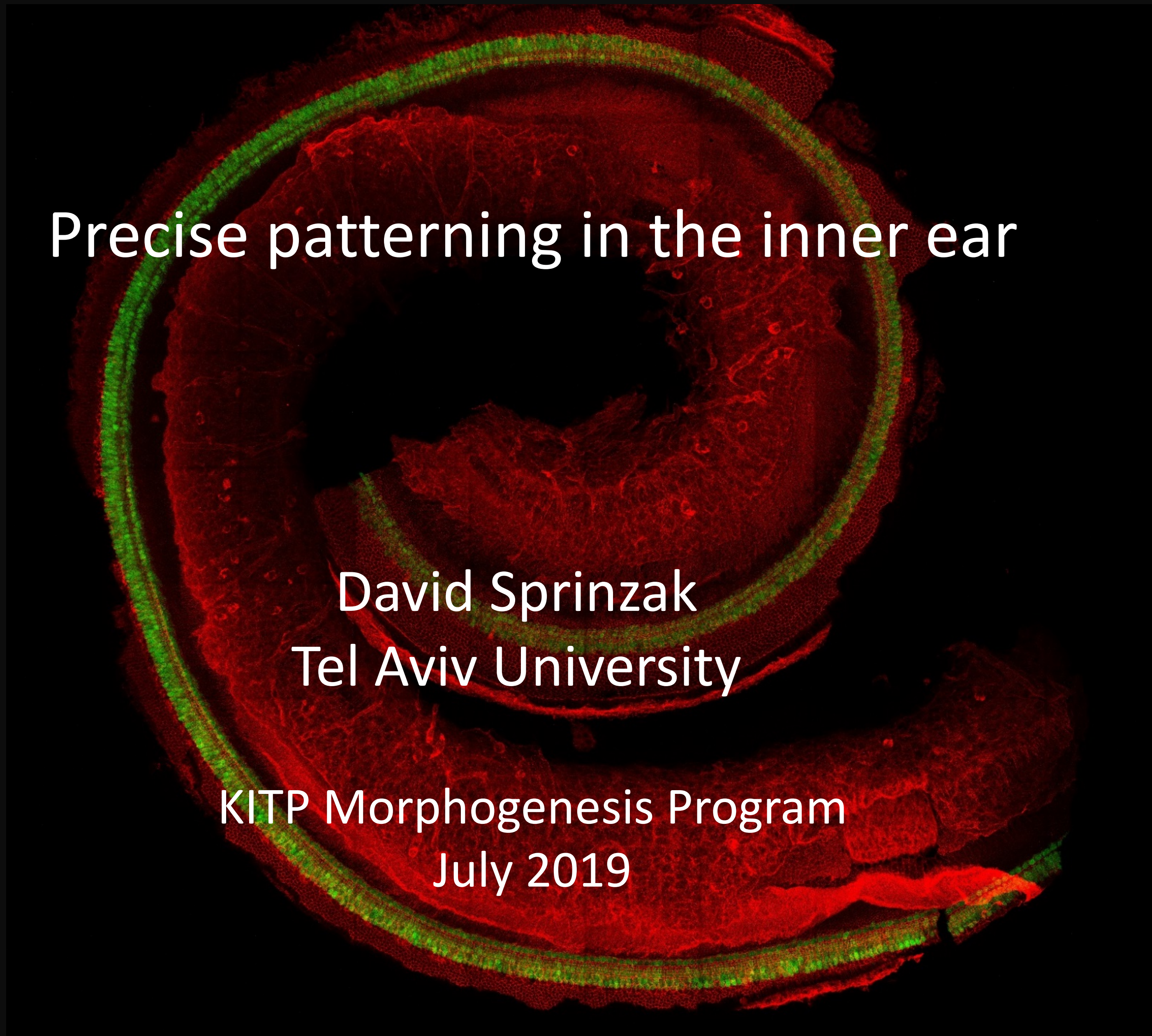
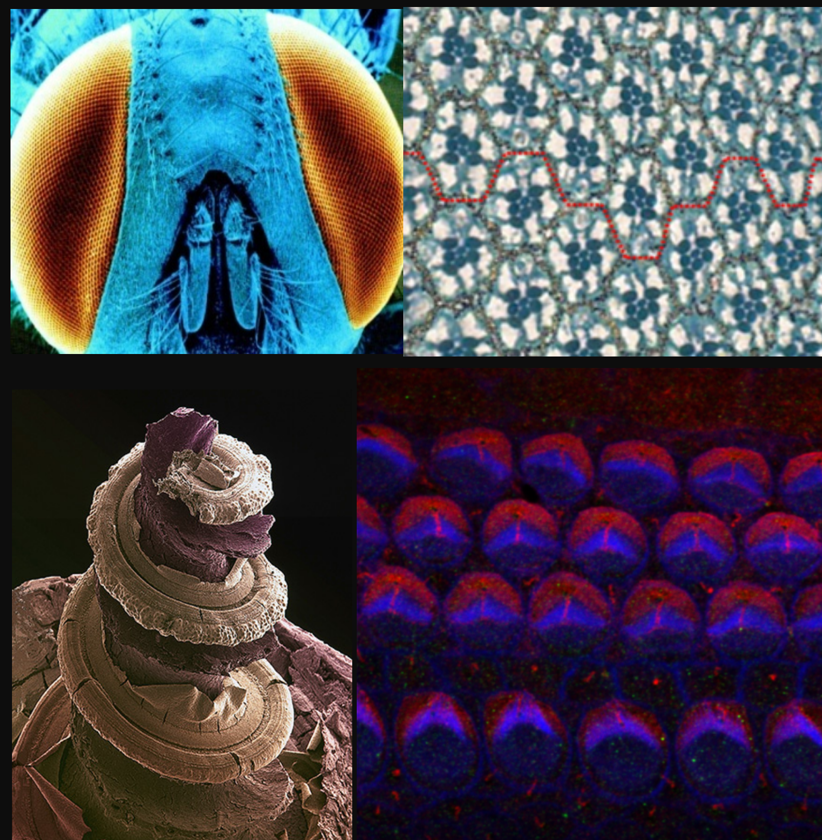


Precise patterning in the inner ear

David Sprinzak
Tel Aviv University

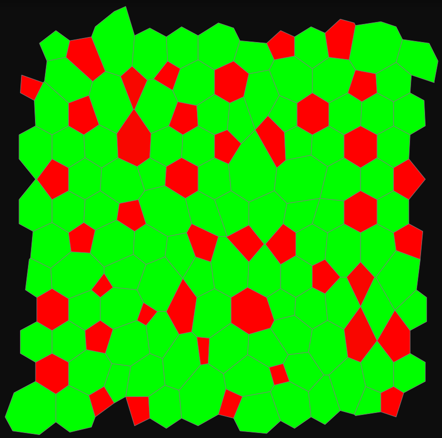
KITP Morphogenesis Program
July 2019

How precise patterns of differentiation emerge during development?

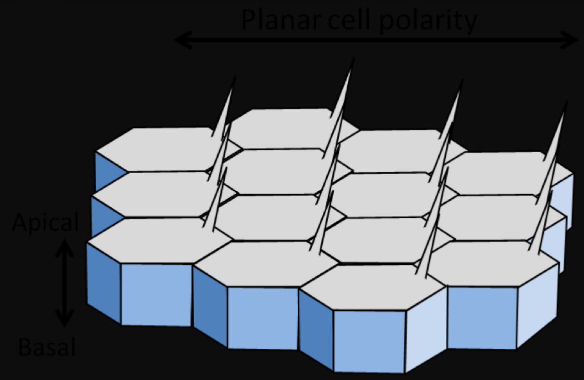


Coordination between neighboring cells

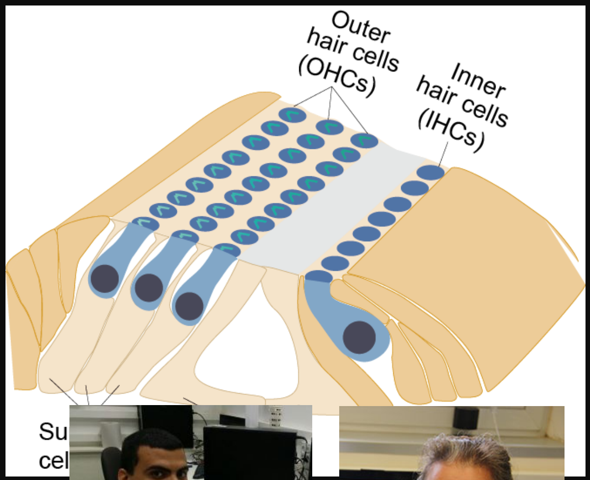
Notch Signaling and Notch mediated patterning	Planar Cell polarity Fat-Dachsous signaling	Interplay between mechanics and signaling in the inner ear



