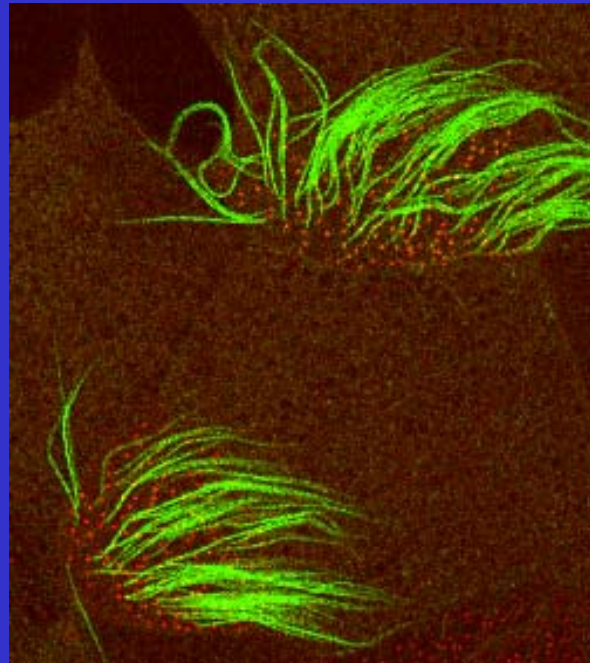# Beating Cilia on *Xenopus* Embryos

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
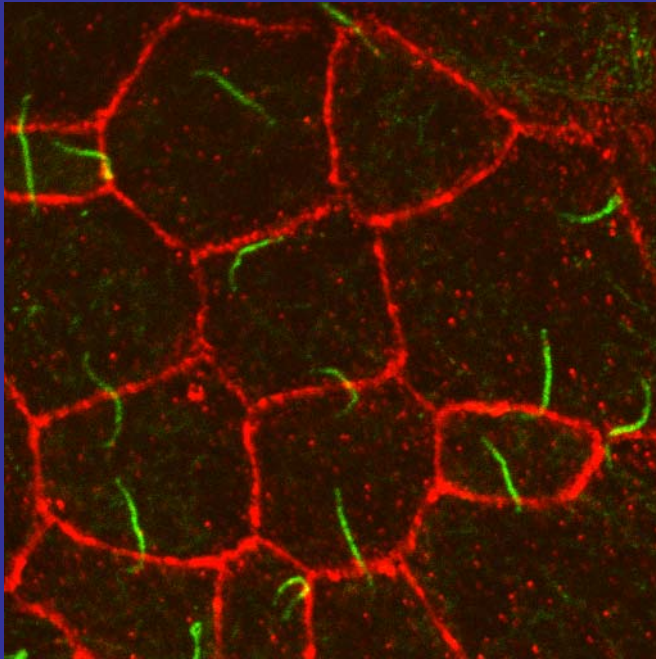3) Northwestern University

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
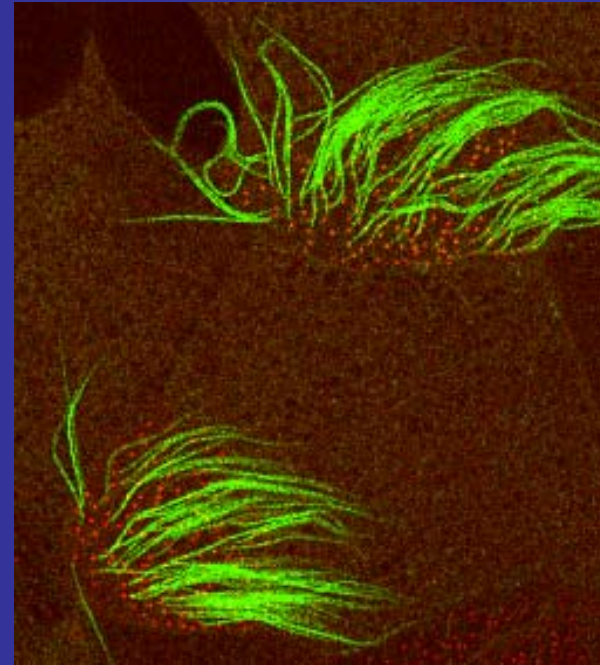- Breaks body symmetry in embryo (e.g., heart on left)

Cilia involved in Fluid Flow

Node-Cilia

Multi-Cilia

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

- 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)

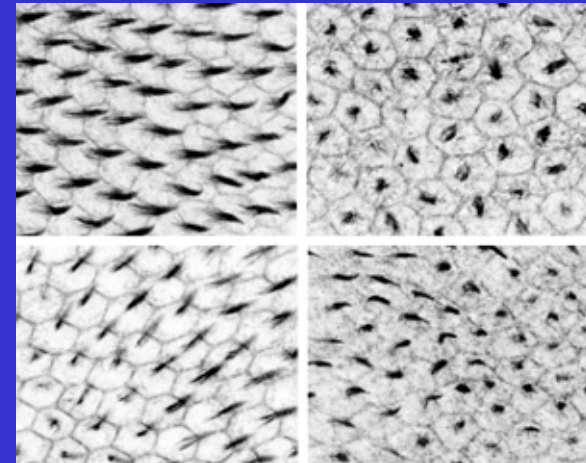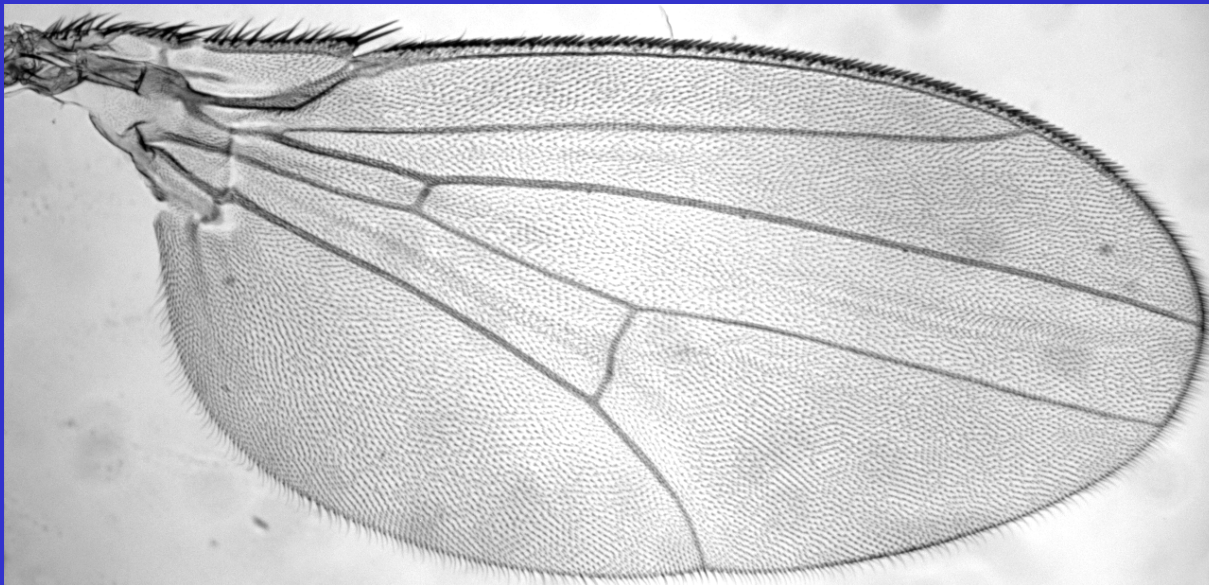Cells in a developing embryo "know" direction (PCP)

Direction:

    Anterior/Posterior (head/tail)

    Dorsal/Ventral (front/back)

    Distal/Proximal and Medial/Lateral

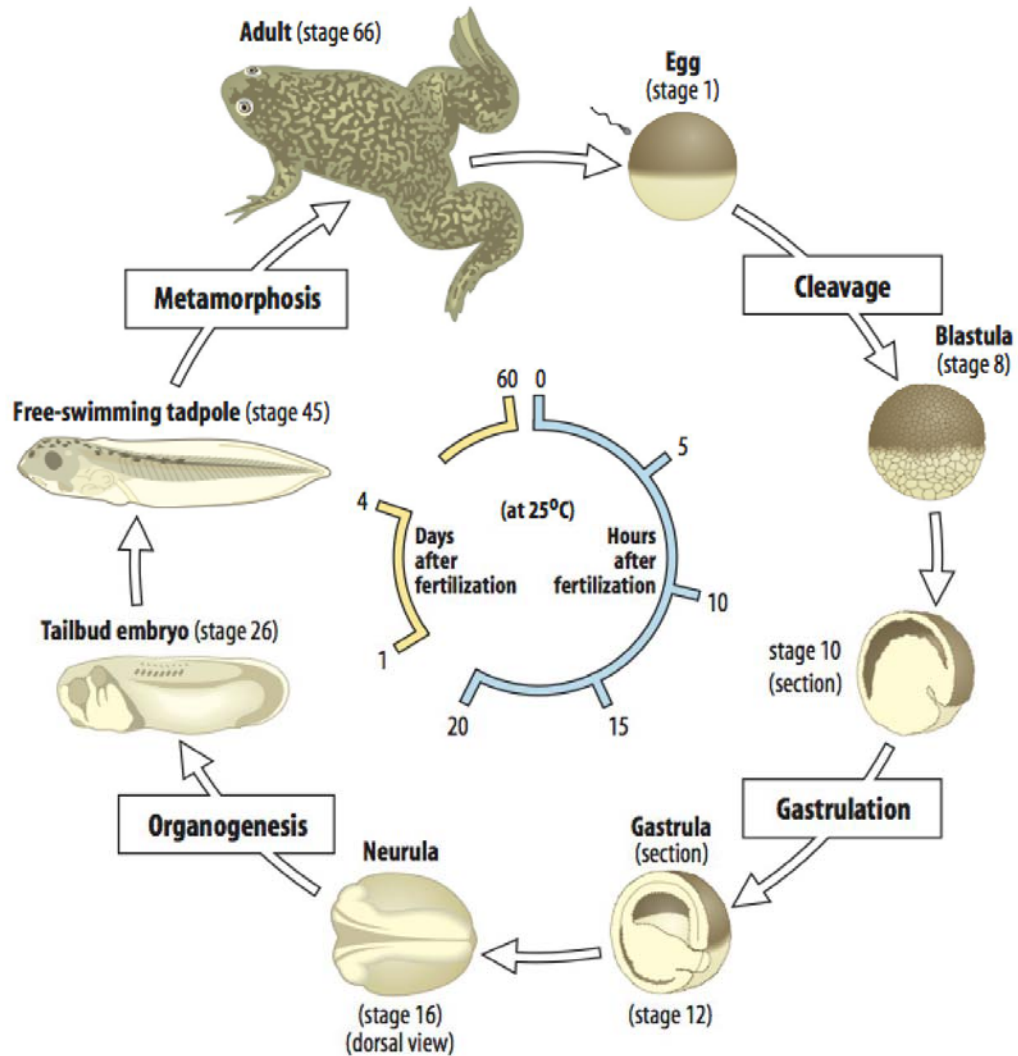Hair on *Drosophila* wing indicate cells' sense of direction

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).
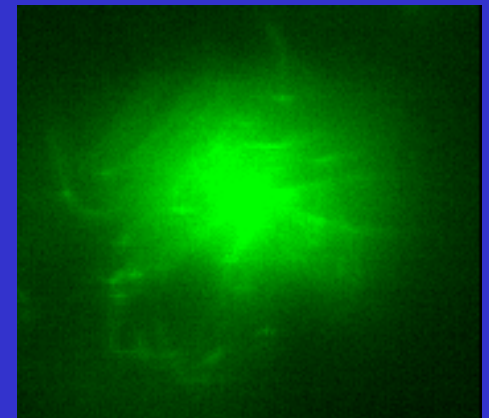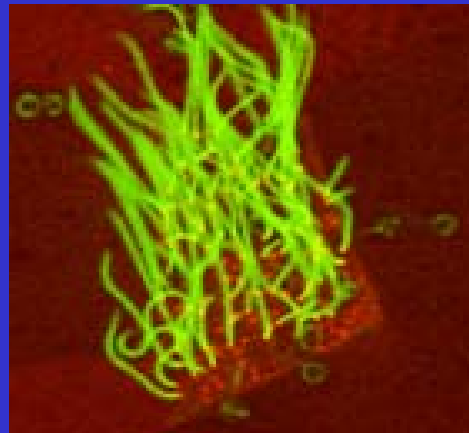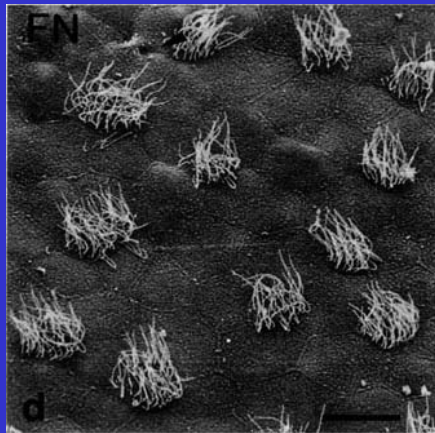
Xenopus Development
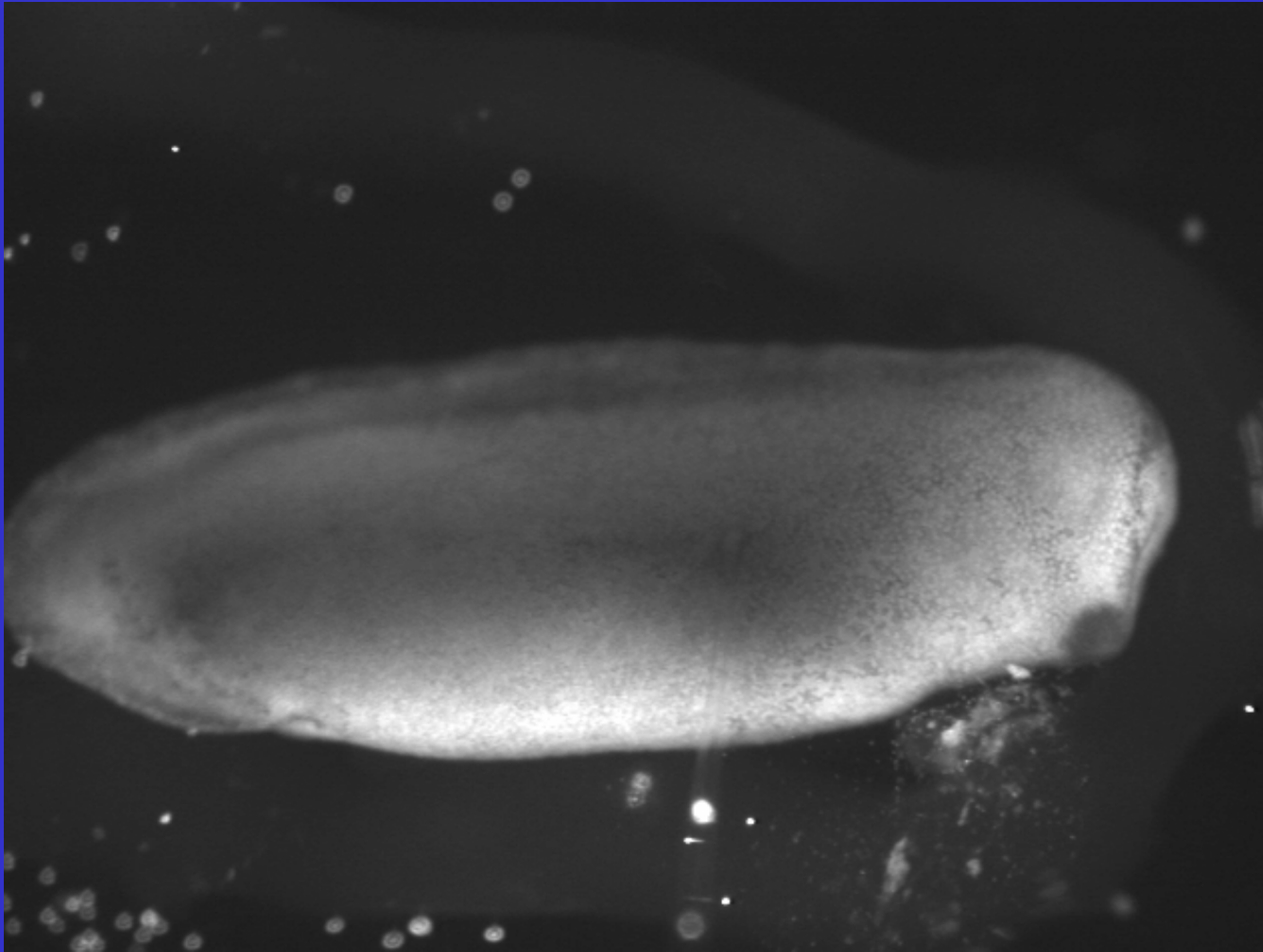
Ciliated Epithelia on *Xenopus* Embryo

