Beating Cilia on *Xenopus* Embryos

Clare Yu\(^1\), B. Mitchell\(^2,3\), J. Stubbs\(^2\), F.M. Huisman\(^1\), P. Taborek\(^1\), C. Kintner\(^2\)

1) Dept. of Physics & Astronomy, University of California-Irvine
2) The Salk Institute for Biological Studies
3) Northwestern University
Importance of Motile Cilia

Motile (beating) cilia found throughout your body:
• Moves mucus up trachea (respiratory system)
• Sense of smell—moves odors to your olfactory system
• Fallopian tubes
• Ventricles of the brain
• Breaks body symmetry in embryo (e.g., heart on left)
Cilia involved in Fluid Flow

Node-Cilia

• Monocilium, 2-5 μm in length
• Rotational beat stroke
• Specialized for producing a transient leftward flow

Multi-Cilia

• 100-200 cilia per cell, 10 μm or greater in length
• Whip like beat pattern
• Specialized for the production of fluid flow along an organ axis, often in combination with mucus
Planar Cell Polarity (PCP)

Cells in a developing embryo “know” direction (PCP)

Direction:
- Anterior/Posterior (head/tail)
- Dorsal/Ventral (front/back)
- Distal/Proximal and Medial/Lateral

Hair on *Drosophila* wing indicate cells’ sense of direction
What about planar cell polarity in vertebrates?

Cilia direction indicate cells’ sense of direction.

Look at *Xenopus* (frog) embryos which have ciliated cells on their epithelium (skin).
Xenopus Development

[Diagram showing the stages of Xenopus development, including Egg (stage 1), Embryo (stage 26), Free-swimming tadpole (stage 45), Adult (stage 66), Gastrula (stage 10), Neurula (stage 16), and Metamorphosis stages.]
Ciliated Epithelia on *Xenopus* Embryo

Head

Tail
Ciliated Epithelia on *Xenopus* Embryo

Fluid is pushed toward posterior.
Why do *Xenopus* embryos have motile cilia on their skin?

• No one really knows.
• Possible reasons:
  – Get oxygen and ions
  – Push away waste products and dirty mucus
  – Spread apart from other embryos to increase survival chances (predators don’t eat everyone at once)
Xenopus Skin is comprised of three principle cell types with different functions.

1. Large epidermal, mucus secreting cells
2. Ciliated cells (CC)
3. Ionocytes which secrete ions (INC)

Skin contains at least three cell types

Ciliary flow produced by ciliated cells allows both mucus producing cells and ionocytes to function.
Ciliated Cell Development

1. Lateral inhibition

2. Radial Intercalation

3. Ciliogenesis
Basal Body orientation dictates direction of ciliary flow
Side View of a Cell with Beating Cilia

- Cilia on a cell like a forest of trees
  - Cilia:
    - ~15 μm long
    - ~1 μm apart
    - ~20 beats/second
    - ~250 nm diameter

How do cilia keep from crashing into each other?

Ciliated cell on *Xenopus* embryo.

- Filmed at 20,000 frames per second
- Cilia diameter ~ wavelength of light
To function in fluid flow, both cilia and ciliated cells need to be polarized along a common planar axis.

**Mechanisms that orient cilia within a cell**

**Mechanisms that orient ciliated cells within a tissue**
Top View of Beating Cilia
What determines the direction that cilia beat?

- Intercellular (chemical) signaling
- Fluid flow
  - Immotile cilia cause cilia disorientation (Mitchell et al, Nature 2007)

Question we would like to answer:

What is the interplay between signaling and fluid flow at various stages of development?
Core components of the PCP Signaling pathway in *Xenopus*

- PK = prickled
- DGO = Diego
- DSH = Dishevelled
- Van Gogh = Strabismus

Adapted from Seifert JR, Mlodzik M. Nat Rev Genet. 2007 Feb;8(2):126-38
1. Polarity is acquired gradually
   - Polarity of skin not fixed before gastrulation

Polarity of skin is not fixed before gastrulation

1. Polarity is acquired gradually
   • Polarity of skin not fixed before gastrulation
2. Patterning establishes a posterior bias
   • Cilia posterior orientation initiated soon after gastrulation

