Characterization of a zebrafish peripheral myelin mutant

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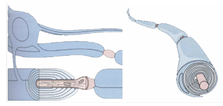
Zebrafish provide an ideal system for studying the development of myelinating glia

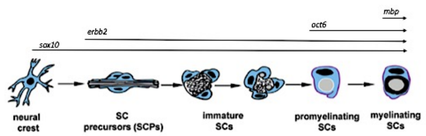
Myelin insulates axons and allows allows for efficient electrical signaling

Myelin is produced by Schwann cells in the PNS and oligodendrocytes in the CNS

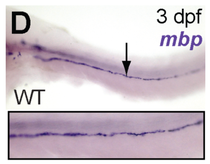


Kandel et al, 2006

Each stage of Schwann cell developmental can be defined by key developmental markers

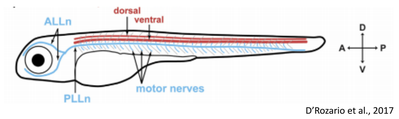


D'Rozario et al, 2017



Petersen et al, 2015

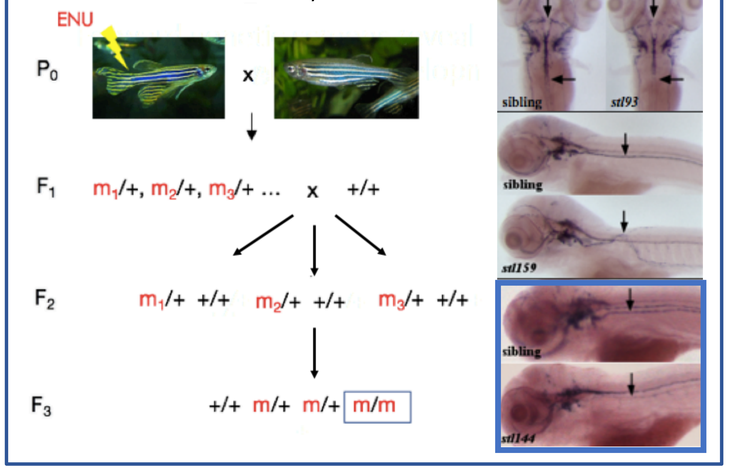
Mechanisms of myelination is conserved in jawed, vertebrate organisms



D'Rozario et al., 2017

Forward genetic screens uncover genes that regulate myelination in vertebrate systems

A screen conducted at Washington University, St. Louis revealed three novel mutants with reduced myelination.



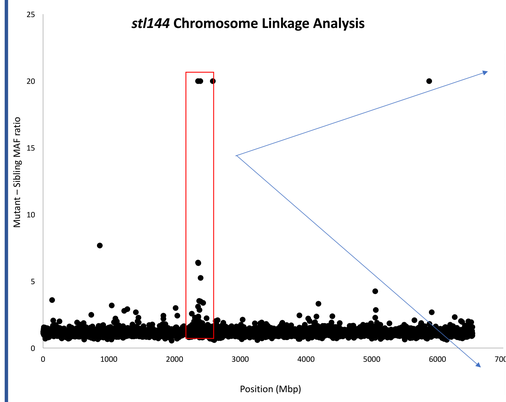
stl144 homozygous mutants have reduced PNS myelination

- Where is the causative lesion? What gene does it affect?
- At what point in development is this gene necessary?
- What are the developmental effects of disrupting the gene?
  1. Reduced number of Schwann cells
  2. Reduced wrapping by Schwann cells
  3. Reduced number of axons present for wrapping

Whole-genome sequencing analysis pipeline to identify affected gene(s) in *stl144*

MAF linkage to chromosome 8 → Delete synonymous mutations → Select for homozygous mutants and heterozygous siblings → Manually filter common variants using SNPFisher → 25 plausible gene candidates

