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Tau/PTL-1 associates with kinesin-3 KIF1A/UNC-104 and affects the motor's motility characteristics in *C. elegans* neurons

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ABSTRACT

Tauopathies are neurodegenerative diseases based on pathological tau-aggregation including Alzheimer's disease, frontotemporal dementia (FTD) and Pick's disease. In general, cargo (e.g., β-amyloid precursor protein, tau, neurofilaments) accumulation is a commonly observed phenomenon in degenerated neurons. Therefore, it is crucial to investigate the interaction between cargo, microtubule-binding proteins and molecular motors. We report the effect of tau/PTL-1 (protein with tau-like repeats) on the transport characteristics of the major axonal transporter kinesin-3 KIF1A/UNC-104 in the nervous system of *Caenorhabditis elegans*. Using confocal spinning disk time-lapse imaging we analyzed the motility of UNC-104::mRFP in *ptl-1* knockout worms and found that predominantly retrograde moving characteristics are affected (rather than the motor's anterograde displacements). A similar motility pattern was observed for synaptobrevin-1-containing vesicles, a major cargo of UNC-104. Moreover, UNC-104 and PTL-1 colocalize and occasionally co-migrate. We further confirmed physical interactions between PTL-1 and UNC-104 in living animals using the bimolecular fluorescence complementation assay (BiFC) as well as in co-immunoprecipitation experiments. Though this study focuses on PTL-1/UNC-104 interactions, we extended our research on monitoring conventional kinesin-1 (UNC-116) as well as dynein motility pattern and found that in *ptl-1* mutants retrograde displacements were also affected for UNC-116, while for dynein, interestingly, its anterograde movements were affected.

Introduction

Increasing findings link neurodegenerative disorders to dysfunctions of the neuronal cytoskeleton and transport systems (for review, see Cairns et al., 2004; Morfini et al., 2009). For instance, tau, a microtubule-binding protein (MAP), is associated with a range of neurodegenerative diseases known as tauopathies including Alzheimer's disease (AD), FTDP17 (frontotemporal dementia and parkinsonism linked to chromosome 17) and Pick's disease (Ballatore et al., 2007). Importantly, tau seems to exist in gradients in neurons with lower concentrations proximally and higher concentrations distally, while, interestingly, in degenerating neurons these gradients are reversed with high concentrations proximally and low concentrations distally (Dixit et al., 2008). Therefore investigating the effect of both low and high tau concentrations (meaning tau depletion versus tau overexpression in experimental approaches) on neuronal viability and cellular mechanisms is critical.

In *C. elegans*, PTL-1 (protein with tau-like repeats) encodes a tau-like protein with 50% similarity to its mammalian analogue with respect to its carboxyl-terminal tandem repeats (basic microtubule-binding domain) (Goedert et al., 1996). *C. elegans* PTL-1 shares several characteristics with human tau as charge distribution, predicted structure and numerous phosphorylation sites (Goedert et al., 1996; McDermott et al., 1996) characteristics that are likely to be important for interacting with microtubules and molecular motors (see also Suppl. Figs. S7 and S8). PTL-1 exists in two isoforms, PTL-1A and PTL-1B, with five or four tandem repeats, respectively. Both PTL-1 isoforms are able to bind to microtubules and promote tubulin assembly in vitro. In addition, PTL-1-transfected Sf9 insect cells grow long cellular extensions similar as seen after MAP2 or tau expression in these cells. In *ptl-1* knockout animals, the hatch ratio of eggs and the response to gentle body touch are slightly reduced. Intriguingly, tubulin immunostaining reveals no significant difference between wildtype and *ptl-1* knockout animals in mechanosensory neurons, suggesting that PTL-1 may not visibly affect microtubule organization in *C. elegans* (Gordon et al., 2008).

PTL-1 is mainly expressed in mechanosensory neurons and their processes (except PVM), but also in some head neurons, motor neurons, in the ventral cord, epidermis (in embryos) and in stomatointestinal muscles (Goedert et al., 1996; Gordon et al., 2008). The six mechanosensory neurons (AVM, ALML, ALMR, PVM, PLML and PLMR) grow long axons along around half of the worms' body length (Suppl. Fig. S1) and sense external gentle body touch (Chalfie et al., 1985). Their axons (positioned just beneath the worm's cuticle) contain

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unusual 15-protofilament microtubules, therefore named "microtubule cells" (e.g., AVM: anterior ventral microtubule cell), generating electrical signals in response to external forces.

