if it is subsequently injured (4, 5). In *Drosophila* sensory neurons, axon injury causes cytoskeletal changes in the entire dendrite arbor, specifically the number of growing microtubules is up-regulated (6). In this study, we investigated the functional significance of the cytoskeletal changes in the dendrite arbor. We present results that suggest the altered microtubule dynamics in dendrites acts to stabilize them, and thus axon injury seems to trigger a neuroprotective pathway that acts on the rest of the cell. However, this neuroprotective pathway is turned on only transiently after axon injury and subsides as axon regeneration initiates.

Axon injury is a very acute neuronal stress. Neurons are also subject to a variety of long-term stresses that have major implications for human health. For example, many forms of neurodegenerative disease, including Alzheimer's and Parkinson diseases, manifest after long periods in which the neurons survive under stress. These long-term stresses include accumulation of misfolded proteins or protein aggregates inside or outside the cell (7). One such set of misfolded protein diseases is CAG-repeat or polyglutamine (poly-Q) repeat diseases (8), including Huntington disease and many forms of spinocerebellar ataxia (SCA). In these diseases stretches of CAG nucleotides in the coding region of specific proteins are expanded in the genome. This results in proteins with long poly-Q spans, which, over time, cause neurodegeneration.

Quite unexpectedly, we found several chronic stresses, including expression of long-poly-Q–containing proteins, induced the same type of cytoskeletal changes as axon injury. We therefore hypothesized that long-term axon stress might trigger the same type of microtubule-based stabilization pathway as acute axon stress. We found evidence to support this hypothesis by examining long-term degeneration in neurons that expressed poly-Q proteins. In this assay, increased microtubule dynamics acted to slow the course of degeneration. The microtubule-based stabilization pathway we describe thus represents an endogenous neuroprotective response to axon stress. This neuroprotective response is turned on transiently after axon injury and for longer periods of chronic stress.

Results

Axon Injury Stabilizes Dendrites. To determine whether axon injury might turn on a pathway to stabilize distant regions of a neuron, we developed an assay to probe dendrite stability after axon injury. We previously showed that dendrites of *Drosophila* larval sensory neurons are cleared rapidly after they are severed from the cell body (9). We reasoned that, if axon injury turned on a stabilization pathway, this might slow down dendrite degeneration after severing. To test this idea, we used a pulsed UV laser to sever axons of GFP-labeled *Drosophila* dendritic arborization (da) neurons (*SI Materials and Methods* includes information about these neurons) in intact animals, and tracked dendrite clearance after severing at subsequent time points.

When dendrites of the ddaE neuron were severed immediately after axons, all dendrites were cleared by 18 h after injury (Fig. 1 *A* and *B*), as in neurons without previous axon injury (9). However, when axons were severed 8 or 24 h before dendrite severing, more than half of dendrites remained 18 h after they were cut from the cell body (Fig. 1 *A* and *B*). This result is consistent with the hypothesis that axon injury stabilizes dendrites. When the time between axon and dendrite severing was increased to 48 h, the stabilization effect was reduced. We also tested whether this stabilization pathway could act on a much larger dendritic arbor. The ddaE neuron (Fig. 1*A*) has a small arbor and ddaC has a large arbor, but was also stabilized by previous axon injury (see Fig. S2*G*). In these experiments, axons were severed near the cell body. When ddaC axons were severed 50 μm or more

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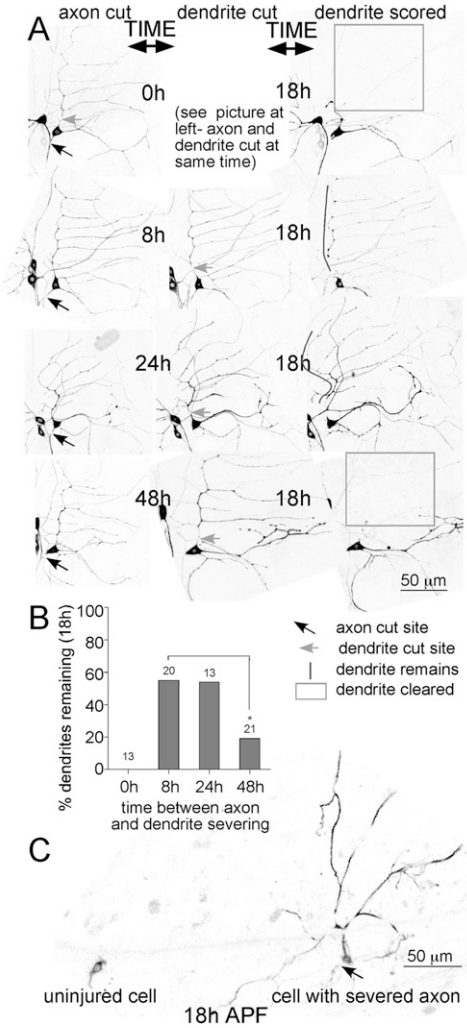

