Microtubule Defects in Neurological Diseases

Overview

Our understanding of neurological diseases has expanded rapidly in the last 20 years. If we looked back at 2002, neurodegenerative diseases like Parkinson's and Alzheimer's disease were treated as separate conditions. Psychiatric diseases like schizophrenia, bipolar disorder, and chronic, severe depression were thought to be more psychosocial conditions, not organic defects, and certainly separate from the neurodegenerative disorders.

Today the emerging model is that **many different neurological problems** can be traced (at least in part) to the same source: **microtubules**. We know neurons depend on microtubule networks in ways we did not understand before. When these networks fail, it leads to or accelerates neurological diseases. Which component(s) of a network have failed, and the cells of the nervous system in which the failure is most severe, determine which disease state occurs.

(This new perspective also could explain why many neurological diseases share similar clinical symptoms. For example, patients with advanced Parkinsonism, Alzheimer's, and white matter syndrome often experience similar bouts of visual and auditory hallucinations. )

Your goal for this week is to build a framework for understanding how microtubule defects lead to neurological disease.

* On Day 1 your team will use information you pull from two pre-class literature readings to work out how microtubules are arranged in differentiated cells, and more specifically, in neurons.
  + The larger learning goal is to practice pulling key concepts out of a noisy background.
  + One reading assignment (Muromoya 2017, pp. 1-6) has way more detailed information than you will need for the case. I recommend that you:
    - Read the guide questions your team needs to answer, then review the figures and read ONLY the first 1-2 paragraphs below each section or subsection heading.
    - See if you can answer some or all of the questions.
    - Only read further if you cannot answer a guide question.
* On Day 2 your team will use the general model you developed on Day 1 to understand what happens to neurons when microtubule-based activities break down.
  + The larger learning goal is to practice synthesizing a model using concepts from **more than one** source.
  + You will not be able to answer all of the Guide Questions for Day 1 or complete Day 2 using just the information in one of the reading assignments.
  + You will need to combine BOTH sources.

Your final assignment will be a summary of what you learned from the case.

Pre-Class Reading Assignment

**You do not have to read both articles.** Half of your team should read Sferra 2020, the others, Muromoya 2017. If you have an odd number, put the extra person on Muromoya 2017.

* [Muromoya 2017 up to p. 6](https://wakeforest.instructure.com/courses/44928/files/2865981?wrap=1)[Download Muromoya 2017 up to p. 6](https://wakeforest.instructure.com/courses/44928/files/2865981/download?download_frd=1), stop before “The stabilization and anchoring of microtubule minus ends”.
* [Sferra 2020 up to p. 10](https://wakeforest.instructure.com/courses/44928/files/2865980?wrap=1)[Download Sferra 2020 up to p. 10](https://wakeforest.instructure.com/courses/44928/files/2865980/download?download_frd=1), stop before “Section 5. Microtubule Dysfunction and Neurodegeneration.”

Both reviews contain information about vertebrates and invertebrates. For this case, you should focus on vertebrates only. The guide questions for the readings are in the next section.

Guide Questions for Day 1

### How are microtubules assembled and regulated?

Get with your team on Wednesday and complete these Guide Questions.

* 1. The proteins listed below are the main structural and regulatory proteins that make up the microtubule network. Using the readings, assign a general function to each of the proteins.
* CDK5RAP2
* CLASPs
* CLIPs
* Dynein
* HDAC6
* Katanin
* Kinesin
* MAP2
* MAPT/Tau
* Nedd1
* Ninein
* Sirtuin-2
* Spastin
* αTAT1
* α-tubulin (*TUBA#* genes)
* β-tubulin (*TUBB#* genes)
* γ-tubulin
* Tubulin tyrosine ligase (TTL)
* γ-TuRCs
  1. Which of the proteins in the list from Qu. 1 are **structural**, meaning they are part of the actual microtubule itself? Which of the proteins are **regulators**, meaning they control or modify stability, assembly/disassembly, etc.?
  2. What types of post-translational modifications do neuronal tubulins undergo? How do these modifications affect tubulin function?
  3. The basic model for microtubules is that the network radiates out from the centrosomes near the nucleus. This is not the only way they can be organized. How can the microtubule network be modified from the radial spokes model to serve differentiated cells? How does the microtubule change in differentiated neurons? Absorptive cells? Secretory cells? How does its arrangement reflect functions of each cell type?
  4. Why is finding a mechanism for nucleation in differentiated cells so important? What needs to happen in order for microtubules to anchor somewhere OTHER than the MTOC?
  5. In terms of regulation, what is the logic in having CDKs control where gamma-tubulin localizes in cells?

Day 2: How do microtubule network defects lead to neurological disease?

Your goal today is to use the core structure and regulation points you built last time to explain how microtubule defects lead to disease states.

Below I provided two lists of options to choose from. Each team gets to choose one (and no, not everyone can do Alzheimer’s!) I recommend picking from the Primary Options, but if you feel daring choose one of the less well-studied diseases in the Alternate Options.

For the disease that you select, use outside sources to supplement your understanding and develop answers to these questions:

1. What are the clinical diagnostic features? What are the cellular diagnostic features?
2. Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?
3. Where is the main defective protein normally? Where is it located during the disease state?
4. How is it possible for several different neurological diseases to arise from the same general defect (i.e., microtubule dysfunction)?

### Primary Options

These are well-established as having associated microtubule defects.

