

Tubulin isotype specificity in neuronal migration: Tuba8 can't fill in for Tuba1a

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Several tubulin isotypes, including Tuba1a, are associated with brain malformations. In this issue, Belvindrah et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201607074>) show that Tuba1a and Tuba8 differentially regulate microtubule organization in neurons, and they provide insights into the mechanisms by which *Tuba1a* mutations disrupt adult mouse brain morphology.

Microtubules regulate essential cellular events, such as cell migration, morphological changes, and cell division. Microtubules consist of laterally connected protofilaments that are constructed from polymerized α - and β -tubulin heterodimers. To date, at least eight α - and nine β -tubulin isotypes have been identified in humans. These tubulin isotypes are encoded by different genes with various cell- or tissue-specific expression. For example, tubulin- $\beta 3$ (Tubb3), an antigen for a Tuj1 monoclonal antibody, is widely used as a neuronal marker. Tubulin- $\alpha 1a$ (Tuba1a) is also predominantly expressed in neurons. Importantly, mutations in several tubulin isotypes, including Tuba1a, tubulin- $\beta 2b$ (Tubb2b), Tubb3, tubulin- $\beta 5$ (Tubb5), and γ -tubulin (Tubg1), result in brain malformation in humans (Bahi-Buisson et al., 2014). A previous study showed that a small deletion in *Tubulin- $\alpha 8$* (*Tuba8*) leads to polymicrogyria with optic nerve hypoplasia, but these patients have an additional homozygous loss-of-function mutation in the *SNAP29* gene, which may be the main cause of the polymicrogyria (Diggle et al., 2017). Tuba8-deficient mice do not exhibit abnormalities in brain morphogenesis (Diggle et al., 2017).

An important question is whether these tubulin isotypes, especially α -tubulins, have redundant or isotype-specific functions. The fact that only a subset of tubulin isotypes is associated with brain malformation may support the latter hypothesis: Tubulins have an isotype-specific function in brain morphogenesis. Nevertheless, we cannot exclude the former possibility, because loss of a tubulin isotype that is predominantly expressed in the brain may cause a reduction in the total amount of tubulin, which may disturb brain morphogenesis. To address this question, we need to understand how tubulin mutations result in brain malformation at molecular and cellular levels and what the structural and functional differences are between tubulin isotypes. In this issue, Belvindrah et al. provide detailed characterizations of a brain malformation-associated mutant of Tuba1a in microtubule organization and neuronal migration in

vivo and provide evidence that Tuba1a and Tuba8 differentially regulate microtubule straightness and polymerization speed in neurons (Belvindrah et al., 2017; Fig. 1). This work suggests that an isotype-specific function of Tuba1a is involved in neuronal migration and its related brain malformation.

Tuba1a was the first tubulin isotype to be associated with brain malformation (Keays et al., 2007). An *N*-ethyl-*N*-nitrosourea-mediated mouse mutagenesis screening revealed that a serine to glycine mutation at residue 140 (S140G) in the *Tuba1a* gene resulted in mild neuronal migration defects in the mouse developing cerebral cortex and hippocampus. In the same paper, two mutations, R402H and R264C, in *Tuba1a* (also known as *Tuba3*) were identified in human patients with lissencephaly, a neuronal migration-related neurological disorder (Keays et al., 2007). To date, many different mutations in *Tuba1a* associated with lissencephaly, microcephaly, or microlissencephaly have been reported (Bahi-Buisson et al., 2014; Chakraborti et al., 2016).

Although neuronal migration is essential for formation of a functional brain during development, it is also observed in the adult brain. The mouse subventricular zone of the lateral ventricles in the adult brain generates new interneurons, which migrate along the rostral migratory stream (RMS) toward the granule and periglomerular cell layers of the olfactory bulb (Lois and Alvarez-Buylla, 1994; Fig. 1 A). Belvindrah et al. (2017) found an abnormal accumulation of neurons in the RMS of Tuba1a S140G mutant mice. In addition, the glial tunnel through which neurons migrating toward the olfactory bulb pass appeared to be dispersed (albeit possibly in a non-cell-autonomous manner) in Tuba1a S140G mutant mice. Interestingly, the migration defect observed in the RMS was more severe than that in a developing cerebral cortex carrying the same *Tuba1a* S140G mutation.

