Evidence for Conserved Mechanisms of Neurulation in the Zebrafish Forebrain

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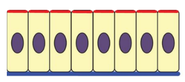
Abstract

The formation of the neural tube, the developmental precursor of the central nervous system, is facilitated by the bending and folding of the neuroepithelium, a process termed neurulation. Neural tube defects (NTD) are the most common type of birth defect in humans, primarily caused by improper neural tube formation. Investigating the cellular and molecular mechanisms that drive neurulation will help identify genetic risk factors for NTDs. The morphogenesis of the neural tube is facilitated by the formation of hingepoints, subsets of neuroepithelial cells that undergo apical constriction to form a wedge shape. Apical constriction occurs when a cell acquires a molecularly defined apical surface through apically polarized tight junction molecules, such as zona occludens (ZO1) and PARD3, in addition to the recruitment of an actomyosin contractile ring. Live imaging of hingepoint dynamics would further advance our understanding of hingepoint formation; however, it is difficult to perform in traditional model organisms. In contrast, the transparency and early accessibility of zebrafish embryos make them amenable to live imaging. Hingepoints were previously not reported in the zebrafish neuroepithelium, but we have recently found evidence for the presence of these structures in the forebrain. Using immunolabeling and confocal microscopy, we show the apical localization of ZO1 and PARD3 in a cluster of medial, wedge-shaped cells in the anterior neuroepithelium. Furthermore, we reveal that disruption of the apical actomyosin contractile ring, using the myosin inhibitor blebbistatin and myosin morpholinos, prevents apical constriction. These findings provide evidence for the presence of hingepoints in zebrafish and highlight the conservation of neural tube morphogenesis in teleosts, which pave the way for future investigations on the cellular and genetic basis of NTDs using zebrafish as a model organism.

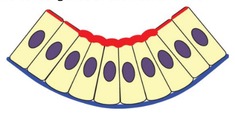
Background

Apical Constriction and Acto-myosin Contractile Ring Facilitate Neural Plate Bending and Folding



* Red indicates an accumulation of polarity molecules which allows for the cell to be apically/basally polarized.

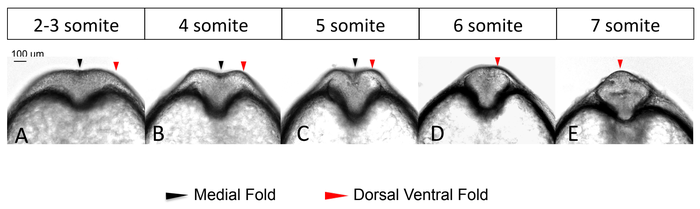
* During this transition state, red indicates an accumulation of actin/myosin allows for the convergence of the neural tissue



Pearl, E. J., Li, J., & Green, J. B. A. 2017

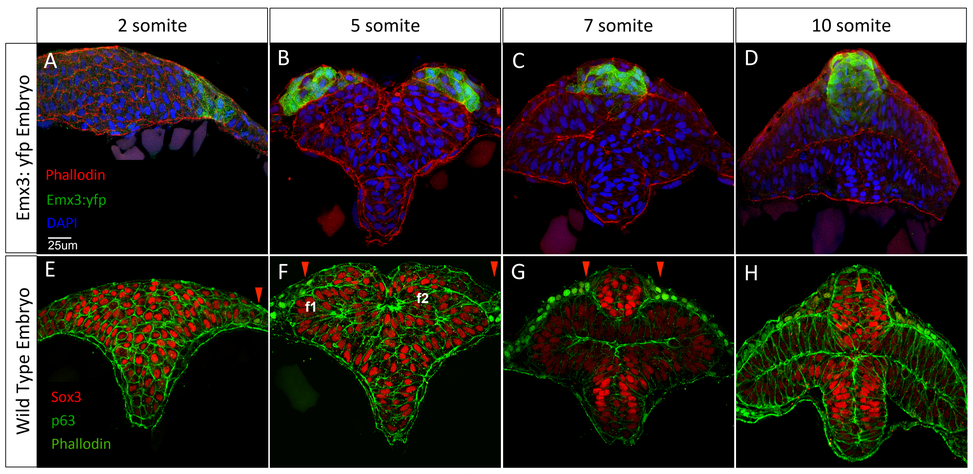
- During apical constriction, cells actively shape change from cuboidal to wedge-shaped.
- An acto-myosin contractile ring is crucial to proper apical constriction, hence essential to neural plate bending and folding.

Medial and Dorsal Lateral Folds are Noticeably Present within The Zebrafish Forebrain

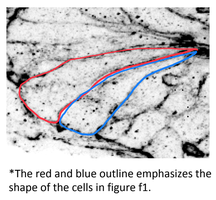


- Optical cross sections showing the developmental progression of the forebrain tissue , highlighting interesting structures in the medial and dorsal lateral regions that appear to be neural folds.

