

Developing a Zebrafish Model of KIF1A Associated Neurological Disorder

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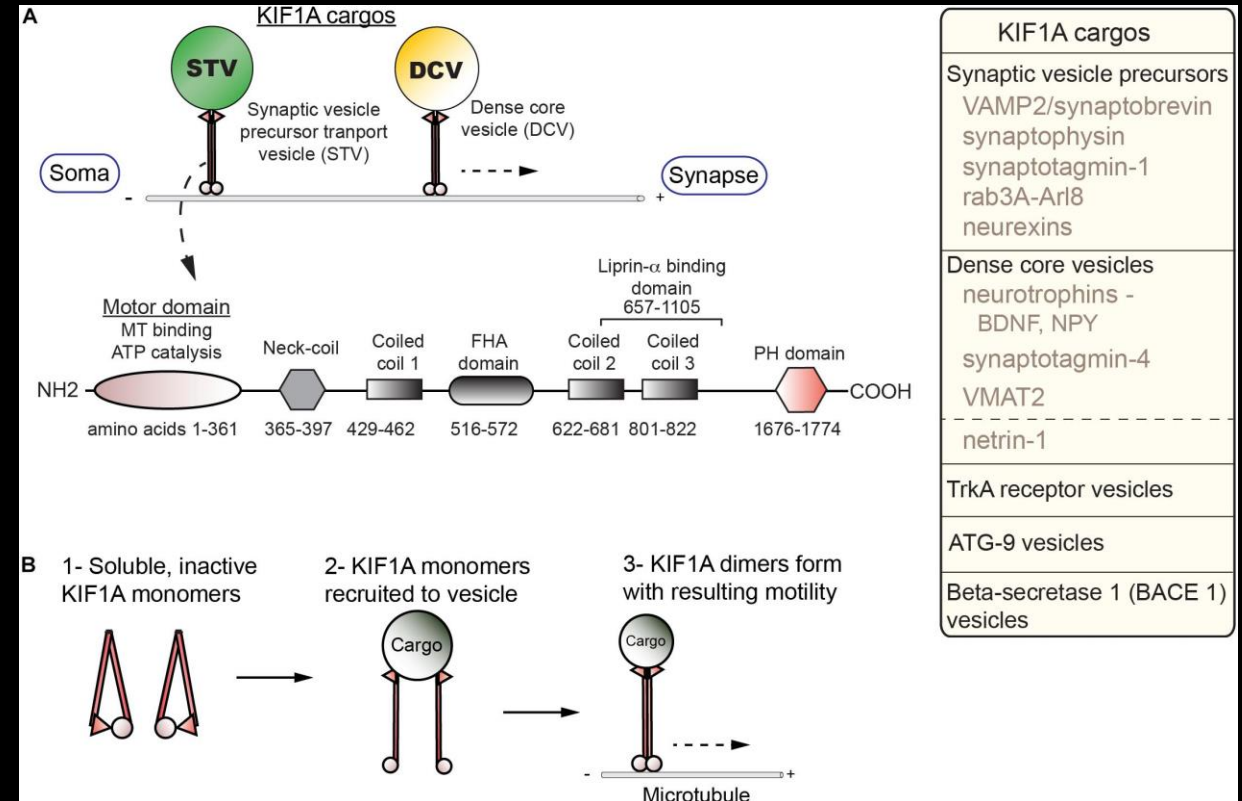
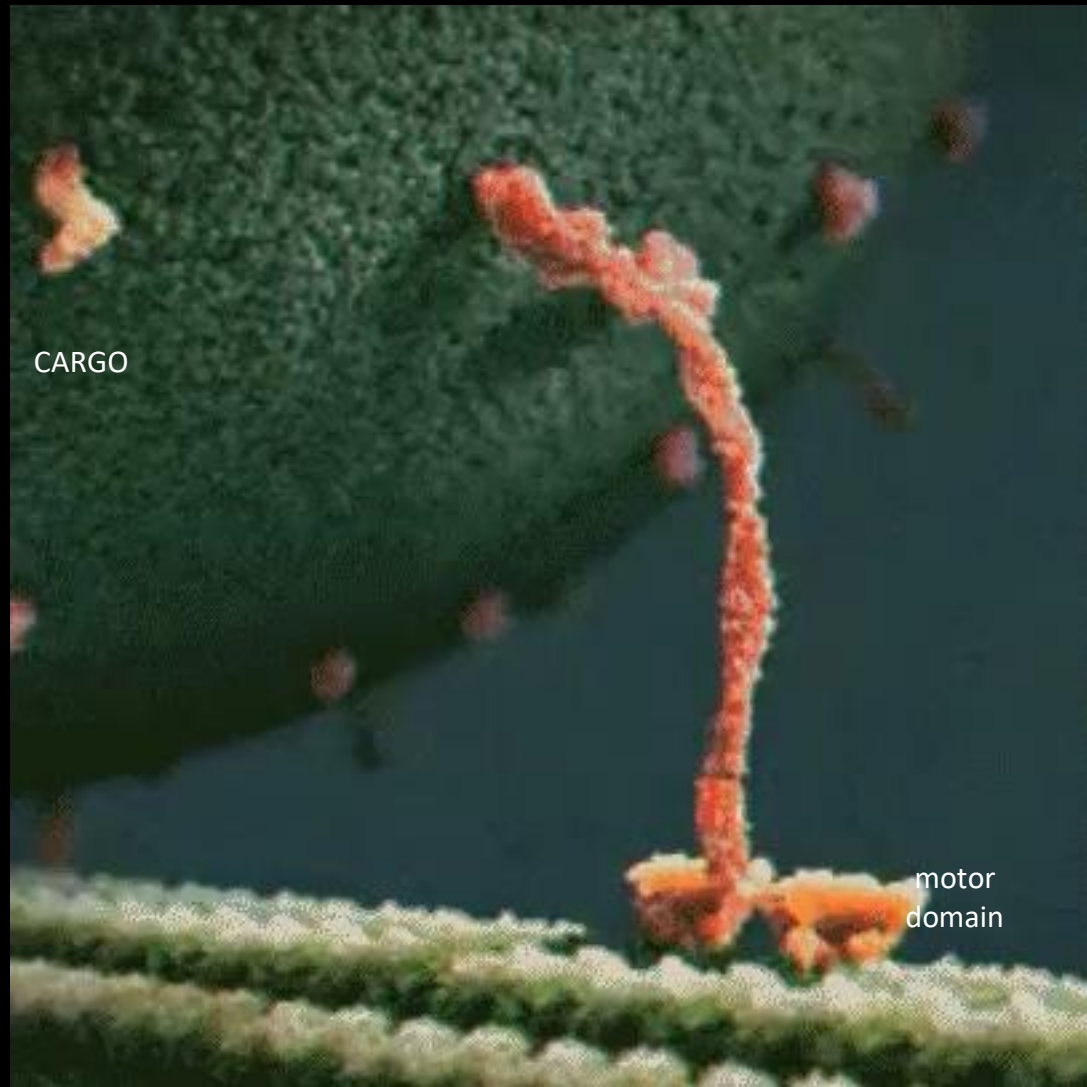
Greg Palardy



