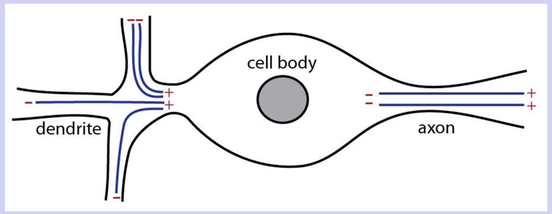
Research Experiences for Urban Science Teachers

Microtubule polarity in dendrites with CDK5 protein knock-down

Lori Piper[1], Alex Weiner[2,3], and Dr. Melissa Rolls[2,3]

[1] Altoona Area School District, Altoona, Pennsylvania; [2]Molecular, Cellular, and Integrated Biosciences Program; [3]*Biochemistry and Molecular Biology, Penn State University*

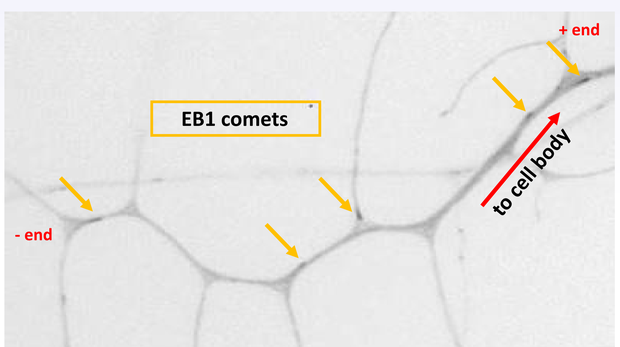
Microtubules have specific organization



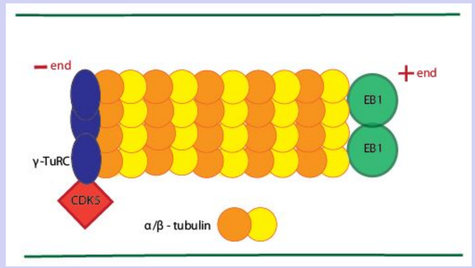
Nervous system health requires proper function of neurons. Each neuron consists of dendrites, an axon, and the cell body. Proper function requires the neuron to track needed proteins from the cell body through the dendrites and axon. Proteins are tracked along microtubules (MT) arranged in a specific polar orientation. Microtubules in fly dendrites are organized in a >90% minus-end-out configuration while axons are ~100% plus-end-out. Because new neurons are not created during an organism's life, there must be mechanisms for repairing damages and reforming microtubules. The microtubules in the dendrite must organize in the absence of a microtubule-organizing center (MTOC) which cellular microtubules use. Several key proteins have been implicated in this formation process.

Microtubule polarity shown by EB1

Polarity is assayed through tagging end-binding protein (EB1) with a fluorescence marker (GFP) and tracking it as it moves throughout the dendrite. Because EB1 always localizes toward the + end of the dendrite, tracking its movement will show the direction of the + end. Quantitating EB1 comets tracking toward the + end indicates the percent polarity of the microtubules within the dendrite.



Proposed model of microtubule growth factors



In dendrites, γ-TuRC complex nucleates new microtubule minus ends. EB1 promotes the addition of free α/β-tubulin to elongate the microtubules at the plus end. This investigation aims to determine whether CDK5 impacts microtubule regulation independent of a MTOC.

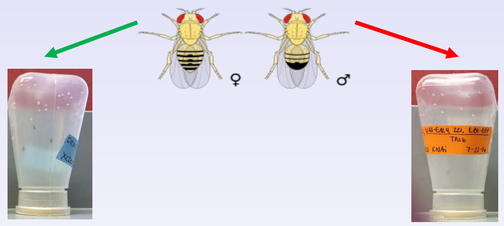
Hypothesis

Knockdown of CDK5 will alter EB1 directionality signaling a change in microtubule polarity in *Drosophila* Class I ddaE neuron dendrites.

