CAMSAP3 maintains neuronal polarity through regulation of microtubule stability

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The molecular mechanisms that guide each neuron to become polarized, forming a single axon and multiple dendrites, remain unknown. Here we show that CAMSAP3 (calmodulin-regulated spectrin-associated protein 3), a protein that regulates the minus-end dynamics of microtubules, plays a key role in maintaining neuronal polarity. In mouse hippocampal neurons, CAMSAP3 was enriched in axons. Although axonal microtubules were generally acetylated, CAMSAP3 was preferentially localized along a less-acetylated fraction of the microtubules. CAMSAP3-mutated neurons often exhibited supernumerary axons, along with an increased number of neurites having nocardazole-resistant/acetylated microtubules compared with wild-type neurons. Analysis using cell lines showed that CAMSAP3 depletion promoted tubulin acetylation, and conversely, mild overexpression of CAMSAP3 inhibited it, suggesting that CAMSAP3 works to retain nonacetylated microtubules. In contrast, CAMSAP2, a protein related to CAMSAP3, was detected along all neurites, and its loss did not affect neuronal polarity, nor did it cause increased tubulin acetylation. Depletion of α-tubulin acetyltransferase-1 (α-TAT1), the key enzyme for tubulin acetylation, abolished CAMSAP3 loss-dependent multiple-axon formation. These observations suggest that CAMSAP3 sustains a nonacetylated pool of microtubules in axons, interfering with the action of α-TAT1, and this process is important to maintain neuronal polarity.

Significance

Each neuron forms a single axon and multiple dendrites, and this configuration is important for wiring the brain. How only a single axon extends from a neuron, however, remains unknown. This study demonstrates that CAMSAP3, a protein that binds the minus-end of microtubules, preferentially localizes along axons in hippocampal neurons. Remarkably, mutations of CAMSAP3 lead to production of multiple axons in these neurons. In attempts to uncover mechanisms underlying this abnormal axon extension, the authors found that CAMSAP3-anchored microtubules escape from acetylation, a process mediated by α-tubulin acetyltransferase-1, and depletion of this enzyme abolishes abnormal axon formation in CAMSAP3 mutants. These findings reveal that CAMSAP3 controls microtubule dynamics, preventing tubulin acetylation; this mechanism is required for single-axon formation.

The authors declare no conflict of interest.

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Supporting Information

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Appendix for updates
pool of microtubules by counteracting αTAT1-mediated tubulin acetylation to retain single axons.

Results

Loss of CAMSAP3 Leads to Supernumerary Axon Formation. Developing brains expressed CAMSAP2 and CAMSAP3, although CAMSAP3 expression in hippocampus was delayed (SI Appendix, Fig. S1A). We collected hippocampal neurons from E16.5 embryos and cultured them for 6 d in vitro (DIV6), followed by double-immunostaining for CAMSAPs and a dendrite or axon marker [MAP2 for dendrite; Tau, ankyrin-G or axonal neurofilaments (SMI312 antigen) for axon]. The results showed that CAMSAP2 was distributed in both axons and dendrites, whereas CAMSAP3 was consistently enriched in axons (Fig. 1A). In earlier stages of culture (stage 2), however, both CAMSAP2 and CAMSAP3 were detected in all neurites, as well as in the cell body (Fig. 1B), and the restriction of CAMSAP3 to axons took place at stage 3 (SI Appendix, Fig. S1B), indicating that the axon-enriched localization of CAMSAP3 coincides with axon differentiation (31). Double-immunostaining for CAMSAP3 and microtubules showed that CAMSAPs were detected as punctate signals that overlap with microtubules (Fig. 1C), as observed in other cell types (23). High-magnification views showed that CAMSAPs were localized only along a subset of microtubule bundles (Fig. 1C, enlarged panels).