Using *in vivo* electroporation, Belvindrah et al. (2017) showed that expression of Tuba1a S140G, but not WT Tuba1a, cell-autonomously retarded the migration of interneurons in the RMS of the postnatal brains. In addition to migration speed defects, migration directionality was perturbed in neurons expressing Tuba1a S140G, which may result from increased neurite branching, because branching rate correlated with altered migration directionality. Thus, the S140G mutation in Tuba1a may lead to abnormally increased neurite branching, which may result in loss of directionality and delayed migration of interneurons in postnatal brains.

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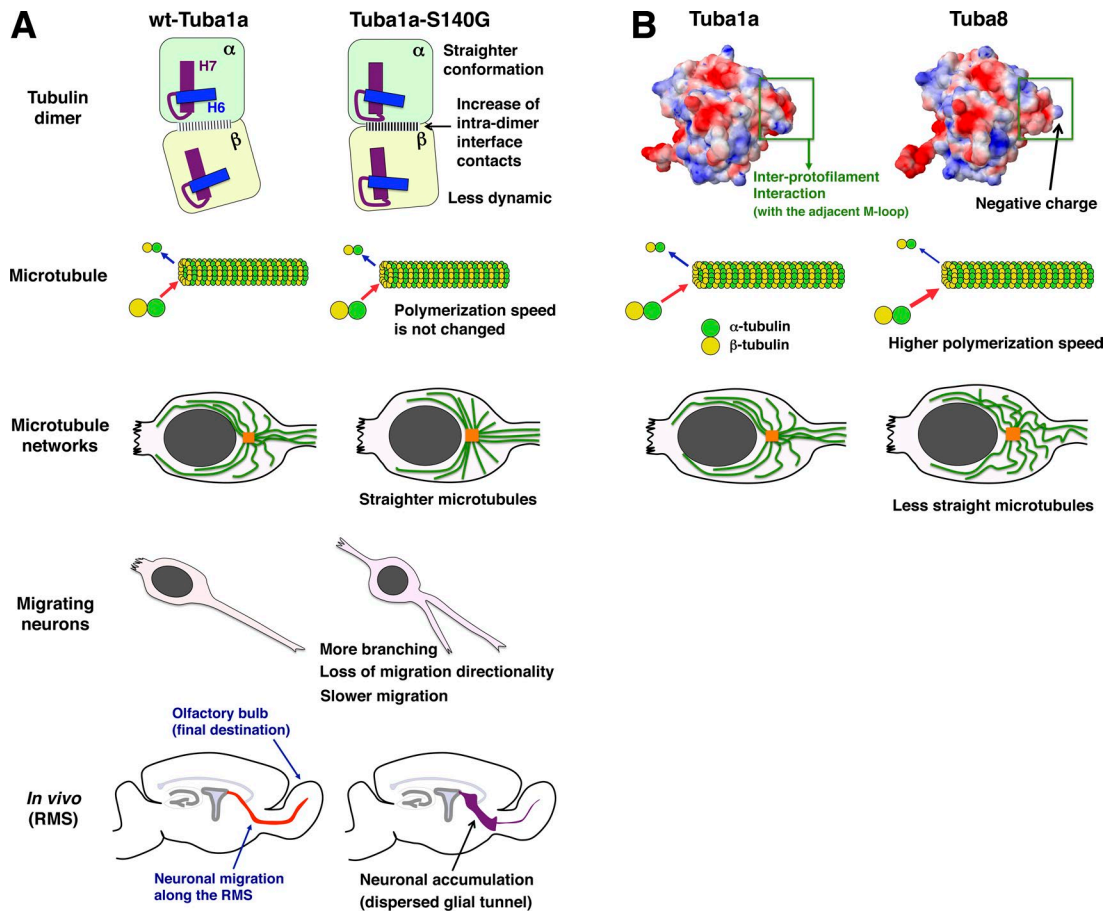