* Schizophrenia
* Progressive Supranuclear Palsy (PSP)
* Primary age-related tauopathy (PART) dementia
* Prion diseases (Crutzfeldt-Jakob disease, chronic wasting disease)
* Parkinson’s disease (PD)
* Hereditary Spastic Paraplegia (HSP)
* Frontotemporal dementia (FTD)
* Amyotrophic Lateral Sclerosis (ALS)
* Alzheimer’s disease (AD)

### Alternate Options

These have less evidence, conflicting data, or both.

* Bipolar disorder
* Chronic traumatic encephalopathy (CTE)
* Corticobasal degeneration (CBD)
* HDAC6 over-activity
* Ischemic stroke
* Lead encephalopathy
* Sirtuin 2 defect
* Tubulin tyrosine ligase deficiency

### What You Will Hand In

1. Your table or illustration summarizing the functions of the main microtubule proteins from Day 1. Make sure to include whether each protein acts mainly as a structural component, or a regulatory component.
2. A summary description of the disease that you selected. It should include:

* What are the clinical diagnostic features? What are the cellular diagnostic features?
* Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?
* Where is the main defective protein normally? Where is it located during the disease state?

1. A short answer to this question: how is it possible for several difference neurological diseases to have similar causes?

Case and Instructor Notes:

This case comes at the end of a junior-level cell and molecular biology course. It is the capstone for several cases in which students have practiced extracting and interpreting information from increasingly complex datasets and primary literature reading assignments. To complete this case students work in teams to assemble a general understanding of how microtubules are organized and function in neurons. Students evaluate the quality and accuracy of their framework by using it to describe the role(s) of microtubule defects in one neurological disease.

The case intentionally is very open ended. Students have spent considerable time developing their skills over the semester. This is their opportunity to test those skills prior to going into the final exams. Many of the students enrolled in this course are on a pre-health track, so this also provides the opportunity to apply course concepts to disease conditions.

*Case Day 1 Questions:*

* 1. The proteins listed below are the main structural and regulatory proteins that make up the microtubule network. Using the readings, assign a general function to each of the proteins.
  2. Which of the proteins in the list from Qu. 1 are **structural**, meaning they are part of the actual microtubule itself? Which of the proteins are **regulators**, meaning they control or modify stability, assembly/disassembly, etc.?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Proteins** | **Structural** | **Mixed** | **Regulatory** | **Function** |
| CDK5RAP2 |  |  | x | Cdk5 regulator. Ties m’tubule stability to cell cycle. |
| CLASPs |  | x |  | +-end protein. Stabilizer |
| CLIPs |  |  |  | + end protin. Stabilizer |
| Dynein | x |  |  | Motor protein for travel to - end |
| HDAC6 |  |  |  | Cytoplasmic histone deacetylase |
| Katanin |  | x |  | Severs microtubules |
| Kinesin | x |  |  | Motor protein for travel to + end |
| MAP2 |  | x |  | Lateral regulator of stability |
| MAPT/Tau |  | x |  | Lateral regulator of stability |
| Nedd1 |  |  | x | Targets γ-tubulin to specific organizer locations |
| Ninein |  |  |  | - end anchor protein for epithelium |
| Sirtuin-2 |  |  |  | NAD-dependent deacetylase |
| Spastin |  | x |  | Severs microtubules |
| αTAT1 |  |  | x | A primary tubulin lysine acetylase |
| α-tubulin (TUBA# genes) | x |  |  | First of 2 proteins in heterodimer |
| β-tubulin (TUBB# genes) | x |  |  | Second of 2 proteins in heterodimer |
| γ-tubulin | x |  |  | Basal – end anchor in MTOCs. Regulates CLIP binding |
| Tubulin tyrosine ligase (TTL) |  |  | x | Addition half of -Y/+Y cycle on tubulin. |
| γ-TuRCs |  | x |  | Gamma tubulin ring complex. |
|  |  |  |  |  |

* 1. What types of post-translational modifications do neuronal tubulins undergo? How do these modifications affect tubulin function?

Tubulins can undergo acetylation and de-acetylation, cyclic tyrosine removal and addition, and glutamylation. Some of these modifications change microtubule stability, either accelerating or blocking catastrophic depolymerization. There is a large amount of evidence to say that these modifications also act as a regulatory coding system, similar to how epigenetic modifications of histones regulate DNA activity.

Many microtubule-related proteins have preferential affinities for microtubules (or domains of a microtubule) that are enriched or deficient in certain modified tubulins. For example, kinesin-1 prefers microtubules rich in detyrosinated and acetylated tubulins, while kinesins 5 and 13 prefer microtubules rich in tyrosinated tubulins. Other proteins sensitive to the tubulin code include microtubule-severing proteins and some + end polymerases.

The tubulin code is regulated by highly conserved and tightly regulated enzymes, which might only be found in selected areas of cells. For example, the enzymes may be differentially enriched or regulated in axons versus dendrites, enabling axonal microtubules to be richer in post-translationally modified tubulin subunits compared to dendritic microtubules.

* 1. The basic model for microtubules is that the network radiates out from the centrosomes near the nucleus. This is not the only way they can be organized. How can the microtubule network be modified from the radial spokes model to serve differentiated cells? How does the microtubule change in differentiated neurons? Absorptive cells? Secretory cells? How does its arrangement reflect functions of each cell type?

How does the microtubule change in:

* Differentiated neurons?
* Absorptive cells?
* Secretory cells?

How does its arrangement reflect functions of each cell type?

Regulates ability to transport vs. remodel.