Khait et al. Cell Reports 2014
Shaya et al. Dev. Cell 2017



Loza et al. Elife 2017

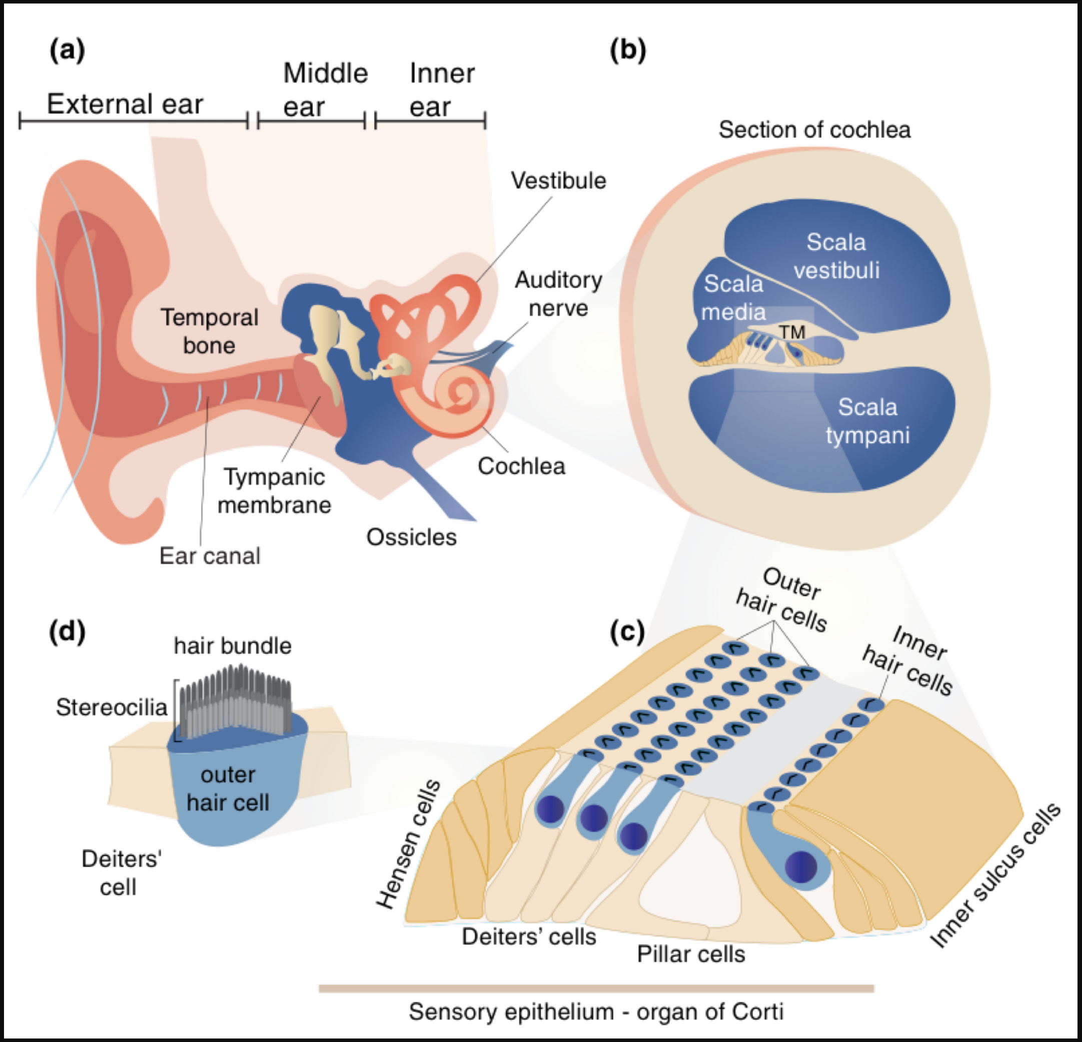


wv /c
10

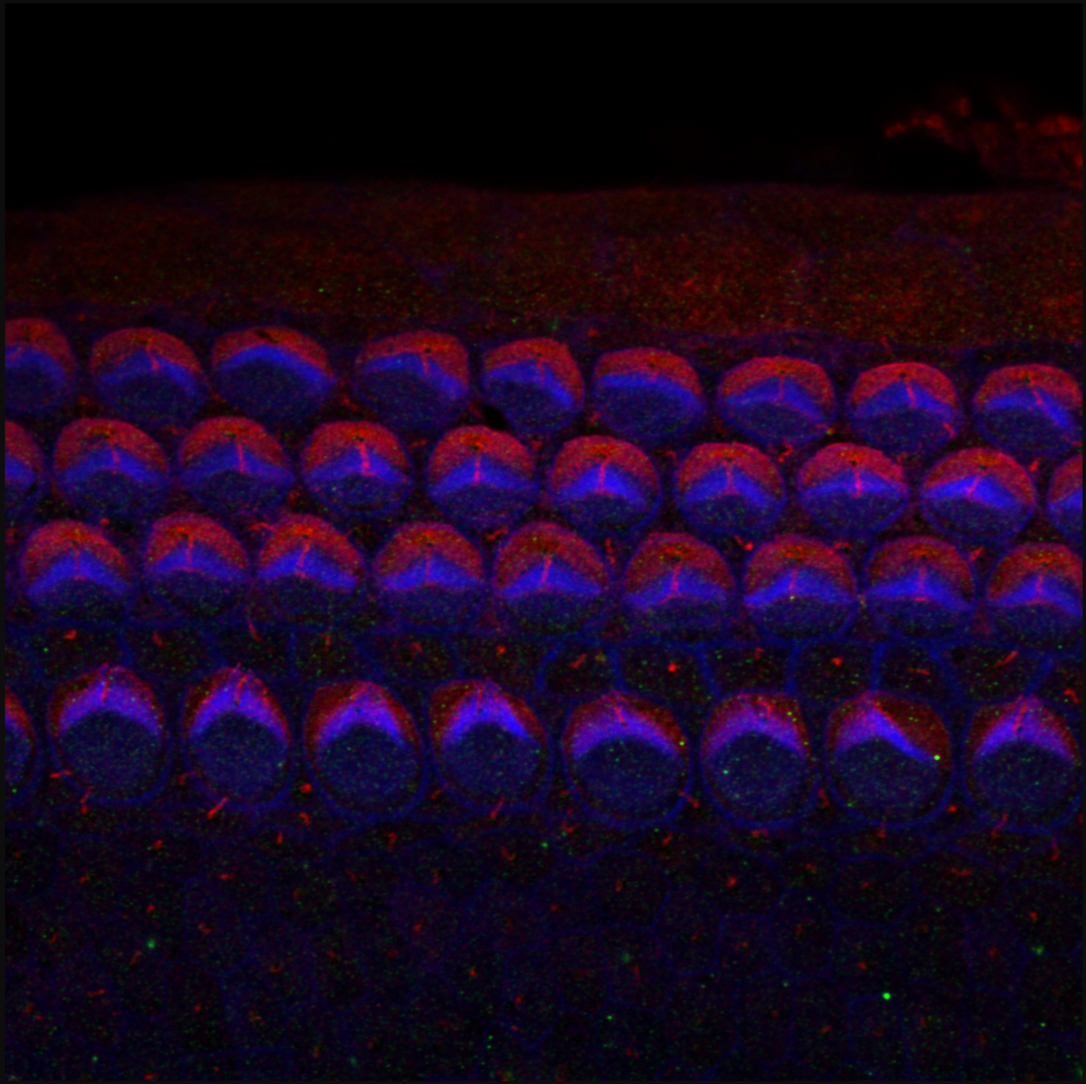
Roie Cohen

Liat Amir

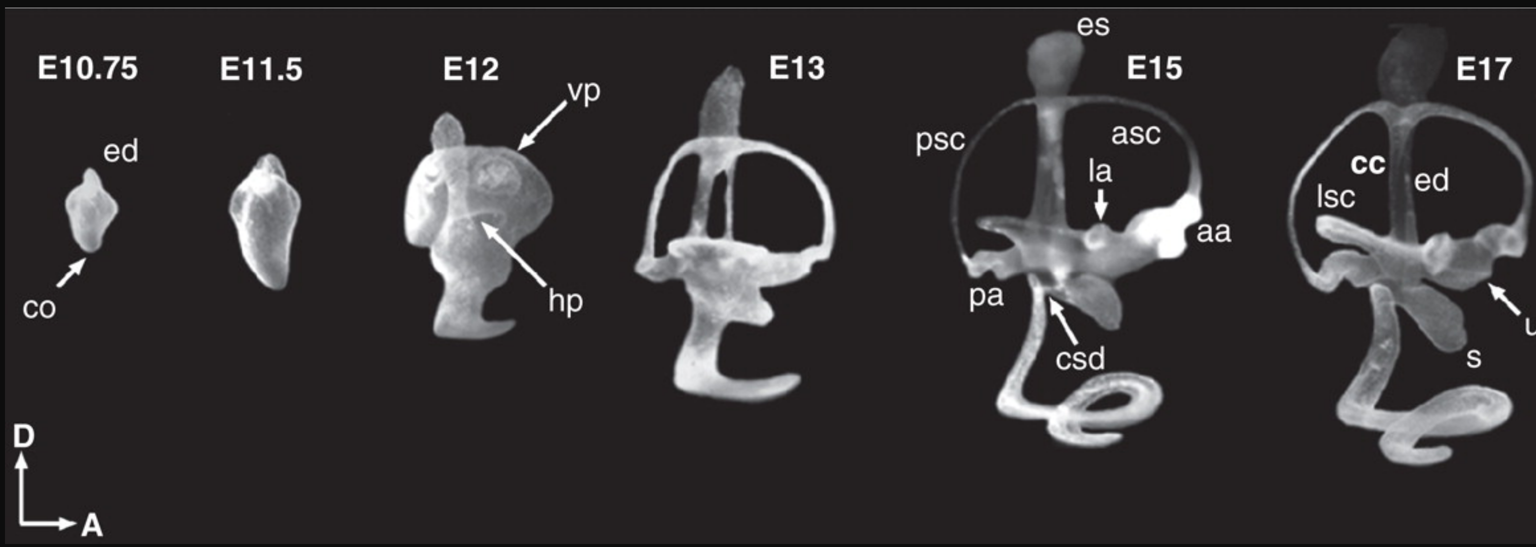
Sculpting the mammalian Organ of Corti



Emergence of organization in the inner ear

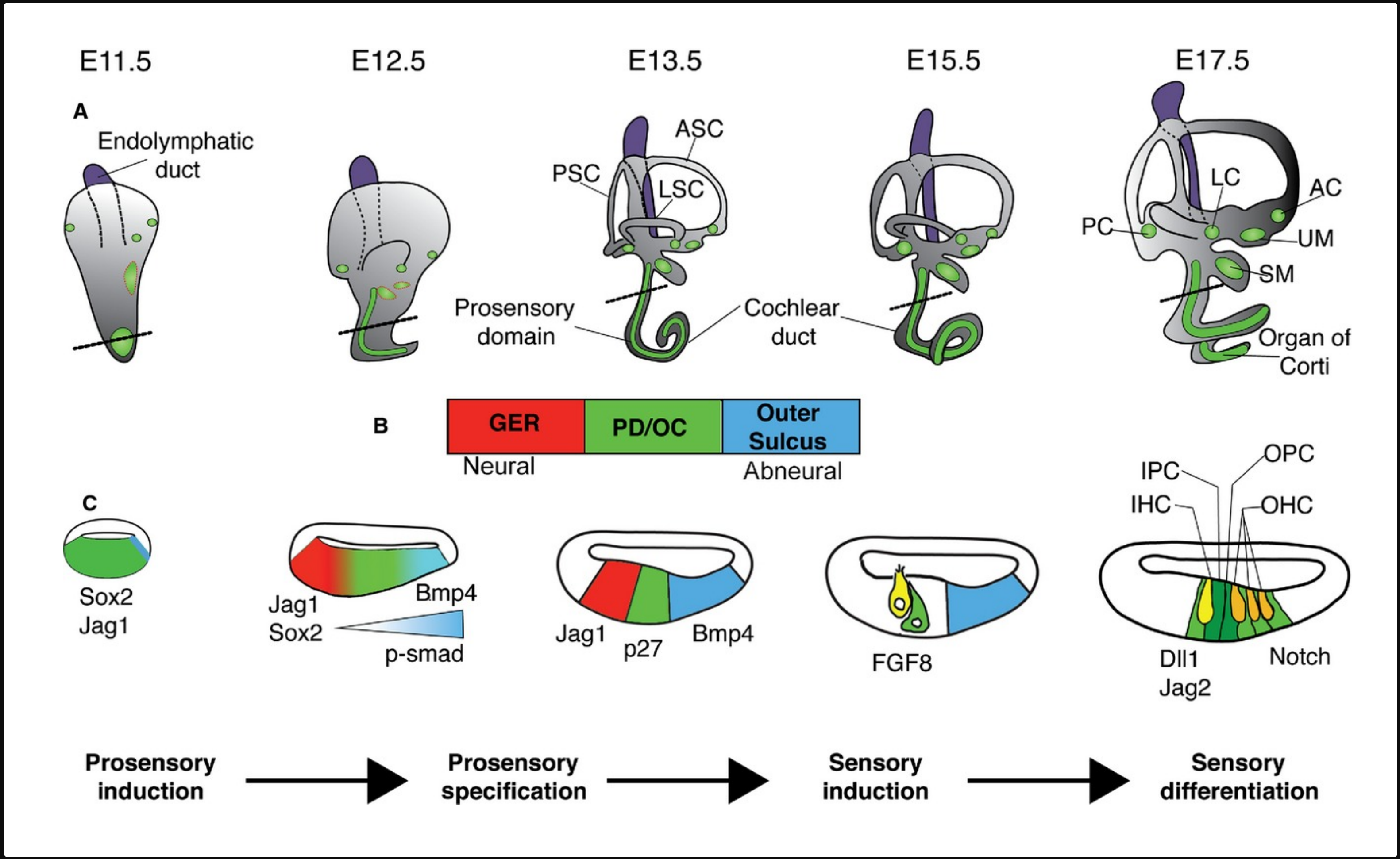


Early development of the inner ear



From Wu and Kelley CSH perspective 2012

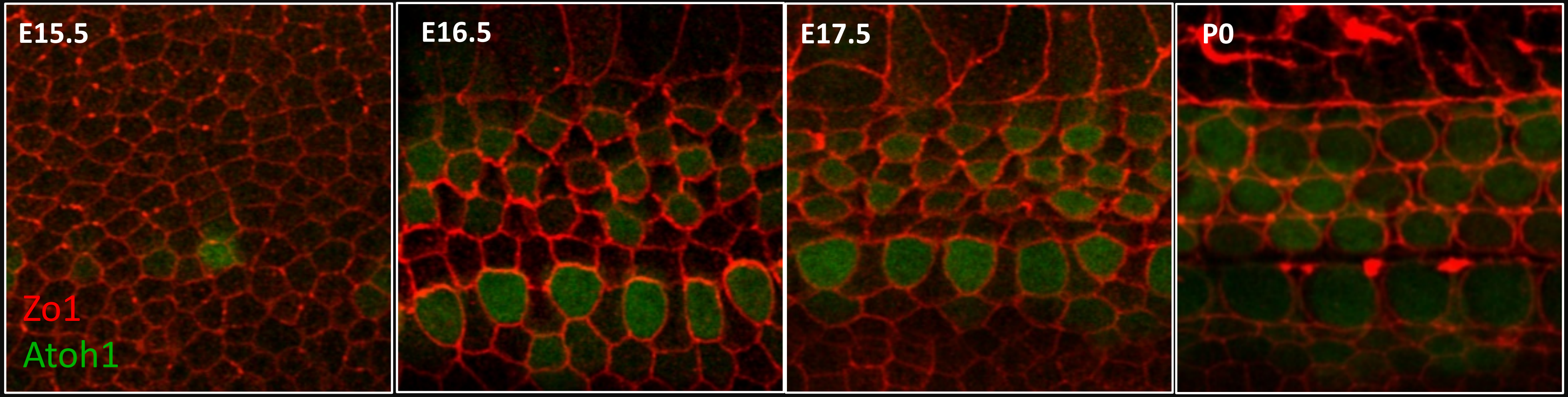
Early development of the inner ear

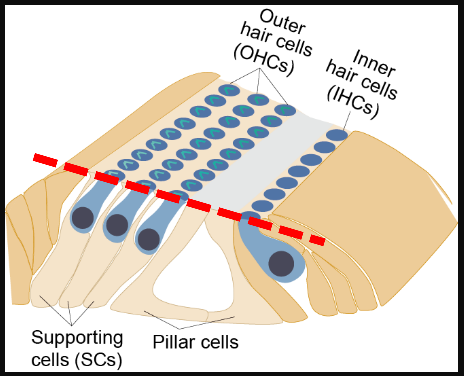


From Basch et al. J Anat. 2016

Focus on the organization at the apical surface

Progression through time





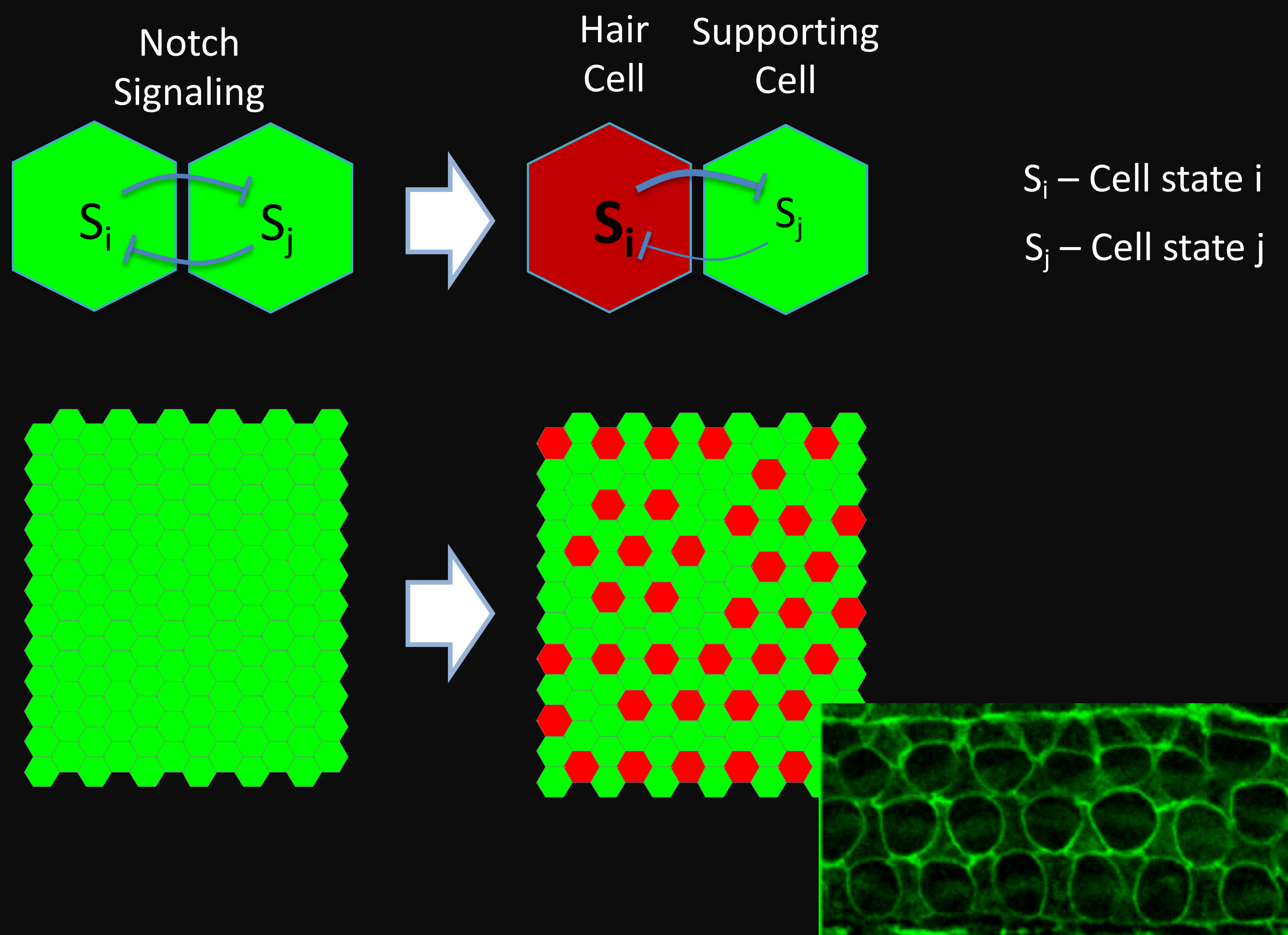
How a perfectly organized pattern of hair cells arises?

1. **Differentiation circuits** – specifying hair cell and supporting cell fates

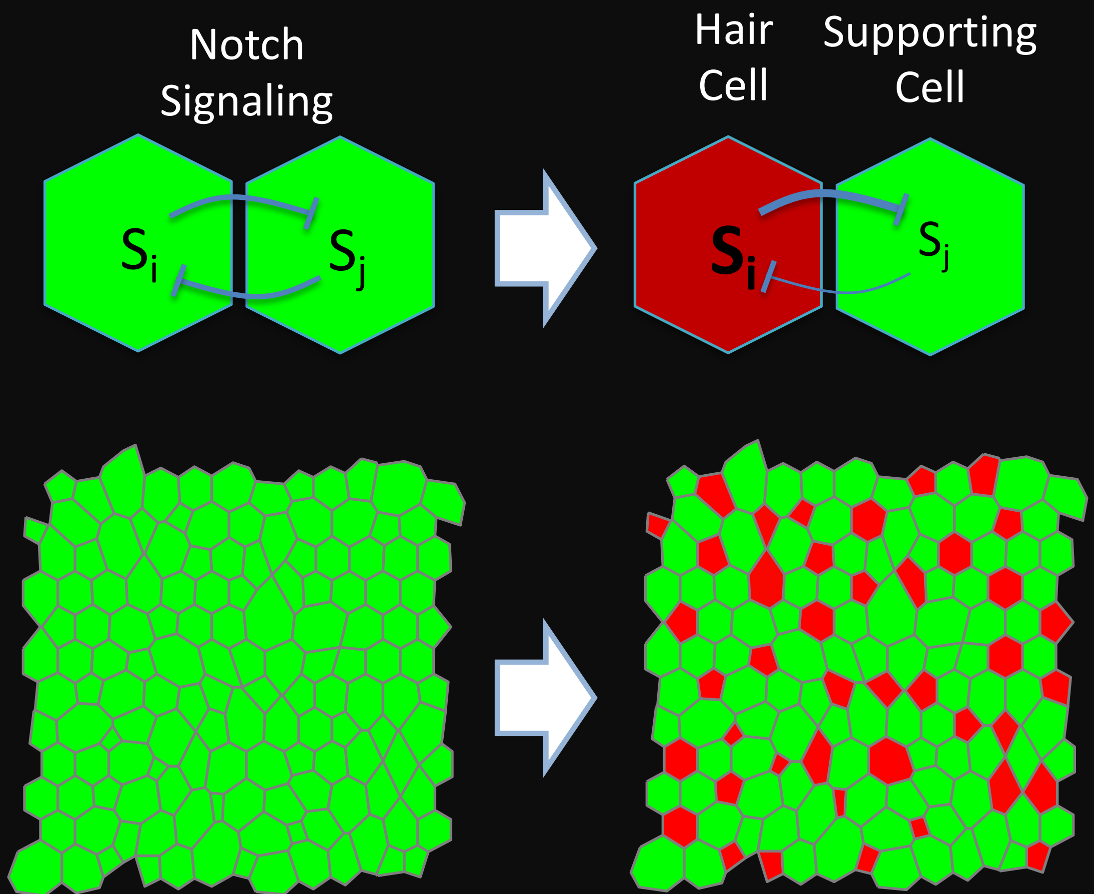
2. **Cellular reorganization** – controlling the shape and the position of cells

(No cell divisions at this stage!)

