

Année de publication : 2020

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Carsten Janke, Maria M Magiera (2020 Feb 27)

**The tubulin code and its role in controlling microtubule properties and functions.**

*Nature reviews. Molecular cell biology* : [DOI : 10.1038/s41580-020-0214-3](https://doi.org/10.1038/s41580-020-0214-3)

**Résumé**

Microtubules are core components of the eukaryotic cytoskeleton with essential roles in cell division, shaping, motility and intracellular transport. Despite their functional heterogeneity, microtubules have a highly conserved structure made from almost identical molecular building blocks: the tubulin proteins. Alternative tubulin isotypes and a variety of post-translational modifications control the properties and functions of the microtubule cytoskeleton, a concept known as the 'tubulin code'. Here we review the current understanding of the molecular components of the tubulin code and how they impact microtubule properties and functions. We discuss how tubulin isotypes and post-translational modifications control microtubule behaviour at the molecular level and how this translates into physiological functions at the cellular and organism levels. We then go on to show how fine-tuning of microtubule function by some tubulin modifications can affect homeostasis and how perturbation of this fine-tuning can lead to a range of dysfunctions, many of which are linked to human disease.

Satish Bodakuntla, Anne Schnitzler, Cristopher Villablanca, Christian Gonzalez-Billault, Ivan Bieche, Carsten Janke, Maria M Magiera (2020 Feb 13)

**Tubulin polyglutamylation is a general traffic-control mechanism in hippocampal neurons.**

*Journal of cell science* : [DOI : jcs241802](https://doi.org/10.1242/jcs.241802)

**Résumé**

Neurons are highly complex cells that heavily rely on intracellular transport to distribute a range of functionally essential cargoes within the cell. Post-translational modifications of tubulin are emerging as mechanisms for regulating microtubule functions, but their impact on neuronal transport is only marginally understood. Here, we have systematically studied the impact of post-translational polyglutamylation on axonal transport. In cultured hippocampal neurons, deletion of a single deglutamylase, CCP1 (also known as AGTPBP1), is sufficient to induce abnormal accumulation of polyglutamylation, i.e. hyperglutamylation. We next investigated how hyperglutamylation affects axonal transport of a range of functionally different neuronal cargoes: mitochondria, lysosomes, LAMP1 endosomes and BDNF vesicles. Strikingly, we found a reduced motility for all these cargoes, suggesting that polyglutamylation could act as a regulator of cargo transport in neurons. This, together with the recent discovery that hyperglutamylation induces neurodegeneration, makes it likely that perturbed neuronal trafficking could be one of the central molecular causes underlying this novel type of degeneration. This article has an associated First Person interview with the

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first author of the paper.

Yanzhang Luo, ShengQi Xiang, Peter Jan Hooikaas, Laura van Bezouwen, A S Jijumon, Carsten Janke, Friedrich Förster, Anna Akhmanova, Marc Baldus (2020 Jan 2)

**Direct observation of dynamic protein interactions involving human microtubules using solid-state NMR spectroscopy.**

*Nature communications* : 18 : [DOI : 10.1038/s41467-019-13876-x](https://doi.org/10.1038/s41467-019-13876-x)

**Résumé**

Microtubules are important components of the eukaryotic cytoskeleton. Their structural organization is regulated by nucleotide binding and many microtubule-associated proteins (MAPs). While cryo-EM and X-ray crystallography have provided detailed views of interactions between MAPs with the microtubule lattice, little is known about how MAPs and their intrinsically disordered regions interact with the dynamic microtubule surface. NMR carries the potential to directly probe such interactions but so far has been precluded by the low tubulin yield. We present a protocol to produce [C, N]-labeled, functional microtubules (MTs) from human cells for solid-state NMR studies. This approach allowed us to demonstrate that MAPs can differently modulate the fast time-scale dynamics of C-terminal tubulin tails, suggesting distinct interaction modes. Our results pave the way for in-depth NMR studies of protein dynamics involved in MT assembly and their interactions with other cellular components.

