Remembering Which Way Is Up: Cell Polarization and Proliferation in the Developing Fruit Fly Wing

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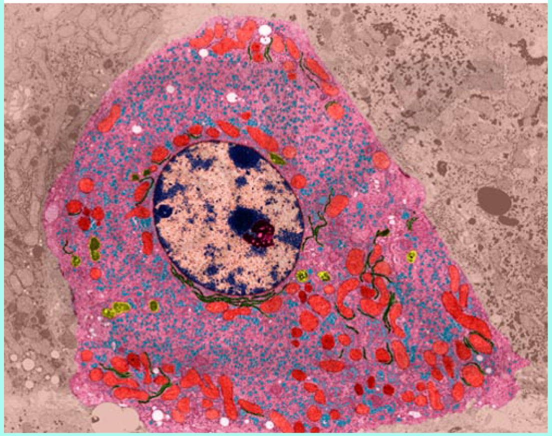
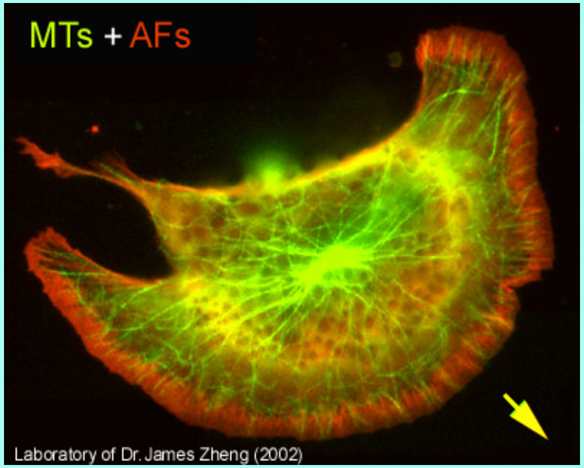
A Cell Is Like a City

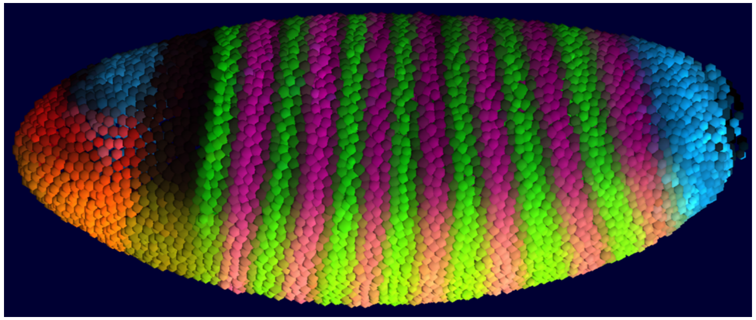
Stained Live Cell



• Workers	• Proteins
• Power Plant	• Mitochondria
• Roads	• Actin fibers, microtubules
• Trucks	• Kinesin, dynein, myosin
• Factories	• Ribosomes
• Library	• Genome
• Recycling center	• Lysosome
• Police	• Chaperones
• Post office	• Golgi apparatus
• Communications	• Chemical signaling networks



What Is Developmental Biology?

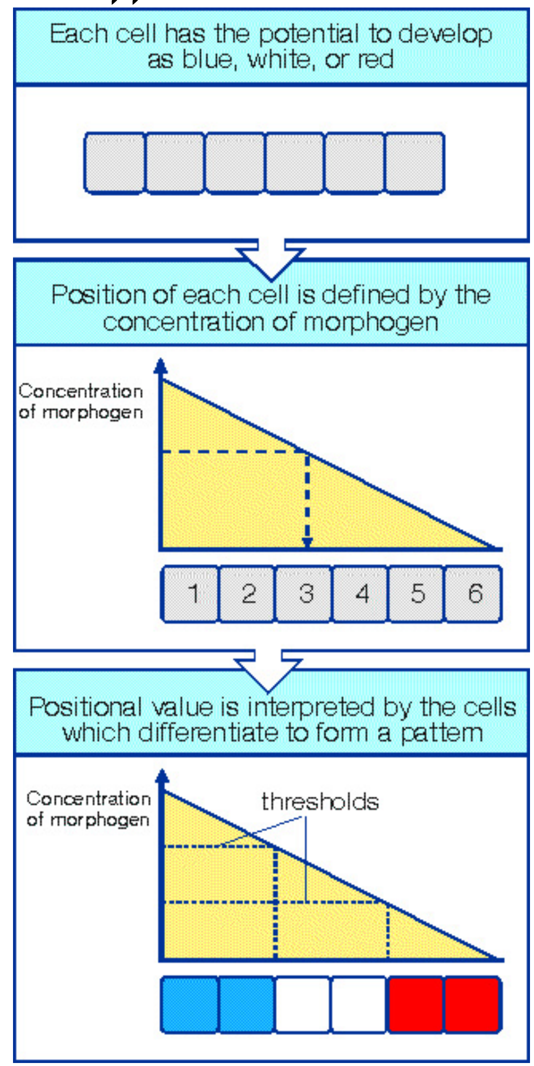


- Developmental biology is the study of the how a fertilized egg develops into a multicellular organism.
- Typical questions:
  - How do different kinds of cells arise?
  - How do cells self-organize into structures and organs like limbs, heart, brain, etc.? Where does this patterning come from?
  - How do cells acquire a sense of direction? How do they know which way is the head, tail, in, out, etc.? (Cell polarization)

French Flag Model of How Morphogens Produce Patterning

(Lewis Wolpert (1969))

- Morphogens are signaling molecules associated with growth and development.
- Concentration of a morphogen determines cell fate.



J. Theoret. Biol. (1969) **25**, 1–47

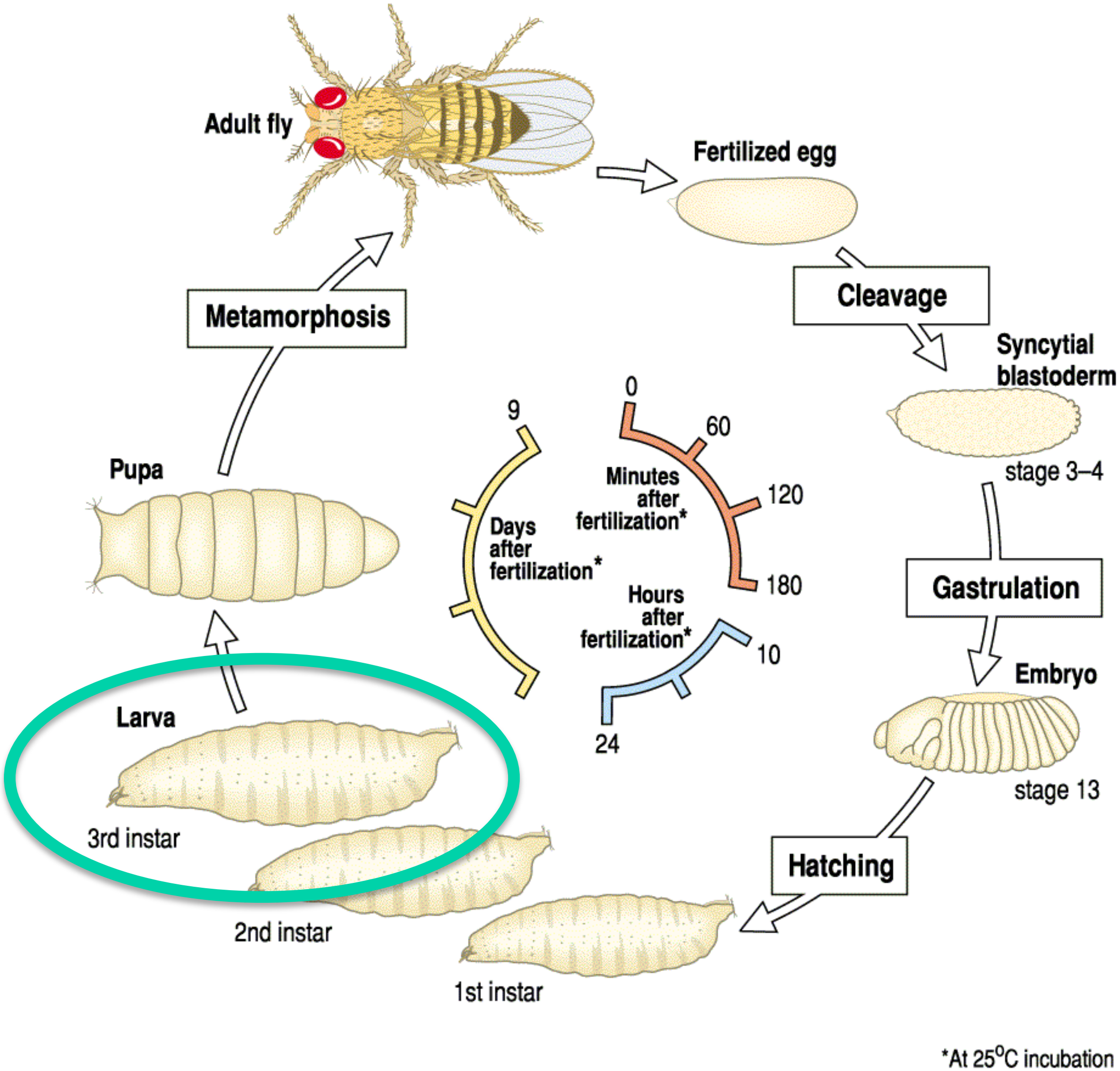
Positional Information and the Spatial Pattern of Cellular Differentiation†

L. Wolpert

Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London, England

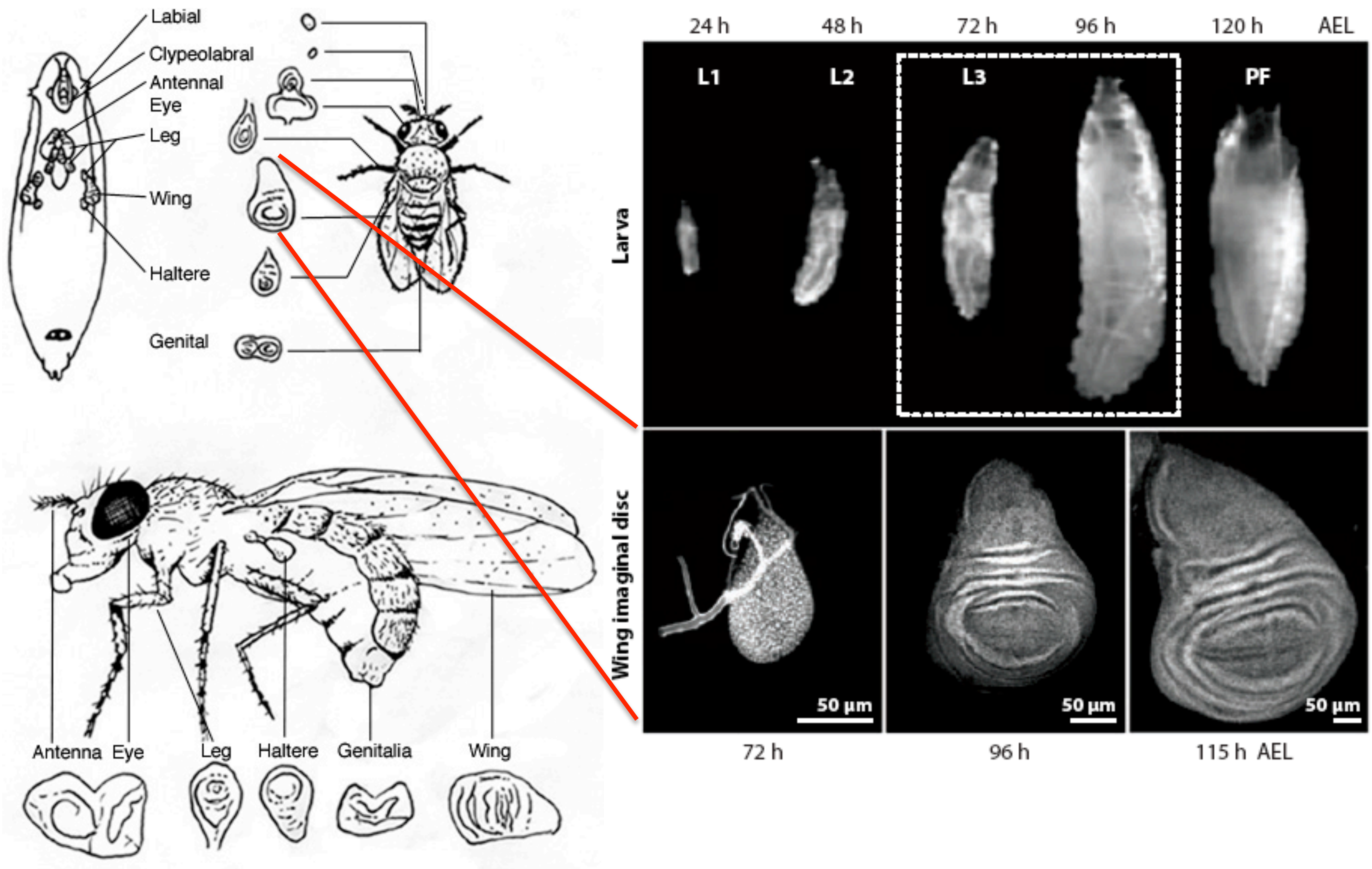
(*Received* 1 *July* 1969)

Overview: *Drosophila* life cycle (*Drosophila* = fruit fly)



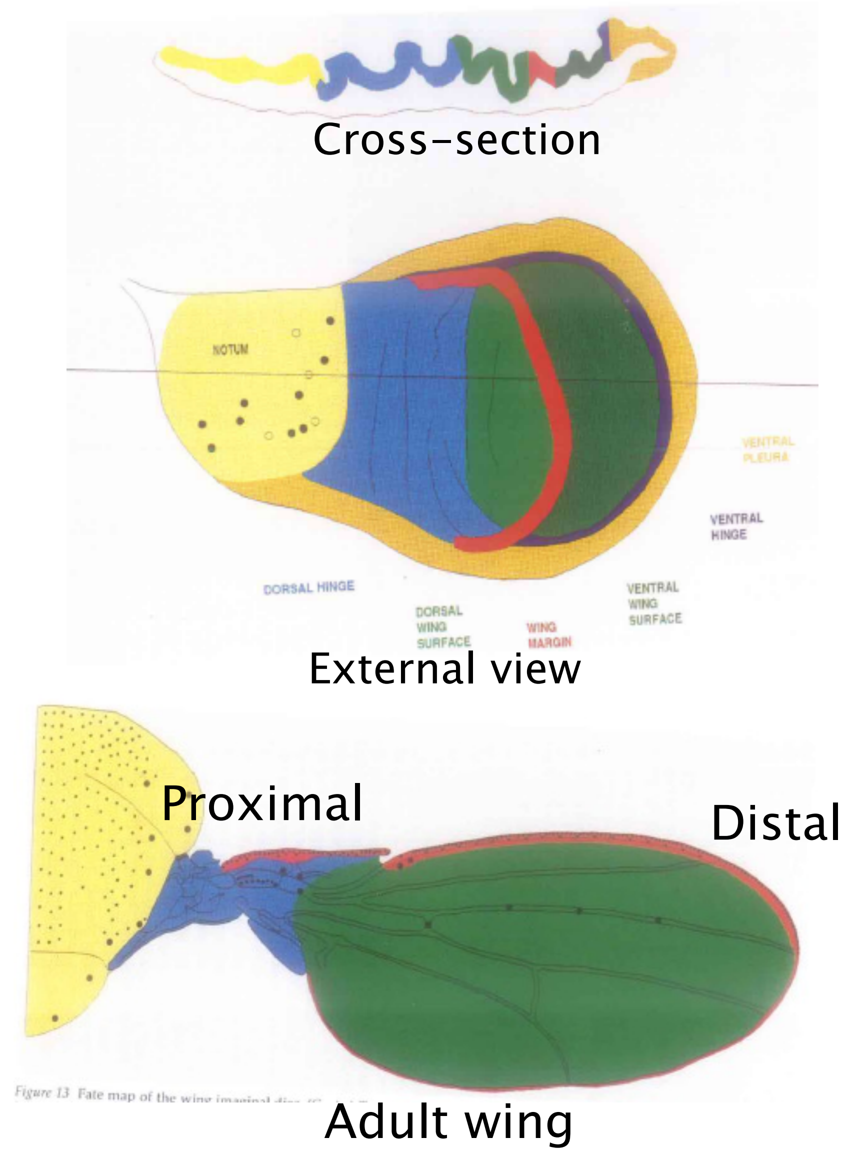
Imaginal Discs

Imaginal discs are patches of cells in the larval insect that will form appendages, e.g., wings, legs, antennae, during metamorphosis.



Overview: The Wing Disc

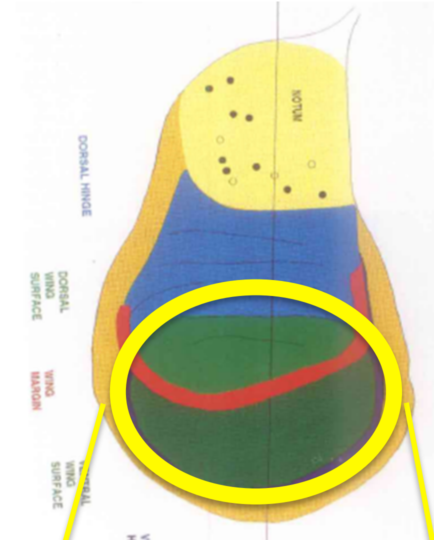
- Cell cycle time ~8 hrs.
- *Drosophila* wing disc grows from about 40 to 50,000 cells over 4 days.
- We study the central wing pouch or primordium (dark green), which forms the wing blade.
- During metamorphosis, the disc everts and “telescopes”.
- The center of the disc forms the wingtip (distal).
- The periphery of the disc forms the base/hinge (proximal).



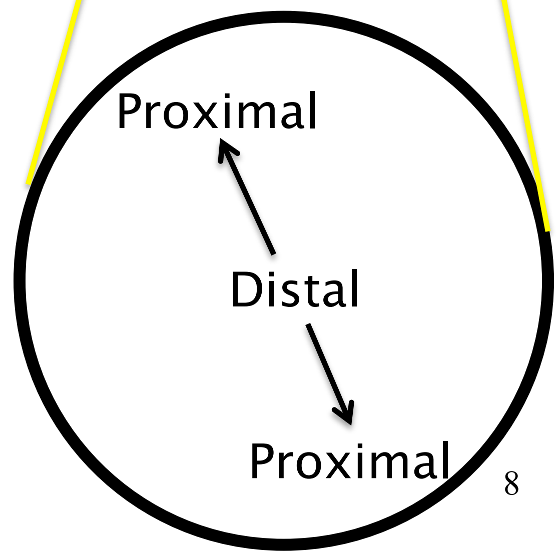
Campuzano and Modolell, 1992; Cohen 1993

The Proximal–Distal Axis

- Central "pouch" forms the wing blade.
- The center of the disc forms the wingtip (distal).
- The periphery of the disc forms the base/hinge (proximal).
- In the wing disc, the proximal–distal axis is in the radial direction.



