Mutations in the most divergent α-tubulin isotype, α8-tubulin, cause defective platelet biogenesis.

Summary

In the panel of genes commonly associated with inherited macrothrombocytopenia, an important fraction encodes key cytoskeletal proteins such as tubulin isotypes, the building blocks of microtubules. Macrothrombocytopenia-causing mutations have been identified in the TUBB1 and TUBA4A genes, emphasizing their importance in the formation of platelets and their marginal band, a unique microtubule ring-like structure that supports the platelet typical disc-shaped morphology. This raised the hypothesis that other tubulin isotypes normally expressed in platelets could play a similar role in their formation.

Distinct roles of α- and β-tubulin polyglutamylation in controlling axonal transport and in neurodegeneration.

Summary

Tubulin polyglutamylation is a post-translational modification of the microtubule cytoskeleton, which is generated by a variety of enzymes with different specificities. The “tubulin code” hypothesis predicts that modifications generated by specific enzymes selectively control microtubule functions. Our recent finding that excessive accumulation of polyglutamylation in neurons causes their degeneration and perturbs axonal transport provides an opportunity for testing this hypothesis. By developing novel mouse models and a new glutamylation-specific antibody, we demonstrate here that the glutamylases TTLL1 and TTLL7 generate unique and distinct glutamylation patterns on neuronal microtubules. We find that under physiological conditions, TTLL1 polyglutamylates α-tubulin, while TTLL7 modifies β-tubulin. TTLL1, but not TTLL7, catalyses the excessive hyperglutamylation found in mice lacking the deglutamylose CCP1. Consequently, deletion of TTLL1, but not of TTLL7, prevents degeneration of Purkinje cells and of myelinated axons in peripheral nerves in these mice. Moreover, loss of TTLL1 leads to increased mitochondria motility in neurons, while loss of TTLL7 has no such effect. By revealing how specific patterns of tubulin glutamylation, generated by distinct enzymes, translate into specific physiological and pathological readouts, we demonstrate the relevance of the tubulin code for homeostasis.
**Controlling Microtubule Dynamics and Function with the tubulin code**

Yanzhang Luo, Shengqi Xiang, Alessandra Lucini Paioni, Agnes Adler, Peter Jan Hooikaas, A S Jijumon, Carsten Janke, Anna Akhmanova, Marc Baldus (2021 May 6)

**Solid-State NMR Spectroscopy for Studying Microtubules and Microtubule-Associated Proteins.**

**Summary**

In this chapter, we describe the preparatory and spectroscopic procedures for conducting solid-state NMR experiments on microtubules (MTs) obtained from human cells and their complexes with microtubule-associated proteins (MAPs). Next to labeling and functional assembly of MTs and MT-MAP complexes, we discuss solid-state NMR approaches, including fast MAS and hyperpolarization methods that can be used to examine these systems. Such studies can provide novel insight into the dynamic properties of MTs and MT-MAP complexes.

Satish Bodakuntla, Carsten Janke, Maria M Magiera (2021 Jan 19)

**Tubulin polyglutamylation, a regulator of microtubule functions, can cause neurodegeneration.**
*Neuroscience letters* : 135656 : [DOI : S0304-3940(21)00034-3]

**Summary**

Neurodegenerative diseases lead to a progressive demise of neuronal functions that ultimately results in neuronal death. Besides a large variety of molecular pathways that have been linked to the degeneration of neurons, dysfunctions of the microtubule cytoskeleton are common features of many human neurodegenerative disorders. Yet, it is unclear whether microtubule dysfunctions are causative, or mere bystanders in the disease progression. A so-far little explored regulatory mechanism of the microtubule cytoskeleton, the posttranslational modifications of tubulin, emerge as candidate mechanisms involved in neuronal dysfunction, and thus, degeneration. Here we review the role of tubulin polyglutamylation, a prominent modification of neuronal microtubules. We discuss the current understanding of how polyglutamylation controls microtubule functions in healthy neurons, and how deregulation of this modification leads to neurodegeneration in mice and humans.