Head

Tail

Ciliated Epithelia on *Xenopus* Embryo

Head

Tail

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)
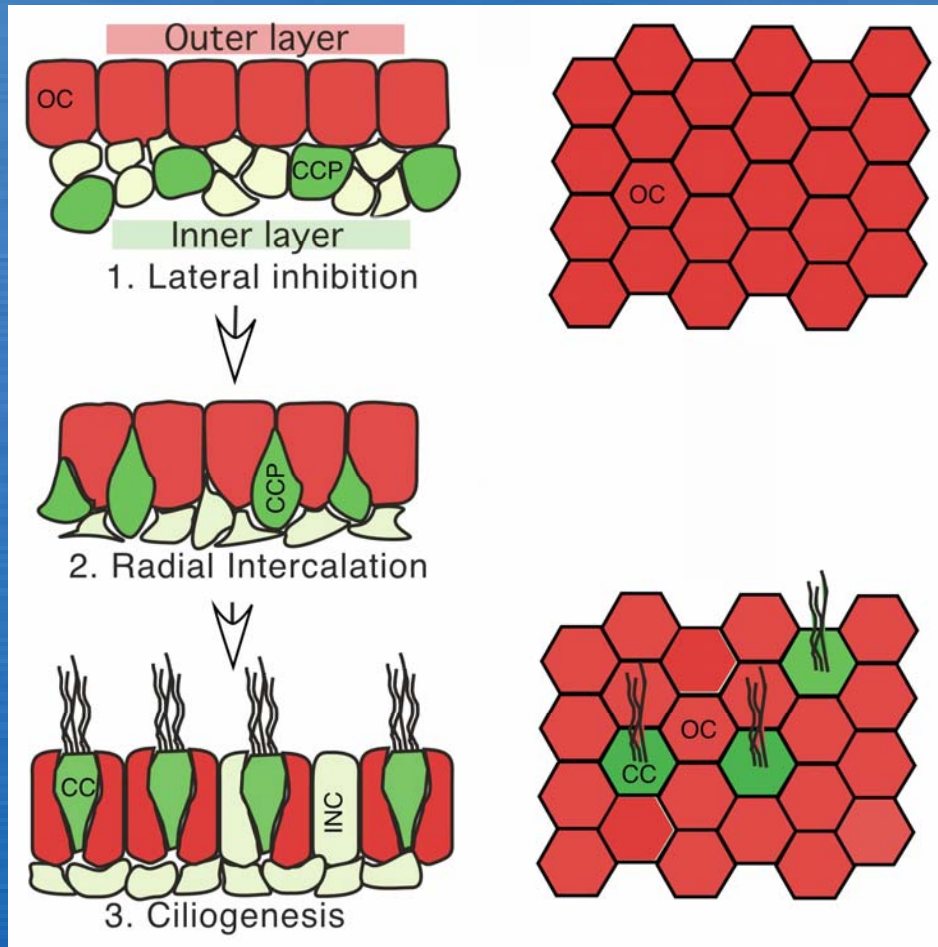
Xenopus Skin is comprised of three principle cell types with different functions
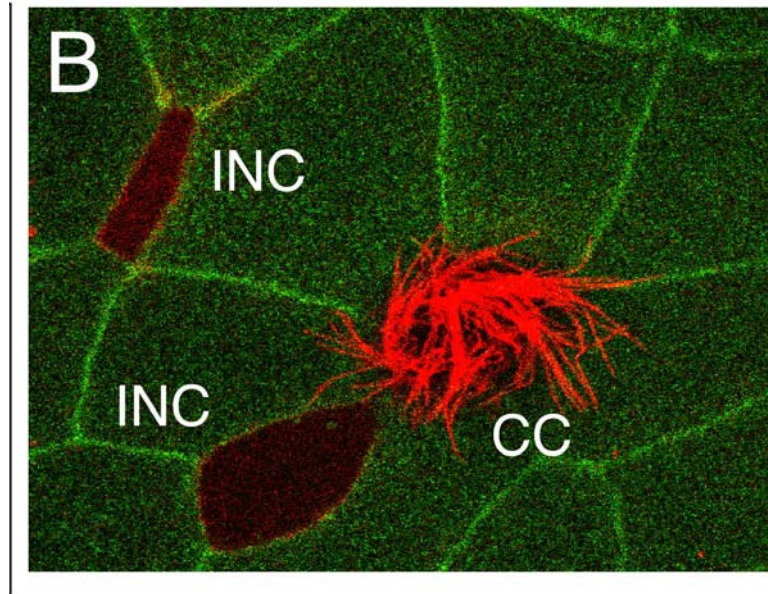

Skin contains at least three cell types

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

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

Ciliated Cell Development

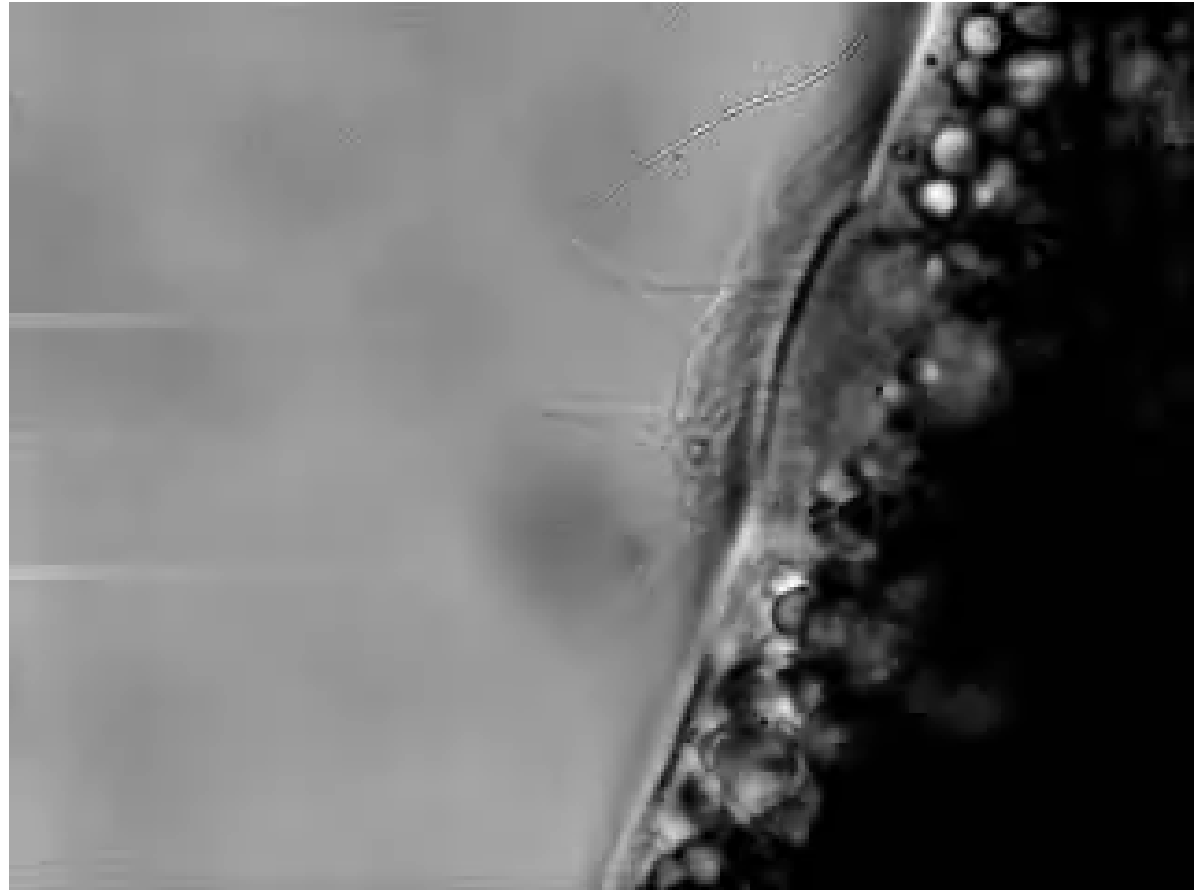# Basal Body orientation dictates direction of ciliary flow

Axoneme

Out

In

Basal body

Basal Foot

Rootlet
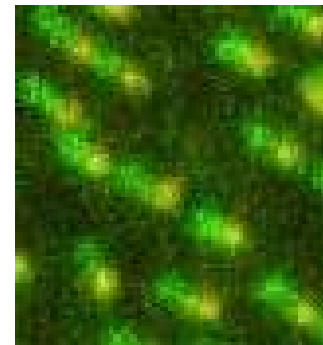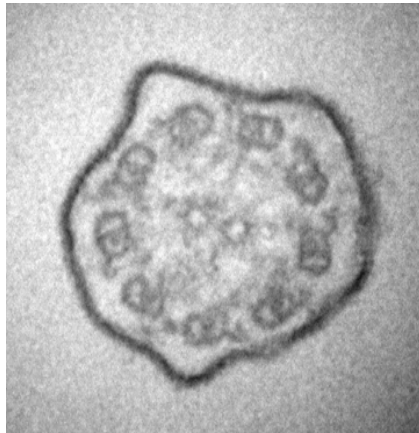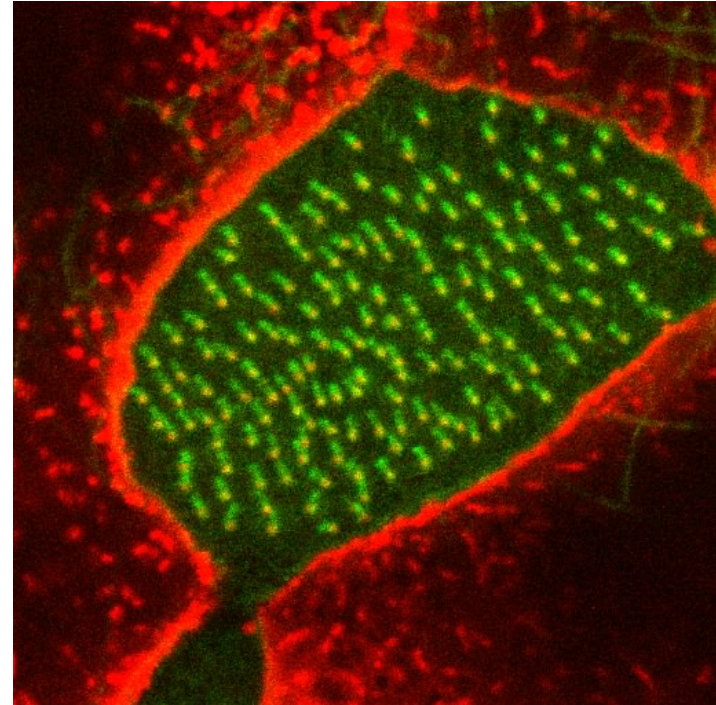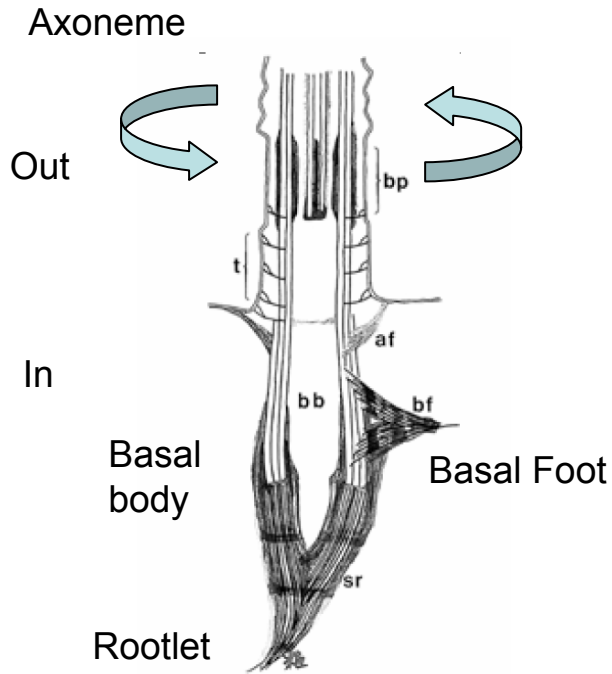
Side View of a Cell with Beating Cilia

• Cilia on a cell like a forest of trees

Cilia:

