Regulation of KIF1A Behavior and Motility Via Tau's Behavioral Dynamics

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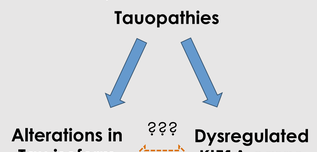
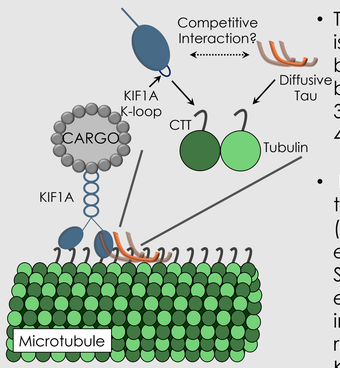
Background

- KIF1A is a superprocessive kinesin-3 family member (Soppina *et al.*, 2014) with characteristic pausing behavior (Lessard *et al.*, 2019) and an important mediator of long-range cargo transport in neurons.
- KIF1A cargo has been shown to irregularly localize within the neurons of certain neurodegenerative diseases (Lassmann *et al.*, 1992; Kandalepas *et al.*, 2013). This abnormal cargo aggregation insinuates a loss of spatiotemporal regulation of cargo delivery.
- Tau, a microtubule associated protein, is one potential regulator of KIF1A motility as it is known to regulate kinesin-1 motility (Vershinin *et al.*, 2007; Dixit *et al.*, 2008; McVicker *et al.*, 2011).
- Tauopathies are a class of neurodegenerative diseases related to Tau dysfunction. Certain Tauopathies such as Alzheimer's disease (AD) and frontotemporal dementia (FTD) present with altered Tau isoform expression as well as impaired KIF1A cargo delivery.



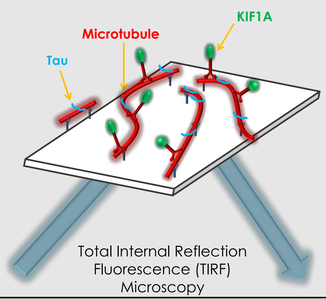


- Tau binds to the microtubule in an isoform-dependent equilibrium between static and diffusive behavior (McVicker *et al.*, 2014). 3RS-Tau is predominantly static while 4RL-Tau is predominantly diffusive.
- Both Tau and KIF1A interact with the microtubule C-terminal tails (CTTs) (Ikegami *et al.*, 2007, Hinrichs *et al.*, 2012; Okada *et al.*, 2000; Soppina and Verhey, 2014, Lessard *et al.*, 2019). These competing interactions present a potential regulatory mechanism of Tau on KIF1A motility.

Question: What is Tau's role in regulating KIF1A behavior and motility?

Experimental design

- A truncated (1-393) *Rattus norvegicus* KIF1A with an added leucine zipper and C-terminal 3xmCitrine (gift from Dr. Kristen Verhey, University of Michigan) was used for all motility experiments. For motor expression, plasmids were transfected into a COS-7 mammalian cell line. Cell lysates were harvested and highly diluted for motility assays (Soppina *et al.*, 2014).
- Wild-type 3RS- and 4RL-Tau constructs were expressed in BL-21 DE3 cells and purified using column chromatography (McVicker *et al.*, 2011).
- For experiments assessing the effects of CTT removal, Taxol-stabilized microtubules were proteolytically digested with subtilisin (Knipling *et al.*, 1999).
- A Q-dot conjugated kinesin-1 motor was used as an experimental control for all *in vitro* motility assays.
- Motility assays were performed on Taxol-stabilized or subtilisin-treated microtubules in the presence or absence of Alexa 647 labelled 3RS- or 4RL-Tau using TIRF microscopy (Hoeprich *et al.*, 2014).
- Fluorescence based TIRF binding assays, motility assays, and pausing parameters were analyzed as described in Stern *et al.*, 2017 and Lessard *et al.*, 2019, respectively.



KIF1A exhibits characteristic pausing behavior

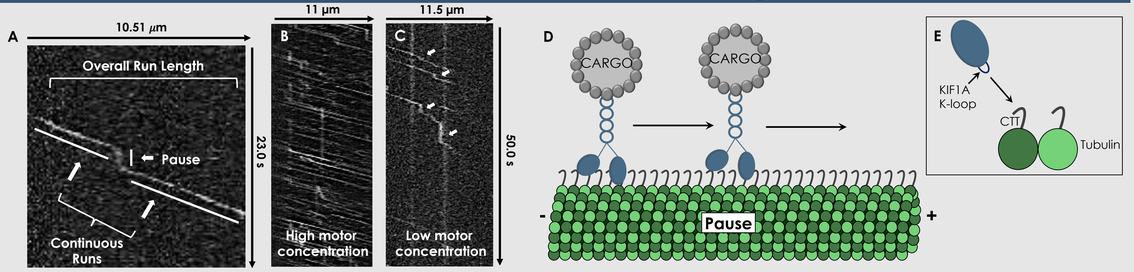


Figure 1. A) Representative kymograph with corresponding nomenclature used to describe novel KIF1A motility behavior. **B,C)** Representative kymographs demonstrating that at **B)** high motor concentrations, pausing becomes obscured by surrounding motor motility while at **C)** low motor concentrations, extensive pausing behavior (white arrows) is revealed at stochastic positions on the microtubule. **D)** KIF1A exhibits an overall run length (RL) composed of continuous runs connected by pauses. **E)** These pauses are reliant upon accessible C-terminal tails to tether the KIF1A K-loop to the microtubule, before initiating another continuous run. Figure 1A-C from Lessard *et al.*, 2019.

