Can targeting growth associated mRNAs into sensory axons enhance growth on inhibitory substrates?

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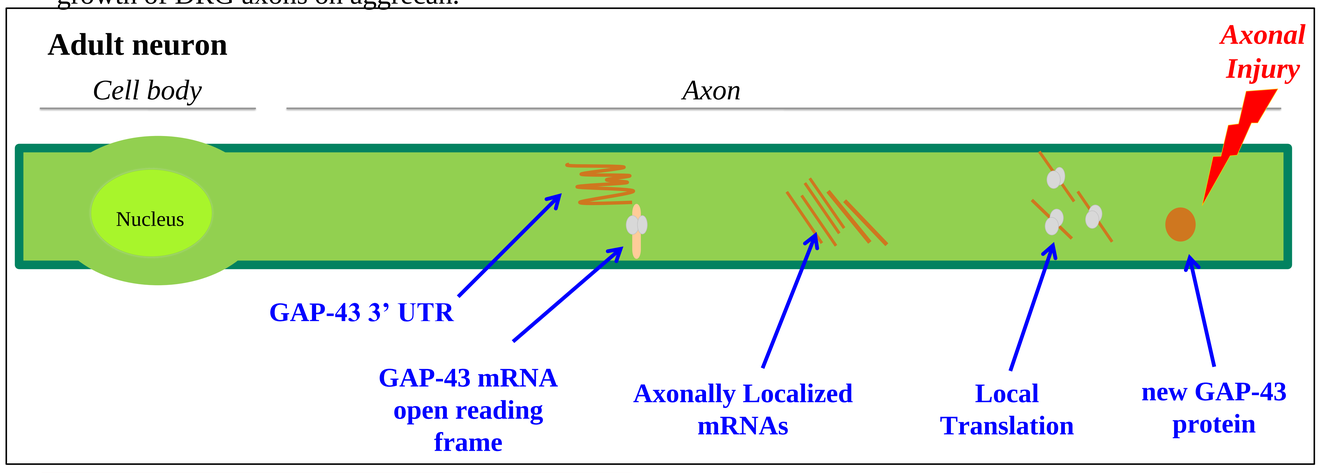
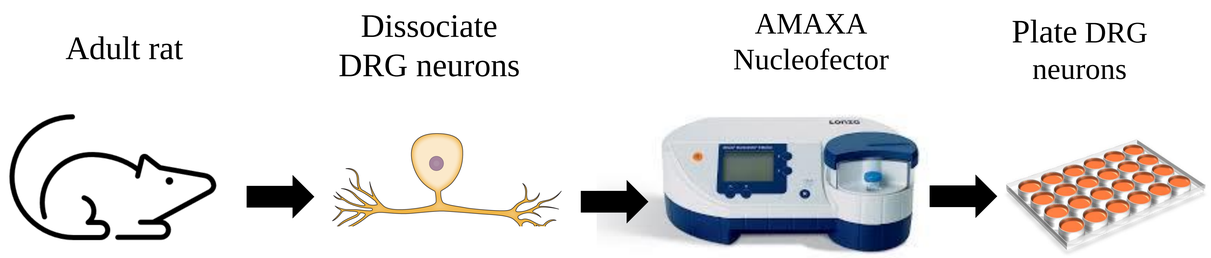
Introduction

Dorsal root ganglia (DRG) contain the sensory neurons of the peripheral nervous system that specialize in relaying sensory information from the periphery to the brain. The peripheral axons of these neurons have the ability to regenerate quite well after injury whereas those in the central nervous system do not. This is due, in part, to the supportive environment in the peripheral nervous system as well as activation of a growth program within the neuron. One component of this growth program is locally synthesized growth-associated proteins within the axon. The axons of DRG neurons can be quite long thereby necessitating a way to provide support for growth at the injury site through translation of mRNAs within the axon. The Twiss lab has previously shown that targeting the mRNAs encoding for the growth-associated proteins GAP-43 and β-actin to the axons leads to increased growth of DRG axons on permissive substrates. It is known that axons in the central nervous system do not regenerate, in part, due to an environment composed of inhibitory components like chondroitin sulfate proteoglycans (CSPGs; e.g., aggrecan). For this project, we hypothesized that targeting GAP-43 and β-actin mRNAs to the axon could increase growth of DRG axons on aggrecan.