~ 15 μm long
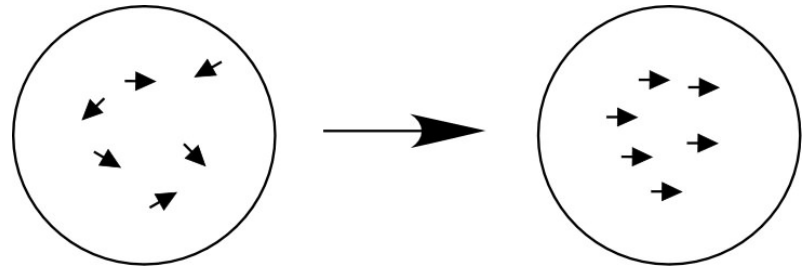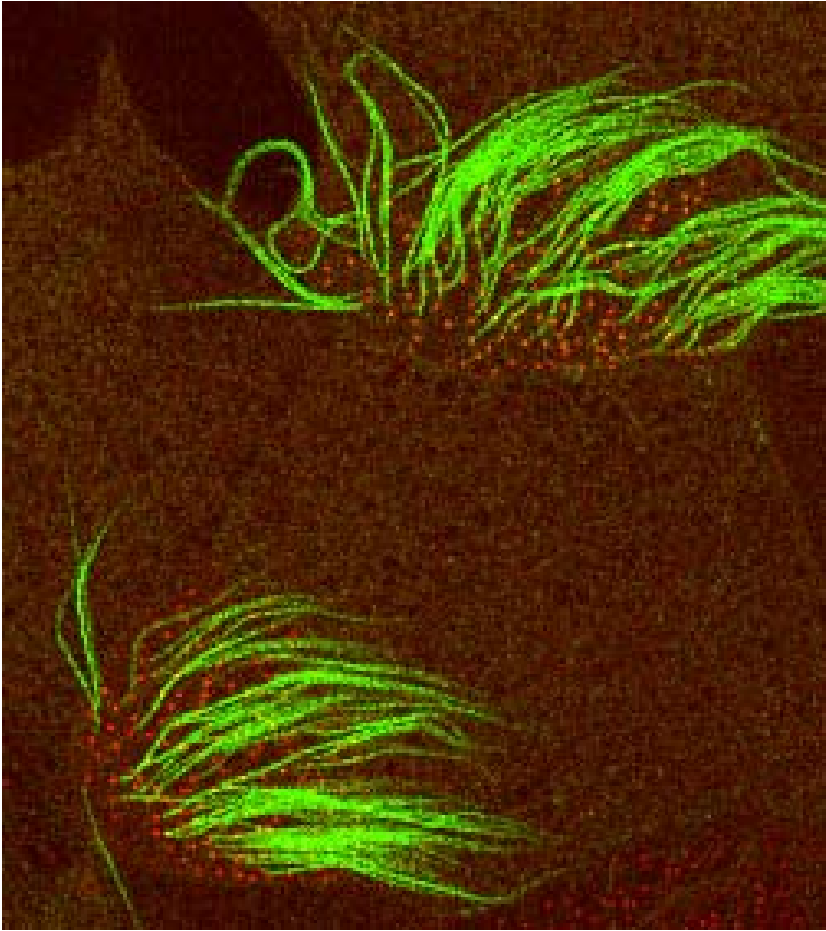
~ 1 μm apart

~ 20 beats/second

~ 250 nm diameter

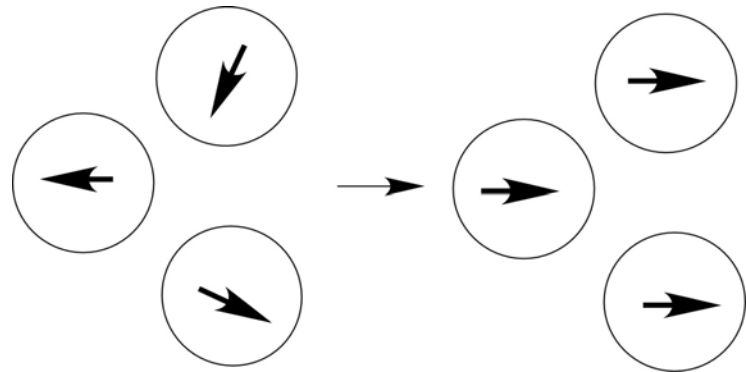How do cilia keep from crashing into each other?

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:

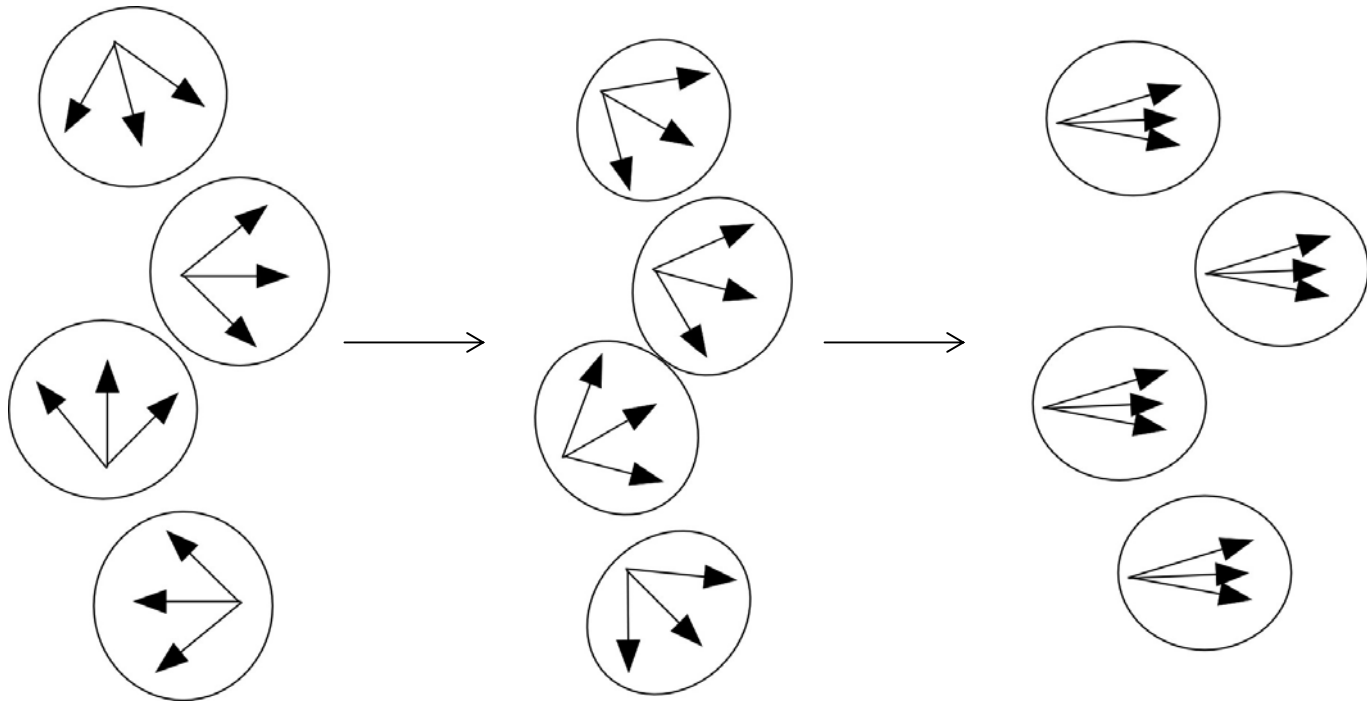What is the interplay between signaling and fluid flow at various stages of development?

Core components of the PCP Signaling pathway in *Xenopus*
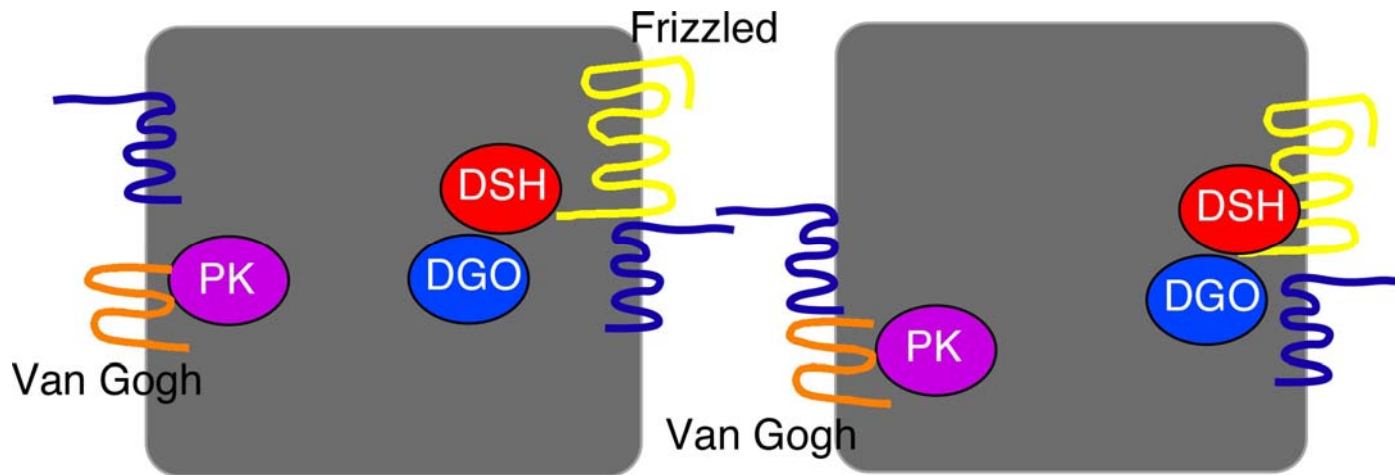
- PK = prickled
- DGO = Diego
- DSH = Dishevelled
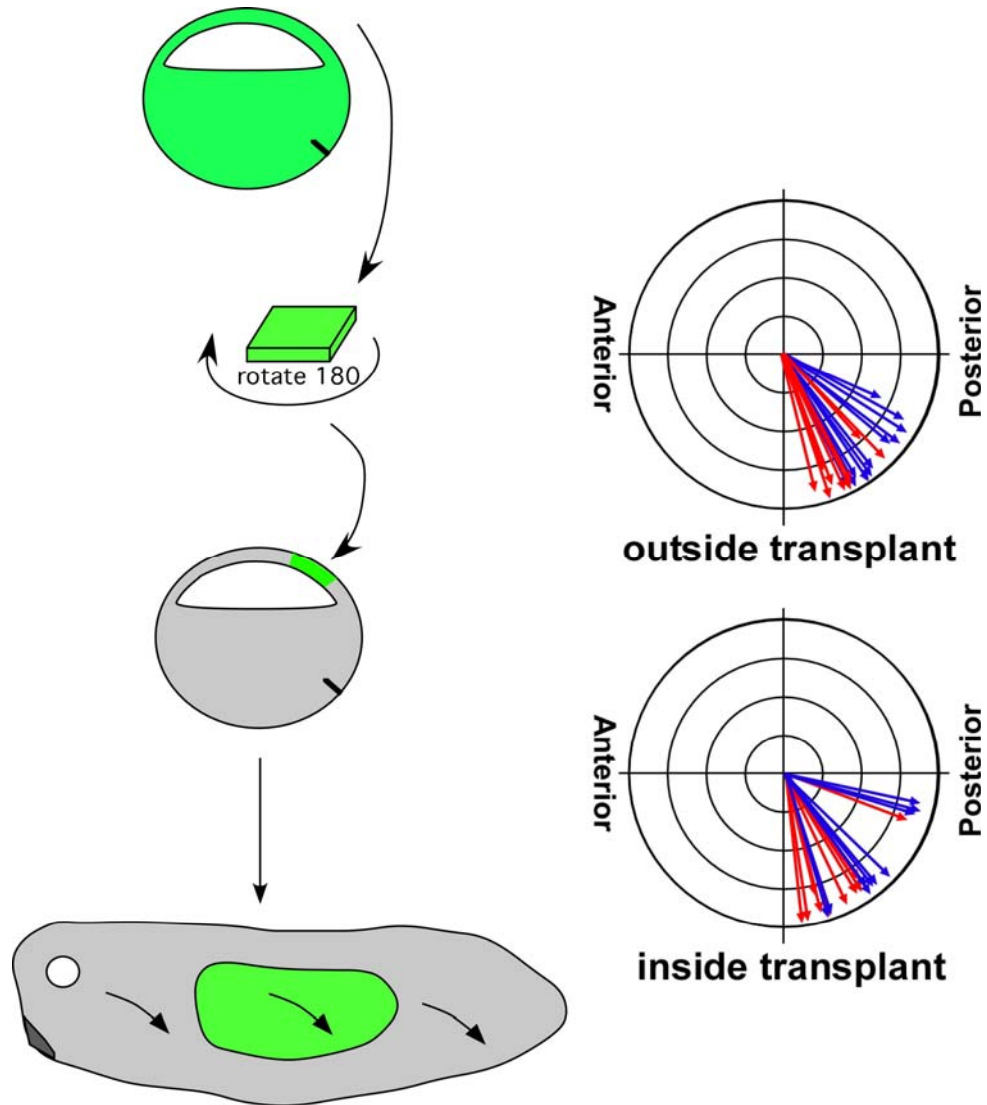- Van Gogh = Strabismus

Patterning　　　　　　Refinement

1. Polarity is acquired gradually
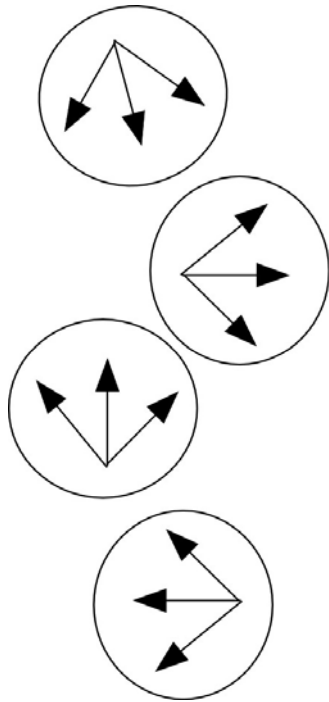   - Polarity of skin not fixed before gastrulation

(Mitchell *et al*. Nature 2007, *Curr. Bio.* 2009)

