The role of Slits and their Roundabout receptors in commissure development within the zebrafish forebrain

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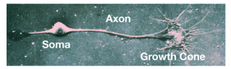
Abstract

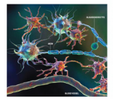
A fundamental characteristic in the formation of the central nervous system is the guidance of axons to their appropriate targets during embryonic development. In bilateral creatures, one common and critical choice point is whether or not to cross the midline to form commissures that functionally connect the two sides of the nervous system. In the zebrafish forebrain, the postoptic commissure (POC) is the first axon pathway to cross the midline. We are interested in the cellular and molecular guidance mechanisms that influence the pathfinding of this first commissure. We have shown that Slits, a family of secreted guidance cues typically known for their roles as repellents, influence not only POC formation but also the position of astroglial cells that might provide a positive growth substrate for POC axons across the midline. Interestingly, in contrast to the repellent effects of Slit2 or Slit3, gene knock down and global misexpression studies of Slit1a support a distinct role in the guidance of POC axons - possibly as an attractant. We are pursing temporally controlled local misexpression experiments to more directly test if Slit1a is truly functioning as an attractant. Slits act on Roundabout receptors (Robos 1-4), which are differentially expressed in commissural neurons and in the astroglial bridge. Using morpholino gene knockdown and mutant analysis, we will determine if Robo receptors 1, 2, 3, and 4 are necessary in commissural axons and/or glial cell guidance in a Slit dependent or independent manner. Current results suggest that Robo1 functions in glial cell positioning by setting up a permissive bridge for the crossing of POC axons, while Robo2 influences POC axon guidance. Robo3 variant2 affects both POC axon guidance and glial cell placement, while Robo3 variant1 plays a Slit2/3 independent role in the guidance of POC axons. Loss of Robo4 results in a reduction of midline astroglia as well as significant POC axon pathfinding errors. Our results suggest that the Robo receptors function distinctly in the guidance of astroglial cells and POC axons by mediating repellent or potentially attractive Slit cues.

Background

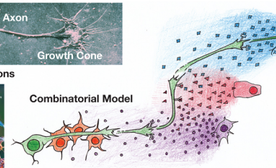
1. Axon Guidance



Axon-Glia Interactions



Combinatorial Model

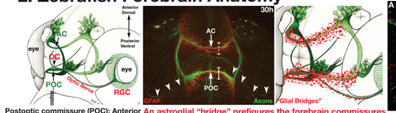


Cell adhesion provides growth substrate

Secreted proteins create gradients of attractant or repellant cues

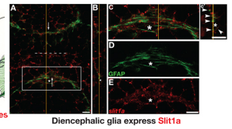
Axon receptors selectively interpret guidance cues

2. Zebrafish Forebrain Anatomy



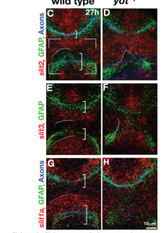
An astroglial "bridge" prefigures the forebrain commissures

Postoptic commissure (POC); Anterior Commissure (AC); Optic Chiasm; Retinal Ganglion Cell (RGC)

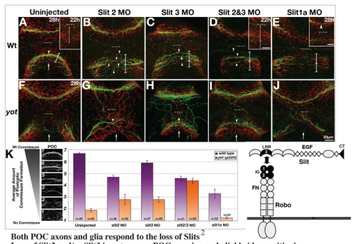


Diencephalic glia express Slit1a

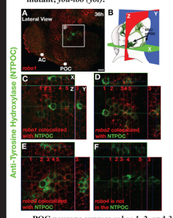
3. Slit/Robo Guidance



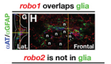
slit2 and *slit3* surround the commissure and unlike *slit1a* are expanded into the POC area in the ipsilateral mutant, *you-too (yot)*.[2]



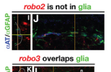
Both POC axons and glia respond to the loss of Slits[2]
Loss of Slit2 and/or Slit3 in *yot* rescues POC crossing and glial bridge positioning.
Loss of Slit1a in yot does not rescue crossing nor glial cell placement.



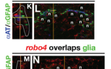
robo1 overlaps glia



robo2 is not in glia



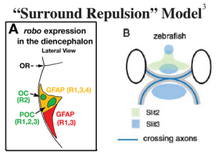
robo3 overlaps glia



robo4 overlaps glia

POC neurons express *robos 1*, *2*, and *3*, but not *robo4* [2]
Diencephalic glia express *robos 1*, *3*, and *4*, but not *robo2*

"Surround Repulsion" Model[3]



Slits 2 and 3 function as repellants that channel the optic nerve and POC axons across the midline.

Slits 2 and 3 also function to condense the diencephalic glial bridge.

