Current Biology, Vol. 15, 2050–2055, November 22, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.10.052

Gigaxonin Interacts with Tubulin Folding Cofactor B and Controls Its Degradation through the Ubiquitin-Proteasome Pathway

Wei Wang,[1] Jianqing Ding,[1] Elizabeth Allen,[1] Ping Zhu,[2] Lan Zhang,[2] Hannes Vogel,[3] and Yanmin Yang[1,*]

[1] Department of Neurology and Neurological Sciences
Stanford University
1201 Welch Road, MSLS P207
Stanford, California 94305

[2] Department of Cell Biology
Shanghai Second Medical University
280 South Chongqing Road
Shanghai 200025
People's Republic of China

[3] Department of Pathology
Stanford University
300 Pasteur Drive, Medical Center R241
Stanford, California 94305

Summary

Gigaxonin is mutated in human giant axonal neuropathy (GAN), an autosomal recessive neurodegenerative disorder. The presence of generalized cytoskeletal abnormalities [1, 2], including few microtubules and accumulated intermediate filaments (IFs), in GAN suggests an essential role of gigaxonin in cytoskeletal organization and dynamics. However, the molecular mechanisms underlying the cytoskeletal pathology remain to be elucidated. Over the years, the ubiquitin-proteasome system (UPS) of intracellular protein degradation has been implicated in the control of many fundamental cellular processes. Defects in this system seem to be directly linked to the development of human diseases, including cancers and neurodegenerative diseases [3, 4]. Here, we show that gigaxonin controls protein degradation of tubulin folding cofactor B (TBCB) [5], a function disrupted by GAN-associated mutations. The substantial TBCB protein accumulation caused by impaired UPS may be a causative factor of cytoskeletal pathology in GAN. Our study provides important insight into pathogenesis of neurodegenerative diseases associated with cytoskeletal abnormalities.

Results and Discussion

Gigaxonin Binds to Tubulin Folding Factor B

Gigaxonin lacks direct binding sites for the cytoskeletal network [6]. We have previously identified the light chain of microtubule-associated protein (MAB-1B-LC) as a neuronal specific binding partner of gigaxonin [6]. However, because of its restricted expression pattern, disruption of the gigaxonin-MAP1B-LC interaction could not account for the generalized cytoskeletal abnormality. The multiple tissue involvement of GAN pathology led us to predict that a widely expressed partner of gigaxonin may exist. In the initial yeast two-hybrid screen, tubulin folding co-factor B (TBCB, amino acids 32–244) was identified as another interacting factor of gigaxonin (Figure 1A). Unlike MAP1B, TBCB is a ubiquitously expressed tubulin chaperone protein and binds to α-tubulin, a microtubule building unit [5, 7, 8]. The specific interaction was confirmed with pulldown assays, revealing that the kelch-repeat domain of gigaxonin contains the site responsible for the interaction with the N-terminal segment of TBCB (Figure 1B, lanes 3 and 5). Furthermore, in COS7 cells that express no detectable gigaxonin [6], the wild-type gigaxonin (Flag-Gig), but not the truncated mutant form (Flag-GigR293X), could coimmunoprecipitate endogenous protein of TBCB (Figure 1C) detected by TBCB specific antibody (Figure S1 in the Supplemental Data available with this article online). To further assess how the GAN-associated mutations affect the specific interaction, we tested four additional mutant gigaxonin, 18insA, R15S, V82F, R545C, and R293X (see diagram in Figure S2) [9], in transfected cells. The expression of the 18insA mutant is barely detectable. Although the BTB domain mutants, R15S and V82F, display no disruptive effect on the specific interaction (Figure 1D, lanes 2 and 3), the mutations in the kelch-repeat domain, R293X (nonsense mutation) and R545C (point mutation), resulted in a complete loss of the binding activity with TBCB in cotransfected cells (Figure 1D, lanes 4 and 5), demonstrating that an effective interaction with TBCB requires a functional kelch-repeat domain. Out of 24 distinct mutations identified in human GAN patients [9–12], 12 nonsense mutations that cause complete or partial loss of the kelch repeats and six point mutations in the kelch-repeat domain may consequently lead to disruption of the gigaxonin-TBCB interaction.