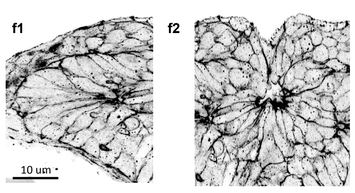
Neural Folds Migrate and Move Medially to Shape the Zebrafish Forebrain



- Neural folds labeled in green with emx3 and in red with Sox3 converge medially and fuse at the dorsal midline.
- The medial aspect of the neural fold becomes the forebrain.
- The non-neural ectoderm labeled with p63 seals medially after the neural folds have fused.



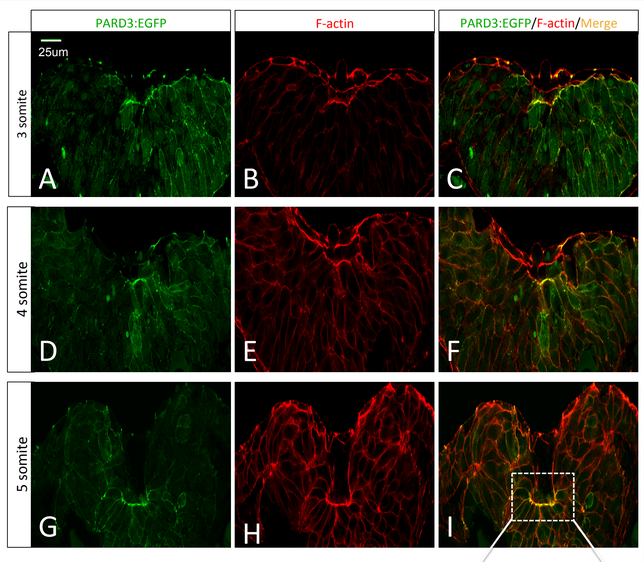
*The red and blue outline emphasizes the shape of the cells in figure f1.

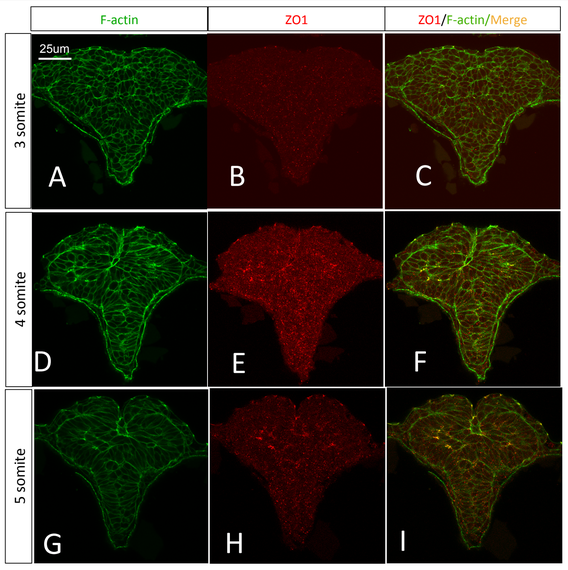


- Taking a closer look, we can see apparent wedge-shaped cells that appear structurally similar to medial and dorsal lateral hingepoints.

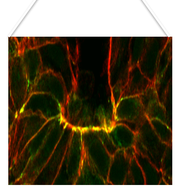
Results

PARD3 and ZO1 are apically localized in a cluster of medial, wedge-shaped cells in the anterior neuroepithelium.



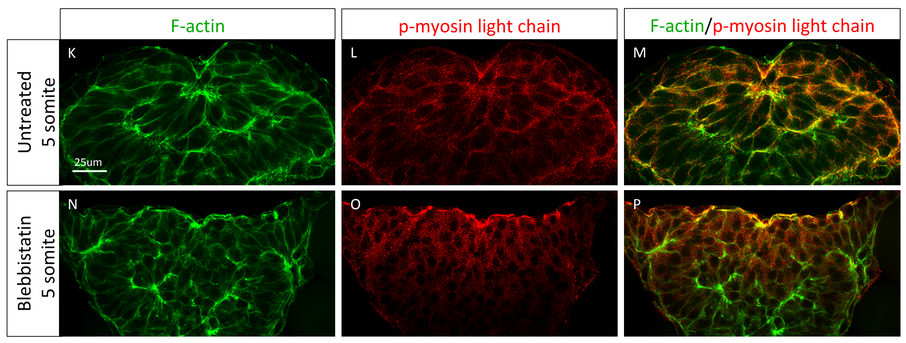


- PARD3 is concentrated in a cluster of medial wedge-shaped cells, demonstrating that these cells are apically polarized.



