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MAP code and regulation of microtubule-based processes
MAP kód a regulace procesů souvisejících s mikrotubuly

Bachelor thesis

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Tímto prohlašuji, že předkládaná bakalářská práce s názvem „MAP kód a regulace procesů souvisejících s mikrotubuly“ je mojí vlastní originální prací. Potvrzuji, že informace získané z odborné literatury jsou citovány v textu a uvedeny v poskytnutém seznamu zdrojů. Tato práce nebyla předložena, zcela ani zčásti, k získání jiného či stejného akademického titulu.

I hereby declare that the present bachelor’s thesis titled “MAP code and microtubule-based processes” is my own original work. I confirm that the information derived from the literature has been fully acknowledged in the text and in a list of references provided. The present thesis has not been submitted, in whole or in part, for any other degree.

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Abstract

Microtubule associated proteins (MAPs) are considered as key regulators of molecular trafficking in cells. Even though their malfunctioning results in severe pathologies, such as neurodegenerative disorders, the regulatory roles of these proteins remain under debate. Since MAPs bind to the cytoskeleton, this structure has to be vital for the function of MAPs. Microtubules, a highly dynamic type of cytoskeletal structure, have been given extra attention due to their association with cell division and vital functions in neurons. Microtubules can undergo post-translational modifications that affect molecular motors as well as binding of other proteins, such as MAPs. Whether post-translational modifications of microtubules regulate the distribution of MAPs is so far not sufficiently documented. However, MAPs have been shown to cooperatively form cohesive envelopes on the microtubules and thereby regulate the access of motors and severing enzymes. As there are many types of MAPs and they are mutually exclusive, a hypothesis of a regulatory 'MAP code' emerged recently in the literature. Using available literature, this review will try to introduce the new model of MAP code and provide some background information on previous research on this topic.

Key words: cytoskeleton, microtubule-associated proteins, MAP code, microtubules, tubulin code, molecular motors, neurodegenerative diseases, post-translational modifications, microtubule severing enzymes

Abstrakt

Proteiny asociované s mikrotubuly (MAPs) jsou považovány za klíčové regulátory molekulárního transportu v buňkách. Ačkoli jejich nesprávné fungování vede k závažným patologiím, jako jsou neurodegenerativní poruchy, regulační role těchto proteinů zůstávají nejasné. Jelikož se MAPs váží na cytoskelet, musí být tato struktura pro funkci MAPs zásadní. Mikrotubulům, vysoce dynamickému typu cytoskeletální struktury, byla věnována zvláštní pozornost kvůli jeho asociaci s buněčným dělením a vitálními funkcemi v neuronech. Mikrotubuly mohou projít post-translačními úpravami, které ovlivňují molekulární motory a také vazbu dalších proteinů, jako jsou MAPs. Doposud není dostatečně zdokumentováno, jestli post-translační úpravy mikrotubulů regulují distribuci MAPs. Ukázalo se však, že MAPs spolupracují na vytváření soudržných obalů na mikrotubulech a regulují přístup molekulárních motorů a enzymů rozdělujících mikrotubuly. Jelikož existuje mnoho typů MAPs a ty se vzájemně vylučují, objevila se nedávno v literatuře hypotéza regulačního ‚MAP kódu '. Pomocí dostupné literatury se tato práce pokusí představit nový model MAP kódu a poskytnout některé základní informace o předchozím výzkumu v tomto tématu.

Klíčová slova: cytoskelet, proteiny asociované s mikrotubuly, MAP kód, mikrotubuly, tubulinový kód, molekulární motory, neurodegenerativní nemoci, post-translační modifikace, enzymy rozdělující mikrotubuly

Contents

1 Introduction

Intracellular environment is a crowded space, yet all cargo transported within the cell gets delivered to its set location. Cytoskeleton represents a vital structure in cells. Not only it gives the cell its shape, but also it provides molecular highways for the transport of proteins, enables cells to carry out functions like movement and is crucial for cell division. The presence of fibrils in cells has been addressed in the second half of the 20th century and has been of interest ever since (Nature, 2008). However, even after almost 100 years of research, scientists are only beginning to understand the complex mechanisms controlling the intricate cytoskeletal network and its countless functions. The cytoskeleton consists of three networks – actin filaments, intermediate filaments, and microtubules. All of them are complex, dynamic networks of filamentous proteins that have distinct purposes in the cell. Actin filaments are crucial in the muscle contraction, strong intermediate filaments provide mechanical support to cells and microtubules are vital in neurons and signal transduction or cell division. Since many proteins are associated with the cytoskeleton, a complex machinery, that regulates functions of these filaments, exists in the cell. Dysfunctions in this machinery have severe consequences and can result in disease, such as cancer and neurological disorders (Binder et al., 2005; Cahill et al., 1998). In this review, microtubules and its associated proteins will be discussed.

2 Microtubules

Microtubules (MTs) are, next to actin and intermediate filaments, crucial components of the eukaryotic cytoskeleton. They play important roles in spatial distribution of organelles, growth cone or filopodia movement, transport of proteins within the cell, chromosomal alignment and parting during mitosis (Costa et al., 2013; Roeles & Tsiavaliaris, 2019). MTs even participate in decoding light or odorant signals (Nachury & Mick, 2019). MTs are on one hand extremely stable, for example in cilia and flagella, where the depolymerization rate is relatively slow (Baas & Black, 1990; Paturle-Lafanechère et al., 1994). On the other hand, MTs in the mitotic and meiotic spindles are very labile and turn over rapidly (Costa et al., 2013; Rusan et al., 2001). MTs also play crucial roles in neuronal functions, as they provide axons with mechanical properties and structure essential for signal transmission (Kelliher et al., 2019). Since MTs fulfil many different functions within the cell, they have been vastly studied in order to better understand the complexity of these functions. However, the question of how MTs adapt to different functions in the cell remains elusive. In this chapter the MT structure, dynamics and lastly, the problematics of tubulin code will be explained.

2.1 Microtubule structure

MTs are polymers of tubulin. Tubulin has a strikingly conserved sequence throughout evolution. As a result, all eukaryotic organisms form almost identical MT structures (Howes et al., 2017). Tubulin is the building block of MTs. Unpolymerized tubulin exists as a heterodimer of globular α-tubulin and β-tubulin molecules bound with guanosine triphosphate (GTP). The polymerization of MTs happens in the presence of GTP. Upon MT nucleation the GTP gets hydrolyzed into guanosine diphosphate (GDP) and forms protofilaments that align together into hollow tubes with approximately 25 nm diameter (Ludueña, 1998). New microscopic techniques, such as cryo-electron microscopy, have been used to investigate the structure and dynamics of MTs (Alushin et al., 2014; Benoit et al., 2018; Howes et al., 2017; Manka & Moores, 2018).

2.1.1 Tubulin isotypes

Tubulin isotypes are generated via alternative gene expression. Although ‘generic’ α-tubulin and β-tubulin are conserved in evolution, less common tubulin isotypes have evolved in different species (Howes et al., 2017). Some isotypes spring into existence together with new MT functions (Howes et al., 2017). β1-tubulin, for example, appears to be connected only with mammalian hematopoietic cells and is thought to be specialized in the assembly of marginal band (Wang et al., 1986), a bundle of MTs that stabilize platelets (Patel-Hett et al., 2008)(Fig.2I).

The evidence that tubulin isotypes directly determine MT structure was found only recently during a study on *Caenorhabditis elegans* and *Bos taurus* tubulin (Chaaban et al., 2018). However, this concept was proposed earlier in a study of *Drosophila melanogaster* and *Heliothis virescens* β-tubulin isoforms from 1997 (Raff et al., 1997). In this study the expression of the moth β-tubulin in *D. melanogaster* resulted in the formation of 16-protofilament MTs common for *H. virescens,* instead of fly-related 13-protofilament MTs. Together these results show that the structure and consequently also the function of MTs depends on the tubulin isotype, regardless of their evolutionary similarities.

2.2 Dynamics of microtubules

MTs represent a highly dynamic but also very rigid structure. Both of these properties are very important in maintaining intracellular MT architecture (Amos, 2004; Kinoshita et al., 2002). The dynamics of MTs is not consistent everywhere in the cell. Even within one cell, MTs display difference in stability (Amos, 2004). This so-called dynamic instability consists of two phases – growth (polymerization) phase and shrinkage (depolymerization) phase (Mandelkow et al., 1991; Manka & Moores, 2018) (Fig. 1A). During the growth (polymerization) phase, new GTP-tubulin is incorporated

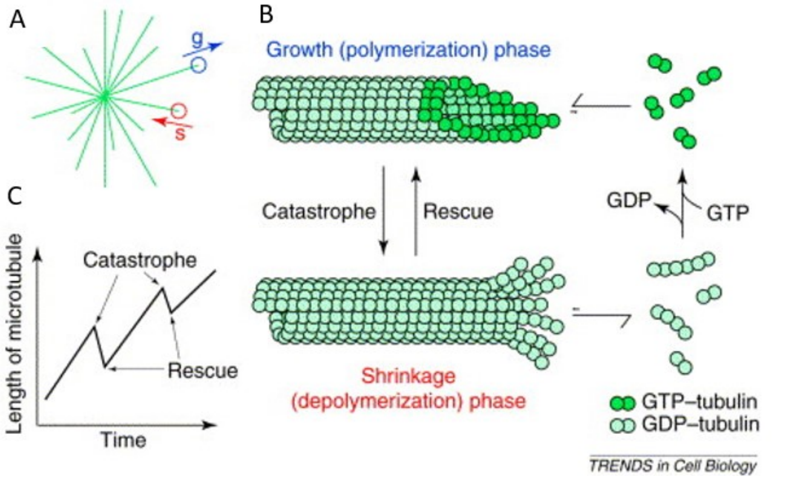

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Fig. 1. Dynamic instability of microtubules. (A) MTs (green) are nucleated in centrosomes. Some MTs are growing (blue arrow), some shrinking (red arrow) at the same time. (B) Growth (polymerization) and shrinkage (depolymerization) of a MT. GTP-tubulin gets hydrolysed into GDP-tubulin after incorporation. (C) Catastrophe and rescue events affect the length and growing speed of a MT. (adapted form Kinoshita et al., 2002)

at the MT end (Alushin et al., 2014) (Fig. 1B). The new GTP-tubulin at the MT end, referred to as the GTP cap, protects the growing tip from catastrophe (Walker et al., 1988). Catastrophe happens when the incorporation of GTP-tubulin is not fast enough and the hydrolyzation to GDP catches up (Fig. 1B). This leads to loss of the GTP cap. Because of the slightly bended conformation of GDP-tubulin in the MT body, the MT without the GTP cap cannot hold together and the MT starts to shrink (Benoit et al., 2018; Meurer-Grob et al., 2001; Walker et al., 1988) (Fig. 1B,C). Depolymerization is stochastically slowed down, giving the MT the ability to switch to the polymerization phase. After that, the MT can either remain the same length or start to grow again, this event is called rescue (Walker et al., 1988) (Fig. 1B,C). The rescue frequency is thought to depend on the concentration of free tubulin in solution (Walker et al., 1988).

