What role do glia play in long-term axon maintenance?

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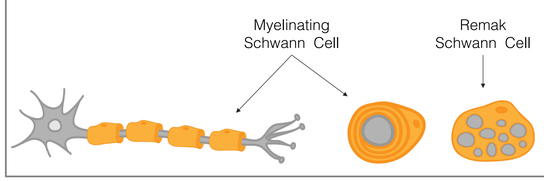
INTRODUCTION

Axons are remarkable structures that can be highly branched and cover great distances, such that most of the volume of the neuron is contained within its axon. This elaborate morphology causes neurons to face unique challenges when it comes to meeting the axon's metabolic and homeostatic needs, especially considering the cost associated with action potential propagation and transport along the axon. The mechanisms that regulate axon maintenance remain poorly characterized. Glia are found throughout the nervous system and there are several specialized glia that associate specifically with axons, going as far as to insulate them along their entire length as in the case of Remak Schwann cells in the peripheral nervous system[1]. This striking relationship between glia and axons has led to the long-held belief that glia contribute significantly to axon maintenance. Further support for this model comes from observations in human neurodegenerative diseases such as multiple sclerosis[2] and Charcot-Marie-Tooth disease[3], where glial cell loss precedes axon degeneration and clinical symptoms. Furthermore, knockout of some glial genes alone can cause axon degeneration even in the absence of glial cell loss[4,5] and recent, exciting work has provided some evidence for a role of glia in metabolic support[6]. However, despite this the widely accepted hypothesis that glia support axons, we know surprisingly little about the extent and molecular nature of glia's role in long-term axon maintenance.

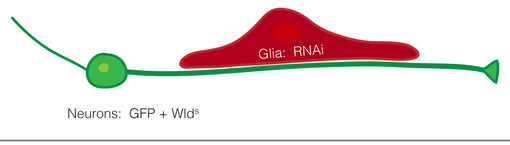
Glia in the Peripheral Nervous System:



OBJECTIVES

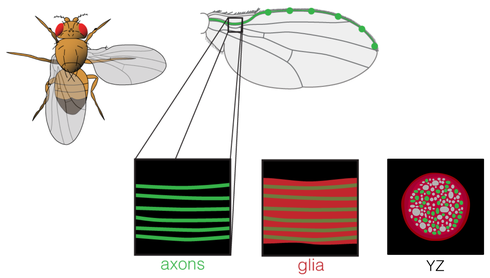
In order to address this gap in our knowledge, we have generated a system aimed to identify novel genes involved in the glial support of axons *in vivo*. By expressing the chimeric protein Wld^s in neurons, we can block Wallerian degeneration in severed axons that are no longer supported by their neuronal cell body[7]. By doing so, this limits the resources available to the axon shifting its dependence onto the surrounding glia. This creates a sensitized system in which we can systematically knockdown genes in glia using RNA interference (RNAi) and evaluate the effect this has on axon integrity. Using this system we are screening thousands of genes in order to identify novel genes involved in long-term axon maintenance. These experiments seek to move the field of glial biology forward by providing a more comprehensive understanding of the molecular mechanisms involved in glia support of axons.

Independent cell-type specific manipulation *in vivo*:

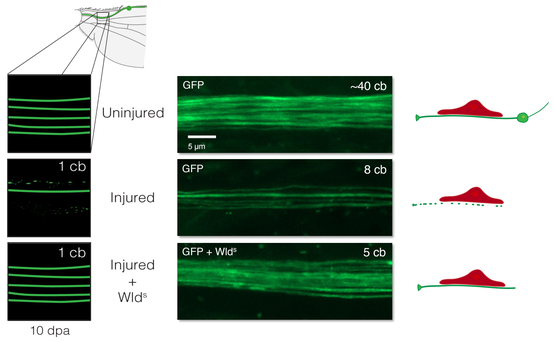


METHODS

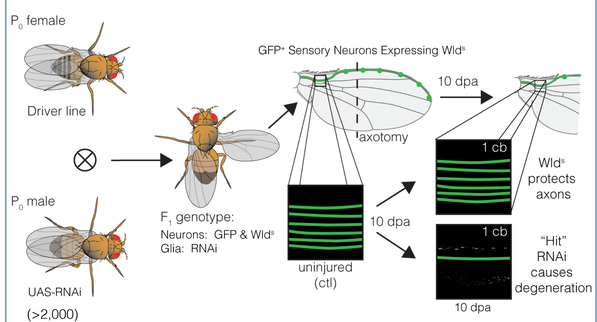
Drosophila provides a genetically tractable model to study axon-glia interactions _in vivo_



Wld^s blocks axon degeneration creating a sensitized system for assaying the glial contribution to axon maintenance



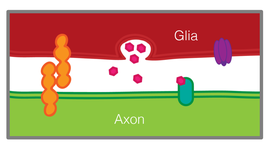
Screening a panel of over 2,000 genes _in vivo_ using RNAi to identify novel genes involved in glial support of axons