To investigate the role of these CAMSAPs in neuron development, we generated a line of Camsap2 mutant mice by a gene knockout method (SI Appendix, Fig. S2 A–C). This mouse line is conventionally called Camsap2−/−, as Camsap2 proteins are not detectable. These mice were born without gross developmental defects, although they showed growth retardation (SI Appendix, Fig. S2D). For the analysis of Camsap3 mutants, we used Camsap3dc mice that express a C terminus-truncated CAMSAP3 protein (24). In addition, we studied mice that express a C terminus-truncated CAMSAP3 protein that is unable to bind microtubule minus-ends (24). In the initial experiments, we stained wild-type and Camsap3dc neurons for the mutated CAMSAP3 protein detected diffuse signals along neurites, which were never enriched in axons (SI Appendix, Fig. S3A), indicating that CAMSAP3 binding to microtubule minus-ends is required for it to concentrate in axons.

Because CAMSAP2 is expressed in Camsap3 mutants, we tested whether CAMSAP2 plays any active role in multiple axon formation. To do this, we obtained double mutants of Camsap2 and Camsap3 by crossing Camsap2−/− and Camsap3dc mice. Neurons derived from double mutants again showed multiple axon phenotypes (Fig. 2 A and C). In addition, the pattern of CAMSAP2 distribution did not particularly differ between wild-type and Camsap3dc neurons (Fig. 2D). These observations suggest that CAMSAP2 was not required for multiple axon formation. Comparisons of axon and dendrite markers (Tau and MAP2, respectively) between control and Camsap3dc neurons indicated that MAP2 tended to spread into Tau-positive neurites more deeply in the mutants than in control patients (Fig. 2E). This suggests that the excessively formed axons might have acquired a hybrid axon/dendrite nature, unlike authentic axons. When Camsap3dc neurons were cultured up to maturation, synaptic proteins came to accumulate along neurites, suggesting that axon and dendrite differentiation took place normally in these neurons, despite the excess number of putative axons (SI Appendix, Fig. S3E).

We also observed neuron morphology in vivo, using sections of postnatal brains stained by the Golgi method, which sporadically labels neurons and their processes. This method allowed us to correctly trace the axons extending from the soma only if labeled neurons were sparsely distributed without overlapping with others. Although such neurons were rare in our specimens, we observed some isolated pyramidal neurons at the cerebral cortex. Pyramidal neurons are known to extend a single axon straight toward the ventricular side. In Camsap3dc brains, we found that at least a few percent of pyramidal neurons produced multiple axon-like processes, and that such neurons were undetectable in wild-type cortices (Fig. 2F). We did not examine neurons in the hippocampus, as they were too densely labeled by the Golgi method.

CAMSAP3 Is Involved in Control of Tubulin Acetylation. We then investigated how CAMSAP3 maintains single axons and why its mutations lead to multiple axon formation. Previous studies showed that stabilization of microtubules is sufficient for driving a neurite to differentiate into an axon (6), and tubulin acetylation is a hallmark of stable microtubules (7). Therefore, we examined whether CAMSAP loss affected microtubule dynamics by observing tubulin acetylation. In the initial experiments, we used the Neuro-2a (N2a) neuroblastoma line (32) as a neuronal model, which allowed us to conduct efficient preliminary analysis. N2a cells expressed both CAMSAP2 and CAMSAP3,
exhibiting their scattered distribution in the cytoplasm with the highest concentration at perinuclear zones (SI Appendix, Fig. S44). When these CAMSAPs were knocked down using siRNAs (SI Appendix, Fig. S3B), acetylated tubulin was increased after CAMSAP3 KD, but it did not change or was slightly decreased in CAMSAP2 KD cells (Fig. 3A). Morphologically, N2a cells exhibited short filopodium-like processes at the peripheries, and this shape of cells was not affected by CAMSAP2 KD. In contrast, ∼30% of CAMSAP3 KD cells produced long neurite-like processes, the number of which varied from cell to cell (SI Appendix, Fig. S4B), as observed in N2a cells whose differentiation was induced by treatment with pharmacological reagents such as retinoic acid (33). Acetylated tubulin was detected from the cytoplasm in control or CAMSAP2 KD cells, but its distribution extended to the elongated processes in CAMSAP3 KD cells (SI Appendix, Fig. S4C).
To check whether acetylated microtubules are stable, we treated control and CAMSAP3 KD cells with nocodazole, a reagent to promote microtubule depolymerization. In the treated cells, although α-tubulin became to diffuse losing its meshwork architecture, acetylated microtubules remained in the cytoplasm, as well as in the neurite-like processes of CAMSAP3 KD cells (SI Appendix, Fig. S4C). Taken together, these observations suggest that CAMSAP3 loss led to an increase of acetylated, stable microtubules, simultaneously promoting neurite-like process formation in N2a cells.

