Lifetime imaging of microtubule functions and their impact on neuronal homeostasis

Introduction

Microtubules are highways for intracellular transport. While transport of organelles, vesicles and molecules is essential in every living cell, it appears to be particularly important in neurons, which must transport cargoes over very long distances, and which must assure homeostasis throughout the entire lifetime of an organism. Subtle perturbations of neuronal transport can lead to neuronal dysfunction and result in neurodegeneration.

Cargo transport takes place on microtubules (MTs). While basic mechanisms of this transport have been well understood, their fine-tuning and adaption to different physiological requirements is less well understood. Here we want to test the hypothesis that tubulin post-translational modifications (PTMs) control MT functions in neurons (Fig. 1A) [1,2]. Importantly, while in most of the so-far observed cases *ex-vivo*, or in cells, tubulin PTMs showed only subtle molecular effects, they lead to dramatic effects *in-vivo* and to disease in humans in a long term (Fig. 1B-D) [3,4]. Thus, the regulation of MT functions by tubulin PTMs fulfils the predictions for mechanisms involved in homeostasis. In this project we will focus on the tubulin PTM polyglutamylation. In a series of studies in mice and human cells, the Janke team has demonstrated that perturbation of this PTM has mostly gradual effects on MT functions, but leads to widespread late-onset neurodegeneration in a variety of brain regions (Fig. 1B-D) [5]. However, how the observed subtle changes in MT functions lead to neurodegeneration in the long term has not been uncovered.

Breaking new ground in this subject requires detailed life-long characterizations of MT functions and their consequence on neuronal morphology at single animal resolution, which has been impossible in mammalian model systems. To achieve this, we propose here to establish a novel interdisciplinary approach using the nematode model organism *C. elegans*. In contrast to currently used vertebrate or mammalian model system, the simplicity of *C. elegans* neural circuits provides us with a tractable neural system to monitor intracellular processes on the single-cell level by life-long *in-vivo* imaging (Fig. 2A).

For this project, the PhD student will develop novel microfluidics-based technologies for long-term high-resolution microscopy and automated analyses of MT structure and MT-based cargo transport in aging *C. elegans* animals. Using these technologies, the student will undertake a detailed characterization of the effects of aberrant tubulin polyglutamylation on MT-based neuronal functions throughout animal lifetime. Most importantly, we will be able to directly correlate axonal transport phenotypes with morphological changes in single, defined neurons in animals altered tubulin polyglutamylation throughout the animal's lifetime.

We anticipate that this new approach will shed long-sought light on the molecular mechanism underlying neurodegeneration caused by tubulin polyglutamylation defects in mice (Fig. 1B-D), opening new promising avenues to cures for neurodegenerative diseases.