Allows different traffic patterns in differentiated cells.

* 1. Why is finding a mechanism for nucleation in differentiated cells so important? What needs to happen in order for microtubules to anchor somewhere OTHER than the MTOC?

Cells cannot change the radial organization of dividing cells if they cannot create new nucleation points.

* 1. In terms of regulation, what is the logic in having CDKs control where gamma-tubulin localizes in cells?

Adding the cdk control ties microtubule stability and structure to cell cycle. Cells cannot form the differentiated organization patterns as long as a cell is getting pro-growth signals.

Overall length is about right for 45 minutes.

**Day 2**

**How do microtubule network defects lead to neurological disease?**

*Case Day 2 Questions:*

Your goal for today is to use the core structure and regulation points you built last time to explain how microtubule defects lead to disease states.

Below I provided two lists of options to choose from. Each team gets to choose one (and no, not everyone can do Alzheimer’s!) I recommend picking from the Primary Options, but if you feel daring choose one of the less well-studied diseases in the Alternate Options.

For the disease that you select, use outside sources to supplement your understanding and develop answers to these questions:

1. What are the clinical diagnostic features? What are the cellular diagnostic features?

2. Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?

3. Where is the main defective protein normally? Where is it located during the disease state?

4. How is it possible for several different neurological diseases to arise from the same general defect (i.e., microtubule dysfunction)?

***What You Should Turn In***

1. Your table summarizing the functions of the main microtubule proteins from Day 1, and whether each protein acts mainly as a structural component, or a regulatory component.

2. A summary description of the disease that you selected.

* What are the clinical diagnostic features? What are the cellular diagnostic features?
* Based on the model you developed for Day 1, how does or could the cellular features explain the clinical features?
* Where is the main defective protein normally? Where is it located during the disease state?

3. A short answer to this question: how is it possible for several difference neurological diseases to share similar causes?

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Disease patterns can be result of:

Microtubule insufficiency overall

Microtubule misalignment, mis-orientation (for example, + ends point wrong way)

Structural defects (g-tubulin or anchor defects, CLASP errors, etc.)

Errors in MAP1b, MAP2, Tau regulatory proteins

In Alzheimer's disease, however, abnormal chemical changes cause tau to detach from microtubules and stick to other tau molecules, forming threads that eventually join to form tangles inside neurons. These tangles block the neuron's transport system, which harms the synaptic communication between neurons.

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Microtubules in health and degenerative disease of the nervous system

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Abstract

Microtubules are essential for the development and maintenance of axons and dendrites throughout the life of the neuron, and are vulnerable to degradation and disorganization in a variety of neurodegenerative diseases. Microtubules, polymers of tubulin heterodimers, are intrinsically polar structures with a plus end favored for assembly and disassembly and a minus end less favored for these dynamics. In the axon, microtubules are nearly uniformly oriented with plus ends out, whereas in dendrites, microtubules have mixed orientations. Microtubules in developing neurons typically have a stable domain toward the minus end and a labile domain toward the plus end. This domain structure becomes more complex during neuronal maturation when especially stable patches of polyaminated tubulin become more prominent within the microtubule. Microtubules are the substrates for molecular motor proteins that transport cargoes toward the plus or minus end of the microtubule, with motor-driven forces also responsible for organizing microtubules into their distinctive polarity patterns in axons and dendrites. A vast array of microtubule-regulatory proteins impart direct and indirect changes upon the microtubule arrays of the neuron, and these include microtubule-severing proteins as well as proteins responsible for the stability properties of the microtubules. During neurodegenerative diseases, microtubule mass is commonly diminished, and the potential exists for corruption of the microtubule polarity patterns and microtubule-mediated transport. These ill effects may be a primary causative factor in the disease or may be secondary effects, but regardless, therapeutics capable of correcting these microtubule abnormalities have great potential to improve the status of the degenerating nervous system.

Keywords: microtubule, neuron, axon, dendrite, katanin, spastin, fidgetin, dynein, kinesin, neurodegeneration, Alzheimer's disease, tau, tauopathy, microtubule-associated proteins, molecular motor proteins, axonal transport, CAMSAP, tubulin, tubulin code, microtubule stability, +tips

The microtubule arrays of axons and dendrites provide a structural backbone that allows them to acquire and maintain their specialized morphologies. In addition to acting as structural elements, microtubules are long-distance railways for proteins and organelles to be actively transported in both directions within axons and dendrites. Microtubules are crucial for early developmental stages of the neuron, such as migration of the soma and the navigation of the growth cone at the tip of the elongating axon. Microtubules are also important throughout the life of the neuron, for it to maintain its proper morphology, to enable axonal and dendritic transport, and to accommodate morphological changes such as alterations in dendritic shape that may correspond with cognitive plasticity, even in old age. Proper functioning of microtubules and their assortment of interacting and regulatory proteins as well as regulatory pathways is crucial for the health of the nervous system. Abnormalities of the microtubule systems of axons and dendrites are a major contributor to neurodegenerative diseases. The purpose of this review is to provide a brief overview of contemporary knowledge of neuronal microtubules and how they may go awry during degeneration of the diseased nervous system.