The function of Slit1a is currently unknown.

Questions

1. Does Slit1a function as an attractant?
2. Which Robos mediate Slit guidance of POC axons and signaling of glial positioning?

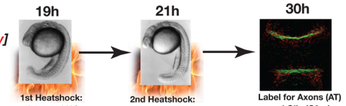
Experimental

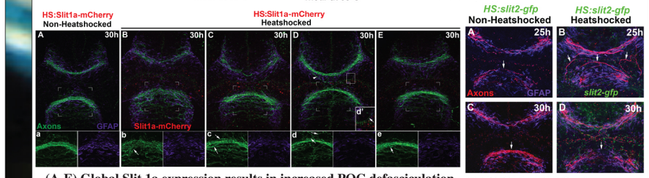
Slit1a acts differently from Slit2 or Slit3.

1a. Global Misexpression of Slits

Heatshock inducible transgenic:

Current Lines:
Tg*[hs:slit1a-mCherry]*
Tg*[hs:slit2-eGfp]*



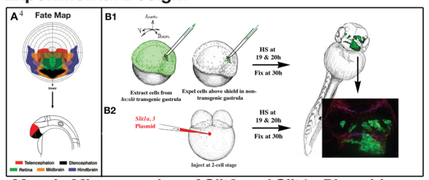


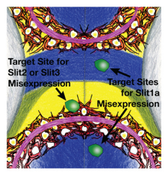
(A-E) Global Slit 1a expression results in increased POC defasciculation.

(A-D) Global Slit2 misexpression causes POC wandering early and loss later.

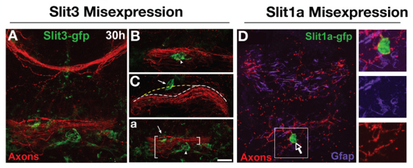
1b. Local Misexpression of Slits

Experimental Design:





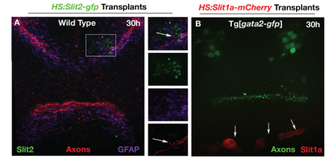
Mosaic Misexpression of Slit3 and Slit1a Plasmid:



(A-C) POC axons and glia avoid the local misexpression of Slit3 in a Slit1a domain, but not within normal Slit2 or Slit3 domains.

(D) Preliminary data suggests that POC axons will not avoid a Slit1a misexpressed cell.

Gastrula Stage Transplantation of Slit1a or Slit2 Cells:

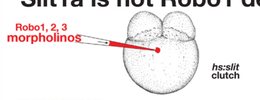


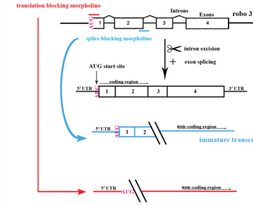
(A) A misexpressing Slit2 cell in the AC region results in a wondering axon.

(B) Visualization of Slit1a misexpressing cells requires transplants into Tg[*gata2-gfp*] fish lines.

Distinct functions exist for Robos in the guidance of POC axons and astroglial.

2a. Preliminary Data shows Slit1a is not Robo1 dependent

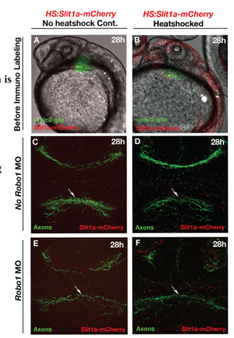




(A-B) Slit1a expression is highly visible before immuno protocol.

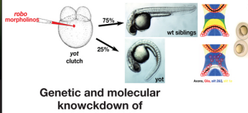
(C-D) Misexpressing Slit1a results in defasciculated POC.

(E-F) *robo1* MO knockdown does not rescue POC defasciculation.

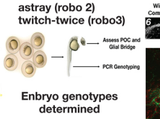


2b. Morphant and Mutant Loss of Function Approach

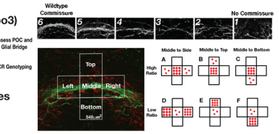
Experimental Design:



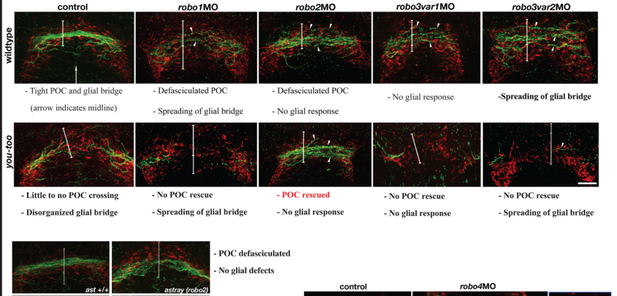
Genetic and molecular knowckdown of Robo1, 2, & 3

