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Report

Axonal Pruning Is Actively Regulated by the Microtubule-Destabilizing Protein Kinesin Superfamily Protein 2A

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SUMMARY

Extensive axonal pruning and neuronal cell death are critical events for the development of the nervous system. Like neuronal cell death, axonal elimination occurs in discrete steps; however, the regulators of these processes remain mostly elusive. Here, we identify the kinesin superfamily protein 2A (KIF2A) as a key executor of microtubule disassembly and axonal breakdown during axonal pruning. Knockdown of *Kif2a*, but not other microtubule depolymerization or severing proteins, protects axonal microtubules from disassembly upon trophic deprivation. We further confirmed and extended this result to demonstrate that the entire degeneration process is delayed in neurons from the *Kif2a* knockout mice. Finally, we show that the *Kif2a*-null mice exhibit normal sensory axon patterning early during development, but abnormal target hyperinnervation later on, as they compete for limited skin-derived trophic support. Overall, these findings reveal a central regulatory mechanism of axonal pruning during development.

INTRODUCTION

The nervous system is shaped during development by progressive and regressive events. Axonal pruning, a strategy used by the nervous system to remove exuberant or misguided connections, often occurs via local axonal degeneration (Coleman, 2005; Low and Cheng, 2006; Luo and O'Leary, 2005). The degeneration process is characterized by a series of discrete steps. Disassembly of microtubules (MTs) is the earliest cellular event, which is followed by degradation of other cytoskeletal elements, such as neurofilaments (NFs) and fragmentation of the axon (Watts et al., 2003; Zhai et al., 2003). This series of steps is also exhibited in injured axons following nerve transection (Zhai et al., 2003). To date, the mechanisms and molecules executing MT breakdown are largely unknown. Moreover, the causal relationship between MT integrity and breakdown of other cytoskeletal components during axonal degeneration is unclear (Saxena and Caroni, 2007).

Here, we show that the MT-stabilizing agent paclitaxel protects against MT disassembly in different paradigms of axonal degeneration, trophic (NGF) deprivation, or axotomy. We further demonstrate that this protection can be largely mimicked by ablation of a single MT-depolymerization protein of the kinesin-13 family, kinesin superfamily protein 2A (KIF2A). Interestingly, degradation of Tau (tubulin-associated unit) precedes MT breakdown by KIF2A. However, this degradation is not sufficient to induce MT disassembly by itself. Last, we demonstrate that the initial growth and arrangement of sensory axons, which are NGF independent, are perfectly normal in *Kif2a*-null mice. However, these mice exhibit severe skin hyperinnervation by sensory axons, a process that is restricted by competition for limited trophic support. Overall, these findings uncover a key regulatory mechanism of axonal pruning during development.

RESULTS

MT Depolymerization Is Inhibited by the MT-Stabilizing Agent Paclitaxel

MT-destabilization drugs, such as vincristine or colchicine, induce axonal degeneration (Saxena and Caroni, 2007). However, whether MT-stabilization drugs can prevent MT breakdown during axonal degeneration is not known. Therefore, we tested whether paclitaxel, a strong MT polymerization agent, can inhibit MT depolymerization in two in vitro models of axonal degeneration; trophic (NGF) deprivation, in which axons degenerate due to lack of trophic support, representing a developmental elimination process, and axotomy in which axons are cut from their cell bodies. Dorsal root ganglia (DRGs) were dissected from mouse embryos at embryonic day 13.5 (E13.5), and, after 48 hr in culture, axonal degeneration was induced and the MTs underwent depolymerization (Figures 1A–1C and 1G). Application of paclitaxel caused inhibition of the MT depolymerization process in both models, with the MTs appearing intact (Figures 1D–1G). To further characterize the cytoskeletal changes during degeneration, we conducted a biochemical analysis of NGF-deprived