Next, we observed tubulin acetylation in hippocampal neurons. Immunostaining showed that, in wild-type neurons, acetylation was broadly detected in various regions of the neuron. Nevertheless, the ratio of acetylated tubulin to the total tubulin tended to be higher in axons compared with dendrites (Fig. 3B, Upper and C). Consistent with previous reports (6, 34), in Camsap2dc neurons, this relative increase of acetylated tubulins came to be observed in multiple neurites (Fig. 3B, Lower and C). When these neurons were treated with nocodazole, nocodazole-resistant microtubules were detectable in various regions of a neuron, regardless of whether CAMSAPs were present or not. However, concerning the thin and long processes extending from the cell body, which are putative axons, only single processes held nocodazole-resistant microtubules in most of Camsap2 knockdown neurons, whereas multiple processes had them in CAMSAP3-mutated neurons (Fig. 3D). These observations support the idea that CAMSAP3 dysfunction leads to an increase of neurites with stable microtubules also in neurons.

Unlike in N2a cells, however, in hippocampal neurons, the total level of acetylated tubulin did not particularly change in Camsap2 or Camsap3 mutants, as assessed by Western blotting (Fig. 3E). This was also the case for cells treated with nocodazole. Immunostaining for acetylated tubulin also did not suggest apparent differences in its overall level between these samples (Fig. 3B and D). To investigate whether CAMSAP3 mutations or overexpression affect tubulin dynamics in hippocampal neurons, we closely observed the distribution of CAMSAPs in relation to that of acetylated and tyrosinated microtubules, which represent stable and dynamic microtubules, respectively, in axons. Microtubules are densely packed in axon shafts, preventing us from resolving individual microtubules; therefore, we purposely observed specific portions of axons, where microtubules happened to be untangled. Our observations showed a tendency for CAMSAP3 puncta to localize along a subpopulation of microtubules that are less acetylated, although this protein well coincided with tyrosinated microtubules (Fig. 3F, Left). In contrast, the distribution of CAMSAP3 in relation to microtubule posttranscriptional modification was not so clear-cut as that of CAMSAP3. Unlike Camsap2, Camsap3 did not avoid acetylated microtubules (Fig. 3F, Right). Given that CAMSAP3 is localized to restricted zones in neurons (Fig. 1C), these observations suggest that, in neurons, CAMSAP3 may affect tubulin acetylation only for a small fraction of microtubules and at levels not detectable using whole-cell lysates.

**CAMSAP3 Actively Suppresses Tubulin Acetylation.** To confirm whether CAMSAP3 is capable of affecting tubulin acetylation in hippocampal neurons, we transiently overexpressed GFP-tagged CAMSAP2 (C2-GFP) or CAMSAP3 (C3-GFP) in them, and observed tubulin acetylation at their cell bodies, as acetylation levels considerably fluctuated between neurites even in untransfected neurons. Our repeated observations showed that tubulin acetylation levels tended to be lower in neurons expressing C3-GFP, whereas this tendency was not observed in those expressing C2-GFP (Fig. 4A and B). These findings support the idea that CAMSAP3 works to suppress tubulin acetylation in axons as well.

For more detailed analysis of CAMSAP3-dependent control of tubulin acetylation, we used Caco2 cells in monolayer cultures, which allowed us to observe individual CAMSAP3 puncta and microtubules at high resolution (23). Double-immunostaining for CAMSAP3 and acetylated tubulins showed that the majority of CAMSAP3 puncta were free from acetylated microtubules, and acetylated microtubules were generally not associated with CAMSAP3 puncta, although a small population of CAMSAP3 puncta attached to an end of these microtubules (SI Appendix, Fig. S4D). We then overexpressed His-tagged CAMSAP3 (C3-His) in Caco2 cells. The results showed that a moderate level of C3-His expression completely eliminated acetylated microtubules (Fig. 4C and D). In contrast, when ectopic C3-His expression was further increased, this protein began to show streak-like distributions along microtubules, probably because of their excess binding to the microtubule lattice; and these microtubules were acetylated again. These observations confirmed that unless CAMSAP3 is overly expressed, CAMSAP3-bound microtubules are prevented from acetylation.