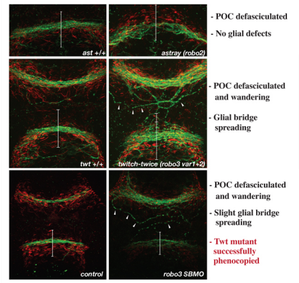


Enbryo genotypes determined

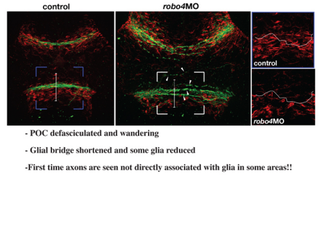


Quantification of POC error and glial bridge formation defects



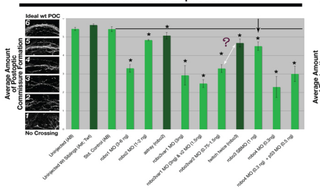


- POC defasciculated
- No glial defects
- POC defasciculated and wandering
- Glial bridge spreading
- POC defasciculated and wandering
- Slight glial bridge spreading
- Twt mutant successfully phenocopied



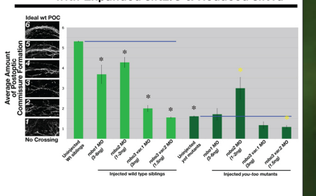
- POC defasciculated and wandering
- Glial bridge shortened and some glia reduced
-First time axons are seen not directly associated with glia in some areas!!

Analysis of Robos in Context of Normal Slit Expression

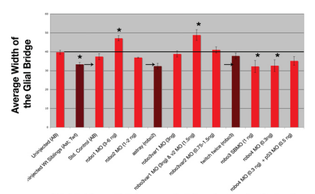


Loss or knockdown of any *robo* reduces POC formation. Robo3 splice blocking morpholino successfully phenocopies *twi* mutant POC formation.

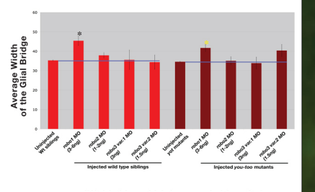
Analysis of Robos in *you-too* Mutants with Expanded *slit2/3* & Reduced *slit1a*



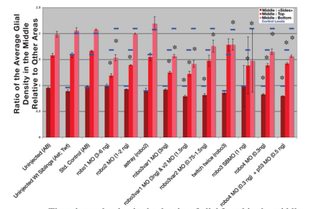
Only knockdown of *robo2* rescues POC crossing, while knockdown of either robo3 variant reduces crossing further in *yot* mutants.



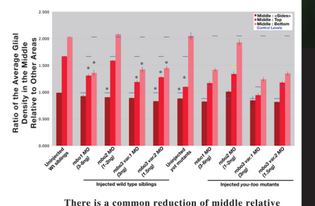
Glial bridge width is expanded in *robo1* and *robo3var2* morphants as well as in *twitch twice (robo3)* mutants. Glial bridge width is reduced in *robo4* and *robo3* splice blocking morphants.



Glial bridge width is expanded in *robo1* and maybe in *robo3var2* morphants.



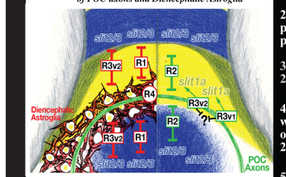
There is a reduction in the density of glial found in the middle domain in *robo1*, *robo3*, *robo3* splice blocking, and *robo4* morphrants, but an increase from the top to the middle in *twitch twice* mutants.



There is a common reduction of middle relative to bottom glia in all experiments in *yot* except in *robo2* morphants which has a slight increase in the middle from the top.

Summary

Model of Robo Mediated Guidance of POC axons and Diencephalic Astroglia



1. Preliminary results with Slit1a misexpression and combined robo1 MO suggest that Slit1a is not mediated by *robo1*, but should be mediated by *robo3*.

2. Robo1 directly mediates Slit 2/3 repulsion in glial bridge positioning, which then indirectly influences POC axon pathfinding.

3. Robo2 functions to repel POC axons by mediating Slit 2/3 surrounging signals.

4. Robo3var2 (not var1) mediates glial bridge positioning, while both regulate POC axon pathfinding by modulating other robos (?) or through mechanism independent of Slit 2/3, perhaps through Slit1a.

5. Robo4 is required for axon-glial interactions necessary for proper POC formation.

References & Acknowledgements

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3. Rashand K, Hardy M, Chien CB. 2003. Generating X: formation of the optic chiasm. Neuron. 39(6):885-8.
4. Woo K, Fraser SE. 1995. Order and coherence in the fate map of the zebrafish nervous system. Development. 121(8):2595-609.

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