Fig. 1. Axon injury delays subsequent dendrite degeneration. (*A* and *B*) Class I da neurons were labeled by using 221-Gal4 to drive expression of EB1-GFP. Whole larvae were mounted on slides for live imaging, and axons of the ddaE neurons were severed where indicated at time 0. The comb-like dendrite that extends dorsally from these neurons was severed immediately afterward (*Top*) or after letting the animal recover for 8, 24, or 48 h. During the recovery period animals were returned to their normal media. In all cases, the presence of the comb dendrite was scored 18 h after it was severed from the cell body. Example images are shown in *A* and quantification is shown in *B*. Numbers above the bars in the graph are number of neurons, one per animal, that were analyzed. A Fisher exact test was used to determine significance. Throughout the figures, the same notation for *P* values is used: $*P < 0.05$, $**P < 0.005$, and $***P < 0.0001$. (*C*) Class IV neurons were labeled with the membrane marker mCD8-GFP under control of ppk-Gal4. The axon of a single ddaC neuron per animal was severed shortly before the onset of pupariation. Pupae were imaged 18 h after pupa formation (APF), and the presence of dendrites was scored. In each animal ($n = 9$), only the injured cell retained dendrites. An example of two neighboring cells in a pupa is shown. The cell body at left has an axon emerging ventrally (toward bottom), but no dendrites are seen dorsal to the cell body. In contrast, its neighbor, at right, has an elaborate dendrite arbor.

from the cell body, dendrite clearance after later dendrite severing was also delayed (Fig. S1). Thus, both proximal and distal axotomy resulted in a cellular response that slowed injury-induced dendrite degeneration.

We next considered two possibilities: (*i*) that axon injury might turn on a response to specifically protect the neuron against subsequent physical trauma or (*ii*) that axon injury might turn on a more general stabilization pathway. To test for a general stabilization pathway, we asked whether axon injury stabilized dendrites against developmental degeneration. Many of the da neurons are pruned during metamorphosis and then regrow dendrite arbors into the remodeled adult body wall (10). To test whether axon injury could delay pruning of the ddaC neuron, we severed axons as larvae were preparing to pupariate. We then assayed for complete clearance of dendrites 18 h after pupariation had initiated. In all cases in which axons were injured first ($n = 9$), dendrites remained (Fig. 1*C*). All dendrites of uninjured cells in these animals (approximately 10 per animal) were successfully pruned. Thus, axon injury stabilized dendrites against both injury-induced degeneration and developmental pruning, suggesting that it turns on a general protective pathway.

Microtubule Nucleation Plays a Role in Dendrite Stabilization. We found previously that the number of growing microtubules is up-regulated in dendrites after axon injury (6). To test whether this change in microtubule dynamics might be related to dendrite stabilization, we compared the timing of these two responses. We assayed the number of dynamic microtubules by expressing the microtubule plus end-binding protein EB1 tagged with GFP and assaying EB1 comet number before and after axon injury. Each EB1-GFP comet marks the plus end of a growing microtubule (11), and so the number of comets is a readout of the number of growing microtubules. The number of comets in dendrites peaked at 24 h after axon injury and decreased thereafter (Fig. 2*A*), similar to dendrite stabilization (Fig. 1*B*).

To further test the relationship between microtubule dynamics and dendrite stabilization, we had to identify the machinery that regulated microtubules in response to axon injury. We hypothesized that either new nucleation of microtubules or severing of existing microtubules could lead to an increase in growing microtubule plus ends labeled by EB1-GFP. We therefore tested whether reduction of nucleation or severing proteins by RNAi could block injury-induced microtubule dynamics. Large RNA hairpins were expressed in the ddaE neurons together with EB1-GFP by using the Gal4-UAS system. Compared with a control hairpin targeting γTub37C, a maternal γ-tubulin that does not play a major role in somatic cells (12), only the hairpin targeting γTub23C, the major somatic nucleation protein, reduced comet number in the cell body 24 h after axon injury (Fig. S2*A*). We concluded that nucleation contributed to increased microtubule dynamics in response to axon injury.

