Aspen Center for Physics Winter Conference on Biological Physics

Growth and Form: Pattern Formation in Biology

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Regulation of Growth in the *Drosophila* Wing Disc via the Fat-Dachsous Pathway

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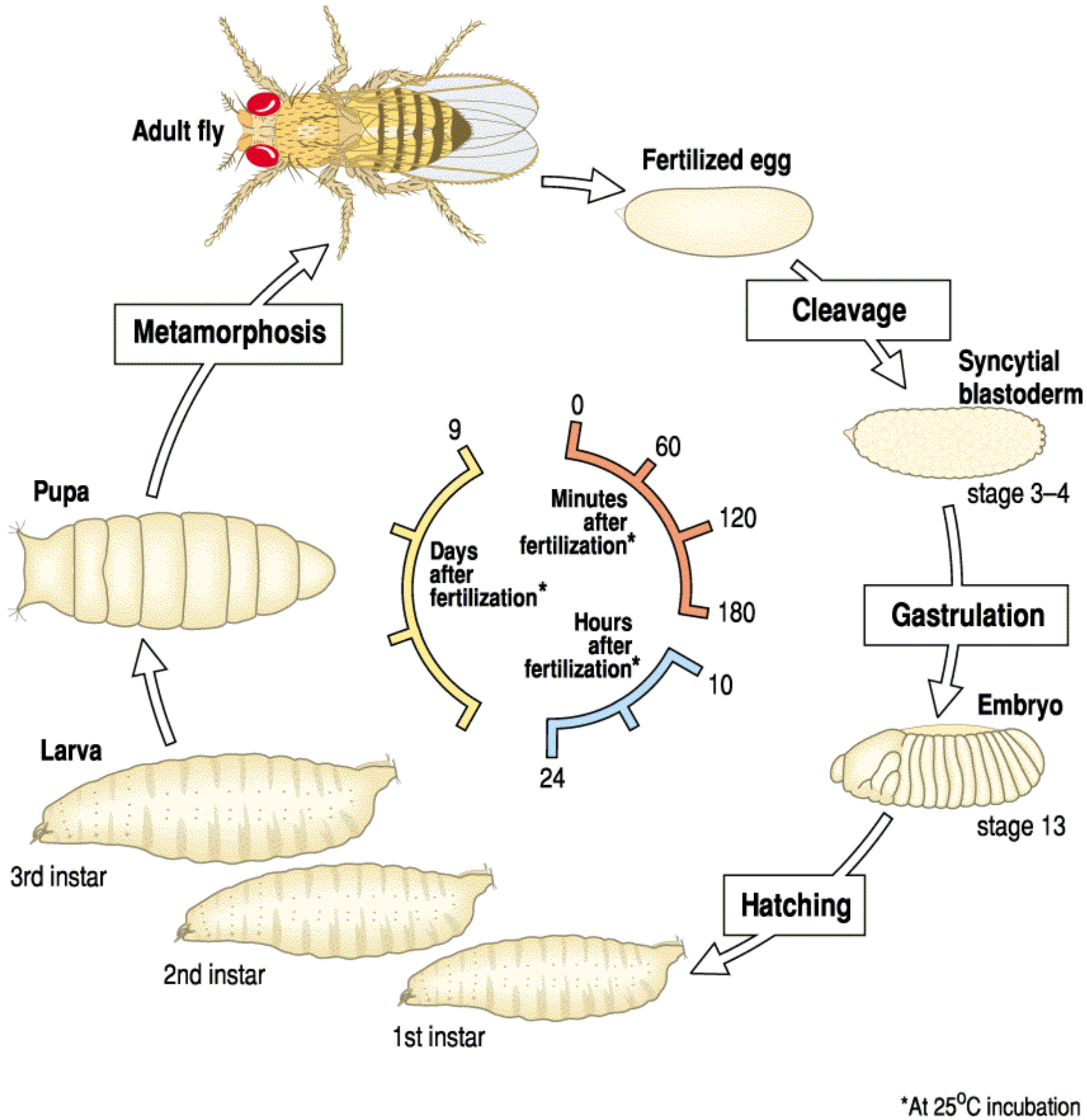
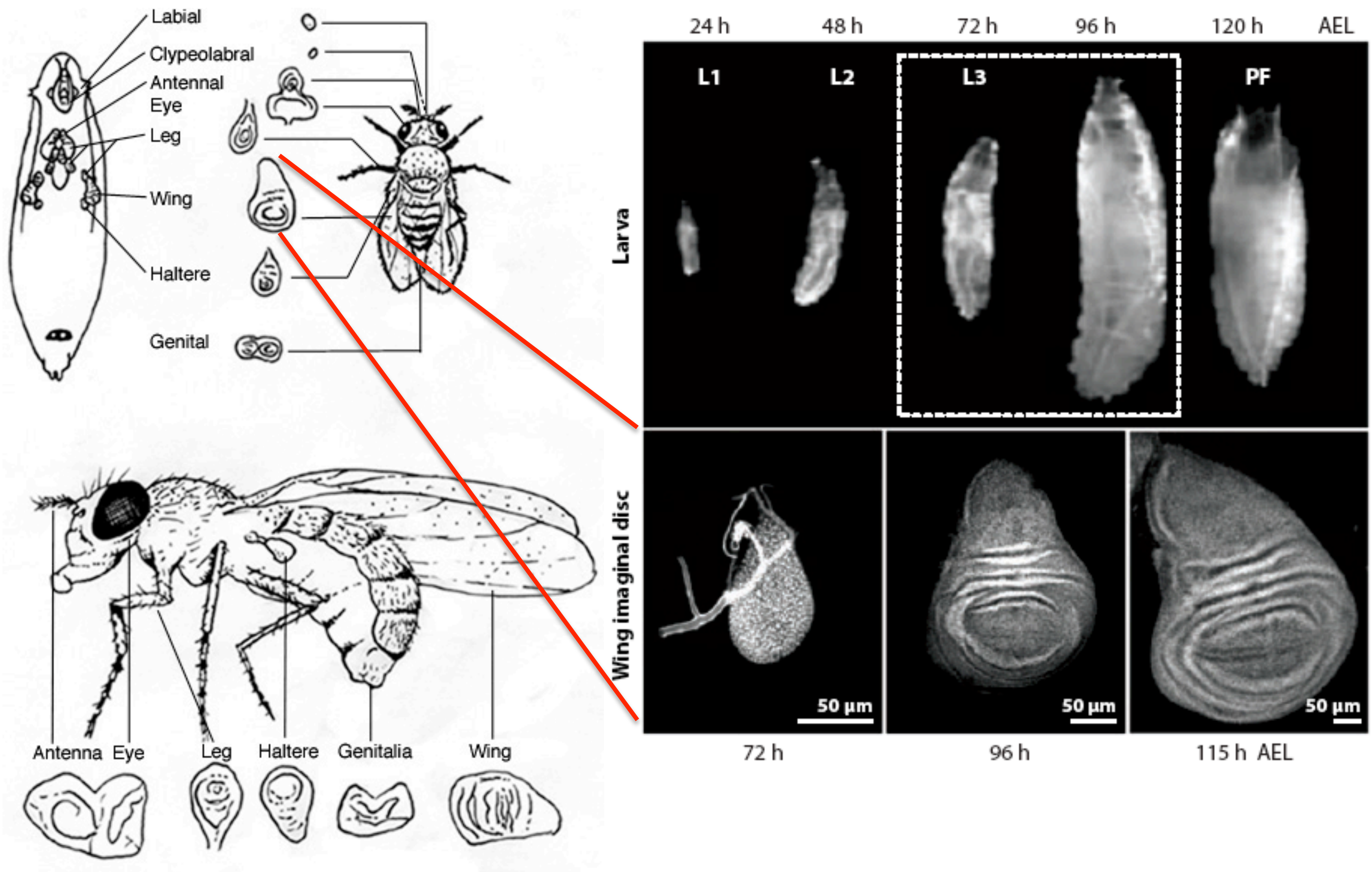
Funded by NIH through UCI Center for Complex Systems Biology

Life cycle of *Drosophila* (fruit fly)