UNC-104 is a neuron-specific, microtubule plus-end directed motor and is a homologue of the mammalian kinesin-3 KIF1A which is the major transporter of synaptic vesicles in axons. Mutations in the UNC-104 gene in worms result in uncoordinated, slow body motion and a slow growth rate with a concomitant increase of synaptic vesicles in cell bodies and decrease at synapses (Hall and Hedgecock, 1991; Vale, 2003). More important, KIF1A knockout mice are lethal with motor and sensory defects and abnormal distribution of synaptic vesicles (also increased in soma and reduced in neuronal terminals) (Yonekawa et al., 1998). In *C. elegans*, fluorescently labeled UNC-104 reveals a bidirectional moving pattern with net transport rates in anterograde directions (Hsu et al., 2011; Kumar et al., 2010; Wagner et al., 2009; Zhou et al., 2001).

Microtubule-associated proteins (MAPs) play important roles in organizing the cellular microtubule network and to maintain the integrity between microtubules and other major cytoskeletal elements (Amos and Schlieper, 2005). Besides this scaffolding function, it has been reported that MAPs can directly affect kinesin motility (Telley et al., 2009) and that phosphorylation of MAPs regulates microtubule-based transport (Mandelkow et al., 2004). A closer look revealed that MAPs, however, sparsely change a motor's velocity or run length but majorly act to reduce the binding/unbinding rates of motors to microtubules (Seitz et al., 2002; Vershinin et al., 2007). Still, there is a discrepancy in the literature regarding the role of tau in axonal transport as there are studies claiming no role for tau in synaptic vesicle transport (Yuan et al., 2008). As we cannot find reports on the effect of tau on a kinesin-3 motor (reported to be a major axonal transporter), we set out to provide an in-depth motor behavioral analysis in *ptl-1* mutants, as well as to investigate whether direct interactions between PTL-1 and UNC-104 exist. For example, we employ the novel BiFC (bimolecular fluorescence complementation assay) method (Hsu et al., 2011) to investigate physical interactions between PTL-1 and the motor in the living worm but also co-immunoprecipitation assays. In addition, we present data on mCherry::PTL-1 movements in the presence or absence of UNC-104. As we have to assume that kinesins and dynein exist in tug-of-war complexes (Gross et al., 2002; Hendricks et al., 2010; Koushika et al., 2004; Muller et al., 2008; Soppina et al., 2009), we furthermore observed the effect of *ptl-1* knockout on dynein motility. Last, as tau/kinesin-1 interactions have been reported (Cuchillo-Ibanez et al., 2008; Utton et al., 2005), we further investigate the effect of *ptl-1* knockout on the motility of the *C. elegans* kinesin-1 (heavy chain) homolog UNC-116.

Materials and methods

Strains and constructs

C. elegans strains were maintained at 22 °C using standard methods (Brenner, 1974). Strains Punc-104::UNC-104::mRFP(*e1265*), Punc-104::UNC-104::GFP;Punc-104::SNB-1::mRFP(*e1265*), Punc-104::UNC-104ΔFHA::GFP and Punc-104::UNC-104ΔPH::GFP have been described elsewhere (Wagner et al., 2009). Strains UNC-104::mRFP(*e1265 ok621*) and Punc-104::UNC-104::GFP; Punc-104::SNB-1::mRFP(*ok621*) were generated by mating males expressing Punc-104::UNC-104::mRFP(*e1265*) or Punc-104::UNC-104::GFP;Punc-104::SNB-1::mRFP(*e1265*) with RB809 *ptl-1(ok621)* hermaphrodites. Males from the F1 generation of these crosses were then backcrossed into RB809 *ptl-1(ok621)* hermaphrodites and further progenies outcloned for highest transmission of the transgene. The strain Punc-104::UNC-104Δ461-1339::GFP (UNC-104ΔFHAΔSTALK) is a kind gift from Dr. Dieter Klopfenstein (Georg-August-University, Göttingen, Germany).