Microtubule dynamics is particularly essential for cell division. During chromosome separation, the balance between the MT shrinkage and growth resolves whether the genetic material is correctly divided between the two daughter cells (Costa et al., 2013). Even small errors in this process can have dramatic consequences such as developmental defects or cancer (Cahill et al., 1998; Lengauer et al., 1997; Michel et al., 2001). To control the process and overcome potential errors, complex control mechanisms exist in cells. Such mechanisms involve tubulin modifications and many molecules such as MT associated proteins (Andersen, 2000; Barisic et al., 2015; Gallaud et al., 2014).

2.3 Tubulin code

The idea that posttranslational modifications of tubulins can modulate the function of MTs was proposed as early as the 1970s. Yet, the concept of 'tubulin code' remained a mystery until the beginning of the twenty-first century with the invention of high-resolution microscopy. Recent advances suggest that the combination of tubulin isotypes and post-translational modifications acts as a fine-tuning mechanism, which regulates interactions of MT-binding proteins with the MT lattice. Disruption in this process can impact the homeostasis and result in disease, such as cancer, retinal degeneration, or muscular dystrophies (Bosch Grau et al., 2017; Kato et al., 2004; Kerr et al., 2015).

Tubulin can be modified in many different ways, from phosphorylation (De et al., 2014) and acetylation (Eshun-Wilson et al., 2019), to tyrosination (Barisic et al., 2015; Erck et al., 2005), glutamylation (Valenstein & Roll-Mecak, 2016), and glycylation (Gadadhar et al., 2017). These modifications are thought to program MTs for different functions, affect flexibility and dynamics, recruit other proteins such as motors (McKenney et al., 2016) and severing enzymes (Valenstein & Roll-Mecak, 2016), and play vital roles in mitosis (Barisic et al., 2015).

The distribution of different tubulin modifications is stereotyped in cells. Different types of modifications are present in different cytoskeletal structures as well as in phases of development and cell cycle. For example, axons tend to be detyrosinated which makes them more stable (Webster et al., 1987) (Fig. 2D). Detyrosination of spindle MTs works as a navigation system that guides kinetochore motors during cell division (Barisic et al., 2015) (Fig. 2B). Growth cones, on the other hand, are enriched in tyrosination which makes them dynamic and contributes to the arrangement of MTs and actin in the growing tip (Marcos et al., 2009; Webster et al., 1987) (Fig. 2D). Finally, glutamylation and acetylation are enriched on MTs with long lifetimes, such as in cilia, flagella or axons (Valenstein & Roll-Mecak, 2016) (Fig. 2E,G,H). For more examples please refer to Fig. 2 (reviewed in Yu et al., 2015).

It is still not understood how the distribution of tubulin isotypes affects MTs and motor functions or how the tubulin code can be deciphered by the cell. Post-translational modifications of tubulin affect the recruitment and velocity of kinesin motors only mildly (Kaul et al., 2014), suggesting that tubulin code may not be the main supervisor of motor-driven transport in cells.

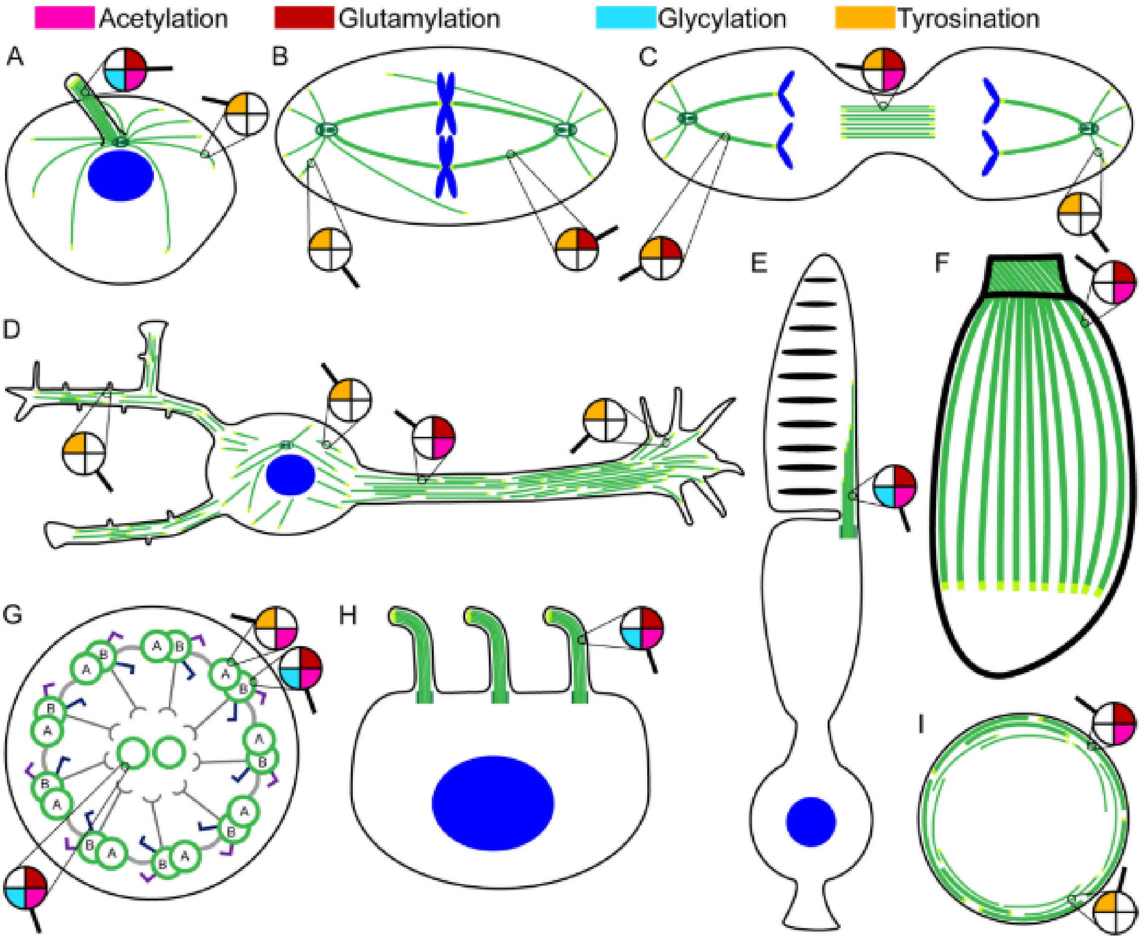

Fig. 2. The tubulin code. (A) Radial MTs in interphase. (B) Mitotic spindle. (C) Midbody array in telophase. (D) Neuron – MTs with parallel polarity in axons and MTs with mixed polarity in dendrites. (E) MTs connect the inner and outer segments of photoreceptor cells and are important for signal transduction (Nachury & Mick, 2019). (F) Special type of MT arrangement called subpellicular microtubules found in some single cell eukaryotes. MTs in these organisms are located immediately below the plasma membrane (Souza & Attias, 2010). On the apical pole there is a hollow cone-like structure consisting of special MTs termed the conoid (Scholtyseck et al., 1970). (G) Cross-sectional view of the MTs in cilia or flagella. Light gray – nexin linkers; dark gray – radial spokes; dark blue – inner arm dyneins; purple – outer arm dyneins. (H) Motile cilia. (I) Marginal band of MTs in blood platelets. Apart from post-translational modifications stated here, tyrosination also localizes with platelet MT coils and marks highly dynamic MTs found in the band (Patel-Hett et al., 2008). MTs – green; MT plus-ends – light green; nuclei – blue. Tubulin post-translational modifications are indicated by magnifying glasses. (adapted from Yu et al., 2015)

3 Cytoskeletal transport

Cytoskeletal transport is very important in maintaining cellular functions since it delivers vital cargo, such as mitochondria, Golgi apparatus vesicles or proteins essential in synapses between neurons. Molecular motors are proteins that mostly provide the molecular trafficking. These motors are traveling along the MT fibers, thus the interaction between those two structures is essential. In this section molecular motors will be discussed.

3.1 Molecular motors

The movement of cargo in cells is driven by anterograde and retrograde transport along the MTs. This long-distance transport is driven by motors from the kinesin family and cytoplasmic dynein that travel towards the MT plus and minus ends, respectively (Paschal & Vallee, 1987; Vale et al., 1985). Molecular motors have the ability to deliver cargo to remote destinations sometimes further than one meter from the cell center. It remains unclear what helps navigate motors within the packed intracellular space and it has been indicated that this information could be encoded on the MT tracks.

3.1.1 The movement cycle of molecular motors

To explain the movement cycle of molecular motors, the mechanical movement of kinesin as a representative molecular motor will be described. Kinesin has two 'heads' and one 'tail'. The tail connects the motor with its cargo and the heads bind to the MT lattice. Apparently, kinesin need both heads to move processively (Hancock & Howard, 1998). It has been proven that kinesin walks according to the hand-over-hand model, in this case the 'head-over-head' model (Hancock & Howard, 1998; Yildiz et al., 2004). In other words, kinesin literally 'steps' along the MT. In the hand-over-hand model at least one head is always attached to the MT surface, allowing kinesin to walk processively (Hancock & Howard, 1998). Kinesin is an ATPase and uses ATP for its movement. While one head is attached to the MT lattice (white in Fig. 3), the other head (black in Fig. 3) is in the ADP (D) state and is not bound to the MT lattice (Fig. 3 (i)). The binding of ATP (T) changes the motor conformation which results in the swing of the ADP-bound head (black) forwards (Fig. 3 (ii)). The ADP-bound head then binds to the MT which creates tension in the coiled-coil stalk and leads to the release of the tailing head (Fig. 3(iii)). The tailing head releases Pi and gets to the ADP bound state (Fig. 3(iv)). This ATP-hydrolysis cycle powers the molecular motor and enables its progressive movement (Carter & Cross, 2005; Hancock & Howard, 1998; Yildiz et al., 2004).

