EXPLORING THE CURVES OF THE ZEBRAFISH INNER EAR

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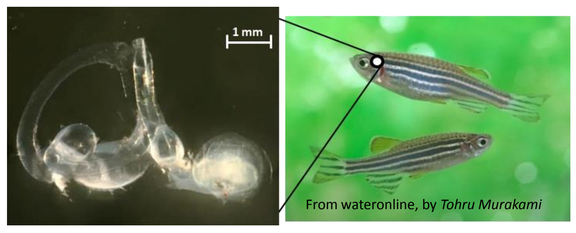
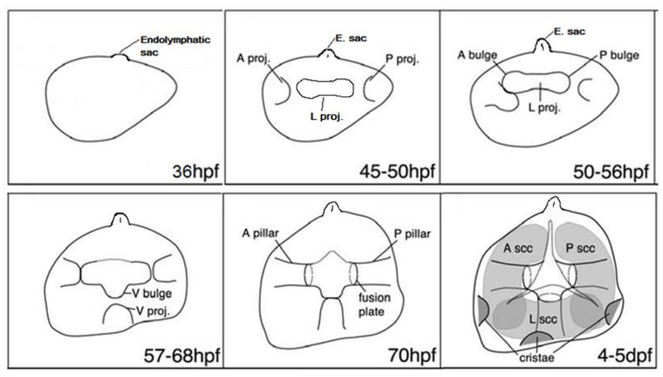
1. Where do the curves in the inner ear come from?

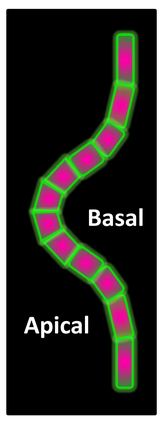
The inner ear is the organ that mediates hearing and balance senses. Its structure comprises a fluid-filled labyrinth with sensitive sensory structures responsible for detecting sound, motion and gravity (1) .



Between one and two days post fertilization, the epithelial projections of the semicircular canal system start to form within the otic vesicle of the zebrafish embryo. These projections are examples of structures generated by epithelial folding and I am studying the mechanisms driving this morphogenetic process.



(Adapted from Geng et al. 2013)



Aim 1

Describing the cell behaviours during otic tissue folding.