To determine whether microtubule dynamics played a functional role in dendrite stabilization, we performed sequential axon and dendrite severing in genetic backgrounds with reduced nucleation. We wished to only partially reduce microtubule nucleation so that normal cellular functions would be unaffected. We therefore used animals heterozygous for a null mutation in *γTub23C* ($\gamma Tub23C^{A15\text{-}2}/+$) or expressed RNA hairpins to reduce γTub23C. Uninjured neurons in both cases appeared normal and had normal numbers of dynamic microtubules (shown for heterozygote in Fig. S2*D*); after injury, the increase in microtubule dynamics in dendrites was reduced at 8 h (Fig. 2*D*) and 24 h (Fig. S2*E*). When we tested heterozygotes for dendrite stabilization after axon injury (experiment schematic shown in Fig. 2*B*), we found that it was impaired compared with control (i.e., *yw*) animals (Fig. 2*E*). To make sure that the phenotype was caused by reduction of γTub23C levels and was cell-autonomous, we performed a rescue experiment with GFP-tagged γTub23C (Fig. 2*E*). We were not able to confirm this result with γTub23C RNAi (Fig. S2*F*), perhaps because partial knockdown of γTub23C was simply not enough to abrogate protection. Consistent with this idea, protection was also not disrupted 24 h after axon injury in the *γTub23C* heterozygous background (Fig. S2*F*). We therefore confirmed that regulation of microtubules was involved in dendrite stabilization by using an independent approach to disrupt microtubule growth. msps, or XMAP215, is proposed to be

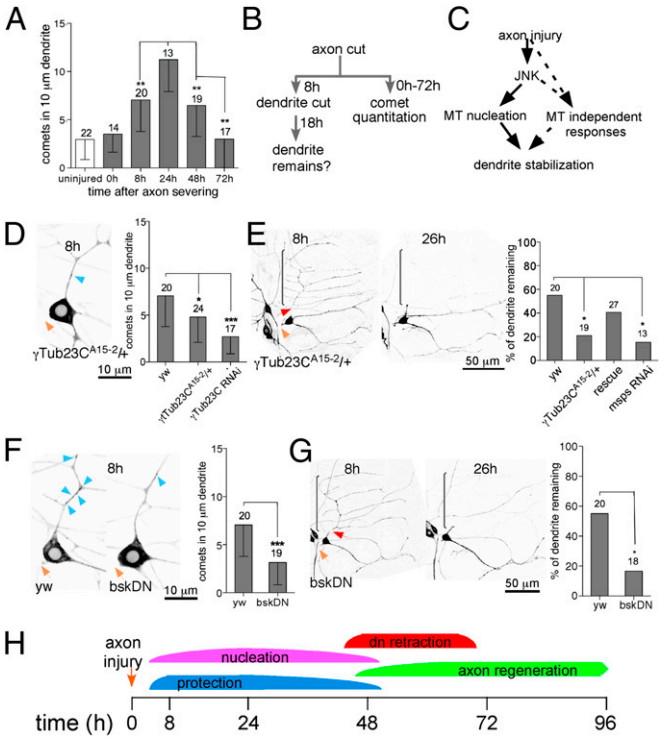

Fig. 2. Injury-induced microtubule dynamics and dendrite protection require γ-tubulin and JNK signaling. (*A*) 221-Gal4 was used to drive EB1-GFP expression in class I neurons. Axons of ddaE were severed at time 0. Movies of EB1-GFP dynamics were acquired at different times after axon severing. The sum of EB1-GFP comets in a 10-µm length of the dendrite is presented in the graph. (*B* and *C*) A schematic of experiments in this figure is shown in *B*, and *C* shows a summary of results in the figure. (*D*) A tester line containing 221-Gal4 and EB1-GFP, and dicer2 for RNAi experiments, transgenes was crossed to either control (*yw*) or *γTub23C* mutant flies or γTub23C RNAi flies. In larval progeny, ddaE axons were severed at time 0 and comet number in dendrites was assayed 8 h later. (*E*) Genotypes used were similar to those in *D*. For the rescue experiment, γTub23C-GFP was added into the *γTub23C*$^{A15-2}$/+ background. For all genotypes, axons were severed at 0 h, the dorsal comb-like dendrite was severed 8 h later, and presence of the severed dendrite was scored 18 h after that. (*F* and *G*) A tester line containing 221-Gal4 and EB1-GFP was crossed to control (*yw*) or UAS-bskDN–containing flies. EB1-GFP comet number was monitored in dendrites 8 h after axotomy (*F*), and dendrite clearance after sequential axon and dendrite severing is also shown (*G*). (*H*) Timeline summarizes the results here and in Fig. S3 is shown. Error bars in all figures indicate SD.