Campuzano and Modolell, 1992; Cohen 1993



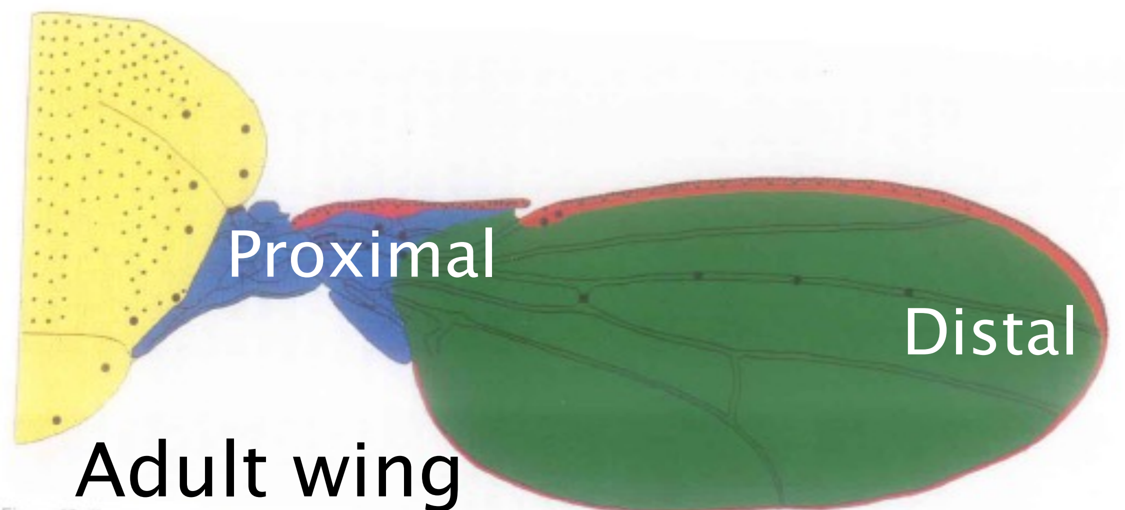
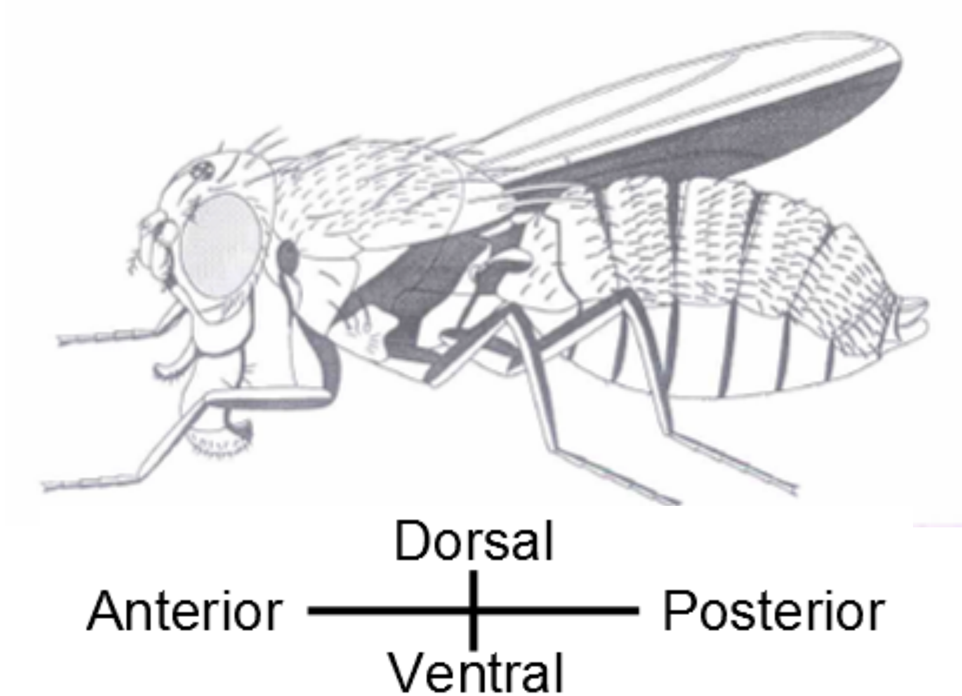
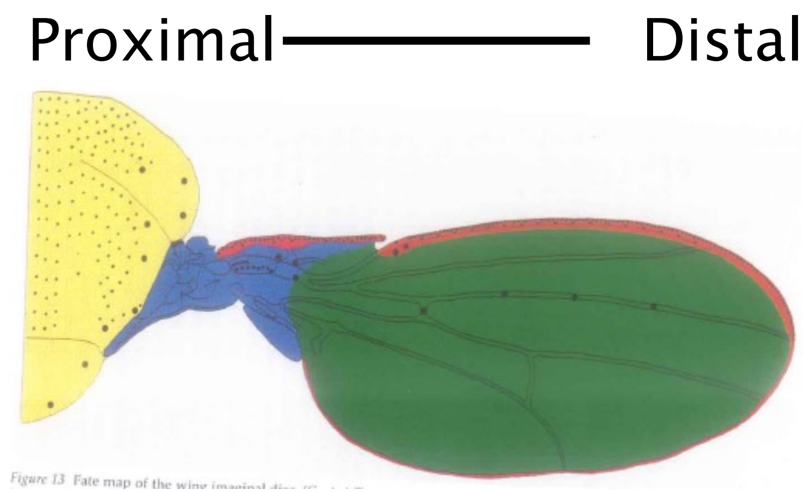


Figure 13 Fate map of the wing imaginal

Overview: Major Questions

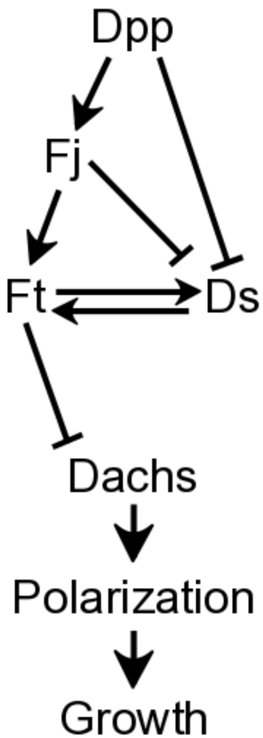
- Polarization: How do cells in a growing tissue acquire a sense of direction with respect to the body axes? (Which way is which)?
- Proliferation: What regulates a cell's rate of growth?



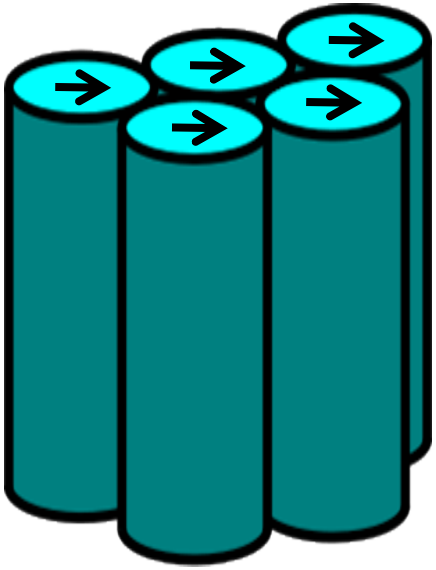


How are growth and polarization regulated in a developing organism?

A Signaling Pathway Involved in Polarization and Growth

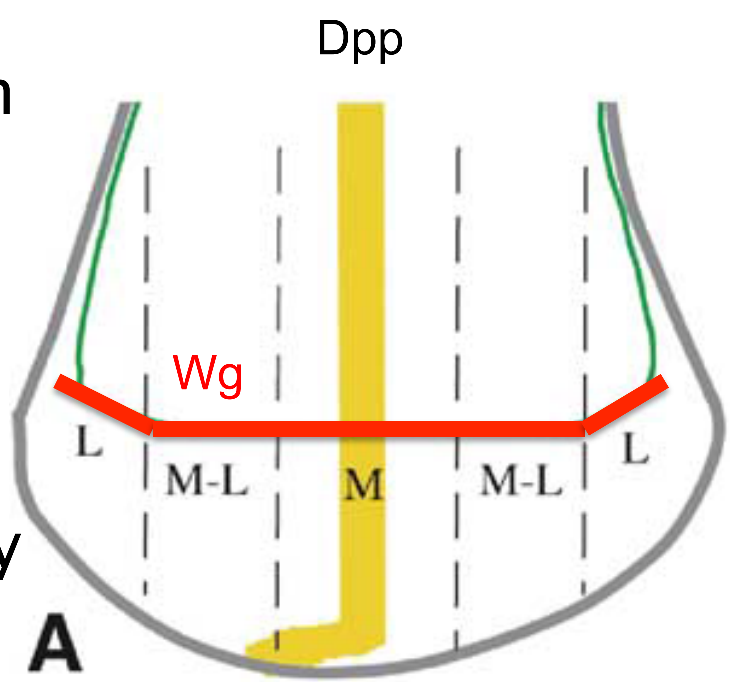


A signaling pathway is a molecular cascade of switches in which various proteins turn other proteins on or off.

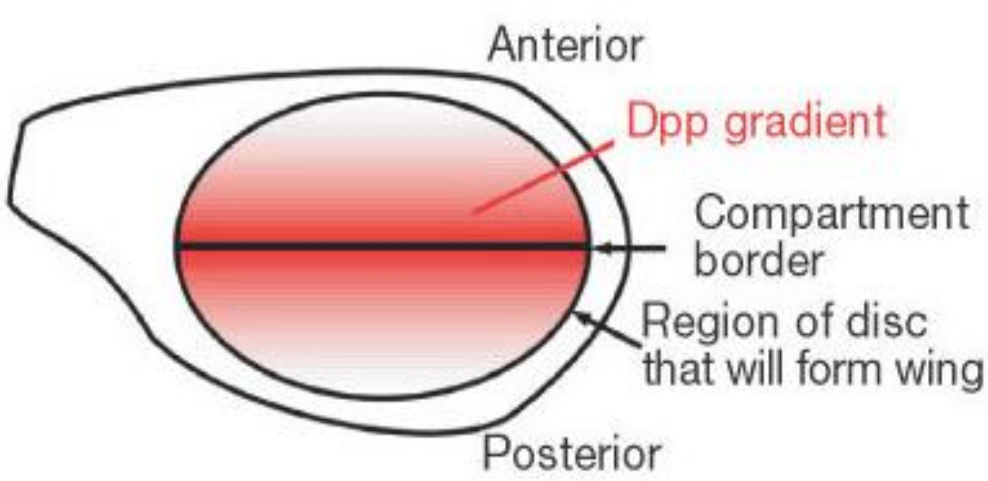


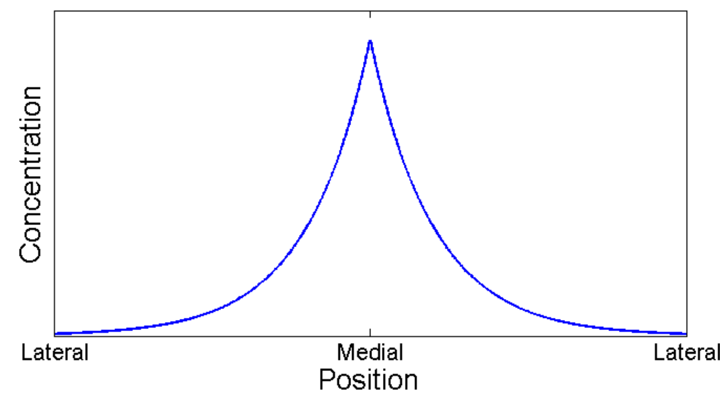
Morphogen: Decapentaplegic (Dpp)

- Morphogens are signaling molecules associated with growth and development.
- Morphogens Decapentaplegic (Dpp) and Wingless (Wg) are necessary for wing growth.
- Dpp and Wg concentrations decay with distance from source.



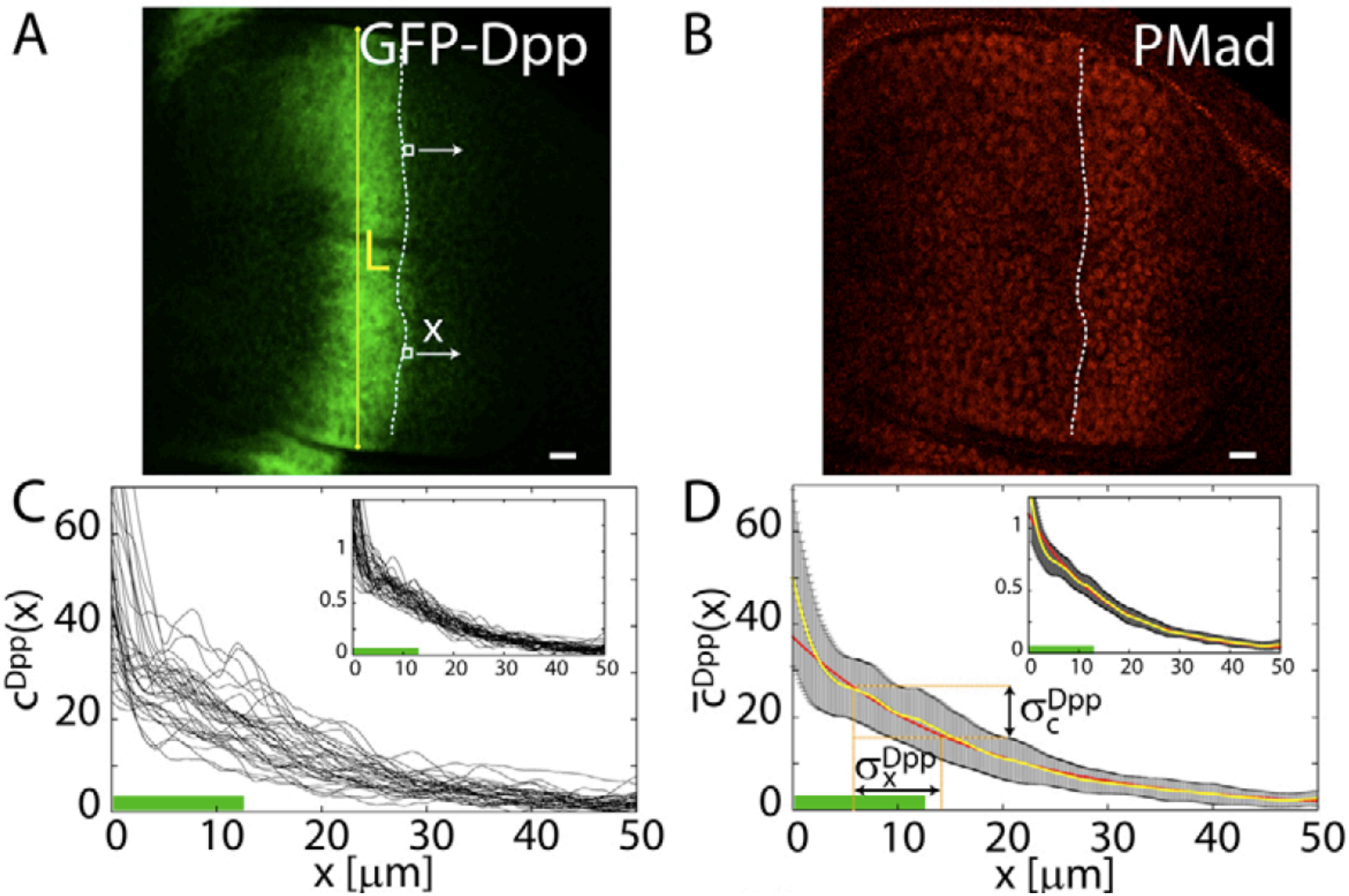
Rogulja and Irvine *Cell* (2005).





The Dpp Concentration Profile Decays Exponentially

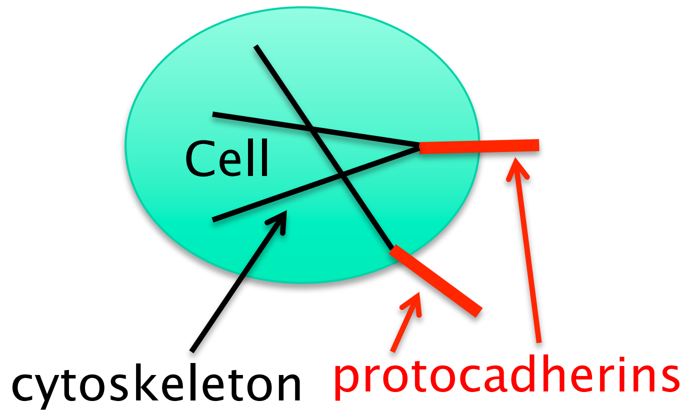
- Growth rate increases with increased Dpp signaling.
  (Zecca *et al.* 1995)

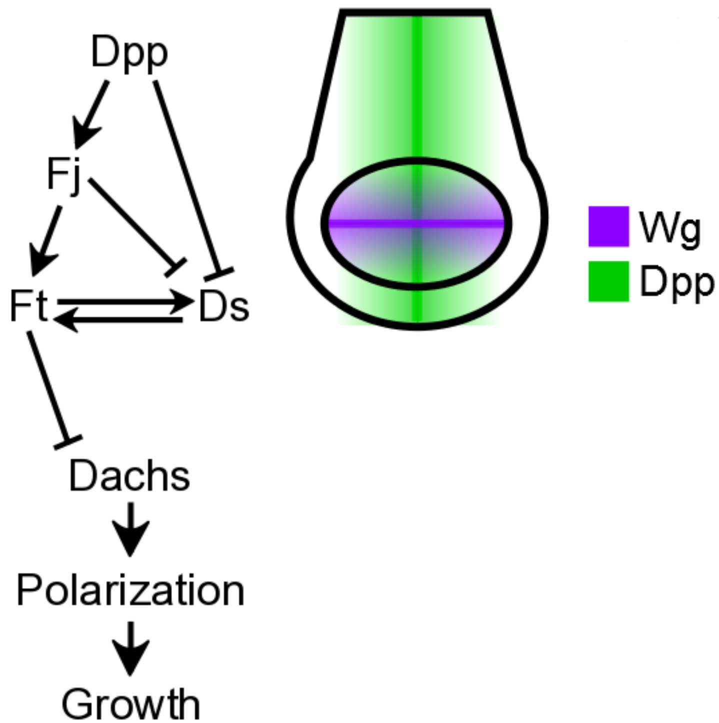


Bollenbach et al. 2008

Key Players Downstream From Dpp

- Protocadherins are transmembrane proteins connected to the cytoskeleton (protein filaments giving cell its shape).
  - Protocadherin Fat (Ft)
  - Protocadherin Dachsous (Ds)
- Golgi kinase Four-jointed (Fj) phosphorylates (adds a phosphate group to) Ft and Ds.
- Unconventional myosin Dachs
  - Location of Dachs inside a cell indicates cell's polarization.

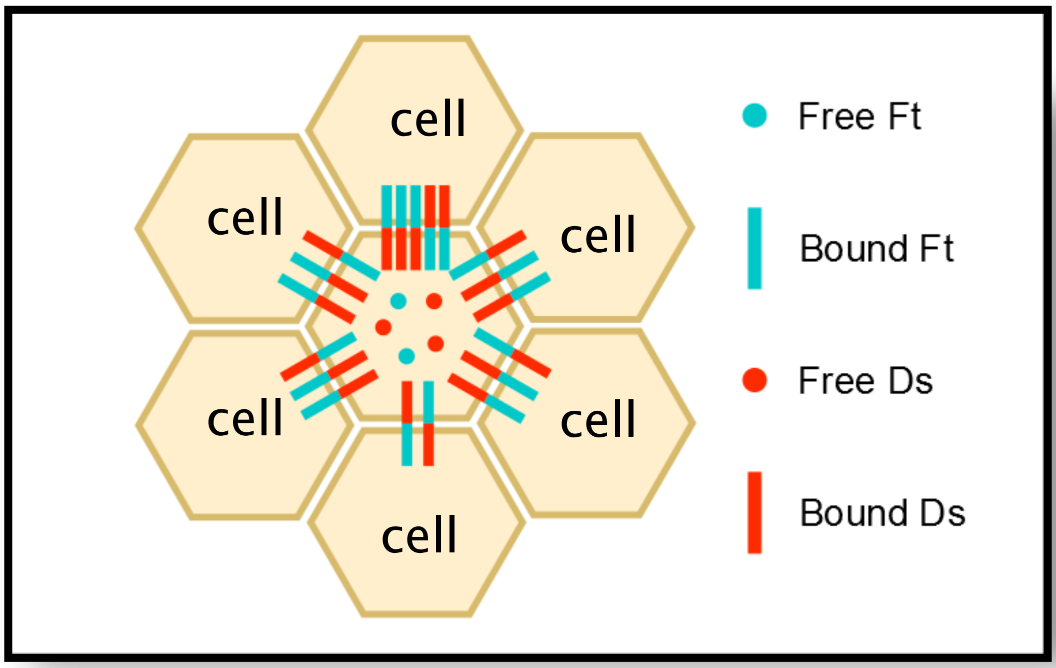




Ft-Ds Binding

(Matakatsu and Blair, 2004)

- Ft and Ds are protocadherins connected to the cytoskeleton.
- They are transmembrane proteins with an intracellular and extracellular domain.
- A Ft on one cell binds to a Ds on an adjacent cell.
- Adjacent cells are mechanically coupled by protocadherins Fat (Ft) and Dachsous (Ds) since protocadherins are connected to the cytoskeleton ("cells hold hands").