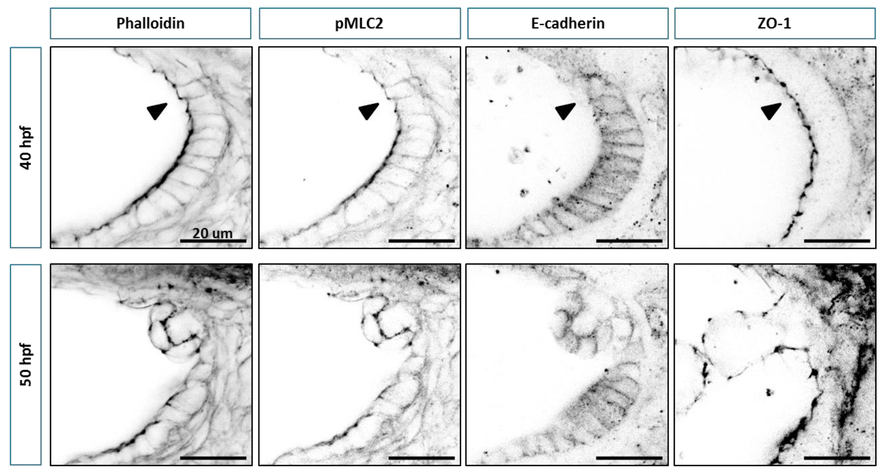
Aim 2

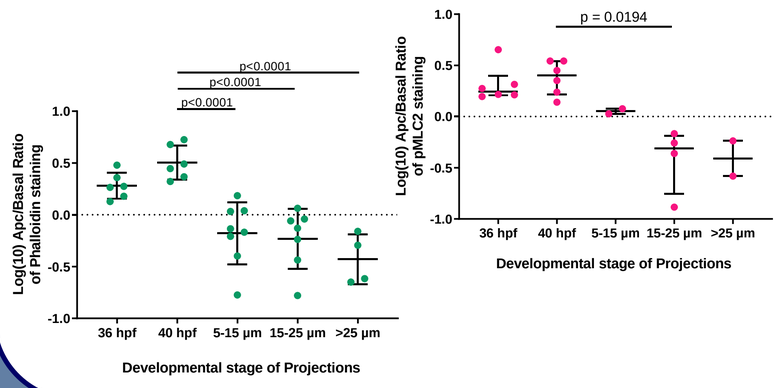
Describing cell shape changes accompanying those cell behaviours.

Aim 3

Understand whether some of these factors are required or sufficient for folding.

2. Cytoskeletal and adhesion proteins change distribution from the apical to the basal domain during epithelial folding



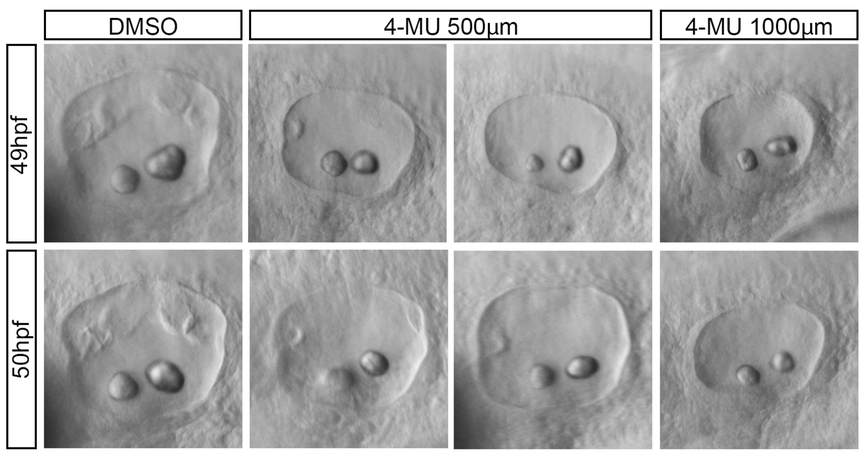


To investigate the distribution of cytoskeletal and adhesion proteins I performed phalloidin and immunofluorescent staining before and after the folding event.

Results:

Actomyosin complex and adherens junctions change location from the apical domain to the basal domain, after the epithelium folds. Tight junctions (ZO-1 staining) remain apically located.

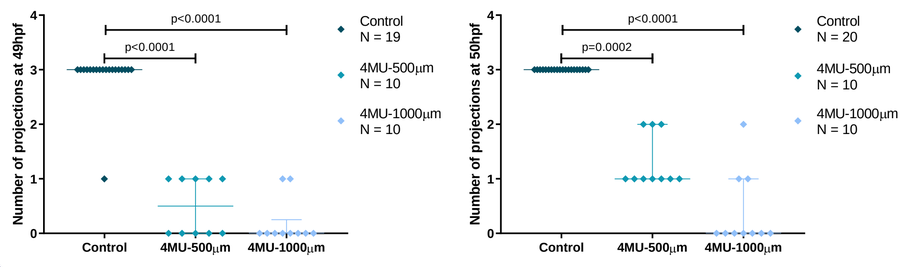
3. The role of extra-cellular matrix in projection formation



To test whether the ECM plays a role in the initial folding of the otic epithelium I performed a 4-MU drug treatment to block hyaluronan production.