Polarity of skin is not fixed before gastrulation

Mitchell *et al. Nature* 2007, *Curr. Bio.* 2009

Patterning   Refinement

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

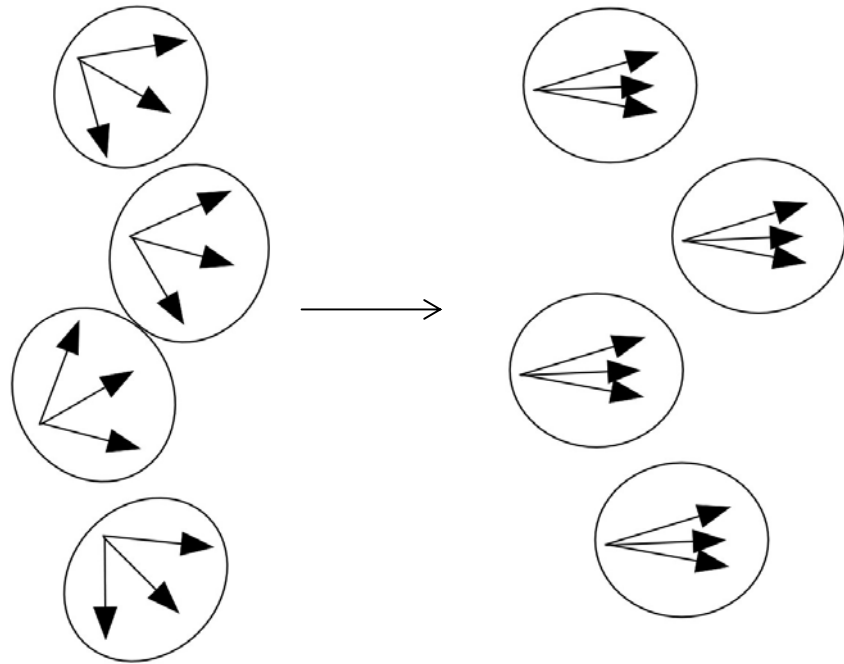(Mitchell *et al.* Nature 2007, Curr. Bio. 2009)

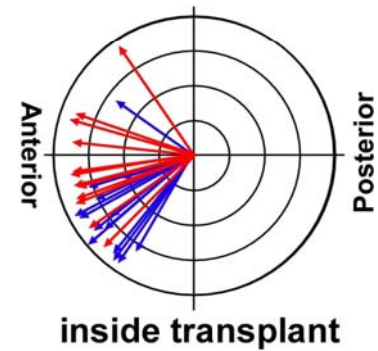Mitchell *et al., Nature* 2007, *Curr. Bio.* 2009.
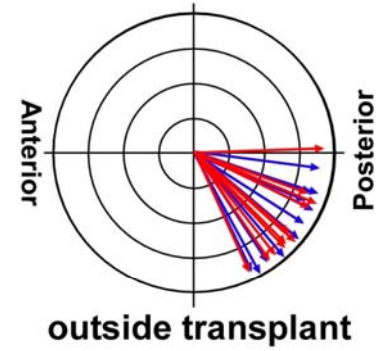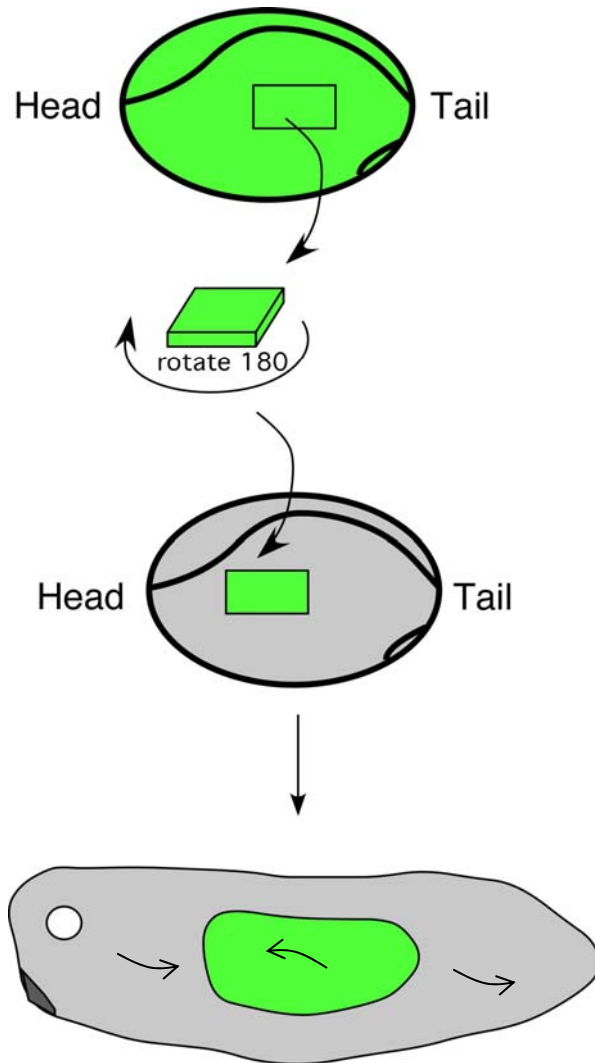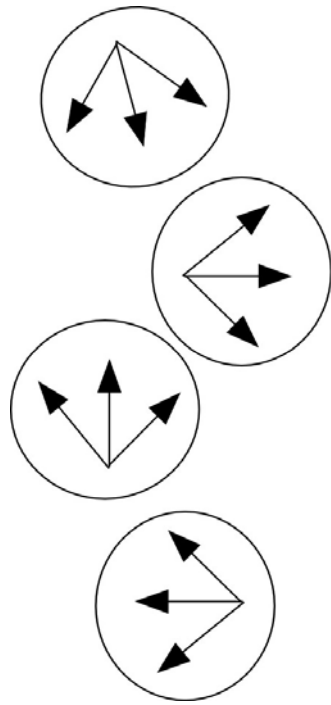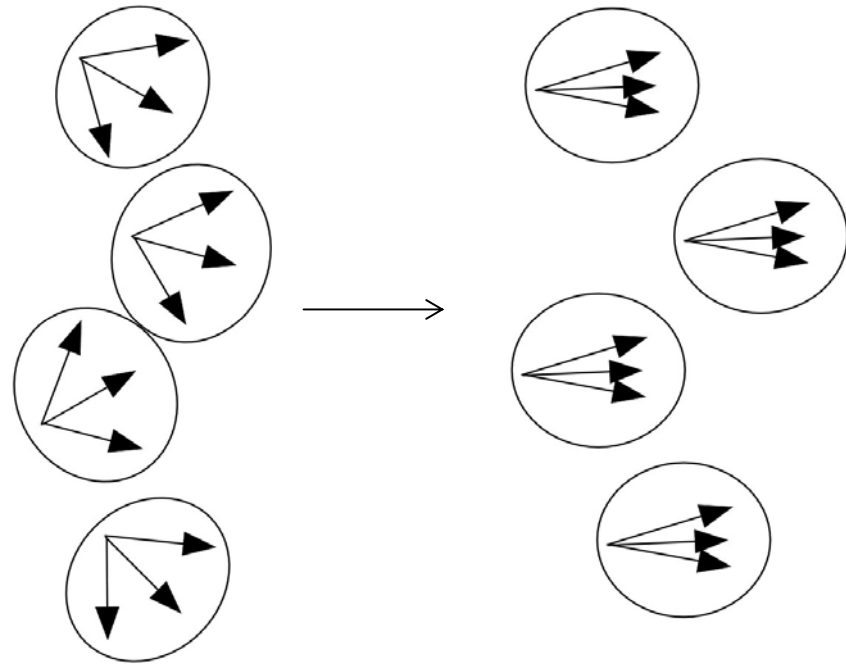
Patterning   Refinement

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
   (Mitchell *et al*. Nature 2007, Curr. Bio. 2009)

Ciliagenesis in *Xenopus* Embryo Explant

- High speed video allows viewing of live cell
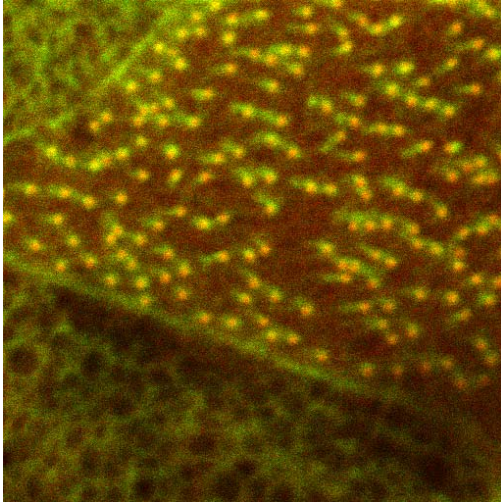 - Explore cilia motion at various growth stages

Core components of the PCP Signaling pathway in *Xenopus*
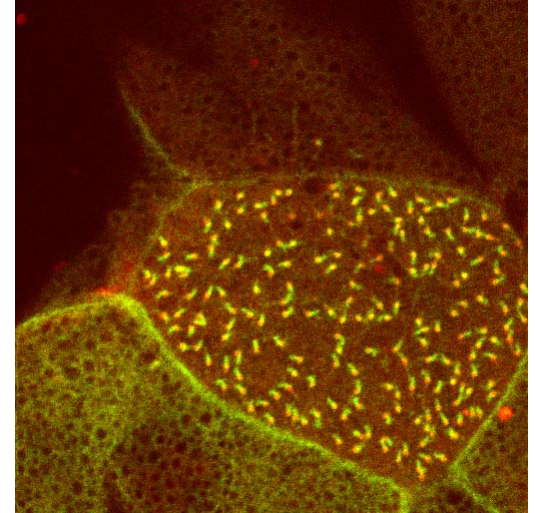

- PK = prickled
- DGO = Diego
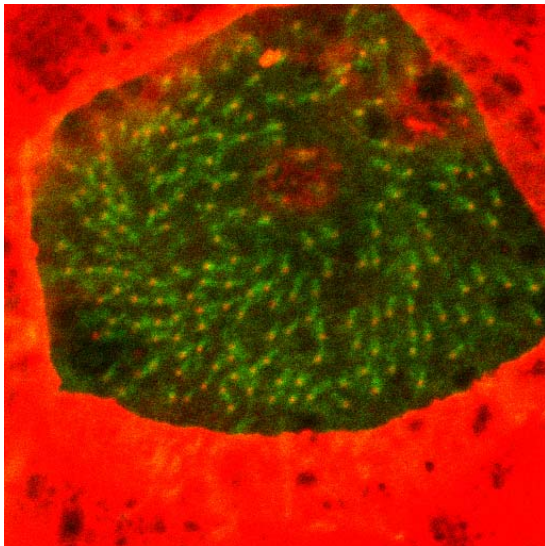- DSH = Dishevelled
- Van Gogh = Strabismus

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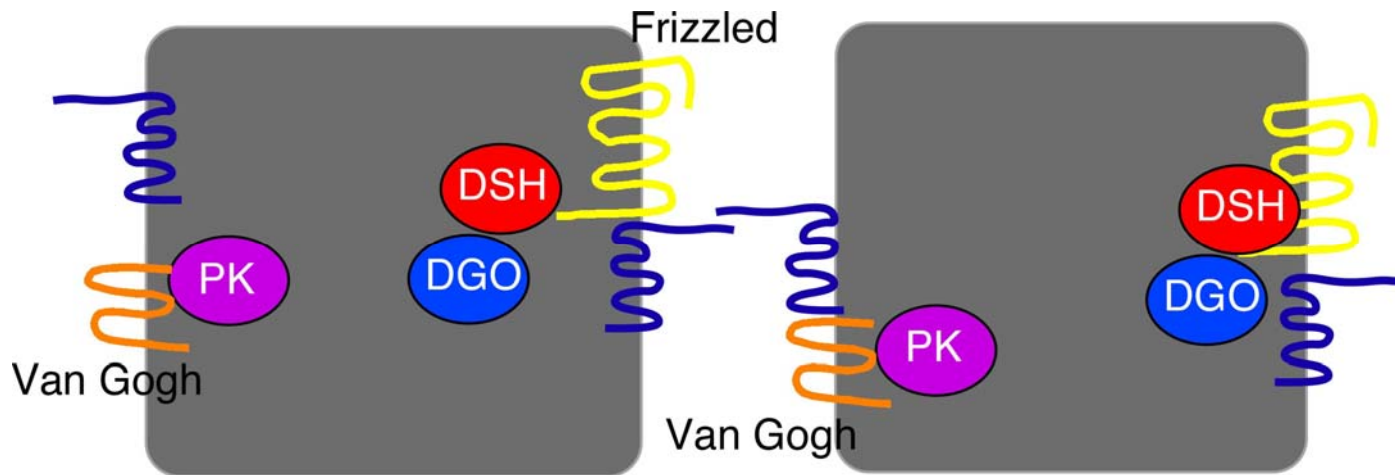
Mutations produce disoriented cilia.