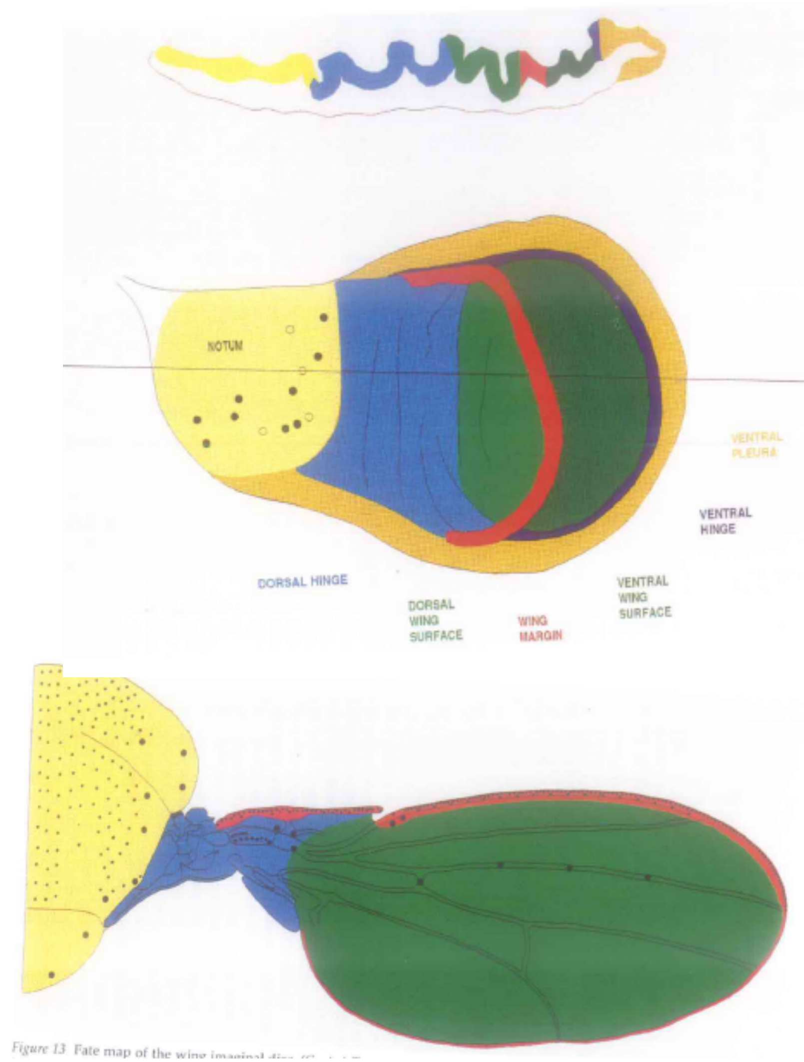
Imaginal Discs

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



Wing Imaginal Disc

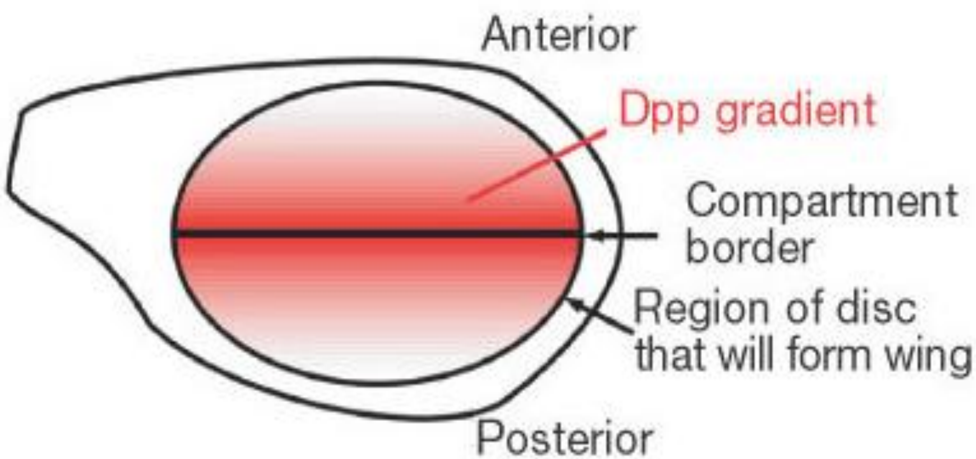
- *Drosophila* wing disc grows from about 40 to 50,000 cells over 4 days



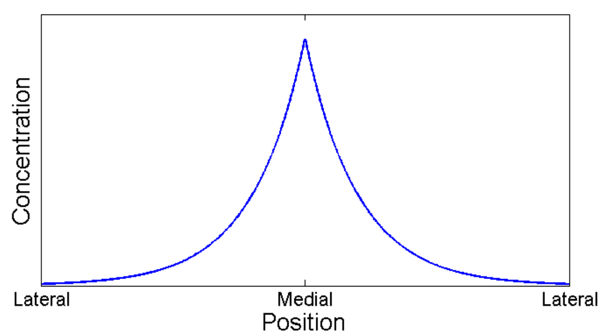
Campuzano and Modolell, 1992; Cohen 1993

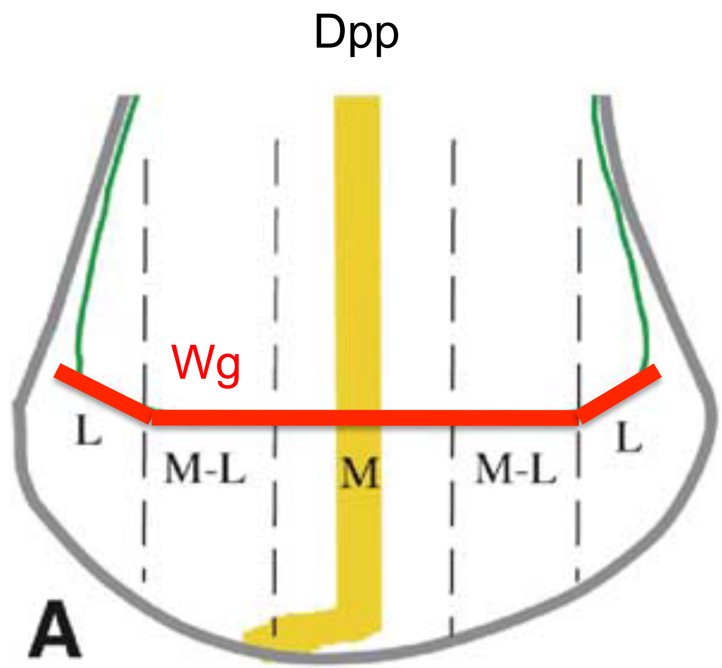
How is growth regulated in a developing embryo?

Morphogen Gradients Produce Growth and Patterning



Gurdon and Bourillot, *Nature* (2001).

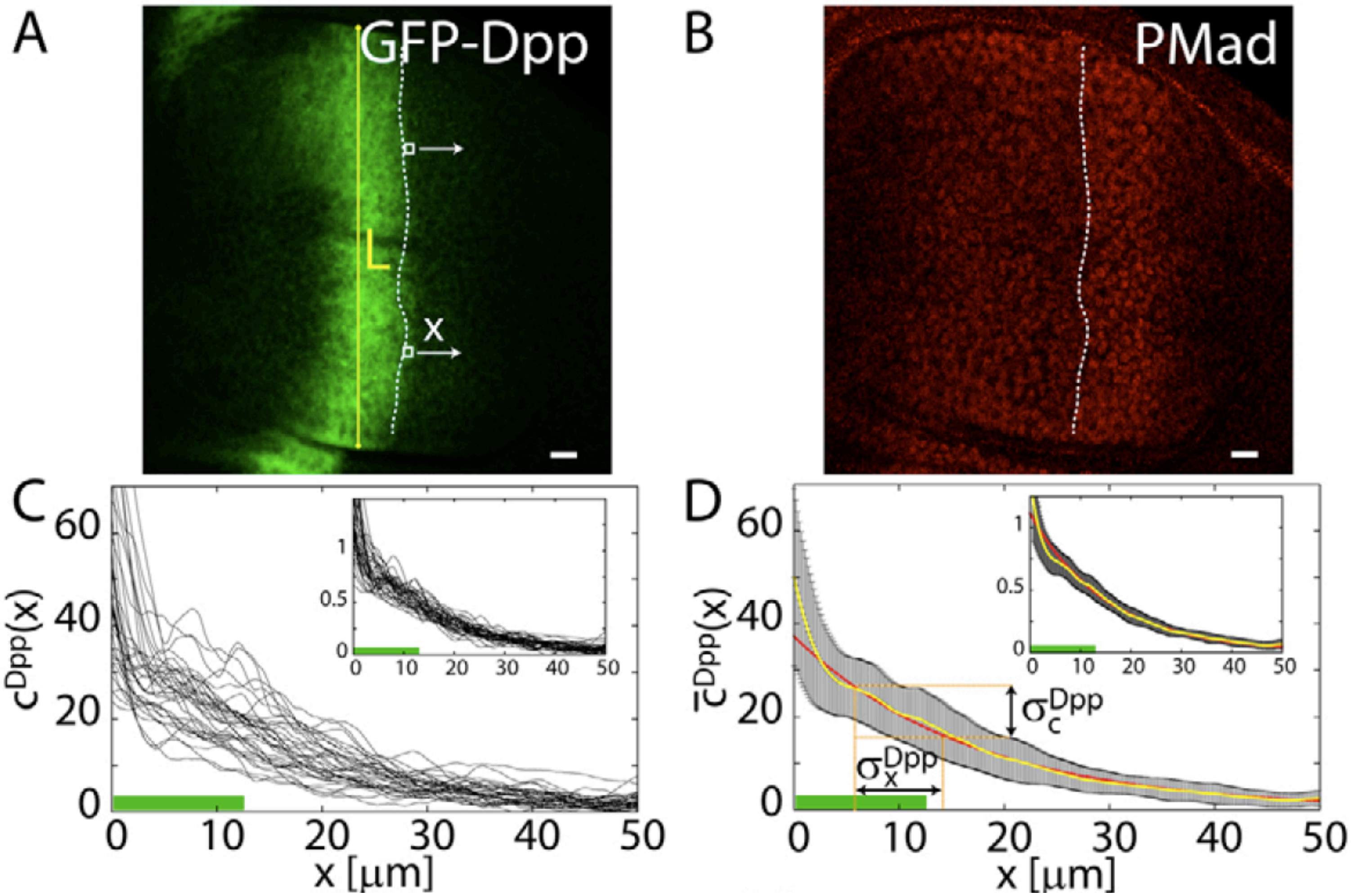




Rogulja and Irvine *Cell* (2005).