a microtubule polymerase (13), and RNAi targeting msps eliminates EB1-GFP comets in neurons (6). We therefore used msps RNAi to perturb the behavior of neuronal microtubule plus ends. This condition blocked the protective effect of axon injury on dendrites in ddaE (Fig. 2*E*) and ddaC (Fig. S2*G*) neurons. We conclude that global changes in microtubule dynamics induced by axon injury play a functional role in dendrite stabilization.

Increased Microtubule Dynamics and Dendrite Stabilization After Axon Injury Depend on JNK Signaling. JNK signaling is implicated in axon injury response in many systems (6, 14–17). We therefore hypothesized that JNK signaling would be required for increased microtubule dynamics and for dendrite stabilization. To test this hypothesis, we compared both responses in control neurons and neurons that expressed a dominant-negative form of the *Drosophila* JNK protein bsk (bskDN), which blocks axon injury signaling in *Drosophila* motor neurons (17). Expression of bskDN dampened the microtubule number increase after axon injury (Fig. 2*F*), and also reduced the protective effect of axon injury on dendrites (Fig. 2*G*). These results are consistent with JNK signaling being upstream of increased microtubule nucleation and dendrite stabilization after axon injury (Fig. 2*C*). We also tested whether elevation of JNK signaling by overexpression of bsk in uninjured neurons was sufficient to increase microtubule dynamics or stabilize dendrites, and found that it was not (Fig. S2 *B* and *C*). Thus, although JNK signaling is required for these injury responses, it most likely requires other pathways that are activated by injury to generate a response.

Axon Regeneration Is Not Dependent on Up-Regulation of Microtubule Dynamics. One of the major injury responses downstream of JNK is axon regeneration. We therefore wished to determine the relationship among axon regeneration, dynamic microtubules, and dendrite stabilization. After proximal axotomy of ddaE, axon regeneration from a dendrite was unperturbed in *γTub23C* heterozygotes or when RNAi was used to target γTub23C (Fig. S3 *A* and *B*). Similarly, regeneration after distal axotomy of ddaC was normal in γTub23C RNAi neurons (Fig. S3*C*). Thus, axon regeneration does not depend on γTub23C in the same way as dendrite stabilization. Importantly, the ability of neurons to initiate axon regrowth when γTub23C levels were reduced indicates that these neurons were not generally sick or unable to respond to injury.

We also considered that altered microtubule dynamics might be required to remodel dendrites after axon injury. In mammalian neurons in vivo, dendrite simplification has been documented after axon injury (18–20). We monitored dendrite shape in ddaC neurons after distal axotomy and found that fine dendrite branches were trimmed back after distal axon severing (Fig. S3*D*), just as in mammals. Most of this trimming occurred between 24 and 72 h after axotomy, and was unchanged when γTub23C levels were reduced (Figs. S3*D* and S4). To compare timing of the responses to axon injury, a timeline is shown in Fig. 2*H*.

Reduction of an Axonal Motor or Expanded Poly-Q Protein Expression Triggers Increased Microtubule Nucleation. Thus far, we have demonstrated that axon injury turns on a pathway that transiently stabilizes dendrites after injury. This pathway seems to be turned off when regeneration begins. We also wished to know whether this type of stabilization pathway could be turned on for more extended times to perhaps protect neurons from long-term degeneration. We tested two types of chronic stress: reduced unc-104 and expression of poly-Q proteins. In both cases, we assayed microtubule dynamics and dendrite stabilization to determine whether a microtubule-based neuroprotective program was activated.