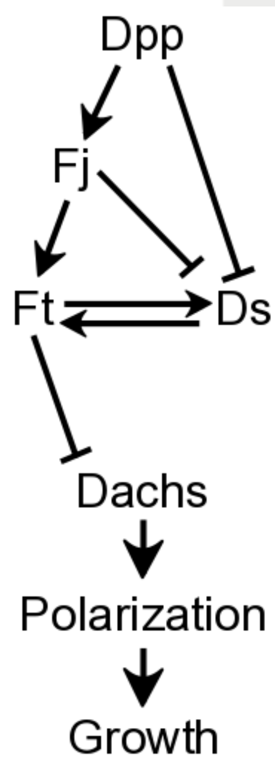


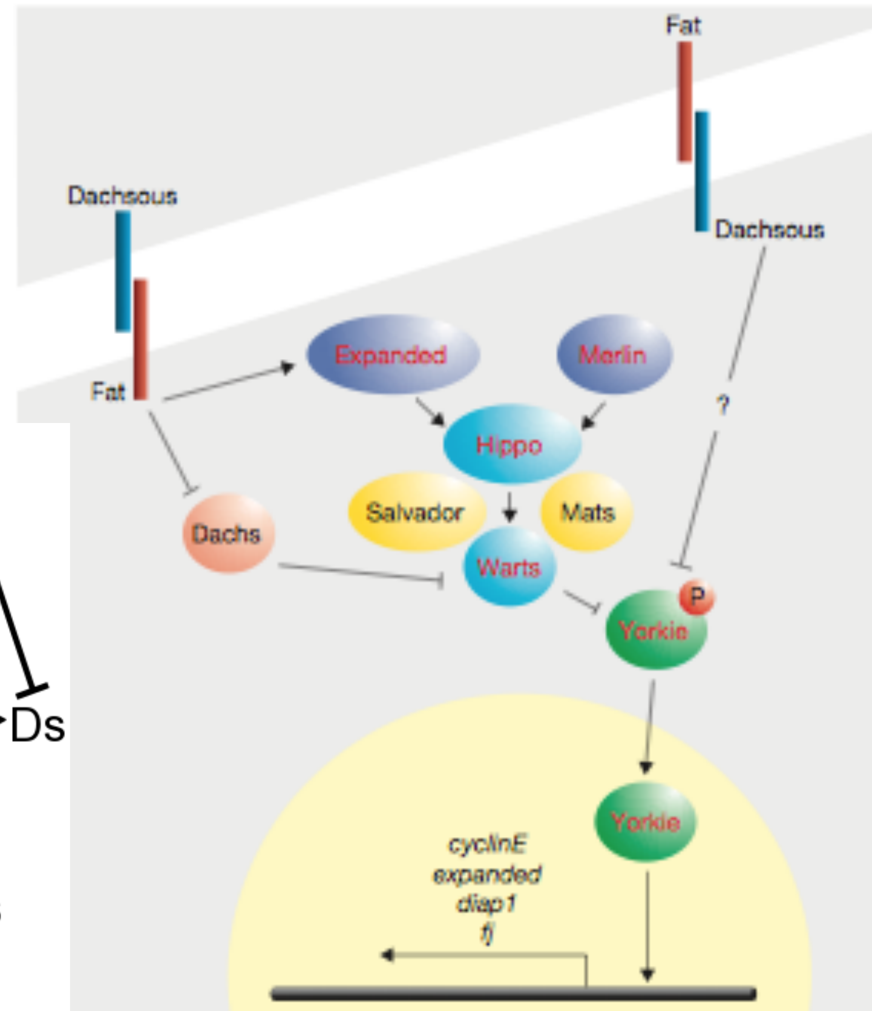
Ft-Ds heterodimers

The Fat (Ft) Signaling Pathway

- Four-jointed (Fj) is a Golgi kinase that phosphorylates (adds a phosphate group to) Ft and Ds. (Ishikawa *et al*. 2008)
- Fj makes
    - phosphorylated Fat (Ft) more likely to bind Ds and
    - phosphorylated Ds less likely to bind Ft.

    (Simon *et al*. 2010, Brittle *et al*. 2010).

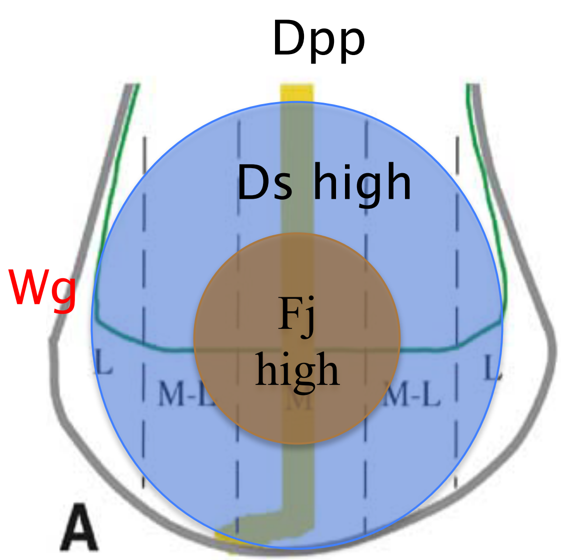




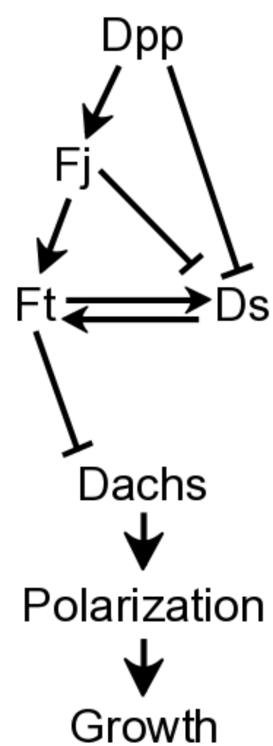
From Lawrence, Struhl, and Casal (2008)

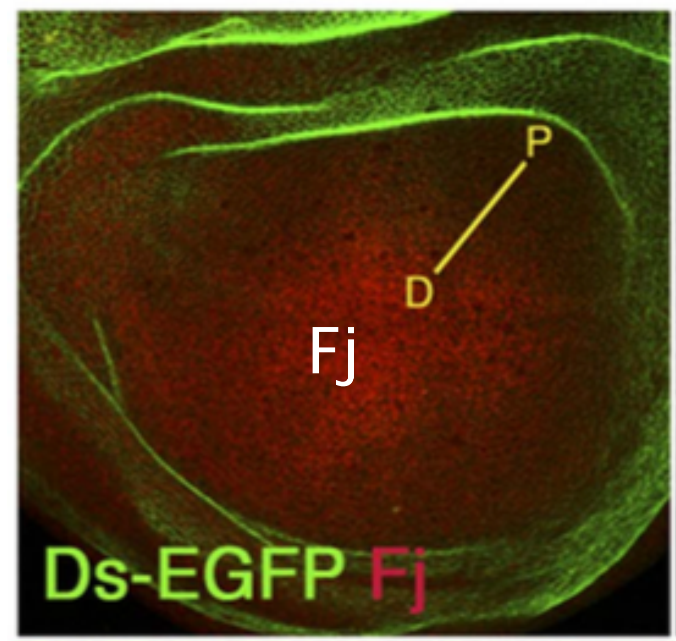
The Ds and Fj Profiles

- Dpp signaling leads to more Fj expression and less Ds expression. (Rogulja *et al*. 2008)
- Fj is expressed at high levels in the wing pouch, and Ds is expressed at high levels outside the wing pouch.
- Ft is expressed comparatively uniformly throughout the pouch. (Mao *et al*. 2009)



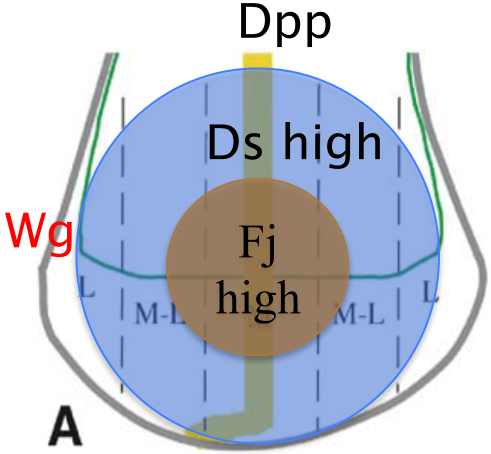
Rogulja and Irvine *Cell* (2005



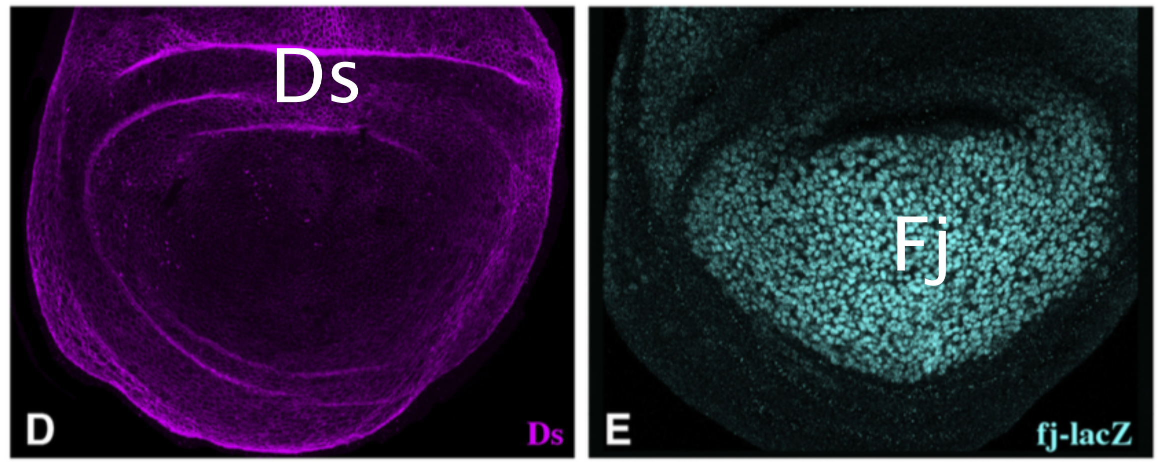


Hale *et al.* 2015

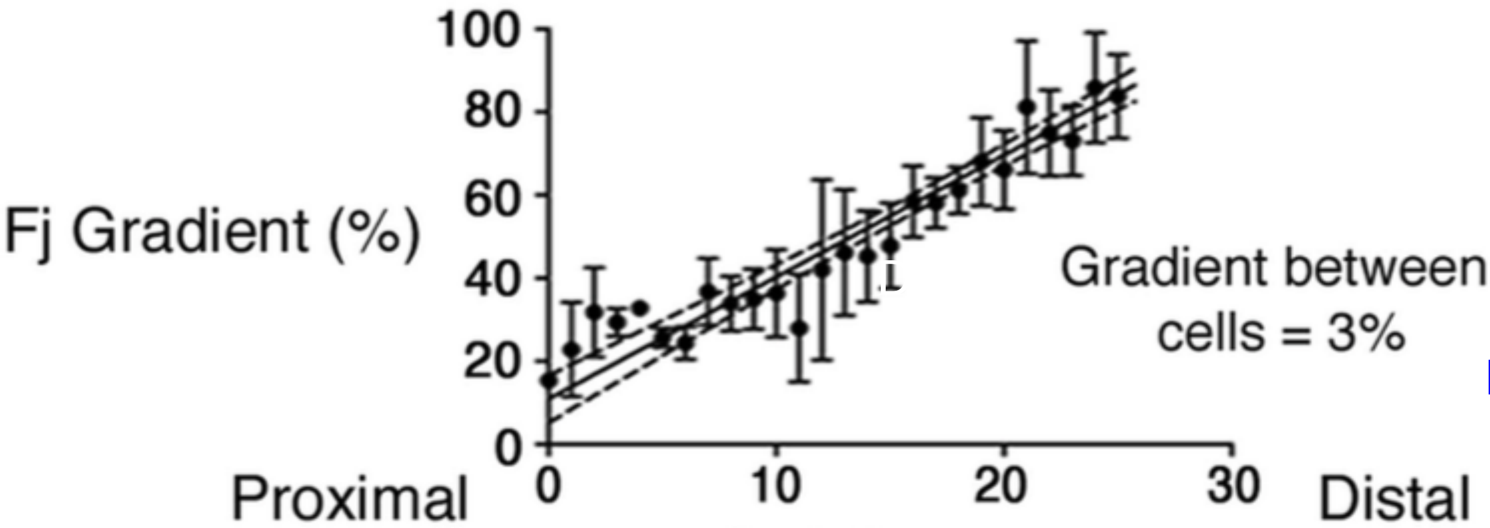
Fj Concentration Has a Linearly Sloping Profile



Rogulja and Irvine *Cell* (2005).



Ambegaonkar *et al*. 2012



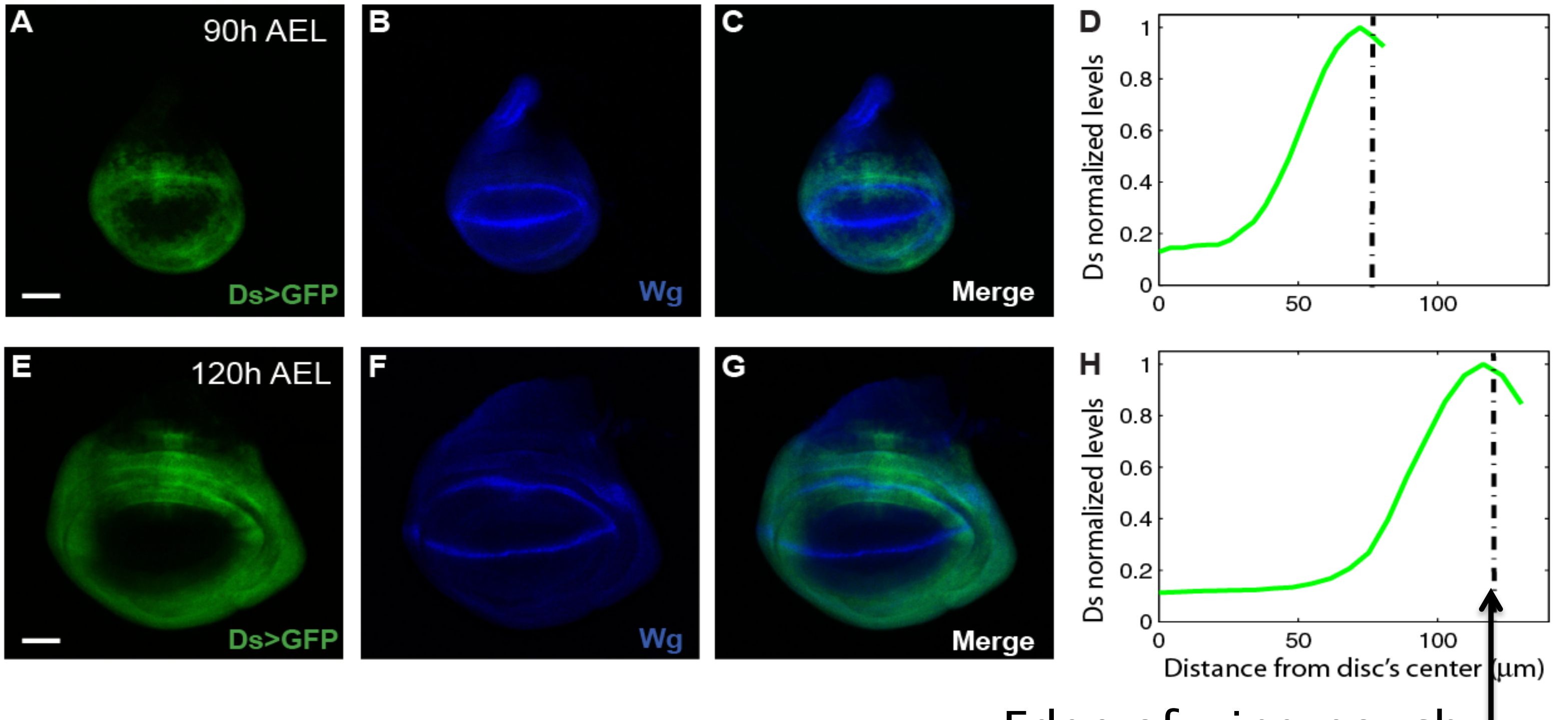
Hale *et al*. 2015

Pouch Periphery

Pouch Center

The Ds Concentration Profile

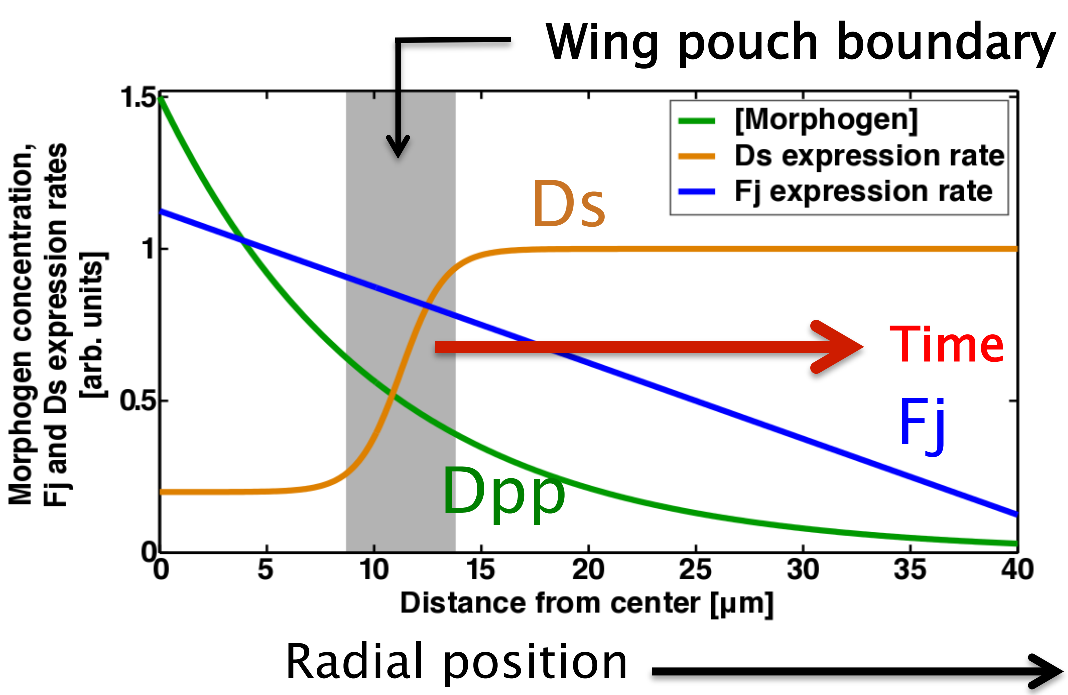
- Ds concentration does not have a constant slope; it is steepest near the edge of the wing pouch and flat in the center.
- Steep Ds region = "Ds Front" = wing pouch boundary.
- Ds front expands outward.