**Tubulin glycylation controls axonemal dynein activity, flagellar beat, and male fertility**
*Science* : [DOI : 10.1126/science.abd4914]
Summary

Abstract

Posttranslational modifications of the microtubule cytoskeleton have emerged as key regulators of cellular functions, and their perturbations have been linked to a growing number of human pathologies. Tubulin glycylation modifies microtubules specifically in cilia and flagella, but its functional and mechanistic roles remain unclear. In this study, we generated a mouse model entirely lacking tubulin glycylation. Male mice were subfertile owing to aberrant beat patterns of their sperm flagella, which impeded the straight swimming of sperm cells. Using cryo-electron tomography, we showed that lack of glycylation caused abnormal conformations of the dynein arms within sperm axonemes, providing the structural basis for the observed dysfunction. Our findings reveal the importance of microtubule glycylation for controlled flagellar beating, directional sperm swimming, and male fertility.

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Year of publication 2020

Satish Bodakuntla, A S Jijumon, Carsten Janke, Maria M Magiera (2020 Nov 5)
Purification of Tubulin with Controlled Posttranslational Modifications and Isotypes from Limited Sources by Polymerization-Depolymerization Cycles.
Journal of visualized experiments : JoVE : DOI : 10.3791/61826

Summary

One important aspect of studies of the microtubule cytoskeleton is the investigation of microtubule behavior in in vitro reconstitution experiments. They allow the analysis of the intrinsic properties of microtubules, such as dynamics, and their interactions with microtubule-associated proteins (MAPs). The “tubulin code” is an emerging concept that points to different tubulin isotypes and various posttranslational modifications (PTMs) as regulators of microtubule properties and functions. To explore the molecular mechanisms of the tubulin code, it is crucial to perform in vitro reconstitution experiments using purified tubulin with specific isotypes and PTMs. To date, this was technically challenging as brain tubulin, which is widely used in in vitro experiments, harbors many PTMs and has a defined isotype composition. Hence, we developed this protocol to purify tubulin from different sources and with different isotype compositions and controlled PTMs, using the classical approach of polymerization and depolymerization cycles. Compared to existing methods based on affinity purification, this approach yields pure, polymerization-competent tubulin, as tubulin resistant to polymerization or depolymerization is discarded during the successive purification steps. We describe the purification of tubulin from cell lines, grown either in suspension or as adherent cultures, and from single mouse brains. The method first
describes the generation of cell mass in both suspension and adherent settings, the lysis step, followed by the successive stages of tubulin purification by polymerization-depolymerization cycles. Our method yields tubulin that can be used in experiments addressing the impact of the tubulin code on the intrinsic properties of microtubules and microtubule interactions with associated proteins.

Genetically encoded live-cell sensor for tyrosinated microtubules.
The Journal of cell biology: DOI: e201912107

Summary

Microtubule cytoskeleton exists in various biochemical forms in different cells due to tubulin posttranslational modifications (PTMs). Tubulin PTMs are known to affect microtubule stability, dynamics, and interaction with MAPs and motors in a specific manner, widely known as tubulin code hypothesis. At present, there exists no tool that can specifically mark tubulin PTMs in living cells, thus severely limiting our understanding of their dynamics and cellular functions. Using a yeast display library, we identified a binder against terminal tyrosine of α-tubulin, a unique PTM site. Extensive characterization validates the robustness and nonperturbing nature of our binder as tyrosination sensor, a live-cell tubulin nanobody specific towards tyrosinated microtubules. Using this sensor, we followed nocodazole-, colchicine-, and vincristine-induced depolymerization events of tyrosinated microtubules in real time and found each distinctly perturbs the microtubule polymer. Together, our work describes a novel tyrosination sensor and its potential applications to study the dynamics of microtubule and their PTM processes in living cells.

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

Summary

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

**Summary**

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 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

**Summary**

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.

Year of publication 2019

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

Summary

Microtubules are polymerized dimers of α- and β-tubulin that underlie a broad range of cellular activities. Acetylation of α-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 α-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 α-tubulin acetylation in axons.


Microtubule-Associated Proteins: Structuring the Cytoskeleton.

Summary

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.
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 isotypes 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 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.
of a particular set of tubulin isotypes that carry specific posttranslational modifications. Although β1-tubulin is known to be essential, no equivalent roles of α-tubulin isotypes in platelet formation or function have so far been reported. Here, we identify α4A-tubulin as a predominant α-tubulin isotype in platelets. Similar to β1-tubulin, α4A-tubulin expression is up-regulated during the late stages of megakaryocyte differentiation. Missense mutations in the α4A-tubulin gene cause macrothrombocytopenia in mice and humans. Defects in α4A-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 -mutant mice. We have, thus, discovered an α-tubulin isotype 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

Summary

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 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.