Morphogens Decapentaplegic (Dpp) and Wingless (Wg) are necessary for wing growth.

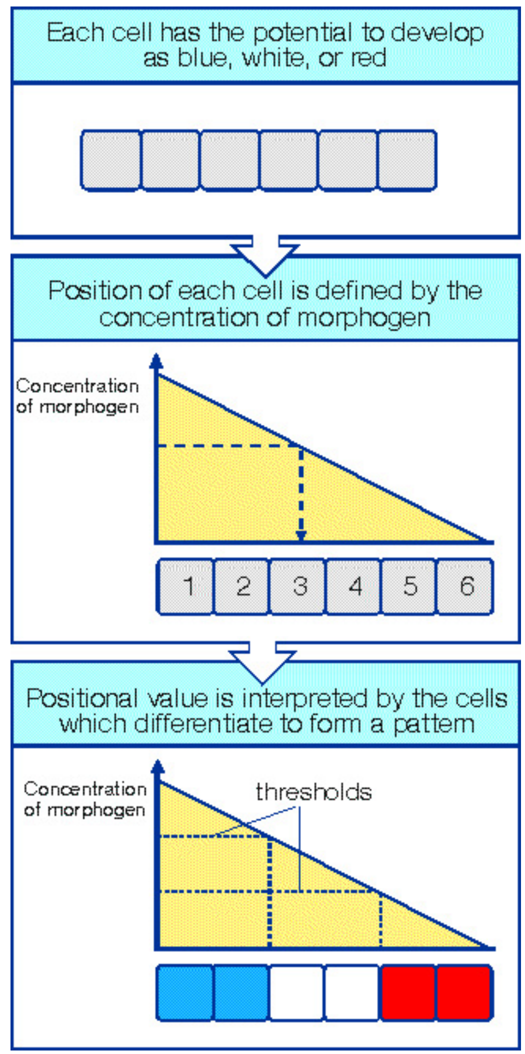
The Dpp Gradient



Bollenbach et al., 2008

French Flag Model of How Morphogens Produce Patterning
(Lewis Wolpert (1969))

- Concentration of morphogen determines cell fate.



J. Theoret. Biol. (1969) **25,** 1–47

Positional Information and the Spatial Pattern of Cellular Differentiation†

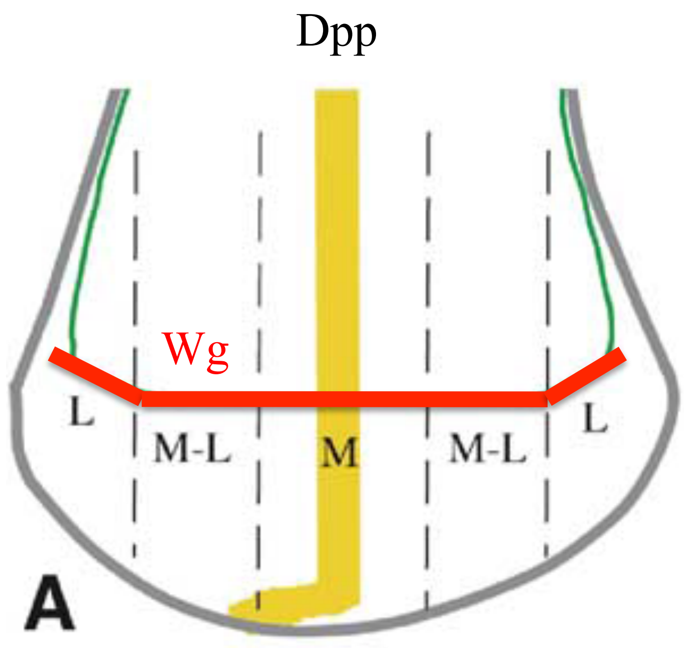
L. Wolpert

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

(*Received* 1 *July* 1969)

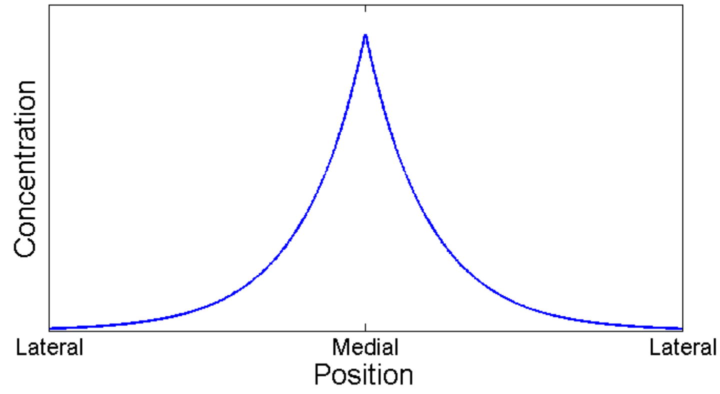
Wing Disc Grows Uniformly

- Dpp and Wg necessary for growth of wing disc
- Dpp and Wg concentrations decay spatially
- Yet wing disc grows uniformly
- Why?



Rogulja and Irvine *Cell* (2005).

How can the wing disc grow uniformly when the morphogens decay spatially?



Previous Models of Growth in the Wing Disc

- French Flag model does not work (Wolpert).
- Spatial morphogen gradient (slope not concentration) determines growth (Ken Irvine).
- Relative rate of change of morphogen signal determines growth (Wartlick, … Julicher,Gonzalez-Gaitan).
- Elastic stress determines growth (Shraiman).

Experiment: Nonautonomous growth around Fj and Ds-overexpressing clones

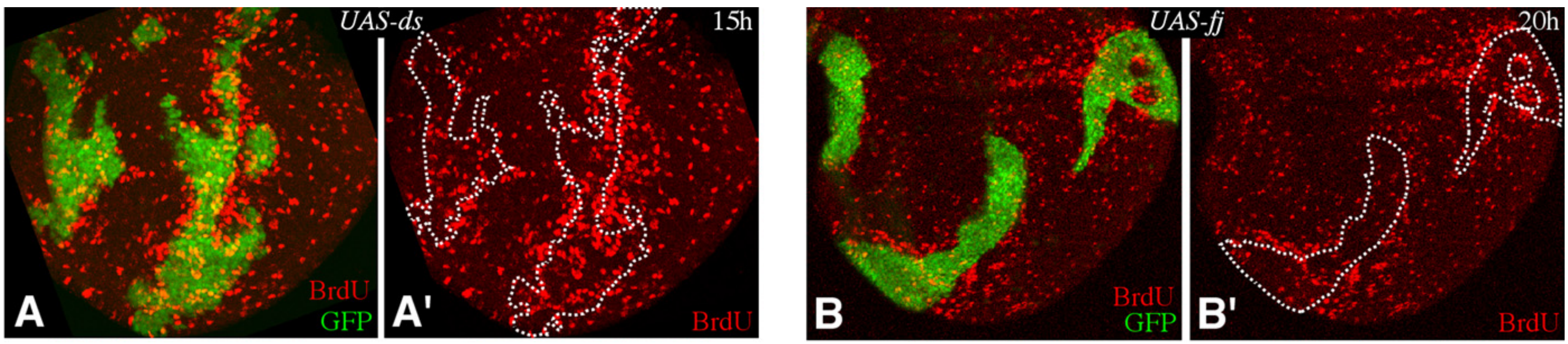
Experiment:

• Nonautonomous growth around the edge of Fj and Ds-overexpressing clones.

• For Ds-overexpressing clones, growth more pronounced in center of disc where there is less Ds than near disc edge where Ds is abundant.

• For Fj-overexpressing clones, growth more pronounced near edge of disc where there is less Fj than in center of disc where Fj is abundant.

Disproves French Flag Model: Differences in morphogen concentration determine growth.