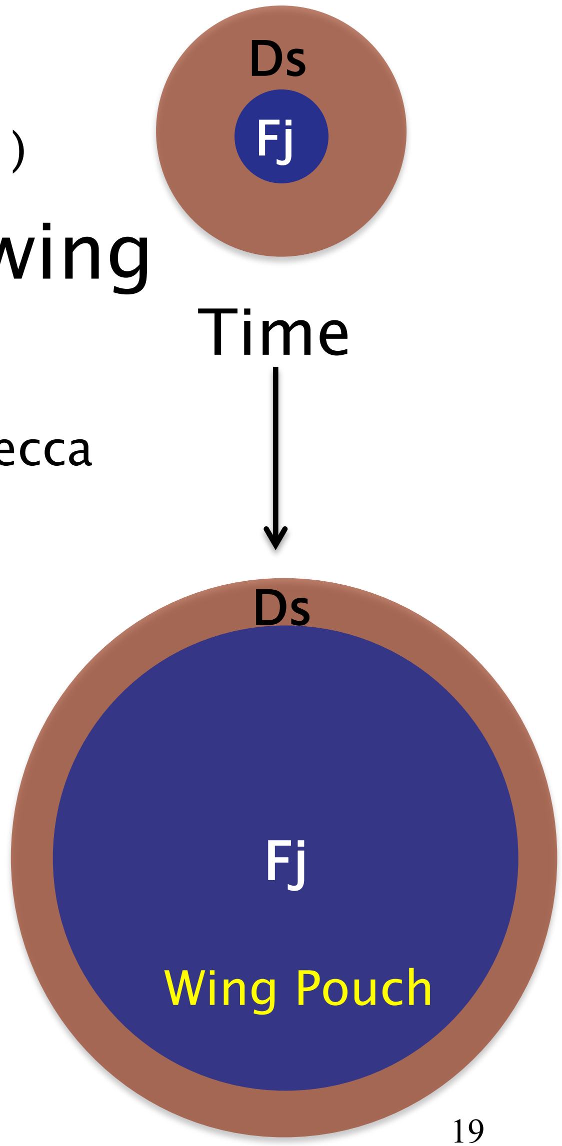


Courtesy of Marcos Nahmad and Peng Cheng Zhang, Lander lab

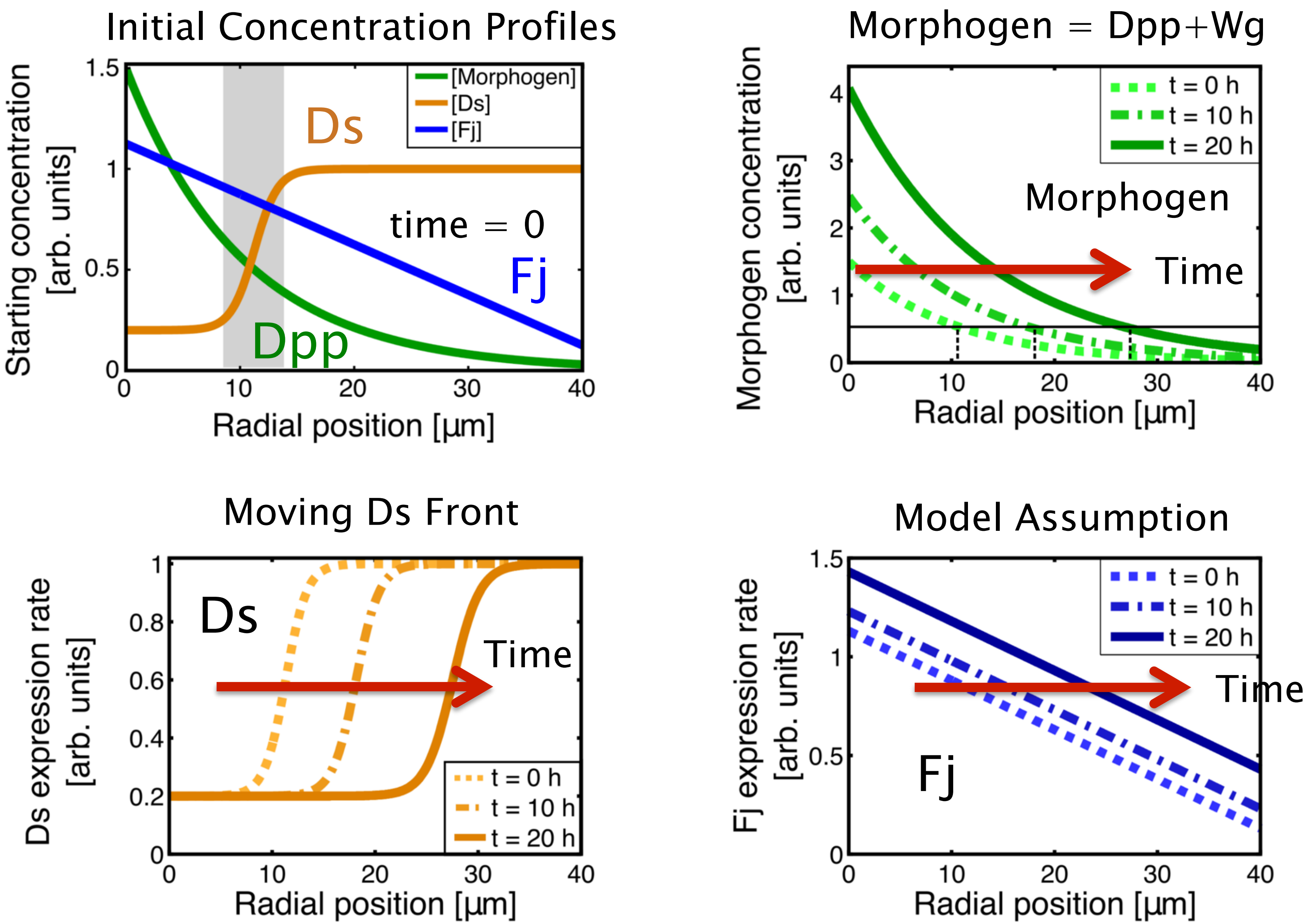
The Moving Ds Front

- The amplitude of the Dpp profile increases with time. (Wartlick *et al.* 2011)
- The Ds front (=boundary of the wing pouch) moves radially outward, recruiting cells into the pouch. (Zecca and Struhl 2010)

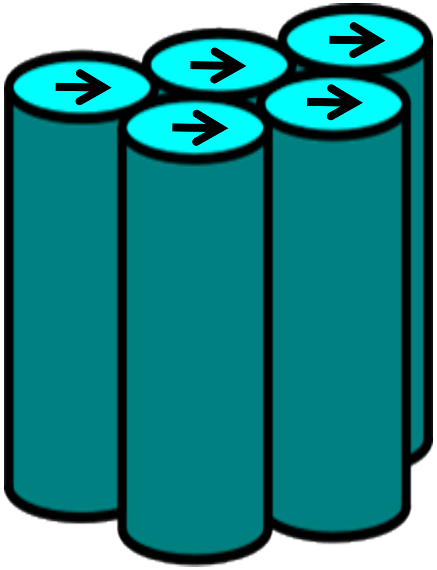




Changing Concentration Profiles



What is cell polarization and how does it arise?



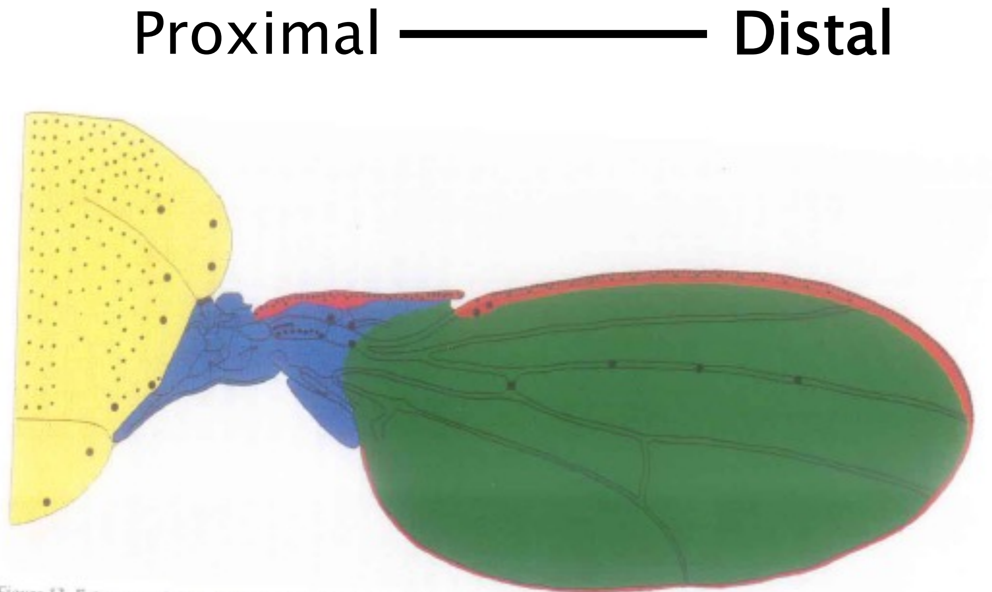
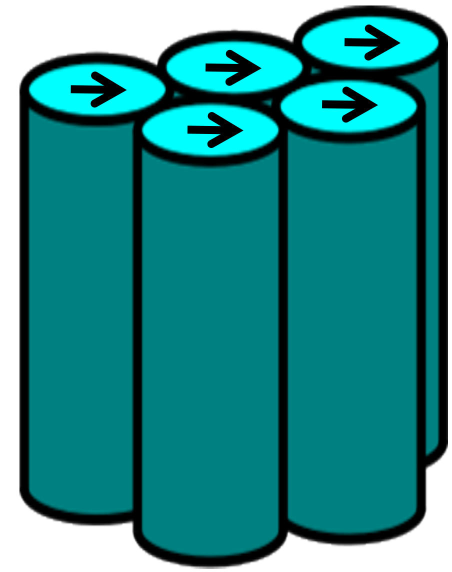


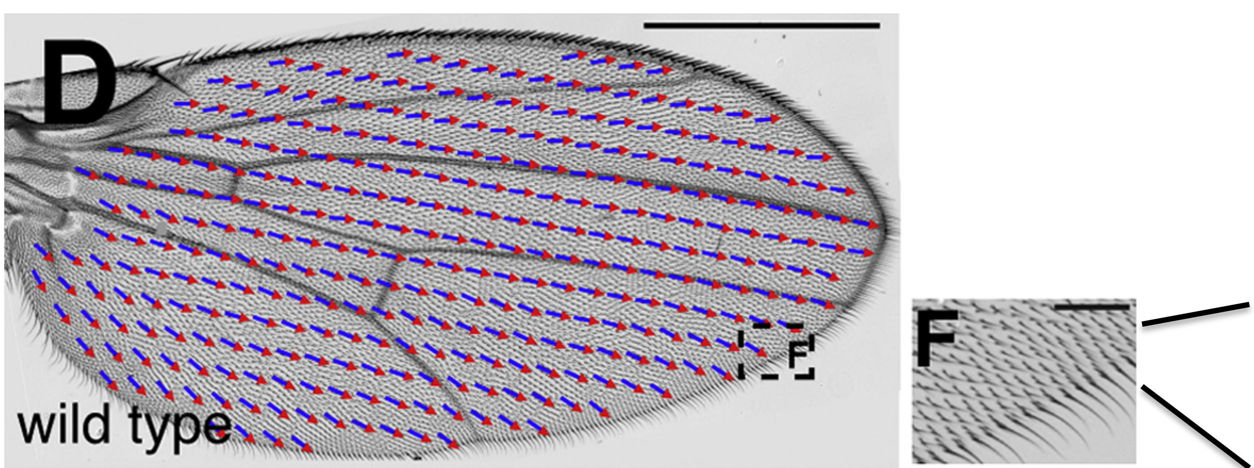
Figure 13 Fate map of the wing imaginal disc

Cell Polarization

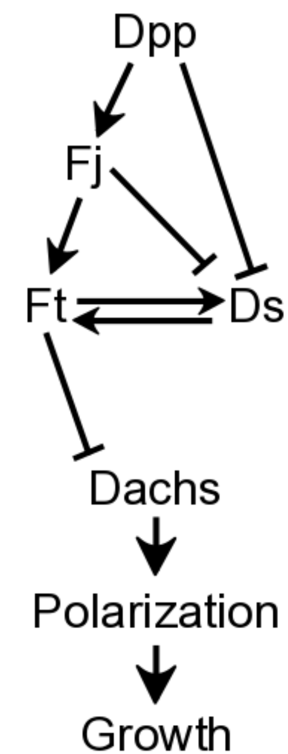
- Cells have a sense of direction, e.g., head vs. tail, in vs. out.
- Readout of polarization: Polarization is associated with wing trichome (hair) orientation (normally point distally).
- Cells in the entire wing pouch are polarized in the plane of the tissue (Dachs localization)



Columnar Cells

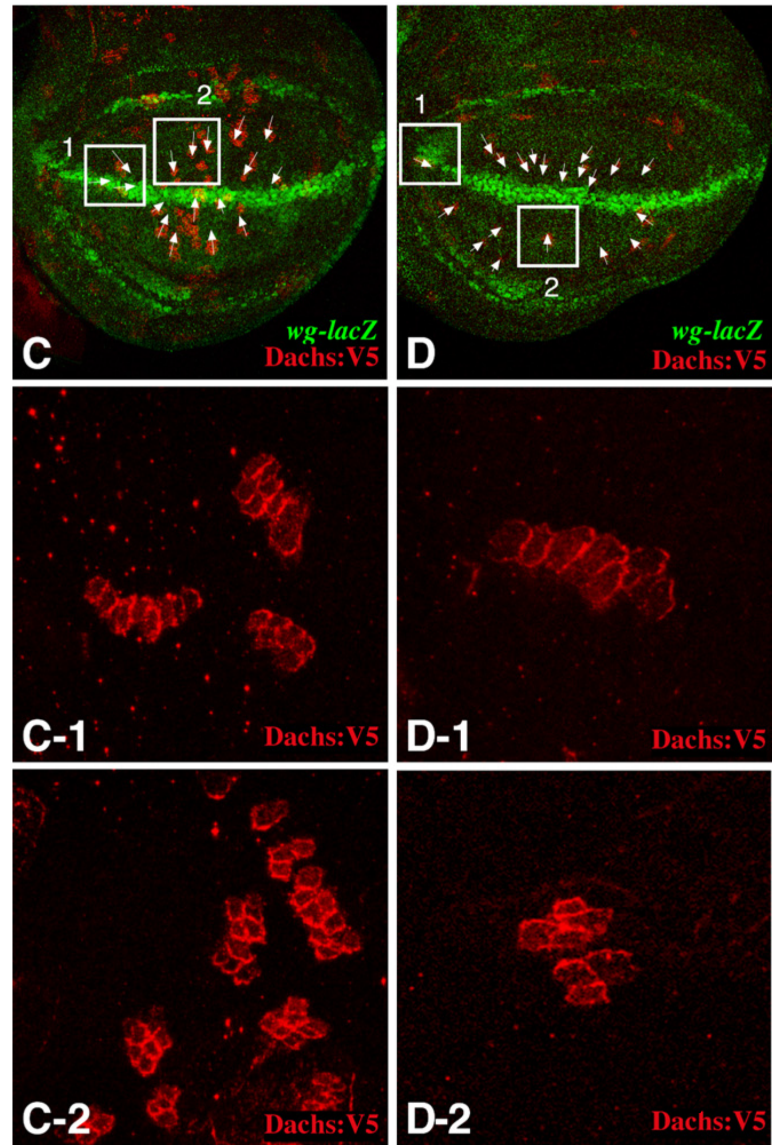


Sagner *et al.*, 2012



Polarization Readout: Dachs Localization

- The unconventional myosin (motor protein) Dachs is asymmetrically localized inside cells in the pouch.
- Dachs normally localizes to the distal side of each cell nearest the center of the wing pouch.
- Dachs localizes on side of cell with least amount of bound Ft.
- More Dachs localization is associated with faster growth.

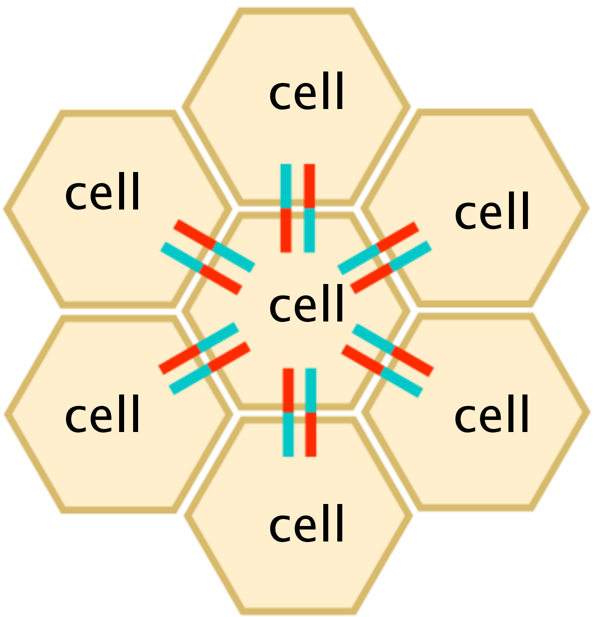


Rogulja, Rauskolb, and Irvine 2008

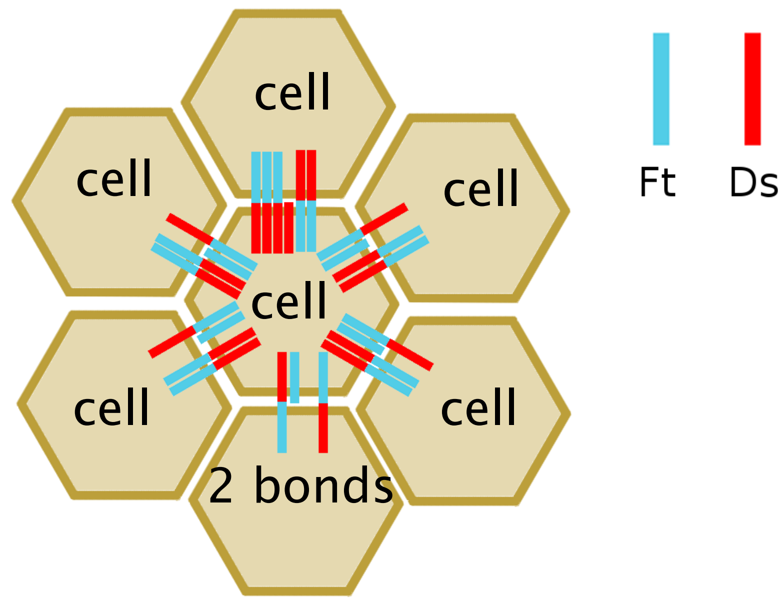
Asymmetry of Ft–Ds Bond Distribution Around a Cell Determines That Cell's Polarization

(Brittle *et al.* 2012)

- Experiment: Unconventional myosin Dachs localizes on side with least amount of bound Ft.
- Asymmetry of the distributions of Ft–Ds bonds around a cell determines the cell's polarization.