Gigaxonin Controls TBCB Degradation via the Ubiquitin-Proteasome Pathway

GAN is postulated to be a disorder of the functional loss of gigaxonin [13]. To understand the pathological effect caused by the disrupted gigaxonin-TBCB interaction, we first examined TBCB expression in the *gan* null mice (a manuscript on the gene-targeting strategy and KO characterization is in process elsewhere). The samples of total mouse-brain lysates were analyzed by sequential immunoblotting with antibodies to gigaxonin, β-tubulin, α-tubulin, TBCB, and actin. As judged by three independent assays, the absence of gigaxonin led to a substantially elevated level of TBCB protein in the null animals (Figure 2A). A modest increase of α- and β-tubulin was also observed in comparison to the unchanged actin control (Figure 2A). In contrast to the elevated protein level, RT-PCR results revealed a moderate downregulation of TBCB transcription in comparison to α-tubulin, GAPDH, and actin in the null samples (Figure 2B). The quantitative real-time PCR has further confirmed this observation (Figure 2C). Thus, the increased level of TBCB was rather due to protein accumulation.

Gigaxonin is distantly related to the evolutionarily conserved BTB/kelch superfamily [9, 13] that may be involved in the ubiquitin-proteasome-dependent protein

*Correspondence: yanmin.yang@stanford.edu

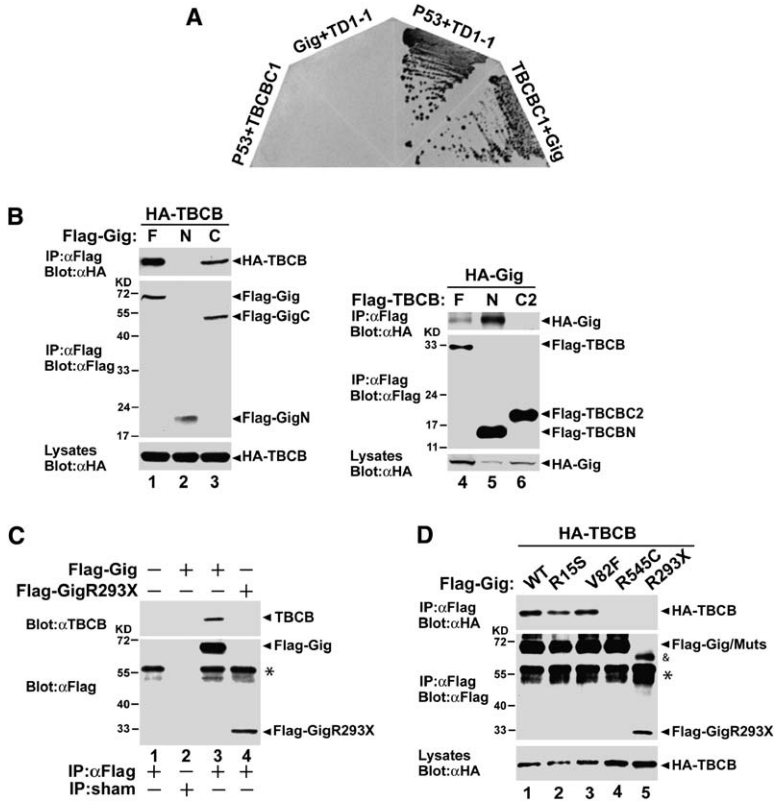

Figure 1. The Kelch-Repeat Domain of Gigaxonin Binds to the N Terminus of TBCB

(A) pGBKT7/gigaxonin (Gig, amino acids 11–584) binds pGADT7/TBCBC1 (amino acids 32–244) in the yeast two-hybrid screen. pGBKT7/P53 with pGADT7/TD1-1: positive control. pGBKT7/P53 with pGADT7/TBCBC1 and pGBKT7/gigaxonin with pGADT7/TD1-1: negative controls.