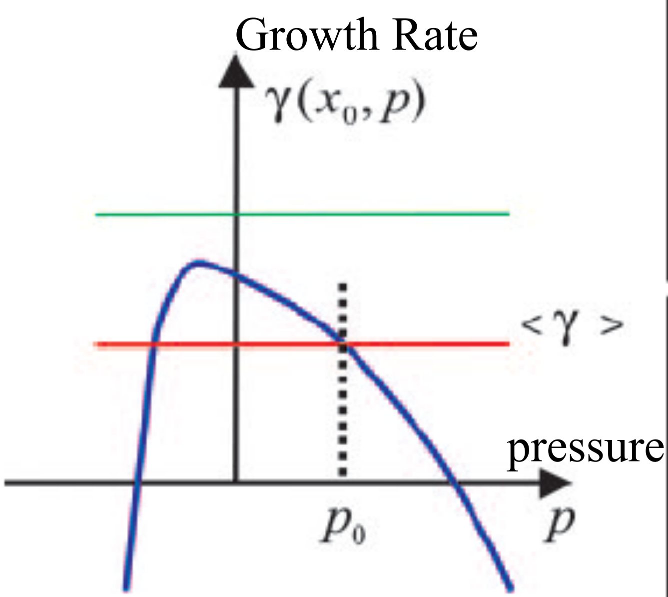


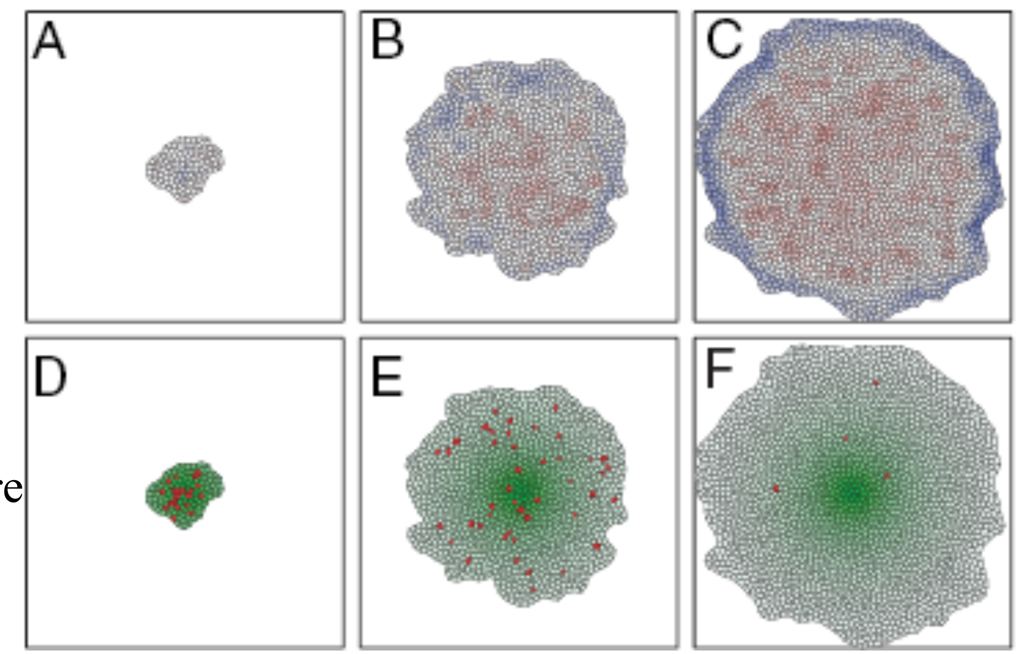
From Rogulja, Rauskolb, and Irvine 2008

Another possibility:
Elastic Stress Affects Growth

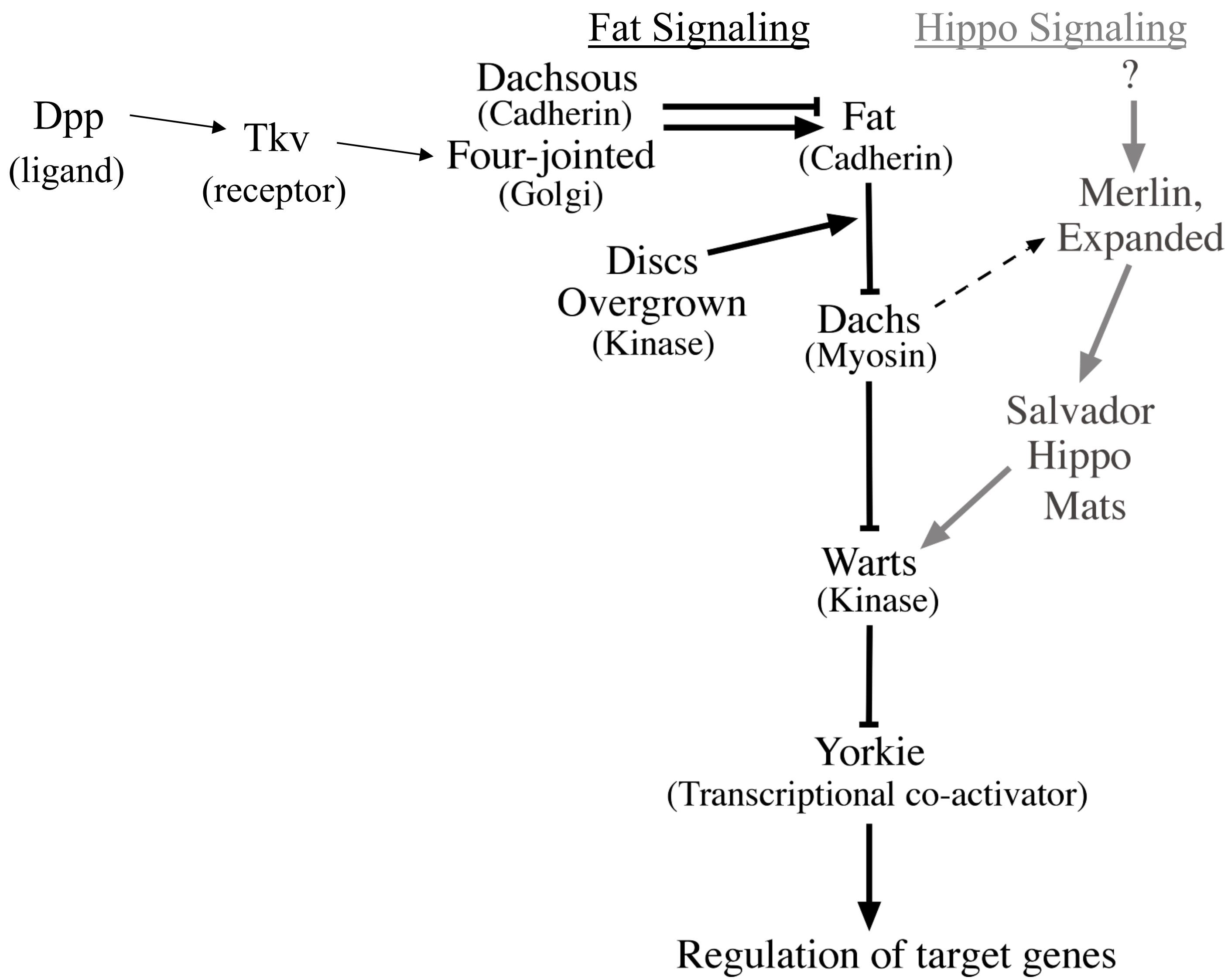
(Shraiman *PNAS (2005)*; Hufnagel *et al. PNAS* (2007))

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



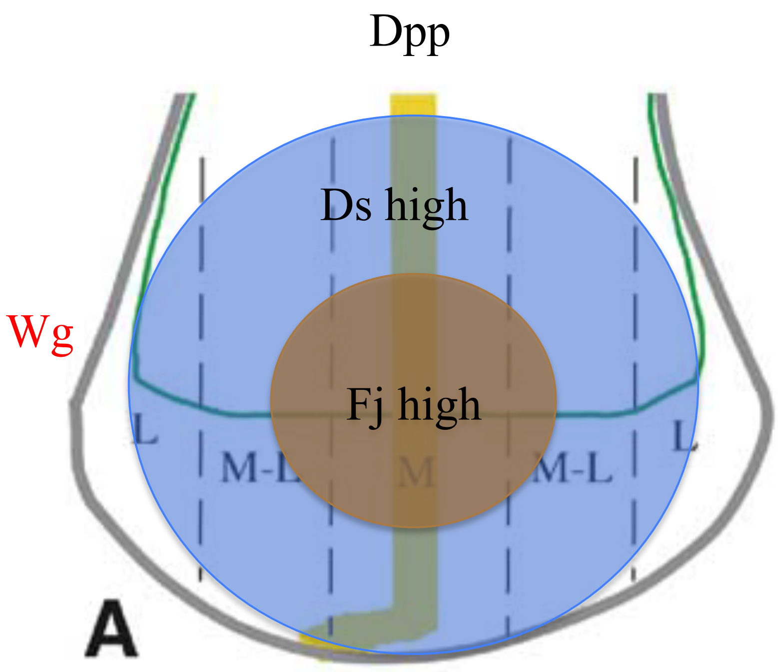


Fat (Hippo) Signalling Pathway

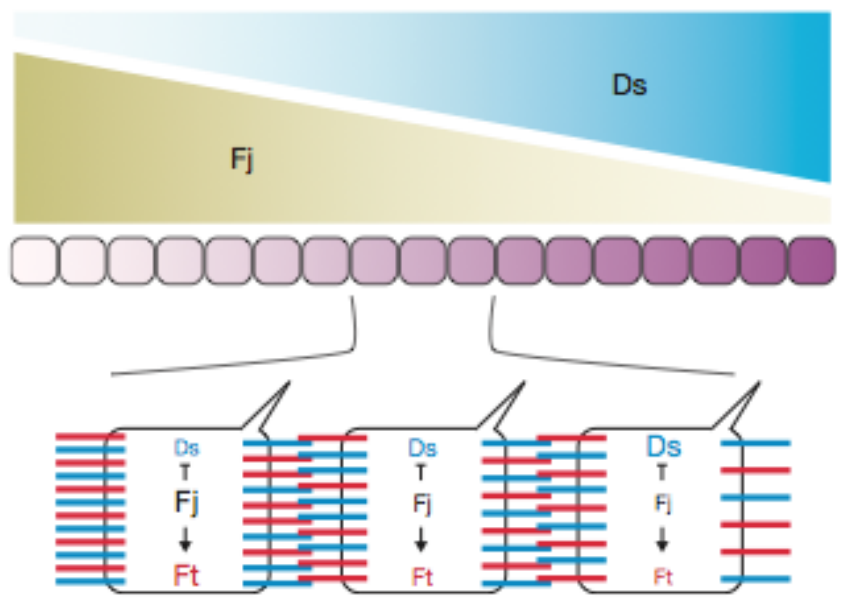


From Ken Irvine

Dpp and Wg Gradients Reflected in Dachsous (Ds) and Four-jointed (Fj) Gradients



Rogulja and Irvine *Cell* (2005).