![Image](https://example.com/image.png)
α-Tubulin Acetyltransferase-1 Is Required for Supernumerary Axon Formation. Next, we examined whether tubulin acetylation itself had any influence on axon behavior. Acetylation of tubulins is mediated with α-tubulin acetyltransferase 1 (αTAT1/MEC-17) (10–12). We confirmed that tubulin acetylation was strongly inhibited by treating N2a cells with αTAT1-specific siRNAs (Fig. S5A), and conversely it was dramatically increased by overexpressing this enzyme in these cells (SI Appendix, Fig. S5A). CAMSAP3 loss-dependent up-regulation of tubulin acetylation was also abolished by depleting αTAT1 in these cells (SI Appendix, Fig. S5B). To confirm whether αTAT1 siRNA-dependent elimination of tubulin acetylation depended on the catalytic activity of the enzyme, we tested whether the catalytic domain-mutated αTAT1 (D157N) (11) could rescue this elimination. The results showed that only wild-type αTAT1 restored tubulin acetylation in CAMSAP3/αTAT1 codepleted cells (SI Appendix, Fig. S5B).

αTAT1 depletion also reduced tubulin acetylation in neurons, although axons still elongated, as assessed by immunostaining for axon markers (Fig. 5 B and C), confirming that axonal tubulin acetylation is αTAT1-dependent. Camasp3ΔΔc neurons similarly responded to αTAT1 depletion, and importantly, in these αTAT1-depleted neurons, supernumerary axon formation was abolished (Fig. 5 B and D). These results suggest that αTAT1 is required to maintain the multiple axons generated by CAMSAP3 mutation. Next, we tested whether excess tubulin acetylation could induce multiple axon formation by overexpressing GFP-tagged αTAT1 or its D157N mutant in wild-type neurons, and found that the expression of either molecule did not particularly increase axon number (SI Appendix, Fig. S5C). Overexpression of αTAT1 in N2a cells also did not affect their process formation, even though tubulin acetylation level was greatly elevated (SI Appendix, Fig. S5D). These results suggest that increase of tubulin acetyltransferase alone is not sufficient for disrupting neuronal polarity or for promoting neurite extension. We also found that CAMSAP3 remained in axons when αTAT1 was knocked down (Fig. 5E), indicating that axonal accumulation of CAMSAP3 occurs independent of tubulin acetylation.

Discussion

Our findings demonstrate that CAMSAP3 is required to maintain single axons in hippocampal neurons in culture. We found CAMSAP3 to be enriched in axons, localizing along a subpopulation of microtubules that are less acetylated. Loss of CAMSAP3 resulted in up-regulation of acetylated tubulins in N2a cells, as found with other cells (23). Conversely, moderate overexpression of CAMSAP3 actively reduced tubulin acetylation in both epithelial and neuronal cells. Given that tubulin acetylation is known to occur in long-lived microtubules, our observations suggest that CAMSAP3-anchored microtubules are not stable when CAMSAP3 density is properly high, thereby escaping from αTAT1-mediated acetylation. CAMSAP3 thus seems to serve the purpose of retaining a pool of dynamic microtubules in axons, although their microtubules are generally acetylated. We suspect that CAMSAP3 removal abolished this system, causing a reduction in dynamic microtubules, and the resultant local increase of stable microtubules induced supernumerary axon formation, as previous studies demonstrated that taxol-induced stabilization of microtubules resulted in multiple axon generation (6) or extra neurite formation (35). We also found that CAMSAP3 depletion in N2a cells induced neurite-like process formation. Although the identity of these processes remains unknown, this observation suggests that CAMSAP3 may have a general ability to control neurite formation, loss of which leads to unregulated growth of neurites, and this might have occurred in the case of hippocampal neurons.