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Kif1a transports cargo away from the cell body toward the synapse

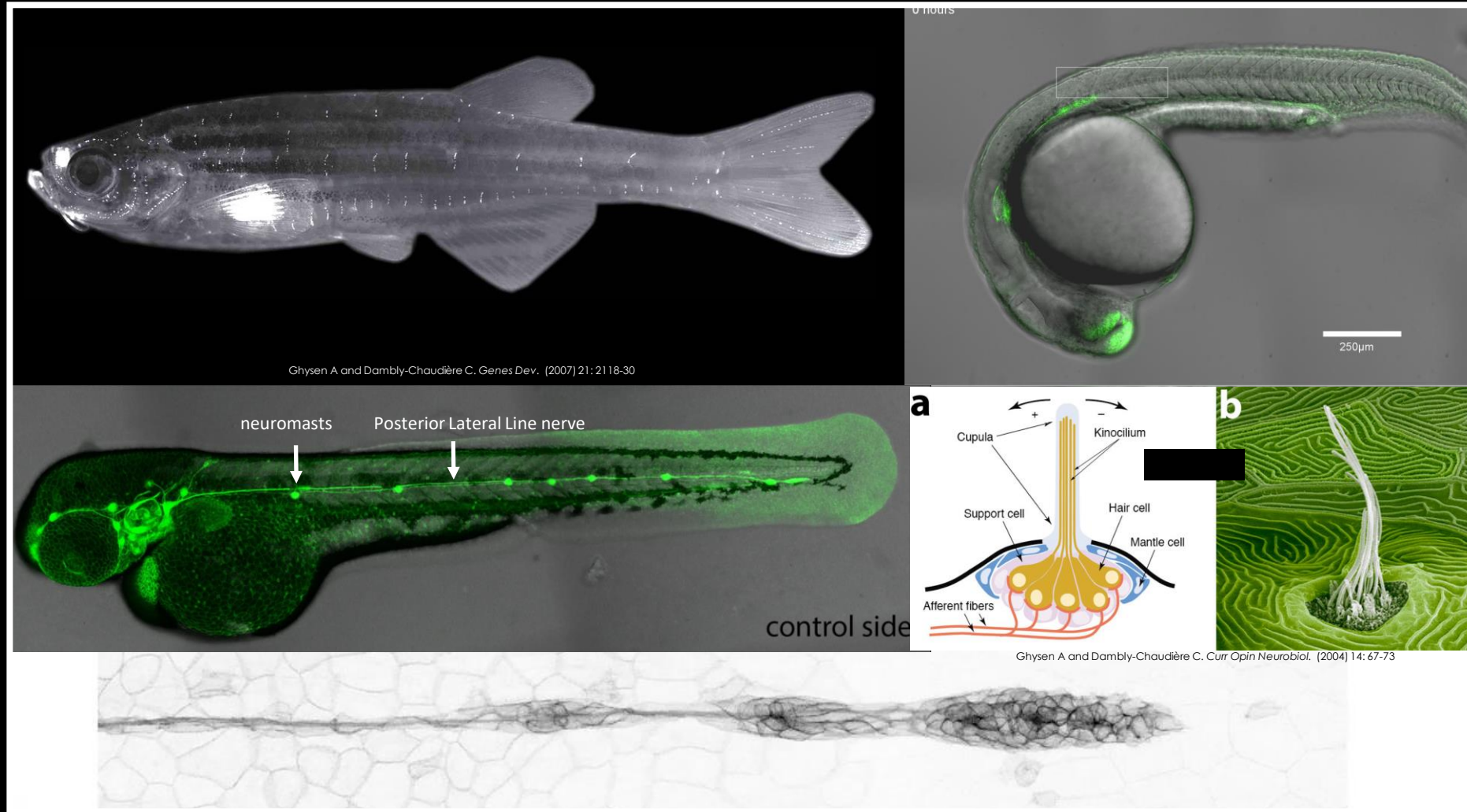


<https://imgur.com/gallery/TKtA28B>

(An animation extracted from The Inner Life of a Cell by Cellular Visions.)