Control (WT)

Random

Swirl

Inward
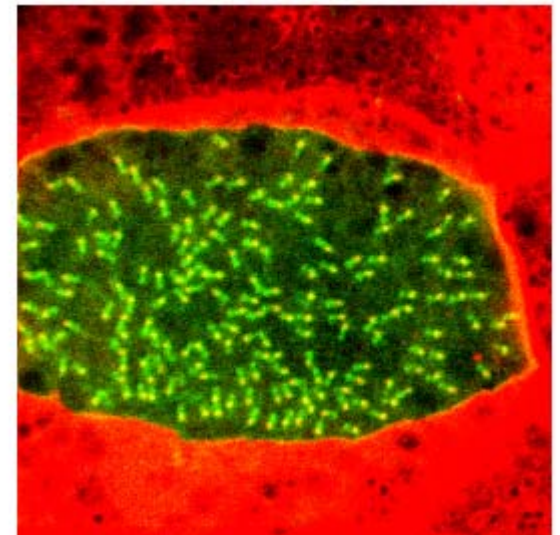
Analogy to Spin Systems

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:
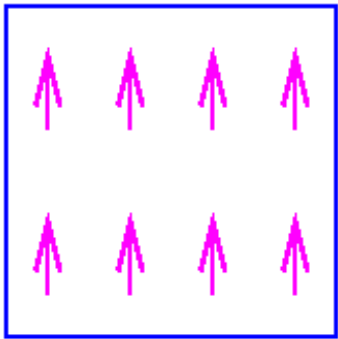
Ferromagnet
J > 0
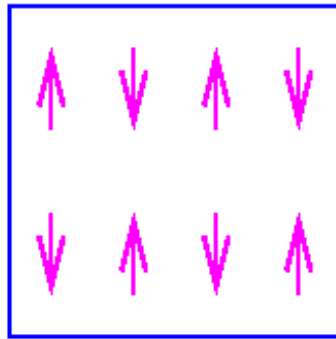
Antiferromagnet
J < 0

Spin Glass
random J_{ij}

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.

$$H = -\sum_{i>j} J_{ij} \vec{S}_i \Box \vec{S}_j - \sum_i \vec{h}_{tot} \Box \vec{S}_i - \sum_i J_{bndy} \vec{S}_i \Box \vec{B}_i$$
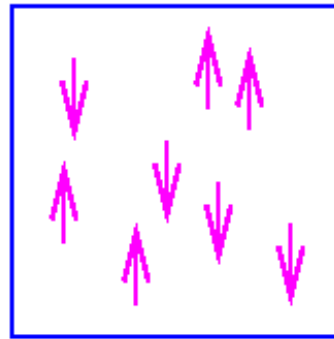
$$\vec{h}_i = \vec{h}_{flow} + \vec{h}_{PCP}$$

- 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.
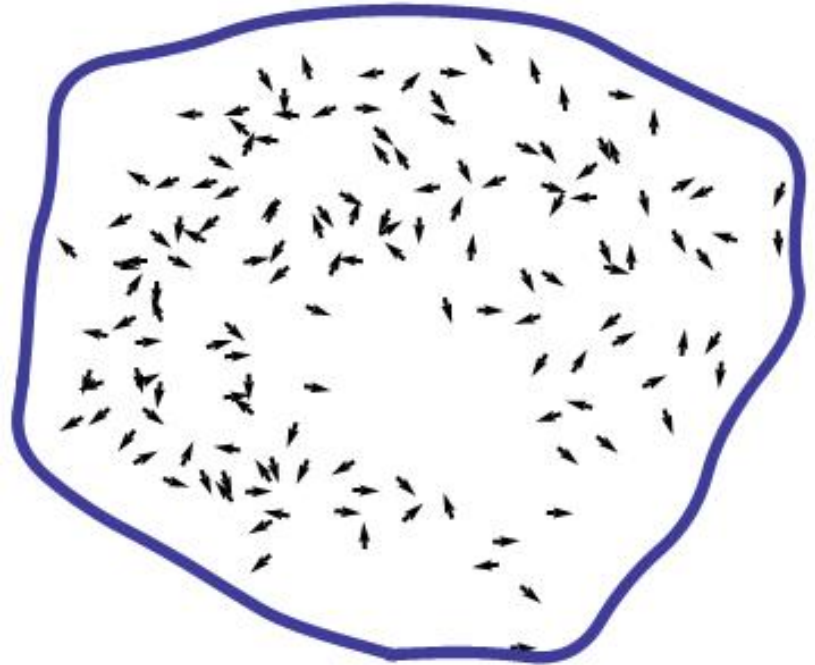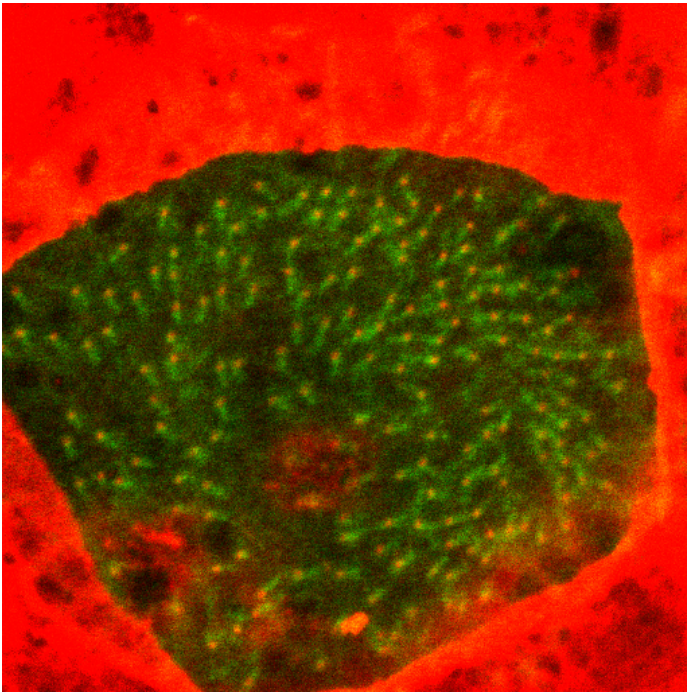
Summary

- 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

Mitchell *et al. Nature* 2007
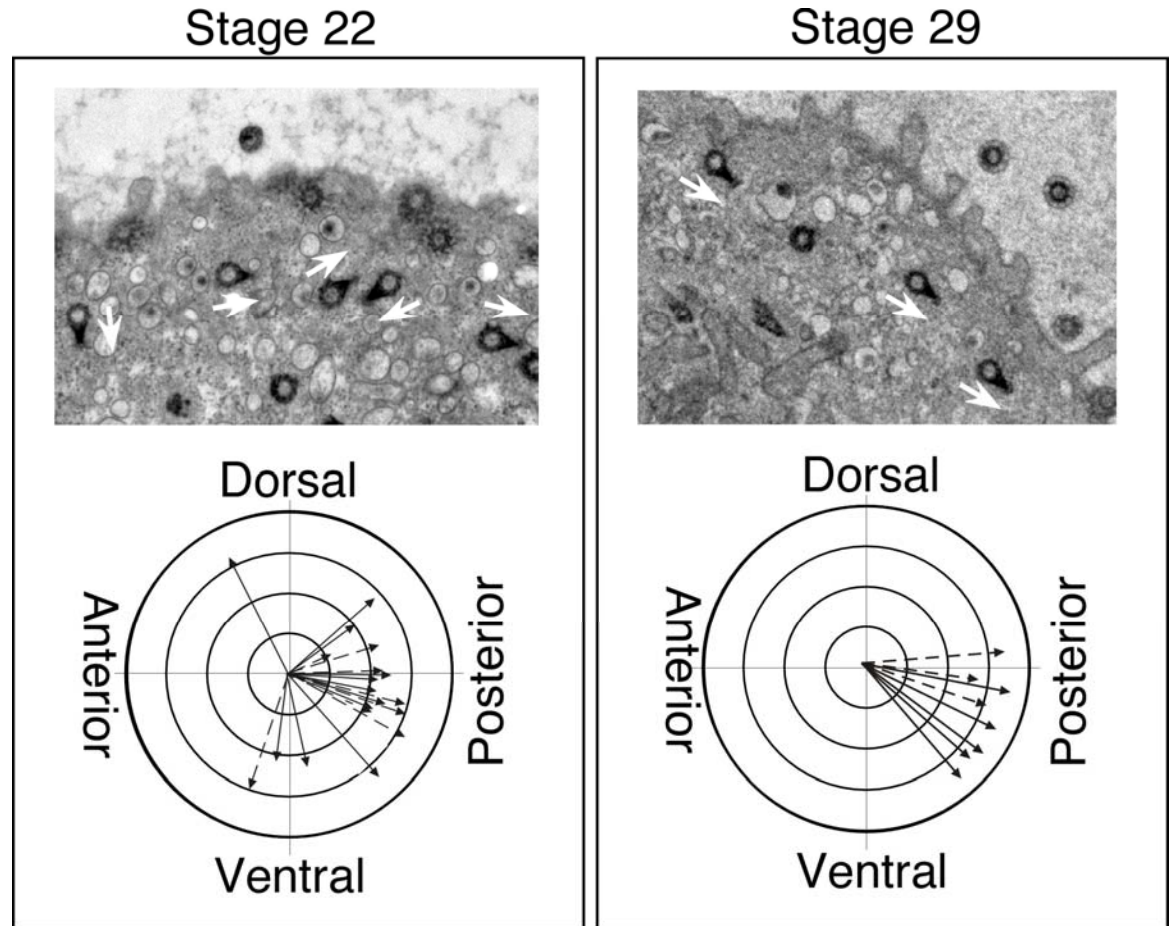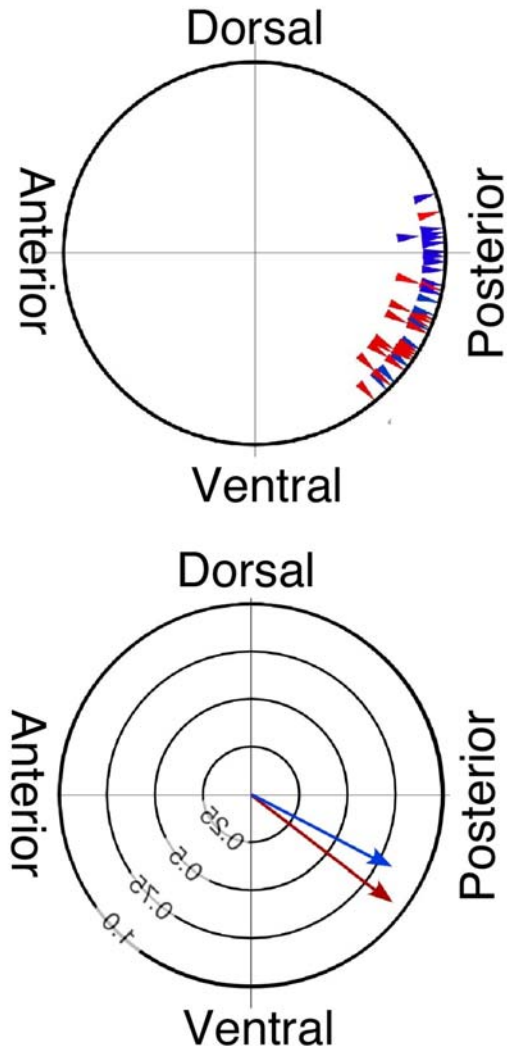
Immotile cilia in Xenopus causes cilia disorientation
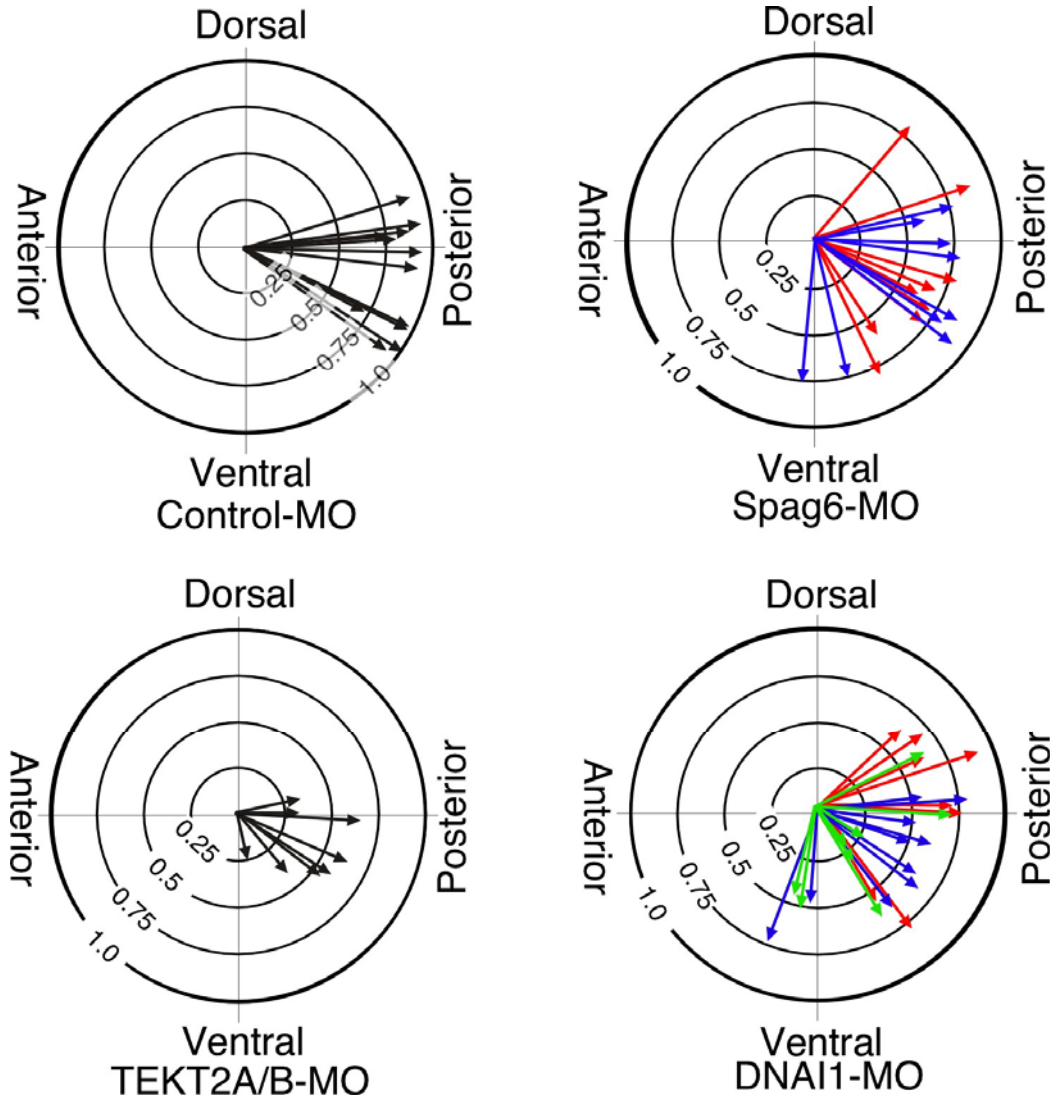
Monte Carlo Simulations of *Drosophila* Wing Disc