Figure 1. **Different features of α -tubulin isoforms and the brain malformation-related mutant.** (A) Tuba1a S140G prefers to exhibit straighter conformation, which is measured by the intradimer angle between the core helix H7 of α - and β -tubulins. The microtubules containing Tuba1a S140G show straighter morphologies, which may result in different neuronal behaviors: more branching, loss of migration directionality, and slower migration. (B) Tuba1a and Tuba8 show different charge distribution in the H1-S2 loop. Tuba8-containing microtubules exhibit higher polymerization speed and less straightness, compared with Tuba1a. The structural images with the charge distribution are adapted from Belvindrah et al. (2017).

The neurons migrating in the RMS are known to exhibit saltatory movement. Neurons extend a leading process toward the direction of migration into which the centrosome moves. Subsequently, the nucleus moves forward and the trailing process is retracted. This coordinated nucleus and centrosome movement (N–C coupling) mainly depends on microtubule organization and is thought to be important for proper neuronal migration in the developing cerebral cortex (Tanaka et al., 2004). Belvindrah et al. (2017) observed that neurons expressing Tuba1a S140G had increased maximum N–C distances and mean durations in the saltatory cycle, compared with WT Tuba1a-expressing control neurons, suggesting that the tubulin mutation slightly disturbs the N–C coupling, possibly because of microtubule abnormalities.

To understand the underlying molecular and cellular mechanisms of these *in vivo* or *ex vivo* phenotypes, Belvindrah et al. (2017) used Neuro-2a mouse neuroblastoma cells transfected with an EB3-mCherry vector to visualize the plus ends of growing microtubules. Tracking of microtubule plus ends revealed that the microtubule straightness was increased in the Tuba1a S140G-transfected neurons, whereas the microtubule polymerization speed was not significantly changed between WT Tuba1a and Tuba1a S140G-transfected neurons. However, it remains unclear whether increased neurite branching is a consequence of straighter microtubules in Tuba1a S140G-transfected neurons. As Belvindrah et al. (2017) discussed,

straighter (less flexible) microtubules are thought to be more breakable, and Spastin, a microtubule-severing protein that induces microtubule breakage, is known to enhance neurite branching. Future studies are required to confirm whether Tuba1a S140G-containing microtubules have increased instability and more easily undergo cleavage mediated by microtubule-severing proteins, thus increasing neurite branching.

The next question is why the point mutation at Ser140 in Tuba1a alters microtubule organization. Ser140 is located in the T4 loop, which interacts with GTP. A previous biochemical study by Keays et al. (2007) revealed that the S140G mutation reduces the efficiency of GTP incorporation and α/β -tubulin heterodimer formation *in vitro* (Keays et al., 2007). However, once folded, the Tuba1a S140G-containing tubulin heterodimers are able to assemble into microtubules, similar to WT Tuba1a-containing tubulins. By using structural modeling and molecular dynamics simulations, Belvindrah et al. (2017) confirmed that the S140G mutation in Tuba1a destabilizes the interaction with GTP. In addition, the authors analyzed the intradimer interface contacts between WT or Tuba1a S140G and β -tubulin and found that the total number of intradimer interactions was increased in the Tuba1a S140G-containing heterodimers. Interestingly, unlike WT Tuba1a, Asp98 on Tuba1a S140G interacted with Arg251 on β -tubulin, resulting in the formation of an additional salt-bridge triad. Thus, Tuba1a S140G has a lower efficiency

of GTP incorporation but forms additional intradimer interface contacts, which may explain the previous biochemical results reported by Keays et al. (2007).

Tubulin heterodimers exhibit curved and straight structures. Conformational transition from curved to straight is required for assembly into microtubules (Ravelli et al., 2004). Simulation studies by Belvindrah et al. (2017) revealed that Tuba1a S140G-containing tubulin heterodimers prefer a straighter conformation, whereas WT Tuba1a-containing heterodimers form a slightly curved conformation in the nonpolymerized state. The conformational flexibility was restricted in the Tuba1a S140G-containing tubulin heterodimers, compared with that of WT Tuba1a. These structural alterations of Tuba1a S140G might affect the polymerized microtubule behavior in cells. However, microtubule morphology and dynamics, including straightness, are also influenced by many external factors as well as tubulin conformation, and it is difficult to predict microtubule behavior in cells solely from the tubulin molecular structure.