(B) HA-TBCB was cotransfected in COS7 cells with either the full-length ([F], lane 1) or N-terminal BTB domain ([N], lane 2) or with the C-terminal kelch-repeat domain ([C], lane 3) of Flag-tagged gigaxonin. Conversely, HA-tagged Gigaxonin was cotransfected with full-length ([F], lane 4), N-terminal ([N], lane 5), and C-terminal ([C2], lane 6) Flag-tagged TBCB. The TBCB-gigaxonin association was examined by co-IPs with anti-Flag M2, followed by immunoblottings either with an anti-HA (upper panels) or with anti-Flag (middle panels). The expression of HA-tagged proteins was also confirmed (bottom panels).

(C) Transfected wild-type Flag-gigaxonin (upper panel, lane 3), but not the mutant R293X (upper panel, lane 4), co-immunoprecipitates endogenous TBCB from COS7 cells. Lanes 1 and 2 are the controls from untransfected cell lysates or sham IPs, respectively. The blot was also probed with anti-Flag to confirm the expression of Flag proteins (lower panel).

(D) The HA-TBCB was cotransfected with wild-type Flag-gigaxonin (WT) or the mutants (R15S, V82F, R545C, or R293X). The TBCB bound gigaxonin was co-IPed with anti-Flag M2 and detected by WB with anti-HA (top panel). The middle and bottom panels were control blots of anti-Flag and anti-HA, respectively. An asterisk indicates some non-specific reactions, and an ampersand indicates the dimerization of the BTB domain.

degradation [14–18]. The accumulation of TBCB protein in the gigaxonin's null mice prompted us to investigate a potential role of gigaxonin in substrate-specific protein degradation. HA-tagged gigaxonin and Flag-tagged TBCB were ectopically coexpressed in COS7 cells. Indeed, in cells transfected with a fixed amount of Flag-TBCB and varying amounts of HA-gigaxonin, the increase of HA-gigaxonin expression was accompanied by a decrease of Flag-TBCB (Figure 2D, left panel). In contrast, TBCA, another tubulin chaperone protein, displayed no such reduction (Figure 2D, right panel). These results suggest that gigaxonin specifically controls TBCB's degradation. To further evaluate TBCB's stability, we employed the strategy of suppressing protein translation by utilizing cycloheximide (CHX) to study TBCB's degradation. As determined by three independent experiments, only half of the ectopic HA-TBCB remained less than 2 hr after the addition of CHX in the presence of Flag-gigaxonin (Figure 2E, lanes 1–5), in contrast to TBCB's marked stability in gigaxonin's absence (Figure 2E, lane 6), supplying additional evidence for its role in TBCB's degradation. Likewise, the overexpression of gigaxonin also significantly reduces in vivo TBCB levels (Figure 2F, lane 2), which could be reversed by applications of proteasome inhibitor (PI) (Figure 2F, lane 4). Take together, our results present gigaxonin as an important player in controlling TBCB protein stability, and the fact that PI reverses TBCB turnover implicates the involvement of the ubiquitin-proteasome system (UPS).

Recombinant gigaxonin was found in immunoprecipitated complex with Cul-3 [16]. If TBCB is a gigaxonin-mediated UPS substrate, TBCB is expected to become covalently conjugated with poly-ubiquitin when gigaxonin is present. To test this hypothesis, we cotransfected Myc-tagged ubiquitin with Flag-TBCB. In the protein complex concentrated by immunoprecipitation with anti-Flag-TBCB, TBCB formed a high-molecular-mass complex consistent with the conjugation of poly-ubiquitin tags in the presence of gigaxonin (Figure 3A, lane 4), and the poly-ubiquitin conjugation of TBCB was dramatically enhanced with PI addition (Figure 3A, lane 5). Similarly, the endogenous TBCB also displayed characteristic high molecular shifts of TBCB in the presence of HA-gigaxonin and PI (Figure 3B, lane 2). In contrast, without gigaxonin, TBCB exhibited no evidence of poly-ubiquitination even when an excess of ubiquitin and PI was supplied (Figure 3B, lanes 1). Moreover, the GAN-associated mutations, V82F, R293X, and R545C, disrupted gigaxonin's function in promoting TBCB's ubiquitination (Figure 3A, lanes 8–10). The R15S mutant, however, functioned similarly to wild-type gigaxonin (Figure 3A, lane 7). Interestingly, although the V82F mutant retained binding activity to TBCB, it failed to