Tubulin is a heterodimer of alpha tubulin and beta tubulin, each of which is a different primary gene product believed to have diverged evolutionarily from a single gene (1). In vertebrates, there are multiple alpha tubulin genes and multiple beta tubulin genes. In the test tube, microtubules can be nucleated de novo if there is a sufficient concentration of free tubulin, as well as the presence of GTP and appropriate temperature and buffer conditions. In living cells, de novo nucleation is suppressed so that nucleation of microtubules is favored to occur from nucleating structures that are usually components of “microtubule-organizing centers” such as the centrosome. The nucleating structures are called gamma-turcs (gamma tubulin ring complexes) because they consist of gamma tubulin, together with other proteins that combine with gamma-tubulin to form a template for the nucleation of new microtubules (2, 3). Gamma tubulin is a separate gene product from alpha or beta tubulin, and binds specifically to beta tubulin to establish the polarity orientation of the microtubule that elongates away from the nucleating structure. Some microtubules may remain attached to their nucleating structures, while others may be released from their nucleating structures and then recaptured by other proteins within the microtubule-organizing center. Still other microtubules may be released and then transported away from the nucleating structure by molecular motor proteins. Which of these options predominates depends on the cell type and stage of development, for example with developing neurons displaying a great deal of transport of microtubules away from the centrosome after their release (4).

Because tubulin is a heterodimer rather than a homodimer, the microtubule is intrinsically polar with beta tubulin at the plus end and alpha-tubulin at the minus end. The plus end of the microtubule is favored for dynamic exchange of tubulin subunits, while the minus end is less favored. The structural polarity of the microtubule exists along its entire length and is recognized by molecular motor proteins, which are enzymes that move along the lattice of the microtubule. Cytoplasmic dynein moves toward the minus end of the microtubule, while most kinesins move toward the plus end. Because different cargoes interact with different motors, the polarity orientation of microtubules in different regions of the neuron is a major determinant of where different cargoes are transported, thus allowing for subcellular localization of cytoplasmic constituents (5). Microtubules in the axon are nearly uniformly oriented, with their plus ends directed away from the soma, while microtubules in vertebrate dendrites are non-uniformly oriented, with roughly equal numbers of microtubules of each orientation (6-8). These distinct microtubule polarity patterns, shown in figure 1, are one of the most essential and earliest developmental differences to arise between axons and dendrites, and are undoubtedly of key importance for many of the compositional and morphological differences that distinguish the two types of processes (8). Other factors that contribute to the delivery of different cargoes to different compartments of the neuron include targeting information within the motor proteins themselves, post-translational modifications of the tubulin subunits that comprise the microtubules in different regions of the neuron, and the microtubule-associated proteins that adorn the microtubules (9).

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Figure 1

Microtubules are oriented differently in the axon and the dendrites

Microtubules in axons are nearly uniformly oriented with plus ends distal to the cell body while microtubules in dendrites are non-uniformly oriented. Microtubules consist of a labile domain (yellow) toward the plus end of the microtubule and a stable domain (red) toward the minus end of the microtubule.

As mentioned above, microtubules in developing neurons are nucleated at the centrosome and then released for transport into axons and dendrites (10-13). Molecular motor proteins transport the microtubules with either plus-end leading (into axons and dendrites) or with minus-end leading (into dendrites only). In this manner, the molecular motor proteins that transport microtubules into axons and dendrites establish the distinct polarity patterns of microtubules in each type of process (14-18). Available data suggest that cytoplasmic dynein is the chief motor protein for transporting plus-end-out (i.e., plus end directed away from the cell body) microtubules into both axons and dendrites, while kinesin-6 and kinesin-12 share the responsibility of transporting minus-end-out microtubules into dendrites but not axons. Recent studies have shown that a protein concentrated in the axon initial segment called tripartite motif-containing protein 46 (TRIM46) may function along the axon's length to preserve the uniform polarity orientation of the microtubules against corruption (19, 20). This function appears to relate to the microtubule-bundling properties of TRIM46. Other studies have shown that the centrosome may become quiescent as the neuron matures (21), such that new microtubules arise only via the severing of existing microtubules or potentially through local nucleation mechanisms.

The microtubule arrays of the neuron consist of individual microtubules that assume a variety of lengths and degrees of stability. Individual microtubules generally do not traverse the entire length of an axon or dendrite. Some microtubules are only a few micrometers in length (or even less), while other microtubules can achieve lengths that exceed a hundred micrometers (22-25). Longer microtubules act both as architectural struts that oppose the retraction of the axon or the dendrite as well as railways for organelle transport. The short microtubules are highly mobile and provide the form by which tubulin is actively transported within the axon and presumably dendrites. A short mobile microtubule may elongate into a long stationary microtubule, or it may entirely or partially depolymerize to yield subunits for other microtubules to elongate.

Microtubule dynamics in cells are governed by a mechanism known as dynamic instability (26-29) that depends on tubulin being a GTPase. Free tubulin associates with hydrolysable GTP. Both alpha and beta tubulin associate with GTP, however the GTP bound to alpha tubulin rests in the region where alpha and beta tubulin interact. Therefore, tubulin heterodimers are only able to hydrolyze the GTP bound to beta-tubulin and this hydrolysis changes the conformation of tubulin dimer-dimer interaction (i.e., the conformation of the protofilaments that make up microtubules). GTP-bound tubulin dimers tend to orient the microtubule in a straight conformation, whereas GDP-tubulin bends the lattice and “spring loads” the microtubule with potential energy that favors catastrophe, which is an explosive bout of disassembly (30). The hydrolysis of GTP-beta-tubulin to GDP-beta-tubulin only happens after the free tubulin has assembled into the microtubule. Because GTP hydrolysis takes time and because dynamics occur principally at the plus end of the microtubule, the region of the microtubule toward the minus end is richer in GDP-tubulin than the region toward the plus end. If GTP hydrolysis catches up to the addition of new tubulin such that there is no longer a ring of GTP-tubulins at the plus end, the microtubule undergoes catastrophe. The microtubule can keep assembling as long as there is a GTP-tubulin “cap” at its plus end. Individual microtubules in a population may undergo assembly while others may at the same time undergo disassembly, depending on whether or not the GTP cap is lost from any individual microtubule.