Collectively, the new results by Belvindrah et al. (2017) shed light on how the disease-associated mutation of *Tuba1a* causes defects in neuronal positioning in the adult brain (Fig. 1 A). At the molecular level, the S140G mutation in Tuba1a enhances the interaction with β -tubulin and induces a straighter conformation of tubulin dimers. At the cellular level, Tuba1a S140G increases the straightness of the growing microtubules without affecting microtubule polymerization speed. These alterations may induce neurite branching of migrating neurons in the RMS, and thereby reduce the migration speed and disturb the directionality of the migrating neurons. The N–C coupling is also perturbed in the migrating neurons. As a result, neurons accumulate in the RMS of the adult brain in Tuba1a S140G mutant mice.

As mentioned above, there are many α -tubulin isotypes, but little is known about an isotype-specific role of α -tubulins in microtubule properties and function. In this issue, Belvindrah et al. (2017) provide evidence that Tuba1a and Tuba8 have different functions in cells (Fig. 1 B). Unlike Tuba1a S140G, Tuba8 suppressed the straightness of microtubules in both cell soma and processes in vitro, compared with WT Tuba1a. Furthermore, Tuba8 increased the microtubule polymerization speed in the cell soma, compared with WT Tuba1a or Tuba1a S140G. Thus, the effects of these α -tubulin isotypes on the microtubule straightness and polymerization speed are different. At the molecular level, the overall structures of Tuba1a and Tuba8 are similar, but, through electrostatic analyses, Belvindrah et al. (2017) report that the charge distributions in the H1-S2 loop of these proteins are different. The H1-S2 loop of tubulins laterally interacts with the M loop of the α -tubulin in an adjacent protofilament. Tuba8 exhibits a unique increase in negative charges at the H1-S2 loop, whereas the charge distribution of the H1-S2 loop in Tuba1a shows positive and roughly complements the M loop. These data indicate the structural and functional differences between α -tubulin isotypes.

What remains to be determined is whether the S140G or other disease-associated mutations of tubulins alter the binding to microtubule-associated proteins or microtubule-mediated cellular events, such as membrane trafficking and cell division. Of note, the Arg402 residue in Tuba1a, whose substitution to His (R402H mutation) results in lissencephaly in humans, is located in the binding site of Dcx and KIF1A, and mutations in Dcx result in X-linked lissencephaly in males and subcortical band heterotopia (also known as double cortex syndrome) in females. Thus, in some cases, defects in the interaction between Tuba1a and its binding partner may be a main cause of brain malformation.

Although Tuba1a is the only α -tubulin reported in association with brain malformation, with the possible exception of Tuba8, mutations in several β -tubulin isotypes are reported to cause brain malformation. Mutations in Tubb2b, Tubb3, and Tubb5 result in similar brain malformations, such as microlissencephaly and polymicrogyria. Interestingly, several β -tubulin isotypes are reported to differentially regulate microtubule dynamics in vitro and in *Caenorhabditis elegans* embryos (Panda et al., 1994; Honda et al., 2017). It would be interesting to test whether mammalian β -tubulins show isotype-specific roles in microtubule organization and brain morphogenesis. Wide spectrum analyses from molecular structures to tissue morphology, like the studies from Belvindrah et al. (2017), will be crucial to investigate these open questions.

Acknowledgments

The author thanks Dr. Ruth T. Yu for English proofreading of the manuscript.

The Kawauchi research group is funded by the Japan Society for the Promotion of Science KAKENHI grant number JP26290015 and the Ministry of Education, Culture, Sports, Science and Technology of Japan, Takeda Science Foundation, and Uehara Memorial Foundation Grant-in Scientific Research on Innovation Areas “Dynamic regulation of Brain Function by Scrap and Build System” (JP17H05757).

The author declares no competing financial interests.

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