Aviel Even, Giovanni Morelli, Loïc Broix, Chiara Scaramuzzino, Silvia Turchetto, Ivan Gladwyn-Ng, Romain Le Bail, Michal Shilian, Stephen Freeman, Maria M Magiera, A S Jijumon, Nathalie Krusy, Brigitte Malgrange, Bert Brone, Paula Dietrich, Ioannis Dragatsis, Carsten Janke, Frédéric Saudou, Miguel Weil, Laurent Nguyen (2019 Dec 18)

**ATAT1-enriched vesicles promote microtubule acetylation via axonal transport.**

*Science advances* : eaax2705 : [DOI : 10.1126/sciadv.aax2705](https://doi.org/10.1126/sciadv.aax2705)

**Résumé**

Microtubules are polymerized dimers of  $\alpha$ - and  $\beta$ -tubulin that underlie a broad range of cellular activities. Acetylation of  $\alpha$ -tubulin by the acetyltransferase ATAT1 modulates microtubule dynamics and functions in neurons. However, it remains unclear how this enzyme acetylates microtubules over long distances in axons. Here, we show that loss of ATAT1 impairs axonal transport in neurons *in vivo*, and cell-free motility assays confirm a requirement of  $\alpha$ -tubulin acetylation for proper bidirectional vesicular transport. Moreover, we demonstrate that the main cellular pool of ATAT1 is transported at the cytosolic side of neuronal vesicles that are moving along axons. Together, our data suggest that axonal transport of ATAT1-enriched vesicles is the predominant driver of  $\alpha$ -tubulin acetylation in axons.

Année de publication : 2019

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Satish Bodakuntla, A S Jijumon, Cristopher Villablanca, Christian Gonzalez-Billault, Carsten Janke (2019 Oct 29)

**Microtubule-Associated Proteins: Structuring the Cytoskeleton.**

*Trends in cell biology* : 804-819 : [DOI : S0962-8924\(19\)30120-5](https://doi.org/10.1016/j.tcb.2019.08.005)

**Résumé**

Microtubule-associated proteins (MAPs) were initially discovered as proteins that bind to and stabilize microtubules. Today, an ever-growing number of MAPs reveals a more complex picture of these proteins as organizers of the microtubule cytoskeleton that have a large variety of functions. MAPs enable microtubules to participate in a plethora of cellular processes such as the assembly of mitotic and meiotic spindles, neuronal development, and the formation of the ciliary axoneme. Although some subgroups of MAPs have been exhaustively characterized, a strikingly large number of MAPs remain barely characterized other than their interactions with microtubules. We provide a comprehensive view on the currently known MAPs in mammals. We discuss their molecular mechanisms and functions, as well as their physiological role and links to pathologies.

Judith Souphron, Satish Bodakuntla, A S Jijumon, Goran Lakisic, Alexis M Gautreau, Carsten Janke, Maria M Magiera (2019 Apr 19)

**Purification of tubulin with controlled post-translational modifications by polymerization-depolymerization cycles.**

*Nature protocols* : [DOI : 10.1038/s41596-019-0153-7](https://doi.org/10.1038/s41596-019-0153-7)

**Résumé**

In vitro reconstitutions of microtubule assemblies have provided essential mechanistic insights into the molecular bases of microtubule dynamics and their interactions with associated proteins. The tubulin code has emerged as a regulatory mechanism for microtubule functions, which suggests that tubulin isoforms and post-translational modifications (PTMs) play important roles in controlling microtubule functions. To investigate the tubulin code mechanism, it is essential to analyze different tubulin variants in vitro. Until now, this has been difficult, as most reconstitution experiments have used heavily post-translationally modified tubulin purified from brain tissue. Therefore, we developed a protocol that allows purification of tubulin with controlled PTMs from limited sources through cycles of polymerization and depolymerization. Although alternative protocols using affinity purification of tubulin also yield very pure tubulin, our protocol has the unique advantage of selecting for fully functional tubulin, as non-polymerizable tubulin is excluded in the successive polymerization cycles. It thus provides a novel procedure for obtaining tubulin with controlled PTMs for in vitro reconstitution experiments. We describe specific procedures for tubulin purification from adherent cells, cells grown in suspension cultures and single mouse brains. The protocol can be combined with drug treatment, transfection of cells before tubulin purification or enzymatic treatment during the purification process. The

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amplification of cells and their growth in spinner bottles takes ~13 d; the tubulin purification takes 6-7 h. The tubulin can be used in total internal reflection fluorescence (TIRF)-microscopy-based experiments or pelleting assays for the investigation of intrinsic properties of microtubules and their interactions with associated proteins.