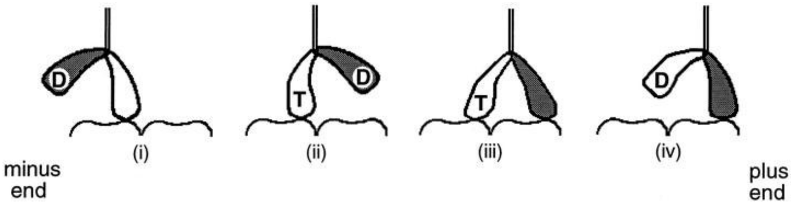

Fig. 3. The hand-over-hand model. Nucleotide states during the motion are presented by letters D (ADP) and T (ATP). The kinesin heads are distinguished by colors (black/white). For the mechanics of the kinesin movement please refer to the text. (adopted from Hancock & Howard, 1998)

3.1.2 Kinesin

Kinesins are a family of molecular motors that mostly drive the movement of cargo from the minus to the plus ends of MTs (Vale et al., 1985). It has been reported that obstacles on the MTs reduce the processivity of kinesin-1, one of the major long-distance transport motors (Telley et al., 2009). Therefore, it remains unclear how these motors are able to walk long distances with great efficiency along densely decorated MTs. Recent studies propose that kinesins are able to work in teams and cooperatively bind to transported cargo (Beeg et al., 2008; Kural et al., 2005; Li et al., 2018). This teamwork strongly increases the run-length (the walking distance) and could partially explain higher transport speeds seen *in vivo* compared to the transport speeds *in vitro* (Beeg et al., 2008; Kural et al., 2005). Kinesin moves forward to the MT plus end and can occasionally take backward steps, yet all movement is only parallel to one MT protofilament. Kinesin was reported to be unable to make sideways steps, which is not true for the retrograde transport motor dynein (Carter & Cross, 2005; Ferro et al., 2019; Ray et al., 1993). However, new studies show that even though single kinesin molecules cannot step around obstacles, cargo carried by multiple kinesins can (Ferro et al., 2019). This suggests that kinesin can bypass obstacles (such as microtubule-associated proteins) the same way that dynein can, by changing the protofilament it walks on (Ferro et al., 2019). However, the mechanism of kinesin cooperation remains largely unexplained.

Proteins associated with MTs have also been reported to affect kinesin recruitment or exclusion, run-lengths and velocity (for example in Chaudhary et al., 2019; Henrichs et al., 2020; Monroy et al., 2018, 2020). The effect of microtubule-associated proteins on molecular motor transport will be covered in further detail in sections 4.2 and 5.

3.1.3 Cytoplasmic dynein

Cytoplasmic dynein (dynein) walks the opposite way kinesin does – from the plus to the minus end of MTs. Although there are many members of the kinesin motor family, there is a single isoform of dynein responsible for nearly all retrograde transport (Ferro et al., 2020). Dynein is an exceptionally large protein complex (~1.5 MDa) consisting of many chains (Burgess et al., 2003). Dynein requires a co-factor dynactin for its activation and processive movement (Fig. 4). Dynactin has been suggested to play a role in cargo binding through the interaction with cargo-adaptor proteins (Qiu et al., 2018). Also, dynactin is believed to recruit and tether dynein to the MT (Ayloo et al., 2014). In summary, dynactin is a crucial component of the retrograde transport complex (Fig. 4).

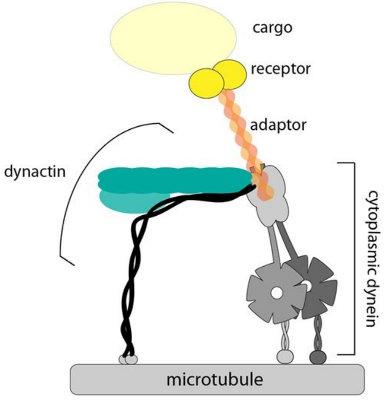

Fig. 4. Cytoplasmic dynein complex. The adaptor refers to cargo-adapter proteins (such as BicD2). The receptor can be for example Rab6. (adapted from Dodding, 2014)

Although yeast dynein displays processive motility on its own, purified mammalian dynein walks poorly by itself, suggesting that its movement is only possible with the presence of an activator (McKenney et al., 2014). Dynein BicD2 is a cargo-adaptor protein which is found on the cargo and facilitates an interaction between dynein and dynactin, forming a stable dynein-dynactin-BicD2 (DDB) complex (Fig. 4). DDB is able to walk robustly and processively towards the minus-end of the MT, exceeding the performance of yeast dynein in both velocity and run length (McKenney et al., 2014). In addition to BicD2, several other cargo-adaptor proteins have been identified, such as Hook (early endosomes), Rab11-FIP3 (Rab11-positive cycling endosomes) or Spindly (kinetochores), that work in similar ways to BicD2 (reviewed in Dodding, 2014). Dynein is recruited to specific cargos by coiled-coil adaptor proteins raising the possibility that regulation of the retrograde transport is driven by cargo-adaptor proteins, rather than dynein itself (Ferro et al., 2020).

Moreover, as mentioned above, dynein is able to take sideways and backwards steps (Can et al., 2014; Ferro et al., 2019). It has been reported, that unlike kinesin, dynein prefers helical trajectory, spinning around the MT as it progresses towards the minus end (Can et al., 2014). This ability may allow dynein to bypass obstacles on MTs and explain partly controversial results of the effect of microtubule-associated proteins on dynein motility (Can et al., 2014; Ferro et al., 2019). This issue will be further discussed in section 4.2.

4 Microtubule-associated proteins

Non-motile proteins with high affinity to MTs are usually referred to as ‘microtubule-associated proteins’ (MAPs). However, all proteins that interact with MTs could be considered MAPs, including molecular motors, plus- and minus-end-tracking proteins or severing enzymes. In this review, however, I will be referring to structural or classical MAPs which solely include non-motor proteins that bind and diffuse on the entire MT lattice and alter the properties of MTs such as polymerization, stability, and bundling.

Cryo-electron microscopy has proved very useful for visualization of MAPs on the MT surface. The footprint of many MAPs has been visualized using this technique, although not all footprints have been determined yet. Single molecule microscopy techniques such as TIRF (total internal reflection fluorescence) microscopy, a special type of fluorescence microscopy, was also used to study the interactions between MTs and MAPs. Such experiments show for example the existence of liquid condensates or sheets of MAPs on the MT, as seen for tau (Siahaan et al., 2019; Tan et al., 2019).

Spatiotemporal association patterns of MAPs are vastly studied because of their possible association to different functions of MAPs. Due to the connection to some neurodegenerative diseases, MAPs have been extensively studied in neurons. In these special cells the proper molecular trafficking is crucial for signal transferring. Neurons consist of a cellular body, multiple dendrites, and a single axon. It has been shown that dendrites and axons possess different MAPs and that the distribution of these MAPs influences the type of molecular motor that is recruited to the MT lattice (Monroy et al., 2018). Consequently, the imbalance of MAP spacial distribution (such as MAP7 or tau overexpression) can lead to abnormal organelle distribution within the neuron (Monroy et al., 2018). Especially in neurons, the perturbations of the organelle distribution has serious consequences and can result in neurodegenerative diseases such as Alzheimer's disease (Ebneth et al., 1998).

4.1 Types of MAPs

All MAP1 members (MAP1A, MAP1B and MAP1S)(Fig. 5) are abundant in the nervous system, where they are believed to function during development and maintenance (Meixner et al., 2000; Takei et al., 2015). MAP1A (Fig. 5) is essential for neuronal function especially in synaptic plasticity (Takei et al., 2015). MAP1A is believed to anchor NMDA receptors to MTs. That results in proper function of long-term potentiation and long-term depression, associated with learning and memory (Takei et al., 2015). MAP1B (Fig. 5) plays an essential role in neurite growth and synapse maturation during development of the nervous system (Benoist et al., 2013; Meixner et al., 2000). Consistent with this notion, MAP1B null allele mice display serious developmental defects, for example absence of corpus callosum (Meixner et al., 2000). Like MAP1A, MAP1B was found to have an effect on synaptic plasticity. It specifically impairs AMPA receptors endocytosis and therefore long-term depression (Benoist et al., 2013). And finally, the third member of the MAP1 family, MAP1S (Fig. 5), can be found in other organs such as the spleen, liver or heart and is believed to be essential for MT stability, nucleation and polymerization velocity during interphase and mitosis. MAP1S was shown to be especially important at the transition between those two stages, ensuring accurate cytokinesis (Tegha-Dunghu et al., 2014). There is a variety of interactions of MAP1 with different receptors and channels in neurons such as with neurotransmitter receptors (ex., GABAc, NMDA, AMPA) (Benoist et al., 2013; Takei et al., 2015). Consequently, the disfunction of MAP1 members has strong impact on neuronal function and is connected with neurological diseases such as Alzheimer's or Parkinson's disease (Chan et al., 2014; Gevorkian et al., 2008).

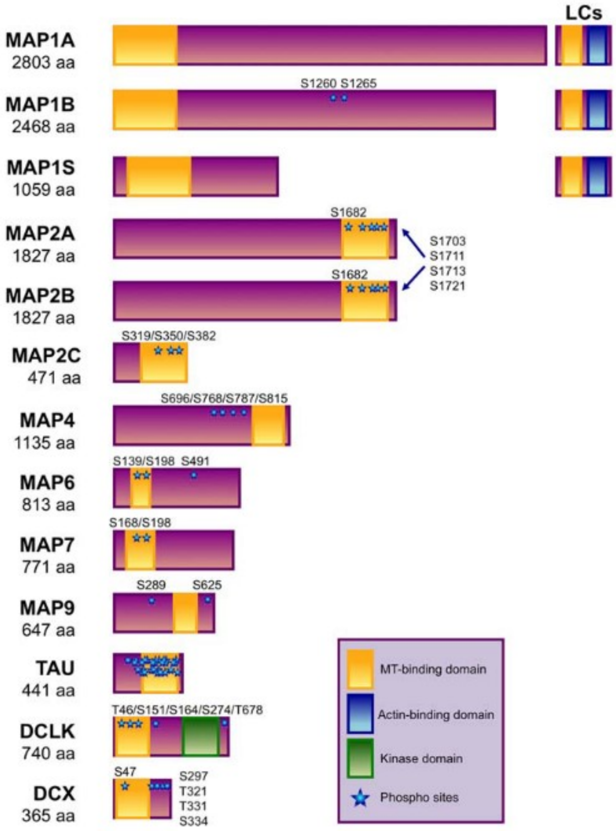

Fig. 5. Visualization of domains of microtubule associated proteins (MAPs). MT-binding domains are showed in yellow, actin-binding domains in blue and kinase domain is shown in green. Blue stars mark the phosphorylation sites. Note that tau can be phosphorylated on many sites. (adapted from review Ramkumar et al., 2018)

The MAP2 family consists of four isoforms (MAP2A – MAP2D)(Chung et al., 1996)(Fig. 5). MAP2 is localized to dendrites and the cell body and is often used as a marker for these parts of neuron (Harada et al., 2002; Lipka et al., 2016). MAP2 is thought to cross-link MTs as well as help distribute organelles such as endoplasmic reticulum in dendrites, both leading to maintenance of dendritic structure and length (Farah et al., 2005; Harada et al., 2002). MAP2 has been shown to stabilize as well as nucleate MTs (Harada et al., 2002; Itoh et al., 1997). It stabilizes MTs mainly by reducing catastrophes, protecting form severing, and increasing rescues (Gamblin et al., 1996; Qiang et al., 2006). Even though recent studies show that MAP2 is also essential in selective axonal cargo trafficking, the sorting of cargo happens near the cell body (Gumy et al., 2017). Therefore, it can still be stated that MAP2 is mainly excluded from the axon. It has been reported that MAP2 can bind not only MTs but also actin and intermediate filaments (neurofilaments), mediating interactions between all cytoskeletal structures (Bloom & Vallee, 1983; Pedrotti et al., 1994). There is a reduction of MAP2 detectable by antibody in individuals with schizophrenia (Shelton et al., 2015). The loss of MAP2 immunoreactivity is known to cause dendritic spine pathology seen in schizophrenia patients (Shelton et al., 2015). Newly published study shows that it is not the reduction of MAP2 protein itself, which causes the loss of immunoreaction, but rather its altered structure (Grubisha et al., 2021). This alteration of MAP2 is believed to be caused by phosphorylation (Grubisha et al., 2021).