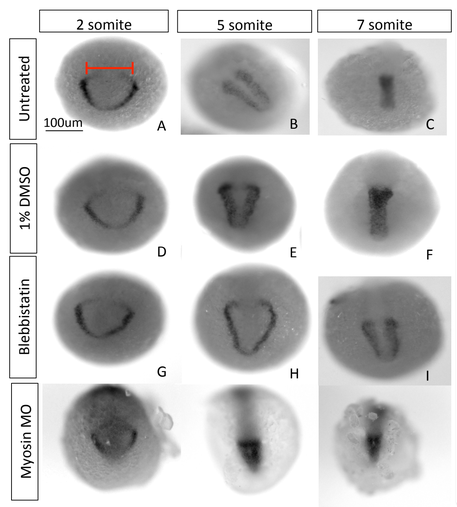
- By 4 som, the lateral hinge points appear to be enriched with ZO-1. By 5 som, the medial deep layer also shows faint enrichment of ZO-1, indicating the cells are apically polarized.
- Establishment of apico-basal polarity is a required step preceding recruitment of contractile machinery.

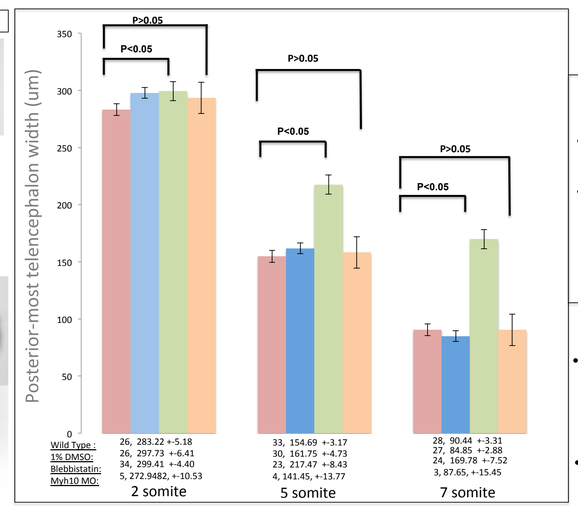
Molecular characterization of wedge-shaped cells: apical localization of actin and myosin



- Apical constriction appears to be facilitated by contraction of an acto-myosin contractile ring.

Myosin Inhibitor Blebbistatin Causes a Delay In Telencephalon Convergence





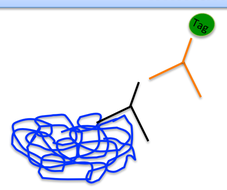
Legend

- The data under the bar graph is as follows: number of embryos, mean +-SEM
- P P-values for T-test
- Untreated
- 1% DMSO
- 50 uM Blebbistatin
- Myh10 Morpholino

- Inhibiting myosin causes a significant decrease in the width of the converging forebrain, highlighting the importance of myosin in telencephalon convergence.
- The delay in convergence is most likely explained by a defect in the medial hinge point formation.
- Embryos were injected at the 1 cell stage with 3ng of myh10 MO.
- Myh10 is the zebrafish homolog of the human non-muscle myosin II proteins, where myh10 encodes for NMIIB.
- Embryos were treated with blebbistatin at tailbud until the desired developmental stages.
- 1% DMSO was used as a control group.

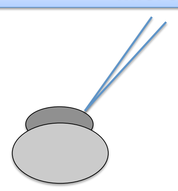
Methods

Immunolabeling



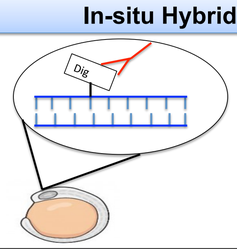
- A primary antibody binds to the desired epitope, and the secondary antibody with a fluorescent tag binds to the primary.

Microinjection



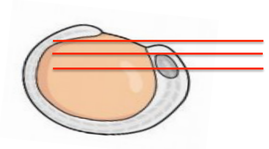
- A molecule, typically RNA or DNA, is injected into a one cell stage embryo.

In-situ Hybridization



- A tagged riboprobe hybridizes to an endogenous mRNA and is labeled with an antibody.

Sectioning



- Transverse sections of the forebrain are made using a vibratome.

Conclusion

- Hinge points are present in the anterior neuroepithelium of the zebrafish forebrain. Hinge points in anterior neural plate present apical markers by 5 som: pard3 and ZO-1, indicating epithelialization.
- Impaired MHP formation delays neural fold convergence.
- Mechanisms of neurulation are more conserved across vertebrates than previously acknowledged.

Future Directions

- Use CRISPR knockdown tools in order to investigate the role of genes that may be associated with neural tube formation.

Acknowledgements

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