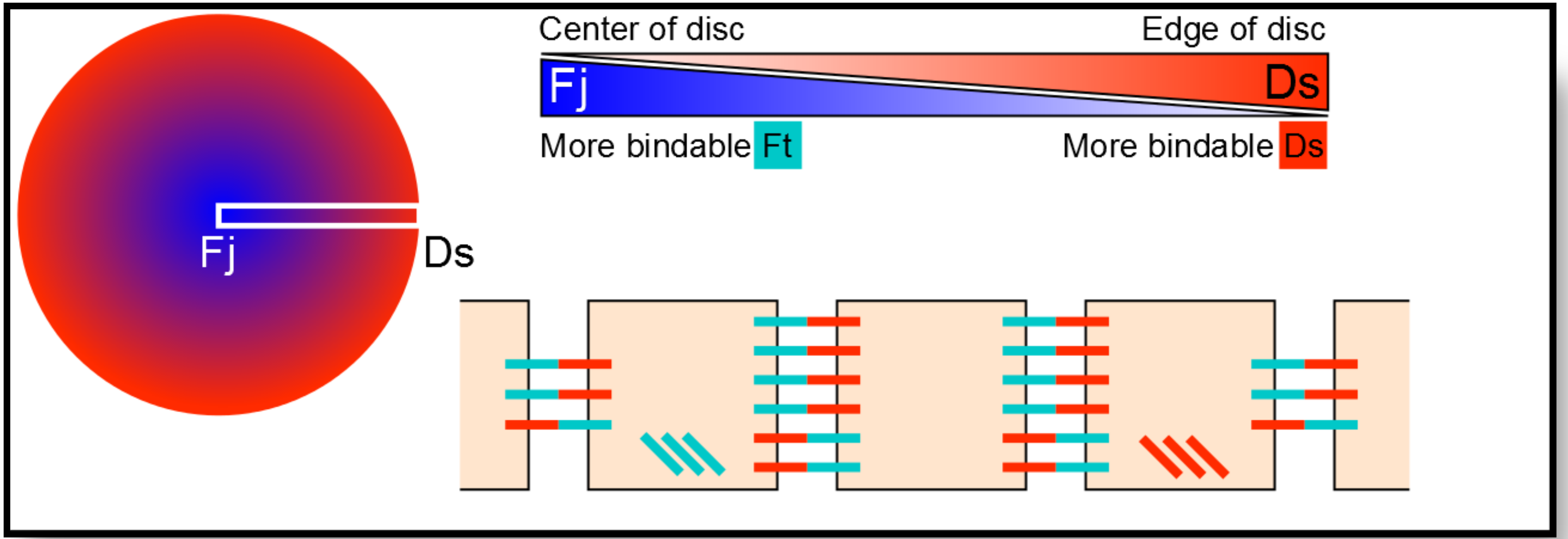
Symmetric Bond Distribution



Asymmetric Bond Distribution

Bond Distribution Asymmetry

- More bond distribution asymmetry means more polarization.
- Bond asymmetry arises when cells have different amounts of Fj and Ds than their neighbors.

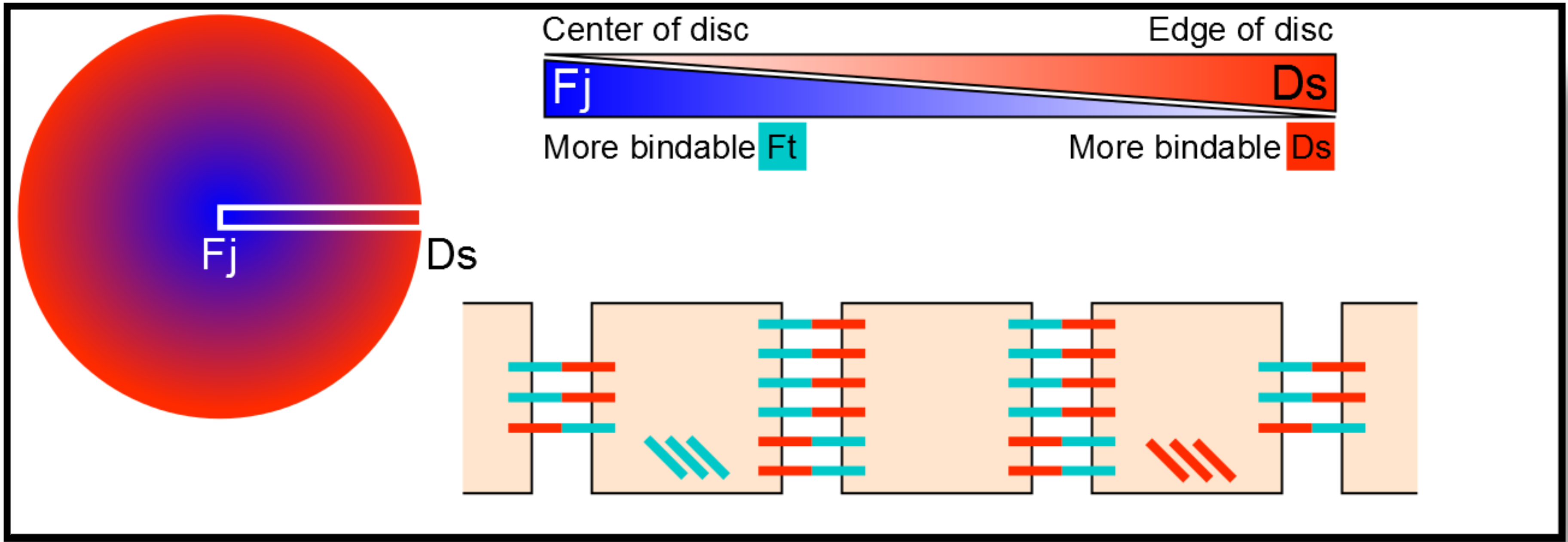


Distal (more Fj) Proximal (more Ds)

Previous Models of Bond Asymmetry

(Jolly *et al.* 2014, Hale *et al.* 2015)

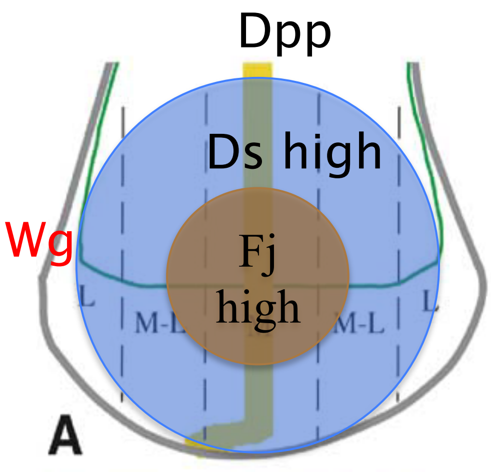
- Assumed the Fj and/or Ds concentration profiles have a constant slope.
- Assumed concentration profiles do not change.
- Ignored the effect of cell division.



Distal (more Fj)

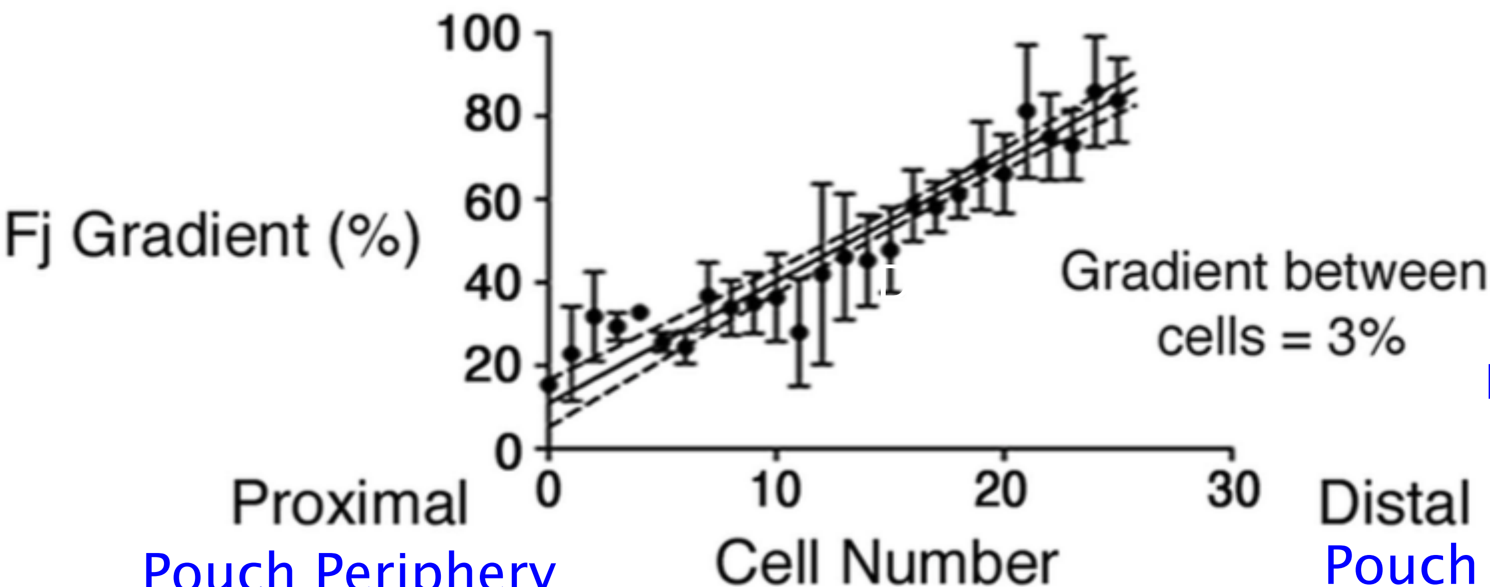
Proximal (more Ds)

Linear Sloping Fj Profile Hard For a Cell To Detect



Rogulja and Irvine *Cell* (2005).

- Small gradient of 3% on single cell level.
- Fj profile is noisy.
- Is the Fj gradient alone responsible for polarization?



Hale *et al*. 2015

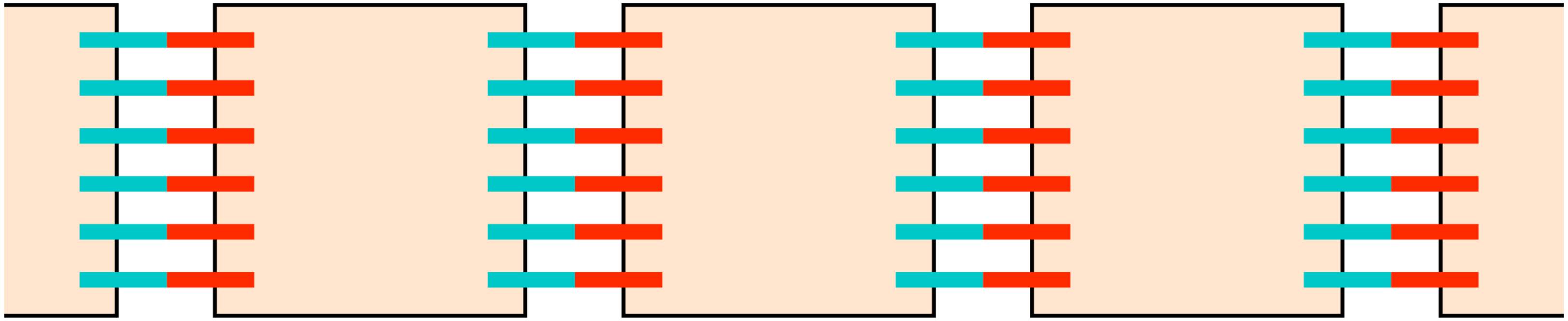
Pouch Periphery

Pouch Center

Previous Models of Bond Asymmetry

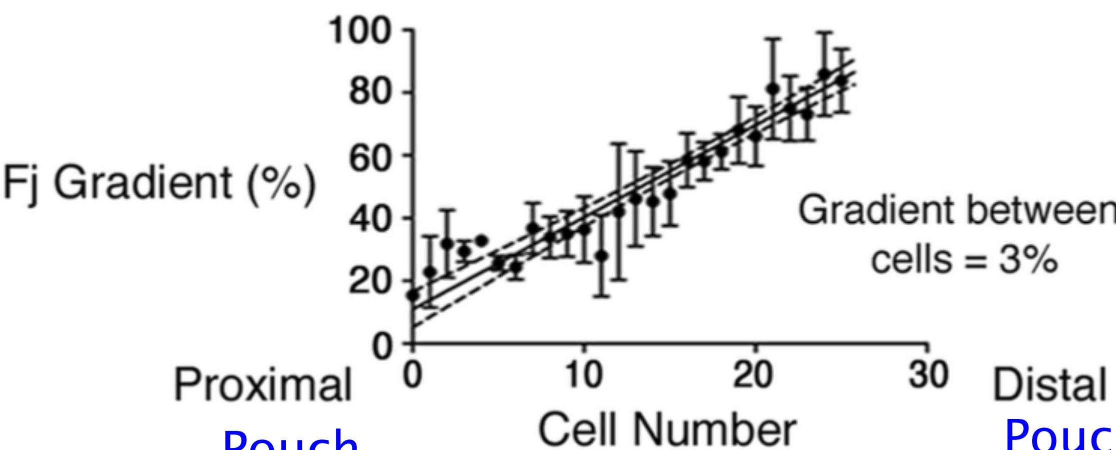
(Mani, Goyal, Irvine, and Shraiman, PNAS 2013)

- Assumed new Ft–Ds bonds at cell–cell interface preferentially form with the same orientation as existing bonds ("ferromagnetic" interactions).
- So shallow (Ds) profile can be amplified to produce bond asymmetry and cell polarization.
- Steady state 1D model that does not include how polarization is retained after cell division.



Cell Polarization

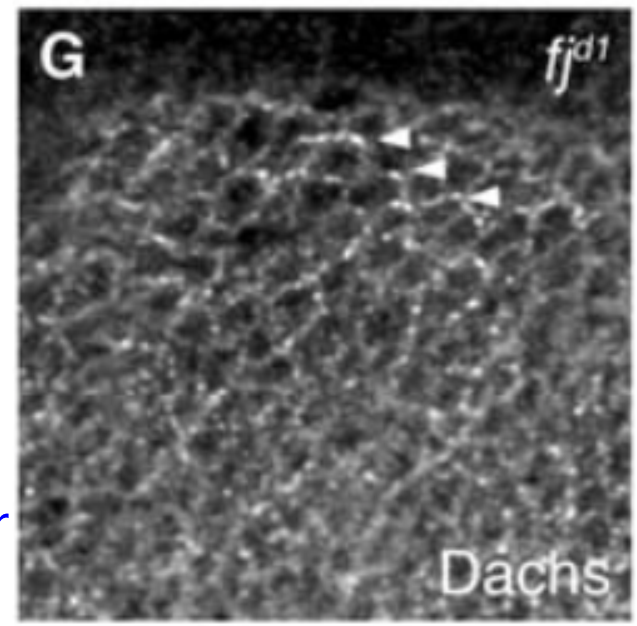
- Is the linearly sloping Fj profile alone responsible for all the polarization?
  - No, experiment without Fj (with wild-type Ds alone) shows weak polarization still exists.



Pouch Periphery

Pouch Center

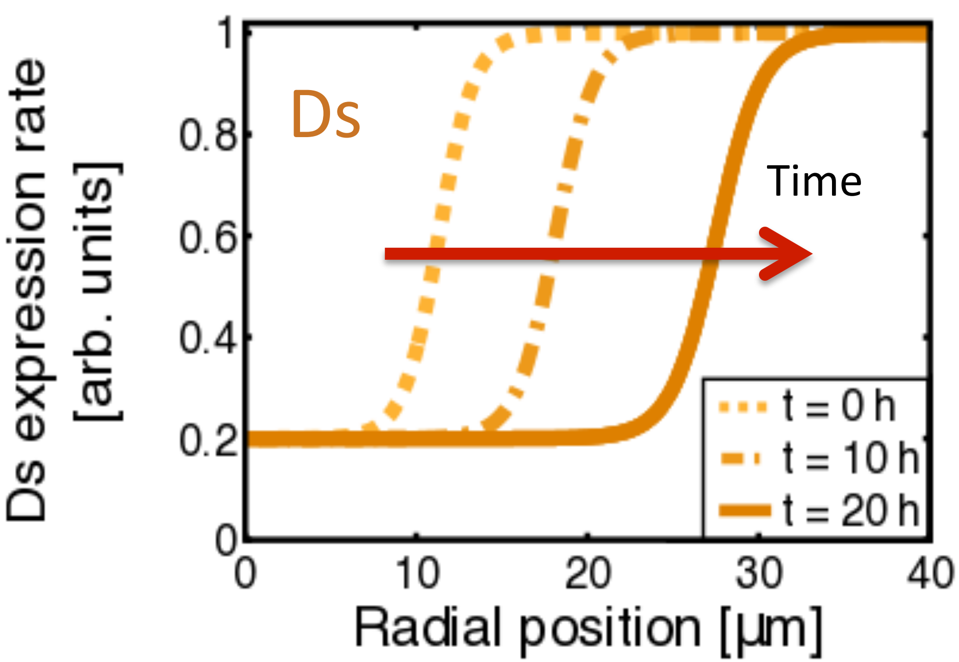
Hale *et al*. 2015



fj⁻ mutant
Brittle *et al*. 2012

Polarization Questions

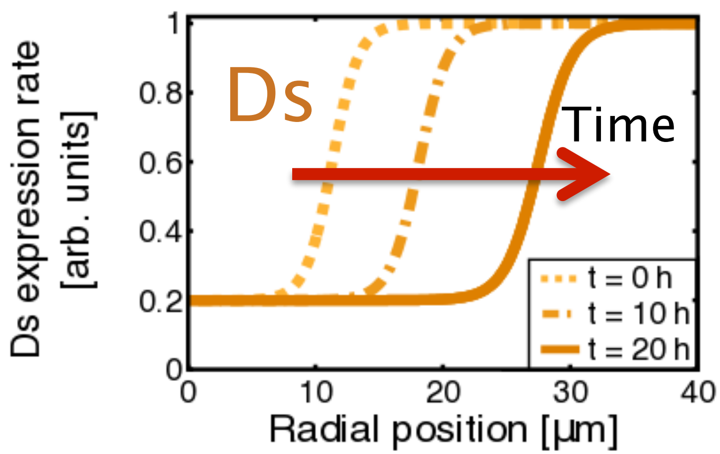
- Fj gradient alone is not enough to produce observed polarization.
- How does the moving Ds front contribute to polarization?
- How do cells retain their polarization after they divide?

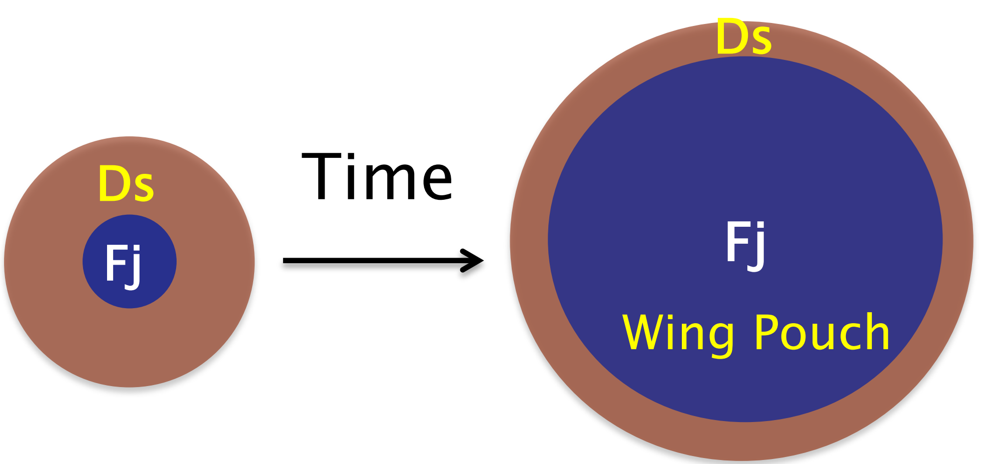


We would expect Ft–Ds bond asymmetry, and hence cell polarization, to be greatest for cells near the Ds front (where Ds is steepest).