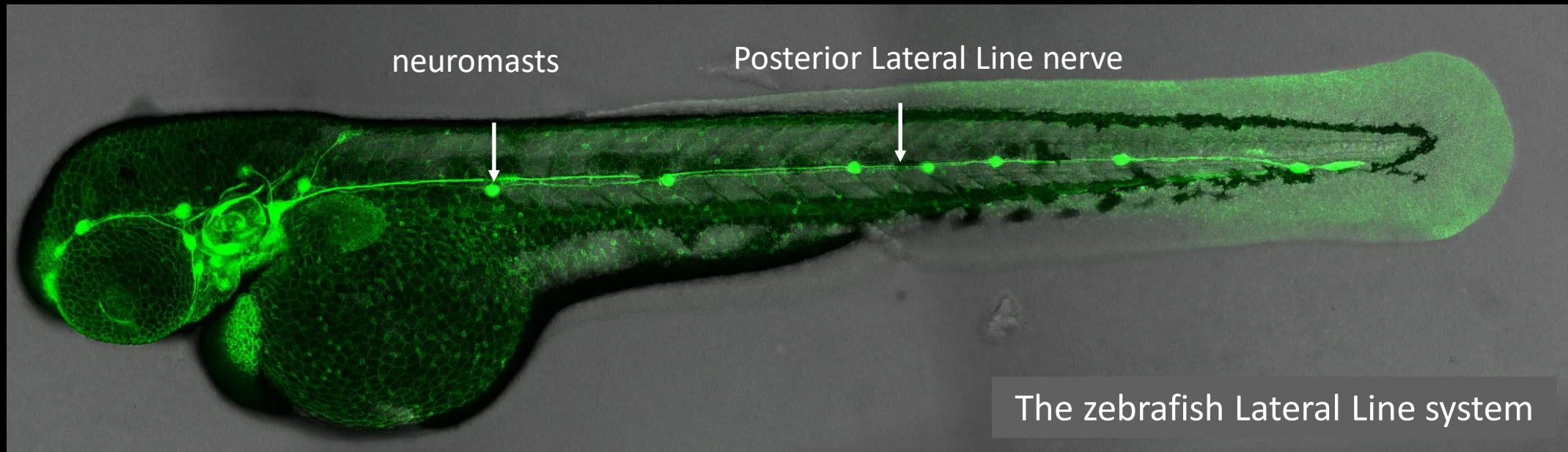
Front. Cell. Neurosci., 26 September 2019 | <https://doi.org/10.3389/fncel.2019.00419>

The Posterior Lateral Line primordium -a model for understanding the self-organization of development in the embryo