Our results also demonstrate that αTAT1 is required to maintain multiple axons in CAMSAP3-deficient neurons. This finding suggests that although enhanced tubulin acetylation might have primarily occurred as a result of microtubule stabilization induced by CAMSAP3 removal, tubulin acetylation per se has a role in extra axon formation. It has been shown that tubulin acetylation alters various mechanical properties of microtubules (13–16), and it keeps microtubules consolidated in axons (17). Such effects of acetylation on microtubule properties could contribute to neurite regulation. In contrast, αTAT1 overexpression did not reduce axon or neurite elongation, suggesting that simple enhancement of αTAT1 activity is not sufficient to bring about these events. Stabilization of microtubules might be a prerequisite for αTAT1 to contribute to the disturbance of neurite genesis.

How, then, does CAMSAP3-dependent regulation of microtubule dynamics retain single axons in normal neurons? CAMSAP3 might merely serve to protect microtubules against overstabilization that would lead to supernumerary axon formation. However, it is equally possible that CAMSAP3 could have other active roles. It has been hypothesized that single axons are maintained by feedback loops between signals that are responsible for axon and dendrite differentiation (3, 36, 37). A recent study showed that a Ca2+ wave derived from the axon terminal suppresses the growth of other neurites (minor neurites) through activation of the CaMKI/GEF-H1/Rho kinase signaling pathway at the cell body region (38). CAMSAP3-bound dynamic microtubules might play a role in the transfer of such axonal signals to the cell body, loss of which leads to uncontrolled growth or differentiation of minor neurites.

Elucidating how CAMSAP3, as a minus-end stabilizer, regulates microtubule dynamics remains an important future subject. It is noteworthy that CAMSAP2 loss did not enhance microtubule acetylation, which suggests that CAMSAP2 and CAMSAP3 differently regulate microtubule dynamics. In fact, some differences in their ability to stabilize the microtubule minus-ends have been suggested by previous in vitro analysis (22). Furthermore, unlike in
the case of CAMSAP3 mutants, polarity defects were not observed in 
Camsap2-knockout neurons, supporting the notion that 
CAMSAP2 and CAMSAP3 differ in biological functions. A pre-
vious study, in contrast, reported polarity defects in CAMSAP2-
deficient neurons (27). In this study, RNAi was used to suppress 
CAMSAP2 expression, and such methodological differences be-
tween the studies might have resulted in nonidentical conclusions. 
Despite this discrepancy, both studies showed reduced branching 
of dendrites in CAMSAP2-deficient neurons, suggesting that this 
CAMSAP subtype plays a role in neurite growth. In C. elegans 
neurons, PTRN-1 works to stabilize microtubules (30), and its 
mutations impair axon regeneration (28), suggesting that PTRN-1 
might be similar to CAMSAP2 rather than CAMSAP3 in function. 
In mammalian epithelial cells, CAMSAP2 and CAMSAP3 show 
overlapping distributions and functions, and their removal simi-
larly up-regulated tubulin acetylation (23). These observations 
suggest that CAMSAP subtypes change their roles in a cell type-
dependent manner. To explore the molecular basis of how differ-
ently CAMSAP2 and CAMSAP3 regulate microtubule dynamics 
is an important future subject. It also remains to be investigated 
how the CAMSAP subtypes localize in axons and dendrites in 
distinct patterns.

The supernumerary axon formation phenotypes were observed 
in vitro, but at lower frequencies than in vitro, suggesting that the 
phenotypes might have been enhanced by in vitro environments. 
As qTAT1 activity has been shown to be promoted under stress 
conditions (39), this enzyme might have responded to in vitro 
environments more sensitively than in vivo, resulting in more 
profound phenotypes of CAMSAP3 mutants in vitro. Given that 
developmental phenotypes were less prominent, it is possible 
that the CAMSAP3/qTAT-dependent mechanisms uncovered by 
the present study are more important for homeostasis of neu-
ronal polarity rather than for development, as suggested in the 
case of PTRN-1 (28). Exploring the potential involvement of 
CAMSAP3 mutation in brain diseases is therefore another in-
triguing subject for future research.