Our Computer Model

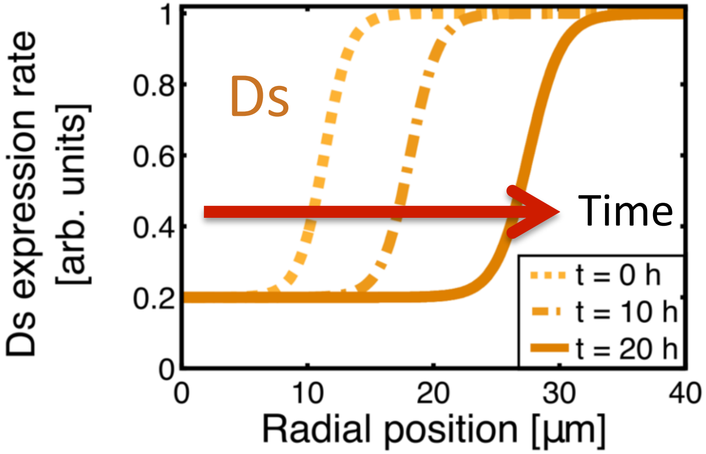
- Ds profile is steeply graded at the *edge* of the wing pouch.
- Ds expression front sweeps over most of the cells in the pouch.
- Cells become polarized when they are near the front, and retain the polarization afterwards.
- After dividing, we find that cells quickly recover polarization.

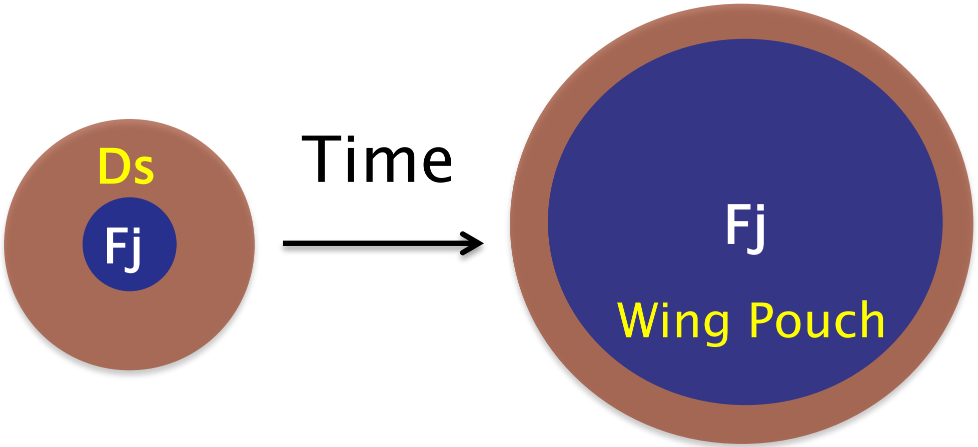




Ds Front Expands Outwards, Leaving Polarized Cells in Its Wake

- The movement of the front is what helps the cells in the pouch to be polarized.
- We propose that cells in the pouch are polarized because they (or their progenitors) were near the Ds front in the past.
- This implies:
  - Cells become more polarized when they are near the front.
  - Memory: They stay polarized after the front has moved on…
  - …even after multiple cell divisions.





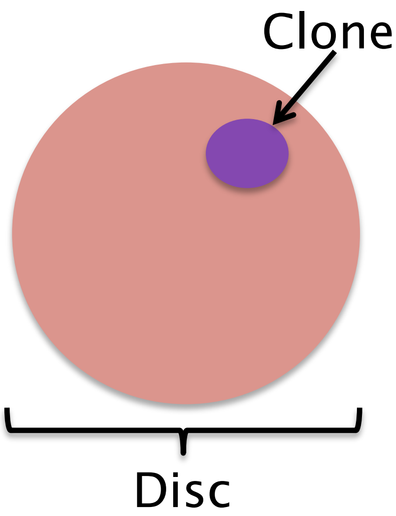
Since our model incorporates cell division and growth, let's talk about what regulates cell growth:

Growth depends on polarization

More polarization → More growth

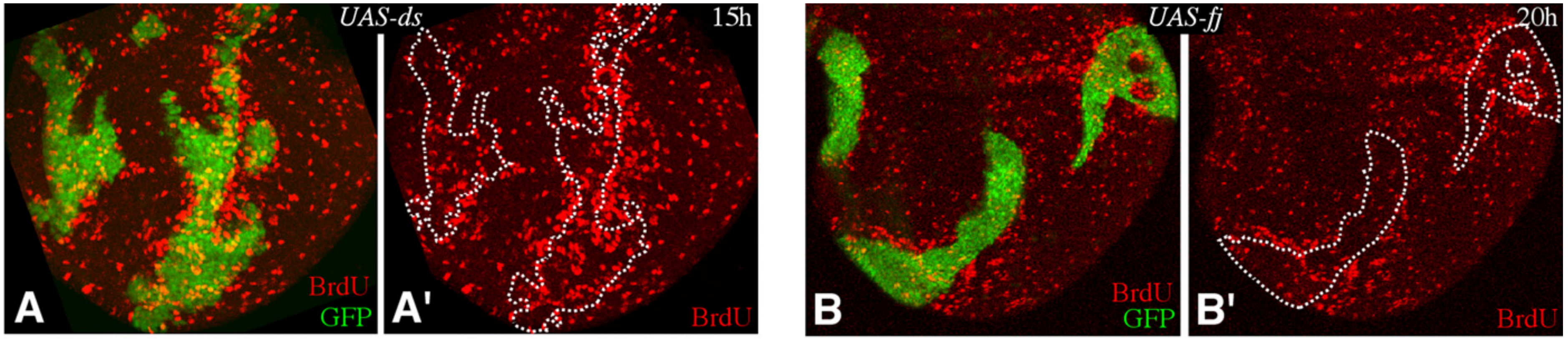
Ft–Ds Bond Asymmetry Promotes Growth

- Clones (clusters of cells with a common progenitor) with Fj or Ds over-expression in a wild-type background have elevated growth near the *edges* of the clones.
- More bond asymmetry around clone edges.
- More Ft–Ds bond distribution asymmetry is associated with more growth.



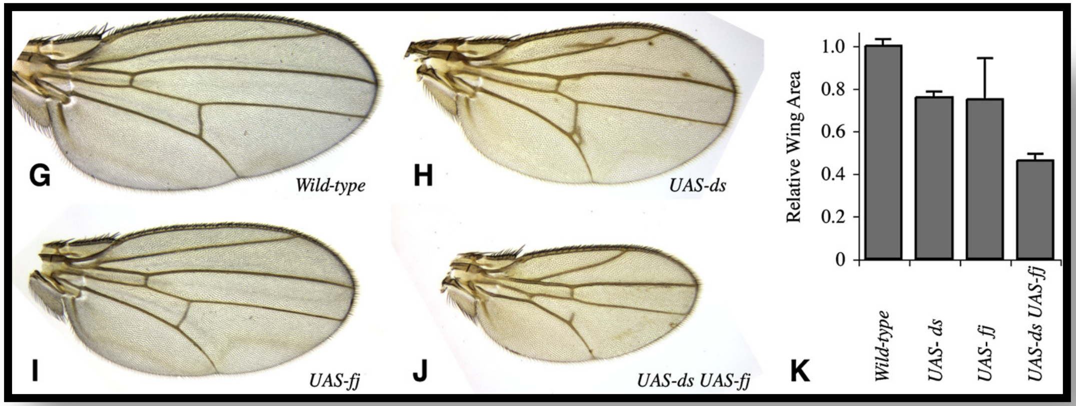
Ds–overexpressing clones

Fj–overexpressing clones



From Rogulja, Rauskolb, and Irvine, 2008

Ft–Ds Bond Asymmetry Promotes Growth

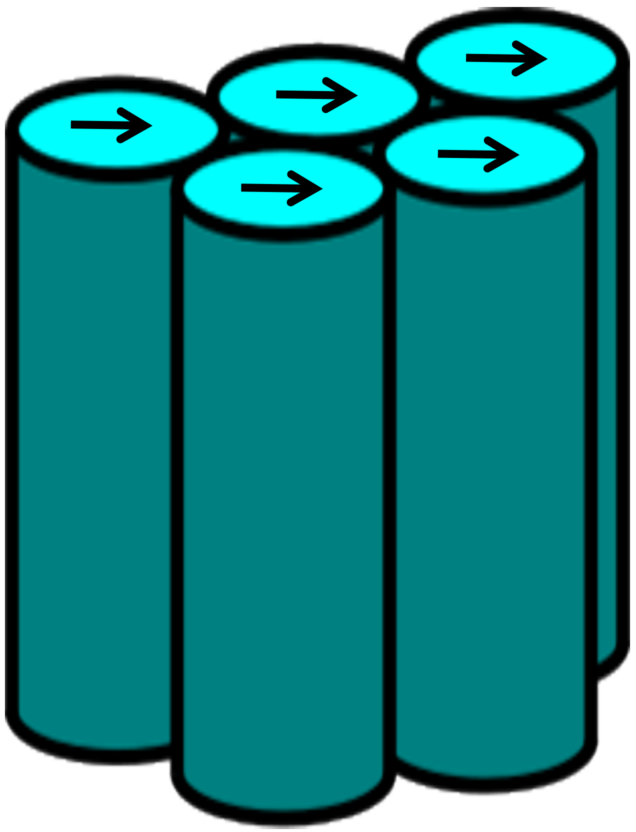


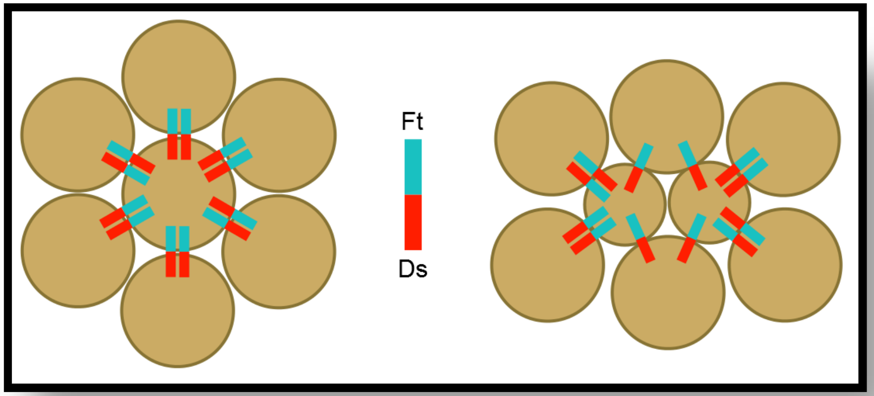
From Rogulja et al., 2008

- Discs that uniformly express Fj or Ds undergrow.
- In wild-type discs, Ft–Ds bonds are asymmetrically distributed on cells throughout the pouch. (Ambegaonkar et al., 2012; Brittle et al., 2012.)
- A cell’s growth rate is determined by both local Dpp signaling and the asymmetry of the Ft–Ds bond distribution. (Rogulja et al., 2008).

Model: Overview

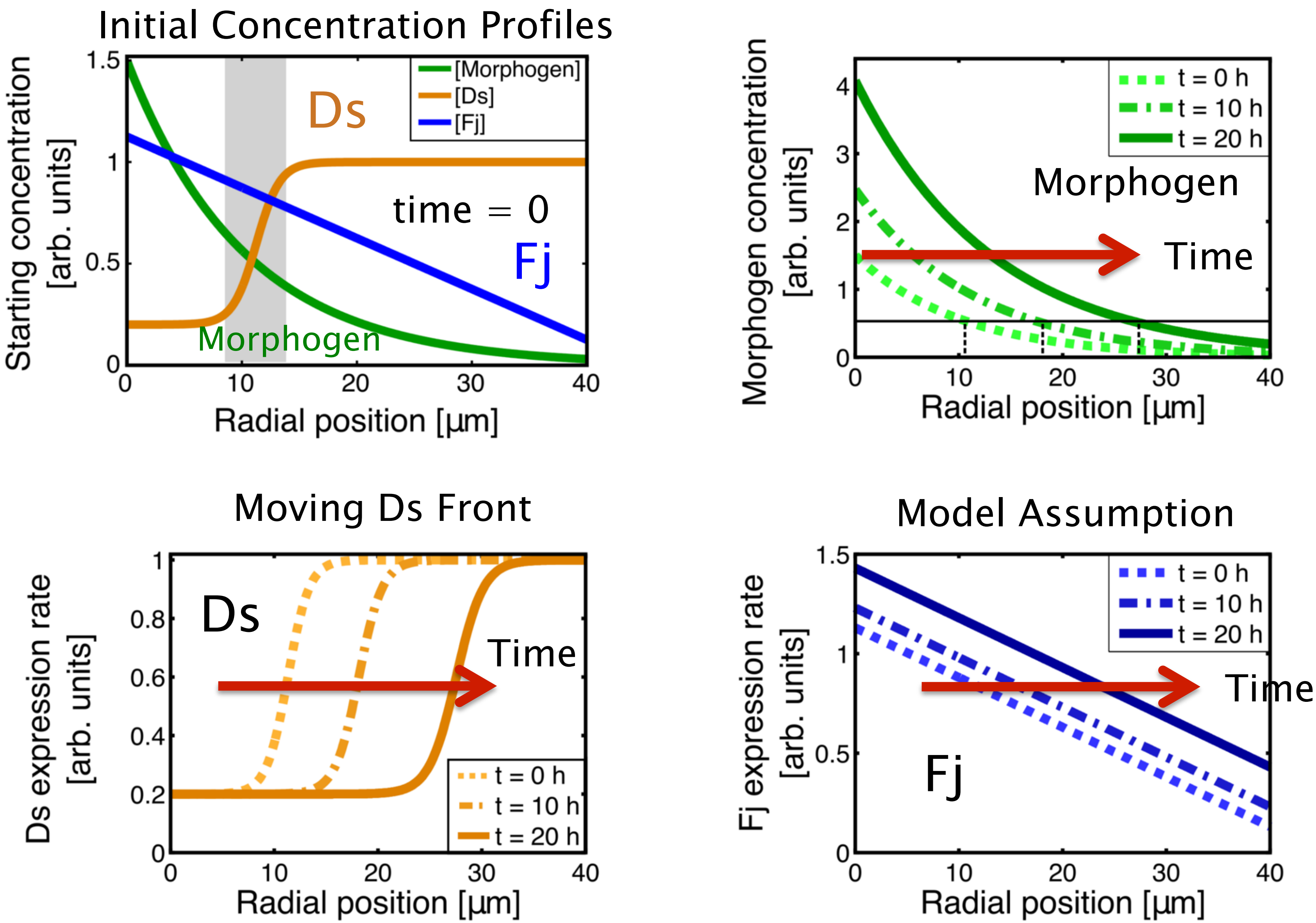
- 2-dimensional model of (columnar) cells in the wing disc.
- Simulate individual cells and individual Ft-Ds bonds.
- Cells grow in response to:
  - Local morphogen concentration
  - Asymmetry of the Ft-Ds bond distribution
- When cells get big, they divide.





Concentration Profiles Used in Model

(Radially Symmetric in Wing Pouch)

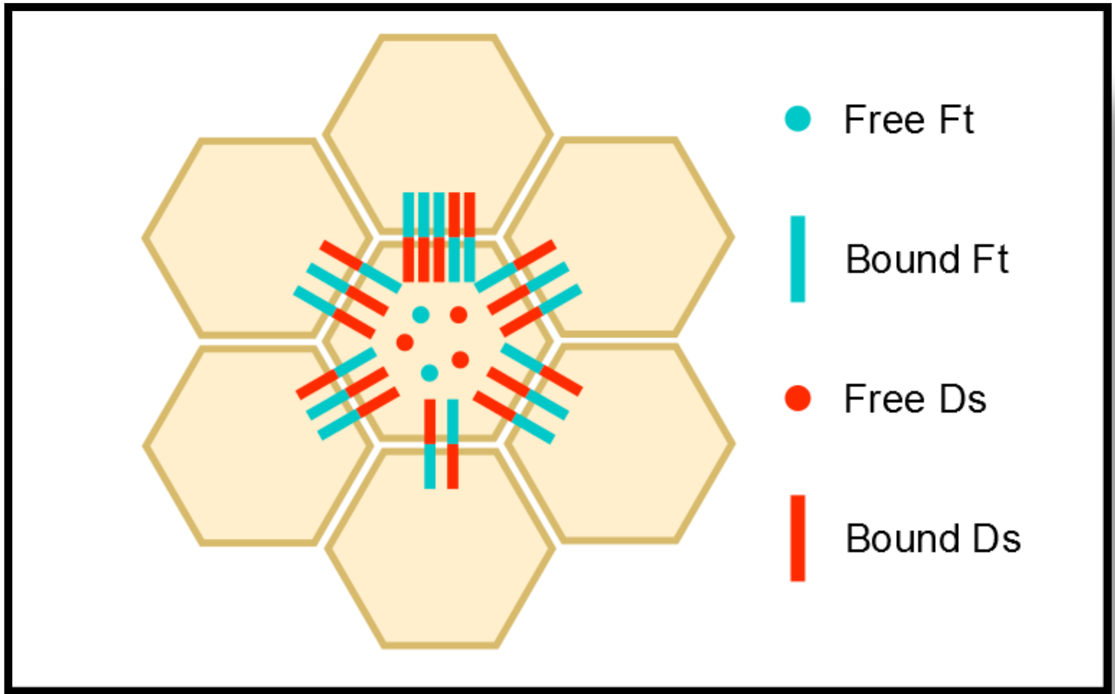


Model Details: Ft–Ds Binding

- Simulation keeps track of which cells are adjacent neighbors.
- Fj phosphorylation makes Ft more likely and Ds less likely to bind.
  - Cells have separate pools of phosphorylated and unphosphorylated Ft and Ds.
- At each time step (1 min):
  - A cell's available (unbound) Ft and Ds are evenly partitioned among all its neighbors, and then the maximum number of possible bonds are formed.
  - Some bonds dissolve.
  - Some unbound Ft and Ds molecules are degraded.
- Number of Ft–Ds bonds at an interface reaches a steady state quickly (~10s of time steps) compared to the cell cycle and Ds front movement (~100s of time steps).

Model: Quantifying Bond Asymmetry

- We measure bond symmetry on a scale of 0 (no bonds with at least one neighbor) to 1 (evenly distributed).
- Asymmetry = (1 – symmetry).

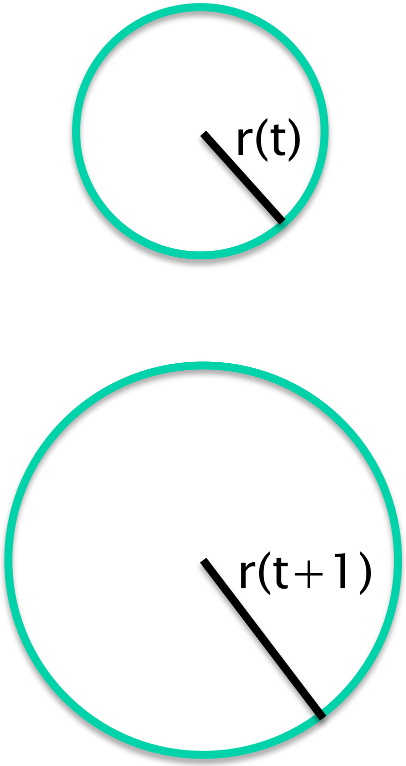


Ft symmetry = (Min. fraction of bound Ft)×(# neighbors)
Ds symmetry = (Min. fraction of bound Ds)×(# neighbors)

In this case, Ft symmetry is 6/7 ⇒ Ft asymmetry is 1/7.
Ds symmetry is 6/12 = 1/2 ⇒ Ds asymmetry is 1/2.