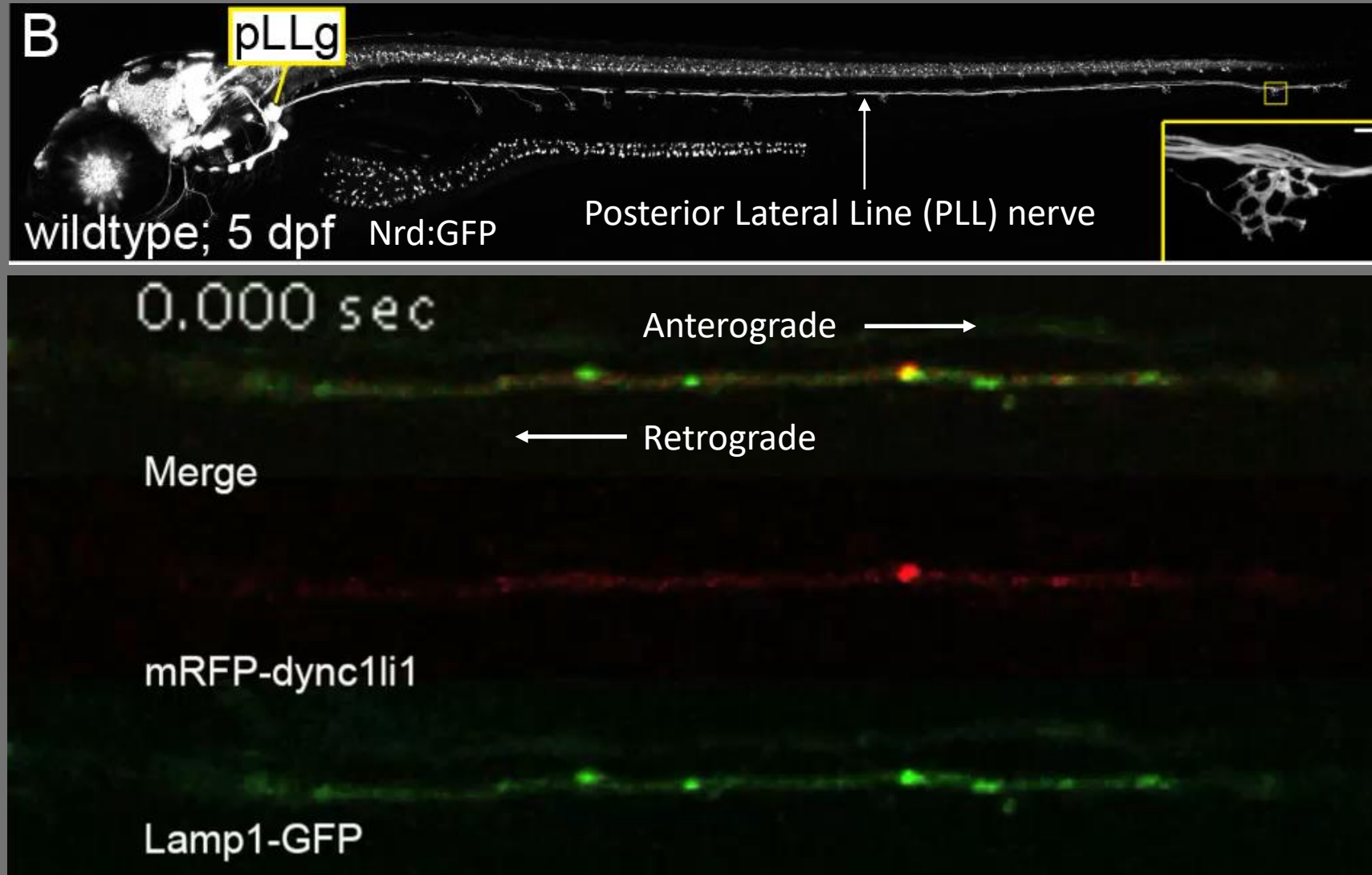


Features of an *in vivo* system to visualize and quantify deficits in anterograde transport and for preliminary evaluation of potential therapeutic approaches in KAND

- Visualize and quantify transport in axons
- Ease of cellular, molecular and genetic manipulation
- Ease of access to manipulation with exposure to drugs and small molecule inhibitors
- Use in high throughput studies



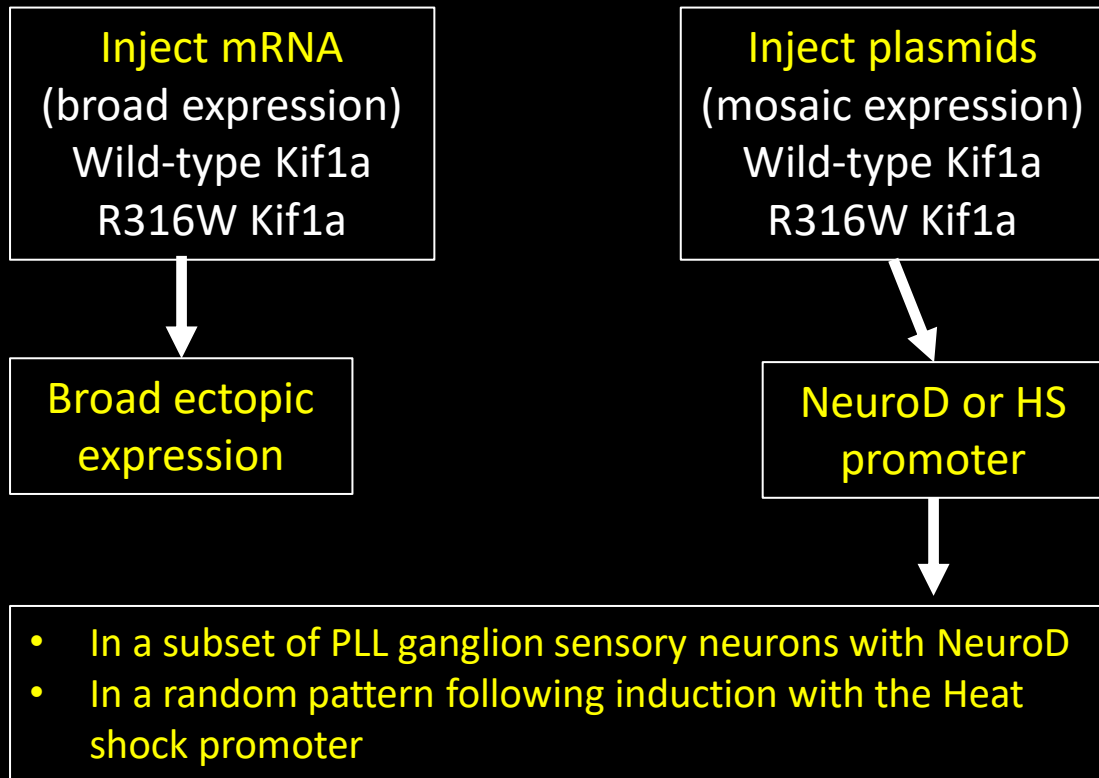
The Lateral Line as a model for studying axonal transport in zebrafish