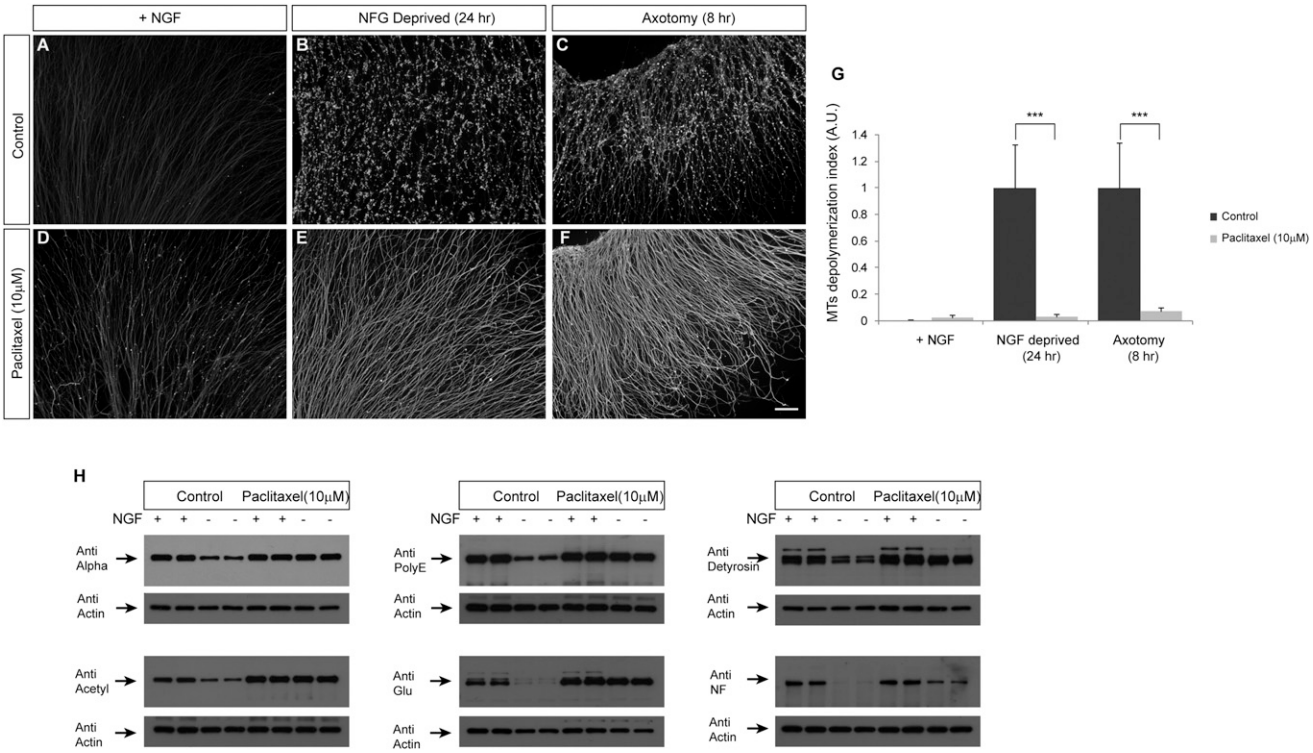

Figure 1. Paclitaxel Protects MT from Degradation upon NGF Deprivation, but NFs Are Only Minimally Protected

(A–G) DRG explants were cultured for 48 hr in NGF-containing medium. For the following 24 hr, they were supplied with NGF (A and D), deprived of NGF (B and E), or axotomized for 8 hr (C and F). The explants were then immunostained for tubulin βIII. Upon NGF deprivation or axotomy, the MTs undergo breakdown, as can be seen by the punctuated form of the MTs (B, C, and G). The addition of 10 μM paclitaxel protected the MTs, as no degradation was detected 24 hr after NGF deprivation (E–G). MT depolymerization average index value ± SD was calculated for each condition (G), Mann-Whitney test, ***$p < 0.001$.

(H) Biochemical analysis of the axonal cytoskeleton. DRG explants were cultured for 48 hr in NGF-containing medium. For the next 20 hr, these explants were either supplied with (+) or deprived of (−) NGF. MT polymerization level was evaluated using immunoblot analysis for α-tubulin and its PTMs antibodies. MTs were both degraded, as detected by total α-tubulin, and depolymerized as revealed by the strong reduction in acetylation, detyrosination, and polyglutamylation state, upon NGF deprivation. In the presence of 10 μM paclitaxel, MTs remained polymerized and were not degraded. NF degradation observed after NGF deprivation was only slightly attenuated in the presence of paclitaxel. Scale bar, 100 μM.

See also Figure S1.

axons (Figure 1H). In agreement with the cellular analysis, paclitaxel inhibited MT degradation after NGF withdrawal, as in its presence there was no reduction in α-tubulin (Figure 1H). Next, we examined the changes in tubulin posttranslation modifications (PTMs), which mark subpopulations of MTs during the degeneration process. Several modifications, including acetylation, detyrosination, and glutamylation, are enriched in stable MTs (Verhey and Gaertig, 2007). Indeed, following NGF withdrawal, we detected a strong decrease in these modifications, indicating MT destabilization. However, in the presence of paclitaxel, the MTs remained polymerized as indicated by all the tubulin PTMs examined (Figure 1H). In contrast to its powerful stabilizing effect on MTs, paclitaxel showed only minor protection of NFs, which are degraded during the axonal degeneration process (Figure 1H). This implies that other pathways operate in parallel to MT depolymerization during axonal elimination. Similar results were obtained using the axotomy model (Figure S1). Overall, these results suggest that elevation of MT polymerization can protect them from disassembly during axonal degeneration.

