Cellular analysis of forebrain morphogenesis in zebrafish shows conservation of mechanisms in vertebrates

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Abstract

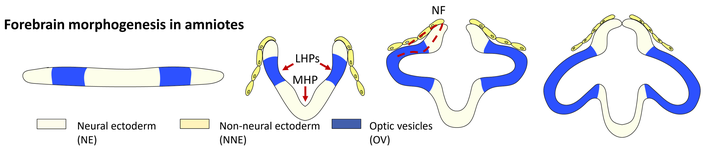
Cranial neurulation is the process via which the brain primordium is shaped during early embryogenesis. Defective neurulation in the cranial region results in the most severe types of neural tube defects (NTDs), exencephaly and anencephaly. Morphogenetic studies of cranial neurulation in the mouse embryo have primarily focused on the midbrain and hindbrain. In contrast, far less is known about neurulation in the forebrain (FB). Fate maps of vertebrate embryos reveal that the prospective FB occupies the lateral edges of the eye field. The optic vesicles evaginate from the neural tube as neurulation proceeds. We explore here the cellular mechanisms that shape the prospective FB in the zebrafish embryo. Preliminary data from our laboratory indicate that FB morphogenesis in zebrafish presents hallmarks of primary neurulation in amniotes, namely medial and lateral hinge point-like structures and neural folds that converge and fuse at the dorsal midline. The medial hinge point (MHP) forms in a superficial layer of mesenchymal like cells, which subsequently undergo epithelialization and intercalate radially between the deep cells, contributing to the expansion of the optic vesicles. Molecular characterization of this MHP reveals apical enrichment of actomyosin. Blebbistatin treatment prevents MHP formation. Disruption of the Planar Cell Polarity (PCP) component Vangl2, previously implicated in MHP formation in amniotes, is required for the formation of the MHP counterpart in zebrafish. Ongoing studies aim to test whether Shroom3 is required for MHP formation. Together, these findings reveal the presence of previously overlooked structures characteristic of primary neurulation in the zebrafish, highlighting conservation of mechanisms. Overall, these studies establish the zebrafish as a model organism to screen for genetic factors that cause NTDs.

Introduction

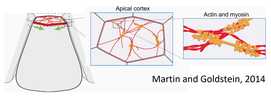
Hallmarks of primary neurulation[1]:

- Hinge points: medial hinge point (MHP) and two lateral hinge points (LHPs) (red arrows)
- Neural folds (NF) (dashed red line)
- Closure points

Forebrain morphogenesis in amniotes

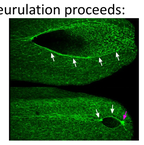


Hinge points are characterized by apically constricted cells, mediated by an actomyosin contractile ring:



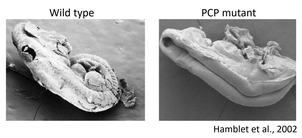
Martin and Goldstein, 2014

Posterior neuropore in mice (white arrows), which closes as neurulation proceeds:



The Planar Cell Polarity (PCP) pathway:

- Conserved in vertebrates
- Regulates hinge point formation, through actin polymerization and actomyosin contractions
- PCP mutant mice display an open neural tube

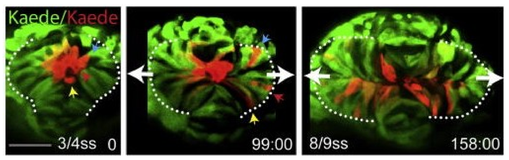


Hamblet et al., 2002

Impaired neurulation results in many NTDs.
Our laboratory aims to establish the zebrafish as a model organism to study NTDs.

Zebrafish anterior neurulation:

- Neural plate starts out bilayered: superficial (orange) and deep layer (green) cells
- As neurulation proceeds, neuroepithelium resolves into a single layer via cellular intercalation: superficial cells intercalate into deep layer[2]



Ivanovitch et al., 2013

- Studies have focused on eye formation, hence little is known about forebrain morphogenesis

Objectives

Characterize forebrain neurulation in zebrafish, to identify if hallmarks of primary neurulation have been overlooked.
Investigate the role of PCP in forebrain morphogenesis.