Methods



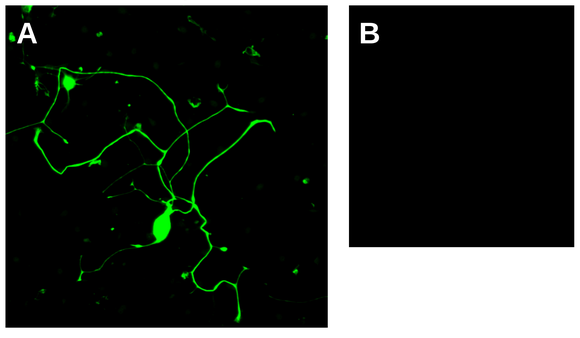
- Experiment 1: Determine best nucleofector program to use for constructs
- Experiment 2: Test aggrecan concentrations
- Experiment 3: Test laminin only + aggrecan and laminin mixture

Results

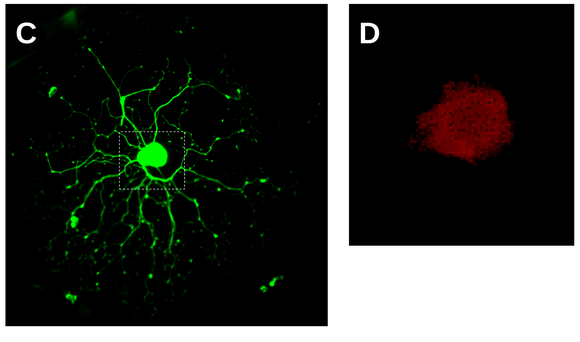
Figure 1. DRG neurons cultured for 48 hours on laminin. A. Neuron that was not transfected with any plasmid DNA. B. The cell body of the untransfected neuron (shown in A.) that does not display any mCherry signal. C. Neuron transfected with the mCherry-GAP-43 3'Amphoterin plasmid construct using the SCN 5 nucleofector program. D. mCherry signal in cell body of neuron shown in B.

Nucleofector Programs	Transfection rate
SCN 1-1	61%
SCN 1-2	72%
SCN 5	90%

Untransfected



mCh-GAP43-3'Amph

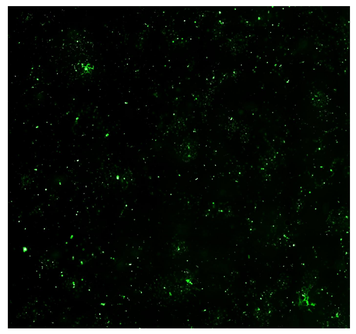


Results

Figure 2:

A

Laminin only



B

Laminin + Aggrecan

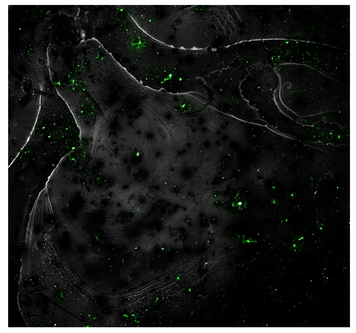


Figure 2. A. This image is a coverslip with only laminin, which is a permissive environment that helps the neurons to grow. B. This is a coverslip with laminin and aggrecan. The aggrecan is acting as an inhibitory substrate, a non-supportive environment, to see if the neurons can still grow even in the present something that is harmful to them.

Future Directions

- To use Program 5 of the nucleofector for another transfection
- Test β-actin constructs
- Combine GAP-43 and β-actin constructs with HuD overexpressing construct or KSRP knockout to see effect on growth (laminin and aggrecan)

References

Donnelly, C. J., et al. "Axonally Synthesized -Actin and GAP-43 Proteins Support Distinct Modes of Axonal Growth." *Journal of Neuroscience*, vol. 33, no. 8, 20 Feb. 2013, pp. 3311–3322., doi:10.1523/jneurosci.1722-12.2013.

Acknowledgements

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