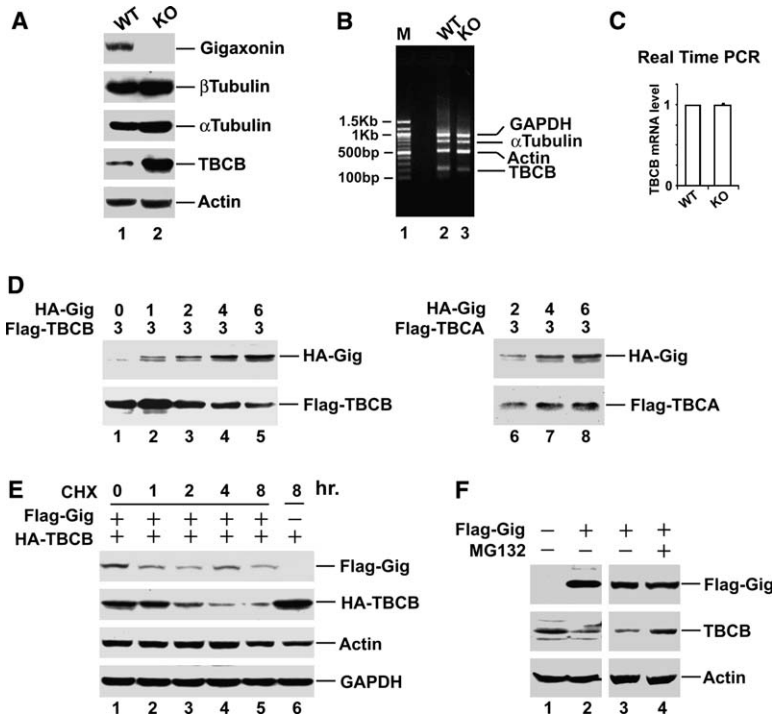

Figure 2. Gigaxonin Controls Protein Degradation of TBCB

(A) Brain lysates (25 μg) from wild-type (WT, lane 1) or gigaxonin-knockout (KO, lane 2) mice were analyzed with antibodies to, sequentially, gigaxonin, β-tubulin, α-tubulin, TBCB, and actin.

(B) RT-PCRs were conducted on mRNAs of WT or KO with multiple primer pairs to TBCB (215 bp), actin (550 bp), α-tubulin (800 bp), and GAPDH (1 Kb).

(C) Real-time PCR from three independent trials confirmed that the TBCB mRNA level remains unchanged in the KO samples. Error bars represent means ± S.D.; $p > 0.05$ with Student's t test.

(D) Increased gigaxonin expression correlates with a decreased level of TBCB. A fixed amount of Flag-TBCB or TBCA (0.75 μg) and an increasing amount of HA-gigaxonin were used (0.25, 0.5, 1, or 1.5 μg for lanes 2–5; 0.5, 1, or 1.5 μg for lanes 6–8). Lane 1: TBCB only.

(E) Stability assays of HA-TBCB. The cotransfections were treated with cyclohexamide (CHX) at 6×10^{-3} mg/ml for 0–8 hr (lanes 1–5). Note the stability of TBCB protein in gigaxonin's absence (lane 6).

(F) Gigaxonin overexpression reduces endogenous TBCB levels (compare lanes 2 and 3 with lane 1). PI addition reversed the effect (compare lane 4 with lanes 2 and 3).

promote TBCB poly-ubiquitination (Figure 3A, lane 8), suggesting that gigaxonin may have additional activities required in controlling TBCB's ubiquitination. These results uncovered the fact that in contrast to its mutant counterpart, gigaxonin is required in linking poly-ubiquitin tags to TBCB. To obtain additional in vivo evidence, we compared the ubiquitination of TBCB in the presence or the absence of gigaxonin in cultured wild-type or knockout (KO) neurons. When Flag-TBCB was transfected for 3 days, overexpressed Flag-TBCB in wild-type neurons apparently activated the endogenous UPS and became costained with ubiquitins (Figures S3A–S3D). In the gigaxonin null neurons, the substantially accumulated Flag-TBCB was negative for ubiquitin staining (Figures S3E–S3H), further supporting the notion that TBCB's ubiquitination requires functional gigaxonin. It is possible that mutations in gigaxonin could cause substrate overstabilization, leading to deleterious consequences.