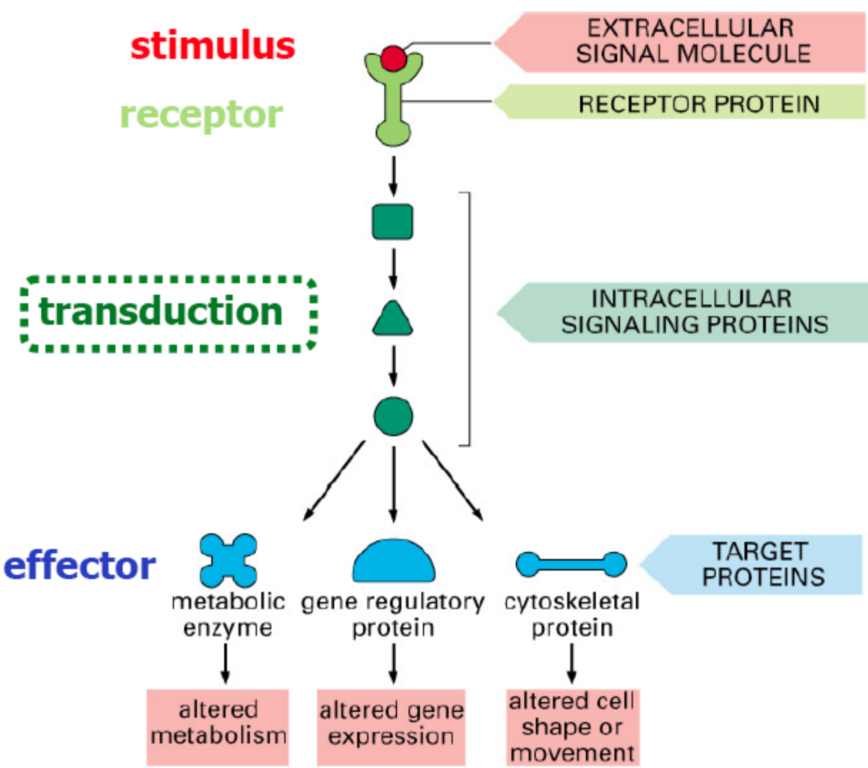


From Lawrence, Struhl, and Casal (2008)

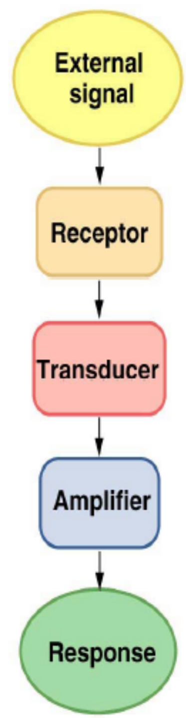
Fj is a Golgi kinase that makes Fat (Ft) more likely to bind and Ds less likely to bind (Simon *et al*. 2010). Ft is uniformly expressed.

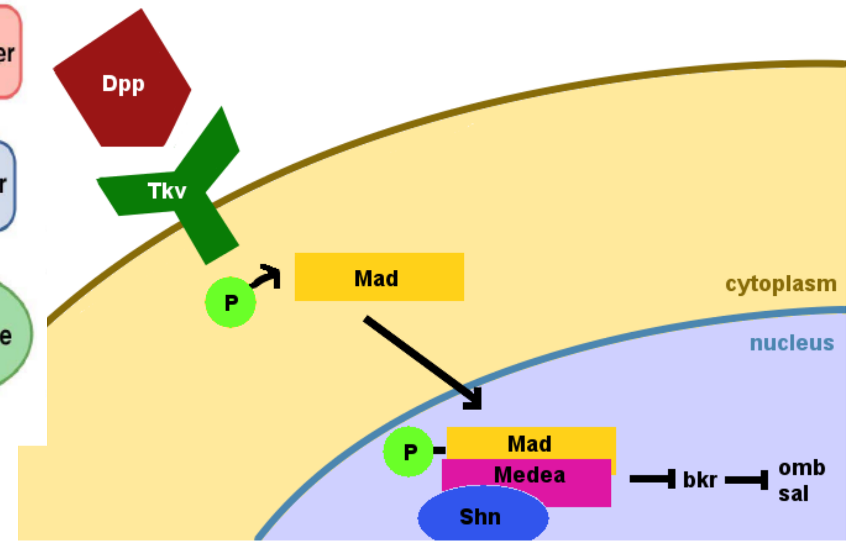
In a signaling pathway, what do the signaling proteins do? How do they act?

- Signaling between cells is usually thought to be chemical, not mechanical
- Chemical signaling: ligand binds to receptor
- What is new and different here is that we consider mechanical signaling



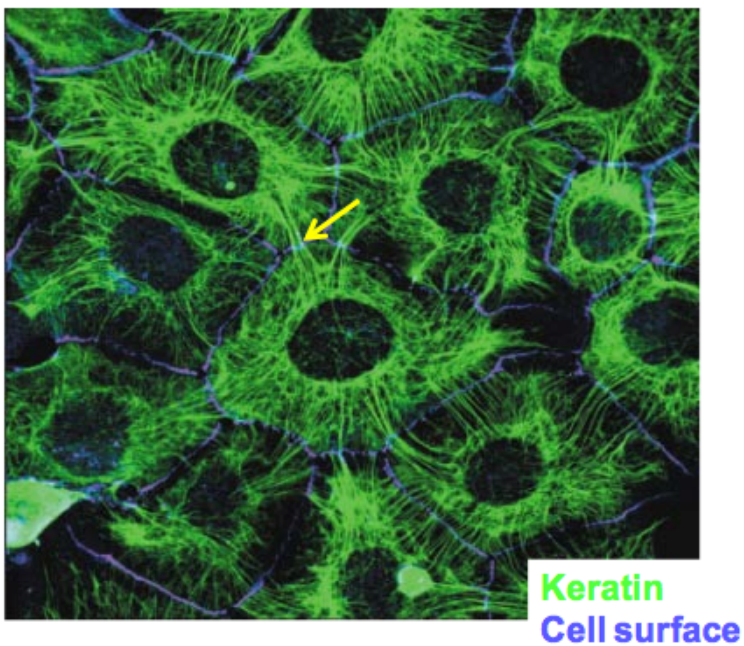
From Lan Huang

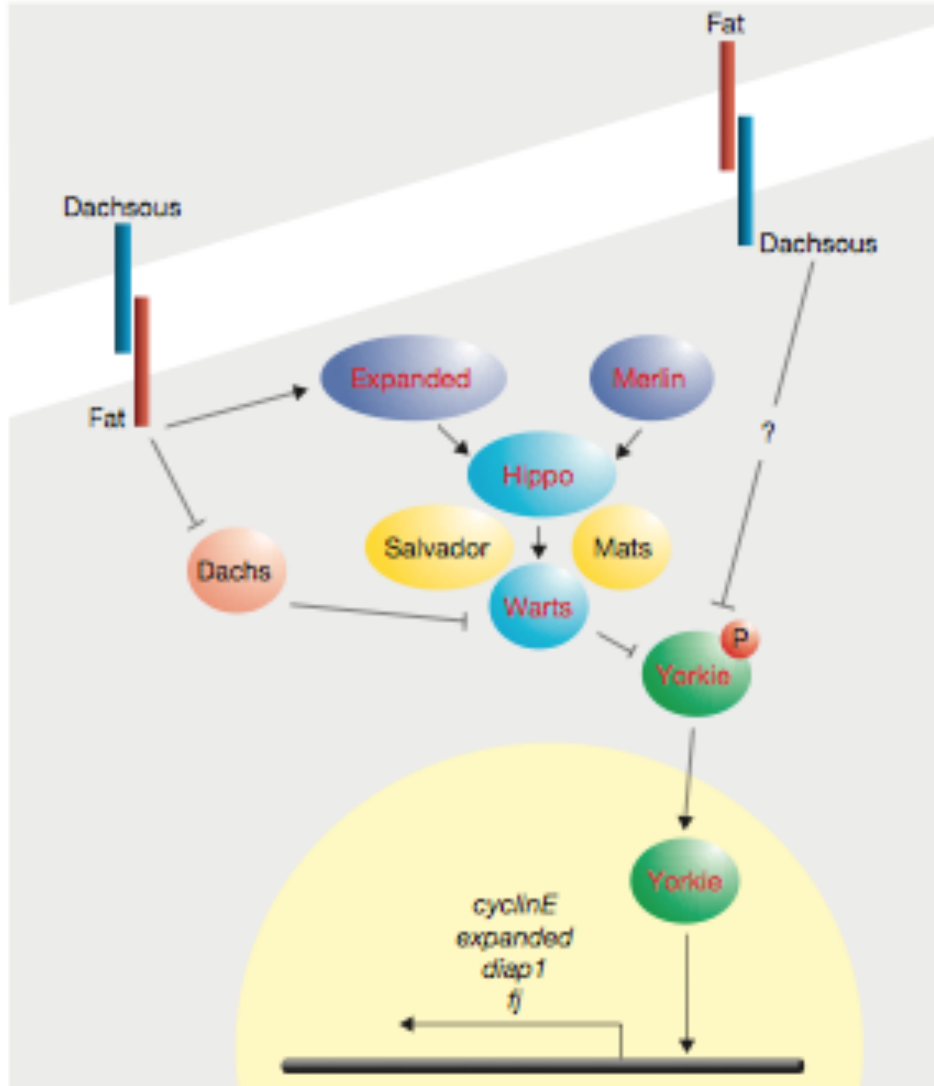




Signaling Pathway

• Adjacent cells mechanically coupled by protocadherins Fat (Ft) and Dachsous (Ds).
• Protocadherins are connected to the cytoskeleton.
• Fj is a Golgi kinase that makes Fat (Ft) more likely to bind and Ds less likely to bind (Simon *et al*. 2010).
• Ft is uniformly expressed.