Model: A Cell's Growth Rate

- More morphogen ⇒ faster growth
- More Ft–Ds bond asymmetry ⇒ faster growth
- More unbound Ft and Ds (U(t)) ⇒ slower growth



$$\frac{r(t+1)}{r(t)} = 1 + G_0 \frac{(1 + C_M x_M(t))\,(1 + C_{Ft} x_{Ft}(t))\,(1 + C_{Ds} x_{Ds}(t))}{1 + U(t)}$$

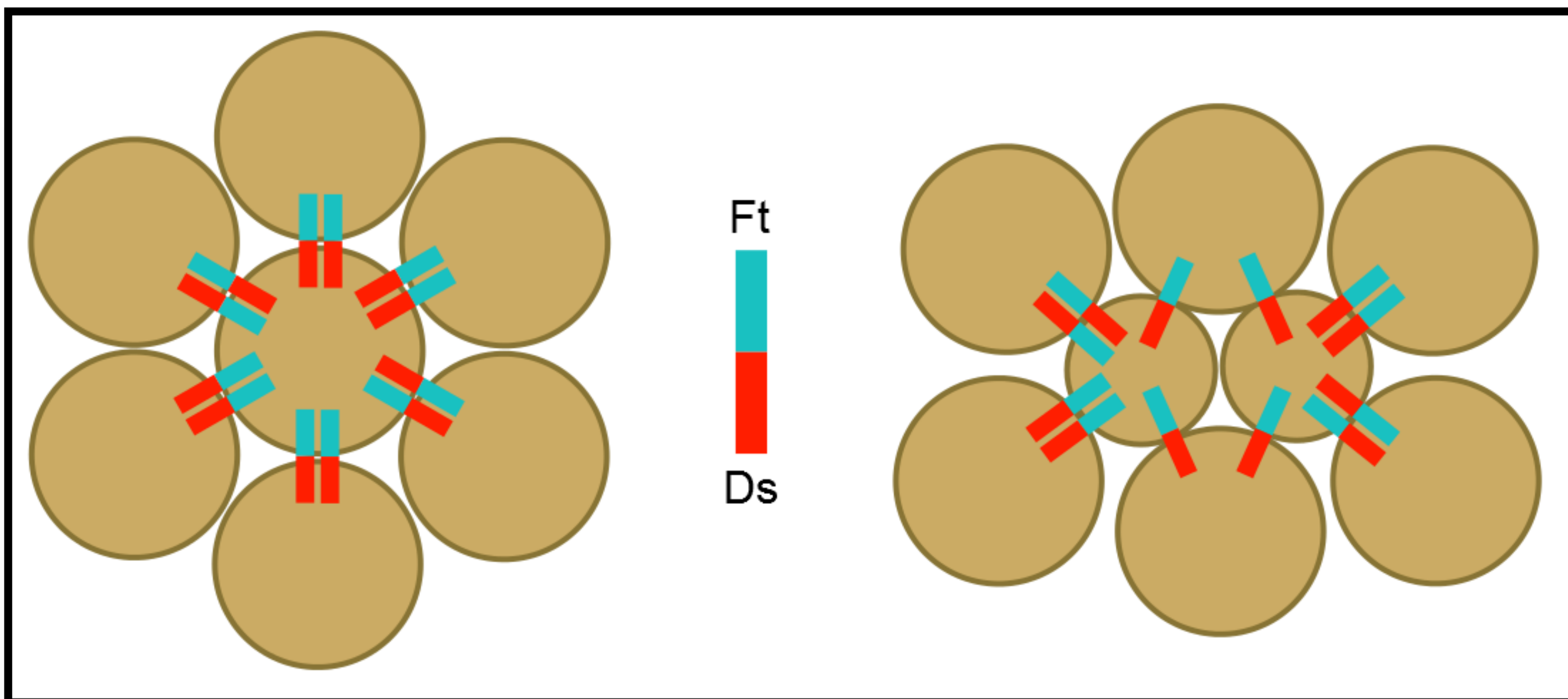
$$x_M(t+1) = G_M \left([M](t+1) - \int_0^t x_M(\tau)\, d\tau \right)$$

$$x_{Ft}(t+1) = G_{Ft} \left(\text{Ft asymmetry}(t+1) - \int_0^t x_{Ft}(\tau)\, d\tau \right)$$

$$x_{Ds}(t+1) = G_{Ds} \left(\text{Ds asymmetry}(t+1) - \int_0^t x_{Ds}(\tau)\, d\tau \right)$$

Model: Cell Division

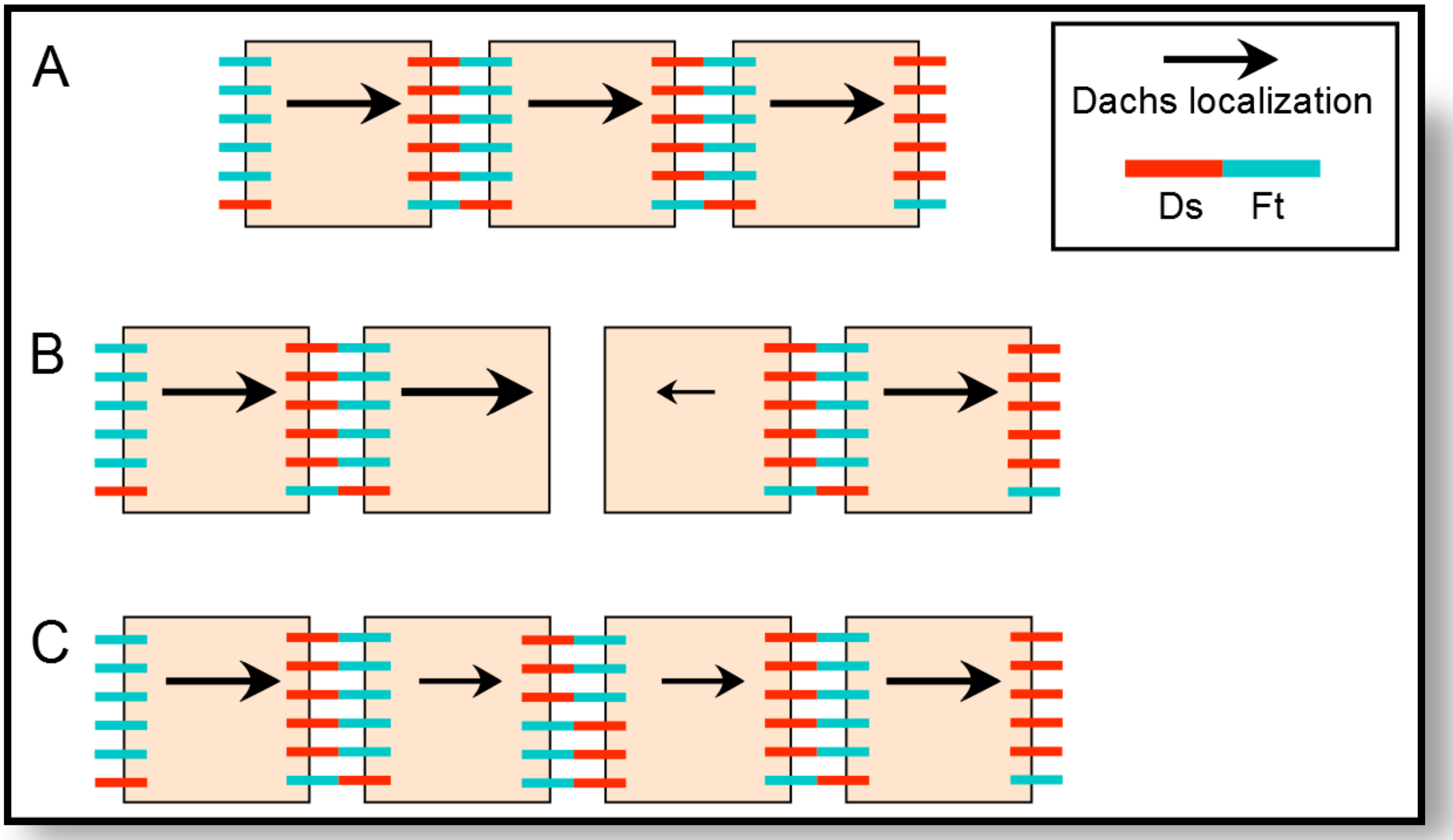
- Cells divide when they grow to a threshold radius, creating a new interface.
- Existing Ft–Ds bonds are distributed to the daughter cells. Bonds with existing neighbors are kept intact as much as possible.



Results

Results: How Cells Remember Polarization After Dividing

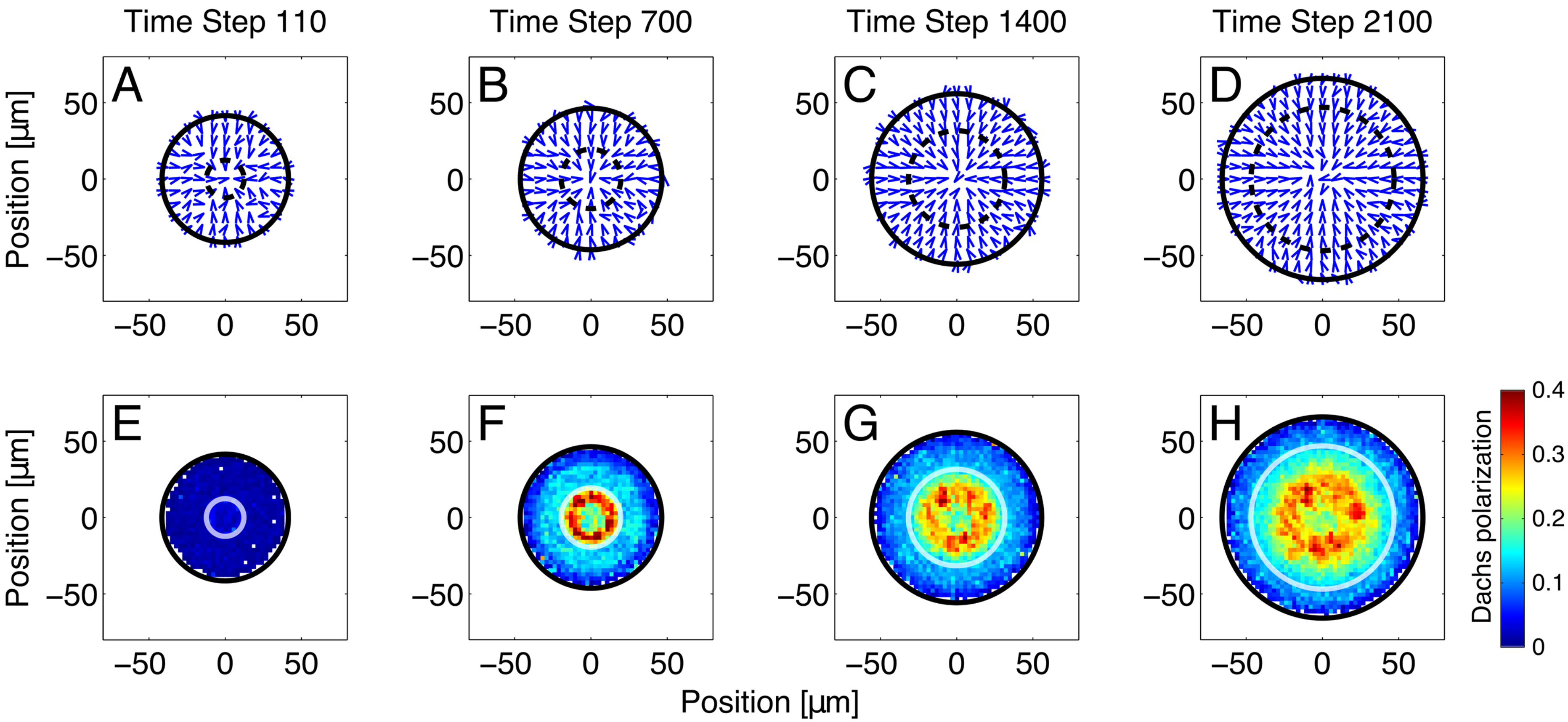
- Cells recover most of their polarization after dividing, since the number of Ft-Ds bonds at an interface quickly reaches a steady state.



Results: Wild Type Polarization

(With Moving Ds Front and Fj Gradient)

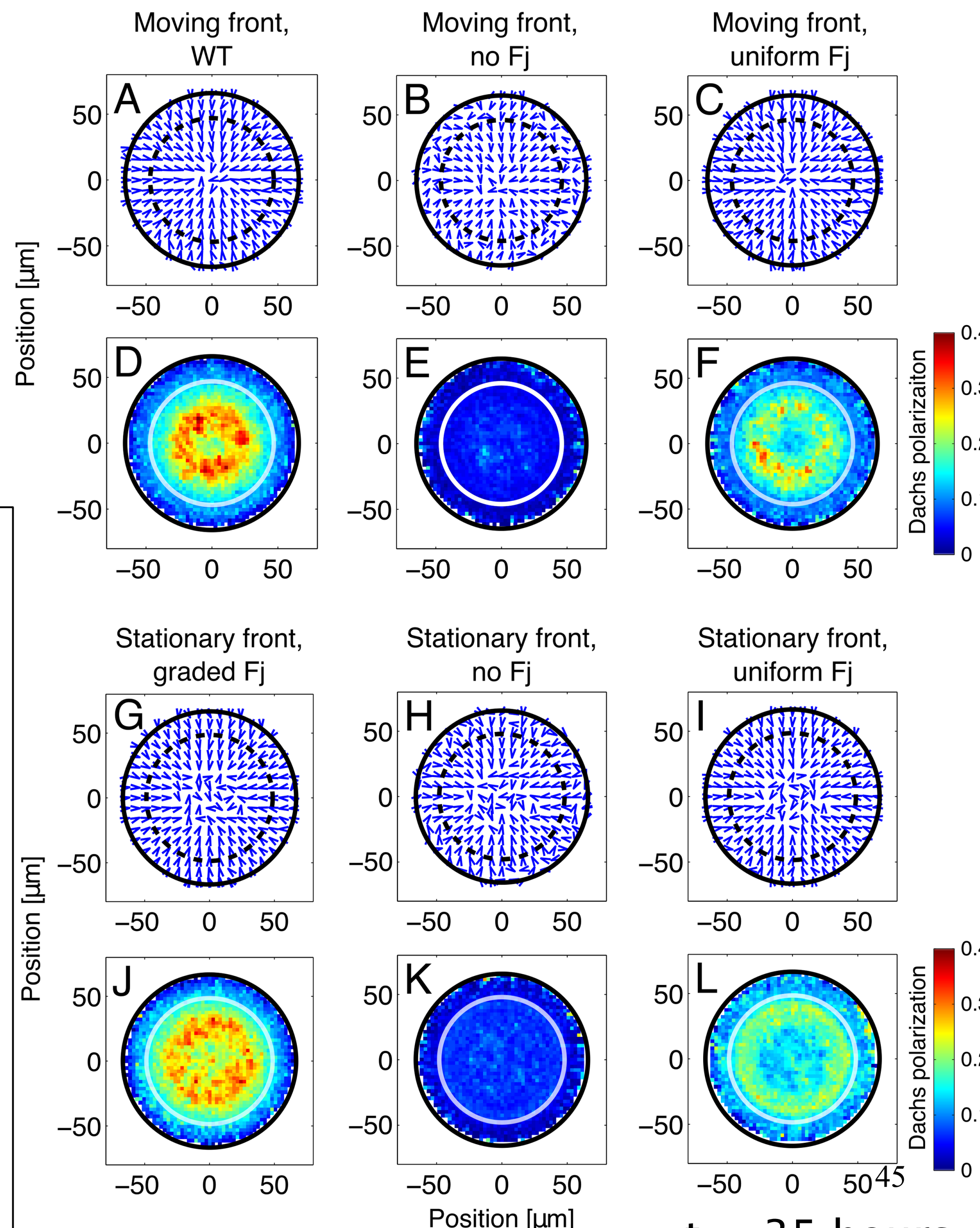
Polarization Direction



Polarization Magnitude

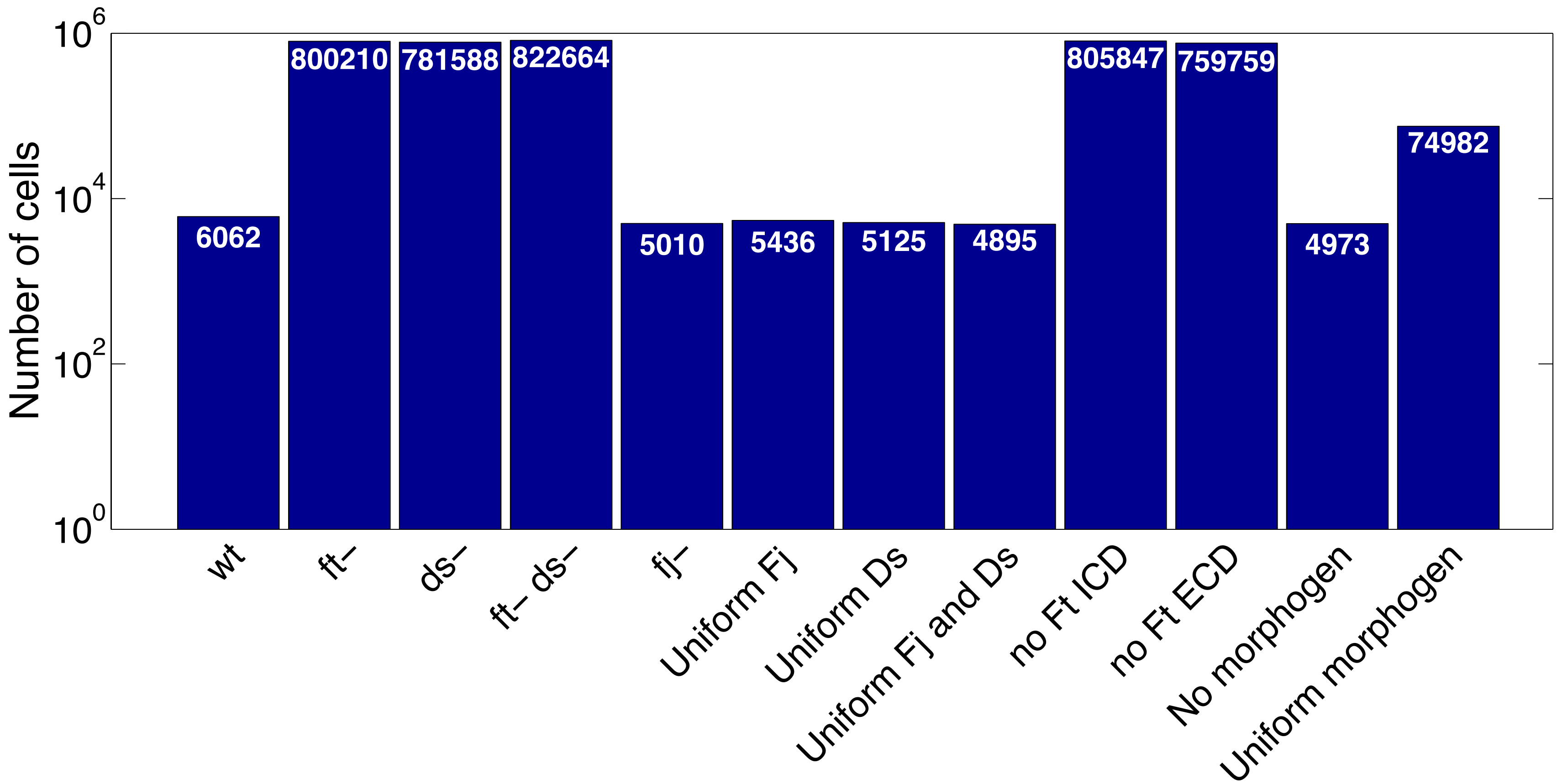
Results:
Dependence of polarization on movement of the Ds front and Fj expression