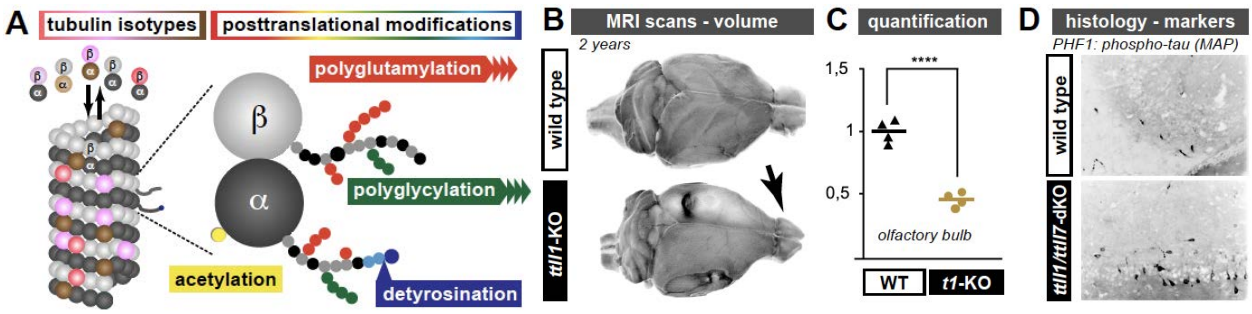

Figure 1: The tubulin code and its impact on MT cytoskeletal function and long-term neuronal homeostasis in mice. (A) The molecular components of the tubulin code: MTs are dynamically assembled from different gene products of α- and β-tubulin, which can freely intermingle. The incorporation of specific isotypes in an MT is expected to gradually change its properties. The key PTMs of MTs are acetylation, polyglutamylation, polyglycylation and detyrosination. (B-D) Analyses of mouse model with reduced polyglutamylation levels: (B) MRI scan shows morphological changes at the olfactory bulb (arrowhead). (C) Quantification of MRI scans (B) show reduced volume of olfactory bulb in 2-year-old ttll1-KO mice. (D) Immuno-histology of hippocampi of 1-year-old ttll1/ttll7-dKO: increased labelling for the phosphorylated MAP tau in neuronal cell bodies – a sign of degeneration.

Model System

The *C. elegans* touch receptor neurons and PTM enzymes

MT polyglutamylation has been recognized as an important modulator of MT function and has been implicated in the regulation of centriole stability, neuronal outgrowth, and cilia motility. Polyglutamylation is catalyzed by a family of tubulin tyrosine ligase-like (TTL) enzymes. Deglutamylation is catalyzed by carboxypeptidases (CCPs). The *C. elegans* genomes encodes several TTLL and CCPP enzymes with significant homology to their mammalian counterparts. While these enzymes have been studied in the context of cilia formation and maintenance in *C. elegans*, links to MT-based cargo transport and neurodegeneration have not been studied.

To determine the effect of aberrant MT polyglutamylation, in this project we will focus on six non-ciliated mechanosensory neurons (ALM-left/right, AVM, PLM-left/right, PVM). These prominent touch receptor neurons extend long neurites across either the anterior or posterior half of *C. elegans.* ALMs and PLMs are the major sensory neurons controling sensation of soft touch in the anterior and posterior half of the animal. The ALM and PLM processes are filled with large diameter 15-protofilament MTs which are required for mechano-sensation and proper neuronal morphology. Touch receptor neurons display the most striking age-related morphologic abnormalities in *C. elegans*, including cytoskeletal disorganization, axon beading, and defasciculation [8,9] (Fig. 2B). These morphological defects start in young adult *C. elegans* (day 4 of adulthood) when animals are still in their reproductive phase and become progressively worse during aging [10]. Moreover, these neurons are located laterally in the animal, allowing optimal access for high-resolution microscopy, making them an ideal model system to investigate the impact of MT polyglutamylation on neuronal morphology and MT-based transport.

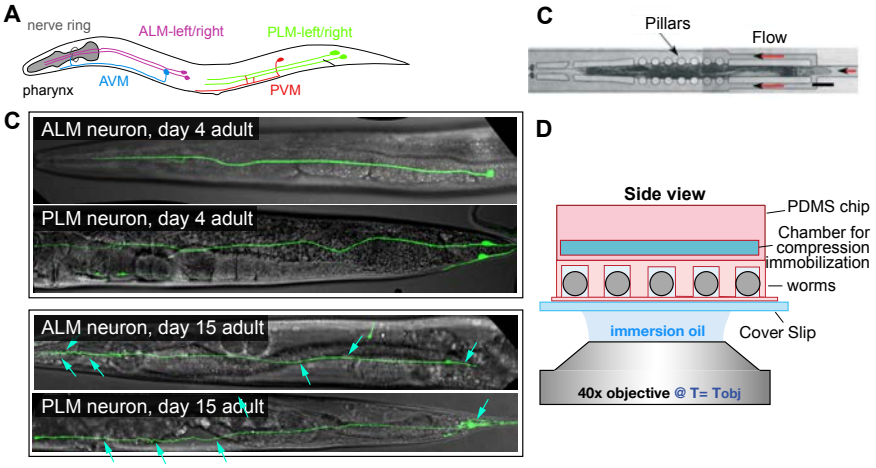

Figure 2: The C. elegans touch receptor neurons combined with microfluidics as a novel system system to study age-related phenotypes associated with aberrant MT polyglutamylation. (A) Schematic representation of the six C. elegans touch receptor neurons. (B) Micrographs of WT C. elegans animals with GFP-labelled touch receptor neurons at different adult ages. Hallmarks of neurodegeneration are indicated with green arrowheads. (C) Image of an adult *C. elegans* animal trapped in a microfluidic channel for long-term observation (adapted from Kopito et al. 2013). (D) Side-view of the proposed microfluidic device with compression mechanism like in (Keil et al., 2017) for occasional complete animal immobilization.