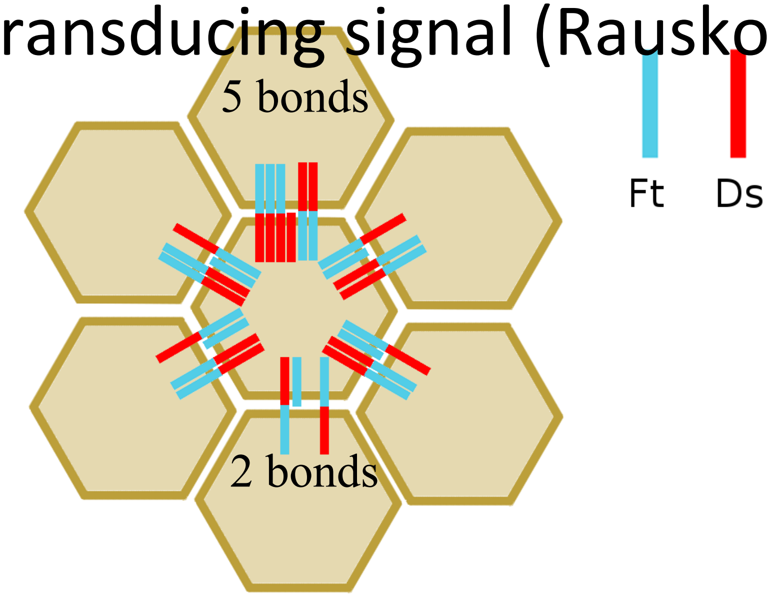




From Lawrence, Struhl, and Casal (2008)

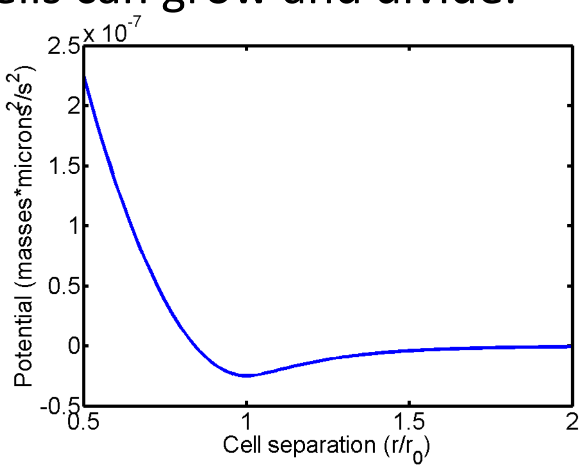
Our Asymmetric Push-Pull Idea

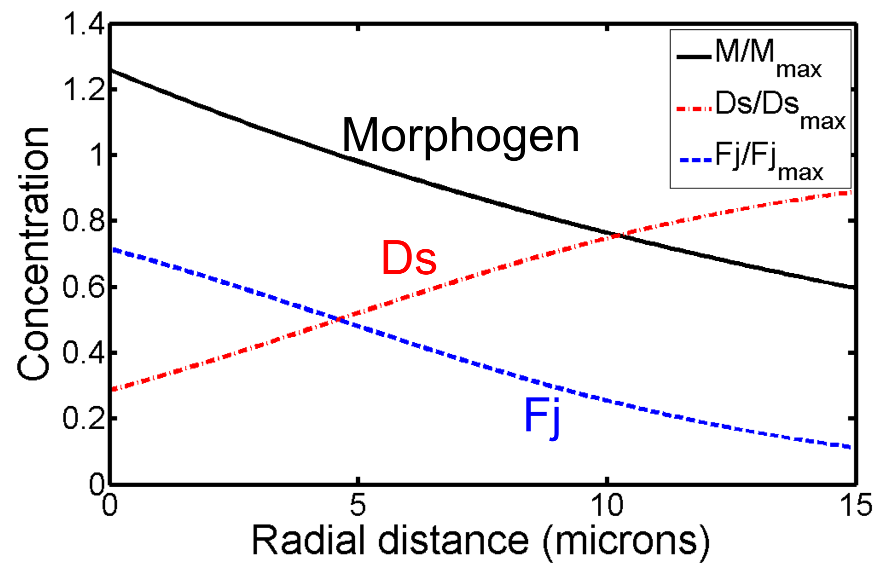
- Mechanical (elastic) interactions via protocadherins (Fat/Dachsous) between cells affect cell growth.
- Protocadherins are connected to the cytoskeleton.
- Asymmetric forces (Ft-Ds bonds) on a cell enhance its growth.
- Speculation: Unconventional myosin Dachs and protein Zyxin may be involved in transducing signal (Rauskolb *et al.* (2011)).



Simulations of Wing Disc

- Single layer of cylindrical cells
- Radial morphogen gradient (Dpp + Wg) decays exponentially with decay length of 20 microns (Kicheva *et al* 2007).
- Gradients in Fj and Ds determined by morphogen.
- Ft uniformly expressed.
- Elastic interactions between neighboring cells (*not* through Ft and Ds) keep cells from drifting apart or overlapping too much. Cells can move in response to forces from neighbors. These forces do not affect growth.
- Cells keep track of number of Ft/Ds bonds with each neighboring cell and number of unbound Ft and Ds.
- Cells can grow and divide.





Cell Growth

Cell radius = R_n at time step n

$$\frac{R_{n+1}}{R_n} = \left(1 + \frac{G_0}{(1+F)(1+D)(1+U)}\right)$$

G_0 = Base growth rate of cell

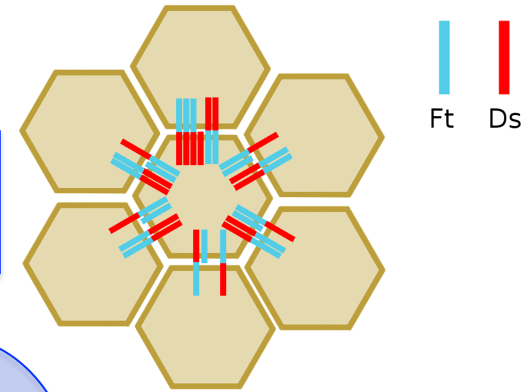
$F \propto$ (Minimum fraction of bound Ft among neighbors) $\times$ (number of neighbors)

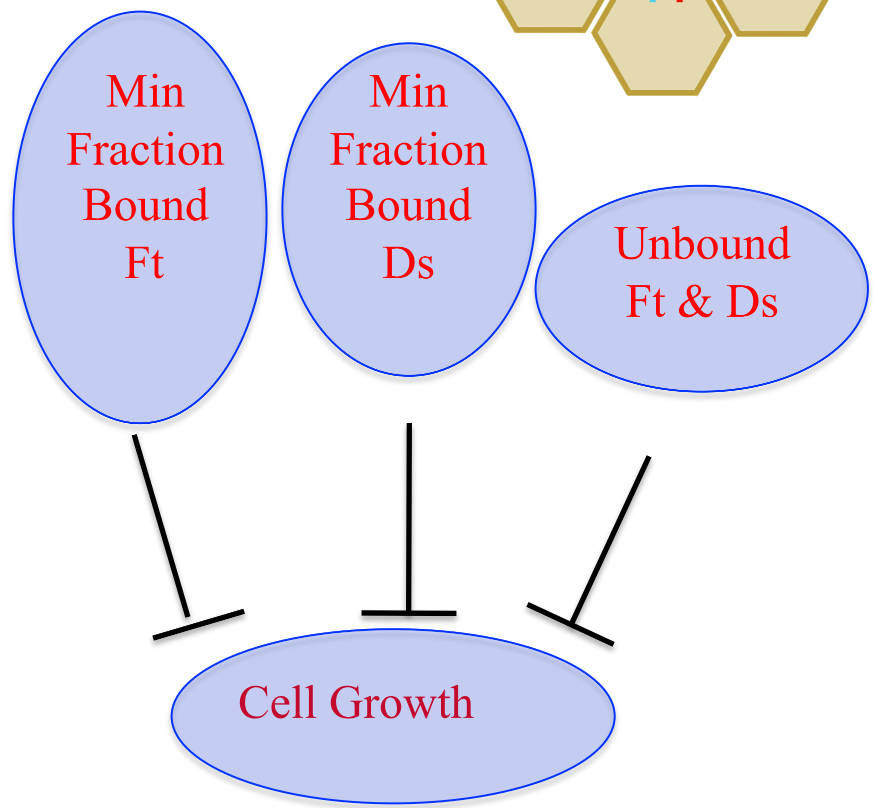
$D \propto$ (Minimum fraction of bound Ds among neighbors) $\times$ (number of neighbors)

$U \propto$ Number of unbound Ft and Ds

$$\underbrace{\frac{\text{1 Ds bond (min)}}{\text{12 total Ds bonds}}}_{\text{Min Frac Bound Ds}} \times (\text{6 neighbors}) = \frac{1}{2}$$