Conditions:

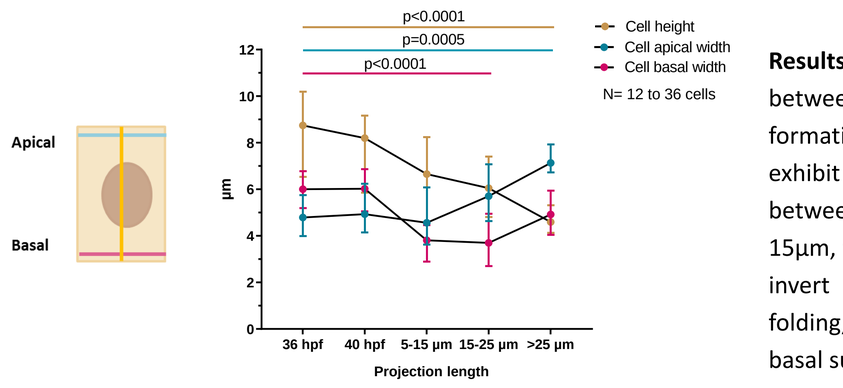
12 hours drug/DMSO treatment from 36hpf to 48hpf. Repeat 1 was imaged at 49hpf and repeat 2 at 50hpf.



Results: Depleting hyaluronan from the embryo seems to prevent the otic epithelium from folding and therefore projection formation.

4. Cell shape changes during epithelium folding

To describe cell shape changes in 2D I measure the apical surface length, basal surface length and height in fixed embryos before and after projection formation, in three cells per projection.



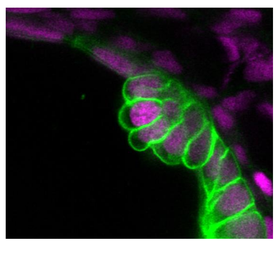
Results: Cell height reduces between throughout projection formation. Before folding, cells exhibit a smaller apical surface; between stages 40hpf and 5-15µm, the cells of the projection invert their skewness; after folding, cells exhibit a smaller basal surface.

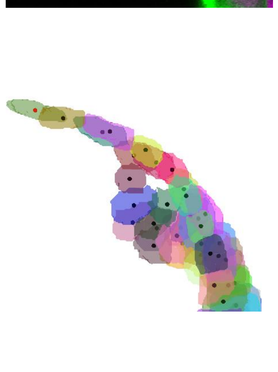
5. Cell segmentation and cell shape analysis in 3D

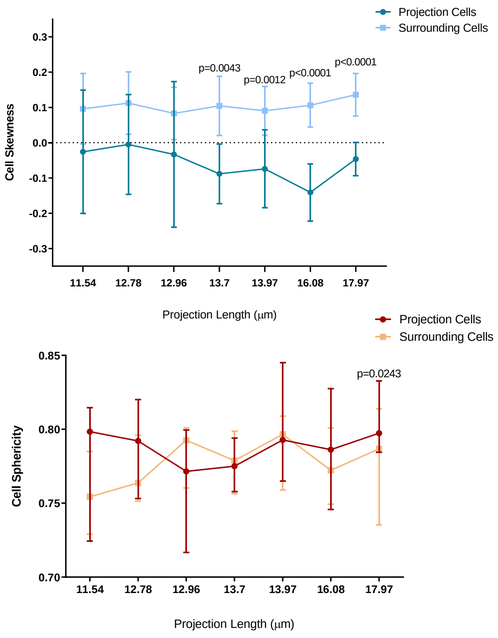
To describe cell shape changes in 3D during projection formation, I performed a timelapse of the zebrafish inner ear in the Airyscan Confocal microscope between 45 and 51hpf.

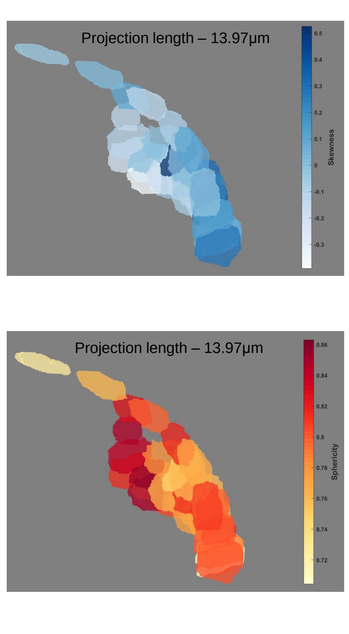
Data analysis: ACME was used to segment cells in 3D; a pipeline in MATLAB was used to extract cell shape metrics by computing geometric moments from 3D meshes of segmented cells.