Microtubules in the population can be stabilized either by the capture of their plus ends, for example by complexes that form at the plus-end of microtubules to promote polymerization and recruit microtubules to the cell cortex (e.g. CLIP and CLASP, respectively), or by proteins that bind to the microtubule lattice (31-33). When a microtubule or a region of a microtubule is stabilized, it may still undergo dynamics, albeit slower. A small portion of the microtubule mass in developing axons is so stable that it appears to undergo no subunit exchange whatsoever, and is resistant to factors such as cold, calcium and microtubule-depolymerizing drugs that cause microtubules to disassemble. This hyper-stable (traditionally called “cold-stable” due to its capacity to resist depolymerization in response to cold in biochemical preparations) portion of the microtubule mass is stabilized by polyamination of the tubulin, and comprises a much larger fraction of the microtubule mass in adult neurons relative to developing neurons (34, 35). Polyamination is different from other known modifications of tubulin associated with microtubule stability, namely acetylation and detyrosination, as these other modifications do not confer stability but rather accumulate on microtubules that are long-lived (36-39). In neurons, available data indicate that different microtubule stability classes exist as domains on individual microtubules, rather than as separate populations of microtubules. As shown in figure 1, a typical microtubule in a developing neuron is, on average, about half stable and half labile, with the stable domain toward the minus end of the microtubule and the labile domain toward the plus end of the microtubule (40). Polyaminated tubulin is sparse in developing neurons, but represents a substantial portion of the total tubulin in adult neurons. It has been posited that the polyaminated regions of microtubules are interspersed as patches along the microtubule (presumably within the stable domain), but this has not been sufficiently explored.

After decades of study, mystery still surrounds the factors and pathways that account for the stability properties of neuronal microtubules (9). Many different proteins have been credited with stabilizing microtubules, including traditional MAPs (microtubule-associated proteins) such as tau, MAP2 and MAP1b, as well as other proteins that bind to the microtubule lattice such as doublecortin (41-43). Most proteins that interact with microtubules can stabilize them in vitro or if overexpressed in cells, assuming the concentration is high enough. Tau and its related family members, MAP2 and MAP4, have repeated microtubule-binding domains that are thought to bind in a series along the microtubule lattice to hold it together and thereby prevent depolymerization (33). However, the on/off rate of these proteins with the microtubule in living cells is only milliseconds (44), which seems inconsistent with tau or its family members stabilizing microtubules in a traditional sense of strong stabilizing protein-protein interactions. Curiously, tau and MAP1b are more enriched on the labile domains of microtubules in the distal region of growing axons than on microtubules in the main shaft of the axon where stable and labile domains intermingle (45-47). This suggests that these proteins may be more important for regulating stability than conferring stability. MAP6 (also called stable tubule only peptide) is more enriched on the stable microtubule domains (48), and hence may be a better candidate for endowing stable domains with their stability properties. One possibility is that microtubule stability in neurons is the result of the combination of many different stabilizers, but another possibility is that many proteins that display microtubule-stabilizing properties in vitro or when overexpressed in cells may not be stabilizers of microtubules in the physiological context.

Certain proteins promote microtubule depolymerization. Examples are stathmin and its neuron-specific variant called SCG10, which shift microtubules toward disassembly by sequestering tubulin subunits and by promoting catastrophe, as well as certain “depolymerizing” kinesins (kinesin-8 and kinesin-13) that use the forces of ATP hydrolysis to remove tubulin subunits from the ends of microtubules (49, 50). Fidgetin, a novel microtubule severing protein, appears to target labile domains of microtubules, creating short microtubule fragments that quickly depolymerize into free tubulin (51). Proteins that depolymerize microtubules can promote reorganization of microtubule networks by liberating tubulin from one microtubule for incorporation into other microtubules elsewhere in the cell. Such transfer of tubulin subunits from one microtubule to another occurs via passive diffusion of free tubulin, but can be augmented by active transport mechanisms, given that tubulin can be incorporated into short polymers that are transportable by motor proteins over longer distances before yielding their subunits for the elongation of other microtubules.

+tips are proteins that associate with the plus end of the microtubule during bouts of assembly. Some have their own affinity for the plus end of the microtubule, by recognizing the GTP cap, while others have an affinity for other +tips and become +tips themselves for that reason. These proteins include EB1, EB3, CLIPs, CLASPs and others (52). Fluorescently conjugated +tips are useful for live-cell imaging of microtubule assembly and organization in cells. In neurons, +tips can influence the assembly dynamics of the microtubule, and integrate its behavior with a variety of proteins and structures (53). +tips are also important players in the “search and capture” mechanism of microtubule-stabilization, wherein a microtubule undergoing bouts of dynamic instability can be selectively stabilized by plus end capture by other cellular structures. This can be important, for example, in stabilizing microtubules on the side of a cell corresponding to the direction of migration, in response to an environmental cue. In this regard, +tips have important roles in growth cone behaviors (54). Recent studies suggest interesting relationships between +tips and more classic microtubule-associated proteins, for example with overexpression of EB1 rescuing the axogenesis impairment phenotype of MAP1B knockout (55).