Asymmetric bonds (forces)

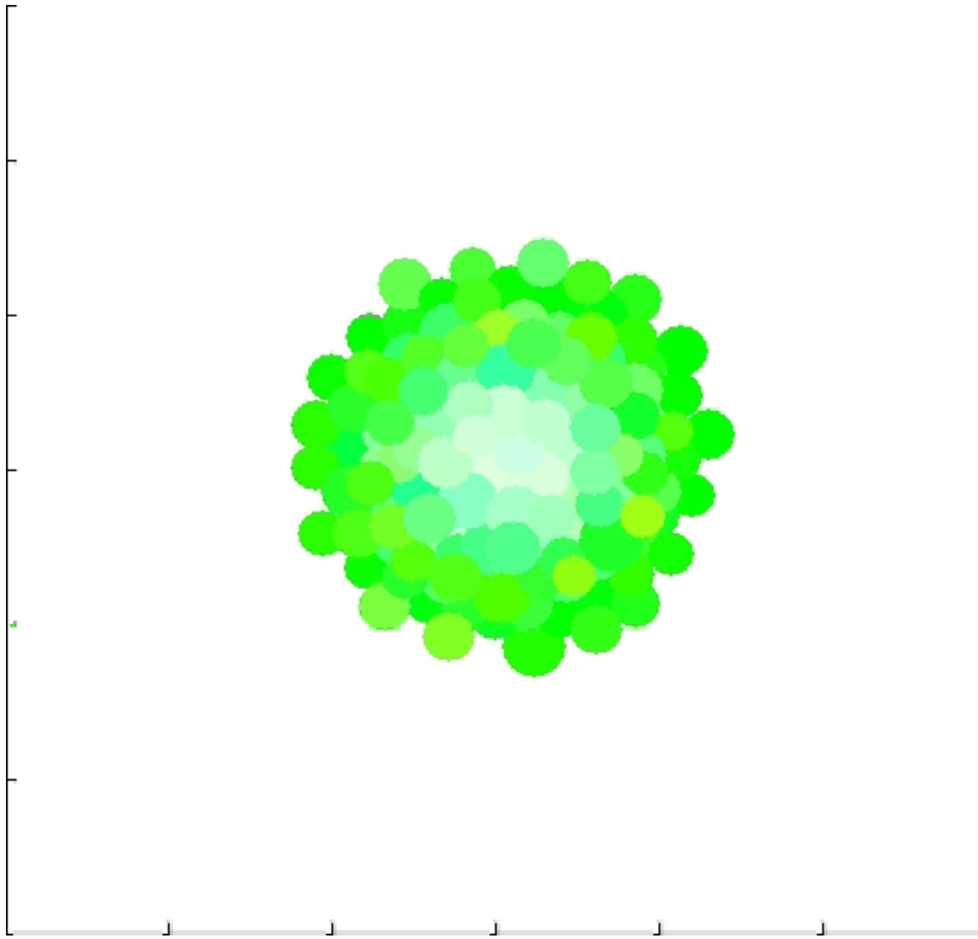




More asymmetric Ft-Ds bond distribution ➔ More growth

Movie of Tissue Growth

- Yellow = Amt of bound Ds
- Blue = Amt of bound Ft
- Blue + Yellow = Green
- Brighter cells have more bonds



Results: ft$^-$ discs overgrow

Experiment: Discs mutant for fat overgrow (Matakatsu and Blair, 2006)

Simulation: Agreement with experiment because

- They have no Ft/Ds bonds
- They have no unbound Ft
- They have small penalty for unbound Ds

A = Ft-Ds bond asymmetry and unbound Ft and Ds

$$A = \frac{1}{(1+F)(1+D)(1+U)} = \frac{1}{(1+U)}$$

$F \propto$ (Minimum fraction of bound Ft among neighbors) $\times$ (number of neighbors) =0

$D \propto$ (Minimum fraction of bound Ds among neighbors) $\times$ (number of neighbors) =0

$U \propto$ Number of unbound Ft and Ds

From Matakatsu and Blair 2006

Results: ds$^-$ discs overgrow but not as much as ft$^-$

Experiment: Discs mutant for dachsous overgrow but not as much as ft$^-$ (Matakatsu and Blair, 2006)

Simulation: Agreement with experiment because

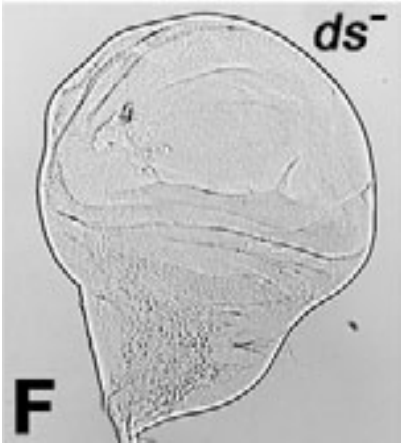
- They have no Ft/Ds bonds
- They have no unbound Ds
- They have large penalty for unbound Ft which is uniformly expressed.

$$A = \text{Bond asymmetry factor} = \frac{1}{(1+F)(1+D)(1+U)} = \frac{1}{(1+U)}$$

$F \propto$ (Minimum fraction of bound Ft among neighbors) $\times$ (number of neighbors)=0

$D \propto$ (Minimum fraction of bound Ds among neighbors) $\times$ (number of neighbors)=0

$U \propto$ Number of unbound Ft and Ds



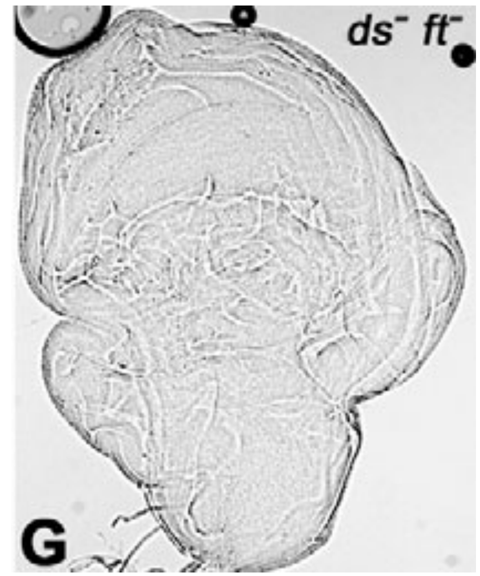
From Matakatsu and Blair 2006

Results: ft$^-$ ds$^-$ discs overgrow more than either ft$^-$ or ds$^-$ discs

Experiment: Discs mutant for both dachsous and fat overgrow more than either ft$^-$ or ds$^-$ discs (Matakatsu and Blair, 2006)

Simulation: Agreement with experiment because

- They have no Ft/Ds bonds
- They have no unbound Ft or Ds

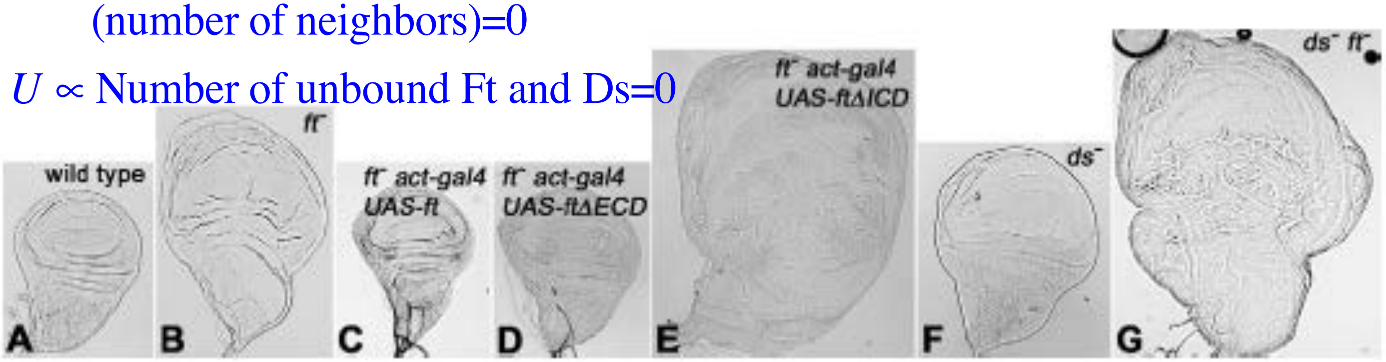


$$A = \frac{1}{(1+F)(1+D)(1+U)} = \text{Bond asymmetry factor=1}$$

$$F \propto \text{(Minimum fraction of bound Ft among neighbors)} \times \text{(number of neighbors)=0}$$

$$D \propto \text{(Minimum fraction of bound Ds among neighbors)} \times \text{(number of neighbors)=0}$$