1. Polarity is acquired gradually
   - Polarity of skin not fixed before gastrulation
2. Patterning establishes a posterior bias
   - Cilia posterior orientation initiated soon after gastrulation
3. Cilia function causes flow, which in turn refines cilia polarity
Ciliogenesis in *Xenopus* Embryo Explant

- High speed video allows viewing of live cell
  - Explore cilia motion at various growth stages
Core components of the PCP Signaling pathway in *Xenopus*

- **PK** = prickled
- **DGO** = Diego
- **DSH** = Dishevelled
- **Van Gogh** = Strabismus

Adapted from Seifert JR, Mlodzik M. Nat Rev Genet. 2007 Feb;8(2):126-38
Mutations produce disoriented cilia.
Analogy to Spin Systems

We can model interactions with spin systems. Spin Hamiltonian $H$:

$$H = -\frac{1}{2} \sum_{i \neq j} J_{ij} \vec{S}_i \cdot \vec{S}_j$$

At high temperatures the spins are in a paramagnetic phase in which the magnetization $M$ can be aligned in an external magnetic field $H$. At low temperatures the spins are frozen in one of the following configurations:

- **Ferromagnet**: $J > 0$
- **Antiferromagnet**: $J < 0$
- **Spin Glass**: $J_{ij}$ random

A spin glass is a collection of spins with random interactions between them.
Spin Glass Model of Cilia

- Inside a ciliated cell, the xy spin $S_i$ points in the direction that a cilium is oriented.

$$H = - \sum_{i \neq j} J_{ij} \vec{S}_i \vec{S}_j - \sum_i \vec{h}_{tot} \vec{S}_i - \sum_i J_{bndy} \vec{S}_i \vec{B}_i$$

$$\vec{h}_i = \vec{h}_{flow} + \vec{h}_{PCP}$$

- Alternatively, in a tissue, $S_i$ represents a ciliated cell and points in net direction of its cilia. ($J_{bndy} = 0$)
- Model not useful to biologists. Unable to specify parameters.
Spin glass model produces random patterns.

But spin glass model is not so useful biologically.
Summary

• Motile cilia are physiologically important.
• Direction determined by PCP signalling and flow.
• Can be visualized with high speed videos.

Questions

1. What is the role of flow in orienting cilia and aligning ciliated cells at different stages of development?
2. How do cilia coordinate beating within a cell?
THE END
Ciliated cells are biased in posterior direction

Immotile cilia in Xenopus causes cilia disorientation

Monte Carlo Simulations of *Drosophila* Wing Disc

Julie Wortman
Clare Yu
Arthur Lander
University of California, Irvine
Funded by NIH through UCI Center for Complex Systems Biology
Life cycle of Drosophila

1. Fertilized egg
2. Cleavage
   - Syncytial blastoderm
   - Stage 3–4
3. Gastrulation
   - Embryo
   - Stage 13
4. Hatching
5. Larva
   - 1st instar
   - 2nd instar
   - 3rd instar
6. Pupa
7. Adult fly
8. Metamorphosis

*At 25°C incubation
Wing Imaginal Disc

- Patches of cells in the larval insect that will form appendages, e.g., wings, legs, antennae, during metamorphosis.
- *Drosophila* wing disc grows from about 40 to 50,000 cells over 4 days.

Campuzano and Modolell, 1992; Cohen 1993
Morphogen Gradients Produce Patterning

Morphogen Dpp (Decapentaplegic) is necessary for wing growth.

Dpp Signalling Pathway

- Morphogen Dpp (Decapentaplegic) binds to and activates thickveins (Tkv) receptor, promoting phosphorylation of Mad (Mothers against Dpp).
Wing Disc Grows Uniformly

- Dpp necessary for growth of wing disc
- Dpp concentration is exponentially decaying gradient
- Wing disc grows uniformly

How can the wing disc grow uniformly when the Dpp has a concentration gradient?
Elastic Stress Affects Growth
(Shraiman *PNAS* (2005); Hufnagel *et al.* *PNAS* (2007))

- Adjacent cells are mechanically coupled through cadherins.
- Tension and compression affect local growth.
- Elastic stresses can produce uniform growth in wing disc.
Questions

- Do elastic interactions affect cell growth?
  - Does a cell grow faster if it is under tension?
- Does result agree with cell packing statistics?
- Is local cell packing correlated with cell growth?
Cell Packing Statistics