Acknowledgement

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Results

Forebrain morphogenesis in zebrafish

Hinge points and neural folds contribute to forebrain morphogenesis

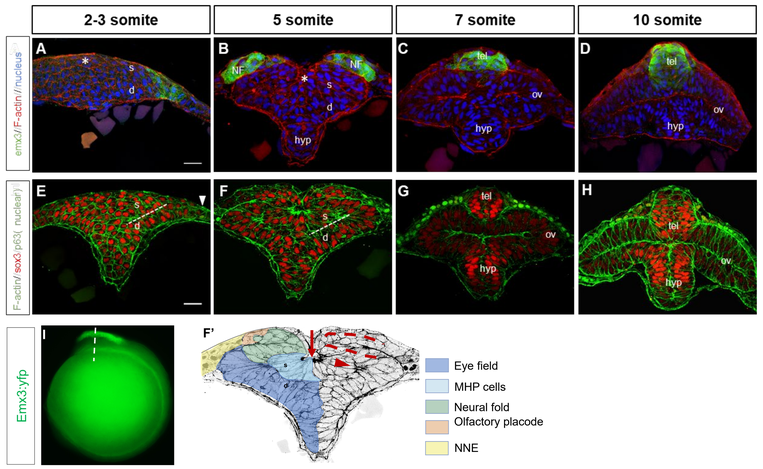

Figure 1: (A-H) Transverse sections through the prospective forebrain at the 2-3, 5, 7 and 10 somite stage. I) Whole mount embryo showing forebrain (emx3 signal) and plane of section. F') A 5 somite cross section illustrates the different areas of the tissue.

Zebrafish anterior neurulation shows conserved structures:
- Neural folds (dashed red line in F')
- Medial hinge point (red arrow in F'), located in the superficial eye field cells, which later intercalate to form single layered optic vesicles and neuroepithelium (D and H).
- Lateral hinge points (red arrowhead in F')

Colocalization of myosin and actin confirms the presence of an actomyosin ring mediating apical constriction.

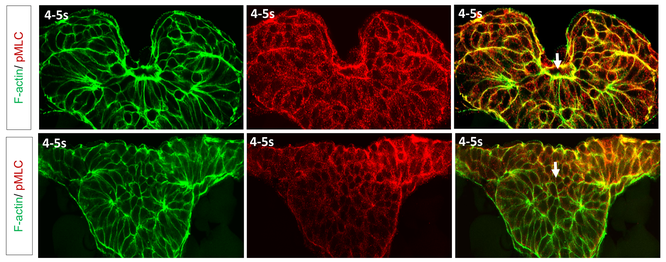

Figure 2: Sections reveal the enrichment of myosin at the MHP. Inhibition of myosin via Blebbistatin impairs MHP formation

Medial hinge point contributes to neural fold convergence

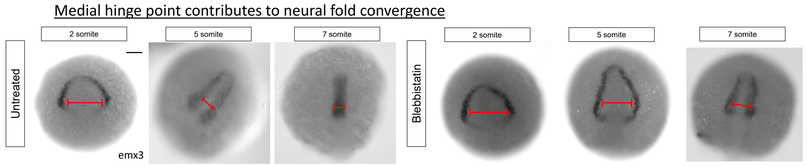

Figure 3: *In situ* hybridization with neural fold marker emx3 reveal impairing the MHP delays neural fold convergence

Neural fold convergence reveals the presence of closure points

Time lapse imaging shows neural fold (NF) elevation and convergence in the zebrafish forebrain

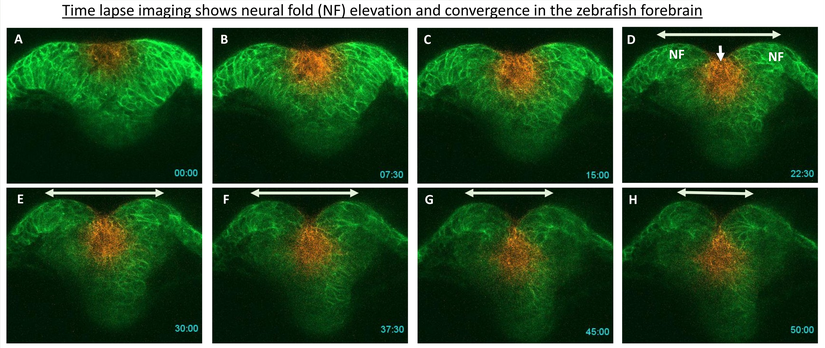

Figure 4: Time lapse imaging showing the neural plate from a transverse view. As the medial hinge point cells (orange; arrow in D) constrict, neural folds (NF) elevate above the eye field (starting in D) and converge towards the midline (double arrow in D-H).