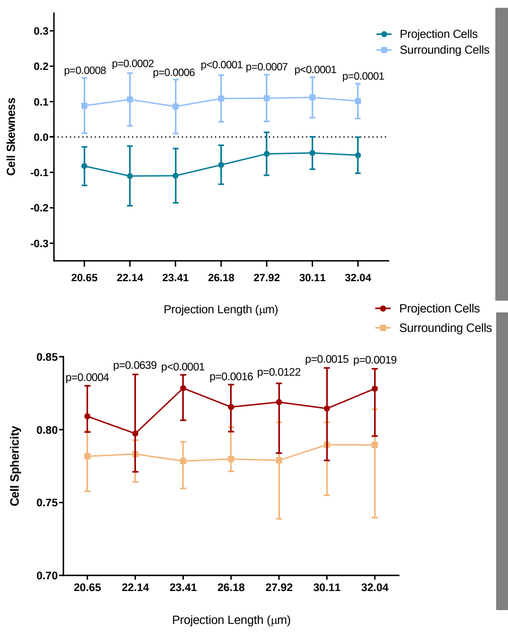
Results: Projection cells exhibit a smaller basal surface (skewness<0) whilst surrounding cells have a smaller apical surface. Projection cells are also more spherical than surrounding ones.

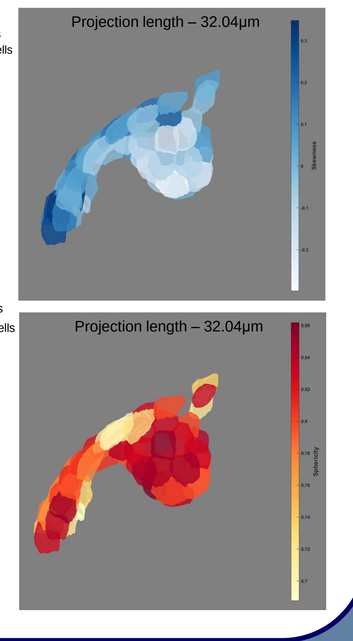






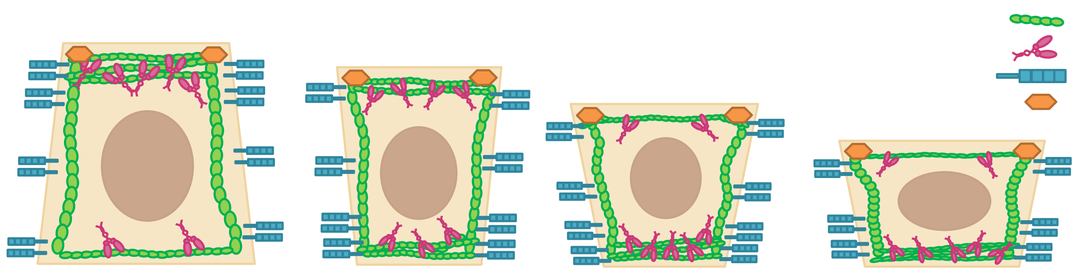






6. Coming Next

- Continue performing automated 3D cell segmentation and measure cell shape changes in WT and abnormal folding tissue (mutant lines will be used).
- Investigate cytoskeleton dynamics in live embryos (Actin, Tubulin and phospho Myosin transgenic lines will be used).
- Explore further the role of ECM-cell interactions in projection formation and see which components are actually required for folding.



References:

(1) Alsina, B. & Whitfield, T.T., 2016. Sculpting the labyrinth: Morphogenesis of the developing inner ear. Seminars in Cell and Developmental Biology, 65, pp.47–59.

(2) Geng, F.-S. et al., 2013. Semicircular canal morphogenesis in the zebrafish inner ear requires the function of gpr126 (lauscher), an adhesion class G protein-coupled receptor gene. Development, 140(21), pp.4362–4374.

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