Julie Wortman

Clare Yu

Arthur Lander

University of California, Irvine

Life cycle of Drosophila

Adult fly

Fertilized egg

Metamorphosis

Cleavage

Syncytial blastoderm
stage 3–4

Pupa

9

0

60

120

180

Minutes after fertilization*

Days after fertilization*

Hours after fertilization*

10

24

Gastrulation

Larva

Embryo
stage 13

3rd instar

2nd instar

1st instar

Hatching
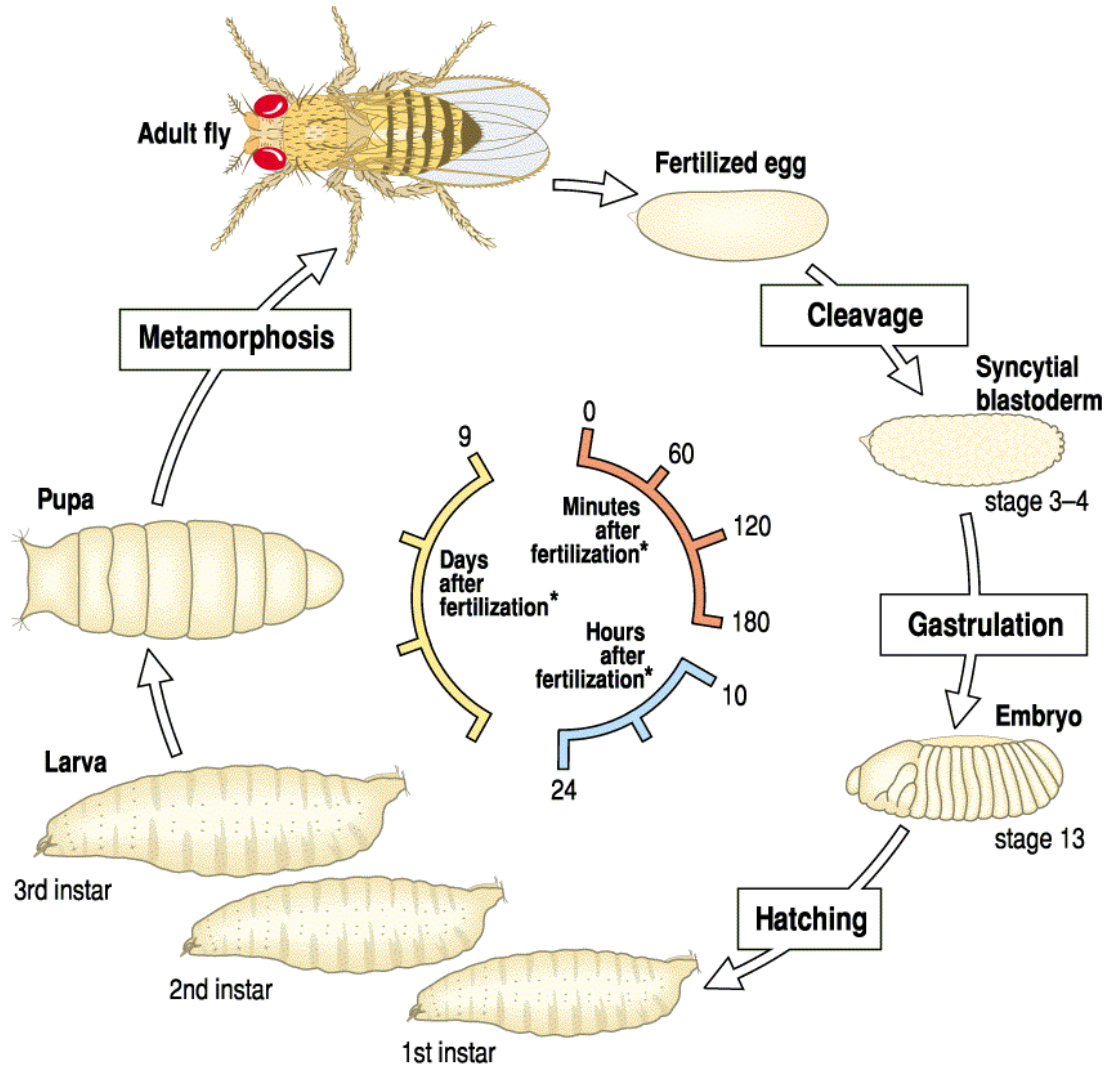
*At 25°C incubation

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
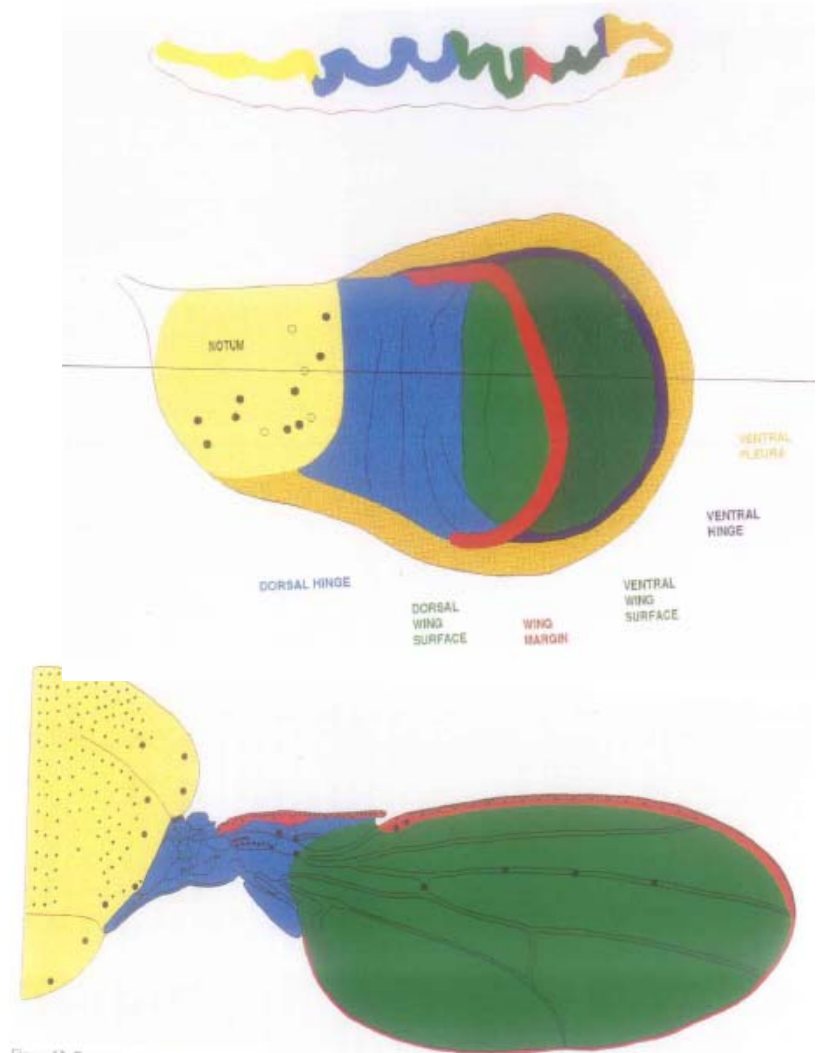

Figure 13. Fate map of the wing imaginal disc...
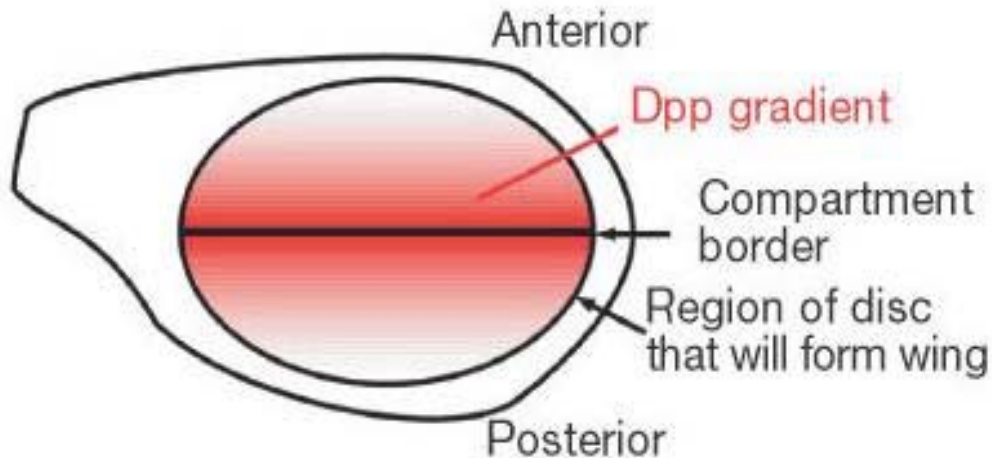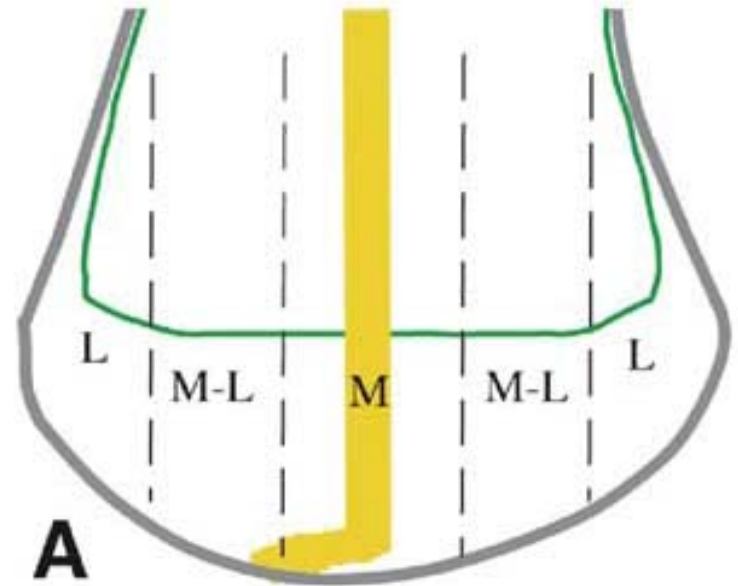
Campuzano and Modolell, 1992; Cohen 1993

Morphogen Gradients Produce Patterning

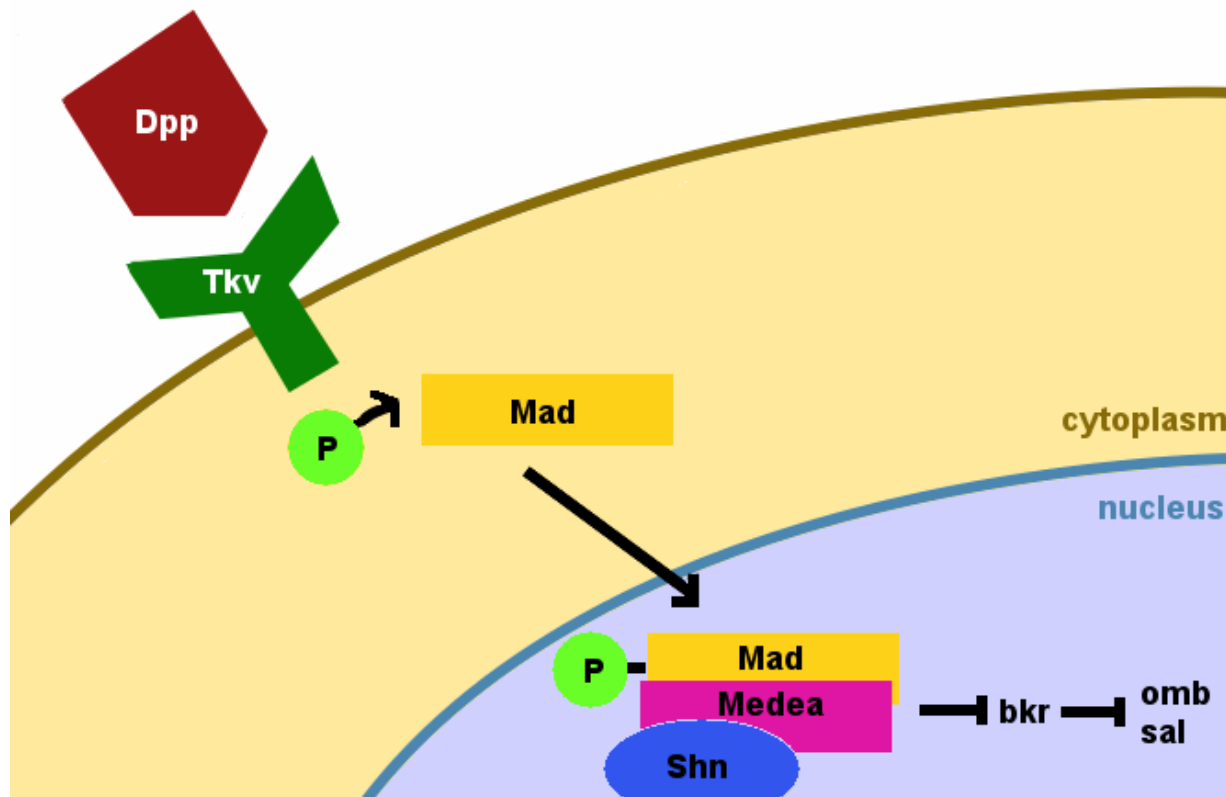

Gurdon and Bourillot, *Nature* (2001).

Rogulja and Irvine *Cell* (2005).

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).
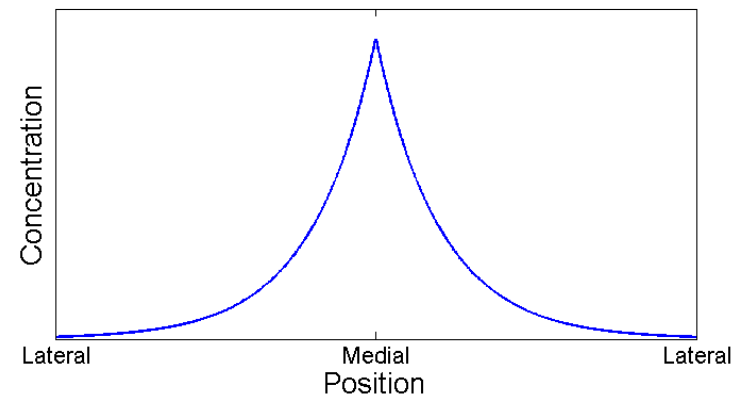
Wing Disc Grows Uniformly