RTLN2 and CDK5 knock-down flies are created and tested

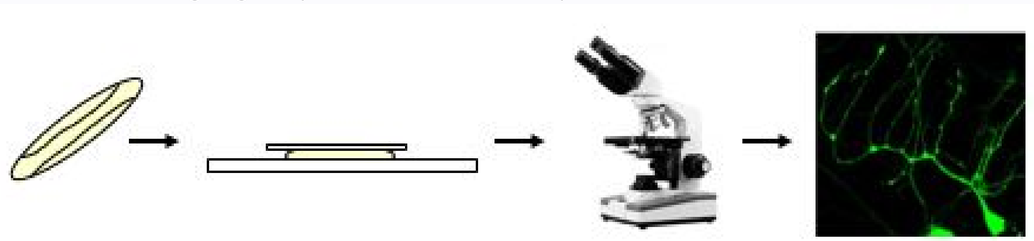
A special fly tester line, EB1-GPF, was used to allow for fluorescence of the EB1 comets. Virgin females were crossed to males with tested protein genes knocked-down. The knocked-down protein will have diminished presence in the fly larva affecting function. Two testing lines were created – one control and one experimental.

Control Line
- RTLN2 knock-down
- No phenotype
- >90% EB1 comets should move toward + end.

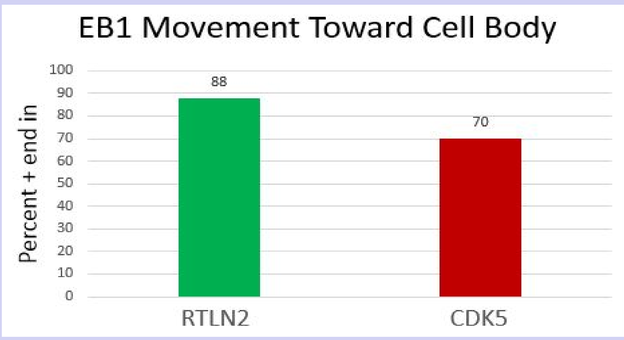


Experimental Line
- CDK5 knock-down
- Should show altered EB1 comet movement

Three day old larva were collected from each cross. Class I ddaE neurons from hemisegments 4/5/6 were live imaged for 5 minutes. EB1 comets tracking through the main dendrite branch were counted as going away or toward the cell body.

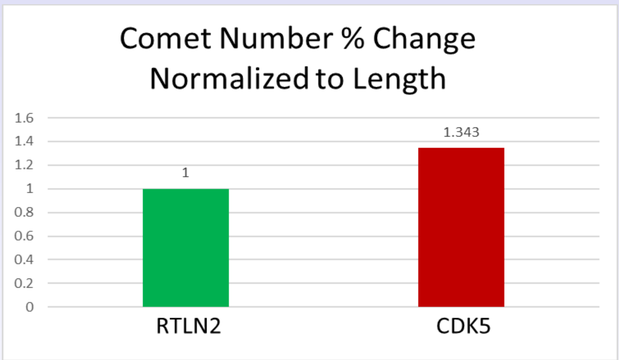


CDK5 knockdown alters MT polarity



Knock-down of CDK5 reduces the percent of EB1 comets tracking toward the +end of the dendrite when compared to the control, RTLN2. This signals a change in polarity of the dendrite following a mutation in CDK5. It suggests CDK5 is important as a microtubule regulator in the absence of MTOC.

CDK5 reduction increases comet number



Knockdown of CDK5 increases the average number of EB1 comets, as compared to the control. This hints at the cell's use of a compensatory mechanism to ensure microtubule growth even in the absence of CDK5.

Further studies

- Does CDK5 affect γ–tubulin localization?
- Does CDK5 affect nucleation dependent injury response?
- Is CDK5 causing a stress response?

Acknowledgments

Special gratitude to Alex Weiner for mentoring, Matthew Shorey for discussions and confocal imaging, and the Melissa Rolls Lab for hosting.

This project was supported by grants from Boeing and NIH, and in coordination with CSATS/PSU.

References:

Baas P.W., Lin S. (2011). Hooks and comets: the story of microtubule polarity orientation in the neuron. *Developmental Neurobiology,* 71(6), 403-18. http://www.ncbi.nlm.nih.gov/pubmed/21557497

Muroyama A., Seldin L., Lechler T. (2013). Divergent regulation of functionally distinct γ-tubulin complexes during differentiation. *Journal of Cell Biology*, 213(6), 679-92. http://www.ncbi.nlm.nih.gov/pubmed/27298324