Degradation of the MT-Stabilizing Protein, Tau, Is Insufficient for Inducing Axonal MT Depolymerization

The experiments with paclitaxel prompted us to hypothesize that specific MT-stabilizing proteins might be degraded during axonal degeneration. Tau (also known as MAPT) belongs to a widespread family of MAPs. It is an abundant protein in the central and peripheral nervous system, where it is expressed predominantly in neurons and enriched in axons and, like paclitaxel, promotes tubulin assembly into MTs (Binder et al., 1985). Importantly, Tau binds to a site on β-tubulin that overlaps the paclitaxel site (Kar et al., 2003). In addition, hyperphosphorylation of Tau and its subsequent degradation prevent it from binding to MTs, leading to a decrease in MT stability (Lindwall and Cole, 1984). Tau is a known substrate of caspase-6, which is activated upon NGF deprivation within axons (Graham et al., 2011).

In light of the above, we monitored the fate of axonal Tau after NGF deprivation or axotomy using an insert culture system that enables effective purification of axonal material (Schoenmann et al., 2010). Degradation of Tau was detected 14 hr after trophic withdrawal, when the axonal MTs are still intact (Figure 2A). The

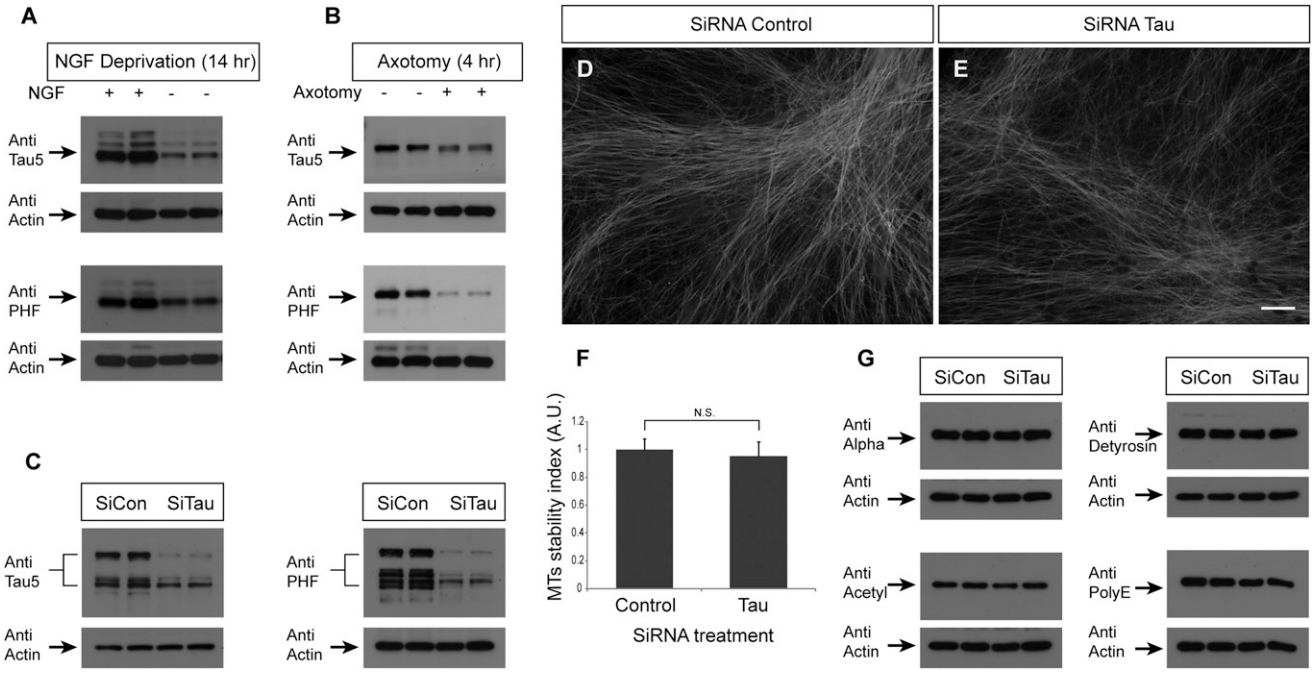

Figure 2. Tau Degradation Is Not Sufficient for Promoting MT Breakdown

(A and B) DRG explants were cultured for 48 hr in NGF-containing medium. These explants were then deprived of NGF for 14 hr (A) or axotomized for 4 hr (B). Axonal Tau was detected by western blot using two antibodies, Tau5 (total Tau) and PHF (phosphorylated Tau). A significant reduction of total Tau was detected using Tau5 antibody 14 hr after NGF withdrawal, while the phosphorylated form was only slightly reduced (A). Both antibodies detected clear reduction of Tau 4 hr after axotomy (B).