Models of lateral inhibition circuits typically give rise to disordered pattern



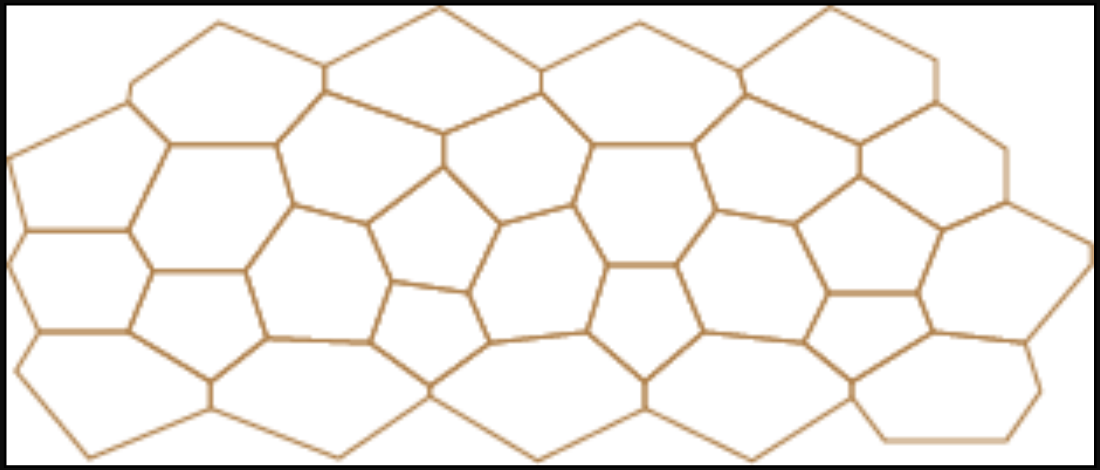
Models of lateral inhibition circuits typically give rise to disordered pattern



S_i – Cell state i

S_j – Cell state j

Cellular mechanics controls shape and organization of cells

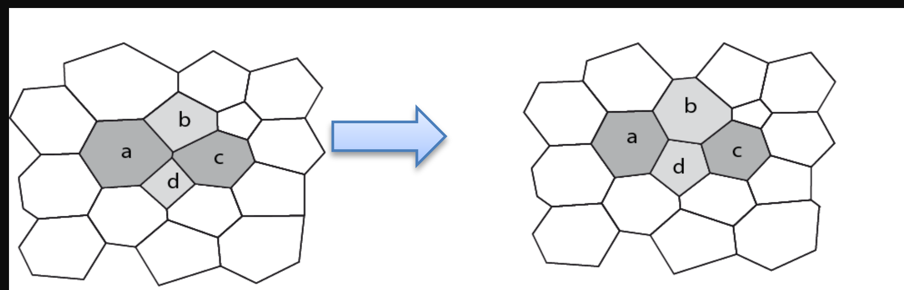


Cellular shapes are determined by local forces acting on the each cell and each bounday

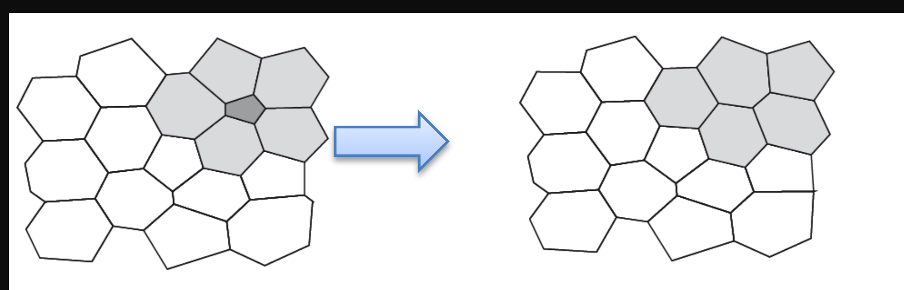
Use mechanical 2D vertex models

Possible morphological transitions in 2D lattices

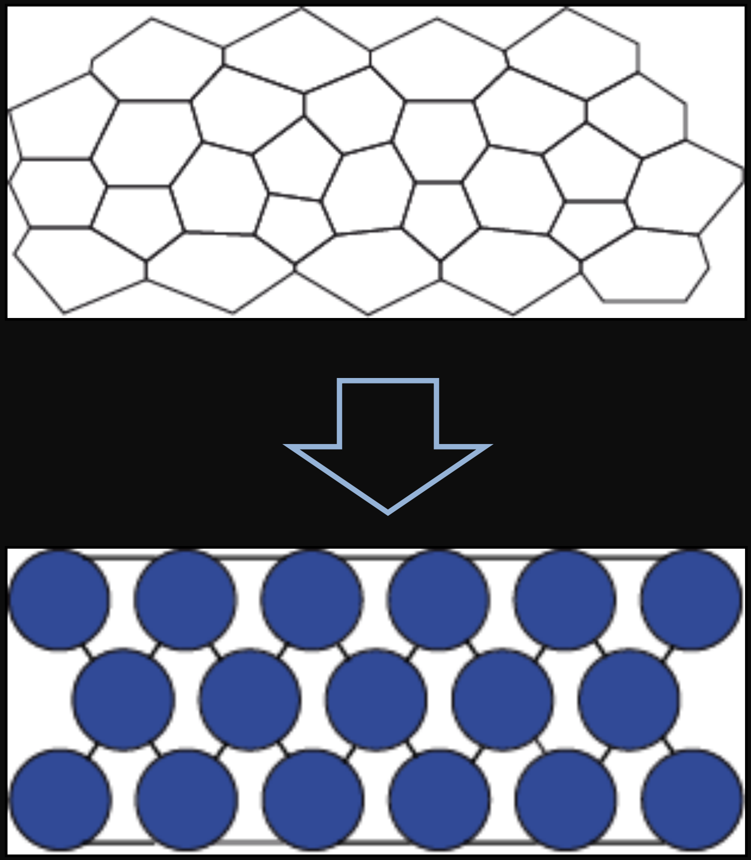
Intercalation



Delamination

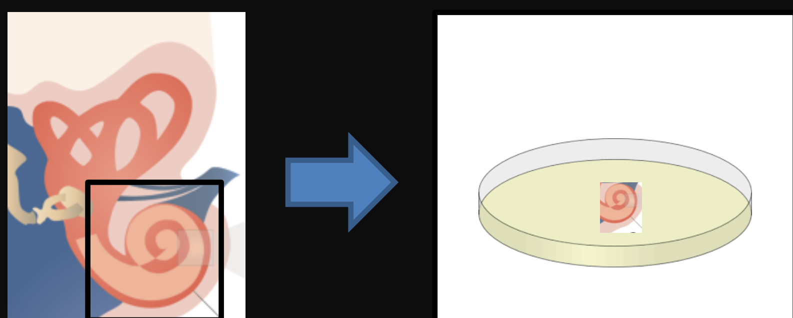


How do the combination of differentiation circuits and cellular reorganization drives transition to organized patterning?



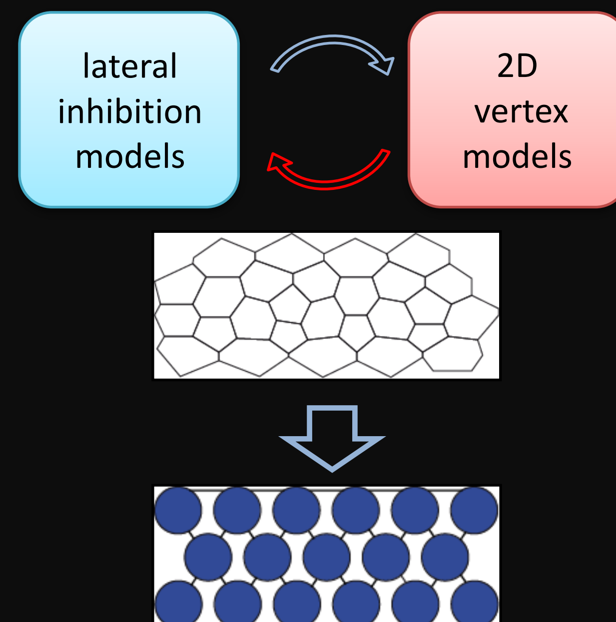
Our approach

Experiments



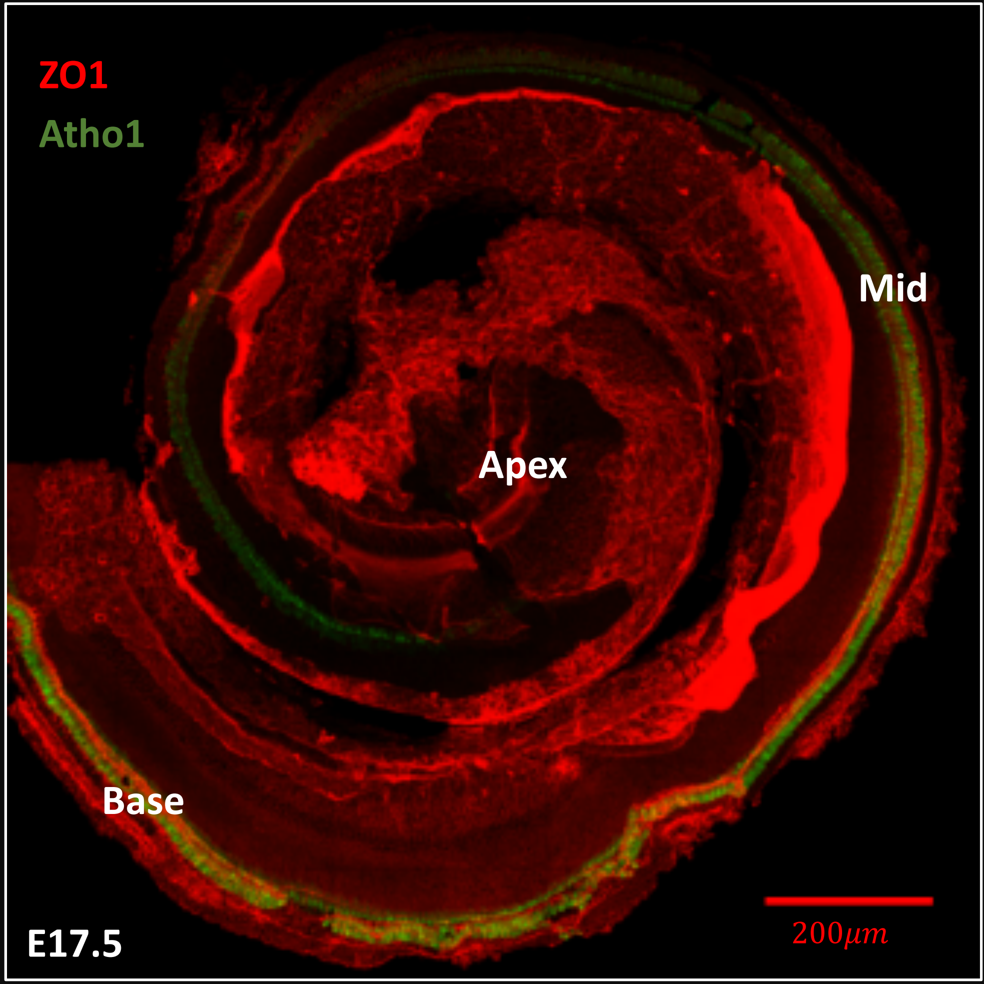
dissection Inner ear explants

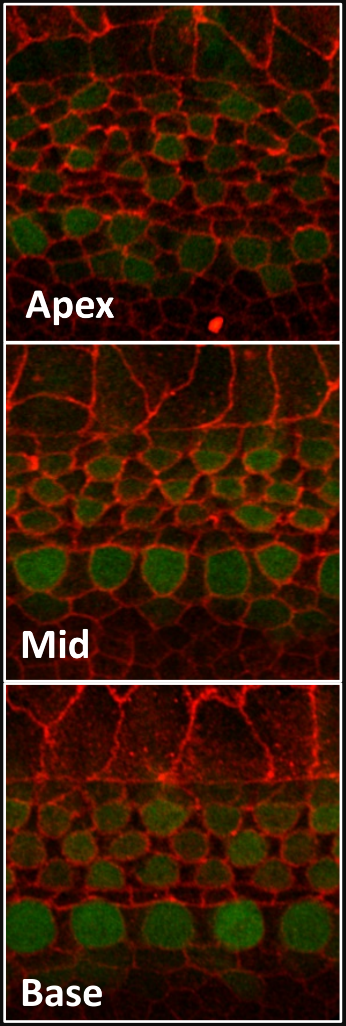
Modeling



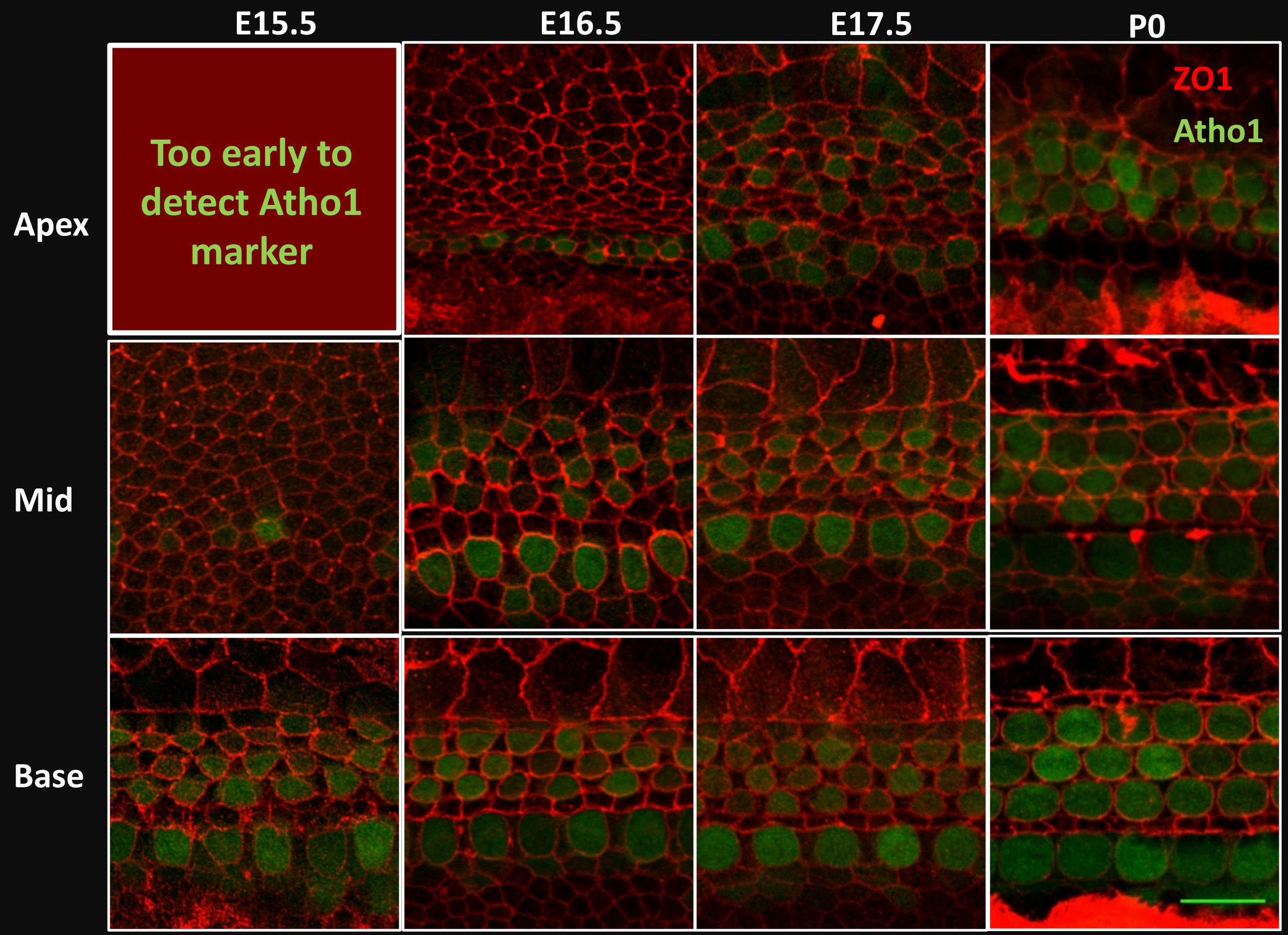
Quantitative analysis of the emergence of organization