Recent studies have revealed a new category of proteins called calmodulin-regulated spectrin-associated proteins (CAMSAPs) (56). CAMSAPs, sometimes referred to as minus-end binding proteins, bind to free minus-ends of microtubules in various cells, and thereby block dynamics at that end of the microtubule (57). Vertebrate neurons express CAMSAP-1, CAMSAP-2, and CAMSAP-3, with CAMSAP2 being the predominant CAMSAP in these cells. Immunostained or ectopically-expressed fluorescently-tagged CAMSAPs appear as short stretches along the microtubule toward its minus end. This suggests that CAMSAPs compete with tubulin subunits for the minus end of the microtubule, until enough CAMSAP accumulates to limit any further dynamics. Depletion of CAMSAPs from neurons has detrimental effects on microtubule levels and stability and results in underdeveloped neuronal morphologies (58). Additional information on CAMSAP distribution in neurons will potentially reveal important insights into microtubule regulation in various neuronal compartments.

Microtubule-related proteins are AAA enzymes that form hexamers on the surface of the microtubule. The hexamers yank on a tubulin subunit to extract it from the microtubule lattice, causing the microtubule to break (59, 60). Such severing of microtubules can occur near their minus ends within the centrosome to release the microtubule so that it can then be transported into an axon or a dendrite. Severing can also occur at the plus end so that subunits are peeled off the more dynamic end of the microtubule. If the severing occurs in the stable domain, the result is two new microtubules, one with a stable and labile domain, and the other exclusively a stable fragment that can then assemble a new labile domain. In this manner, severing in the stable domain creates new microtubules. This is important for axonal and dendritic growth and branching to supply new microtubules for elongation and engorgement; thus reducing the dependence on new microtubules from the centrosome. Mobile microtubules observed in the axons of cultured neurons are generally about 7 micrometers in length and stable (61, 62), and hence presumably arise from the severing of longer microtubules in their stable domains. If the severing occurs in the labile domain, the result would be one microtubule with a stable domain and a shorter labile domain, as the microtubule fragment without a stable domain would presumably vanish as it would completely depolymerize (63). Thus, severing in the labile domain would not create new microtubules, but rather would pare down the labile domains, keeping them shorter than they would otherwise be. It is likely that the existence of multiple severing proteins allows for certain ones to target the stable domain and others to target the labile domain. Tight regulation of the expression levels and activities of the various severing proteins could thereby impose control over the expansion or tamping back of the microtubule array during axon growth, non-growth or retraction. This would be reminiscent of the circumstances in Drosophila cells undergoing mitosis, wherein katanin, spastin, and fidgetin play unique roles in the microtubule-mediated movement of chromosomes (64).

Katanin and spastin are microtubule-severing proteins with a preference for severing in the stable domain of the microtubule, resulting from a preference for tubulins that have been post-translationally acetylated or polyglutamylated (65, 66). Suppressing katanin or spastin can be deleterious to axonal growth and branching (13, 67-69), as would be expected, given the important roles these proteins play in generating new microtubules via the severing of existing ones. Recent data suggest that fidgetin targets labile domains of microtubules through a preference for tubulins that are not post-translationally acetylated (51). Knocking down fidgetin causes axons of fetal cortical neurons to grow longer, potentially due to elongation of the labile domains of microtubules (51). Thus knocking down or inhibiting proteins that prune the labile domain, such as fidgetin, may be a useful therapeutic approach for elevating labile microtubule mass in injured or diseased axons.

Why do cells so tightly regulate tubulin post-translational modifications that do not impose stability on microtubules directly, but often reflect their stability? A great deal of evidence suggests that post-translational modifications of tubulin impose a “code” on the microtubule that is read by other proteins (37, 70). The idea is that many microtubule-related proteins have preferential affinities for microtubules (or domains of a microtubule) that are enriched or deficient in certain modified tubulins. For example, kinesin-1 prefers microtubules rich in detyrosinated and acetylated tubulins, while kinesins 5 and 13 prefer microtubules rich in tyrosinated tubulin (71-73). Thus, the post-translational modifications of a microtubule may make it a more or less favored substrate for proteins such as molecular motors. Other examples of proteins sensitive to the tubulin code include microtubule-severing proteins, as discussed earlier (51, 65, 66), and certain +tips that have a preference for tyrosinated over detyrosinated tubulin (74). This is important because when experiments demonstrate a functional role for stable or labile microtubules (or domains of a microtubule), it may not be the stability properties of the microtubule, per se, that is relevant so much as the tubulin modifications that accompany the microtubule's stability properties.

The tubulin code is regulated by highly conserved and tightly regulated enzymes, and for some modifications, the enzymes orchestrate a cycle wherein the modification is imposed only after assembly of the tubulin into the microtubule and only reversed when the tubulin subunits are liberated from the microtubule during bouts of disassembly. For example, the enzymes may be differentially enriched or regulated in axons versus dendrites, enabling axonal microtubules to be richer in post-translationally modified tubulin subunits compared to dendritic microtubules. These enzymes provide a hub for regulation of the tubulin code, and may also provide an avenue for therapeutics, as they can be experimentally or clinically manipulated to change the tubulin code in a manner that might be conducive to reversing the ill effects of disease.