(C–G) Tau expression was reduced using siRNA treatment as detected by Tau5 and PHF (C), yet this reduction did not induce MT depolymerization, as evaluated by both the immunostaining for βIII tubulin (D and E) and immunoblotting for α-tubulin, acetylation, detyrosination, and polyglutamylation (G). MT stability average index value ± SD was calculated for each siRNA treatment (F), Mann-Whitney test. NS, nonsignificant.

Scale bar, 100 μM. See also Figures S2 and S3.

hyperphosphorylated form of Tau was only slightly reduced in this time frame (Figure 2A). This might indicate that Tau is hyperphosphorylated during trophic deprivation. Tau was completely degraded by 20 hr (Figure S2). In addition, at 4 hr postaxotomy the level of Tau was reduced, and by 6 hr it was eliminated (Figures 2B and S2). However, treatment of the sensory neuronal cultures with small interfering RNA (siRNA) against Tau did not induce axonal MT depolymerization (Figures 2D–2G), even though Tau was markedly reduced (Figure 2C). These results indicate that the degradation of Tau during axonal degeneration is not in itself sufficient to induce MT depolymerization.

Knockdown of *Kif2a*, but Not Other MT-Severing Proteins, Inhibits MT Depolymerization

The inability of Tau knockdown to induce axonal MT depolymerization may be due to lack of strong MT depolymerization activity. Therefore, we examined the role of MT-destabilizing proteins in axonal MTs depolymerization, using siRNA knockdown in combination with the NGF-deprivation model. We screened several candidates, including the four *Stathmin* family members (*Stmn1–4*), *Katanin*, *Spastin*, and *Kif2a* (Conde and Cáceres, 2009). Strikingly, only the knockdown of *Kif2a* strongly inhibited MT depolymerization, revealing MT preservation 24 hr after NGF withdrawal (Figure S3).

Genetic Ablation of *Kif2a* Delays MT Disassembly and Axonal Degeneration

To further establish the role of *Kif2a* in initiating axonal fragmentation, we performed a temporal analysis of axonal degeneration after NGF deprivation using neurons from the *Kif2a* knockout (KO) mice (Homma et al., 2003). Clear inhibition in MT depolymerization and its subsequent degradation can be detected at all time points 18–27 hr after NGF deprivation (Figures 3A–3K). However, by 30 hr the MTs from the *Kif2a* KO neurons were completely depolymerized as well, representing a delay of up to 9 hr. These cellular results are also supported by our biochemical analysis as ablation of *Kif2a* preserved MT integrity from both depolymerization and degradation, while NF degradation was only slightly attenuated in *Kif2a* KO mice (Figure 3L). Importantly, the protection is not due to inhibition of caspase-3, as it is fully active in the *Kif2a* KO axons (Figure S4). Tau degradation was also attenuated, in both paclitaxel-treated and *Kif2a* KO neurons (Figures 3M and 3N). Therefore, under conditions in which the MTs are preserved, Tau might still be bound to the polymerized MTs, this shade Tau from the degradation machinery.

In the *Kif2a* KO neurons, a clear protection of the MTs was observed 8 hr after axotomy (Figures 3O–3Q). Yet by 12 hr the MTs were depolymerized. In addition, MT degradation was attenuated, as indicated by the preservation of α-tubulin, while