We found, fortuitously, that RNAi hairpins targeting unc-104 caused a dramatic increase in the number of EB1-GFP comets in dendrites (Fig. 3*A* and Movie S1), similar to that seen after axon injury. Comet number in these cells was compared with that in neurons expressing a control hairpin (Rtnl2). Unc-104 has previously been linked to synaptic vesicle transport (21–23) and thus might cause stress. To test whether other long-term axon stress might cause changes in dendrite microtubules, we assayed microtubule dynamics in neurons that expressed proteins used to model neurodegenerative disease in *Drosophila*.

When human expanded poly-Q proteins are expressed in fly eyes, they can cause degeneration (24–26). We expressed three matched pairs of short and expanded poly-Q proteins in *Drosophila* sensory neurons. Expression of a truncated version of SCA3 with a long poly-Q repeat (SCA3tr-Q78), SCA1 with long repeat (SCA1-Q82), or a truncated huntingtin protein with long repeat (httex1p-Q93) resulted in increased microtubule dynamics (Fig. 3*B* and Movie S2). For SCA3 and htt, the neurons expressing the short poly-Q proteins had similar comet numbers to control cells. For SCA1-Q30–expressing cells, comet

number was slightly elevated at the normal incubation temperature (25 °C), consistent with previous results indicating the Q30 form has some deleterious effects (26). Both unc-104 RNAi and expression of SCA3tr-Q78 caused reduced complexity of the highly branched class IV ddaC neuron (Fig. S5). The effect of SCA3tr-Q78 on cell morphology is similar to that reported in a recent study (27).

To test whether increased microtubule dynamics resulting from unc-104 RNAi and poly-Q protein expression relied on microtubule nucleation and JNK signaling, we made strains that paired SCA3tr-Q78 with control RNA hairpins (γTub37C and Rtnl2), hairpins to target γTub23C, or bskDN. We used a similar strategy for unc-104. In both cases, expression of bskDN or the hairpin targeting γTub23C reduced microtubule dynamics compared with controls (Fig. 3 *C* and *D* and Movies S3, S4, S5, and S6). We conclude that, like axon injury, reduction of the unc-104 motor or expression of expanded poly-Q proteins caused neurons to up-regulate microtubule nucleation through a JNK-dependent pathway.

Expression of an Expanded Poly-Q Protein Stabilizes Dendrites Against Injury-Induced Degeneration. The activation of a common JNK-dependent stress response pathway by axon injury and poly-Q proteins suggested that poly-Q protein expression might also stabilize dendrites. To test this idea, we again used clearance of dendrites after severing from the cell body as a reporter of dendrite stability. In control ddaE neurons, most dendrites were cleared 18 h after severing from the cell body (Fig. 4 *A* and *C*). In contrast, more than half of dendrites in ddaE neurons expressing SCA3tr-Q78 were still present 18 h after removal from the cell body (Fig. 4 *B* and *C*). Expression of SCA3tr-Q78 also delayed degeneration in ddaC cells (Fig. S6*A*), and similar results were obtained with unc-104 RNAi (Fig. S6).

To determine the timing of increased microtubule dynamics and dendrite stabilization in larvae that expressed poly-Q proteins, we assayed both throughout larval life. Larvae were assayed 1, 2, and 3 d after hatching (Fig. 4*C*). On the first day, both microtubule dynamics and dendrite stabilization were similar to control levels, presumably because poly-Q proteins had not accumulated at high enough levels to trigger a response. Microtubule dynamics and protection levels were similar to those of injured neurons at days 2 and 3 (Fig. 4 *C* and *E*).

For technical reasons, after axon injury, we only assayed stabilization and microtubule dynamics in dendrites. In poly-Q–expressing neurons, we were able to ask whether both responses also affected axons. Poly-Q protein expression increased the number of dynamic microtubules in axons, and delayed axon beading after severing (Fig. 4*D*), suggesting that this is a very general pathway that can affect all parts of the cell.

Microtubule Nucleation Reduces Neurodegeneration Induced by Poly-Q Proteins. Thus far, we assayed neuronal stabilization by monitoring the timing of axon or dendrite disassembly after removal from the cell body. We also wished to determine whether dynamic microtubules could counteract longer-term progressive degeneration. We therefore compared dendrite retraction in SCA3tr-Q78–expressing ddaC neurons with normal and reduced levels of nucleation. Neurons expressing a control RNA hairpin (Rtnl2) or γTub23C RNAi had complex dendrite arbors that increased the number of branches during larval life (Fig. 5). Similarly, neurons expressing SCA3tr-Q78 with Rtnl2 RNA hairpins had complex dendrite arbors that increased in branching during larval life (Fig. 5). However, when the γTub23C hairpin RNA was paired with SCA3tr-Q78, dendrite complexity was reduced as larvae aged, and, in late larvae, only the main dendrite trunks were present (Fig. 5 and Fig. S7*A*). Strikingly similar results were found when unc-104 RNA hairpins were paired with a control RNA hairpin or γTub23C hairpin (Fig. S7*B*).