MAP4/MAP3 has been shown to promote MT stability by enhancing MT bundling and by exclusion of severing enzymes from the MT (McNally et al., 2002; Mogessie et al., 2015). MAP4 was reported to promote MT nucleation, polymerization and longitudinal affinity of protofilaments (Katsuki et al., 1999). Moreover, MAP4 is believed to cross-link MT and actin cytoskeletal networks (Matsushima et al., 2012). Apart from the brain, MAP4 can also be found in muscles where it establishes MT bundles during myogenesis and it is thought to prevent motor-driven gliding during the myotubule elongation (Mogessie et al., 2015). Furthermore, MAP4 is critical for maintaining balance in forces, ensuring proper cell division (Grubisha et al., 2021; Samora et al., 2011).

MAP6 (also known as STOP, Stable-Tubule-Only Polypeptide) is believed to play an essential role in stabilizing MTs in cold conditions (Delphin et al., 2012). Consistent with this idea, MTs depolymerize rapidly in temperatures below 20°C in the absence of MAP6. However, they are stable in the presence of MAP6 (Delphin et al., 2012). It has been proposed that the observed stabilization is possible by bridging tubulin heterodimers (Lefèvre et al., 2013). Moreover, MAP6 binding is temperature-dependent and may serve as a temperature sensor (Delphin et al., 2012). MAP6 plays a key role in neurotransmission and synaptic plasticity, the dysfunction of which are thought to cause schizophrenia, depression, and memory problems (Fournet et al., 2012). Furthermore, MAP6 can also be found in other than neuronal tissues, such as in skeletal muscles, where it is believed to change MT organization and sarcoplasmic reticulum morphology (Sébastien et al., 2018). That leads to reduced calcium release which results in muscle weakness, one of possible schizophrenia symptoms (Sébastien et al., 2018). MAP6 is associated not only with MTs but also with actin and whether MAP6 interacts with MTs or actin cytoskeleton seems to be regulated by phosphorylation (Baratier et al., 2006).

MAPT, also known as tau, is a soluble intrinsically disordered protein that can be found in many cell types but is especially abundant in neurons (Samsonov et al., 2004). It is one of the most studied MAPs due to its association with neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia, and traumatic brain injury (Kondo et al., 2015; Tran et al., 2011). Tau is believed to increase MT polymerization, decrease the occurrence of catastrophes and inhibit depolymerization (Drechsel et al., 1992; Duan et al., 2017). Tau plays a role in remodeling MT cytoskeleton during neuronal plasticity as well as in MT-based molecular transport and MT severing (Ebneth et al., 1998; Qiang et al., 2006; Samsonov et al., 2004). Tau strongly supports MT stability primarily by laterally crosslinking GDP-tubulin protofilaments, even though the presence of longitudinal binding was not precluded and could contribute to overall MT stability (Duan et al., 2017). When tau is bound to a MT, the N-terminal half stays unstructured, giving the possibility that this "tau tail", which has been shown to act as a binding site for some kinases, may contribute to interactions with other MAPs, motor proteins or severing enzymes (Reynolds et al., 2008; Wegmann et al., 2018).

Using *in vitro* reconstitution of tau and MTs it has been demonstrated that tau forms condensates, or islands, on the MT lattice (Siahaan et al., 2019; Tan et al., 2019)(Fig. 6). These islands could be yet another MT stabilizing factor apart from longitudinal binding mentioned above (Duan et al., 2017). Cohesive islands are formed by tau molecules that bind cooperatively and occupy the total available MT surface within the island boundaries (Tan et al., 2019). Consequently, the turnover inside those high-density tau islands is slow, whereas outside those regions low-density tau undergoes rapid turnover (Samsonov et al., 2004; Siahaan et al., 2019; Tan et al., 2019)(Fig. 6C). With sufficient tau

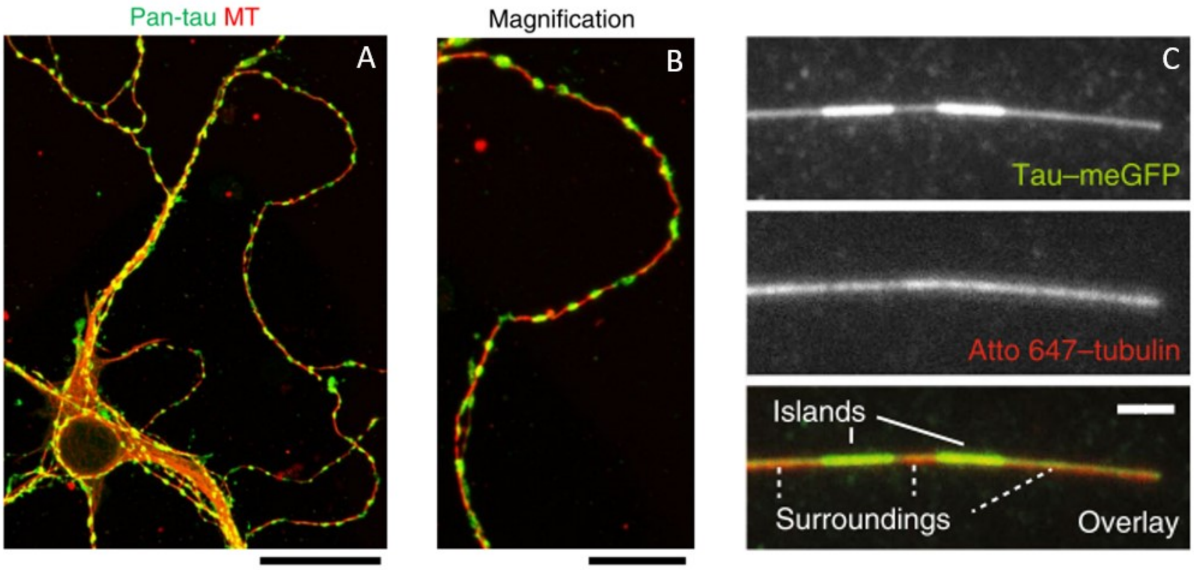

Fig. 6: Tau islands (bright green) on MTs (red) *in vivo* (A, B) and *in vitro* (C). (C) Fluorescence micrograph shows areas of low-density (surroundings) and high-density (islands) tau on the MT. Image was taken 5 min after the addition of 20 nM tau. (adapted from Siahaan et al., 2019; Tan et al., 2019)

concentrations in solution, islands grow from one or both ends and merge with other condensates on the MT lattice. Nevertheless, after the removal of tau from solution, first the low-density regions dissolve and eventually also the high-density tau islands disassemble (Siahaan et al., 2019; Tan et al., 2019). Furthermore, tau islands have been documented not only *in vitro*, but also *in vivo* in mouse hippocampal neurons (Tan et al., 2019)(Fig. 6A,B). Since tau exhibits island formation it is not unlikely that other MAPs might display similar behavior.

Tau has been shown to bind with high affinity to curvatures on MTs both *in vitro* and *in vivo* (Samsonov et al., 2004; Siahaan et al., 2019). Both studies reported higher tau densities in curved MT regions. However, *in vitro* experiments show that unlike islands, MT curvatures are not resistant to katanin severing and thus tau may play a different role in this case (Siahaan et al., 2019). Nevertheless, these experiments show, that tau is able to intrinsically recognize tubulin lattice conformation (Tan et al., 2019).

MAP7 (also known as ensconsin – from *Drosophila melanogaster* or E-MAP-155) has been hypothesized to modulate MT functions or interaction with other proteins rather than stabilize MTs under physiological concentrations (Faire et al., 1999). MAP7 has been identified as a vital cofactor of kinesin-1 (Barlan et al., 2013). Together they are essential for centrosome separation and spindle assembly as well as oocyte polarity (Gallaud et al., 2014; Sung et al., 2008). Consequently, ensconsin-null mutant flies display defective centrosome separation and positioning as in kinesin-1 mutants (Barlan et al., 2013; Faire et al., 1999; Monroy et al., 2018).

MAP7 and tau are considered as antagonists since they compete for binding to the MT (Monroy et al., 2018) (Fig. 7). It has been documented that full-length MAP7 inhibits tau binding and even displaces tau from the MT, even though they occupy different sites on the lattice (Ferro et al., 2020; Monroy et al., 2018, 2020). This suggests that the removal of tau by MAP7 is not driven by the binding overlap model (Monroy et al., 2018). Rather, MAP7 has a higher MT-binding affinity and binds approximately 40-times longer than tau (Monroy et al., 2018). These factors might be the reason why MAP7 is able to invade tau-rich regions (Monroy et al., 2018).