Developmental gradient along the base-apex axis





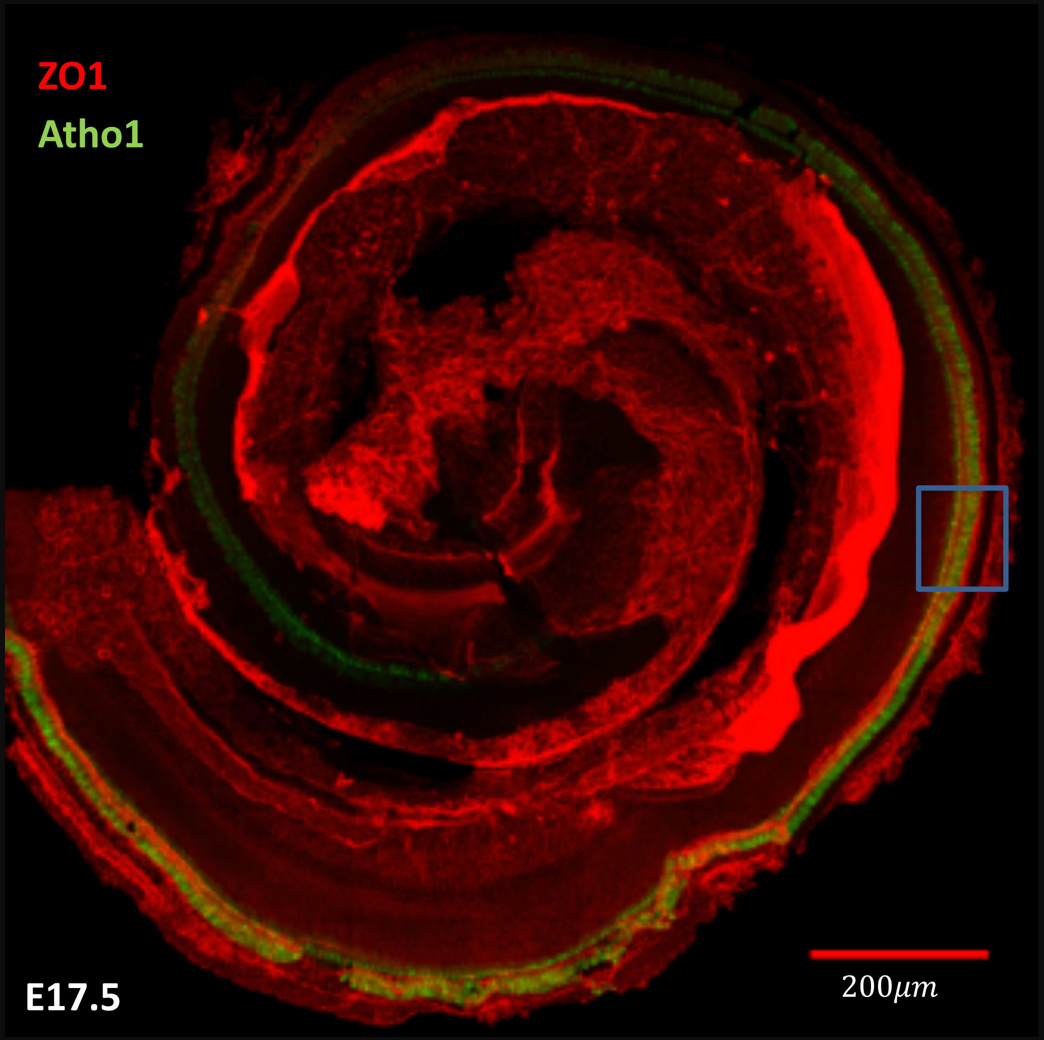
The Organ of Corti reorganizes to a checkerboard pattern in space and time

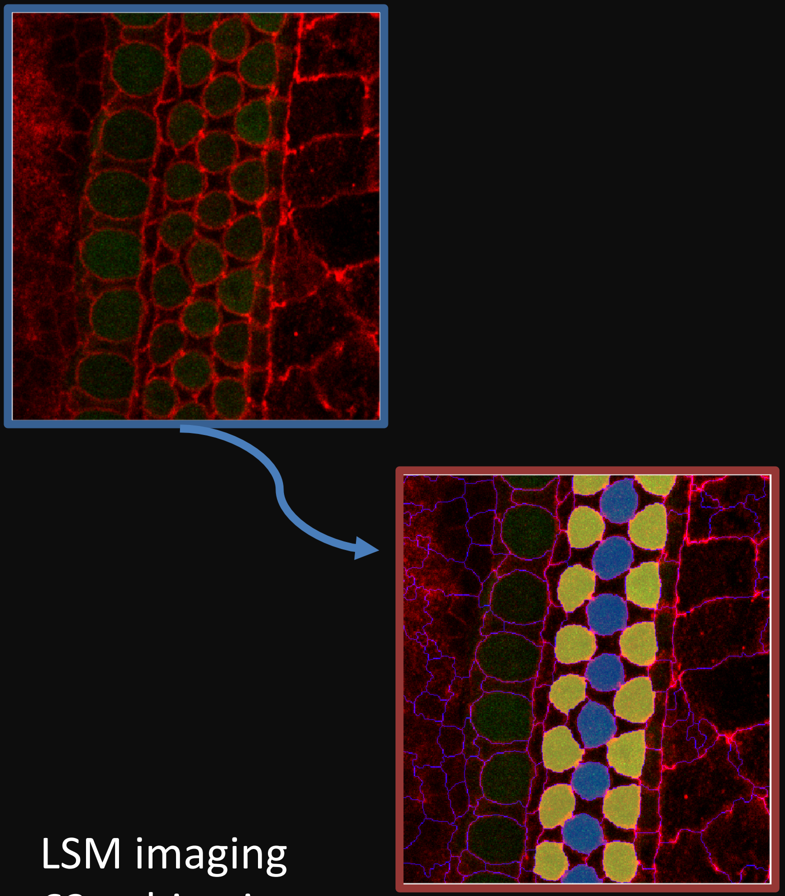


Few observations:

- Inner row of HCs differentiate first
- Initially disordered HC patterning becomes more ordered

Designed code to segment and analyze fixed samples of full cochleae

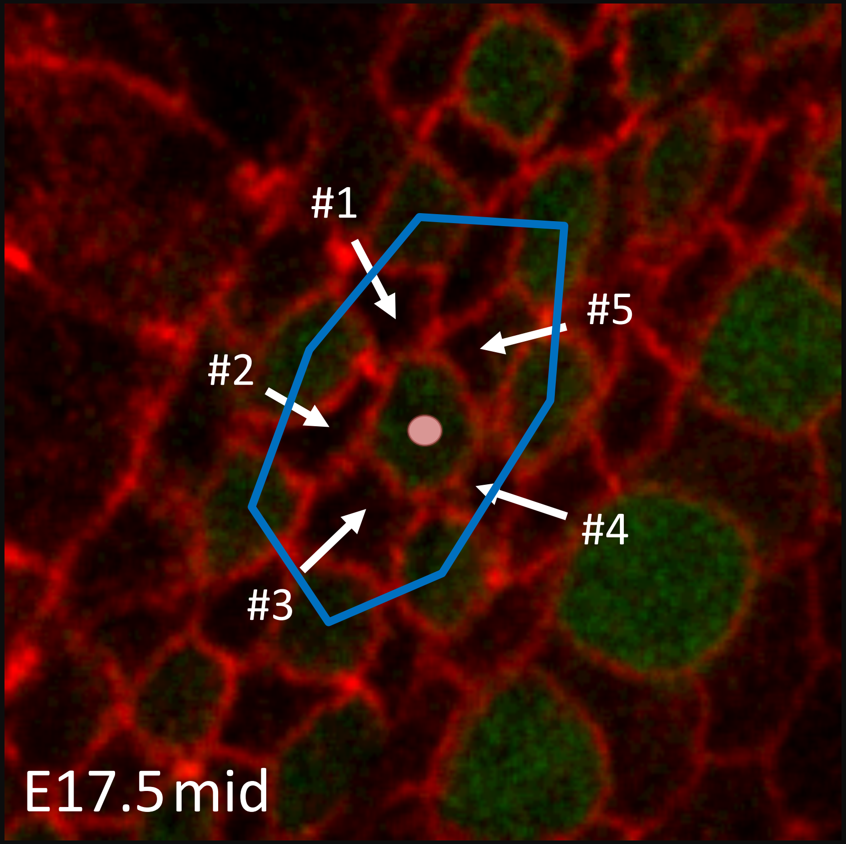


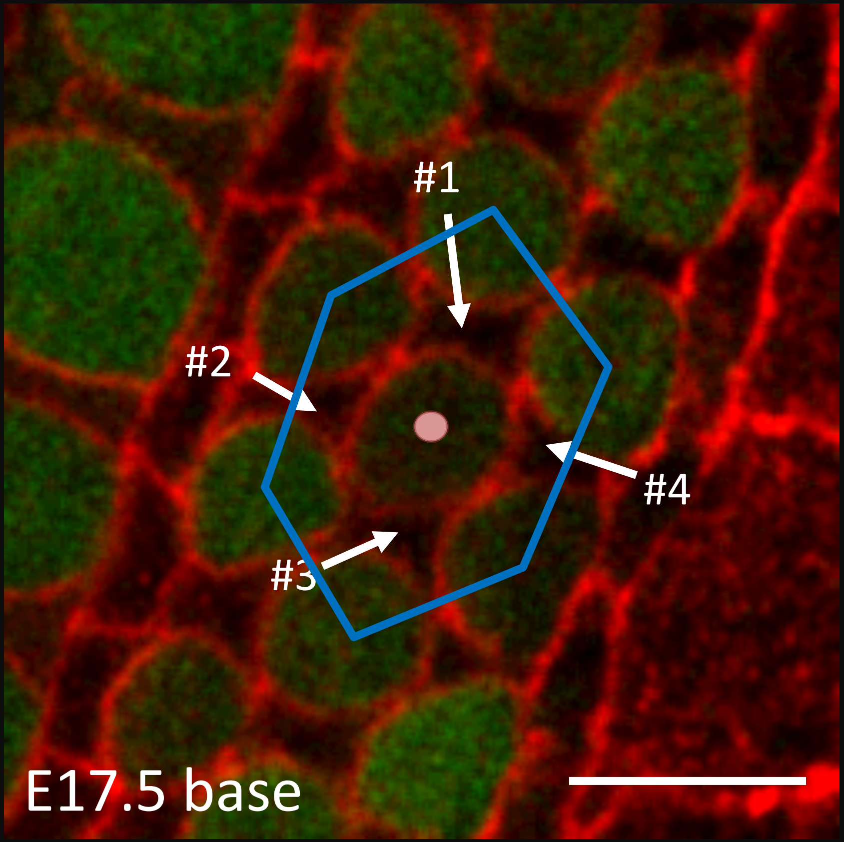


- LSM imaging
- 63x objective
- ~9x9 stitched tiles

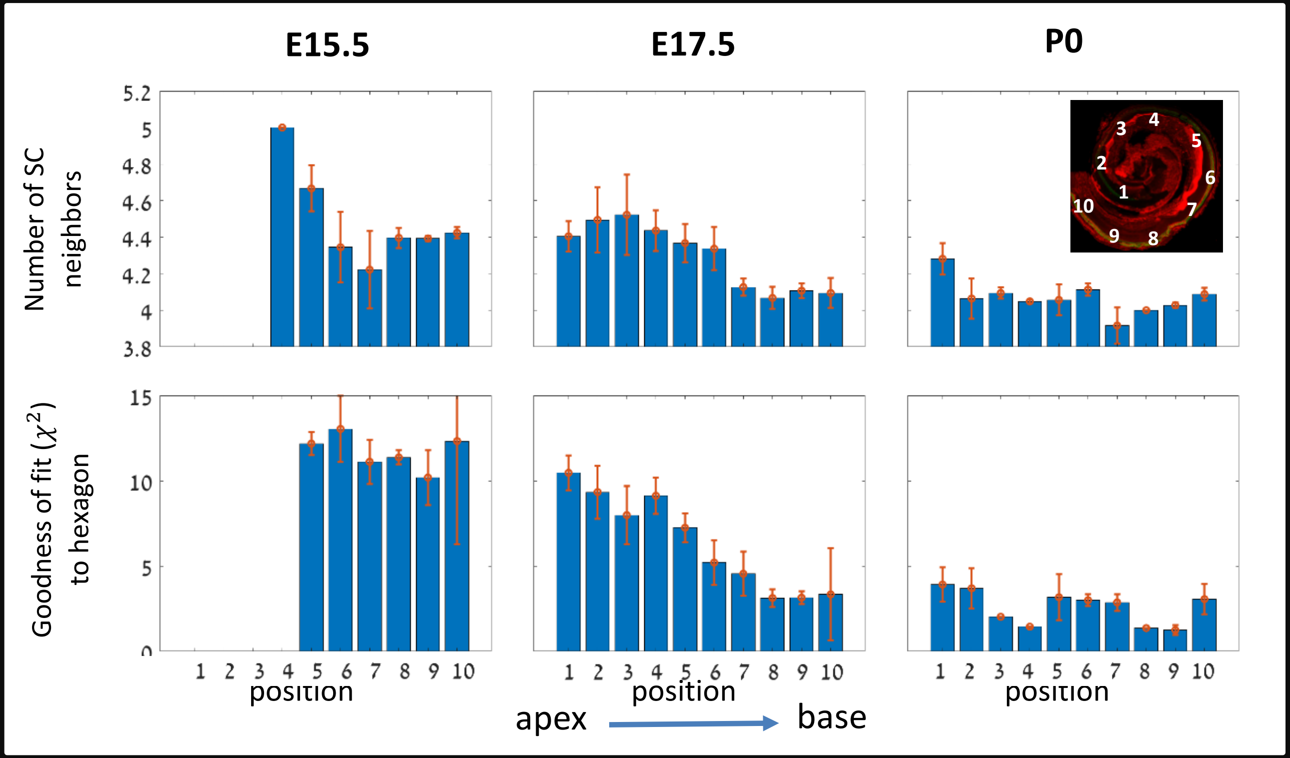
Defining quantitative order parameters

- Number of supporting cells (SCs) neighbors
- The goodness of fit to symmetric hexagon