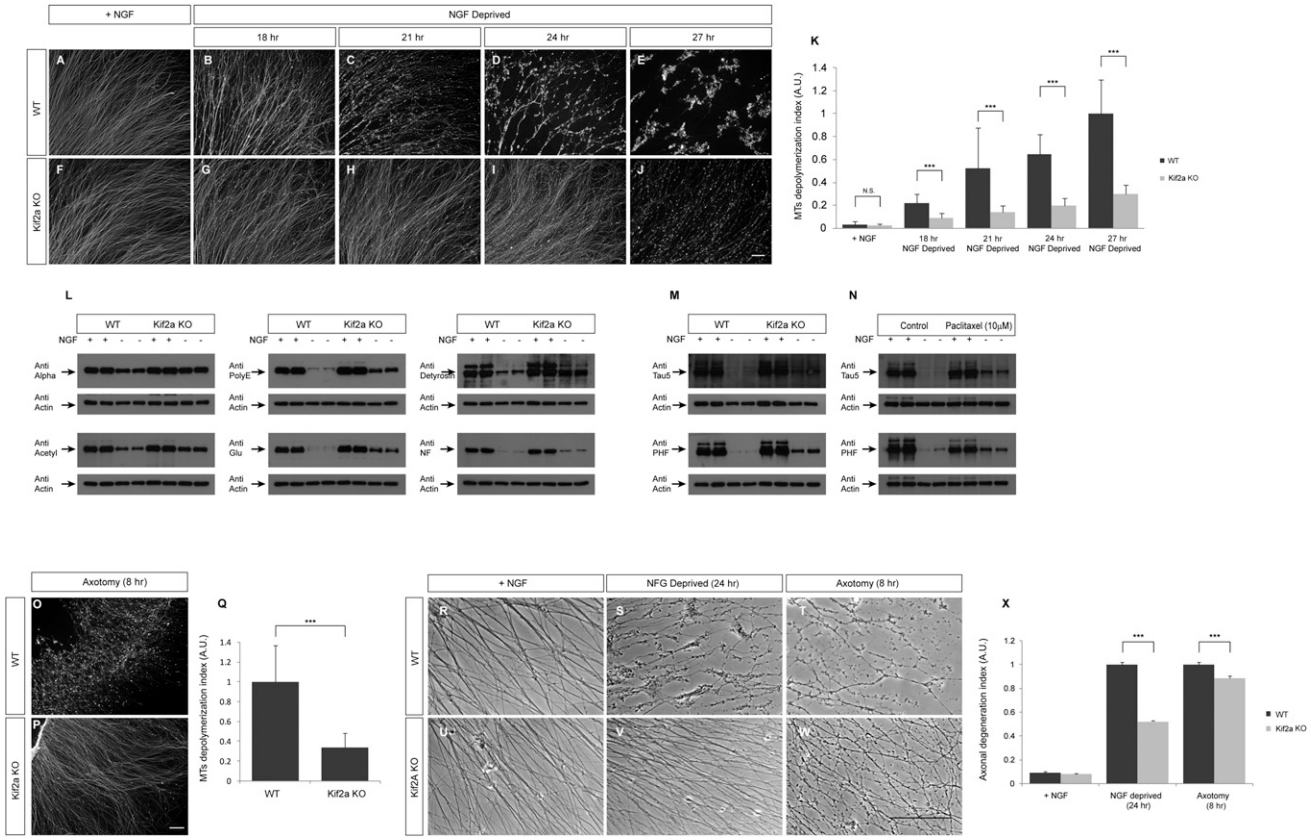

Figure 3. Genetic Ablation of *Kif2a* Delays MT Depolymerization upon NGF Deprivation

(A–K) DRG explants of either *Kif2a* KO embryos (F–J) or WT littermate (A–E) were cultured for 48 hr in NGF-containing medium. These explants were either supplied with (A and F) or deprived of (B–E and G–J) NGF for an additional 18 (B and G), 21 (C and H), 24 (D and I), and 27 hr (E and J) and then immunostained for tubulin βIII. Deprived of NGF, the axonal MTs of the WT neurons were depolymerized and degraded, as can be seen by the punctuated form of the MTs (B–E). In contrast, the axonal MTs breakdown of the *Kif2a* KO neurons was inhibited after NGF deprivation (G–J). MT depolymerization average index value ± SD was calculated for each condition and genotype (K), Mann-Whitney test, ***$p < 0.001$.

(L) Genetic ablation of *Kif2a* protects MT but hardly protects NFs from degradation upon NGF deprivation. DRG explants were cultured for 48 hr in NGF-containing medium. Then they were deprived of NGF for the next 20 hr. MT polymerization level was evaluated using immunoblot analysis for α-tubulin and its PTM antibodies. In WT axons upon NGF deprivation, MTs were both degraded, as detected by α-tubulin and depolymerized, as detected by the reduction in acetylation, detyrosination, and polyglutamylation. In the *Kif2a* KO axons, MTs were more polymerized and less degraded. NF degradation upon NGF deprivation was only slightly attenuated in the *Kif2a* KO neurons, compared to the WT controls.

(M and N) Tau degradation following NGF deprivation is attenuated by either *Kif2a* ablation or paclitaxel. Tau levels were evaluated by western blot using Tau5 and PHF antibodies. In the presence of 10 μM paclitaxel (N), or the genetic ablation of *Kif2a* (M), the degradation of Tau was attenuated.

(O–Q) *Kif2a* ablation protects MTs from degradation after axotomy. DRG explants were cultured for 48 hr in NGF-containing medium. These explants axons were transected and immunostained for tubulin βIII at 8 hr after the transaction (O and P). Upon transection, the MTs undergo breakdown, as can be seen by the punctuated form of the MTs (O). While in the *Kif2a* KO, MTs were protected and only a small fraction was degraded 8 hr after transaction (P). Quantification of a MTs depolymerization average index value ± SD was calculated for each condition and genotype (P), Mann-Whitney test, ***$p < 0.001$.

(R–X) *Kif2a* ablation delays axonal degeneration. DRG explants from WT (R–T) and *Kif2a* KO (U–W) embryos were cultured, treated as described above, and imaged by phase microscopy. Axonal degeneration average index value ± SEM was calculated from each condition and genotype (X), Mann-Whitney test, ***$p < 0.001$. Scale bar, 100 μM.

