Identification of axon-oligodendrocyte preferential interaction sites preceding initial myelin sheath formation

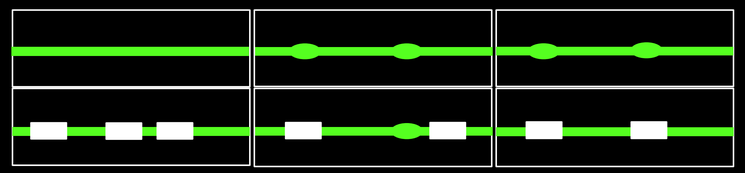
Taryn Mallon and Rachael Sawyer
Biology Department, Winona State University, Winona, MN

Abstract

Oligodendrocytes are matured oligodendrocyte progenitor cells that serve to myelinate axons within the central nervous system. The myelination process is complex and is orchestrated by many unknown mechanisms, of particular interest, the causal relationship between axon diameter and myelination. Previous studies suggest that oligodendrocytes preferentially myelinate axons with larger diameters. Due to the variation in axon diameter along the length of individual axons, we first hypothesized that oligodendrocytes interact with and initiate myelination at thicker domains of axons known as varicosities. To test this hypothesis, we performed in vivo time-lapse in zebrafish larvae to determine if oligodendrocytes preferentially interact with and sustain interactions at varicosities compared to intervening, thin axonal segments. These larvae expressed green and red fluorescent proteins in each cell type, enabling direct observation of oligodendrocyte-axon interaction over several hours. Oligodendrocytes more frequently interacted with varicosities as compared to intervening axonal segments. In addition, oligodendrocytes interacted with varicosity domains for more sustained periods of time as compared to intervening segments.
Varicosities serve as sites of enriched synaptic vesicle release, which is instrumental in oligodendrocyte and axonal communication. Therefore, ongoing experiments are testing whether synaptic vesicle release is necessary to direct and stabilize oligodendrocyte interactions at these sites.

Introduction

Oligodendrocytes (OL) function to wrap axons with myelin sheaths that are essential for proper axonal functioning. Previous studies show that OL's wrap axons of larger diameter as compared to those smaller in diameter. This knowledge led us to come up with following three working models exemplifying the initial OL-axon interactions and myelination processes.



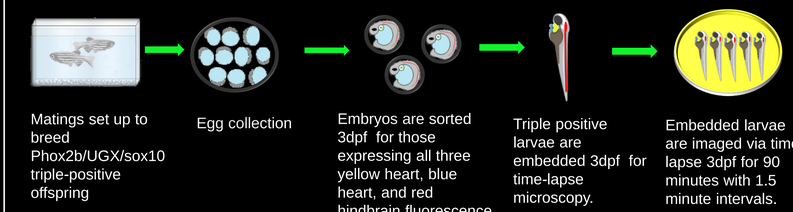
Working Model 1: Axons do not have varicosities and OL's wrap without preference.

Working Model 2: Axons have varicosities, but OL's wrap without preference

Working Model 3: Axons have varicosities and wrap such domains preferentially

We hypothesize that Working Model 3 is what will be observed because varicosities are sites of enriched vesicle release which is believed to play a roll in axon-OL signaling and communication.

Methods



1. Do oligodendrocytes preferentially interact with varicosities as compared to intervening segments?

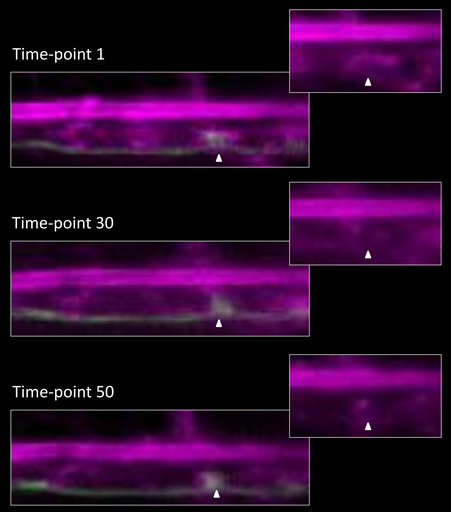


Fig. 1a. OL's preferentially interact with varicosities.
We preformed in vivo time-lapse microscopy to observe preferential OL-axon interaction domains. Over the course of 90 minutes with 1.5 minute intervals, it was observed OL's interact with varicosity domains more readily than intervening segments.