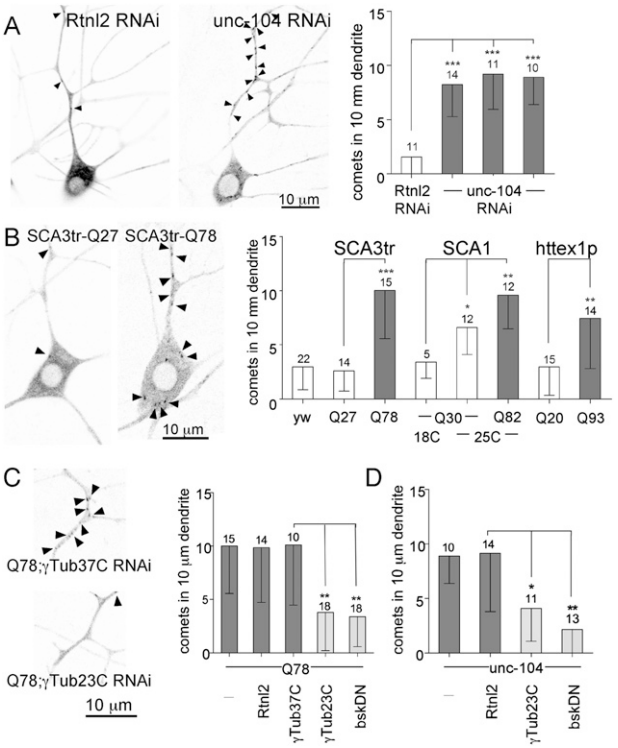

Fig. 3. Reduction of unc-104 or expression of expanded poly-Q proteins triggers nucleation-based increases microtubule dynamics. (*A*) ddaE neurons expressing EB1-GFP, dicer2, and either unc-104 or Rtnl2 RNAi hairpins were imaged in whole larvae. Single frames from movies are shown. The cell body and part of the comb dendrite is visible. Arrowheads indicate EB1-GFP comets. Comets in a region of the dendrite were counted, and averages are shown. Each of the unc-104 bars in the graphs represents data obtained from animals expressing a different RNA hairpin. (*B*) Human proteins containing short or long poly-Q repeats were expressed in ddaE neurons together with EB1-GFP. All data were obtained at 25 °C unless indicated otherwise. (*C* and *D*) The SCA3tr-Q78 transgene or unc-104 hairpin was expressed together with a control RNA hairpin (γTub37C or Rtnl2), a hairpin targeting the major nucleation protein in somatic cells, γTub23C, or bskDN. In all graphs, numbers above the bars indicate the number of neurons analyzed. Significance was calculated by using unpaired *t* tests. Error bars indicate SD.

It is important to note that dendrite complexity was also reduced when SCA3tr-Q78 or unc-104 RNAi was expressed in the absence of additional RNA hairpins (Fig. S5). This is most likely because all the transgenes, including SCA3, rely on the Gal4-UAS expression system. The reduction in phenotype severity when additional transgenes are added is consistent with dilution of the Gal4 protein between more UAS-driven transgenes. We therefore controlled for transgene number in our experiments.

To test whether the microtubule-based stabilization pathway acted to globally protect from poly-Q–induced neurodegeneration in adult neurons, we used a well-established neurodegeneration model. Expression of human proteins that induce degeneration in *Drosophila* eyes has proven to be a powerful system in which to study neurodegeneration (28). Expression of SCA3tr-Q27 or γTub23C RNA hairpins in photoreceptors did not disrupt eyes (Fig. S8). Expression of SCA3tr-Q78 caused a variety of eye phenotypes including lack of pigmentation (mild), eye collapse (moderate), and appearance of completely disrupted black areas on the eye (severe). Phenotypes were slightly more severe in males than females. When microtubule nucleation was reduced in the background of SCA3tr-Q78 expression, severe