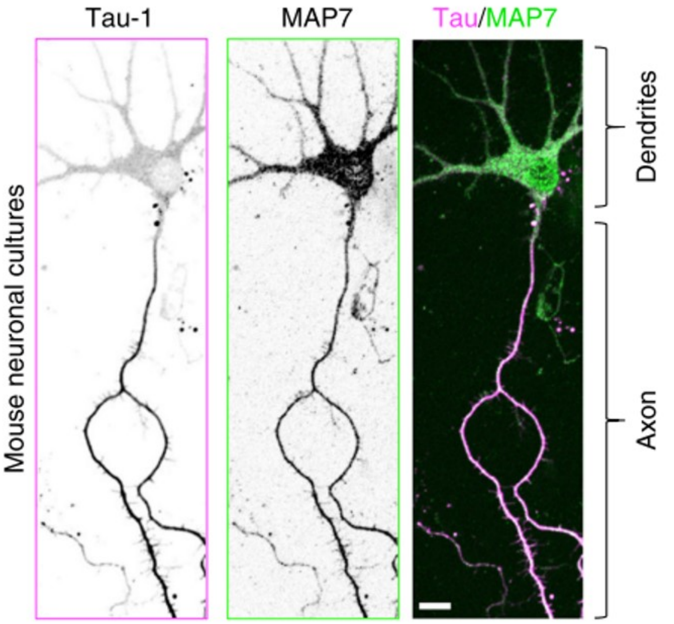

Fig. 7. Immunohistochemistry of mouse neurons. Both tau and MAP7 are expressed within axons, however unlike MAP7, tau is not expressed within dendrites and the cell body. Tau-1 refers to dephosphorylated tau. Scale bar is 20 µm. (adapted from Monroy et al., 2018)

Another reason may be the possibility of weak MT-binding interaction of tau described before (Butner & Kirschner, 1991). The binding dynamics *in vivo* is likely modulated by external factors such as phosphorylation (reviewed in Ramkumar et al., 2018). Moreover, new studies have shown that MAP7 binds to a novel site and can coexist with tau on the MT to some extent (Ferro et al., 2020). This raises the possibility that the regulation of MAPs might be more complex in cells and that other MAPs, or perhaps tubulin code, might play an important role in the distribution and maintenance of MAP condensates. Consistent with this idea, tau competes with EB1, a MT plus-end associated protein, for binding to lattice on stabilized MTs. However, *in vivo* and on dynamic MTs, tau binds only to GDP-tubulin whereas EB1 prefers GTP-tubulin on the growing MT tip (Duan et al., 2017). Consequently, although tau and EB1 compete for the same MT binding site in *in vitro* conditions, *in vivo* the tubulin state determines which protein binds to the MT lattice and thanks to the tubulin code enables the cell to regulate the dynamics of MT tip and lattice independently (Duan et al., 2017).

MAP9 (or ASAP – ASter-Associated Protein) is associated with proper bipolar spindle assembly, mitotic progression, and cytokinesis. It has been proposed that phosphorylation of MAP9 is essential for proper bipolar spindle assembly (Venoux et al., 2008). MAP9 is equally important for MT dynamics and stability during interphase, when it is thought to stabilize MTs and prevent depolymerization (Saffin et al., 2005). MAP9 deficiency results in defective cytokinesis or cell death and is associated with colorectal cancer (Rouquier et al., 2014; Venoux et al., 2008).

Fig. 8. Transversal cut of a MT. Binding of doublecortin (yellow), MAP2 (orange) and tau (green) to the MT (blue). DCX binds in the valley between protofilaments, whereas MAP2 and tau bind on the protofilament ridge. The white asterisk marks fenestration in the MT wall. (adapted from Moores et al., 2004)

Doublecortin family consists of doublecortin (DCX), and doublecortin-like kinase (DCLK) and behaves a little differently from other MAPs because it binds in the valleys between MT protofilaments, whereas other MAPs bind on the ridges (Al-Bassam et al., 2002; Moores et al., 2004) (Fig. 8). DCX can be mainly found on dynamic MTs in migrating neurons during embryonic and postnatal development and is thought to direct neuronal migration through MT stabilization as DCX decreases MT depolymerization (Gleeson et al., 1999; Lipka et al., 2016; Moores et al., 2004). DCX binds selectively to 13-protofilament MTs and promotes the assembly of also 13-protofilament MTs (Moores et al., 2004). DCLK regulates the formation of bipolar spindle and the transition to M-phase during neurogenesis (Shu et al., 2006). Moreover, DCLK overexpression, commonly found in patients with pancreatic cancer, has been documented to promote amoeboid morphology, which greatly enhances the invasive and metastatic properties of pancreatic cancer stem cells (Ito et al., 2016). Both DCX and DCLK are thought to regulate MT stability and path-finding in growth cones, promote growth of dendrites and facilitate kinesin-3 transport (Jean et al., 2012; Lipka et al., 2016; Liu et al., 2012). Consequently, mutations in the DCX family cause disorganized neocortex layers, affect the formation of corpus callosum, show abnormal dendritic structure and impede kinesin-3 synaptic vesicle proteins (Deuel et al., 2006).

4.2 The effects of MAPs on intracellular transport

From all MAP functions, the effect on intracellular transport has been studied most excessively, so far. *In vitro* reconstitution studies of molecular motors have contributed to the current understanding of how motors are regulated by different MAPs. However, the mechanism how MAPs interact with motors remains largely unexplained. MAPs organize recruitment and exclusion of kinesin and dynein motors from MTs, directing specific cargoes to particular locations and could help prevent collisions between motors and their cargoes (Monroy et al., 2018). However, the questions of how motors differentiate between specific MAPs and what causes the spatial distribution of MAPs remain elusive.

MAPs exhibit distinct influences on the motility of the three main classes of transport motors: kinesin-1, kinesin-3, and cytoplasmic dynein. These proteins are crucial for the establishment and maintenance of order and for the polarization in highly polarized cells, such as neurons. Nevertheless, they are equally important in other cell types and cellular processes, such as mitosis (MAP7, MAP9, DCLK1)(Gallaud et al., 2014) or nuclear positioning in muscle cells (MAP7)(Metzger et al., 2012). In neurons some MAPs are found predominantly on both axons and dendrites, such as tau, MAP7, and MAP9, whereas other MAPs, like MAP2, DCX, and DCLK1 are connected preferably to dendrites (Lipka et al., 2016; Monroy et al., 2020). This spacial pattern of MAPs could play a major role in sorting cargoes transported by specific motors. Nevertheless, the principle of interaction between MAPs, motors, and MTs remains largely unexplained.

BINDING-SITE OVERLAP MODEL

MAPs are thought to function as a selective barrier giving access to the MT only to specific motors (Siahaan et al., 2019). It has been thought that the interaction of MAPs and motors could be predicted solely on the overlap with the microtubule-binding site (Monroy et al., 2020; Siahaan et al., 2019). This concept works for some motor-MAP pairs, such as kinesin-1 and MAP9 (Ferro et al., 2020). However, new studies have shown that MAPs can inhibit motors even when they do not overlap the motor binding site (Ferro et al., 2020)(Fig. 9B). This has been seen before with DCX and DCLK1 which did not need a shared binding site with the kinesin-1 motor domain to interfere with its landing on the MT (Monroy et al., 2020). Tau does not compete for the MT binding site with dynein (Ferro et al., 2020)(Fig. 9B). Nevertheless, tau still prevents this motor from processing along the MT as it forbids dynein to switch to a high-affinity state, resulting in fast dynein dissociation from the MT (Ferro et al., 2020). Moreover, the overlapping MT binding site does not necessarily mean that the motor is excluded from the MT. In fact, the situation is opposite with MAP7, which despite sharing a MT binding site with kinesin-1, recruits this motor to the MT lattice (Monroy et al., 2018, 2020). How the interaction between MAPs, motors, and MTs was provided was shown using molecular dynamics simulations. MT-binding domains of MAPs are positively charged whereas the MT surface is negatively charged. The change of charge initiated by binding of MAPs to tubulin disfavors the interaction between motor and MT and causes their disassociation (Ferro et al., 2020). Together, these studies show that the binding-site overlap model cannot be applied generically and that some motors do not need shared MT binding sites with MAPs to obstruct their function.

MAP7 ACTIVATES **KINESIN-1** AND ENHANCES ITS MOTILITY

The only MAP that has been reported to increase kinesin-1 recruitment and motility is MAP7 (Hooikaas et al., 2019; Monroy et al., 2018, 2020; Sung et al., 2008)(Tab. 1). What is more, kinesin-1 function is severely impeded without MAP7, showing that this MAP serves as a kinesin-1 activator and is specifically required for kinesin-driven transport (Hooikaas et al., 2019; Sung et al., 2008). MAP7 also has the ability to restore kinesin-1 ATPase activity (walking) in the presence of inhibitory MAPs (e.g., tau, DCX, MAP9) (Monroy et al., 2018). Subsequent *in vitro* studies have shed a light on the interactions between MAPs and motors and described that the projection domain of MAP7 interacts with the stalk of kinesin-1 (Hooikaas et al., 2019; Monroy et al., 2018, 2020)(Fig. 9A). *In vitro* studies have shown that kinesin-1 is inhibited by all studied MAPs except for MAP7 (Gumy et al., 2017; Monroy et al., 2018; Siahaan et al., 2019)(Tab. 1).

At low concentrations, MAP7 activates kinesin-1 on MTs and strongly favors motor-driven transport to the plus end of MT (Hooikaas et al., 2019; Monroy et al., 2018)(Fig. 9A). However, MAP7 inhibits kinesin motility at higher concentrations, acting as a biphasic regulator (Ferro et al., 2020)(Fig. 9A). MAP7 directly interacts with the coiled-coil stalk of kinesin-1 through ionic interactions with its projection domain (Monroy et al., 2018). These interactions initiate the processive walking along the MT and prevent from disassociation, increasing the run length (Ferro et al., 2020)(Fig. 9A). At high MAP7 concentrations, however, the affinity of kinesin-1 for MAP7 gets higher due to higher density of

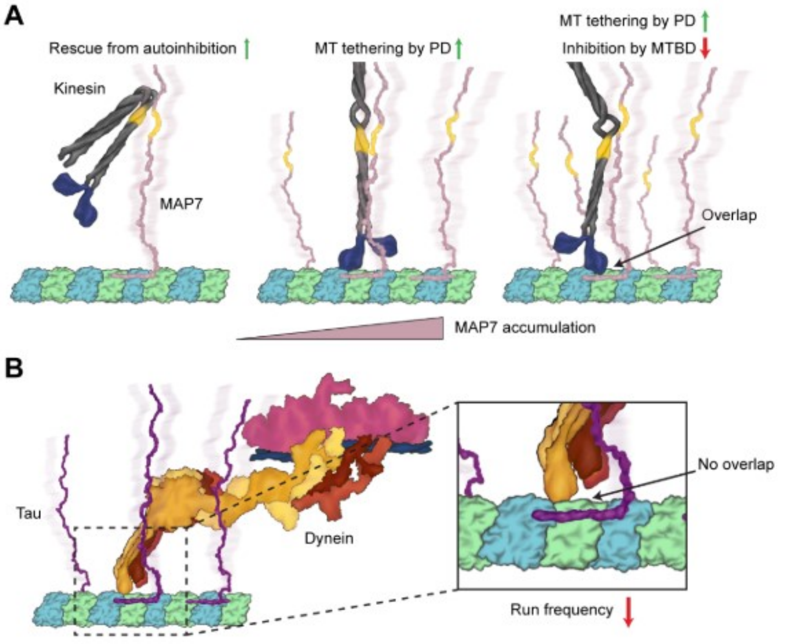

Fig. 9. Model for regulation of kinesin and dynein motors by MAP7 and tau. (A) Kinesin-1 is auto-inhibited when not bound to cargo (cite!). MAP7 binds with the MAP7 stalk. Interaction with MAP7 activates kinesin-1 and tethers the motor to the MT. Kinesin-1 is able to walk on the MT even though it shares binding site with the MT-binding domain of MAP7.
(B) Dynein does not directly interact with tau. Even though there is no overlap between the binding sites of dynein and tau, tau still inhibits the run frequency of dynein. Tau inhibits dynein by competing for the negative charge on the MT. (adapted from Ferro et al., 2020)

MAP7 on the MT. As a result, kinesin-1 cannot take another step before MAP7 detaches or rearranges on the MT surface since their MT binding sites overlap (Ferro et al., 2020)(Fig. 9A). In summary, these results indicate that there is a possibility that MAPs do not always act as a binary switch, but that they, like tubulin code, resemble more a fine-tuning mechanism. Moreover, it can be hypothesized that the tubulin code might play a role in the number of MAP7 molecules decorating the MT lattice and thus have an impact on the kinesin-1 processivity.