mRFP-Dync1li1/Lamp1-GFP cotransport in a pLL axon at 2dpf. Cell bodies are to the left meaning that particles moving toward the left are moving in the retrograde direction.

Strategies for engineering expression of Wild-type and mutant Kif1a in zebrafish larvae

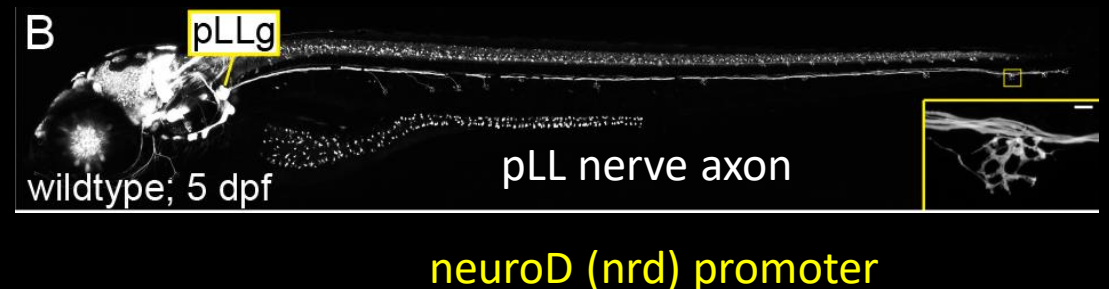
Transient expression



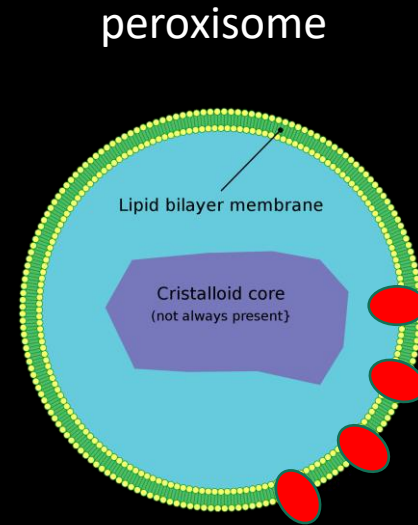
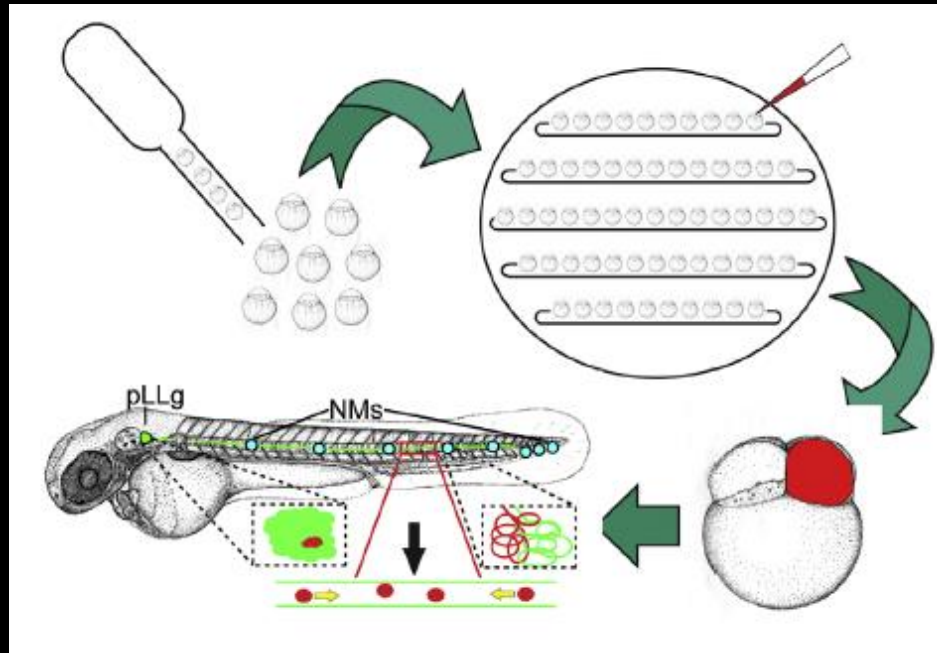
Stable transgenics