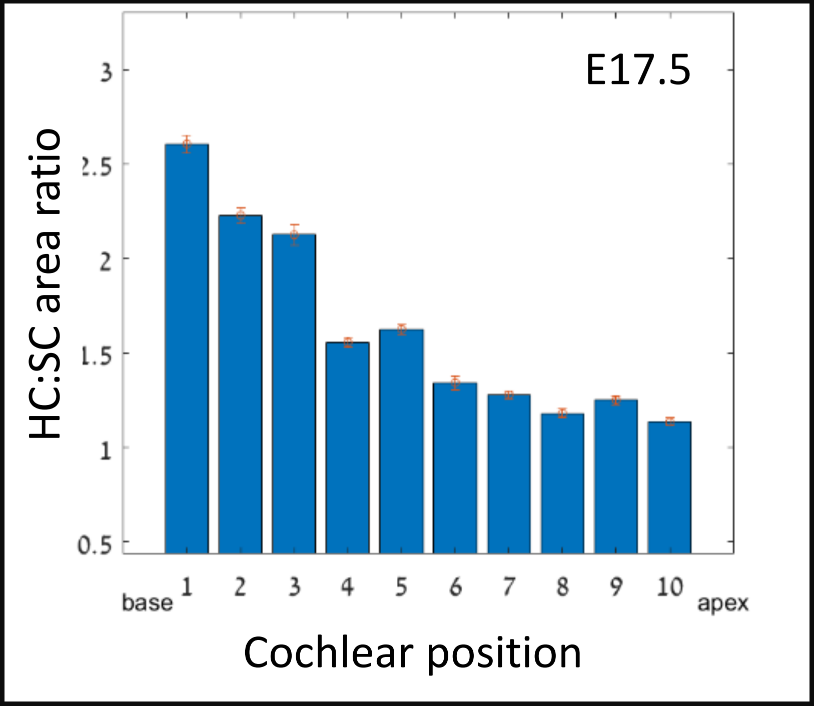


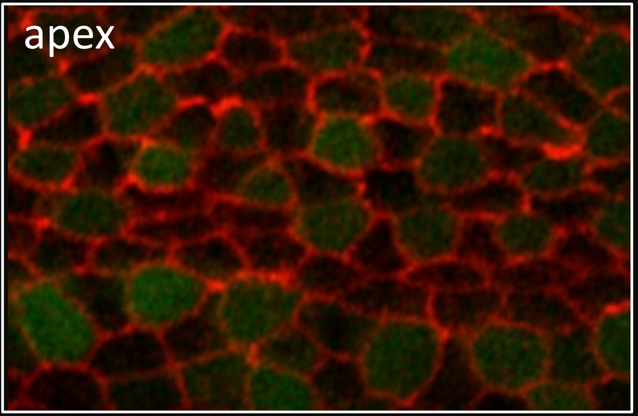
Analysis shows gradual change in order parameters

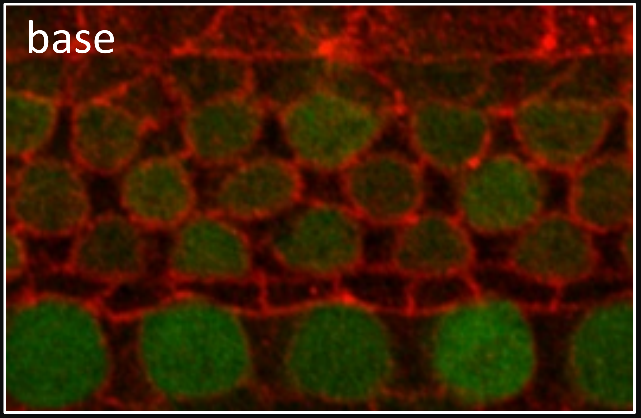


- Number of SC neighbors decreases
- Hexagonal order increases

Analysis shows gradual increase in Hair cell area with developmental stage

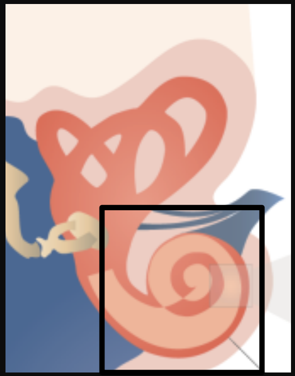




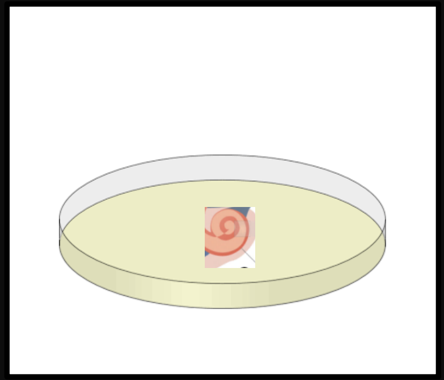


- HC area increases
- SC area decreases

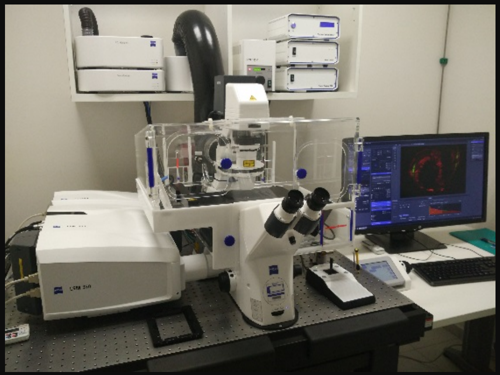
Live inner ear explant imaging



dissection



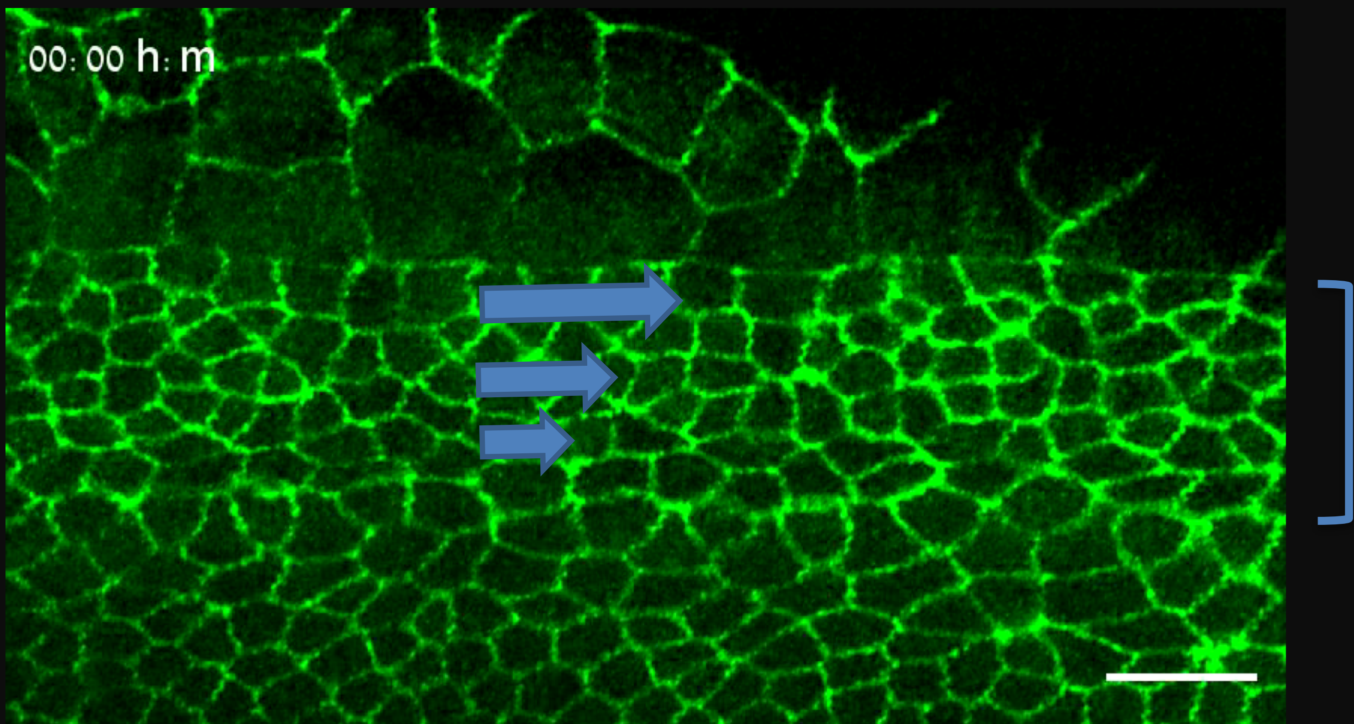
embed explant



live imaging

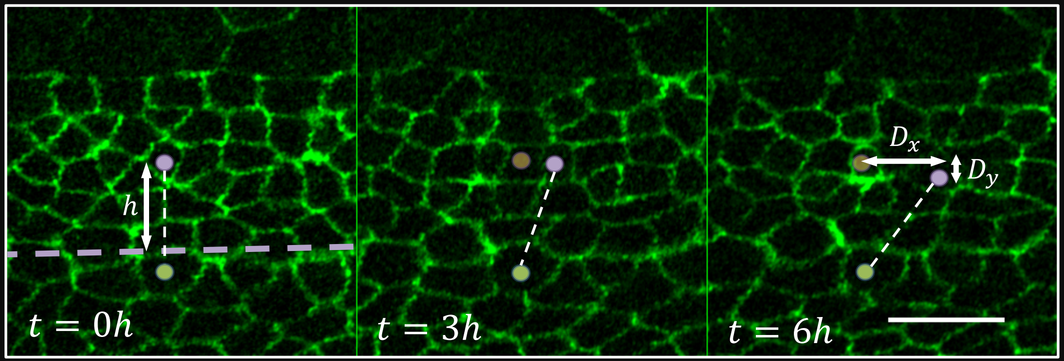
Transgenic mice:
ZO1-GFP – tight junction marker

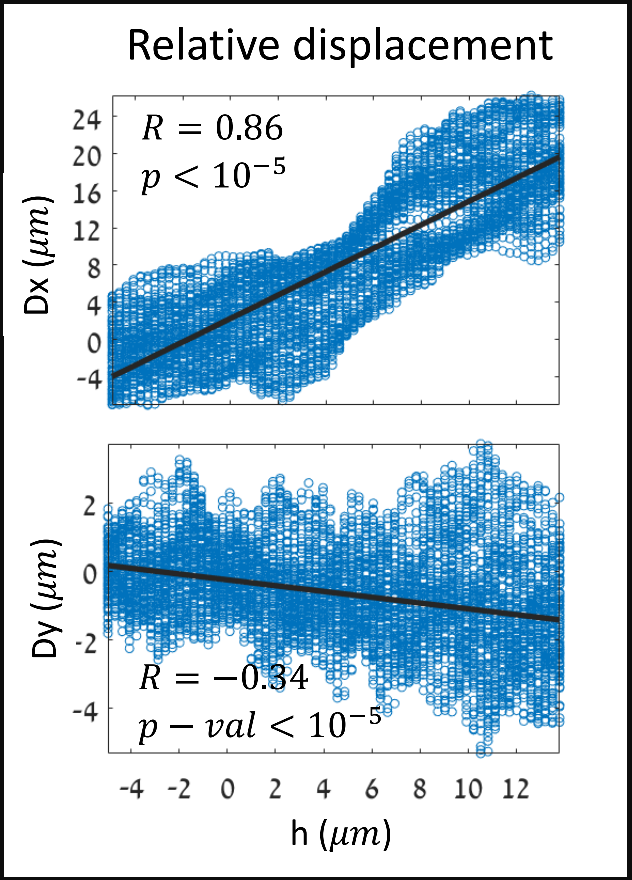
Live imaging of cochlear explants captures shear movement of HCs.



* E15.5 explant,
15 min frame interval

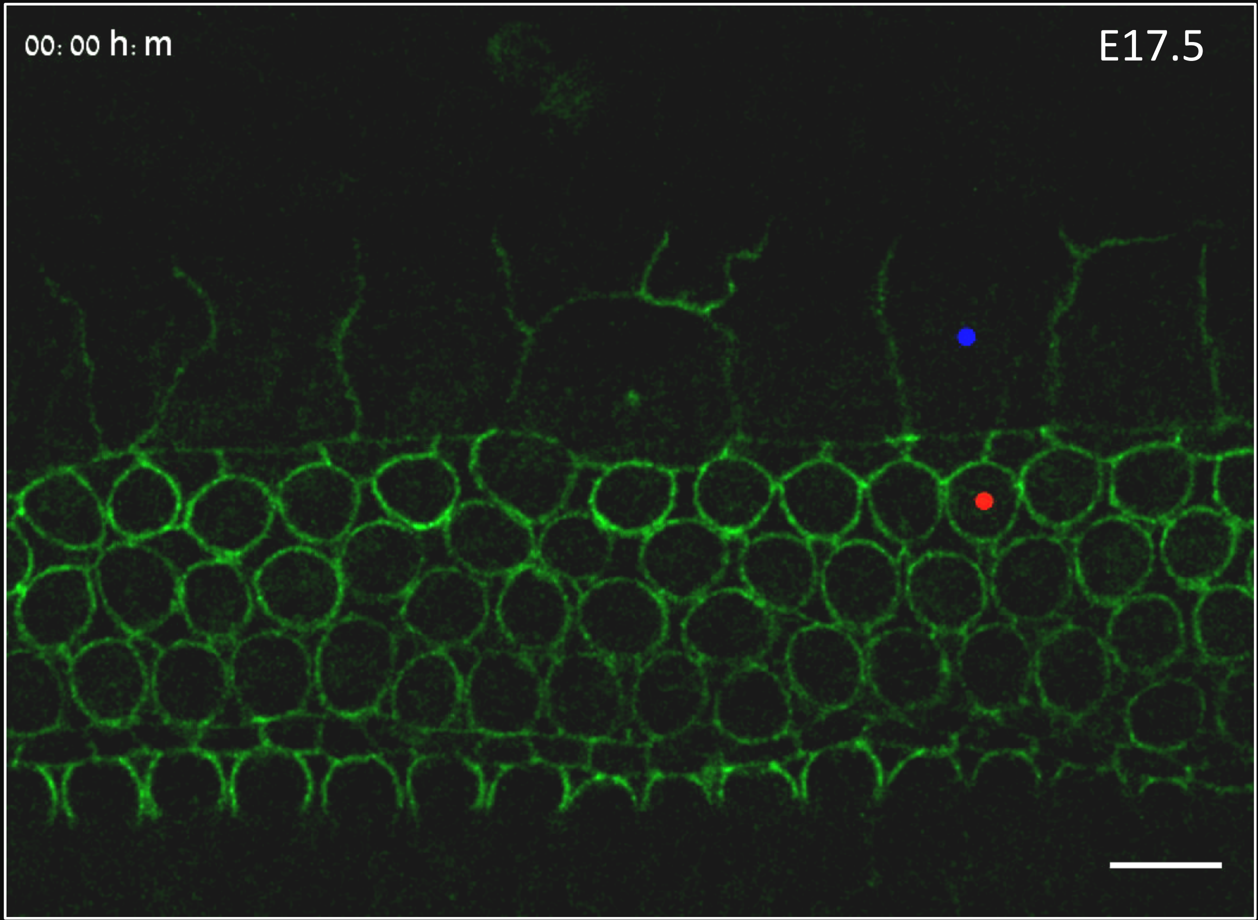
Analysis of shear motion





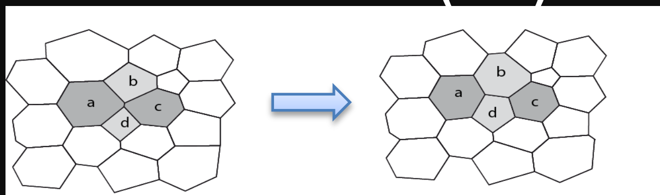
- Analysis based on image registration algorithm

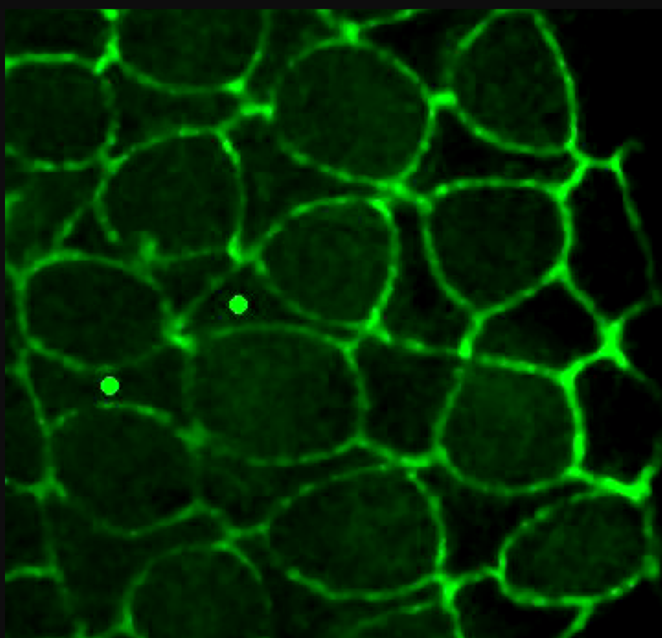
Hensen cells slide and divide



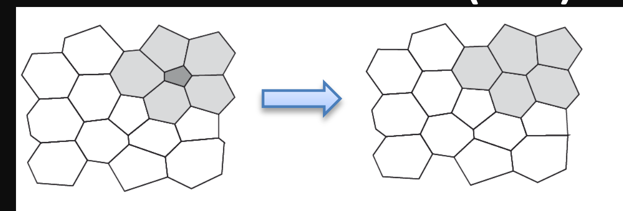
Live imaging of cochlear explants captures local reorganization processes