See also Figures S4 and S5.

its PTM levels were preserved only to a small extent (Figure S5). Finally, the overall axonal structure was preserved as observed by phase microscopy in the *Kif2a* KO axons 24 hr after trophic withdrawal and, to a small degree, 8 hr after axotomy (Figures 3R–3X). In summary, these results demonstrate that, upon trophic withdrawal and (to a lesser extent) after axotomy, KIF2A plays a key role in MT depolymerization and axonal degeneration.

KO of *Kif2a* Causes Skin Hyperinnervation

Gain- and loss-of-function studies have established skin-derived NGF as a key regulator of innervation by sensory axons during development (Albers et al., 1994; Patel et al., 2000; Wickramasinghe et al., 2008). Therefore, we postulated that the resistance of Kif2a KO sensory axons to NGF deprivation would lead to hyperinnervation of the skin by these axons. To test this hypothesis, we stained sections of E15.5 embryos, *Kif2a* KO, and

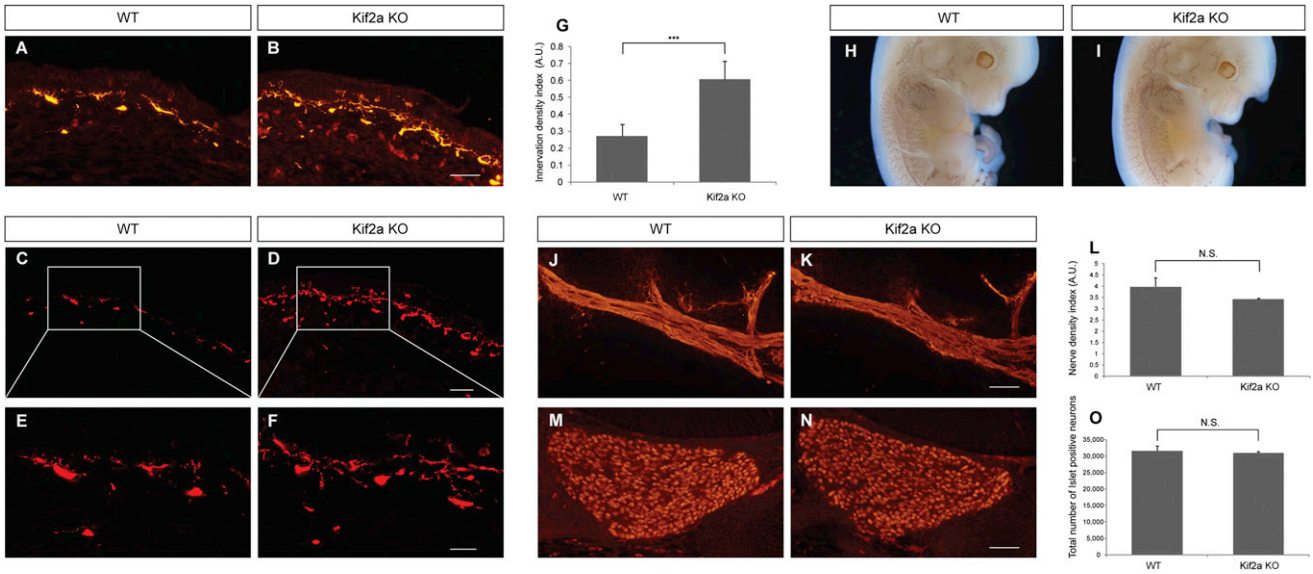

Figure 4. *Kif2a* KO Mice Exhibit Developmental Skin Hyperinnervation by Sensory Axons

(A–G) Paraffin sections of E15.5 embryos. Shown here are peripheral axons adjacent to the skin labeled with antitubulin βIII antibody (A and B) and confocal analysis of the same sections (C and E) of (A) and (D and F) of (B). Hyperinnervation of the sensory axons was detected in *Kif2a* KO (B, D, and F) when compared to their WT littermate embryos (A, C, and E). Innervation density index value ± SD was calculated from 180 sections of four embryos from each genotype (G), Mann-Whitney test, ***$p < 0.001$.

(H–L) The peripheral nervous system pattern in the *Kif2a* KO mice is normal early during development. As can be detected in whole-mount anti-NF analysis of E12.5 WT (H) and *Kif2a* KO (I) and in the section of nerves, labeled with tubulin βIII antibody, exiting the DRG at E12.5 (J and K). Nerve density index value ± SD was calculated from 360 sections of four embryos from each genotype (L), N.S., nonsignificant.