1. Heat shock promoter
2. neuroD promoter

CRISPR induced R316W mutation



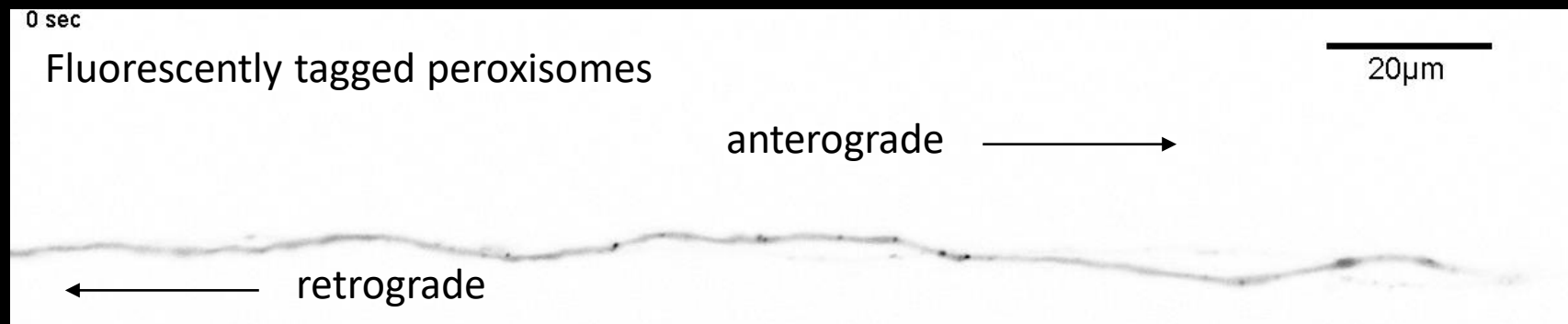
Monitoring anterograde and retrograde transport with fluorescently labelled cargo



● PTS1-mCherry

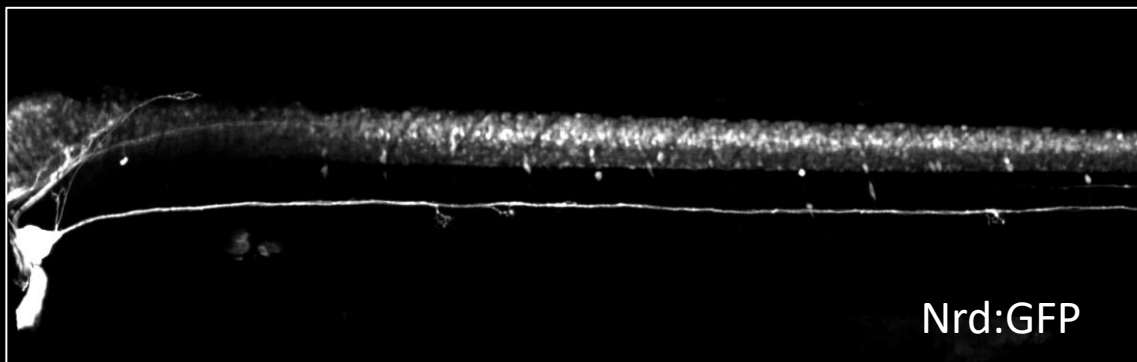
Inject embryos at the 1-2 cell stage with with plasmids driving PTS1-mCherry expression under control of a *neuroD* promoter (*nrd: PTS1-mCherry*)