Materials and Methods

Generation of CAMSAP knockout mice are described in SI Appendix, Ma-
terials and Methods. Cell cultures, RNA interference, and CDNA transfection 
are described in SI Appendix, Materials and Methods. Immunoblotting, im-
munocytochemistry, and Golgi staining are described in SI Appendix, Ma-
terials and Methods. Image analysis and statistical analysis are also described 
in SI Appendix, Materials and Methods.

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1. Dotti CG, Sullivan CA, Banker GA (1988) The establishment of polarity by hippocampal 
nurons in culture. J Neurosci 8:1454–1468.
2. Schelski M, Bradke F (2017) Neuronal polarization: From spatiotemporal signaling to 
cytoskeletal dynamics. Mol Cell Neurosci 80:11–28.
3. Arimura N, Kaibuchi K (2007) Neuronal polarity: From extracellular signals to in-
tracellular mechanisms. Nat Rev Neurosci 8:194–205.
4. Yoge S, Shen K (2017) Establishing neuronal polarity with environmental and in-
trinsic mechanisms. Neuron 96:638–650.
5. Kapitein LC, Hoogenraad CC (2015) Building the neuronal microtubule cytoskeleton. 
Neuron 87:492–506.
6. Witte H, Neukirchen D, Bradke F (2008) Microtubule stabilization specifies initial 
nearonal polarization. J Cell Biol 180:619–632.
7. Janke C, Bulinski JC (2011) Post-translational regulation of the microtubule cyto-
skeleton: Mechanisms and functions. Nat Rev Mol Cell Biol 12:773–786.
8. Palazzo A, Ackerman B, Gunderson GG (2003) Cell biology: Tubulin acetylation and 
cell motility. Nature 421:239.
9. Wehner DR, Gunderson GG, Bulinski JC, Borisy GG (1987) Differential turnover of 
tyrosinated and detyrosinated microtubules. Proc Natl Acad Sci USA 84:9040–9044.
10. Kaledin N, et al. (2013) αTAT1 is the major α-tubulin acetyltransferase in mice. Nat 
Commun 4:1962.
11. Shiina T, Cueva JG, Xu Z, Goodman MB, Nachury MV (2010) The major alpha-tubulin 
K40 acetylation transferase αTAT1 promotes rapid cilogenesis and efficient mecha-
no-pneumation. Proc Natl Acad Sci USA 107:21517–21522.
12. Akella JS, et al. (2010) α-ECAT-1 is an alpha-tubulin acetyltransferase. Nature 467: 
218–222.
13. Cueva JG, Hsin J, Huang KC, Goodman MB (2012) Posttranslational acetylation of 
α-tubulin contains profilinulfim component in native microtubules. Curr Biol 22: 
1066–1071.
14. Topalidou I, et al. (2012) Genetically separable functions of the MEC-17 tubulin ac-
etyltransferase affect microtubule organization. Curr Biol 22:1057–1065.
15. Portman D, Schedel I, Xu Z, Thery M, Nachury MV (2017) Tubulin acetylation protects 
long-lived microtubules against mechanical ageing. Nat Cell Biol 19:391–398.
16. Xu Z, et al. (2017) Microtubules acquire resistance from mechanical breakage through 
intraluminal acetylation. Science 356:328–333.
17. Dan Wei, et al. (2017) α-Tubulin acetylation restricts axon overbranching by damp-
ening microtubule plus-end dynamics in neurons. Cereb Cortex 1–15.
18. Stevens M, et al. (2010) Axon extension occurs independently of neuronal micro-
tubule nucleation. Science 327:704–707.
19. Meng W, Mushika Y, Ichi T, Takeihi M (2008) Anchorage of microtubule minus ends 
that adheres junctions regulates epithelial cell–cell contacts. Cell 135:948–959.
20. Goodman SS, Vale RD (2010) Patronin regulates the microtubule network by pro-
tecting microtubule minus ends. Cell 143:263–274.
21. Hendershot MC, Vale RD (2014) Regulation of microtubule minus-end dynamics by 
CAMSAPs and Patronin. Proc Natl Acad Sci USA 111:5860–5865.
22. Jiang K, et al. (2014) Microtubule minus-end stabilization by polymerization-driven 
CAMSAP deposition. Dev Cell 28:295–309.