Overexpressing TBCB Could Recapitulate the Microtubule Pathology in GAN

GAN is featured pathologically by generalized cytoskeletal abnormalities, including few microtubules [2]. Aberrations in the cytoskeletal network are often observed prior to other signs of severe neurodegeneration. The microtubule pathology in human GAN was also confirmed in the pre-phenotypic *gan* null animals. Regardless of

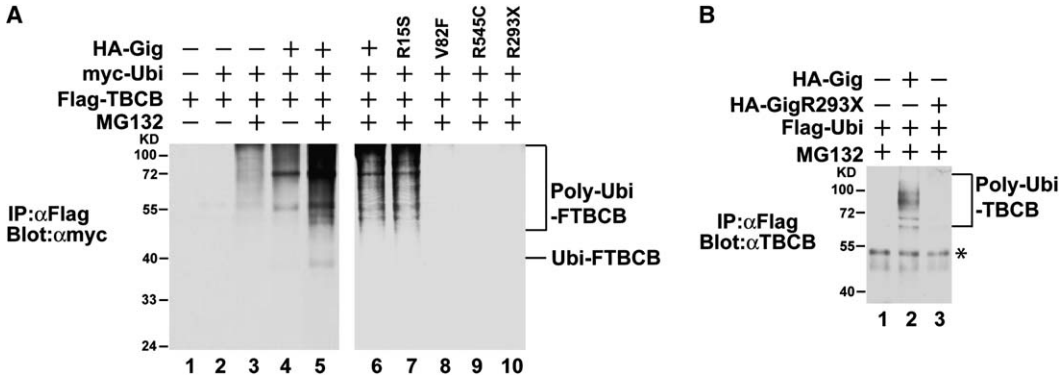

Figure 3. Gigaxonin Is Required for TBCB's Ubiquitination

(A) The cotransfected cells were subjected to co-IPs with anti-Flag (Flag-TBCB) and immunoblotted with anti-Myc (Myc-Ubiquitin) to detect Flag-TBCB ubiquitination. Note: wild-type gigaxonin and R15S promoted TBCB's ubiquitinations (lanes 4–7), but other mutants did not (lanes 8–10). (B) Poly-ubiquitin conjugations of endogenous TBCB in the presence of PI (MG132) and gigaxonin (lane 2). Note: without functional gigaxonin, TBCB shows no poly-ubiquitinations (lanes 1 and 3). An asterisk indicates some non-specific background reactions.