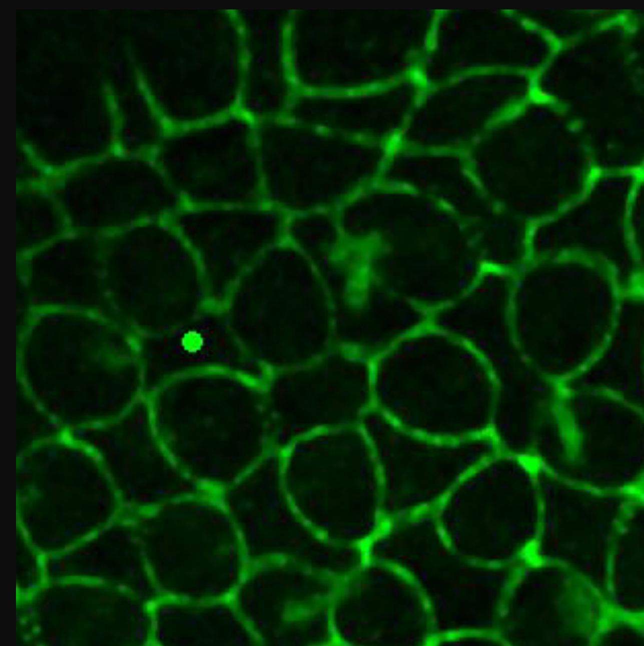
Intercalation (T1)





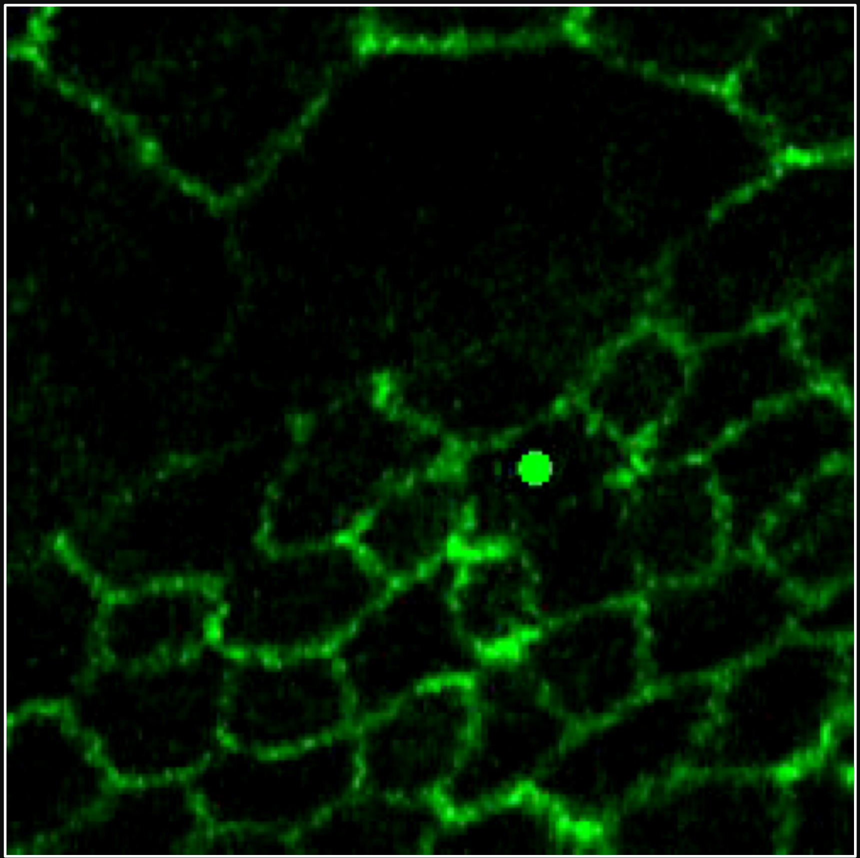
Delamination (T2)





* E17.5 explant, 15 min frame interval

Supporting cells are 'squeezed out' of the HC region



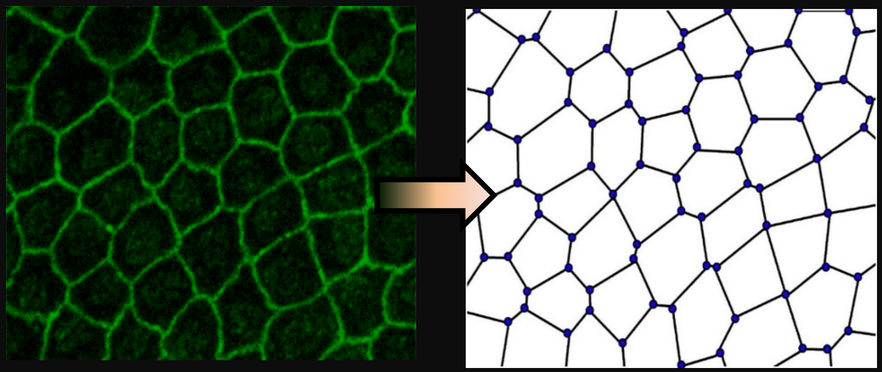
E15.5

Intermediate summary

- Morphological analysis on fixed samples:
  - # of SC neighbors around each HC is reduced
  - HCs gradually reorganize into hexagonal pattern
  - HCs become larger

- From live explant imaging:
  - Organ of Corti exhibits shear motion
  - Multiple intercalations and delaminations are observed
  - SCs delaminate or squeeze out from the organ of Corti

Simulations are done using 2D vertex model

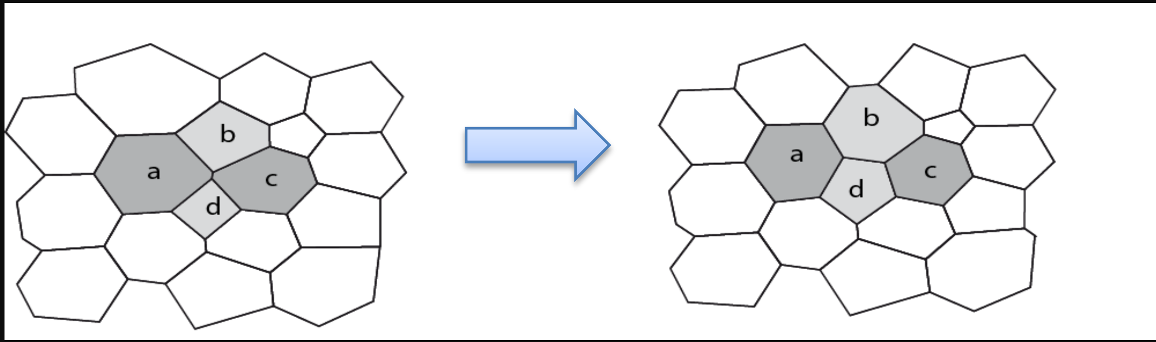


- Cells are defined as polygons
- Mechanical properties:
  - Bond tension (adherens/tight junction)
  - Preferable area (internal pressure)
  - Circumference rigidity (cytoskeleton)
  - External forces acting on cells
- Described by an energy function:

$$E = \frac{1}{2}\alpha \sum_{n=1}^{N_c} (A_n - A_0)^2 + \sum_{\langle ij \rangle} \gamma_{ij} l_{ij} + \frac{1}{2}\Gamma \sum_{n=1}^{N_c} L_n^2 + E_{ext}$$

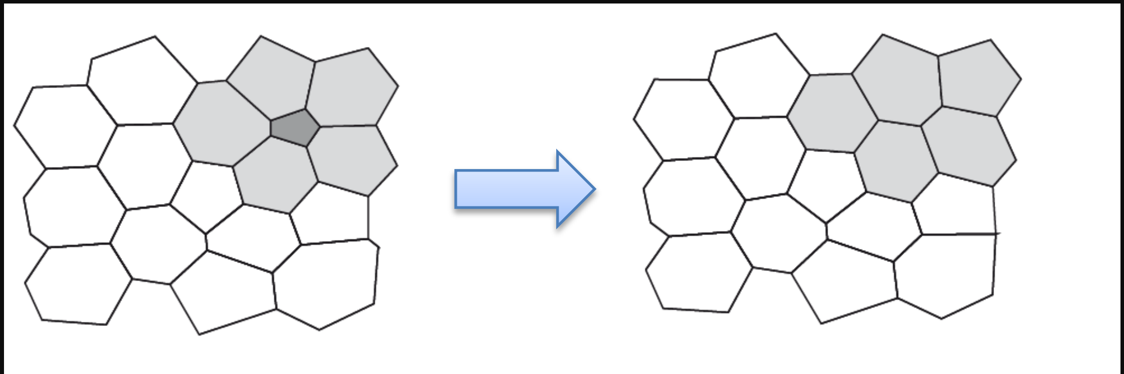
Model incorporates morphological transitions

Intercalation (T1)



For each bond smaller than threshold length with some probability

Delamination (T2)



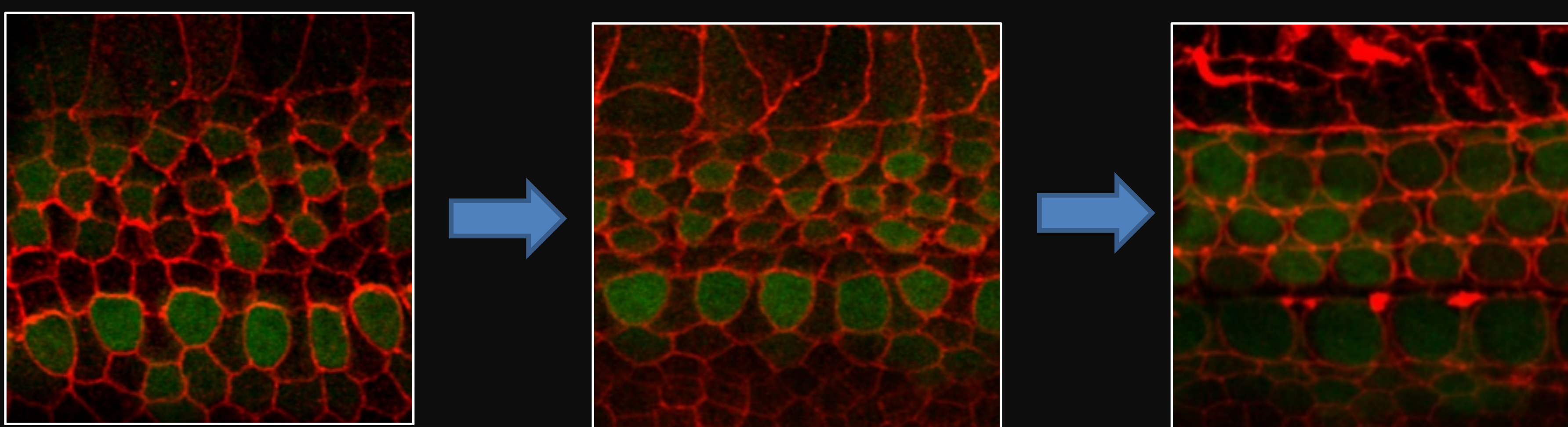
For each cell smaller than threshold size

Modeling the organization of the outer hair cells

- At this stage we focus on the organization of the outer HC region.
- We consider two processes/stage:

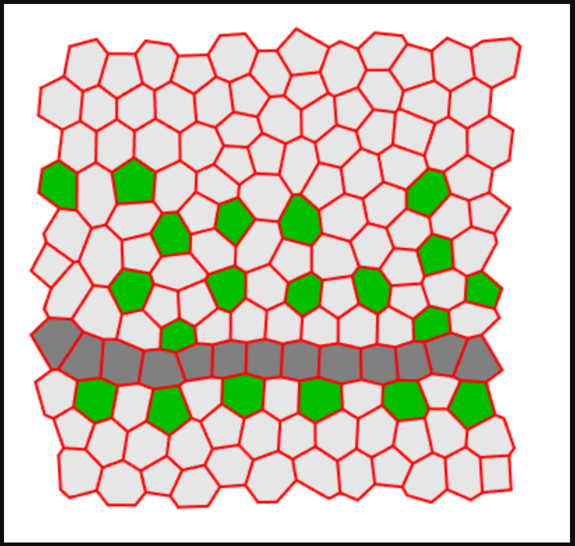
Stage 1 – initial compaction

Stage 2 – refinement



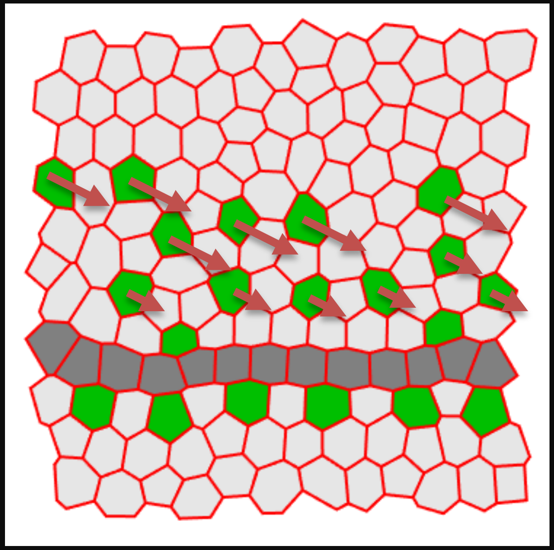
Defining initial and boundary conditions

- The inner HCs row is predefined with the row of pillar cells.
- A region above the pillar cells differentiates in a lateral inhibition pattern

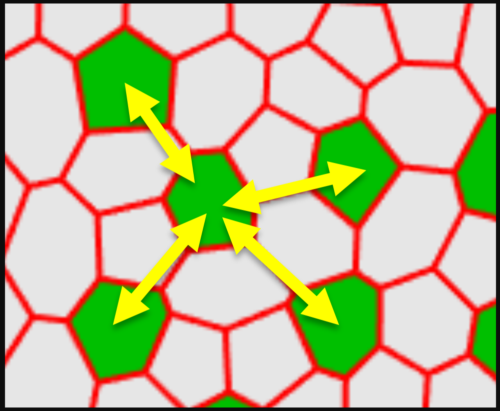


Model assumptions (stage 1)
Global Shear and local repulsion

1. Global shear on HCs towards pillar cell row



2. Local repulsion between HCs



3. HCs are more rigid than SCs

Analogous to crystallization under external pressure

Details of the model

Dynamics is determined by minimizing the system's energy under external forces:

$$E = \sum_{n=1}^{N_c} \left[\frac{1}{2}\alpha_n \left(A_n - A_{n,0}\right)^2 + \sum_{\langle ij \rangle_n} \gamma_n^{ij} l_n^{ij} + \frac{1}{2}\Gamma_n L_n^2 + \sum_{m=1}^{N_c} \sigma_{nm} \left(\frac{D_{nm}}{R_{nm}}\right)^{\kappa} \right]$$

$$\left[\vec{F}_n^{ext}\right]_i = \eta_n y_n^{CM} \hat{x} + \zeta_n y_n^{CM} \vec{\nabla}_i y_n^{CM}$$

For each vertex:

$$-\vec{\nabla} E + \vec{F}^{ext} = 0$$

Where n, m are cell indices, N_c is the total number of cells and $\langle ij \rangle_n$ are the pairs of adjacent vertices in cell n.

