TBA-7 is not a microtubule-destabilizing tubulin

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To the Editor:

Eukaryotic genomes contain multiple tubulin genes that code for different tubulin isotypes. Whether the incorporation of certain tubulin isotypes into the microtubules (MTs) can lead to the destabilization of MTs has been a long-standing question. In a previous paper that analyzed the effects of tubulin mutations on neurite growth in *Caenorhabditis elegans* touch receptor neurons (Zheng *et al.*, 2017), we reported that the α-tubulin TBA-7 may be such a MT-destabilizing tubulin because the loss of *tba-7* resulted in increased MT stability and excessive neurite growth. In light of our more recent data, however, we realized that the above conclusion is not warranted.

Previously, in a genetic screen (Zheng *et al.*, 2017), we isolated a mutant allele, *u1105*, that induced the growth of an ectopic neurite in the ALM neurons (we called this neurite ALM-PN for the posterior neurite of ALM). Whole genome sequencing of strain TU4696, *u1105; uIs134[mec-17p::TagRFP]*, found a missense mutation, G92D, in the α-tubulin gene *tba-7*. To confirm that *tba-7(G92D)* was the phenotype-causing mutation, we obtained a million-mutation project strain (VC40740) containing *tba-7(gk787939; Q230*)*, a presumptive null allele of *tba-7*. We crossed VC40740 with *uIs115[mec-17p::TagRFP]* to create the TU5198 strain and found the same extended ALM-PN phenotype. (Both *uIs115* and *uIs134* transgenes specifically labeled the touch receptor neurons.) We also performed a complementation test and found that TU4696 failed to complement with VC40740. Finally, we overexpressed *tba-7(+)* in the touch receptor neurons using the cell-specific *mec-17* promoter and observed the rescue of the ALM-PN phenotype. These results led us to conclude that *u1105* was a loss-of-function mutation of *tba-7* and that the inactivation of *tba-7* resulted in the growth of ectopic ALM-PN.

In a continuation of this research on *tba-7*, we created knockout alleles of *tba-7* through CRISPR/Cas9-mediated gene editing (see *Materials and Methods*) and found that the deletion of *tba-7* did not create the ALM-PN phenotype observed in TU4696 and TU5198. Neither of the two independently generated *tba-7* deletion alleles (*unk70* and *unk74*) resulted in a prominent ALM-PN (Figure 1). To test whether *u1105* and *gk787939* were gain-of-function alleles, we deleted the *tba-7* gene using CRISPR/Cas9 gene editing in the TU5198 strain. The resulted strain (CGZ549) still showed a long ALM-PN, suggesting that the ectopic neurite phenotype was not induced by the *tba-7(gk787939)* mutation. Finally, we used gene editing to introduce the G92D and Q230* mutations into the *tba-7* locus in the wild-type background but failed to produce the ALM-PN phenotype (*unk77* and *unk78* in Figure 1).

Being puzzled by the results, we decided to reanalyze the whole genome sequencing data of the TU4696 strain and found a *klp-7(E95K)* mutation closely linked to the *tba-7(G92D)* mutation. The genetic distance between *klp-7* (III:5.41) and *tba-7* (III:8.79) is 3.38 cM, making them difficult to separate during crossing. We also analyzed the sequence of VC40740 and found that it also contained a missense mutation in *klp-7*, named *gk787931(E334K)*. We then found that both *u1105* and the strain that contained *gk787939* failed to complement with the *klp-7(tm2143)* deletion allele in inducing the growth of ALM-PN. These results indicate that *u1105* should be mapped to *klp-7* and not *tba-7*. Thus, we have no evidence that the loss of *tba-7* causes any morphological defects in the touch receptor neurons; our interpretation of the previous *tba-7* results published in Zheng *et al.* (2017) is incorrect. All the phenotypes observed for *tba-7* are likely caused by the loss-of-function allele *klp-7(E95K)*.

Coincidently, this effect of *klp-7* was also reported in Zheng *et al.* (2017), which examined the *klp-7(tm2143)* allele. *klp-7* codes for a kinesin-13 protein that promotes the destabilization of MTs. So, the loss of *klp-7* is expected to increase MT stability and cause excessive neurite growth in the ALM neurons. It is worth noting that Kim *et al.* (2018) reported enhanced axonal regeneration in *tba-7* mutants using the *u1105* and *gk787939* alleles, but we believe that this enhanced regeneration phenotype is likely caused by mutations in *klp-7*, because the loss of *klp-7* is known to enhance axonal regeneration (Ghosh-Roy *et al.*, 2012).

We do not know why overexpression of *tba-7(+)* in *klp-7(E95K)* animals suppressed the ALM-PN phenotype but suspect that it may have triggered the down-regulation of other tubulins, such as *mec-7* and *mec-12*, by tubulin autoregulation (Lin *et al.*, 2020). Such down-regulation could suppress the ALM-PN growth, because we had already shown that the loss-of-function mutations in *mec-7* and *mec-12* suppressed the ALM-PN growth in *klp-7(tm2143)* mutants (Zheng *et al.*, 2017).

In summary, a rare incidence of secondary mutations in the same gene in two independently isolated strains misled us in previous studies. Recent data with the *tba-7* knockout alleles clearly indicate that the loss of *tba-7* does not induce increased MT stability and excessive neurite growth. Whether the *C. elegans* genome contains MT-destabilizing tubulins still remains unclear.