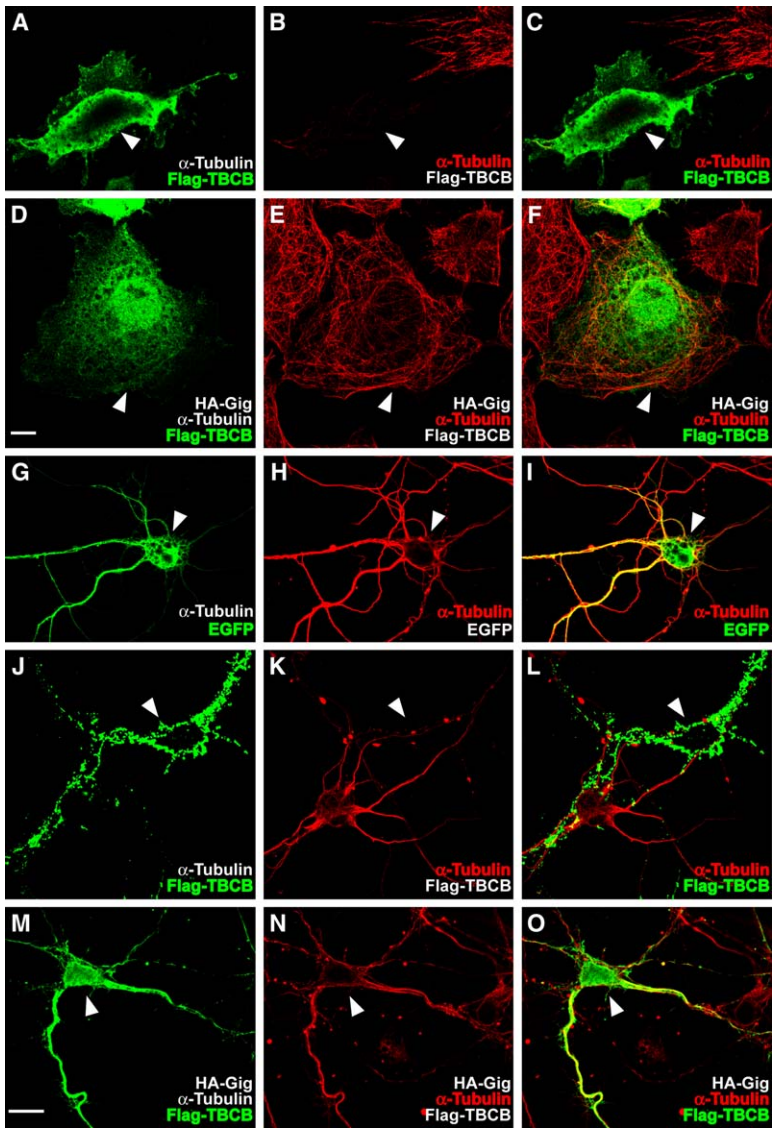

Figure 4. Overexpressing TBCB Recapitulates Microtubule Pathology in Cells

(A–F) COS7 cells were transfected with Flag-TBCB (A–C) or cotransfected with Flag-TBCB and HA-gigaxonin (HA-Gig) (D–F) and were then costained with antibodies to tubulin and Flag. (G–O) wild-type cortical neurons transfected with either GFP (G–I) or Flag-TBCB (J–L) or cotransfected with HA-Gig and Flag-TBCB (M–O) were processed for double immunofluorescence microscopy with GFP and mouse anti-tubulin (G–I) or rabbit anti-Flag and mouse anti-tubulin (J–O). Arrowheads point to the transfected cells. The scale bar represents 30 μm in all panels.

neurofilament density or myelination, the common and consistent feature found in all areas is a significant reduction of microtubules (Figures S4C and S4F). The observations at the EM level were further sustained by immunostaining on cultured neurons, revealing that microtubule staining in the null neurons was notably weakened (Figures S5D–S5F), in comparison with that in the wild-type controls (Figures S5A–S5C). Quantitative analysis in the null animals confirmed the microtubule pathology, revealing an average decrease of approximately 51% of microtubule density both in and outside of the nervous system at postnatal weeks 20–26.

TBCB is one of the five tubulin folding cofactors (TBCA, TBCB, TBCC, TBCD, and TBCE) that coordinately control functional heterodimerizations of α/β-tubulin to form the microtubule network [5, 8, 19]. Recently, a direct involvement of tubulin chaperones in human diseases of protein folding has become increasingly evident [20–23]. It has been demonstrated that overexpression of various tubulin folding cofactors destabilizes microtubules [19, 24, 25]. To understand the pathogenic contributions by TBCB protein accumulation to the substantial reduction of microtubules in *gan* null mice, we first manipulated COS7 cells by overexpressing TBCB and thus forcing its cellular accumulation. We found a dramatic decrease in the intensity of microtubule staining in the transfected cells by using antibodies to α-tubulin (Figures 4A–4C) or β tubulin (not shown) after 48 hr transfection, and the disappearance of microtubules correlates with TBCB's accumulated level. The microtubule-network disruption could be restored when wild-type gigaxonin was coexpressed with TBCB in the cells (Figures 4D–4F). Consistent with the finding in vitro, only the coexpression of R15S mutant with TBCB displayed restoration of the microtubule network (Figures S6A–S6C), whereas V82F, R293X, and R545C mutants exhibited no rescuing effects (Figures S6D–S6L). The observed functional retaining of the R15S mutant implies that controlling TBCB's degradation is not gigaxonin's only function and that this mutation works by a different mechanism than the others. We next sought to further assess the correlation between accumulated TBCB and abnormal microtubules in cultured primary neurons. After the initial 4–5 days, the