The Keil team has already constructed *C. elegans* strains in which the mechanosensory neurons are labelled with GFP in various null-mutant combinations of TTLL/CCPP enzymes. In addition, using CRISPR/Cas9 and clones of the *C. elegans* TTLL/CCPP enzymes obtained in the Janke Lab, we have engineered strains in which these enzymes are overexpressed exclusively in the mechanosensory neurons. These strains will form the basis for Aim 1 of the proposed PhD project.

Specific Aims & Methodology

Our overall approach rests on two main axes that, together, specifically harness *C. elegans*' key model organism strengths and combine the expertise in the Keil and Janke teams:
(1) Cutting edge genetics and genome-editing tools to manipulate the expression of (de)glutamylating enzymes specifically in the touch receptor neurons.

(2) Microfluidics and image analysis to allow life-long high-resolution imaging and quantitative analysis of MT-based transport dynamics as well as neuronal morphology.

Aim 1 Characterize touch receptor neuron homeostasis in *C. elegans* animals with perturbed polyglutamylation

In addition to the strains mentioned above, the student with construct *C. elegans* lines in which tubulin-modifying enzymes can be downregulated in touch receptor neurons through auxin-inducible

degradation (AID) [11]. The choices of tubulin-modifying enzymes will be guided by the published and ongoing biochemical characterizations in the Janke team. Using the established CCPP/TTLL mutants, the CRISPR overexpression lines as well as the AID lines, the student will then image and quantify touch receptor neuron morphologies at various ages, to dissect whether aberrations in MT polyglutamylation exacerbate/accelerate or rescue the wild-type aging-induced morphological changes (see Fig. 2B). An automated analysis pipeline to quantify features of touch receptor neuron morphology such as turns, axonal beading, process lengths based on [10] has already been established in the Keil team.

Aim 2 Develop a microfluidics setup and image analysis pipeline for life-long analyses of MT function at single animal resolution

To follow subcellular morphologies and axonal trafficking in adult aging *C. elegans*, animals must be confined permanently while still allowed to feed and lay eggs. Microfluidics is an ideal means to overcome this challenge and has been extensively used to study various aspects of *C. elegans* cellular physiology, development, and behaviour (e.g. [12–14]). For long-term imaging, instead of the circular chambers that the Keil lab has been developed to confine growing young larva [13], adult animals can be confined in elongated channels whose widths are specifically adapted to their size [15] (Fig. 2C). However, this only allows for imaging at low resolution and animal motion is not inhibited enough to image MT-based transport. We will solve this problem in two ways. First, we will add the possibility to pressurize animals for brief periods of time during which MT transport can be imaged (Fig. 2D). Second, to correct for any residual animal motion, we will implement deformable image registration using Deep Learning. We anticipate that MT-based transport can be periodically imaged for episodes of 2-3 min every 8h in up to 15 animals simultaneously throughout their lifetimes on one microfluidic chip. Combining microfluidics with the AID lines obtained in Aim 1 enables us to alter tubulin PTMs at precise stages of life by adding/removing auxin to/from the feeding medium during live imaging, thus uncoupling developmental defects from those of aging. Taken together, Aim 2 will put the student in a position to quantify neural morphologies together with microtubule-based transport parameters throughout the *C. elegans* adult lifespan at high-throughput.

Aim 3 Monitoring the effects of aberrant tubulin polyglutamylation on MT-based functions throughout animal lifetime in *C. elegans* neurons

Using the system developed in Aim 2, the student will determine changes in MT architecture and dynamics and analyse the transport of functionally different cargos (mRNAs, vesicles, protein complexes) in the six touch receptor neurons following AID mediated knockdown of tubulin-modifying enzymes at specific stages of life within the same animal. This approach will allow measuring direct correlations between aberrant neural transport and changes in neural morphology, with high statistical power, potentially revealing direct causal links between the two phenomena. Aim 3 will for the first time directly relate neuronal cargo transport to long-term changes in neural morphologies at single axon resolution, providing the first *in-vivo* analysis of the impact of the tubulin code on neuronal homeostasis and neurodegeneration during aging.

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