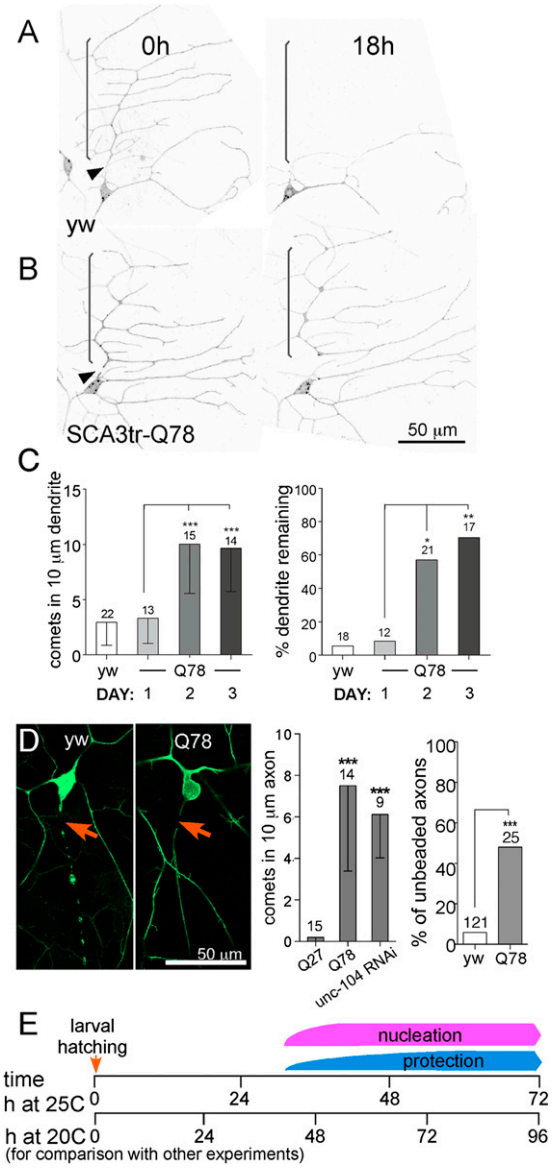

Fig. 4. Expression of SCA3tr-Q78 delays injury-induced dendrite degeneration. (*A–C*) The comb dendrite of ddaE neurons expressing mCD8-RFP alone (*A*) or with SCA3tr-Q78 (*B*) was severed in whole living animals with a pulsed UV laser (arrowheads) at different times after larval hatching. The presence of the distal dendrite was scored 18 h later (*C, Right*). Example images in *A* and *B* are from larvae severed at 2 d after hatching. Comet number in uninjured neurons expressing SCA3tr-Q78 was scored (*C, Left*). Significance was calculated with a Fisher exact test. Numbers above bars are numbers of neurons tested. (*D*) Control larvae expressing mCD8-GFP under control of 477-Gal4 or flies expressing SCA3tr-Q78 in addition to mCD8-GFP had axons of the ddaC neuron severed at 0 h. Pictures are images of neurons 12 h after severing. Arrows indicate cut sites. The graph shows the percentage of animals that had smooth unbeaded axons 12 h after severing. Numbers above the bars are the numbers of animals in each group. Statistical significance was calculated with a Fisher exact test. The number of EB1-GFP comets was quantitated as in dendrites in the genotypes indicated. (*E*) Results from the figure are summarized in a timeline.

disruption of eye morphology was very common (Fig. S8). This result is consistent with poly-Q proteins turning on a stabilization pathway that counteracts progressive degeneration (diagram in Fig. S8*A*). We conclude that microtubule-based neuroprotection can counter degeneration in different types of neurons in larvae and adults.