Catherine Strassel, Maria M Magiera, Arnaud Dupuis, Morgane Batzenschlager, Agnès Hovasse, Irina Pleines, Paul Guéguen, Anita Eckly, Sylvie Moog, Léa Mallo, Quentin Kimmerlin, Stéphane Chappaz, Jean-Marc Strub, Natarajan Kathiresan, Henri de la Salle, Alain Van Dorselaer, Claude Ferec, Jean-Yves Py, Christian Gachet, Christine Schaeffer-Reiss, Benjamin T Kile, Carsten Janke, François Lanza (2019 Feb 15)

### **An essential role for $\alpha$ 4A-tubulin in platelet biogenesis.**

*Life science alliance* : [DOI : e201900309](https://doi.org/10.1038/e201900309)

#### Résumé

During platelet biogenesis, microtubules (MTs) are arranged into submembranous structures (the marginal band) that encircle the cell in a single plane. This unique MT array has no equivalent in any other mammalian cell, and the mechanisms responsible for this particular mode of assembly are not fully understood. One possibility is that platelet MTs are composed of a particular set of tubulin isoforms that carry specific posttranslational modifications. Although  $\beta$ 1-tubulin is known to be essential, no equivalent roles of  $\alpha$ -tubulin isoforms in platelet formation or function have so far been reported. Here, we identify  $\alpha$ 4A-tubulin as a predominant  $\alpha$ -tubulin isoform in platelets. Similar to  $\beta$ 1-tubulin,  $\alpha$ 4A-tubulin expression is up-regulated during the late stages of megakaryocyte differentiation. Missense mutations in the  $\alpha$ 4A-tubulin gene cause macrothrombocytopenia in mice and humans. Defects in  $\alpha$ 4A-tubulin lead to changes in tubulin tyrosination status of the platelet tubulin pool. Ultrastructural defects include reduced numbers and misarranged MT coils in the platelet marginal band. We further observed defects in megakaryocyte maturation and proplatelet formation in  $\alpha$ 4A-tubulin mutant mice. We have, thus, discovered an  $\alpha$ -tubulin isoform with specific and essential roles in platelet biogenesis.

Tiziana Giordano, Sudarshan Gadadhar, Satish Bodakuntla, Jonas Straub, Sophie Leboucher, Guillaume Martinez, Walid Chemlali, Christophe Bosc, Annie Andrieux, Ivan Bieche, Christophe Arnoult, Stefan Geimer, Carsten Janke (2019 Feb 7)

### **Loss of the deglutamylase CCP5 perturbs multiple steps of spermatogenesis and leads to male infertility.**

*Journal of cell science* : [DOI : jcs226951](https://doi.org/10.1042/jcs226951)

#### Résumé

Sperm cells are highly specialized mammalian cells, and their biogenesis requires unique intracellular structures. Perturbation of spermatogenesis often leads to male infertility. Here, we assess the role of a post-translational modification of tubulin, glutamylation, in

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spermatogenesis. We show that mice lacking the tubulin deglutamylase CCP5 (also known as AGBL5) do not form functional sperm. In these mice, spermatids accumulate polyglutamylated tubulin, accompanied by the occurrence of disorganized microtubule arrays, in particular in the sperm manchette. Spermatids further fail to re-arrange their intracellular space and accumulate organelles and cytosol, while nuclei condense normally. Strikingly, spermatids lacking CCP5 show supernumerary centrioles, suggesting that glutamylation could control centriole duplication. We show that most of these observed defects are also present in mice in which CCP5 is deleted only in the male germ line, strongly suggesting that they are germ-cell autonomous. Our findings reveal that polyglutamylation is, beyond its known importance for sperm flagella, an essential regulator of several microtubule-based functions during spermatogenesis. This makes enzymes involved in glutamylation prime candidates for being genes involved in male sterility.

Pedro Guedes-Dias, Jeffrey J Nirschl, Nohely Abreu, Mariko K Tokito, Carsten Janke, Maria M Magiera, Erika L F Holzbaur (2019 Jan 21)

### **Kinesin-3 Responds to Local Microtubule Dynamics to Target Synaptic Cargo Delivery to the Presynapse.**

*Current biology* : CB : 268-282.e8 : [DOI : S0960-9822\(18\)31595-1](https://doi.org/10.1016/j.cub.2018.12.011)

#### Résumé

Neurons in the CNS establish thousands of en passant synapses along their axons. Robust neurotransmission depends on the replenishment of synaptic components in a spatially precise manner. Using live-cell microscopy and single-molecule reconstitution assays, we find that the delivery of synaptic vesicle precursors (SVPs) to en passant synapses in hippocampal neurons is specified by an interplay between the kinesin-3 KIF1A motor and presynaptic microtubules. Presynaptic sites are hotspots of dynamic microtubules rich in GTP-tubulin. KIF1A binds more weakly to GTP-tubulin than GDP-tubulin and competes with end-binding (EB) proteins for binding to the microtubule plus end. A disease-causing mutation within KIF1A that reduces preferential binding to GDP- versus GTP-rich microtubules disrupts SVP delivery and reduces presynaptic release upon neuronal stimulation. Thus, the localized enrichment of dynamic microtubules along the axon specifies a localized unloading zone that ensures the accurate delivery of SVPs, controlling presynaptic strength in hippocampal neurons.