(M–O) The number of sensory neurons in the DRGs does not differ in the *Kif2a* KO mice, as detected by islet1 antibody, in E15.5 WT (M) and *Kif2a* KO (N). Total Islet1-positive neurons ± SD was calculated from 110 sections of three embryos of each genotype (O). N.S., nonsignificant.

Scale bars, 50 μM (A–D, J, K, M, and N) and 20 μM (E and F).

wild-type (WT) littermates and examined the innervation of the skin in the ventral body surface. Significant enhancement was detected in the amount of axons innervating the skin in the *Kif2a* KO embryos (Figures 4A–4G). The pattern of cutaneous sensory axons early during development at E12.5 is NGF independent (Davies et al., 1987; Lumsden and Davies, 1983; Wickramasinghe et al., 2008). Indeed, we did not detect any abnormalities in the growth, branching, or nerve density of these axons in the *Kif2a* KO embryos at E12.5 (Figures 4H–4L). Moreover, the number of sensory neurons in the DRG at E15.5 (when the skin hyperinnervation is observed) is identical between WT and *Kif2a* KO (Figures 4M–4O). Thus, *Kif2a* KO enables sensory axons to hyperinnervate the skin between E12.5 and E15.5 in the presence of limited trophic support.

DISCUSSION

Microtubule breakdown is a hallmark of axonal degeneration, both during development and after injury (Watts et al., 2003; Zhai et al., 2003). However, the mechanism of MT breakdown during the degeneration process has yet to be elucidated. Here, we show that MT depolymerization is an active process that is regulated by a specific disassembly protein, KIF2A. Whether other severing proteins control MT disassembly during axonal pruning remains an open question. Studies in the fly did not reveal, so far, a role for Stathmin, Katanin, or Spastin in neurite pruning and degeneration (Graf et al., 2011; Lee et al., 2009; Stone et al., 2012; Tao and Rolls, 2011). Interestingly, it was recently suggested that stathmin2/SCG10 delays axonal degeneration upon axotomy (Shin et al., 2012).

How is KIF2A regulated during axonal degeneration? We did not observe any elevation in KIF2A protein levels, suggesting that it is not regulated at the transcriptional or translational level upon trophic withdrawal (Figure S3). In principle, KIF2A might be constitutively active, and simple elimination of MAPs would then enable its action. However, our results argue against this model, as in such case axonal degeneration should have been observed by knockdown of Tau or Crmp2. Instead, we propose that signaling events that initiate the degeneration process stimulate the activity of KIF2A. In agreement, several reports have shown that KIF2A activity is controlled by phosphorylation (Jang et al., 2009; Mennella et al., 2009; Noda et al., 2012). Therefore, kinases that regulate the axonal self-destruction program may activate KIF2A.

Previous studies and our work suggest that the cell employs parallel degeneration pathways to disassemble the axonal cytoskeleton. The calpain inhibitor ALLN or inhibition of the IκB kinase (IKK) specifically prevent NF degradation (Gerdts et al., 2011; Zhai et al., 2003). In contrast, paclitaxel treatment and *Kif2a* ablation were found to protect MTs. However, it seems that some level of coregulation of these pathways does exist as protecting the MTs very partially preserves the NFs after

NGF withdrawal. More importantly, we observed that axonal structure is largely preserved after trophic deprivation in *Kif2a* KO neurons. This suggests that although the cell employs several parallel pathways to eliminate cytoskeletal and regulatory proteins, depolymerization of MTs is a critical event that regulates other destruction pathways. This may also explain why depolymerization of MTs but not actin or NFs elimination induces axonal degeneration (Walker et al., 2001; Zhai et al., 2003).

The neurotrophic hypothesis argues that proper skin innervation by sensory axons is achieved through competition for a limited amount of NGF (Davies, 1996; Harper and Davies, 1990). Our results suggest that axonal pruning by KIF2A is an important part of this process. The hyperinnervation we detect in the *Kif2a* KO mice is not due to interference with the apoptotic machinery, which is not impaired in these neurons, as the number of DRG neurons in the *Kif2a* KO is indistinguishable from the number in the WT. Moreover, the NGF-independent initial growth and pattern of sensory axons is normal early in development, suggesting that there is no general alteration of these axons as was previously observed in the CNS (Homma et al., 2003). Therefore, *Kif2a* is a pruning factor that regulates target innervation by sensory axons. Whether *Kif2a* controls axonal pruning in the CNS or pathological degeneration of the nervous system remains to be discovered.