cultured cortical neurons displayed abundant neurites. The neurons were then transiently transfected with TBCB or GFP for various numbers of days. Notably, it takes longer for Flag-TBCB protein to be accumulated in neurons than in COS7 cells. However, usually 3–4 days after transfection, the microtubules in more than two-thirds of the TBCB-overexpressing neurons became barely detectable (Figures 4J–4L), similar to the results in TBCB-overexpressing COS7 cells. Again, the microtubule staining could be restored by gigaxonin coexpression (Figures 4M–4O), further implicating the importance of gigaxonin in the maintenance of the microtubule network. As a control, GFP-overexpressing neurons retained their strong microtubule staining over the entire time course up to two weeks (Figures 4G–4I). By day 6–7 post-transfection, approximately 60% of the TBCB-overexpressing neurons began to lose their normal morphology, suggesting that disruption of the microtubule network precedes neurodegeneration or cell death. Our data add important evidence that accumulated TBCB is a microtubule-destabilizing factor and strongly suggest that accumulation of TBCB in cells may be a causative factor responsible for microtubule pathology in human GAN.

Conclusions

We have characterized the specific interaction of gigaxonin with TBCB and demonstrated that gigaxonin controls protein degradation of TBCB via the ubiquitin-proteasome pathway; this function is critical to the maintenance of the microtubule network. Gigaxonin is a distinct cytoskeleton regulatory protein that may represent a general pathological target for other neurodegenerative disorders with cytoskeleton alterations, as well as defects in UPS. Many questions, including how accumulated TBCB causes low microtubule density, remain to be addressed. In addition, future studies should elucidate whether the densely accumulated NFs in GAN are a compensatory response to the abnormal microtubule network or whether gigaxonin controls the stability of a yet-unknown partner that primarily involves in IF network.

Supplemental Data

Supplemental Data including six additional figures are available with this article online at http://www.current-biology.com/cgi/content/full/15/22/2050/DC1/.

Acknowledgments

We thank Dr. Susanna M. Grzeschik for technical advice on real-time PCR; we also thank other colleagues of the Yang Lab for scientific discussions. This work was supported by research grants from the Muscular Dystrophy Association and from the National Institutes of Health (NS42791 and NS43281) to Y.Y.

Received: June 13, 2005
Revised: October 5, 2005
Accepted: October 7, 2005
Published: November 21, 2005