- Dpp necessary for growth of wing disc

- Dpp concentration is exponentially decaying gradient

- Wing disc grows uniformly
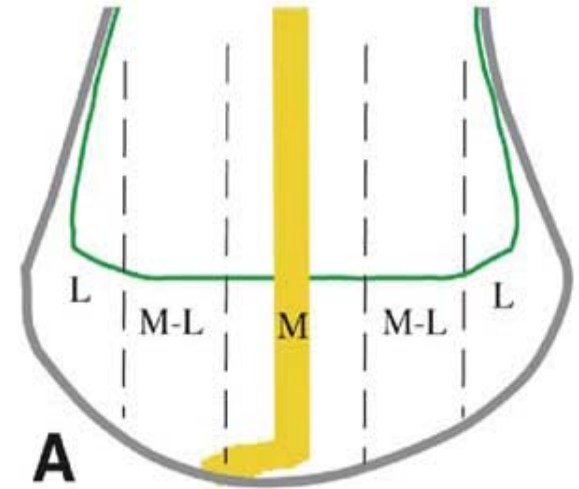
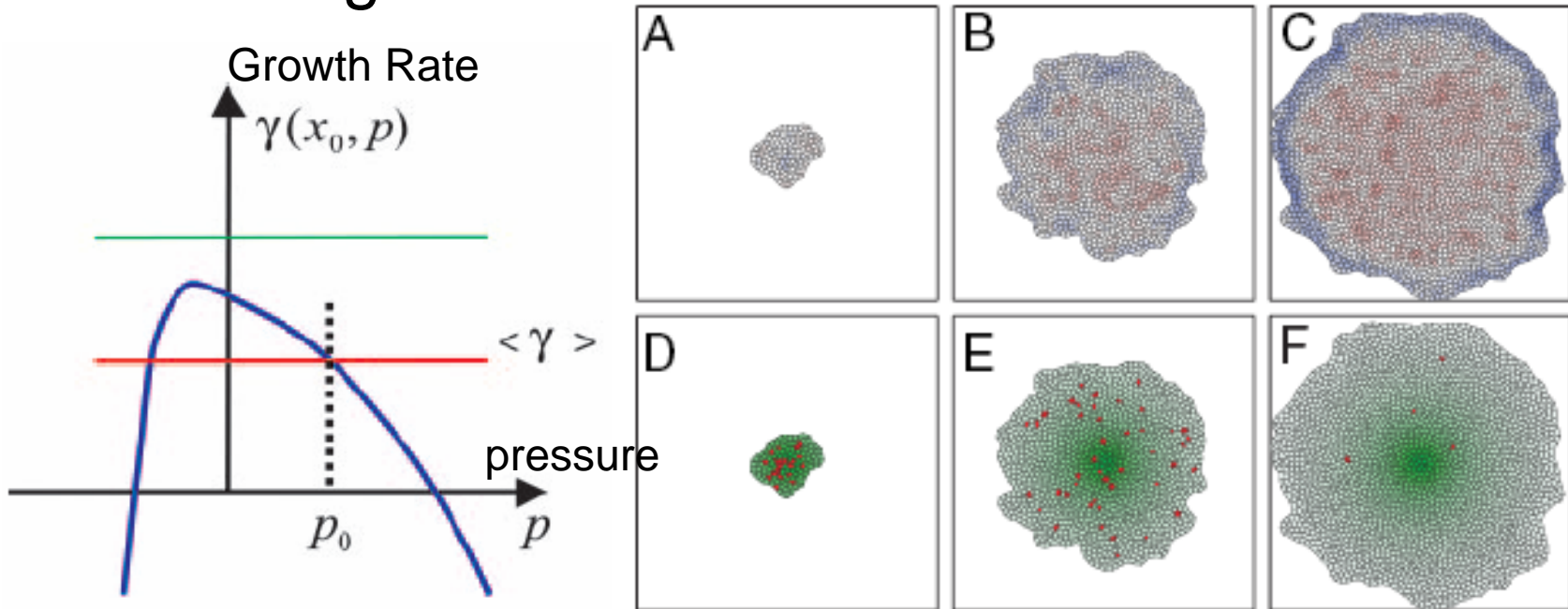Rogulja and Irvine *Cell* (2005).

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

| Number of Nearest Neighbors	Fraction of Cells
5	~ 29%
6	~ 46%
7	~ 21%

•Geometric arguments
•Cell division
•No cell rearrangement
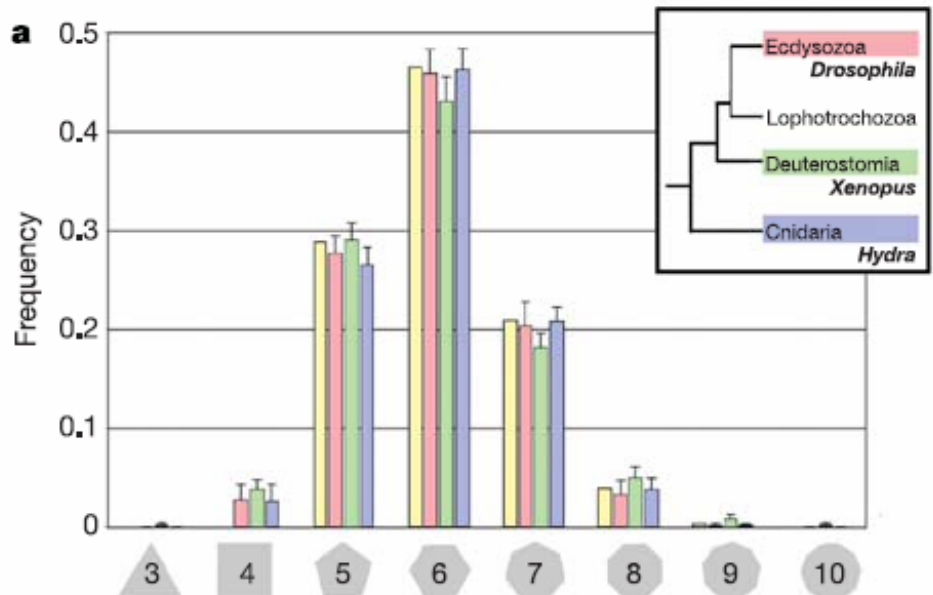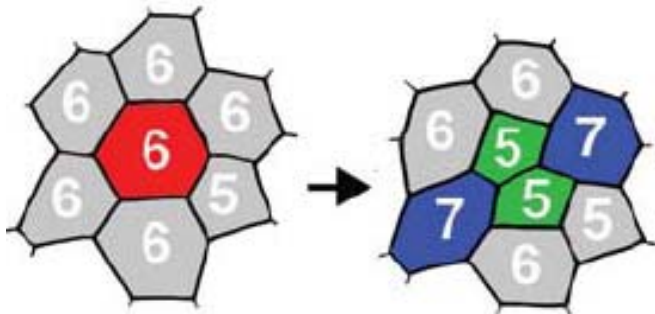
Gibson *et al.*, *Nature* (2006); Farhadifar *et al.*, Curr. Bio. (2007).

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
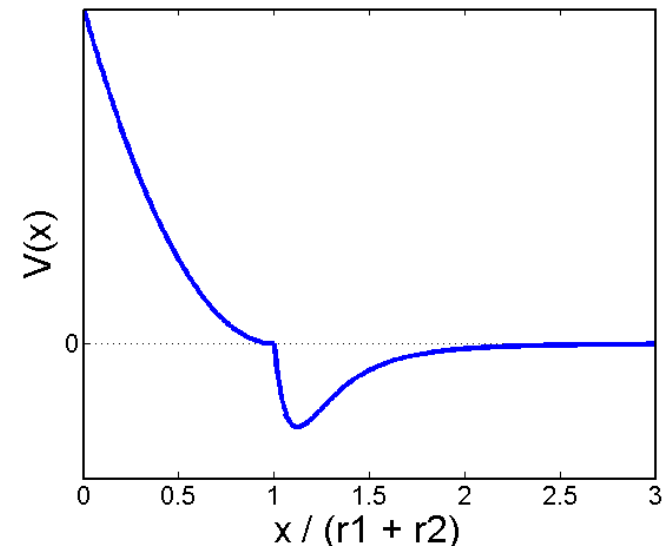- Cells can divide
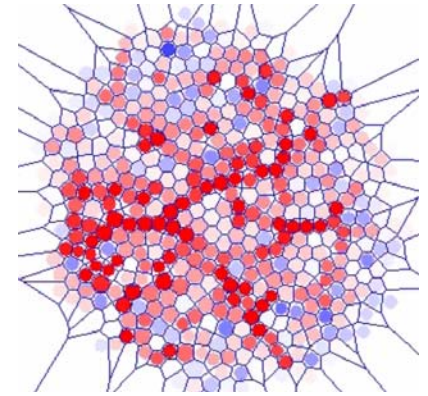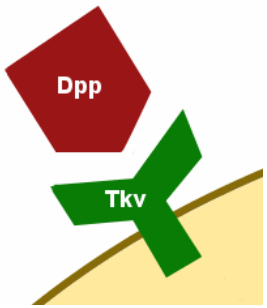
Elastic Interaction Between Adjacent Cells

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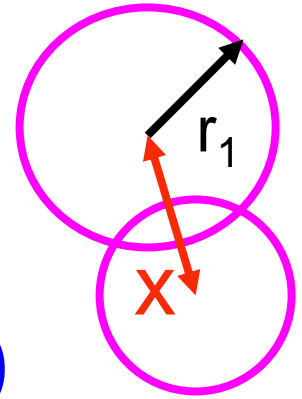
$$V(x) = \begin{cases} k\left[x - (r_1 + r_2)\right]^2 & \text{if } x < (r_1 + r_2) \\ \varepsilon\left[\left(\dfrac{r_1 + r_2}{x}\right)^{12} - \left(\dfrac{r_1 + r_2}{x}\right)^{6}\right] & \text{if } x > (r_1 + r_2) \end{cases}$$

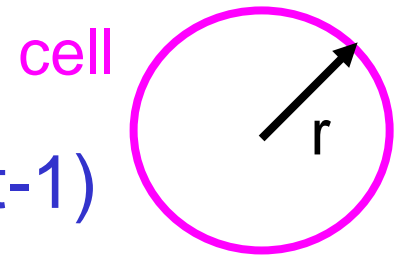Compression or tension from neighbors affects a cell's growth.
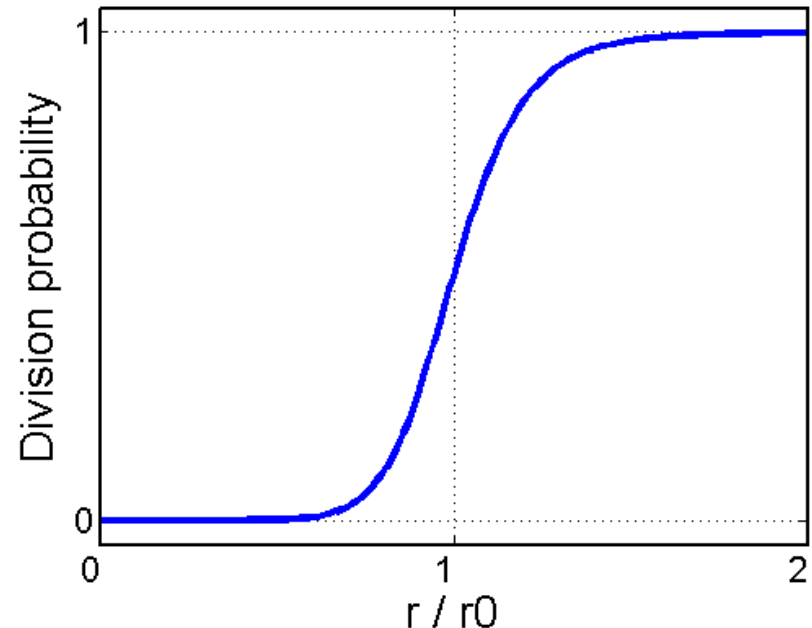
Dpp Concentration Affects Growth

Let B = # bound receptors on a cell

- Linear growth: radius r(t) = r(t-1) + a·B
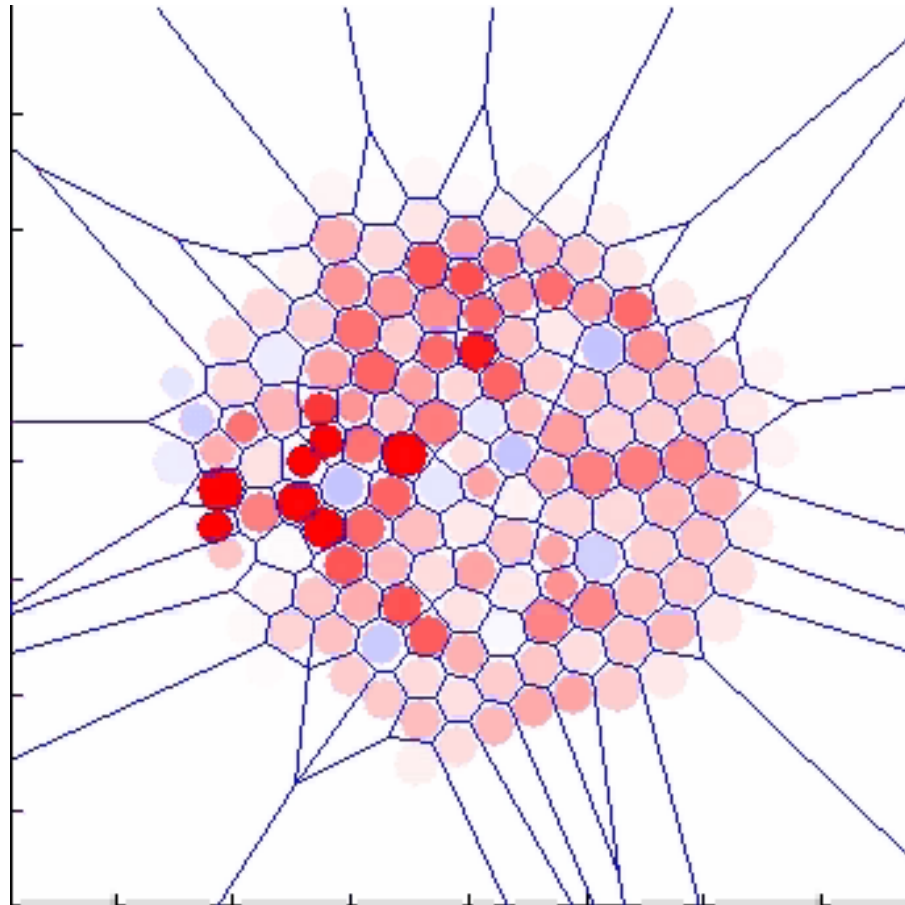- Exponential growth: radius r(t) = b·B·r(t-1)

cell

A Cell's Probability to Divide Increases with Size

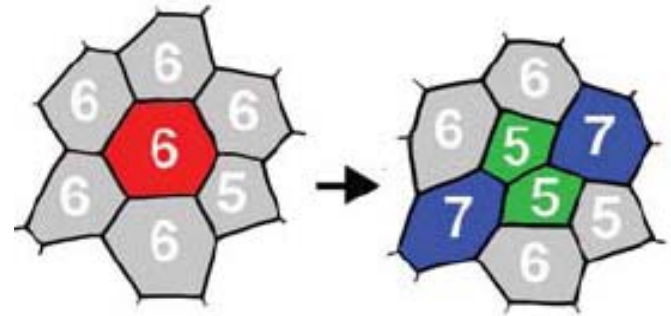$$P = 1 - \frac{1}{1 + \left(\dfrac{r}{r_0}\right)^n}$$