EXPERIMENTAL PROCEDURES

Antibodies

Antibodies and dilutions used for immunofluorescence staining: tubulin β-III (Covance, MRB-435P, 1:1,000), cleaved caspase-3 (Cell Signaling, 9664, 1:100). Anti-mouse or anti-rabbit antibodies conjugated with either Alexa 549 or Alexa 488 fluorophores were used at 1:200 (Jackson ImmunoResearch Laboratories). For immunoblotting, the following antibodies and dilutions were used: tubulin βIII (1:2000), tubulin α (Millipore, 05-829, 1:1,000), tubulin detyrosinated (Millipore, AB3201, 1:1,000), tubulin acetylated (Convance, MMS-413R, 1:1,000), tubulin glutamylated: GT335 and PolyE (kindly provided by Prof. C. Janke, CRBM, 1:2,000). Tau antibodies: Tau5 and PHF1 (kindly provided by Dr. E. Elliott, produced at the Lab of Prof. I. Grinzburg, Weizmann Institute of Science, Israel, 1:1,000), NF (2H3) and Islet1 (Developmental Studies Hybridoma Bank, 1:10 and 1:1,000, respectively), actin (MP Biomedicals, 691001, 1:5,000), STMN1 (Abcam, ab52906, 1:1,000), STMN2 (kindly provided by Prof. E Coffey, Turku University, 1:1,000), and KIF2A (Abcam, ab37005, 1:500). Immunoblots were developed using horseradish-peroxidase-labeled donkey anti-rabbit or anti-mouse IgG followed by detection with chemiluminescence.

Mouse Strains

The *Kif2a* KO mouse strain was previously described (Homma et al., 2003). All of the animal experiments were done according to the protocols that were approved by the Weizmann Institute of Science Institutional Animal Care and Use Committee.

Explant Culture

Dorsal root ganglion (DRG) explants of E13.5 mice were aseptically removed and cultured on poly-d-lysine (PDL)-laminin-coated plates. The explants were grown in neurobasal (NB) medium supplemented with 2% B-27, 1% glutamine, 1% penicillin-streptomycin, and 25 ng/ml mNGF 2.5S (Alomone Labs; N-100) for 48 hr before treatments. For NGF deprivation, the medium was exchanged for medium lacking NGF with addition of 0.1 μg/ml rabbit anti-NGF neutralizing antibodies (Alomone Labs; AN-240). Other conditions required the addition of 10 μM paclitaxel (Sigma-Aldrich; T7191).

DRG-Dissociated Neurons

DRGs from E13.5 mice were aseptically removed and pelleted in Hank's balanced salt solution (Biological Industries, Beit Haemek, Israel) for 10 min and dissociated with 5% trypsin 37°C for 2 min. The trypsin was neutralized with 10 ml L15 medium supplemented with 5% fetal calf serum. The cells were then centrifuged at 2,800 rpm 21°C for 4 min and resuspended in NB medium supplemented with B-27, glutamine, and 12.5 ng/ml NGF. The dissociated cells were cultured onto 18 mm PDL-laminin-coated coverslips in 12-well plates.

siRNA Treatment

siRNA oligonucleotide sequences were used to target the following proteins: *Stmn1*, *Stmn2*, *Stmn3*, *Stmn4*, *Katanin*, *Spastin*, and *Kif2a* (Dharmacon, ON-TARGETplus SMARTpool). For negative control, a nontarget sequence was used (Dharmacon, ON-TARGETplus Non-Targeting Pool, D-001810). DRG-dissociated cells were transfected with siRNA, using the protocol supplied with DharmaFECT 4 (Dharmacon, T-2004-03). Briefly, siRNA and the transfection reagent were each diluted separately into NB medium without serum and antibiotics for 5 min; then, the siRNA was added to the medium with the transfection reagent. After an additional 20 min incubation, the transfection reagent-siRNA complex was added to the dissociated cells, and grown in NB medium containing 25 ng/ml NGF, without serum and antibiotics. Fifteen hours later, the transfection reagent was removed by replacing the medium with a complete medium and the neurons were cultured for an additional 48 hr. NGF deprivation was performed as described above. The final concentration of the siRNA was 0.1 μM. The level of the target protein, reduced by its specific siRNA treatment, was evaluated by western blot analysis.

Axonal Caspase Activity Assay

Axonal caspase activity assay was preformed as described in Schoenmann et al. (2010).

Immunohistochemistry of Mouse Embryos

E15.5 or E12.5 embryos were fixed for 24 hr in 4% formaldehyde. The following day, the embryos were embedded in paraffin, and 4 μm transversal sections were taken. The slides then underwent deparaffinization with xylene and ethanol. Antigen retrieval was performed in a pressure cooker in a sodium citrate buffer at 125°C. The slides were stained with tubulin β-III antibody for axon visualization and with anti-islet1 for sensory neuron detection.

Whole-Mount Staining

E12.5 mouse embryos (*Kif2a* KO and WT littermates) were stained as described (Yaron et al., 2005).

Axonal Quantification In Vitro and In Vivo

All the data were quantified using computerized algorithms; for details, please see Extended Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.03.005.

LICENSING INFORMATION

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