A recent article on nerve regeneration after injury provides a good example of the potential for therapeutics, based on manipulating the tubulin code. Transgenic mice with constitutively active GSK3 displayed decreased detyrosination of microtubules in growth cones of injured axons as well as improved sciatic nerve regeneration (75). Drugs that inhibit tubulin detyrosination resulted in similar regenerative enhancing effects, whereas microtubule stabilization negated these effects. These findings support the notion that manipulating the tubulin code has different effects than simply manipulating microtubule stability, thus providing different tools to achieve changes in the microtubule array that may assist in treating various disease or injury scenarios.

The various aspects of the neuronal microtubule arrays thus far discussed can be corrupted over the life of the neuron by the aging process as well as by neurodegenerative disease mechanisms. For example, the axon must preserve a nearly uniform polarity orientation of microtubules in order for the anterograde and retrograde transport of various cargoes to be properly orchestrated. Corruption of the microtubule polarity pattern (i.e., appearance of too many mal-oriented microtubules) would send cargoes moving in the wrong direction and potentially cause traffic jams. Preserving the microtubule polarity pattern of the axon is ongoing work for the neuron, requiring cytoplasmic dynein to transport mal-oriented microtubules back to the cell body (76). This clearing mechanism could be overwhelmed by pathogenic factors that promote the formation of mal-oriented microtubules or the flipping of short microtubules. The clearing mechanism itself could also be degraded by pathology. This has not yet been explored in the context of known diseases, but mutation of the fly ortholog of an epilepsy gene causes notable microtubule polarity flaws in the axon (77).

Microtubule loss (i.e. reduction in microtubule mass) from axons and dendrites is often associated with neurodegenerative diseases (78, 79). This is best documented in diseases called tauopathies, in which tau dissociates from microtubules as a result of abnormal phosphorylation (80, 81). Pure tauopathies result from mutations that render tau prone to such effects. Tau is not mutated in Alzheimer's disease, but rather becomes hyper-phosphorylated in response to abnormal amyloid-β (82). Popularly, microtubules are said to disintegrate as they lose tau, due to the microtubules being destabilized (27). Such a mechanism would suggest that stable domains of microtubules become labile, which would then somehow lead to a degradation of the microtubule mass. Another theory posits that detachment of tau from microtubules causes them to become more sensitive to microtubule-severing proteins, mainly katanin (83, 84). There has also been evidence that tau tangles themselves can promote microtubule depolymerization (85), and the same could be true of soluble abnormal tau. For example, abnormal tau, either in the form of soluble protein or in the form of abnormal filaments, is known to produce toxicity that could theoretically contribute to microtubule loss via Tau's phosphatase-activating domain (86). When tau abnormally enters dendrites during Alzheimer's disease, recent evidence suggests that dendritic microtubules degrade because they become more polyglutamylated and hence more sensitive to spastin (87). Loss-of-function and gain-of-function mechanisms may apply to various neurodegenerative disorders in which loss of microtubule mass or a change in microtubule dynamics or stability has been observed, including Amyotrophic Lateral Sclerosis, Hereditary Spastic Paraplegia, Parkinson's disease, and others (88-91). Figure 2 shows potential mechanisms of microtubule loss during axonal degeneration, as well as a potential contribution to axonal degeneration of microtubule polarity flaws, as discussed earlier.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| TUBB3 | R262H, R262C | Intermediate | Mouse | Defects in axon guidance | Tischfield et al., 2010 |
| TUBB2B | E421K | C-terminal | Mouse | Axonal dysinnervation | Cederquist et al., 2012 |
| TUBB2B | S172P | N-terminal | Rat | Impaired microtubule assembly, defective migration of cortical neurons | Jaglin et al., 2009 |
| TUBB2B | F265L | Intermediate | Rat | Impaired microtubule assembly, defective migration of cortical neurons | Jaglin et al., 2009 |
| TUBB2A | N247K | Intermediate |  | Cortical dysplasia | Cushion et al., 2014 |
| TUBB3 | T178M, E205K | N-terminal |  | Malformation of cortical development and neuronal migration defect | Poirier et al., 2010 |
| TUBB3 | A302V, M323V | Intermediate |  | Malformation of cortical development and neuronal migration defect | Poirier et al., 2010 |
| TUBB4A | D249N | Intermediate |  | Leukodystrophy | Simons et al., 2013 |
| TUBB4A | R2Q, T178R | N-terminal |  | Leukodystrophy | Miyatake et al., 2014 |
| TUBB4A | R53Q | N-terminal |  | Hypomyelinating leuko-encephalopathies | Miyatake et al., 2014 |
| TUBA4A | W407X | C-terminal |  | ALS | Smith et al., 2014 |
| TUBB4A | R2G, R53G | N-terminal |  | DYT4 dystonia dysphonia | Lohmann et al., 2013 |
|  |  |  |  |  |  |

DYT4 dystonia dysphonia

TDP-43

Genetic mutations in the microtubule-severing enzyme Spastin, is most commonly associated with hereditary spastic paraplegia (HSP) (Hazan et al., 1999). Spastin loss of function results in local accumulation of detyrosinated microtubules (Tarrade et al.,2006) and reduced number of dynamic plus-ends marked by EB3 along the axon shaft (Fassier et al.,2013). These mutations also result in axonal swellings which can be rescued by treatment with microtubule-destabilizing drugs such as Nocodazole (Fassier et al.,2013). This hyper-stability of microtubules likely triggers the progressive degeneration of corticospinal tracts in the Spastin-dependent HSP cases(Hazan et al., 1999; Evans et al.,2005). Collectively these studies demonstrate that reduced dynamics of microtubules is also detrimental to neuronal health.