4RL-Tau more strongly regulates KIF1A run length than 3RS-Tau

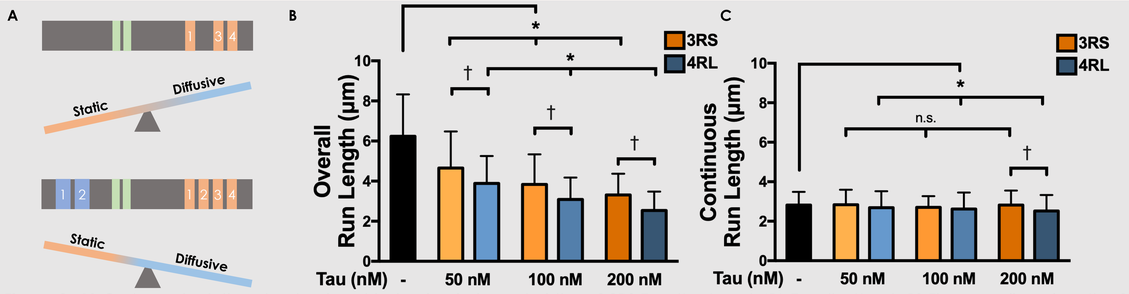


Figure 2. A) Cartoon depicting structural and behavioral characteristics of 3RS-Tau (top) and 4RL-Tau (bottom). **B)** The overall run length of KIF1A was reduced upon the addition of 3RS- or 4RL-Tau in a dosage-dependent manner. When 3RS- and 4RL-Tau are compared at specific concentrations, 4RL-Tau is more regulatory than 3RS-Tau. **C)** Continuous run length was not significantly reduced between 3RS- and 4RL-Tau at 50 nM and 100 nM concentrations, but was significantly reduced at 200 nM Tau. Run length values are reported as mean ± standard deviation and were calculated as previously reported in Thompson et. al, 2013. *,† p <0.05

4RL-Tau more strongly regulates KIF1A pausing behavior than 3RS-Tau

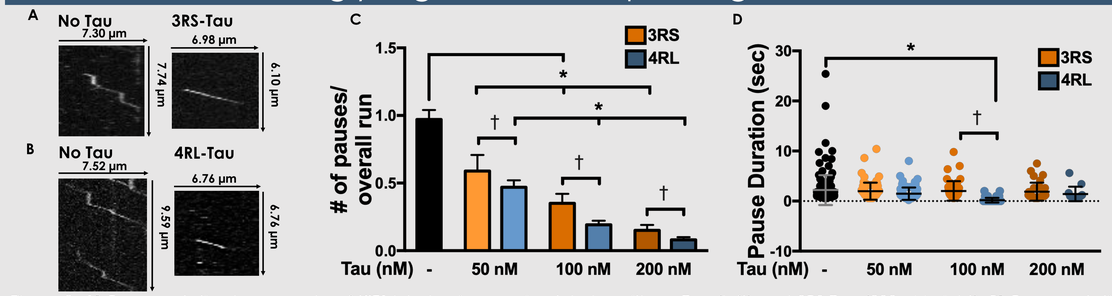


Figure 3. A) Representative kymographs of KIF1A behavior on microtubules with no Tau (left) and 3RS-Tau (200 nM; right). **B)** Representative kymographs of KIF1A behavior on microtubules with no Tau (left) and 4RL-Tau (200 nM; right). **C)** KIF1A pause frequency (pauses/overall run) decreases in a dosage-dependent manner upon addition of 3RS- and 4RL-Tau. At individual concentrations of Tau (50 nM, 100 nM, or 200 nM), 4RL-Tau significantly reduces the pause frequency of KIF1A when compared to 3RS-Tau. **D)** Pause duration was not significantly reduced between 3RS- and 4RL-Tau at 50 nM and 200 nM concentrations, but was significantly reduced at 100 nM Tau. *,† p <0.05