#### Année de publication : 2018

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Athanasia Stoupa, Frédéric Adam, Dulanjalee Kariyawasam, Catherine Strassel, Sanjay Gawade, Gabor Szinnai, Alexandre Kauskot, Dominique Lasne, Carsten Janke, Kathiresan Natarajan, Alain Schmitt, Christine Bole-Feysot, Patrick Nitschke, Juliane Léger, Fabienne Jabot-Hanin, Frédéric Tores, Anita Michel, Arnold Munnich, Claude Besmond, Raphaël Scharfmann, François Lanza, Delphine Borgel, Michel Polak, Aurore Carré (2018 Nov 18)

### **TUBB1 mutations cause thyroid dysgenesis associated with abnormal platelet**

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### physiology.

*EMBO molecular medicine* : [DOI : e9569](#)

#### Résumé

The genetic causes of congenital hypothyroidism due to thyroid dysgenesis (TD) remain largely unknown. We identified three novel gene mutations that co-segregated with TD in three distinct families leading to 1.1% of mutations in TD study cohort. (Tubulin, Beta 1 Class VI) encodes for a member of the  $\beta$ -tubulin protein family. gene is expressed in the developing and adult thyroid in humans and mice. All three mutations lead to non-functional  $\alpha/\beta$ -tubulin dimers that cannot be incorporated into microtubules. In mice, knock-out disrupted microtubule integrity by preventing  $\beta$ 1-tubulin incorporation and impaired thyroid migration and thyroid hormone secretion. In addition, mutations caused the formation of macroplatelets and hyperaggregation of human platelets after stimulation by low doses of agonists. Our data highlight unexpected roles for  $\beta$ 1-tubulin in thyroid development and in platelet physiology. Finally, these findings expand the spectrum of the rare paediatric diseases related to mutations in tubulin-coding genes and provide new insights into the genetic background and mechanisms involved in congenital hypothyroidism and thyroid dysgenesis.

Maria M Magiera, Satish Bodakuntla, Jakub Žiak, Sabrina Lacomme, Patricia Marques Sousa, Sophie Leboucher, Torben J Hausrat, Christophe Bosc, Annie Andrieux, Matthias Kneussel, Marc Landry, André Calas, Martin Balastik, Carsten Janke (2018 Nov 12)

### **Excessive tubulin polyglutamylation causes neurodegeneration and perturbs neuronal transport.**

*The EMBO journal*. : [DOI : e100440](#)

#### Résumé

Posttranslational modifications of tubulin are emerging regulators of microtubule functions. We have shown earlier that upregulated polyglutamylation is linked to rapid degeneration of Purkinje cells in mice with a mutation in the deglutamylating enzyme CCP1. How polyglutamylation leads to degeneration, whether it affects multiple neuron types, or which physiological processes it regulates in healthy neurons has remained unknown. Here, we demonstrate that excessive polyglutamylation induces neurodegeneration in a cell-autonomous manner and can occur in many parts of the central nervous system. Degeneration of selected neurons in CCP1-deficient mice can be fully rescued by simultaneous knockout of the counteracting polyglutamylase TLL1. Excessive polyglutamylation reduces the efficiency of neuronal transport in cultured hippocampal neurons, suggesting that impaired cargo transport plays an important role in the observed degenerative phenotypes. We thus establish polyglutamylation as a cell-autonomous mechanism for neurodegeneration that might be therapeutically accessible through manipulation of the enzymes that control this posttranslational modification.

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Vandana Shashi, Maria M Magiera, Dennis Klein, Maha Zaki, Kelly Schoch, Sabine Rudnik-Schöneborn, Andrew Norman, Osorio Lopes Abath Neto, Marina Dusl, Xidi Yuan, Luca Bartesaghi, Patrizia De Marco, Ahmed A Alfares, Ronit Marom, Stefan T Arold, Francisco J Guzmán-Vega, Loren Dm Pena, Edward C Smith, Maja Steinlin, Mohamed Oe Babiker, Payam Mohassel, A Reghan Foley, Sandra Donkervoort, Rupleen Kaur, Partha S Ghosh, Valentina Stanley, Damir Musaev, Caroline Nava, Cyril Mignot, Boris Keren, Marcello Scala, Elisa Tassano, Paolo Picco, Paola Doneda, Chiara Fiorillo, Mahmoud Y Issa, Ali Alassiri, Ahmed Alahmad, Amanda Gerard, Pengfei Liu, Yaping Yang, Birgit Ertl-Wagner, Peter G Kranz, Ingrid M Wentzensen, Rolf Stucka, Nicholas Stong, Andrew S Allen, David B Goldstein, , Benedikt Schoser, Kai M Rösler, Majid Alfadhel, Valeria Capra, Roman Chrast, Tim M Strom, Erik-Jan Kamsteeg, Carsten G Bönnemann, Joseph G Gleeson, Rudolf Martini, Carsten Janke, Jan Senderek (2018 Nov 12)