Results cont'd

Time lapse imaging reveals the presence of two closure points in the zebrafish forebrain

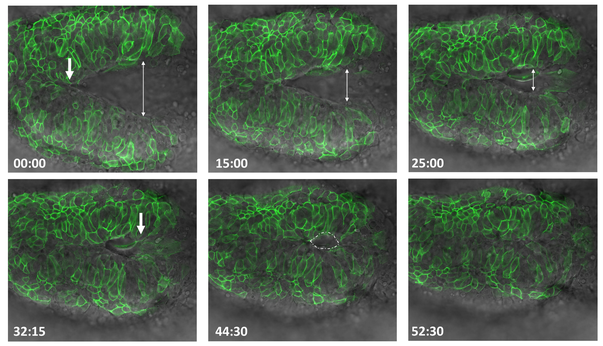

Figure 5: Time lapse imaging showing the neural plate from a dorsal view reveals the presence of two closure points in the zebrafish forebrain, represented by the white arrows. A zippering-like closure is observed. Embryo was mosaically expressing GFP.

Role of Planar Cell Polarity component Vangl2 in forebrain morphogenesis

Mutations in vangl2 disrupts hinge point formation, neural fold convergence and forebrain morphogenesis

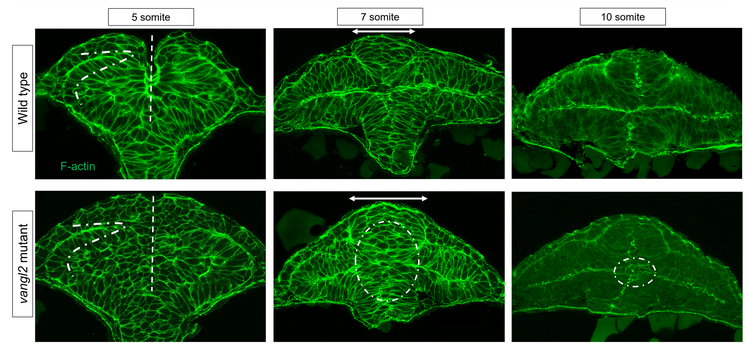

Figure 6: Transverse sections through the prospective forebrain of WT and vangl2 mutants.

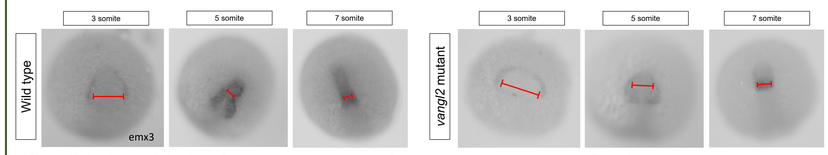

Figure 7: *In situ* hybridization with neural fold marker emx3 reveal delayed convergence at 5 somites

At 4 somites, *vangl2*mutants fail to form a MHP.
At 7 somites, *vangl2* mutants fail to undergo radial intercalation, resulting in an accumulation of cells medially, as well as less expanded optic vesicles.
Morphometric analysis indicates approach of neural folds towards the midline correlates with presence of a MHP.

Conclusion

- Hallmarks of primary neurulation: conserved in the anterior neural anlage of zebrafish
- MHP: localized actomyosin that promotes apical constriction and neural fold convergence
- Two novel closure points in the prospective forebrain identified
- MHP: PCP dependent
- PCP mutant: failed radial intercalation and neural fold convergence

Future directions

- Build on conservation of hallmarks of primary conservation to show genetic and molecular conservation
  - Investigate bias in localization of PCP components
  - Elucidate the role of PCP in neural fold convergence and closure points
  - Test whether shroom3 plays a role in medial hinge point formation
- If mechanisms are conserved, establish zebrafish as a model to screen variants identified in GWAS, using CRISPR/CAs9 technology

References

1. Nikolopoulou, E., Galea, G. L., Rolo, A., Greene, N. D., & Copp, A. J. (2017). Neural tube closure: cellular, molecular and biomechanical mechanisms. *Development, 144*(4), 552-566.
2. Ivanovitch, K., Cavodeassi, F., & Wilson, S. W. (2013). Precocious acquisition of neuroepithelial character in the eye field underlies the onset of eye morphogenesis. *Dev Cell, 27*(3), 293-305.
3. Nishimura, T., Honda, H., & Takeichi, M. (2012). Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell, 149*(5), 1084-1097.
4. Shindo A, (2017). Models of convergent extension during morphogenesis. Wiley Interdiscip Rev Dev Biol 7:e293.
5. Hamblet, N. S. et al. Dishevelled 2 is essential for cardiac outflow tract development, somite segmentation and neural tube closure. Development 129, 5827–5838 (2002).