KINESIN-3 INTERACTS WITH DCX/DCLK1 AND MAP9

The most important MAPs for kinesin-3 movement are DCX/DCLK1 and MAP9 (Lipka et al., 2016; Liu et al., 2012; Monroy et al., 2020)(Tab. 1). Kinesin-3 is necessary for the transport of postsynaptic vesicles in dendrites and DCX as well as DCLK1 has proven important for this kinesin-3 function (Lipka et al., 2016; Monroy et al., 2020). This importance has been supported by the observation that the deficiency of kinesin-3 and DCX/DCLK1 deficiency show similar morphology in neurons, both effecting neuronal migration (Liu et al., 2012). MAP9 has been shown to increase the number of processive kinesin-3 motors on a MT. However, unlike MAP7 which increases the landing rate of kinesin-1 even when the MT is occupied with other inhibitory MAPs, MAP9 is not able to tether kinesin-3 to the MT is similar conditions (Monroy et al., 2020). Like kinesin-1, kinesin-3 contacts MAP9 directly (Monroy et al., 2020). However, unlike kinesin-1 which binds the stalk of MAP7, kinesin-3 interacts with both MAP9 and DCX through its motor domain (Ferro et al., 2020; Lipka et al., 2016).

Why do kinesin-1 and kinesin-3 motor proteins behave so differently despite their apparent similarity is an intriguing question (Kikkawa et al., 2001). According to Monroy et al. (2020), the K-loop of kinesin-3 represents the primary difference between these two kinesins (Monroy et al., 2020). The author proves this theory on several experiments which all happen to support it. For example, a kinesin-1 chimera (KIF5B_K) with the K-loop insertion from kinesin-3 showed behavior similar to kinesin-3 rather

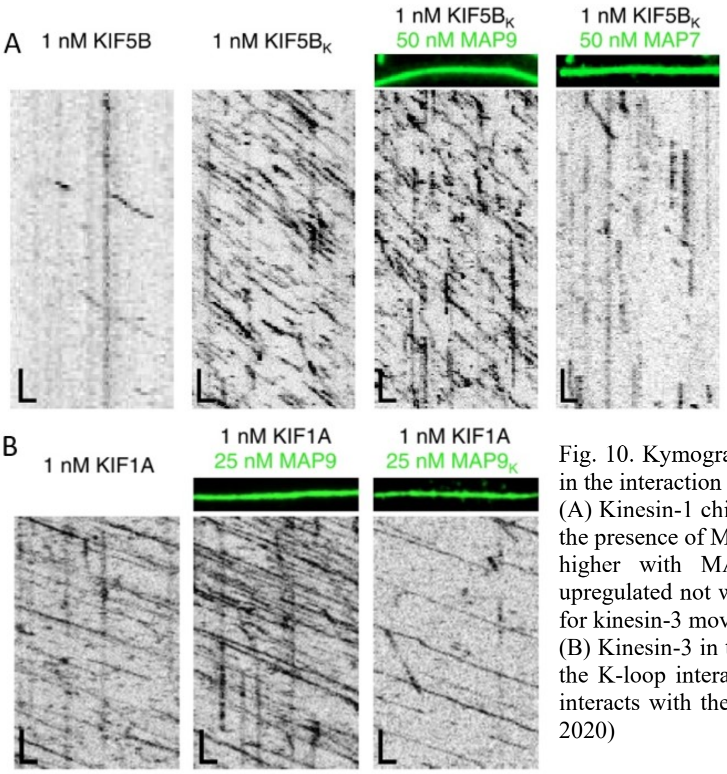

Fig. 10. Kymographs showing the importance of kinesin-3 K-loop in the interaction with MAP9.
(A) Kinesin-1 chimera (KIF5B_K) with inserted kinesin-3 K-loop in the presence of MAP9 or MAP7. Number of kinesin-1 molecules is higher with MAP7. However, the mutants landing rate is upregulated not with MAP7, but with MAP9. Since MAP9 allows for kinesin-3 movement, the mutant behaves similarly to kinesin-3.
(B) Kinesin-3 in the presence of MAP9 or MAP9 with mutation in the K-loop interacting domain (MAP9_K). This shows that MAP9 interacts with the kinesin-3 K-loop. (adapted from Monroy et al., 2020)

than kinesin-1 (Monroy et al., 2020) (Fig. 10A). Also, to prove that the interaction of kinesin-3 and MAP9 is mutual, a part of MAP9 that interacts with the kinesin-3 K-loop has been mutated ($MAP9_K$). This mutation resulted in severe reduction of landing rate and the number of processive kinesins on the MT compared to kinesin-3 in the presence of unmutated MAP9 or even kinesin-3 alone (KIF1A) (Monroy et al., 2020) (Fig. 10B). Furthermore, even highly homologous kinesins from one subfamily (ex., kinesin-3 subfamily) display distinct targeting preferences for axons or dendrites (Lipka et al., 2016). These results indicate that even seemingly minor differences in molecular motors can have prominent effect on their behavior and MAP preference. Since MAPs and tubulin are often regulated by post-translation modifications which represent such minor changes, it rases a question whether slight differences in MAPs and tubulin post-translational modifications could also contribute to similar outcome.

MAP-DEPENDENT REGULATION OF **DYNEIN**

Unlike kinesin-1 and MAP7, or kinesin-3 and MAP9, dynein does not interact with MAPs directly. However, dynein competes with the MAP for negative charge on the MT surface. Therefore, MAPs do not need an overlapping MT binding site with dynein to disrupt its motility (Ferro et al., 2020)(Fig. 9B). Even though MAPs differentially regulate the anterograde transport, the evidence for retrograde transport is less clear (Tab. 1). Previous studies have shown that dynein motility is not strongly inhibited by tau or MAP7 *in vitro* (Monroy et al., 2018; Tan et al., 2019)(Tab. 1). However, it has been shown recently that tau, MAP7, and DCX have the ability to inhibit dynein motility as strongly as kinesin under physiological salt concentrations (Ferro et al., 2020; Siahaan et al., 2019)(Tab. 1). Furthermore, the reason for such differences in dynein motility reports may be due to dynein-dynactin complex formation with different cargo-adaptor proteins such as BicD2N (DDB) or Hook3 (DDH) (Tan et al., 2019). This raises the possibility that regulation of the retrograde transport by MAPs is driven by cargo-adaptor proteins, rather than dynein itself (Ferro et al., 2020).

In summary, as the cytoplasm is an extremely crowded environment, both anterograde and retrograde motors seem to require interactions with MAPs decorating the MT in order to walk in either direction (Ferro et al., 2020). MAPs and molecular motors differentiate not only in their cellular distribution but also in the way they interact with each other on the MT lattice. The binding-site overlap model cannot be applied to all MAPs since some MAPs do not need an overlapping binding site to impede the motor function. Moreover, even minor differences in motors and perhaps MAPs have significant impact on their distribution in cells. This raises the opportunity that tubulin code and post-translational modifications of MAPs can also participate in the directional motor transport. In conclusion, all studies mentioned in this section support the existence of a regulatory code generated by various MAPs on the MT surface that has the capacity to direct motor transport in cells – this code is often referred to as ‘MAP code’.

Tab. 1. The effects of different MAPs on molecular motors. 'Inhibits' refers to situations when a MAP obstructs the motor from accessing the MT or from processing along the MT. 'Recruits' means that the motor recruitment and movement are improved in the presence of the particular MAP. Finally, 'allows' refers to situations when the motor is not effected by the MAP on the MT surface and can move along MTs decorated with this MAP. For more information refer to the main text.

	kinesin-1	kinesin-3	dynein	
MAP2	inhibits	inhibits/allows	inhibits	(Gumy et al., 2017; Monroy et al., 2020)
MAP7	recruits	inhibits	inhibits/allows	(Ferro et al., 2020; Monroy et al., 2018, 2020)
MAP9	inhibits	recruits	inhibits	(Monroy et al., 2020)
tau/MAPT	inhibits	inhibits	inhibits/allows	(Ferro et al., 2020; Monroy et al., 2020)
DCX/DCLK	inhibits	recruits	inhibits/allows	(Ferro et al., 2020; Liu et al., 2012; Monroy et al., 2020)

4.3 Interaction of MAPs and severing enzymes

Severing enzymes such as spastin or katanin represent another type of proteins that could be *sensu lato* considered as MT associated proteins. Considering that MT severing and consequential axon shrinkage and neuronal cell death are the cause of neurodegenerative diseases such as Alzheimer's disease, the studies about regulation of the MT severing mechanisms are of critical importance (Qiang et al., 2006).

Similar to molecular motors, access of severing enzymes to MTs could be regulated by MAPs. The simple presence of MAPs *in vivo* could explain why previous studies showed that katanin does not colocalize with MTs in interphase cells, while purified katanin binds MTs *in vitro* (McNally et al., 2002). Although MAP2c and MAP4 have displayed some degree of protection against severing by katanin, the best performance in shielding axonal MTs from the activity of severing enzymes has been shown with tau (McNally et al., 2002; Qiang et al., 2006). Tau needs the flexible projection domains to cooperatively bind and form islands (Tan et al., 2019). The ability of tau to form islands seems to play a vital part in the shielding task (Siahaan et al., 2019). The idea is also supported by the fact that the tau MT-binding domain alone is not sufficient for proper protection against severing (Qiang et al., 2006). Consistent with this idea, katanin (Siahaan et al., 2019) and spastin (Tan et al., 2019) are able to severe predominantly outside the islands. Moreover, tau islands enable the MTs to prevail longer in depolymerizing conditions compared to areas of low-density tau outside islands (Siahaan et al., 2019). Since the depletion of tau is not sufficient to disturb the severing protection in neurons, other MAPs must contribute to the overall stability and MT shielding *in vivo* (Qiang et al., 2006). Nevertheless, the dissociation of tau from MTs is one of the hallmarks for human diseases termed tauopathies, which supports the idea that tau may be the chief MT protector (Siahaan et al., 2019).