The worm Punc-104::mCherry::PTL-1;UNC-104::GFP(*e1265*) was generated by microinjecting an Punc-104::mCherry::PTL-1 plasmid (28 μg/ml) into UNC-104::GFP*(e1265*) expressing hermaphrodites using standard microinjection methods (Fire, 1986; Mello et al., 1991). The transgenic animal Punc-104::mCherry::PTL-1(*ok621*) was generated by microinjecting an Punc-104::mCherry::PTL-1 plasmid (14 μg/ml) into RB809 ptl-1(*ok621*) hermaphrodites. The worm expressing UNC-116::GFP was described elsewhere (Wagner et al., 2009) and was crossed into RB809 *ptl-1(ok621)* hermaphrodites. Punc-104::DLC-1::YFP(*ok621*) worms were generated by microinjecting a Punc-104::DLC-1::YFP plasmid at 50 μg/ml into RB809 *ptl-1(ok621)* hermaphrodites (the DLC-1 insert was amplified from genomic DNA and subcloned into an existing vector containing the promoter and the fluorophore). Note, that for technical reasons, DLC-1::YFP observation was carried out in isolated primary *C. elegans* neurons as described below. Using the strain BC12648, expressing a ptl-1::GFP transcriptional fusion, we found that 90% of isolated neurons express PTL-1 (data not shown).

To extract genomic DNA from worms, we picked 2–3 young adults per μl into a worm lysation buffer (0.45% Tween-20, 0.45% NP-40, PCR buffer, 20 mg/ml proteinase K) and first incubated the solution at 65 °C for 2 h, then at 95 °C for 25 min and finally used it as a PCR template.

Bimolecular fluorescence assay

For bimolecular fluorescence complementation assay (BiFC) experiments, we used the BiFC control vector kit (pCE-bJUN-VN173 and pCE-bFOS-VC155) from Dr. Chang-Deng Hu's Lab (Purdue University, USA) and replaced the heat shock promoter *hsp16.41* with the pan-neuronal Punc-104 promoter. Positive control vectors for nucleus expression Punc-104::bJUN::VN173 (40 μg/ml) and Punc-104::bFOS::VC155 (30 μg/ml), respectively, were microinjected into N2 hermaphrodites using the pRF4 rol-6(su1006) co-injection marker. Positive control vectors for investigating cytoplasmic expression of two known interacting proteins (UNC-104/UNC-104) Punc-104::UNC-104::VN173 (60 μg/ml) and Punc-104::UNC-104::VC155 (60 μg/ml) were microinjected into CB1265 *unc-104(e1265)* hermaphrodites rescuing the highly uncoordinated and paralytic phenotype (note, that the need of high plasmid dosages to rescue the *unc-104* phenotype has been reported previously (Wagner et al., 2009)). Negative control vectors Punc-104::UNC-104::VC155 (60 μg/ml) and Punc-104::bJUN::VN173 (60 μg/ml) were microinjected into CB1265 *unc-104(e1265)* hermaphrodites rescuing the *unc-104* phenotype. The Punc-104::bJUN::VN173 plasmid was detected using a forward primer (GGAAAGATCGCAATGAGCTT) covering 20 bp near the 3′-terminus of the promoter and a reverse primer (ATGTAGGGATGTTGAAGAGTAAT) covering 23 bp near the 5′-terminus of the VN173 insert. To detect UNC-104/PTL-1 interactions, the vectors Punc-104::UNC-104::VC155 (60 μg/ml) and Punc-104::VN173::PTL-1 (60 μg/ml) were microinjected into CB1265 *unc-104(e1265)* hermaphrodites rescuing the *unc-104* phenotype. For details using the BiFC assay in the nervous system of *C. elegans* and further positive and negative controls refer to Hsu et al. (2011).

Motor motility analysis

For imaging, worms were immobilized by treatment with 5 mM levamisole (Sigma-Aldrich) before being placed on 2% agarose-coated objective slides. We used an Olympus IX81 microscope with a DSU Nipkow spinning disk unit connected to an Andor iXon DV887 EMCCD camera for high-resolution and high-speed time-lapse imaging (at 1–2 frames per second). To convert recorded time-lapse sequences into kymographs we used the imaging analysis software NIH ImageJ 1.43 software (NIH, http://rsb.info.nih.gov/ij/). Here, a line over the axon of interest is drawn, followed by the application of the "reslice