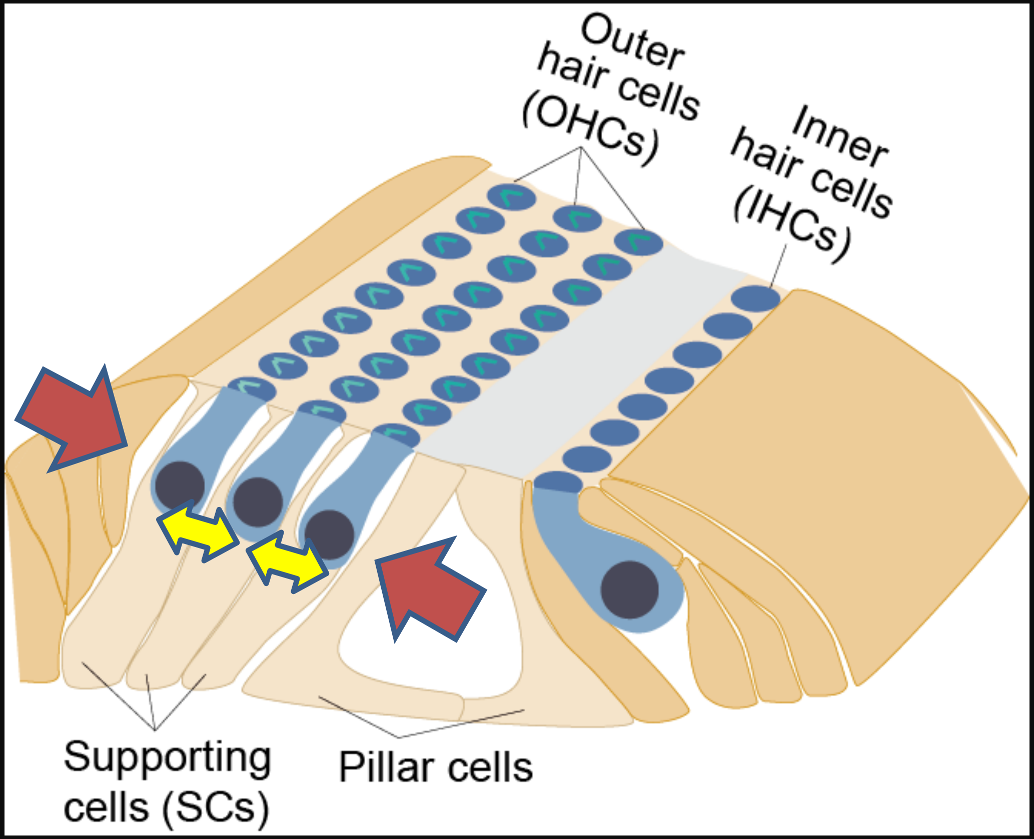
Variables (determined by vertices):

A_n - area of cell *n*
l_n^{ij} - length of bond ij
L_n - circumference of cell *n*
y_n^{CM} - y coordinate of center of mass (relative to PCs)
R_{nm} - distance between cells n and m

Parameters:

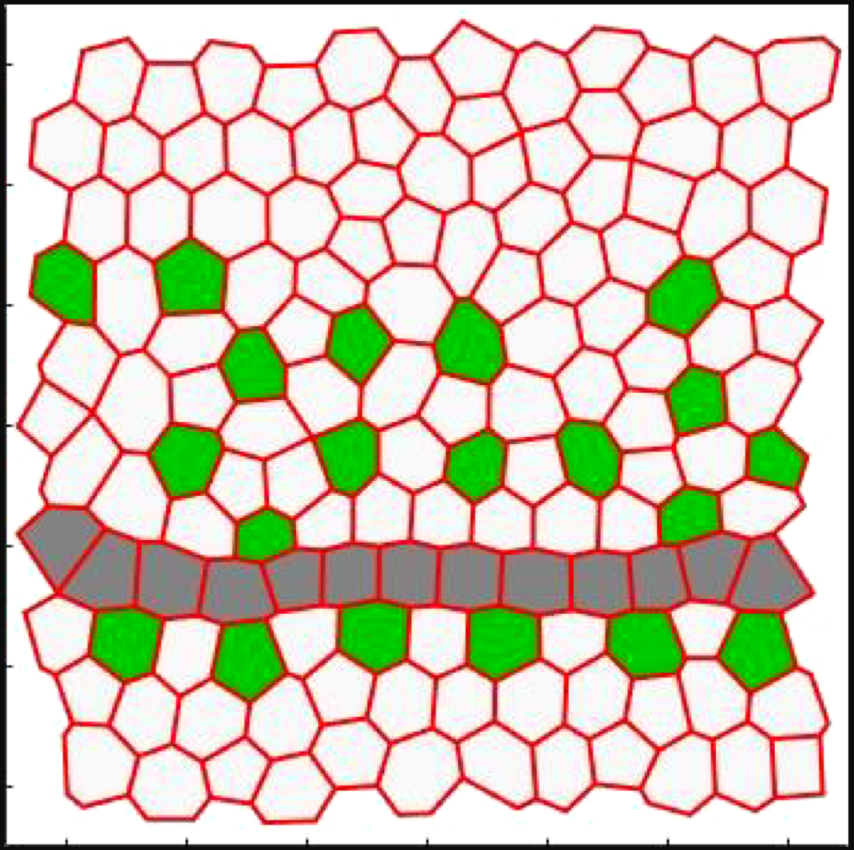
α_n - incompressibility
$A_{n,0}$ - preferable area
γ_n^{ij} - tension of bond ij
Γ_n - structural rigidity
η_n - shear force
ζ_n - compression coefficient
σ_{nm} - repulsion coefficient
κ- repulsion coefficient
D_{nm} - repulsion distance

Physical basis for global shear and local repulsion (hypothesis)



Important: There is a separation between nuclei of HCs and SCs in apical-basal axis!

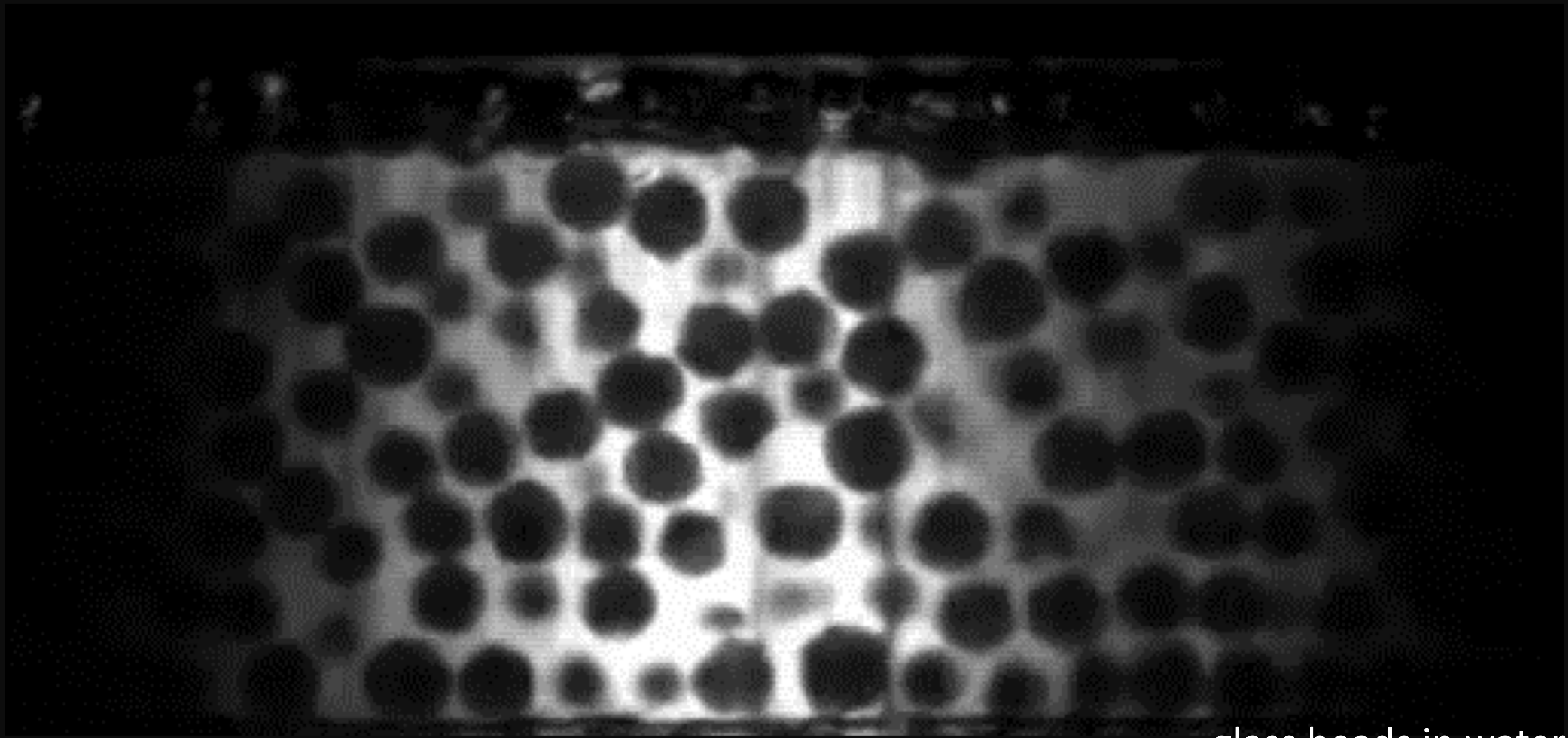
Simulation of stage 1



Model captures:

- Decrease in # of SC neighbors
- Increase degree of hexagonal order

Analogy – shear induced crystallizations



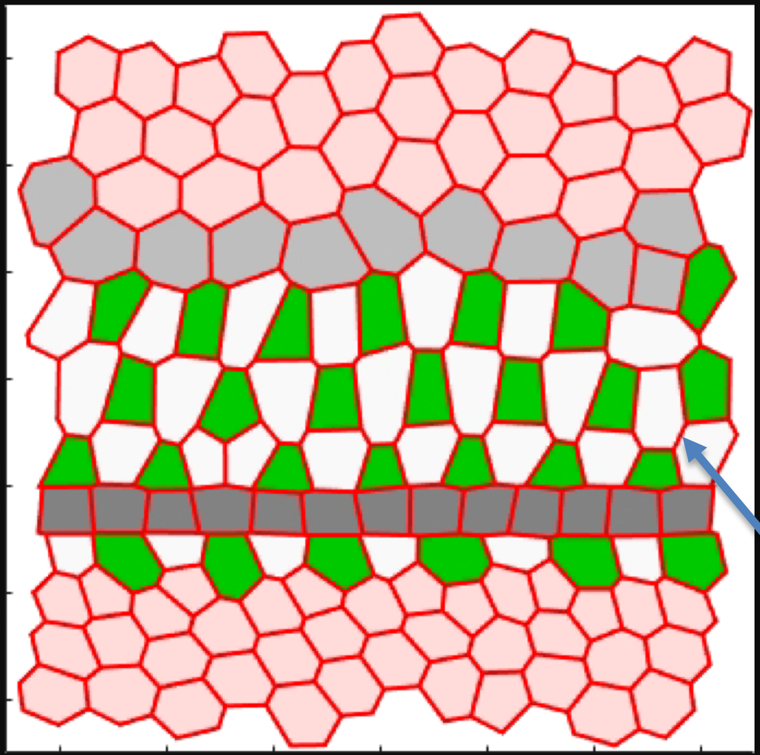
glass beads in water

Tsai et al., PRL 2003

Stage 2 – refinement process

Assumptions:

- Starts when stage 1 ends
- Tension increases for SC:SC bonds (but not for other bonds)
- Top boundary is defined



Increased SC:SC tension

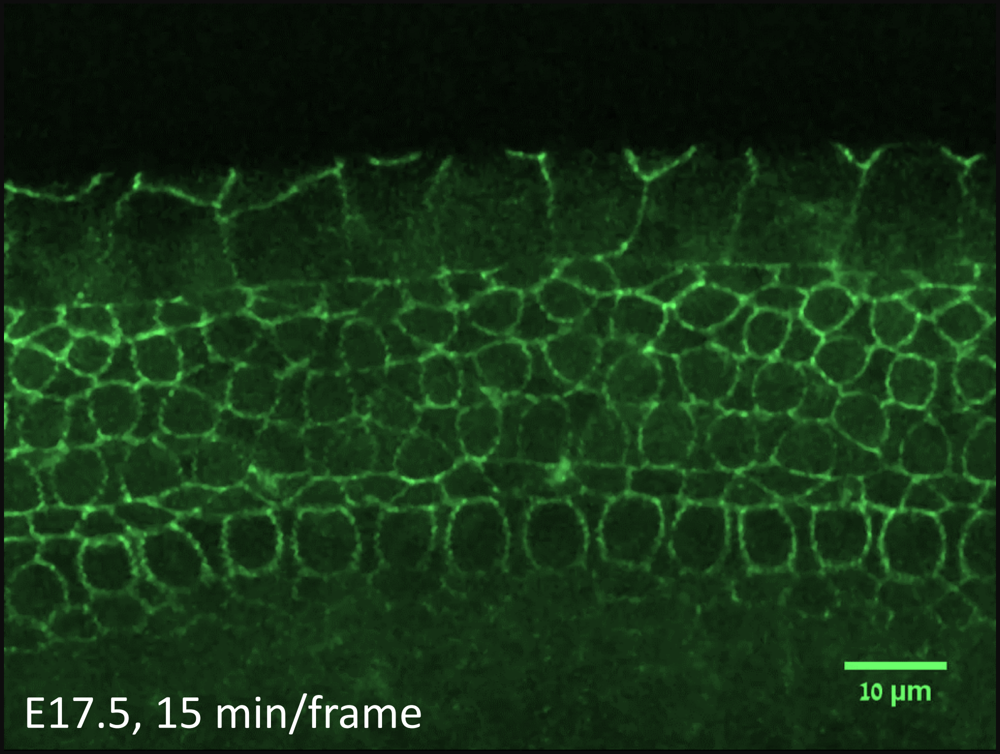
Model captures:

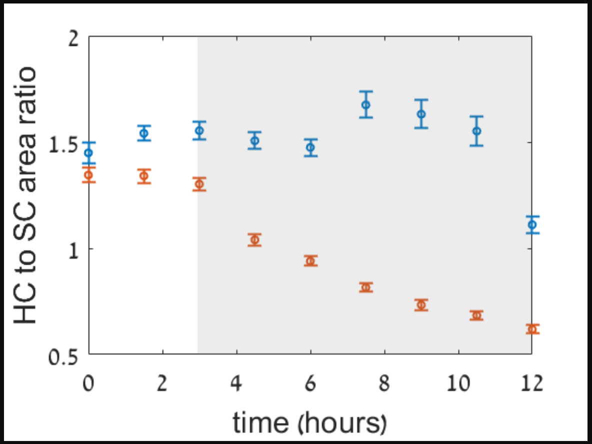
- Increase in HC area
- Decrease in SC area
- Almost perfect square lattice

Testing the model
Applying mechanical perturbations

Prediction I: Reducing SC:SC bond tension should revert increase of HCs areas and decrease of SCs areas