There are two kinesin subfamilies that have been reported to work as MT depolymerases, also referred to as super-processive motors or Kin I kinesins – kinesin-8 and kinesin-13. Kinesin-8 was reported to serve a role in controlling MT length during cell division and it was reported to be associated with cancer (Nagahara et al., 2011). However, the depolymerization mechanism of kinesin-8 is still poorly understood. The bump-off model suggests that kinesins push each other off the MT end, but instead of dissociating they take the tubulin dimer with them, thus causing catastrophe (Varga et al., 2009). The switching model proposes another mechanism. In this model kinesin-8 serves as a curvature-sensing enzyme and accumulates at the unstable MT end where it promotes MT depolymerization (Arellano-Santoyo et al., 2017). Since the traffic jams, observed at the ends of MTs, can be explained by either model, the exact mechanism remains under debate. A recent study unites both models and suggests that the removal of tubulin dimers from the MT end is facilitated by force generated by multiple kinesin-8 motors (Bugiel et al., 2020). Kinesin-8 has been shown to accumulate not only at the MT ends but also in front of a tau island. These traffic jams are then able to displace tau from the island edge and gradually disassemble the island (Siahaan et al., 2019). Kinesin-13 has a role for example in the cell cycle and in ciliogenesis. Like kinesin-8, Kinesin-13 is thought to induce depolymerization from the MT end (Asenjo et al., 2013; Benoit et al., 2018). The depolymerization is assumed to be initiated by a conformational change caused by binding of kinesin-13 to curved MTs (Asenjo et al., 2013). This change further bends the MT and leads to catastrophe (Benoit et al., 2018). Since kinesin motors have been shown to be regulated by MAPs, it can be speculated that unique kinesins, such as kinesin-8 and kinesin-13, could also be regulated by the MAP code (Desai et al., 1999). However, evidence supporting this idea is scarce and therefore represents an interesting field for future studies.

4.4 Post-translational modifications of MAPs

Given the fundamental importance of MTs, it is unsurprising that defects in MT-based processes such as motor transport, often lead to neurodegenerative or neurodevelopmental diseases in humans. Tauopathies are a group of neurodegenerative diseases connected to tau and its malfunction (Ishihara et al., 1999). Even though other proteins are associated with pathologies such as dementia, tau is believed to be essential for the onset of neurotoxicity induced by amyloid-β plaques and tau tangles (Rapoport et al., 2002). MAPs other than tau also undergo post-translational modifications and these modifications have impact on MAP functions. However, this review will focus on tau phosphorylation and mention other MAPs only briefly.

It is well documented that post-translational modifications of MAPs affect the MT cytoskeleton (Baratier et al., 2006; Drewes et al., 1997; Saffin et al., 2005). Although MAPs can be affected by many post-translational modifications, phosphorylation is by far the most excessively studied. Phosphorylation affects many MAPs and has a pronounced effect on rearrangements of the MT network in all stages of development (reviewed in Ramkumar et al., 2018). The most prominent effect of this modification is the dissociation of MAPs from MTs (Drewes et al., 1997). MAP2 phosphorylated in the microtubule-binding domain results in inhibition of the stabilizing and nucleating activity of this MAP (Itoh et al., 1997). MAP2 phosphorylation has also recently been connected to dendritic spine pathologies in individuals with schizophrenia (Grubisha et al., 2021). MAP4 phosphorylation has been reported to be important in the regulation of mitosis (Ookata et al., 1995). Phosphorylation of this MT associated protein leads to MAP4 detachment from the MT, activation of MT severing, and decrease of rescue frequency, all leading to MT destabilization (McNally et al., 2002). The detachment of MAP4 from MTs also affects anterograde and retrograde transport since MAP4 allows for the movement of kinesin-2, while it precludes dynein motility (Semenova et al., 2014). The phosphorylation of MAP6 determines whether it will interact with MTs (dephosphorylated MAP6) or actin cytoskeleton (phosphorylated MAP6) (Baratier et al., 2006). Furthermore, phosphorylation of MAP9 is crucial for MAP9 stability, mitosis and cytokinesis (Saffin et al., 2005). In summary, phosphorylation has been reported to affect many physiological functions of MAPs.

In this section, tau phosphorylation will be discussed in further detail since it plays a key role in tau biology and disease. As tau relates to human diseases, understanding the regulation and function of tau is of high importance. Although tau is subject for various post-translational modifications, tau phosphorylation has received the most attention. Tau phosphorylation is thought to be the main cause for abnormal behavior and aggregation of tau in neurodegenerative disorders, collectively termed tauopathies. The phosphorylation of tau impedes its vital functions, such as increasing elongation rate or suppressing the catastrophe rate, thus interfering with tau's ability to stabilize MTs (Drechsel et al., 1992). It has been documented that tau phosphorylation plays a role in tau static-dynamic equilibrium on the MT (Stern et al., 2017). Phosphorylated tau seems to be more dynamic and binds the MT with decreased affinity (Stern et al., 2017). Moreover, dynamic phosphorylated tau allows kinesin-1 to walk along the MT, whereas dephosphorylated tau is more static and inhibits kinesin-1 (Stern et al., 2017). This study adds yet another layer of regulation to the molecular trafficking system and foreshadows the importance of post-translational modifications of MAPs in physiological conditions. It also raises a question, whether the static-dynamic state can be the mechanism that determines if tau forms islands on the MT.

Tau hyperphosphorylation is thought to be one of the main causes of Alzheimer's disease (Rapoport et al., 2002). According to the World Health Organization (WHO), Alzheimer's disease is the most common type of dementia in humans (60-70% of all types of dementia). Worldwide, around 50 million people suffer from dementia with nearly 10 million new cases each year. In brains of Alzheimer's disease patients, two main histopathological hallmarks can be found: clots of amyloid-β called amyloid-β (neuritic, senile) plaques and tau aggregates termed paired helical filaments, which later form neurofibrillary tangles (Ebneth et al., 1998; Rapoport et al., 2002)(Fig. 11). Dysfunctional tau and amyloid-β cause neurotoxicity and eventually cell death, leading to symptoms associated with dementia, such as forgetfulness, difficulty in navigating familiar places or even behavioral changes in more advanced stages (WHO)(Rapoport et al., 2002; World Health Organization, 2020). What triggers the transition of highly soluble tau into aberrantly folded and aggregating tau remains to be determined.

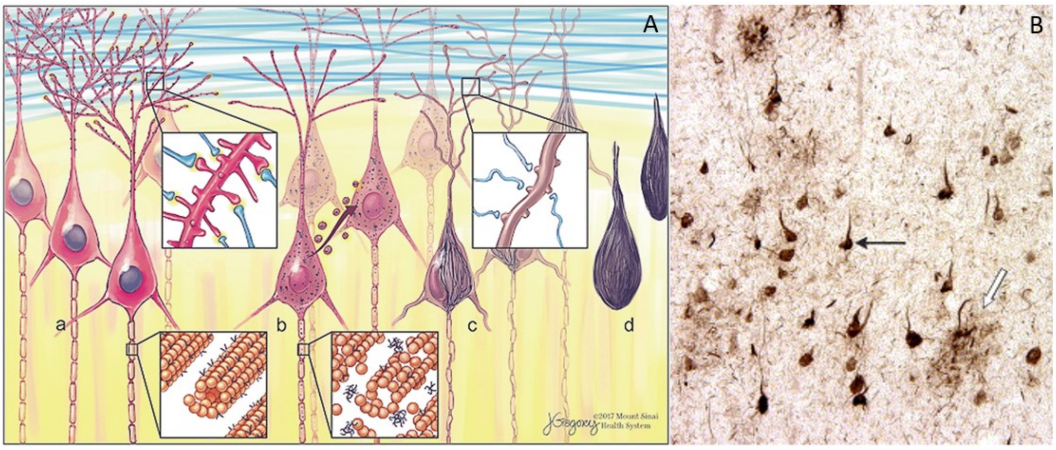

Fig. 11. The hallmarks of Alzheimer's disease.
(A) (a) A healthy neuron with MTs stabilized by tau. (b) The onset of tauopathy, tau hyperphosphorylation, formation of paired helical filaments and spread to other neurons. (c) The formation of neurofibrillary tangles. Shrinkage of the axon. (d) The formation of dense neurofibrillary tangles also seen in bright-filed immunochemistry in (B) (black arrow). Amyloid-β plaques (white arrow). (adapted from Binder et al., 2005; Signaevsky et al., 2019)

Recent studies suggest that tau (unphosphorylated and hyperphosphorylated) can undergo liquid-liquid phase separation (Wegmann et al., 2018). Liquid-liquid phase separation droplets of phosphorylated tau resemble aggregates found in neurodegenerative diseases. Although liquid-liquid phase separation is important in healthy cells, since it generates membrane-less organelles that create a specific environment for particular functions, phosphorylation may favor the droplet state of tau and enhance its aggregation (Wegmann et al., 2018). In those aggregates, tau is in highly concentrated and is more likely to form "crystals" also known as neurofibrillary tangles (Ciryam et al., 2015; Wegmann et al., 2018). A possible mechanism of the build-up of phosphorylated tau may be as follows: Tau gets hyperphosphorylated and undergoes liquid-liquid phase separation and oligomerization, forming paired helical filaments. Consequently, there is not enough tau available for MT stabilization in axons (Tan et al., 2019)(Fig. 11). The cell tries to rescue the situation by producing more tau, which would explain elevated tau levels in brains of Alzheimer's disease patients (Ebneth et al., 1998; Wegmann et al., 2018). However, the additional tau gets hyperphosphorylated as well which only leads to the formation of more neurofibrillary tangles. This leads to neurotoxicity which results not only in cell death, but also in dementia and eventually death of the patient.

Another interesting feature of tau observed in the brain of an Alzheimer's disease patient is the ability to spread among neurons. There is strong evidence that tau (and what is more, aggregated tau) can be transferred from one neuron to another and spread even to distant neurons, causing the spread of the pathology (Clavaguera et al., 2009; Wu et al., 2016). How this transfer is accomplished in cells is unknown. However, it seems to explain the widespread distribution of tau pathology in the brain of Alzheimer's patients (Wu et al., 2016). It raises the question, whether other MAPs, post-translationally modified or not, could also undergo cell-to-cell transport, or if tau is the only one able to be transported due to its ability to aggregate.