Modified from *Neukomm et al. PNAS 2014*

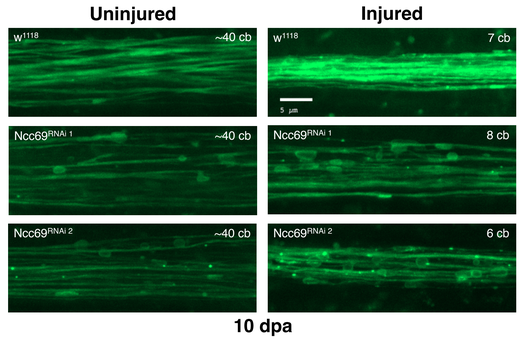
Testing genes that are likely involved in cell-cell signaling based on predicted protein domains

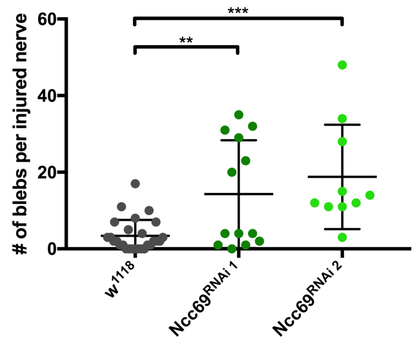
- Transmembrane domain
- Signal peptide
- Genes enriched in glia



RESULTS

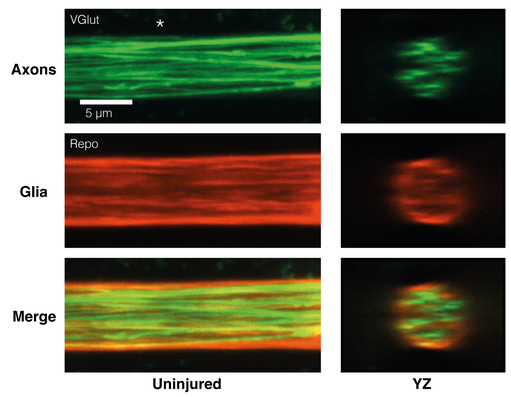
RNAi knockdown of sodium chloride cotransporter 69 (Ncc69) in glia causes increased formation of blebs in axons





At 10 days post axotomy (dpa), there are an increased number of blebs in nerves when Ncc69 has been knocked down in glia. Statistics: ordinary 1-way ANOVA with Dunnett's multiple comparisons test, n = 27, 13, and 10 wings respectively, mean ± SD, ** = p <0.01, *** = p < 0.001

Assessing glial integrity by independent labeling of axons and glia _in vivo_ using genetically encoded fluorescent proteins



CONCLUSIONS

- We have generated a powerful tool to screen thousands of genes *in vivo* for their involvement in glial support of axons
- Our preliminary hits suggest that there are many genes from a variety of molecular classes that cause defects in axon maintenance when knocked down in glia
- This system can identify genes that have conserved biological function

FUTURE DIRECTIONS

- ❑ Finish screening the remaining genes
- ❑ Validate initial hits from screen using second RNAi and mutants with glial-specific rescue
- ❑ Identify pathways and gene families that are enriched for from the screen
- ❑ Adult-specific knockdown of top candidate genes to identify roles in development versus roles in the adult
- ❑ Identify glial subtypes involved and assess glial integrity in knockdown animals
- ❑ Characterize precise changes in axon maintenance

REFERENCES

1. Jessen, K. R. and Mirsky, R. The origin and development of glial cells in peripheral nerves. *Nat Rev Neurosci* **6**, 671–682 (2005)
2. Rivers, T. M. and Schwentker, F. F. Encephalomyelitis accompanied by myelin destruction experimentally produce in monkeys. *J. Exp. Med.* **61**, 689-702 (1935)
3. Dyck, P. J. and Lambert, E. H. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. *Arch Neurol.* **18**, 603-618 (1968)
4. Griffiths, I., Klugmann, M., Anderson, T., Yool, D., Thomson, C., Schwab, M., Schneider, A., Zimmermann, F., McCulloch, M., Nadon, N., and Nave K. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science.* **280**, 1610-1613 (1998)
5. Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P., Griffiths, I., and Nave, K. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nat. Genet.* **33**, 366-374 (2003)
6. Saab, A. S., Tzetavona, I. D., Trevisiol, A., Baltan, S., Dibaj, P., Kusch, K., Möbius, W., Goetze, B., Jahn, H. M., Huang, W., Steffens, H., Schomburg, E. D., Pérez-Samartin, A., Pérez-Cerda, F., Bakhtiari, D., Matute, C., Löwel, S., Griesinger, C., Hirrlinger, J., Kirchhoff, F., and Nave, K. A. Oligodendroglial NMDA Receptors Regulate Glucose Import and Axonal Energy Metabolism. *Neuron.* **91**, 119-132 (2016)
7. Mack, T.G.A, Reiner, M., Beirowski, B., Mi, W., Emanualli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., Fernando, F. S., Tarlton, A., Andressen, C., Addicks, K., Magni, G., Ribchester, R. R., Perry, V. H. and Coleman, M. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat Neurosci.* **4**, 1199-1206 (2001)
8. Neukomm L. J., Burdett, T. C., Gonzalez, M. A., Züchner, S. and Freeman, M. R. Rapid *in vivo* forward genetic approach for identifying axon death genes in Drosophila. *Proc. Natl Acad. Sci.* USA **111,** 9965-70 (2014)

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