- What if Ds front is stationary (relative to the radius of the disc)?
- What if there is no Fj?
- What if Fj is spatially uniform (no gradient)?



t = 35 hours

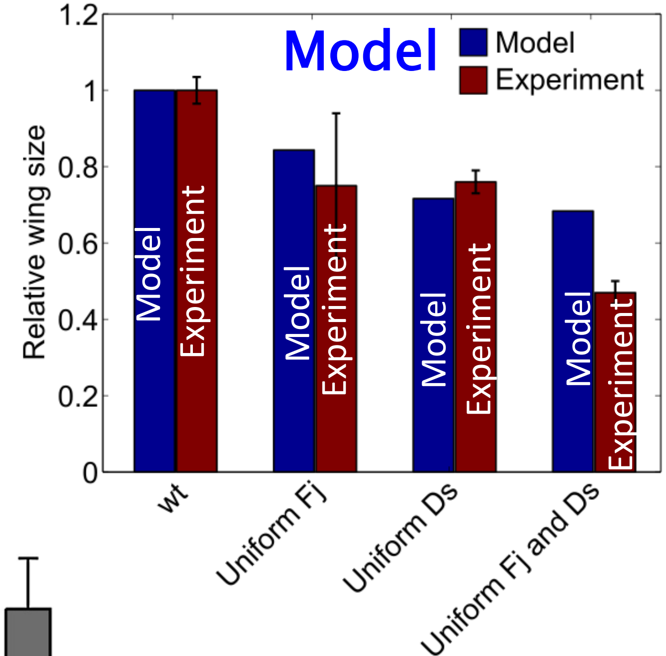
Results: Growth
Size of Disc

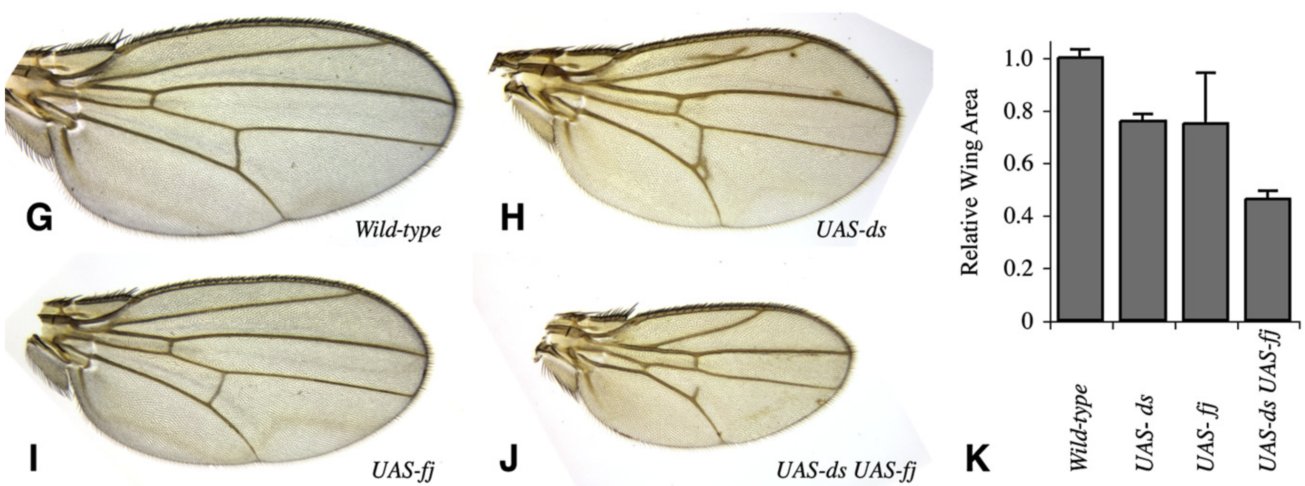


- Disc sizes are qualitatively consistent with experiment.

Results: Discs Uniformly Expressing Ds and/or Fj Undergrow

Experiment: Discs uniformly expressing Ds and/or Fj undergrow. Model: Agreement with experiment because uniform Ds/Fj expression means Ft/Ds bonds are more symmetrically distributed than in wild type (WT).

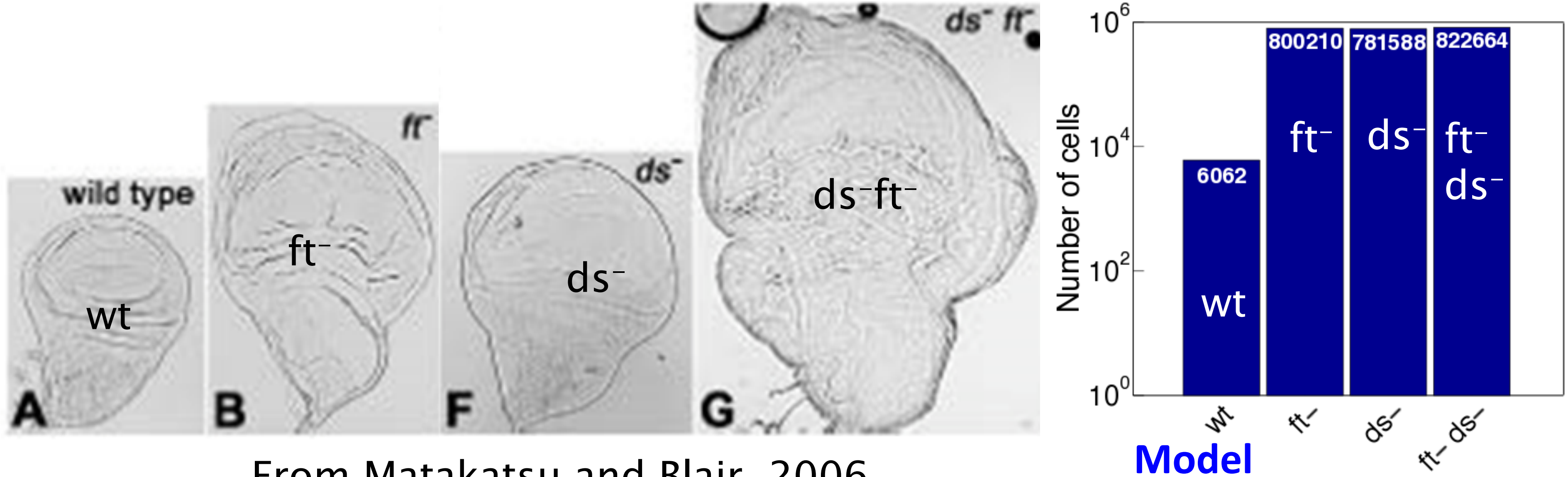




From Rogulja, Rauskolb, and Irvine 2008

Experiment

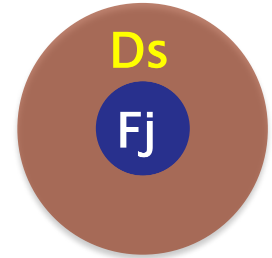
Results: *ft⁻* and *ds⁻* Mutants Overgrow



From Matakatsu and Blair, 2006

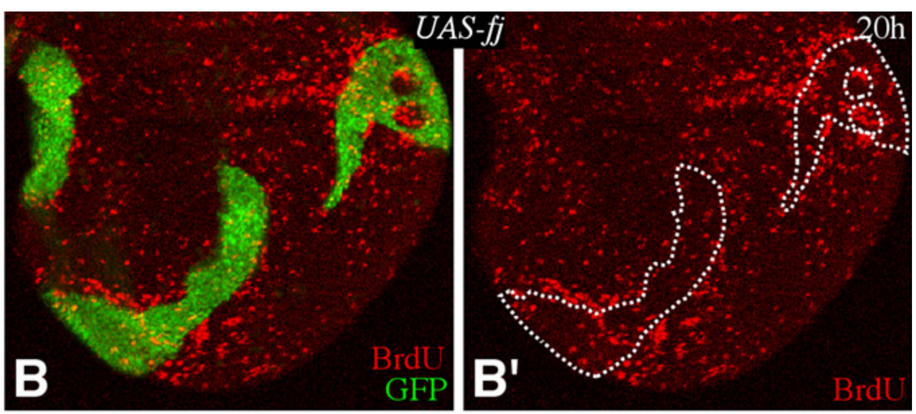
- Model: Lack of Ft–Ds bonds produce overgrowth due to high bond asymmetry.
- Experiment and model agree:
  - ft⁻ mutant overgrows. (Model: Unbound Ds hinders growth)
  - ds⁻ mutant overgrows. (Model: Unbound Ft hinders growth)
  - ds⁻ft⁻ double mutant overgrows even more. (Model: No unbound Ft or Ds to hinder growth.)

Results: Growth Around Fj–Overexpressing Clone Boundary

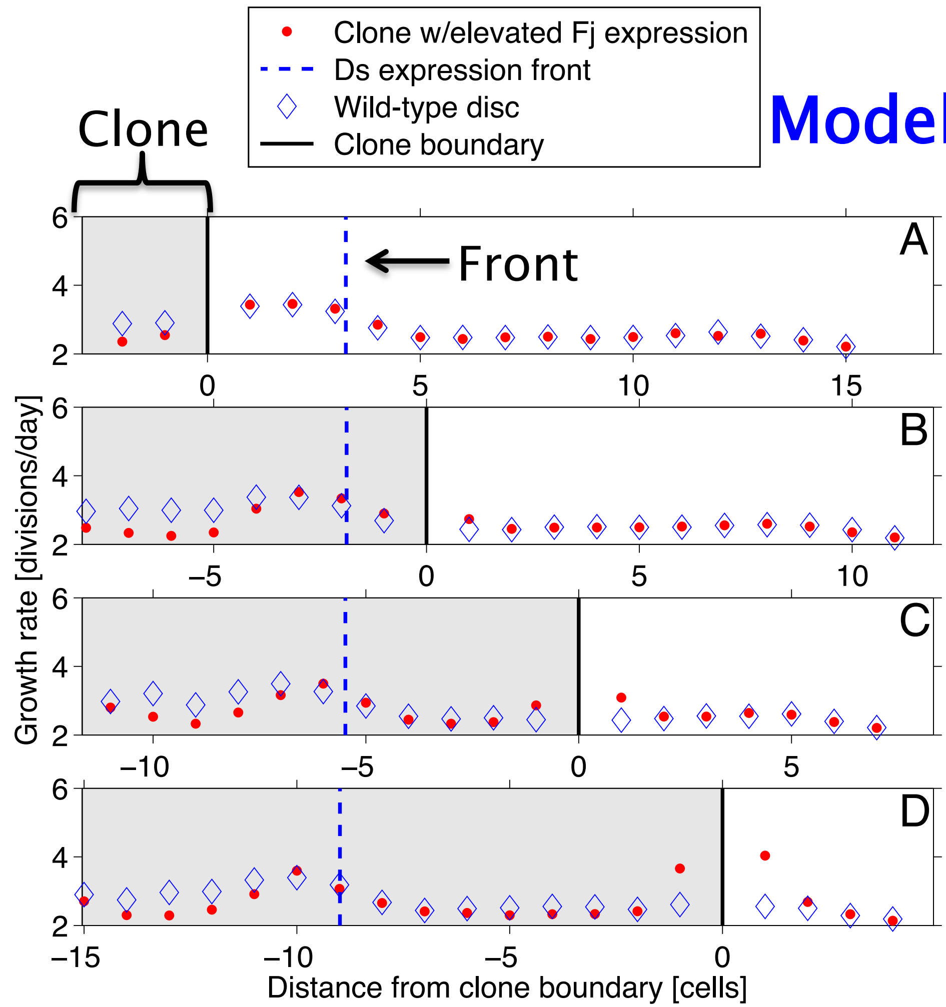


- Qualitative agreement between experiment and model.
- Growth is fastest around the Fj clone boundary when clone is outside the front (wing pouch), where there is the greatest discrepancy in Fj.
- Model: Neighboring cells with large differences in Fj have strongly polarized (asymmetric) bonds and hence tend to grow faster.

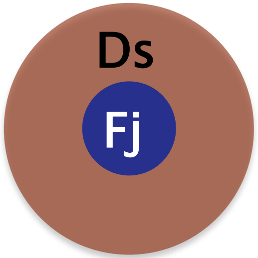
Fj–overexpressing clones



From Rogulja, Rauskolb, and Irvine 2008

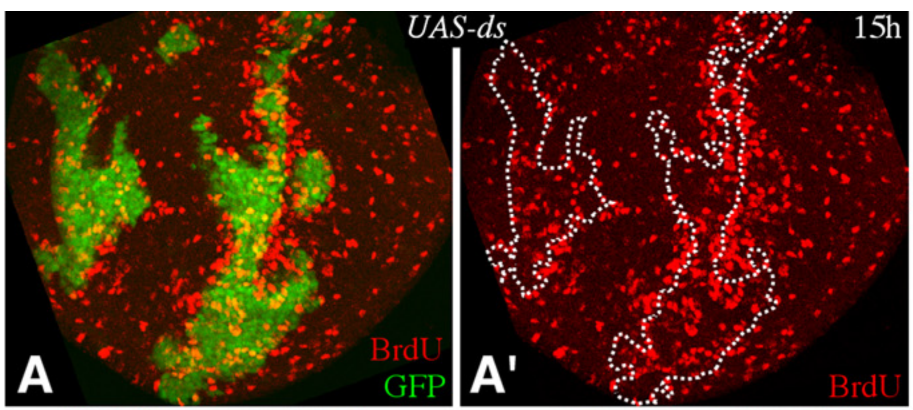


Results: Growth Around Ds–Overexpressing Clone Boundary

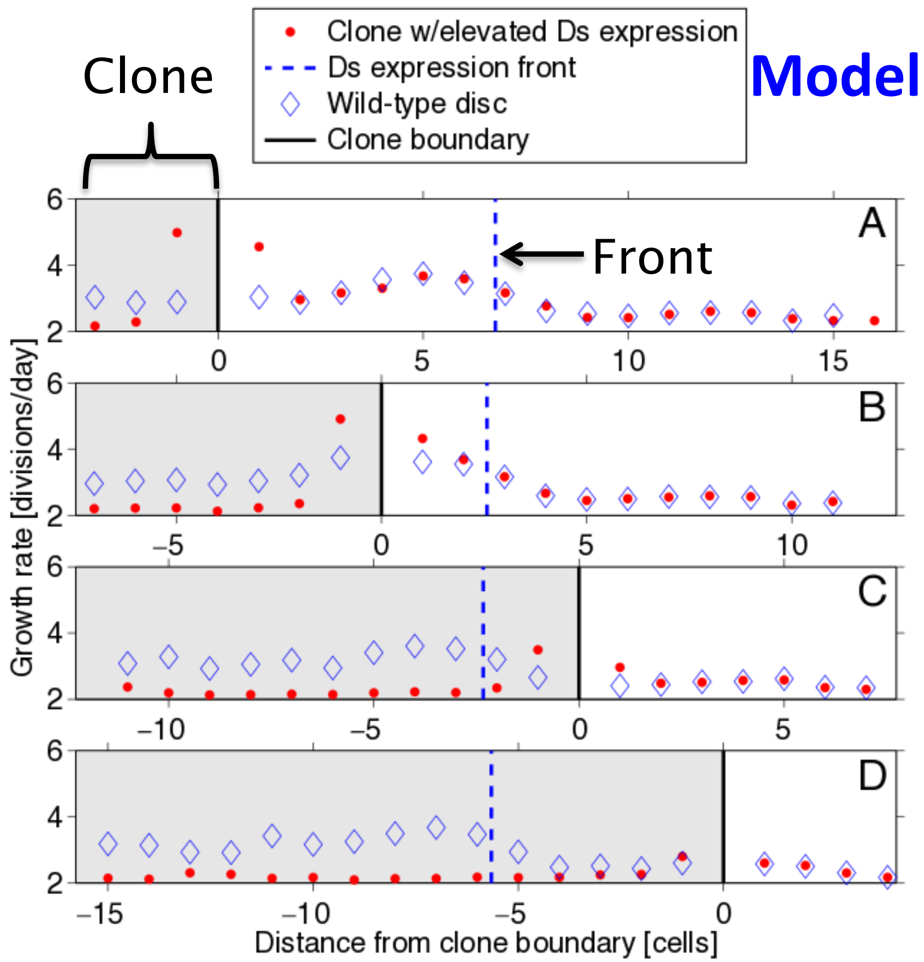


- Qualitative agreement between experiment and model.
- Growth is fastest around the clone boundary when clone lies inside the front (wing pouch), where there is the greatest discrepancy in Ds.
- Model: Neighboring cells with large differences in Ds have strongly polarized (asymmetric) bonds and hence tend to grow faster.

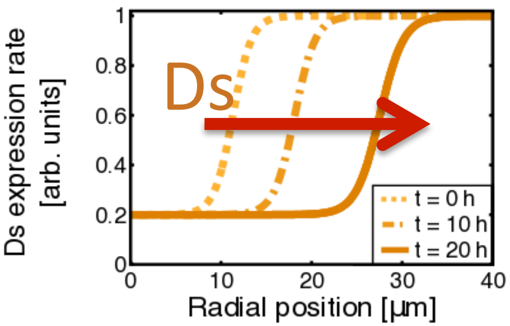
Ds–expressing clones

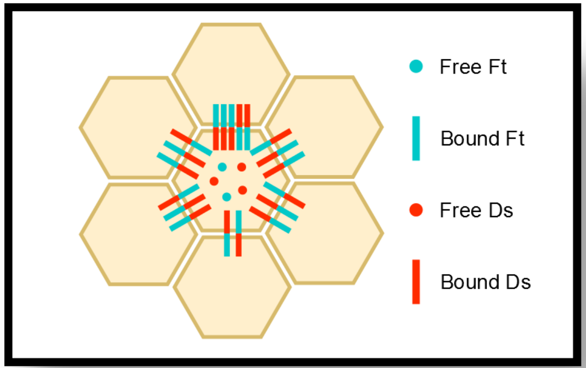


From Rogulja, Rauskolb, and Irvine 2008



Summary





- Polarization:
  - Asymmetric distribution of Ft–Ds bonds around a cell's periphery produces Dachs polarization.
  - Our model indicates that
    - The Fj gradient together with the expanding Ds expression front can polarize cells throughout the wing pouch.
    - Cells can recover their polarization naturally after dividing.
    - Cell polarization retains a memory of the expanding Ds front that passed by, even after cell division.
- Growth:
  - More cell polarization produces more growth.
  - Our model for growth gives results that are consistent with experiment.

THE END

Extra Slides

Model Parameter Dependence

- Initial wing disc: 1000 cells
- Run for 48 hours
- Slow Ft-Ds bond dissociation: 10x slower than WT.
- Slow degradation of Ft and Ds: 10x slower than WT.
- Fast Ft-Ds bond dissociation: 10x faster than WT.
- Fast degradation of Ft and Ds: 10x faster than WT.