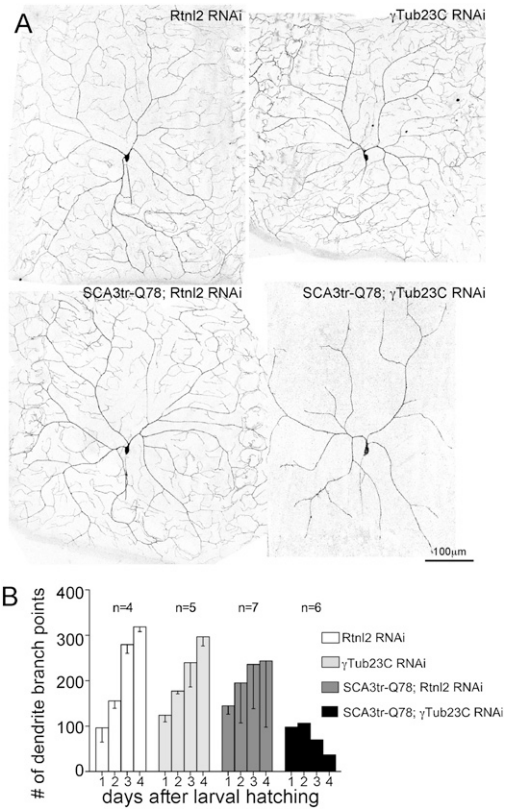

Fig. 5. Reduction of microtubule nucleation increases dendrite degeneration in SCA3tr-Q78–expressing neurons. (*A*) Morphology of ddaC neurons expressing dicer2 and mCD8-GFP with hairpin RNAs only (*Upper*) or hairpin RNAs with SCA3tr-Q78 (*Lower*) was assayed over 4 d. Images from the final day are shown. (*B*) Complexity of ddaC dendrite arbors was assayed over time by counting the number of branch points.

Discussion

We have shown that axon injury, reduction of a synaptic vesicle motor, or expression of expanded poly-Q proteins triggers dramatic changes in the cytoskeleton. Each of these cellular stresses results in a huge increase in the number of dynamic microtubules throughout the neuron. Moreover, in all cases, this increase in dynamic microtubules is blocked if levels of a microtubule nucleation protein are reduced. We therefore propose that axon injury, as well as more long-term neuronal stresses, triggers a global cellular response that alters microtubules. This response is mediated by JNK signaling, suggesting that these three diverse stresses activate a common pathway with cytoskeletal regulation as its output.

After axon injury, the increase in microtubule dynamics was transient, and it tapered off by 48 h after the injury. Dendrite stabilization followed the same time course. This time course is also similar to protection after axon injury in a recently described model for conditioning lesion in *Drosophila* (29). Although dependence on JNK could not be tested in this model, an upstream JNK regulator, wallenda, was required (29), hinting that there may be mechanistic overlap between these protective pathways.

In contrast to the brief activation of protection after axon injury, expression of SCA3tr-Q78 resulted in increased microtubule dynamics and dendrite stabilization over several days. The extended activation of this pathway suggested that it might be able to protect against long-term degeneration. We found support for this idea in both da neurons and eyes: in both cases, SCA3tr-Q78–induced degeneration was more severe when microtubule nucleation was partially reduced.

Based on these results, we speculate that this pathway might function in different scenarios. In the case of axon trauma, the stabilization pathway might prevent further damage during the initial response to the injury. During long-term neurodegenerative disease, activation of this stabilization pathway could extend the time that neurons can maintain their normal structure.

We do not yet know how increased microtubule dynamics might reinforce dendrites to protect them from long- and short-term degeneration. One possibility is that nucleation of new microtubules plays a structural role. Rebuilding microtubule rods down the center of the dendrite could prevent beading, which is an early step in most types of axon and dendrite degeneration (9, 30) In support of this idea, microtubule disassembly has been proposed to be an early step in Wallerian axon degeneration (31, 32) and developmental pruning of dendrites (33). In fact, for the developmental pruning pathway, a putative microtubule severing protein is required at an early step (34). Thus, microtubule nucleation could very directly battle the microtubule disassembly that is activated during pruning or degeneration. It is also possible that microtubules could contribute to dendrite stability less directly, for example by altering intracellular transport.

If increased microtubule dynamics are a conserved general feature of neuronal response to axon stress, it could be developed into a diagnostic tool for early stages of neurodegenerative disease, perhaps before overt dysfunction becomes obvious. Identification of this pathway also offers some novel ideas about therapies for neurodegenerative disease. Turning on this pathway earlier in patients who express expanded poly-Q proteins could delay the onset of symptoms. It is possible that other neurodegenerative diseases do not activate this protective pathway. If this is the case, then strategies to activate it could result in improvement of the disease course.

Materials and Methods

All imaging was performed in living whole-mount larvae or pupae expressing GFP-tagged proteins in neuronal subsets. Axon and dendrite severing was performed with a pulsed UV laser as described (6). For information about genotypes and additional experimental details see *SI Materials and Methods*.

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