<table>
<thead>
<tr>
<th>Number of Nearest Neighbors</th>
<th>Fraction of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>~ 29%</td>
</tr>
<tr>
<td>6</td>
<td>~ 46%</td>
</tr>
<tr>
<td>7</td>
<td>~ 21%</td>
</tr>
</tbody>
</table>

- Geometric arguments
- Cell division
- No cell rearrangement

Monte Carlo Simulations of Wing Disc

- Single layer of cylindrical cells
- Dpp concentration decays exponentially
- Each cell has Dpp receptors (Tkv)
- Number of receptors varies in time due to degradation and production
- Number of receptors can vary from cell to cell
- Elastic interactions between neighboring cells
- Cells can move in response to forces from neighbors
- Cells grow in size and in number of receptors
- Cells can divide
Elastic Interaction Between Adjacent Cells

- Spring if cells overlap
- 6-12 potential if cells separated

$$V(x) = \begin{cases} 
  k \left[ x - (r_1 + r_2) \right]^2 & \text{if } x < (r_1 + r_2) \\
  \varepsilon \left[ \left( \frac{r_1 + r_2}{x} \right)^{12} - \left( \frac{r_1 + r_2}{x} \right)^6 \right] & \text{if } x > (r_1 + r_2) 
\end{cases}$$

Compression or tension from neighbors affects a cell’s growth.
Dpp Concentration Affects Growth

Let \( B = \# \) bound receptors on a cell

- Linear growth: radius \( r(t) = r(t-1) + a \cdot B_{\text{cell}} \)
- Exponential growth: radius \( r(t) = b \cdot B \cdot r(t-1) \)

A Cell’s Probability to Divide Increases with Size

\[
P = 1 - \frac{1}{1 + \left(\frac{r}{r_0}\right)^n}
\]
Movies of Tissue Growth

- Red cells under compression from neighbors.
- Blue cells under tension from neighbors.
- Lines are from Voronoi tessellation
Cell Packing Statistics

Our Monte Carlo simulations find:

- Cells with fewer neighbors (e.g., 5) are under compression
- Cells with more neighbors (e.g., 7) are under tension

Previous experiments and geometric arguments:

<table>
<thead>
<tr>
<th># Neighbors</th>
<th>Fraction of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>~ 28%</td>
</tr>
<tr>
<td>6</td>
<td>~ 46%</td>
</tr>
<tr>
<td>7</td>
<td>~ 20%</td>
</tr>
</tbody>
</table>

Gibson et al., Nature (2006); Farhadifar et al., Current Biology (2007).
How is cell growth affected by elastic stress?

3 Possibilities:
1. Grow faster under tension (Shraiman et al.)
2. Grow faster under compression
3. Elastic stress has no effect (control case)

Strategy:
- Run simulations with these possibilities and compare to cell packing statistics.
- No morphogen gradient.
If Cells Under Tension Grow Faster

- Suppose cells under tension (blue) grow and divide faster
- Their neighbors are compressed (red) and grow slower
- Cell rearrangement fast compared to proliferation
- Control case similar (growth independent of stress)
If Compressed Cells Grow Faster

• Suppose compressed cells (red) grow and divide faster
• Their neighbors are compressed and grow faster
• Proliferation of cells faster than rearrangement (like Gibson et al. assumed in geometric argument)
• Better agreement with cell packing seen experimentally
Is Tension Better for Uniform Growth?

If disc initially at radius \( r_0 \) grows uniformly to a final radius \( r_{\text{max}} \), the number of divisions goes as

\[
\# \text{ Divisions} \propto \begin{cases} 
\ln \left( \frac{r_{\text{max}}}{r_0} \right) & \text{if } r \leq r_0 \\
\ln \left( \frac{r_{\text{max}}}{r} \right) & \text{if } r > r_0 
\end{cases}
\]

Growth independent of Dpp concentration and elastic stress is most uniform.

Caveat: Simulations do not yet include morphogen gradient.
Conclusions

• Monte Carlo simulations of cell growth in *Drosophila* wing disc.
• If cells under compression grow faster, we obtain better agreement with observed cell packing.
• Fast growing cells do not have enough time to rearrange. This is consistent with the assumption used in the geometric argument for cell packing (Gibson *et al.*, *Nature* (2007)).
THE END