Frontotemporal dementia (FTD):

Loss of nuclear-cytoplasmic transport due to tau accumulation in cell body, leading to track errors.

Dysfunction of MAP2/tau family proteins in prion disease

There are more than 80 Ser/Thr phosphorylation sites and 5 potential tyrosine phosphorylation sites in human tau. The biological activity of tau is regulated by the degree of its phosphorylation. Low phosphorylation level of tau in normal brain is required for its interaction with tubulin, in order to promote its assembly into microtubules and help stabilize their structure.

Some kinases and phosphatases have been implicated in hyperphosphorylation of tau. The main aberrantly hyperphosphorylated sites on tau include the phospho-sites Ser202/Thr205, Thr-231/Ser-235, Ser-262, Ser-396/404 and Ser422.11-14 Phosphorylation of Ser202/ Thr205, Thr-231/Ser-235 and Ser-262 significantly decreases the interaction of tau with microtubules. Phosphorylation of Ser-396/404 increases the propensity of tau to oligomerize and eventually form filamentous aggregates.

Cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β) have been identified as major candidates mediating tau phosphorylation at disease-associated sites. Ser-396/404 is a major target of GSK3β, while Ser202/Thr205, Thr-231/Ser-235 and Ser-262 are major targets for CDK5. CDK5 is an atypical cyclin-dependent kinase and its activity is dependent upon interaction with p35/p39.

Expression of GSK3β in the brains of scrapie-infected animals is downregulated and its target p-tau (Ser396/Ser404) is depressed as well. In contrast, the activities of both CDK5 and GSK3β are demonstrated to be increased, which are involved to regulate tau hyperphosphorylation in Alzheimer disease.

The different pattern of two kinases between AD and prion disease may lead

to different pathological effects of tau. In prion disease, the predominant phosphorylation of tau are those that decrease the ability of tau to bind microtubules by CDK5 rather than those increase the ability of tau to self-associate and aggregation by GSK3β. That might explain why it is hard to observe NFTs in most types of prion diseases, except in some GSS cases.

End-binding proteins (+ end)

Regulator Proteins

Tubulin modifier enzymes

Acetylation

Tyrosinylation

Motor Proteins

Kinesin

Dynein

Anchoring proteins (- end)

γ-TuRCs

CDK5RAP2

Nedd1

ninein

Tubulins

α-tubulin (TUBA genes)

β-tubulin (TUBB genes)

γ-tubulin

End-binding proteins (+ end)

CLIPs

CLASPs

Regulator Proteins

Lateral Stabilizer Proteins

MAP2

~~MAP4~~

MAPT/Tau

Destabilizers/severing proteins

Katanin

Spastin

Tubulin modifier enzymes

Acetylation

Tyrosinylation

Motor Proteins

Kinesin

Dynein

Acetylation/deacetylation of a-tubulin K40 occurs within the microtubule lumen; catalyzed by acetyltransferases aTAT and Atat-2, whereas b-tubulin can be acetylated on K252 by the Sun acetyltransferase leading to a block of free tubulin assembly [Magiera and Janke, 2014]. Deacetylation, on the other hand, is executed by the histone deacetylase 6 (HDAC6) and nicotine adenine dinucleotide-dependent deacetylase sirtuin-2 (SIRT2) [Hubbert et al., 2002]. The anti-depressant action of HDAC inhibitors in a variety of rodent tests for depression has been recently reviewed [Fuchikami et al., 2015]. Although most HDACs act on histones in the nuclear compartment, HDAC6 is cytosolic and acts on tubulin.

Tyrosination/detyrosination of a-tubulin occurs in cycles. Detyrosination confers stability whereas tyrosination increases microtubule dynamics. Catalyzed by tubulin tyrosine ligase (TTL), tyrosination is important in neurons during axonal growth and transport [Magiera and Janke, 2014]. Detyrosination refers to the removal of Tyr from the C-terminal of a-tubulin to expose a Glu residue. Glutamylation takes place when the side chains of glutamates are added to the carboxy terminal tails of a– and b-tubulin by enzymes belonging to the tubulin

tyrosine ligase-like (TTLL) family. This occurs at high levels in the nervous system. Deglutamylation is catalyzed by cytosolic carboxypeptidase enzymes, which are capable of removing glutamate side chains. Polyglutamylation changes the surface charge of tubulin, and influences the binding of MAPs and motor proteins [Janke, 2014].

Indeed impaired protein transport and disrupted binding of MAPs is proposed to contribute to the underlying pathology in schizophrenia [Ikegami et al., 2007]. The TTLL11 polyglutamylase is linked to schizophrenia where a combined disruption in TTLL11 (a balanced t(9;17) (q33.2;q25.3) translocation, and a microduplication at 16p13.1) is associated with an additive predisposing effect [Fullston et al., 2011]. Interestingly, this same chromosomal aberration is also linked to bipolar disorder [Rajkumar et al., 2014].

Polyglycylation of a- and b-tubulin is analogous to polyglutamylation except that of glycines are added instead of glutamates. This modification is limited to cilia and flagella. The responsible enzymes are again members of the TTLL family [Fukushima et al., 2009]. Tubulin glycylases and glutamylases are important for stabilization and motility of ependymal cilia, which line the ventricles of the brain [Grau et al., 2013], while interestingly, neuropsychiatric risk genes have been shown to converge on regions encoding proteins found in cilia [Marley and von Zastrow, 2012].