Adding blebbistatin (NMII inhibitor) to explant

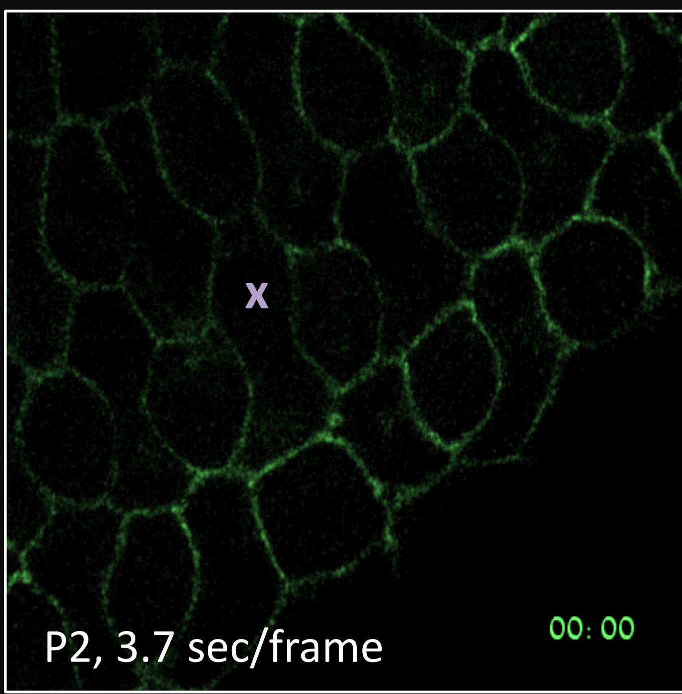


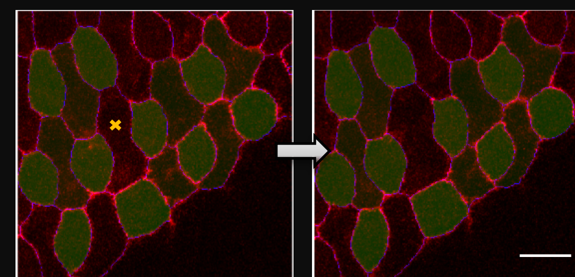


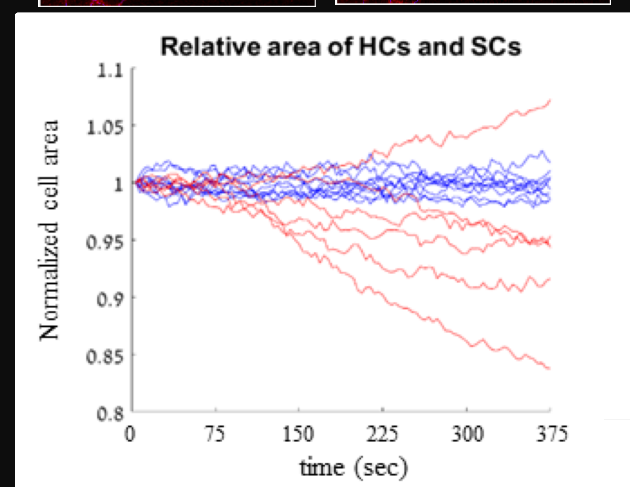
Testing the model
Applying mechanical perturbations

Prediction II: HCs are ‘rigid’ objects flowing in ‘liquid like’ SC environment

Laser ablation in explant







* P2 explant, 3.7 sec frame

Summary

- Reorganization driven by mechanical forces underlie patterning in the inner ear.
- We propose that global shear and local repulsion between HCs controls precise hair cell patterning
- Increase in SC:SC bond tension leads to pattern refinement
- Shear forces may also underlie spiral shape of cochlea.

Open questions and ongoing directions

- What are molecular regulators controlling the mechanical properties of cells and boundaries?
- What drives the global shear?
- What happens to cells that delaminate?
- Role of Notch signaling in maintaining cellular morphology
- How the system reorganize in response to local perturbations?

Acknowledgement

Lab members

Liat Amir
Roie Cohen
Shahar Taiber, TAU
Shiran Woland

Bassma Khamaisi
Udi Binshtok
Natanel Eafergan
Yathreb Issa
Rose Mamistvalov

Liat Amir

Roie Cohen

Micha Hersch

Collaborators:

Micha Hersch, UNIL
Sven Bergmann, UNIL
Karen Avraham, TAU

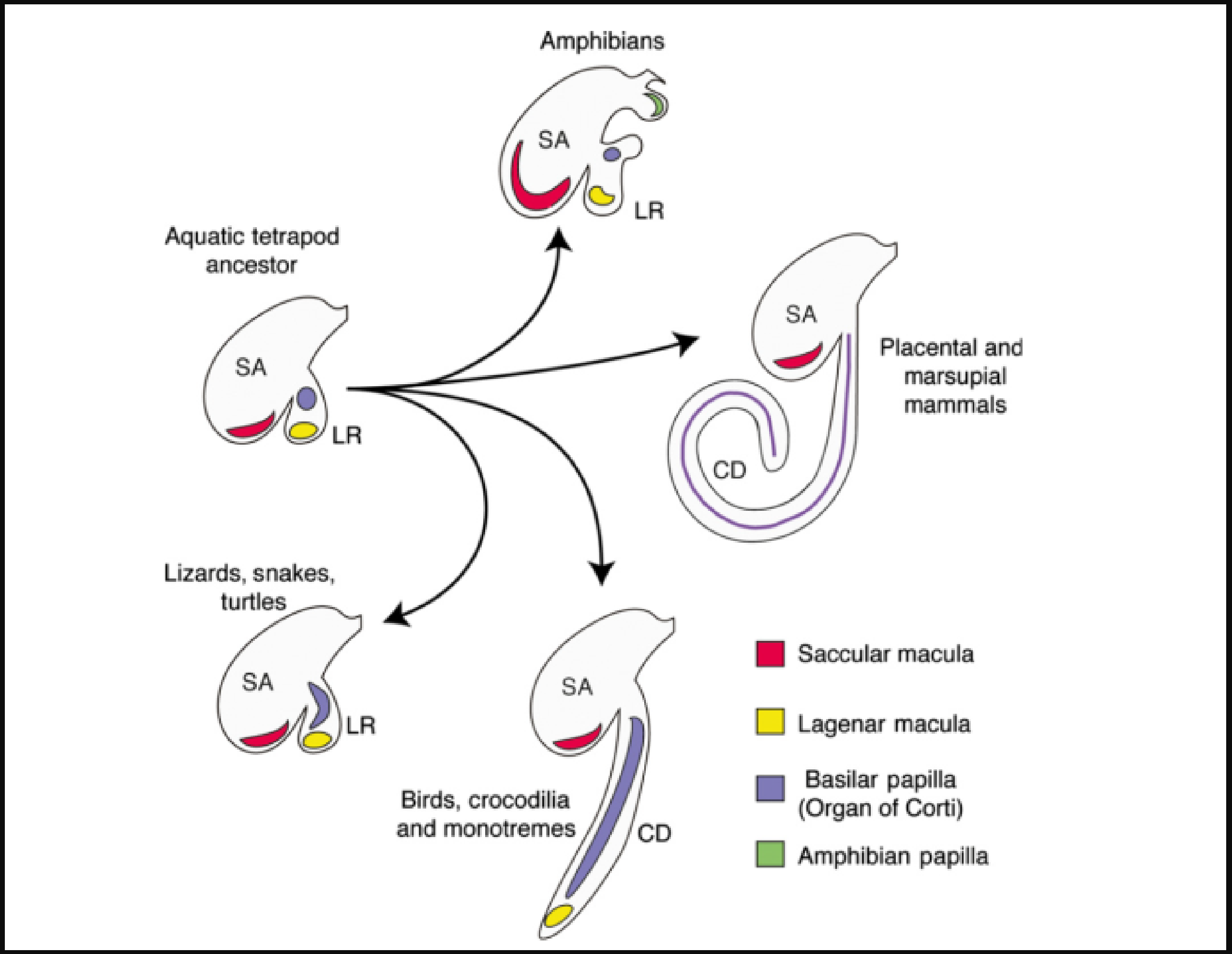
Funding

erc

ISF

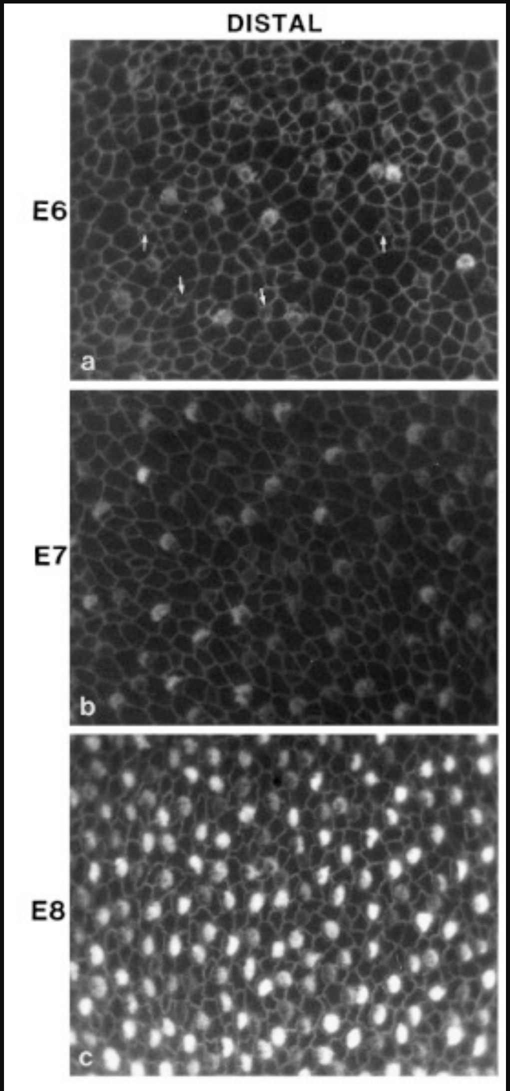
BSF
United States – Israel
Binational Science Foundation

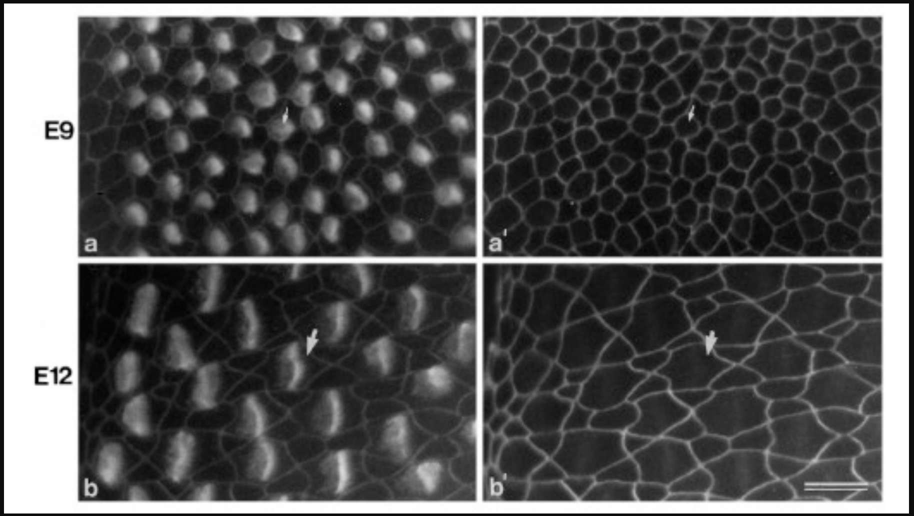
Evolutionary perspective

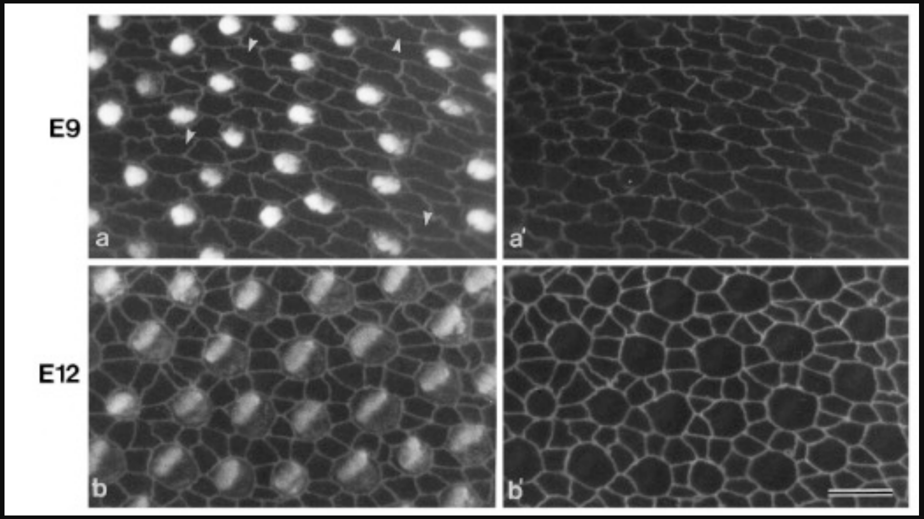


From Basch et al. J Anat. 2016

Development of chick Basilar Papilla

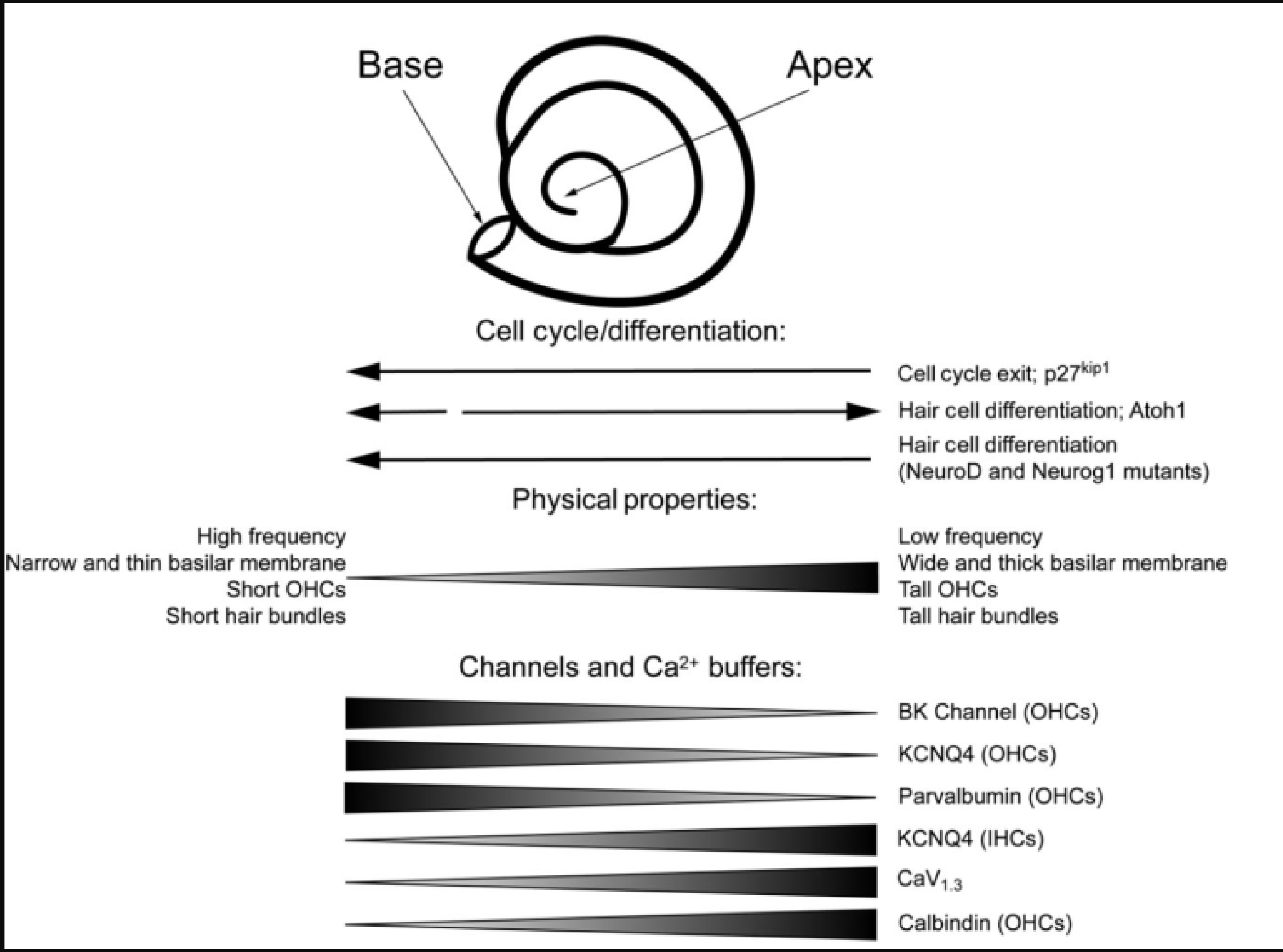






Goodyear and Richardson, J. Neurscience 1997

Differences along the Base-to-Apex axis



From Basch et al. J Anat. 2016