Whole Genome Sequencing of *stl144* shows linkage to chromosome 8



Mutation	Gene	Gene Ontology
A/C	fabp1b.2	fatty acid binding
G/T	adam9	integrin-mediated signaling pathway
A/C	**ogg1**	**early brain development**
V/M	ciao1	iron-sulfur cluster assembly
Q/E	igsf9b	unknown
W/C	irak1	protein phosphorylation
L/V	**mon1b**	**vesicle-mediated transport**
A/T	plat	plasminogen activation
T/A	cb607	unknown
R/H	ufd1l	response to xenobiotic stimulus
I/V	ptpn18	dephosphorylation
V/L	fam208b	unknown
H/Q	brpf3	unknown
D/N; N/I	**ntng2a**	**axon guidance**
A/E	**skia**	**dorsal/ventral pattern formation**
G/A	tnni3k	protein phosphorylation
S/N	glis1b	metal ion binding
T/M	obscna	muscle development
N/S	**nphp4**	**positive regulation of Wnt signaling pathway; planar cell polarity**
C/G	**prex1**	**Rac1 exchange factor**
Q/STOP	**opn7z**	**g-protein coupled signaling pathway**
Y/C	PRRT3	proline-rich transmembrane protein 3
S/C	**mfn2**	**axon transport; peripheral axon guidance; neuromuscular junctions**
E/K	prickle3	metal ion binding
I/T	**gpr144**	**g-protein coupled signaling pathway**

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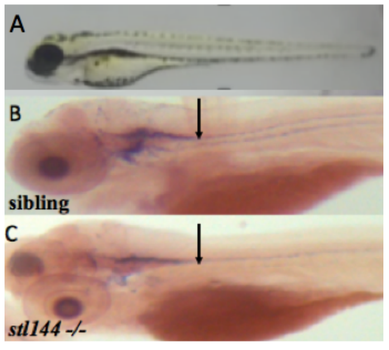
Figure 1. Whole genome sequencing of mutants and siblings shows linkage to chromosome 8. Regions of linkage were located using mutant-sibling MAF ratio mapping. 22 candidate genes were identified.

Identifying Carriers of *stl144* By Cross



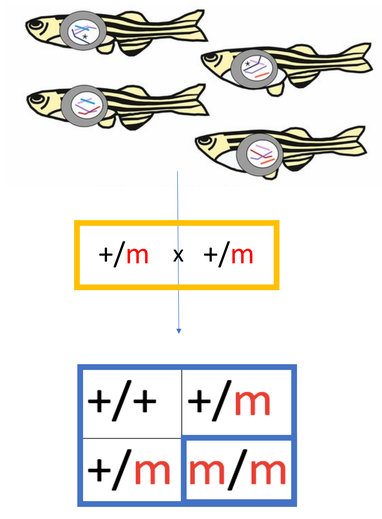
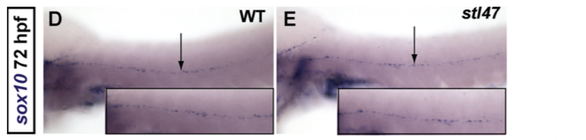


Figure 2. Reduced expression of mbp reveals *stl144* homozygous mutants in the progeny of a random in-cross (n=6/23). **(A)** Live progeny showing no visible deformities. **(B)** *stl144* sibling with wild-type expression of mbp along the pLLn (arrow). **(C)** *stl144 -/-* mutant with reduced expression of mbp along the pLLn.

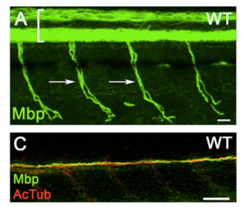
Future Directions

1. Are neural crest cells present and migrating properly?
   - *sox10* marks neural crest cells and can reveal if early development is affected by the mutation.



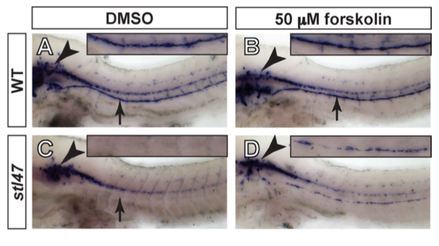
Petersen et al, 2015

2. Are axons present and migrating normally?
   - Acetylated microtubules mark PNS axons and can be used to determine if axon development is affected in *stl144* mutants.



Monk et al., 2009

3. Is the *stl144* mutation affecting a GPCR, upstream of cAMP?
   - Rescuing *stl144* mutants with forskolin, a cAMP elevator, may reveal the genetic pathway affected by *stl144*.



Petersen et al., 2015

Molecular characterization of *stl144* mutants may shed light on which candidate gene is most likely responsible for the reduction in PNS myelination.

Acknowledgments

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