Finally, other studies show that the delicate intracellular environment can be quite easily disturbed merely because of the overexpression of tau, not necessarily by tau post-translational modification. For example it has been documented, that an increased level of tau leads to disproportions in cellular transport, endoplasmic reticulum mislocalization, mitochondrial disfunctions, and cell shape change (Ciryam et al., 2015; Ebneth et al., 1998). Similar results have been obtained *in vivo* as well. In those experiments, mice overexpressing tau developed pathology similar to tauopathies observed in humas (Ishihara et al., 1999). As in the brains of Alzheimer's disease patients, tau concentration is elevated. Elevation of tau might contribute to the development of the disease for example by enhancing amyloid-β overexpression or by impeding the molecular transport (Ciryam et al., 2015; Ebneth et al., 1998). Consistent with this idea, spontaneous liquid-liquid phase separation was observed with tau from healthy individuals at high concentrations even in the absence of phosphorylation or other agents promoting aggregation (Wegmann et al., 2018). These results indicate that mechanisms regulating the motor trafficking and intracellular maintenance are quite complex and are probably controlled by multiple mechanisms.

4.5 MAP code

All studies mentioned above support the idea of so called MAP code – a hypothesis that has emerged only recently in literature (Monroy et al., 2018, 2020). In those studies, the MAP code refers to functional compartmentalization of MT lattice in which different MAPs form cohesive envelopes on the MT in order to regulate access of other proteins and enzymes and thus dictate the MT function. MAPs can exhibit opposing or synergistic activities and provide local signals to guide for example polarized cargo transport or MT dynamic instability (Lipka et al., 2016; Tan et al., 2019). Although the MAP code shows more dramatic effects on motor landing and motility than tubulin post-translational modifications, the possibility of essential counterplay between those two systems cannot be ruled out. Nevertheless, recent evidence shows a strong potential for the MAP code in directing motor transport *in vivo* (Ferro et al., 2020; Monroy et al., 2020). The MAP code hypothesis thus introduces a plausible mechanism of regulation of molecular transport and other processes associated with MTs. Moreover, there are many MAPs that have been given less attention a thus could represent an interesting topic for future studies.

5 Possible connection between Tubulin code and MAP code

Tubulin code is hypothesized to affect MAP binding and contribute to gating motor access to MTs. The ability of tubulin post-translational modifications to determine which MAPs bind to the MT lattice could directly generate the MAP code and establish a multilayer regulatory mechanism (Monroy et al., 2020). This mechanism could then control the access to MTs for other proteins and enzymes and may contribute to tight regulation of molecular transport in cells.

Subtilisin is a protease that removes the tubulin C-terminal tail. Tubulin tails are thought to contribute to proper function of motors. Consistent with this idea, subtilisin-cleaved MTs show decreased kinesin and dynein motor landing and run-length rates (Ferro et al., 2020; McKenney et al., 2014). DDB motility is strongly reduced on MTs consisting of tubulin lacking the C-terminal tail (McKenney et al., 2016). Moreover, these results have been seen only with the cleavage of C-terminal tail on α-tubulin, but not on the β-tubulin, proposing another possible regulation that could contribute to complex distribution of motors *in vivo* (McKenney et al., 2016). Furthermore, it has been demonstrated that not only motors are affected by C-terminal cleavage, MAP tau is unable to form cohesive islands on subtilisin treated MTs (Tan et al., 2019). These experiments show that tubulin tails are required for tau condensation, facilitating direct contact of tau with the tubulin dimer surface (Tan et al., 2019).

It has been shown that tau is able to intrinsically recognize the tubulin lattice conformation, which supports the idea of cooperation between tubulin code and MAP code (Duan et al., 2017; Samsonov et al., 2004; Siahaan et al., 2019; Tan et al., 2019). It is thought that tau islands may act as a readout of post-translational tubulin modifications, moderating the accessibility of this region to other MAPs (Siahaan et al., 2019). Islands condensation seems to be gated by the nucleotide state of the MT lattice (Tan et al., 2019). However, it remains elusive, whether the spacing between tubulin dimers regulates tau condensation and island formation or whether tau itself regulates tubulin spacing and thus stabilizes the lattice (Duan et al., 2017; Tan et al., 2019).

The effect of tubulin post-translational modifications has been studied not only with tau, but also with other MAPs. It has been documented that MAP1S, a member of MAP1 family, is required for tubulin acetylation (Tegha-Dunghu et al., 2014). When MAP1S was knocked down, acetylation of α-tubulin was decreased by 60%. However it did not affect other tubulin post-translational modifications in any way (Tegha-Dunghu et al., 2014). Also MAP1S knockdown in cells yielded MTs that grew faster but expressed shorter half-lives (Tegha-Dunghu et al., 2014). As acetylated MTs are more resistant to bending and thus more stable than those not acetylated (Eshun-Wilson et al., 2019), the impaired ability to acetylate MTs could lead to faster growth and shorter half-lives (Tegha-Dunghu et al., 2014). This proves that MAPs can have a direct effect on tubulin post-translational modifications and opens up the possibility that other MAPs could have an impact on the tubulin code.

6 Conclusion

In cells, MTs fulfill many functions. However, the question of how MTs adapt to these functions remains elusive. To control the process of cell division or organelle distribution, complex control mechanisms exist in cells. Such mechanisms involve tubulin modifications and many molecules such as MT associated proteins (Andersen, 2000; Barisic et al., 2015; Gallaud et al., 2014). Especially in neurons, the perturbations of the organelle distribution has serious consequences and can result in neurodegenerative diseases such as Alzheimer’s disease (Ebneth et al., 1998).

Post-translational modifications of tubulin create the 'tubulin code' on the MT surface. Even though evidence that molecular motors can be regulated by the tubulin code exist, the regulation seems only mild (Kaul et al., 2014). Consequently, the tubulin code may not be the main supervisor of motor-driven transport in cells. On the contrary, this role could belong to the MAP code. However, in cells, a complex multilevel regulatory system could be created if both coding systems cooperate. Tubulin code is hypothesized to affect MAP binding and contribute to gating motor access to MTs (Monroy et al., 2020). Nevertheless, whether post-translational modifications of MTs regulate the distribution of MAPs has, so far, not sufficiently been documented. Super-resolution imaging studies in cells are required to test whether tubulin code regulates the recruitment of specific MAPs to MTs (Ferro et al., 2020). However, recent evidence shows a strong potential for tubulin post-translational modifications in establishing MAP code (Ferro et al., 2020; Monroy et al., 2020). It has been shown that tau is able to intrinsically recognize the tubulin lattice conformation, which supports the idea of cooperation between tubulin code and MAP code (Duan et al., 2017; Samsonov et al., 2004; Siahaan et al., 2019; Tan et al., 2019). Using *in vitro* reconstitution of tau and MTs, it has been demonstrated that tau is able to form condensates, or islands, on the MT lattice (Siahaan et al., 2019; Tan et al., 2019). Tau islands have the ability to affect molecular motors and severing enzymes. Moreover, the fact that MAP1S is required for tubulin acetylation proves that MAPs can have direct effect on tubulin post-translational modifications and opens up the possibility that other MAPs could have an impact on the tubulin code (Tegha-Dunghu et al., 2014).

Since obstacles on the MTs reduce the processivity of transport motors (Telley et al., 2009), it remains unclear how these motors are able to walk along densely decorated MTs *in vivo*. Recent studies propose that kinesin teams are able to cooperatively bind to transported cargo and bypass obstacles (Beeg et al., 2008; Ferro et al., 2019; Kural et al., 2005; Li et al., 2018). Furthermore, the helical trajectory and the ability to take sideway steps may explain partly controversial results of the effect of MAPs on dynein motility. Kinesin shows clear differences in interactions with different MAPs, in other words, it can be said whether a particular MAP inhibits the binding of kinesin or not. However, the results are not so clear for dynein. (Monroy et al., 2018, 2020). However, it has been shown recently that MAPs have the ability to inhibit dynein as strongly as kinesin motility (Ferro et al., 2020; Siahaan et al., 2019). Moreover, since results on the effect of MAPs on dynein motor are debatable (Ferro et al., 2020; Monroy et al., 2018, 2020), the regulation of the retrograde transport was proposed to be driven by cargo-adaptor proteins, rather than dynein itself (Ferro et al., 2020).

Using kinesin-1 and kinesin-3 motor proteins as an example, it has been shown that even seemingly minor differences in molecular motors can have prominent effects on their behavior and MAP preference (Kikkawa et al., 2001). MAPs and tubulin are often regulated by post-translation modifications which represent such minor changes and thus could have a great impact on the behavior of MAPs and motors. This further supports the idea of complex regulatory mechanisms of tubulin and MAP code.

The fact that post-translational modification can have crucial consequences can be seen with tau phosphorylation. Tau is one of the most studied MAPs due to its association with neurodegenerative diseases collectively termed tauopathies (Kondo et al., 2015; Tran et al., 2011). In tauopathies, tau is abnormally hyperphosphorylated, which results in tau dissociation from MTs, MT destabilization and the formation of tau aggregates, one of the hallmarks of Alzheimer's disease. In the light of new studies, the loss of tau islands might be critical for the death of neurons. MT severing and consequential axon shrinkage and death of neurons are the cause of neurodegenerative diseases (Qiang et al., 2006). Katanin (Siahaan et al., 2019) and spastin (Tan et al., 2019) are unable to severe inside the tau islands, showing that MAPs can shield MTs from these enzymes. Since kinesin motors have been shown to be regulated by MAPs, it can be speculated that MT depolymerizing kinesins (kinesin-8 and kinesin-13) could also be regulated by the MAP code (Desai et al., 1999). However, evidence supporting this idea is scarce and therefore represent an interesting field for future studies.

Combined, all results discussed in this review show that there is vast evidence supporting the existence of complex mechanisms regulating MT-based processes. Regulation of MTs is essential for proper cell function. MTs are involved in axonal maintenance and cell division and their disfunction leads to severe pathologies, such as neurodegenerative disorders or cancer. Post-translational modifications of tubulin have been proposed to work as a regulatory mechanism of MT functions. However, recent studies suggest that this role could be passed to MAPs. MAPs have been documented to have the ability to recognize tubulin conformation, direct motor transport, and protect MTs from severing. Moreover, MAPs show spatial-temporal distribution within the cell and are mutually exclusive. Further work will be however necessary to test further the hypothesis of the 'MAP code' and understand its interplay with other MT-based regulatory processes.

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