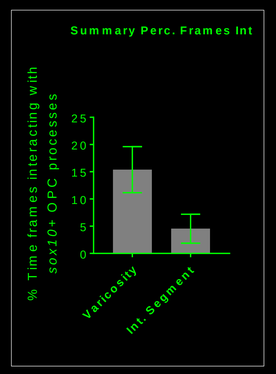


Fig. 1b. Quantitative representation. We observed varicosities are being interacted with 15.386% of the time frames as compared to an intervening segments 4.54343% of time frames.

2. Do oligodendrocytes preferentially sustain interactions at specific axonal domains?

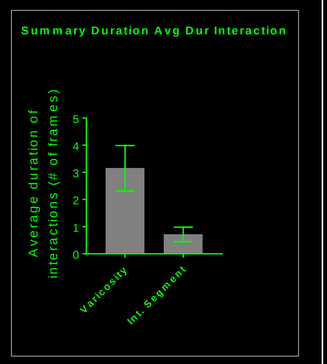


Fig. 2b. Quantitative Representation
Varicosities with an average duration of 3.15 frames interacting is higher than that of the intervening segments average duration of .7125 frames spent interacting.

Fig. 2a. OL's preferentially sustain interactions at varicosities. Time-lapse 12252015 is showing a sustained interaction at the varicosity through listed time points. Time-lapse 02232017 exemplifies an interaction at an intervening segment that is not sustained through the time points.

3. Does synaptic vesicle release direct oligodendrocytes to axonal varicosities for myelination?

Figure 3a. Varicosity expression and oligodendrocyte interaction along fluorescently tagged axons. *Phox2b*+ axons are labelled with UAS:memGFP whereas oligodendrocyte processes and nascent sheaths are labelled sox10:memRFP.

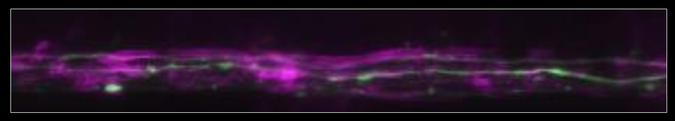
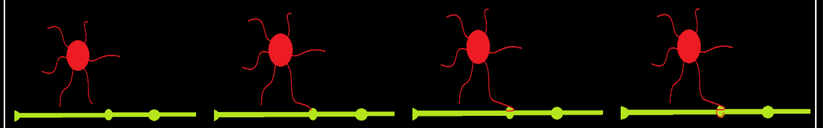


Figure 3b. TeNT expression in single *phox2b*+ axons and nascent sheath expression marked by sox10:memRFP. Preliminary results indicate blockage of synaptic vesicle release causes OLs to bind without preference along an axon.

Conclusions

Oligodendrocytes preferentially interact with varicosities.

Oligodendrocytes sustain interactions preferentially at varicosities as compared to intervening segments.



OL extends processes to interact with axon.

OL processes preferentially dancing on axon at varicosities

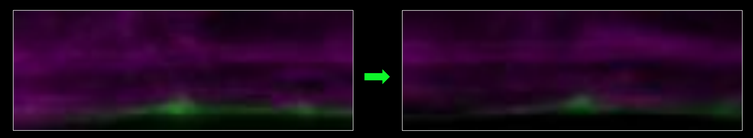
OL interacts with varicosity preferentially

OL preferentially sustains interaction at varicosity

Future Directions

Knowing OLs preferentially interact with varicosities, we next want to determine what comes first. Does axon diameter increase precede initial wrapping or does initial wrapping precede diameter increase? We continue to explore this chicken or the egg question to determine which comes first and why.

We hypothesize that axonal diameter will increase prior to initial wrapping events.



In order to continue studying the importance of synaptic vesicle release in the myelination process, a future experiment of ours would include the relocation or overexpression of vesicle release in normally unmyelinated axons. This would help identify the role vesicle release plays in OL axon recognition for myelination.

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