Static Tau obstacles do not regulate KIF1A motility

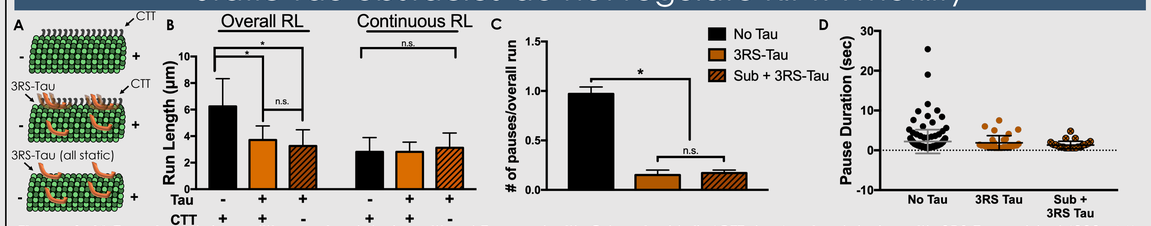


Figure 4. A) Experimental conditions: microtubules without Tau and with C-terminal tails (CTT; top), microtubules with 3RS-Tau added (200 nM) and with CTTs (middle), or microtubules with with 3RS-Tau added (200 nM) and no CTTs (bottom). **B)** KIF1A overall run length was significantly reduced upon the addition of 3RS-Tau, or the addition of Tau on subtilisin (Sub)-treated microtubules. KIF1A continuous run length was not significantly changed. **C)** KIF1A pauses/overall run decreased on subtilisin-treated + 3RS-Tau microtubules (200 nM 3RS-Tau) when compared to control (no Tau, no subtilisin treatment) but not when compared to untreated microtubules with 3RS-Tau (200 nM 3RS-Tau). **D)** KIF1A pause duration on control microtubules, across experimental conditions. * p <0.05

3RS-Tau binding on subtilisin microtubules

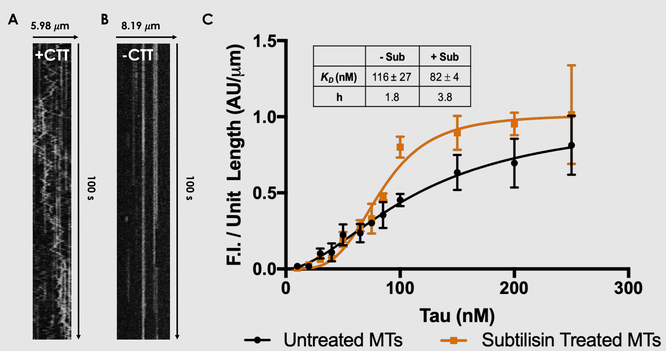


Figure 5. A) Representative kymograph of 3RS-Tau on untreated microtubules (MTs) demonstrating the static (straight lines) and diffusive (jagged lines) behavioral states of Tau. **B)** Representative kymograph of 3RS-Tau on subtilisin-treated MTs demonstrating that C-terminal tail removal shifts Tau to the static state. **C)** Upon subtilisin treatment of MTs, Tau's affinity and cooperativity increased.

Model of Tau-mediated KIF1A regulation

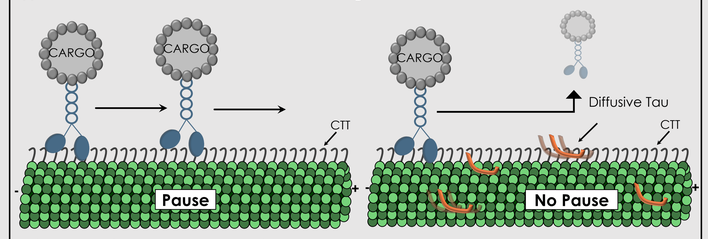
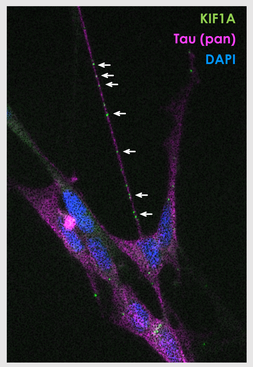


Figure 6. A) In the absence of Tau, KIF1A exhibits an overall run length composed of continuous runs connected by pauses. These pauses are reliant upon accessible CTTs to tether KIF1A to the microtubule, before initiating another continuous run. **B)** When Tau is bound to the microtubule surface, the diffusive state of Tau interacts with and occupies the CTTs. KIF1A can still achieve an initial continuous run, and navigate around static Tau obstacles, but cannot interact with the CTTs due to diffusive Tau occupancy. This reduction in pausing manifests in KIF1A's inability to string together multiple continuous runs within an overall run length event.

Future Directions



Shifts in Tau isoform expression are observed in Tauopathies such as AD and FTD.

How do changes in Tau expression affect KIF1A localization and motility in cellular models of axonal transport?

SH-SY5Y cellular model → How do changes in Tau expression affect KIF1A localization and motility in a structurally "neuron-like" cell type?

Figure 7. SH-SY5Y neuroblastoma cells, 7 days post retinoic acid differentiation. Punctate KIF1A staining on neurites is observed (white arrows). KIF1A, green puncta; Tau (pan), magenta; DAPI, blue. 40x.

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