**Loss of tubulin deglutamylase CCP1 causes infantile-onset neurodegeneration.**

*The EMBO journal.* : [DOI : e100540](https://doi.org/10.1093/emboj/kdy340)

**Résumé**

A set of glutamylases and deglutamylases controls levels of tubulin polyglutamylation, a prominent post-translational modification of neuronal microtubules. Defective tubulin polyglutamylation was first linked to neurodegeneration in the () mouse, which lacks deglutamylase CCP1, displays massive cerebellar atrophy, and accumulates abnormally glutamylated tubulin in degenerating neurons. We found biallelic rare and damaging variants in the gene encoding CCP1 in 13 individuals with infantile-onset neurodegeneration and confirmed the absence of functional CCP1 along with dysregulated tubulin polyglutamylation. The human disease mainly affected the cerebellum, spinal motor neurons, and peripheral nerves. We also demonstrate previously unrecognized peripheral nerve and spinal motor neuron degeneration in mice, which thus recapitulated key features of the human disease. Our findings link human neurodegeneration to tubulin polyglutamylation, entailing this post-translational modification as a potential target for drug development for neurodegenerative disorders.

Nirakar Basnet, Hana Nedožralova, Alvaro H Crevenna, Satish Bodakuntla, Thomas Schlichthaerle, Michael Taschner, Giovanni Cardone, Carsten Janke, Ralf Jungmann, Maria M Magiera, Christian Biertümpfel, Naoko Mizuno (2018 Oct 20)

**Direct induction of microtubule branching by microtubule nucleation factor SSNA1.**

*Nature cell biology* : 1172-1180 : [DOI : 10.1038/s41556-018-0199-8](https://doi.org/10.1038/s41556-018-0199-8)

**Résumé**

Microtubules are central elements of the eukaryotic cytoskeleton that often function as part of branched networks. Current models for branching include nucleation of new microtubules from severed microtubule seeds or from  $\gamma$ -tubulin recruited to the side of a pre-existing microtubule. Here, we found that microtubules can be directly remodelled into branched

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structures by the microtubule-remodelling factor SSNA1 (also known as NA14 or DIP13). The branching activity of SSNA1 relies on its ability to self-assemble into fibrils in a head-to-tail fashion. SSNA1 fibrils guide protofilaments of a microtubule to split apart to form daughter microtubules. We further found that SSNA1 localizes at axon branching sites and has a key role in neuronal development. SSNA1 mutants that abolish microtubule branching in vitro also fail to promote axon development and branching when overexpressed in neurons. We have, therefore, discovered a mechanism for microtubule branching and implicated its role in neuronal development.

Maria M Magiera, Puja Singh, Carsten Janke (2018 May 31)

### **SnapShot: Functions of Tubulin Posttranslational Modifications.**

*Cell* : 1552-1552.e1 : [DOI : 10.1016/j.cell.2018.05.032](https://doi.org/10.1016/j.cell.2018.05.032)

#### **Résumé**

Post-translational modification of tubulin offers a mechanism for functional diversification of microtubules and regulation in a variety of physiological contexts. This SnapShot recaps the current state of understanding of tubulin posttranslational modifications and their functions in the regulation of biological processes. To view this SnapShot, open or download the PDF.

Maria M Magiera, Puja Singh, Sudarshan Gadadhar, Carsten Janke (2018 May 31)

### **Tubulin Posttranslational Modifications and Emerging Links to Human Disease.**

*Cell* : 1323-1327 : [DOI : 10.1016/j.cell.2018.05.018](https://doi.org/10.1016/j.cell.2018.05.018)

#### **Résumé**

Tubulin posttranslational modifications are currently emerging as important regulators of the microtubule cytoskeleton and thus have a strong potential to be implicated in a number of disorders. Here, we review the latest advances in understanding the physiological roles of tubulin modifications and their links to a variety of pathologies.