References

1. Berg, B.O., Rosenberg, S.H., and Asbury, A.K. (1972). Giant axonal neuropathy. Pediatrics *49*, 894–899.
2. Taratuto, A.L., Sevlever, G., Saccoliti, M., Caceres, L., and Schultz, M. (1990). Giant axonal neuropathy (GAN): An immunohistochemical and ultrastructural study report of a Latin American case. Acta Neuropathol. (Berl.) *80*, 680–683.
3. Ciechanover, A., and Brundin, P. (2003). The ubiquitin proteasome system in neurodegenerative diseases: Sometimes the chicken, sometimes the egg. Neuron *40*, 427–446.
4. Sherr, C.J. (2004). Principles of tumor suppression. Cell *116*, 235–246.
5. Tian, G., Lewis, S.A., Feierbach, B., Stearns, T., Rommelaere, H., Ampe, C., and Cowan, N.J. (1997). Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. J. Cell Biol. *138*, 821–832.
6. Ding, J., Liu, J.J., Kowal, A.S., Nardine, T., Bhattacharya, P., Lee, A., and Yang, Y. (2002). Microtubule-associated protein 1B: A neuronal binding partner for gigaxonin. J. Cell Biol. *158*, 427–433.
7. Watanabe, T.K., Shimizu, F., Nagata, M., Kawai, A., Fujiwara, T., Nakamura, Y., Takahashi, E., and Hirai, Y. (1996). Cloning, expression, and mapping of CKAPI, which encodes a putative cytoskeleton-associated protein containing a CAP-GLY domain. Cytogenet. Cell Genet. *72*, 208–211.
8. Cowan, N.J., and Lewis, S.A. (1999). A chaperone with a hydrophilic surface. Nat. Struct. Biol. *6*, 990–991.
9. Bomont, P., Cavalier, L., Blondeau, F., Ben Hamida, C., Belal, S., Tazir, M., Demir, E., Topaloglu, H., Korinthenberg, R., Tuysuz, B., et al. (2000). The gene encoding gigaxonin, a new member of the cytoskeletal BTB/kelch repeat family, is mutated in giant axonal neuropathy. Nat. Genet. *26*, 370–374.
10. Kuhlenbaumer, G., Young, P., Oberwittler, C., Hunermund, G., Schirmacher, A., Domschke, K., Ringelstein, B., and Stogbauer, F. (2002). Giant axonal neuropathy (GAN): Case report and two novel mutations in the gigaxonin gene. Neurology *58*, 1273–1276.
11. Bomont, P., loos, C., Yalcinkaya, C., Korinthenberg, R., Vallat, J.M., Assami, S., Munnich, A., Chabrol, B., Kurlemann, G., Tazir, M., et al. (2003). Identification of seven novel mutations in the GAN gene. Hum. Mutat. *21*, 446–447.
12. Bruno, C., Bertini, E., Federico, A., Tonoli, E., Lispi, M.L., Cassandrini, D., Pedemonte, M., Santorelli, F.M., Filocamo, M., Dotti, M.T., et al. (2004). Clinical and molecular findings in patients with giant axonal neuropathy (GAN). Neurology *62*, 13–16.
13. Timmerman, V., De Jonghe, P., and Van Broeckhoven, C. (2000). Of giant axons and curly hair. Nat. Genet. *26*, 254–255.
14. Pintard, L., Willis, J.H., Willems, A., Johnson, J.L., Srayko, M., Kurz, T., Glaser, S., Mains, P.E., Tyers, M., Bowerman, B., et al. (2003). The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature *425*, 311–316.
15. Xu, L., Wei, Y., Reboul, J., Vaglio, P., Shin, T.H., Vidal, M., Elledge, S.J., and Harper, J.W. (2003). BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature *425*, 316–321.
16. Furukawa, M., He, Y.J., Borchers, C., and Xiong, Y. (2003). Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nat. Cell Biol. *5*, 1001–1007.
17. Geyer, R., Wee, S., Anderson, S., Yates, J., and Wolf, D.A. (2003). BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol. Cell *12*, 783–790.
18. Wilkins, A., Ping, Q., and Carpenter, C.L. (2004). RhoBTB2 is a substrate of the mammalian Cul3 ubiquitin ligase complex. Genes Dev. *18*, 856–861.
19. Radcliffe, P.A., Hirata, D., Vardy, L., and Toda, T. (1999). Functional dissection and hierarchy of tubulin-folding cofactor homologues in fission yeast. Mol. Biol. Cell *10*, 2987–3001.
20. Schwahn, U., Lenzner, S., Dong, J., Feil, S., Hinzmann, B., van Duijnhoven, G., Kirschner, R., Hemberger, M., Bergen, A.A., Rosenberg, T., et al. (1998). Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat. Genet. *19*, 327–332.
21. Martin, N., Jaubert, J., Gounon, P., Salido, E., Haase, G., Szatanik, M., and Guenet, J.L. (2002). A missense mutation in Tbce causes progressive motor neuronopathy in mice. Nat. Genet. *32*, 443–447.
22. Parvari, R., Hershkovitz, E., Grossman, N., Gorodischer, R., Loeys, B., Zecic, A., Mortier, G., Gregory, S., Sharony, R., Kambouris, M., et al. (2002). Mutation of TBCE causes

hypoparathyroidism-retardation-dysmorphism and autosomal recessive Kenny-Caffey syndrome. Nat. Genet. *32*, 448–452.

23. Bommel, H., Xie, G., Rossoll, W., Wiese, S., Jablonka, S., Boehm, T., and Sendtner, M. (2002). Missense mutation in the tubulin-specific chaperone E (Tbce) gene in the mouse mutant progressive motor neuronopathy, a model of human motoneuron disease. J. Cell Biol. *159*, 563–569.
24. Bhamidipati, A., Lewis, S.A., and Cowan, N.J. (2000). ADP ribosylation factor-like protein 2 (Arl2) regulates the interaction of tubulin-folding cofactor D with native tubulin. J. Cell Biol. *149*, 1087–1096.
25. Martin, L., Fanarraga, M.L., Aloria, K., and Zabala, J.C. (2000). Tubulin folding cofactor D is a microtubule destabilizing protein. FEBS Lett. *470*, 93–95.