DOI:10.1091/mbc.E21-04-0157

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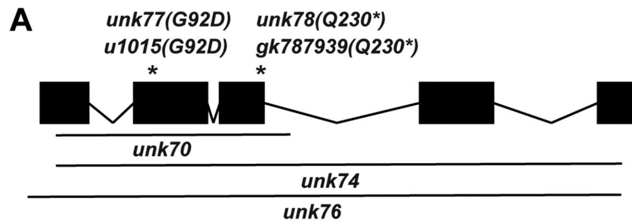

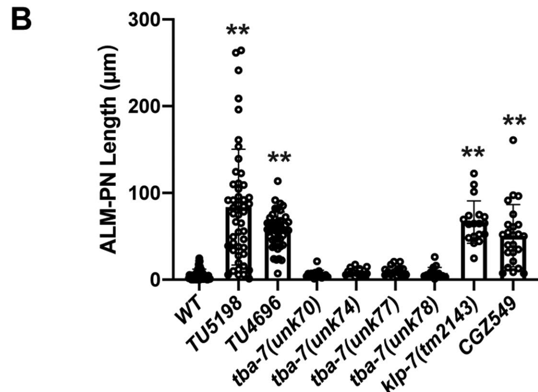

TU4696 ***tba-7(u1015; G92D) klp-7(E95K); uIs134***
TU5198 ***tba-7(gk787939; Q230*) klp-7(gk787931; E334K); uIs115***
CGZ549 ***tba-7(unk76) klp-7(gk787931; E334K); uIs115 IV;***

FIGURE 1: Deletion of *tba-7* did not induce ALM-PN growth. (A) Gene structure of *tba-7* and the position of various alleles. *u1015* and *gk7879393* were previously analyzed alleles. *unk70* and *unk74* are deletions alleles created in the wild-type background through CRISPR/Cas9 gene editing. *unk77* and *unk78* were missense mutations generated by CRISPR/Cas9 gene editing with repair donor ssDNA. Single asterisks indicate the position of the missense mutation in the gene; the lines indicate the deleted regions in the knockout aleles. (B) ALM-PN length in various strains. TU4686 and TU5198 carried closely linked *tba-7* and *klp-7* alleles. CGZ549 was generated by creating the *tba-7(unk76)* deletion allele in the TU5198 strain. Thus, the *tba-7(gk787939)* allele but not *klp-7(gk787931)* was removed in the resulting CGZ549 strain.

MATERIALS AND METHODS

CRISPR/Cas9 gene editing was performed according to a published protocol (Dokshin *et al.*, 2018). Recombinant spCas9 (catalogue #M0646T) was purchased from New England Biolabs (NEB), and single guide RNA (sgRNA) was synthesized using the sgRNA synthesis kit (catalogue #E3322S) from NEB. To generate *unk70*, which deleted exon 1-3 of *tba-7*, 5′-aagtaatctcaatccacgtg-3′ in exon 1 and 5′-cgatttatgatatctgccgg-3′ in exon 3 were chosen as the targets. To generate *unk74* and *unk76*, which deleted the whole *tba-7* gene, 5′-aagtaatctcaatccacgtg-3′ in exon 1 and 5′-ttccgatgctaatgataatg-3′ in exon 5 were chosen as the targets. Cas9 assembled with two different sgRNAs were injected together, and progeny with large deletions were screened for by PCR. For precise editing to generate *unk77(G92D)* and *unk78(Q230*)* in the wild-type background, 5′-gcaaatgctcactggcaaag-3′ and 5′-attatttctcaggtttggag-3′ were used as targets, respectively. Synthetic single-stranded oligodeoxynucleotide (ssODN) donors containing the desired mutations and 35-base-pair flanking sequences were used as the repair templates and injected together with the Cas9/sgRNA complex. We screened for successfully edited progeny by worm PCR using primers that bound specifically to the edited site. For each edited line, at least 25 adults were imaged for their ALM-PN length, and double asterisks (Figure 1B) indicate a significant difference in a two-way analysis of variance with Tukey's post hoc analysis.

ACKNOWLEDGMENTS

We thank Martin Chalfie at Columbia University for comments and advice. This work is supported by grants from the Research Grant Council of Hong Kong (ECS 27104219 to C.Z.) and the Food and Health Bureau of Hong Kong (HMRF 07183186 to C.Z.) and seed funds from the University of Hong Kong (201812159005 and 201910159087 to C.Z.).

REFERENCES

Dokshin GA, Ghanta KS, Piscopo KM, Mello CC (2018). Robust genome editing with short single-stranded and long, partially single-stranded DNA donors in *Caenorhabditis elegans*. Genetics 210, 781–787.

Ghosh-Roy A, Goncharov A, Jin Y, Chisholm AD (2012). Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. Dev Cell 23, 716–728.

Kim KW, Tang NH, Piggott CA, Andrusiak MG, Park S, Zhu M, Kurup N, Cherra SJ 3rd, Wu Z, Chisholm AD, Jin Y (2018). Expanded genetic screening in *Caenorhabditis elegans* identifies new regulators and an inhibitory role for NAD(+) in axon regeneration. eLife 7, e39756.

Lin Z, Gasic I, Chandrasekaran V, Peters N, Shao S, Mitchison TJ, Hegde RS (2020). TTC5 mediates autoregulation of tubulin via mRNA degradation. Science 367, 100–104.

Zheng C, Diaz-Cuadros M, Nguyen KCQ, Hall DH, Chalfie M (2017). Distinct effects of tubulin isotype mutations on neurite growth in *Caenorhabditis elegans*. Mol Biol Cell 28, 2786–2801.