Movies of Tissue Growth

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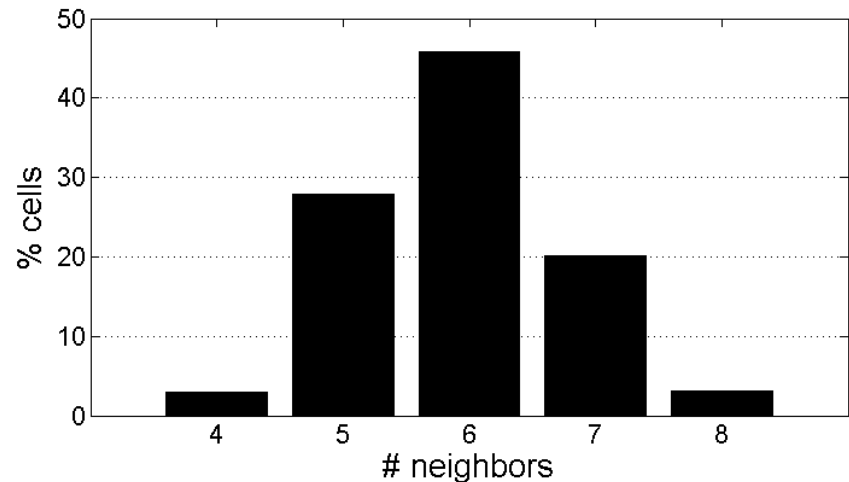
Cell Packing Statistics

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:

# Neighbors	Fraction of Cells
5	~ 28%
6	~ 46%
7	~ 20%

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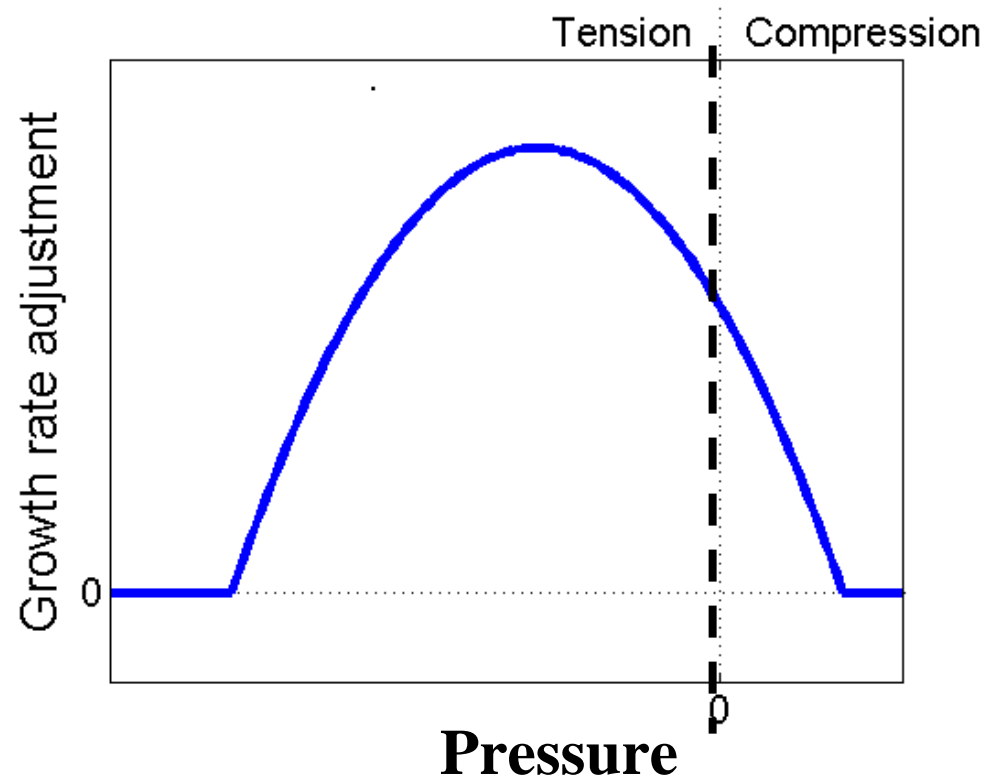
How is cell growth affected by elastic stress?

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)
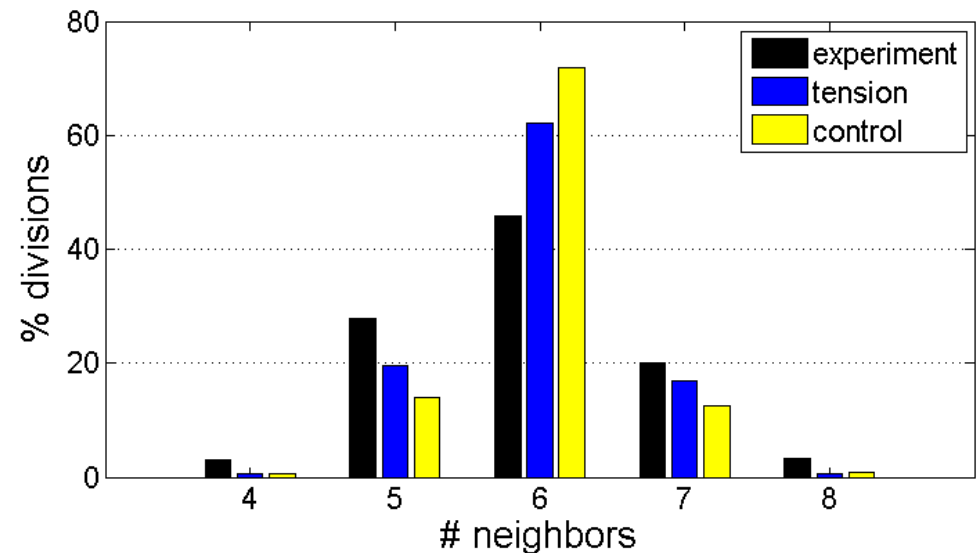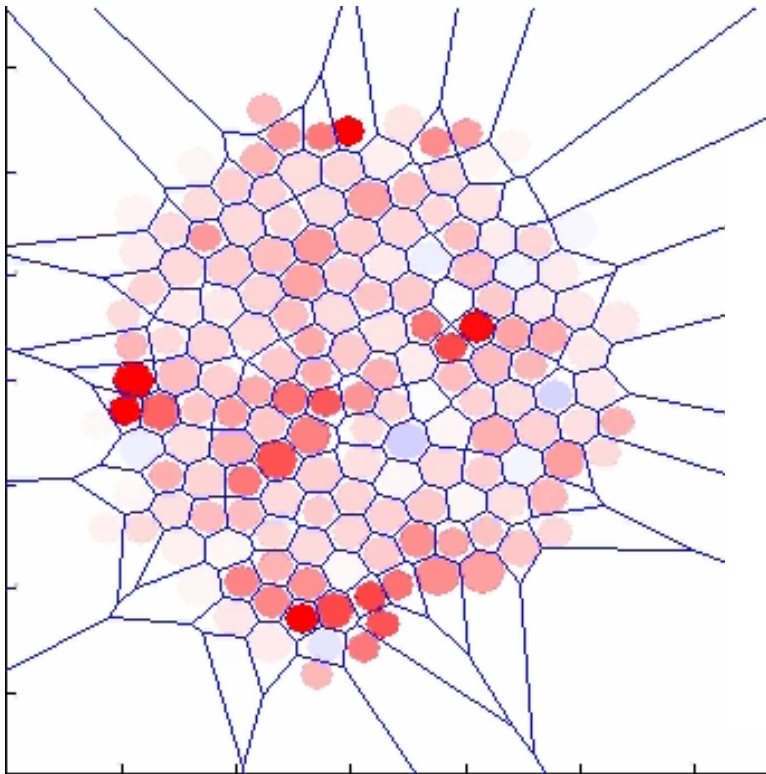
If Compressed Cells Grow Faster

- 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)
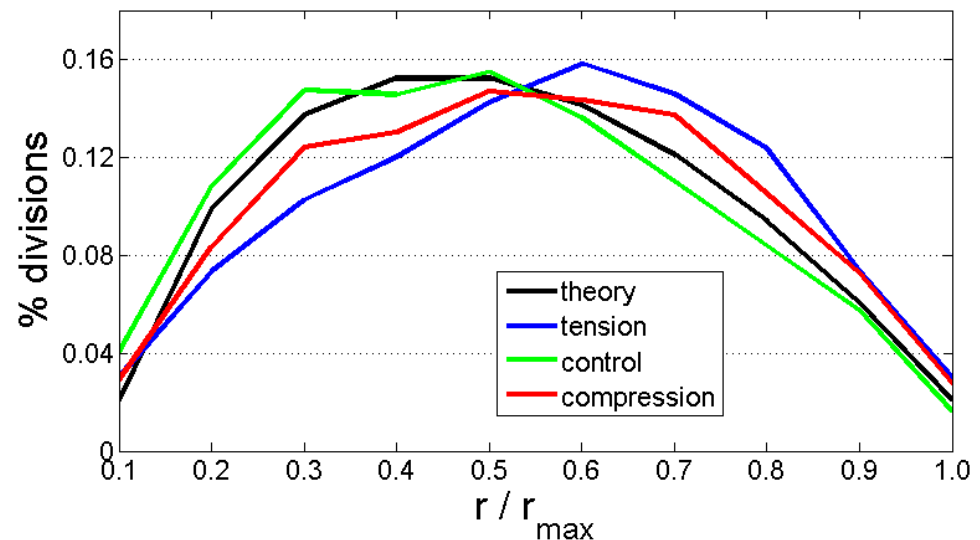- Better agreement with cell packing seen experimentally
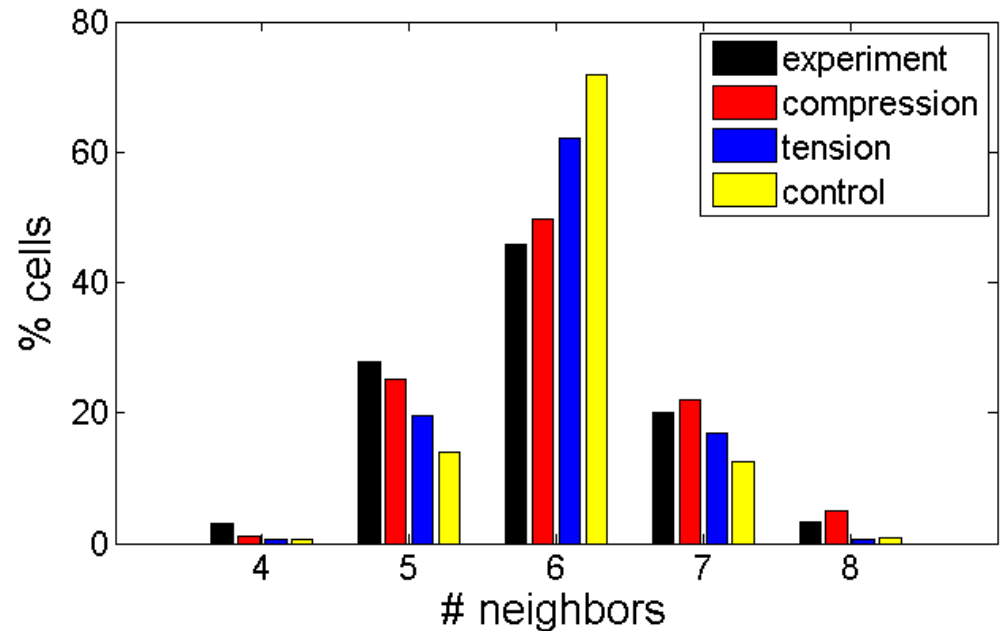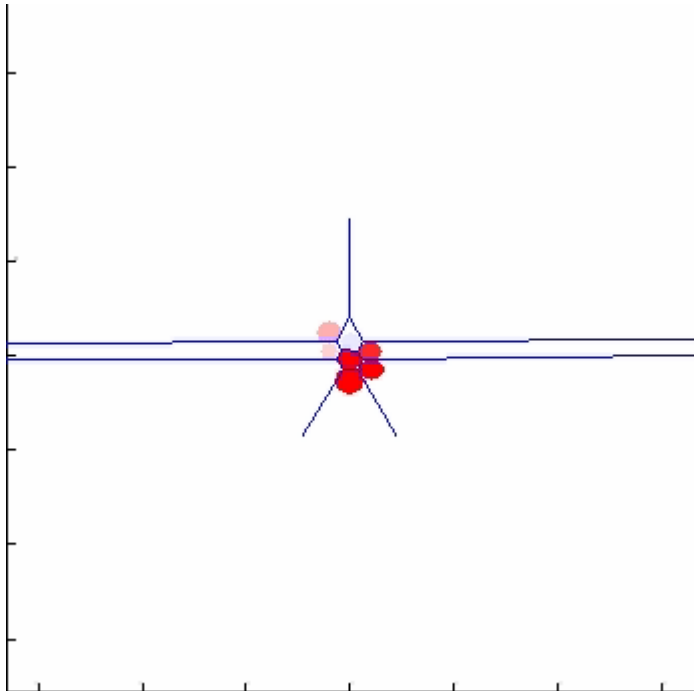
Is Tension Better for Uniform Growth?

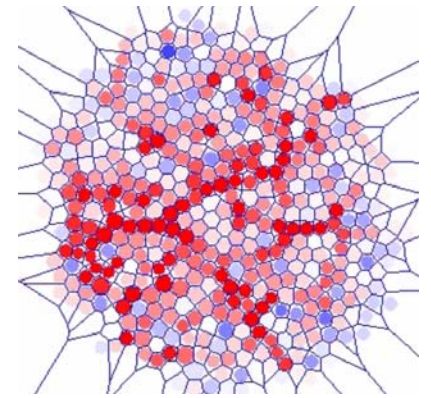If disc initially at radius r_0 grows uniformly to a final radius r_{max}, the number of divisions goes as
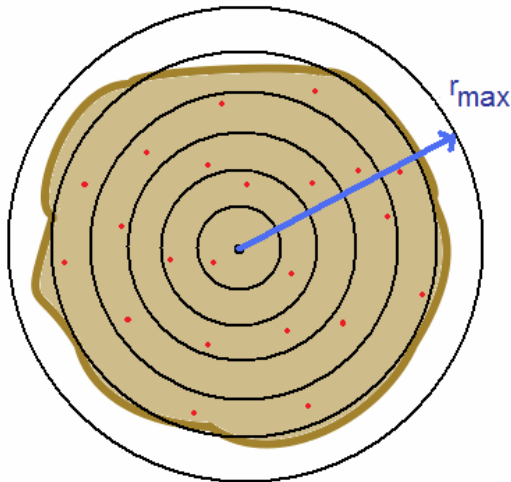
$$\text{\# Divisions} \propto \begin{cases} \ln\left(\dfrac{r_{max}}{r_0}\right) & \text{if } r \le r_0 \\[2em] \ln\left(\dfrac{r_{max}}{r}\right) & \text{if } r > r_0 \end{cases}$$

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