From Drerup and Nechiporuk (2016)

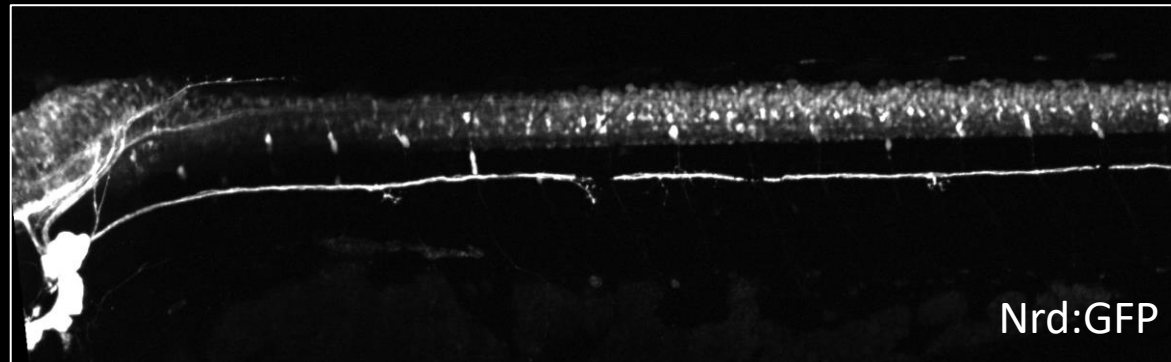


The Peroxisome Targeting Signal 1 (PTS1) is used to target mCherry tp peroxisomes

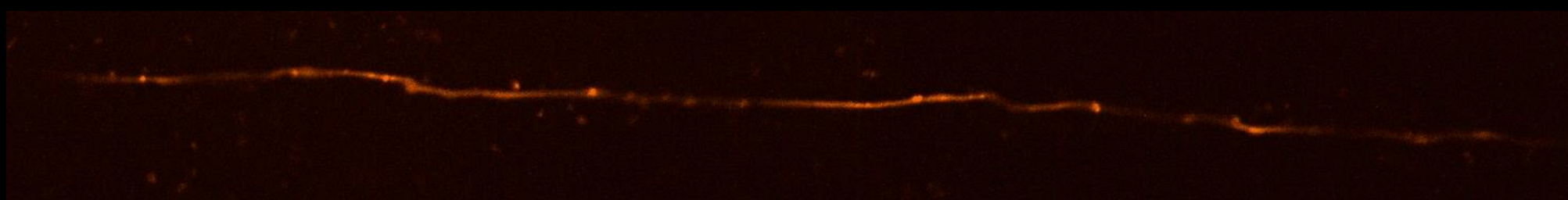
R316W Kif1a mRNA does not affect Lateral line morphology but stalls peroxisome movement



200 pg Wt Kif1a mRNA injected



200 pg R316W Kif1a mRNA injected



200 pg Wt Kif1a mRNA injected



200 pg R316W Kif1a mRNA injected

Future Plans

- Quantify effects of injecting different concentrations of Wt and R316W Kif1a mRNA –
at what concentration does anterograde and retrograde transport get affected
- Does additional expression of Wt Kif1a reduce slowing due to R316W Kif1a
- How is transport of other cargoes affected?
- Establish R316W mutant line
- See if knockdown of Kif1a or ectopic expression of Wt Kif1a reduces problems.
- Can CRISPR be used to repair the endogenous mutation
- Make our system available for testing of any potential therapeutic agents or other strategies suggested by the Kif1a scientist's network

Thank You!



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Abhishek
Mukherjee



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Rachel Dansereau



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