$$U \propto \text{Number of unbound Ft and Ds=0}$$



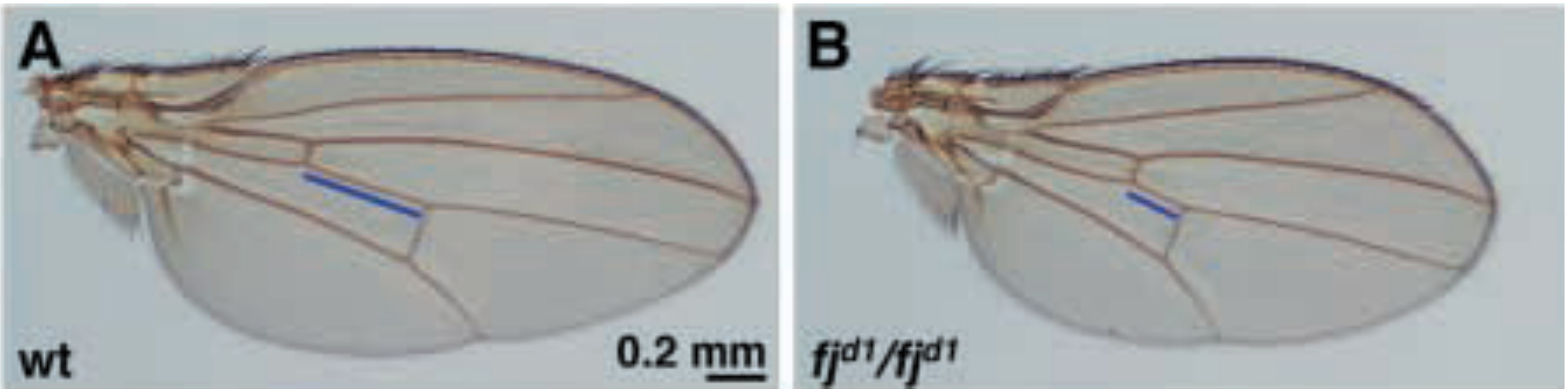
From Matakatsu and Blair 2006

Results: fj⁻ discs undergrow

Experiment: Discs mutant for four-jointed undergrow

Simulation: Agreement with experiment because

- Ft/Ds binding probabilities are uniform throughout disc so Ft/Ds bonds are more symmetrically distributed



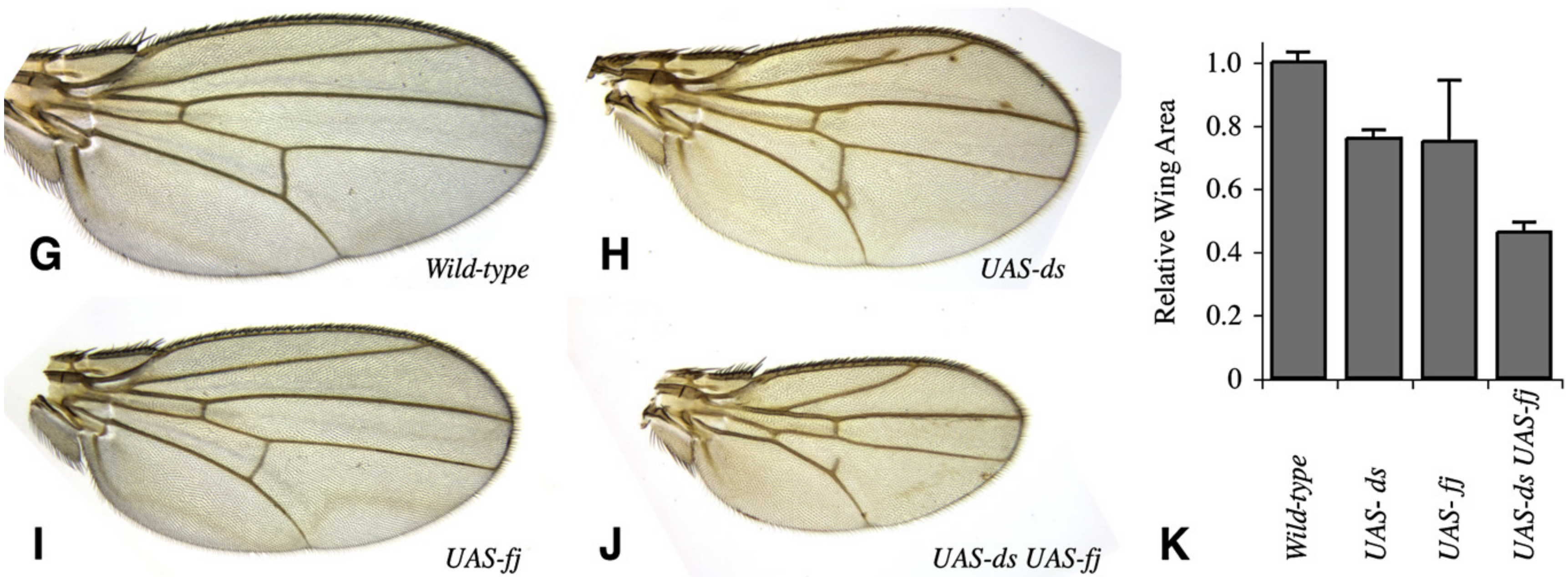
From Strutt *et al.* 2004

Results: Discs uniformly expressing Ds and/or Fj undergrow

Experiment: Discs uniformly expressing Ds and/or Fj undergrow

Simulation: Agreement with experiment because

- Ft/Ds bonds are more symmetrically distributed than in wild type (WT)



From Rogulja, Rauskolb, and Irvine 2008

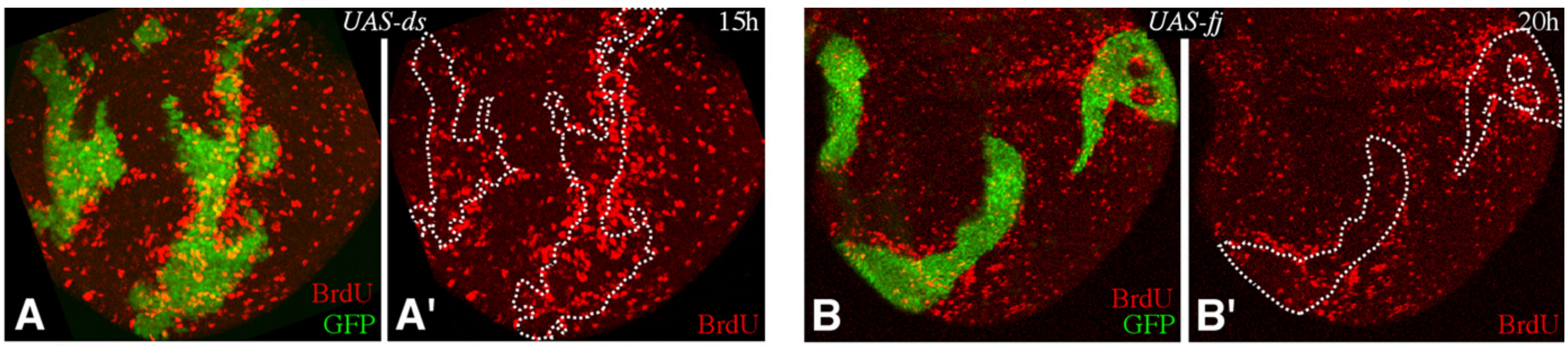
Results: Nonautonomous growth around Fj and Ds-overexpressing clones

Experiment:

- Nonautonomous growth around the edge of Fj- and Ds-overexpressing clones.
- For Ds-overexpressing clones, growth more pronounced in center of disc where there is less Ds than near disc edge where Ds is abundant.
- For Fj-overexpressing clones, growth more pronounced near edge of disc where there is less Fj than in center of disc where Fj is abundant.

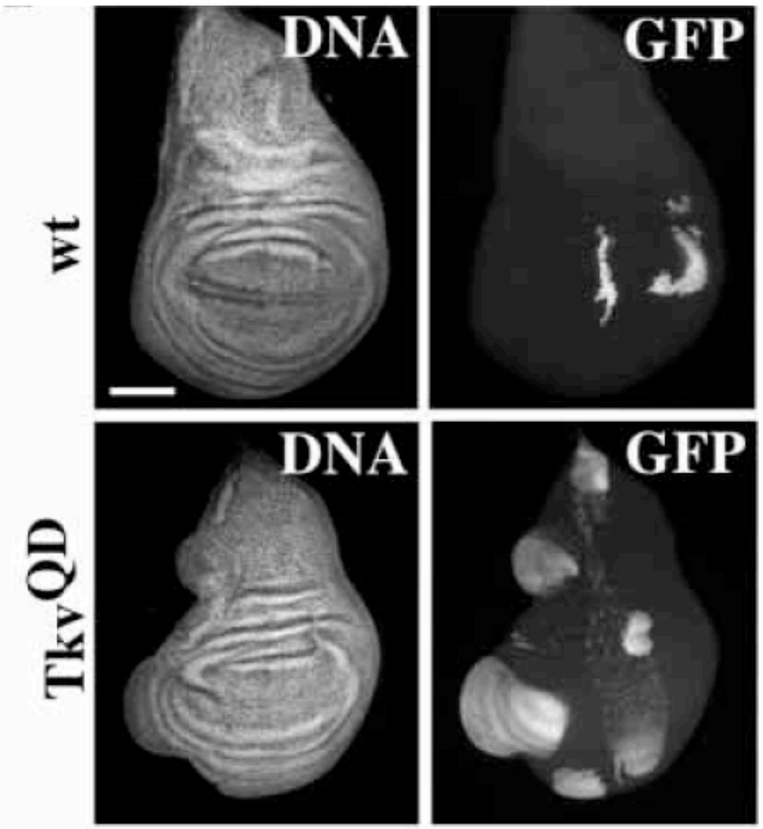
Simulation: Agreement with experiment because

- Neighboring cells with very different amounts of Ds or Fj have strongly polarized (asymmetric) bonds and hence tend to grow faster.
- More growth both inside and outside edge of clone.



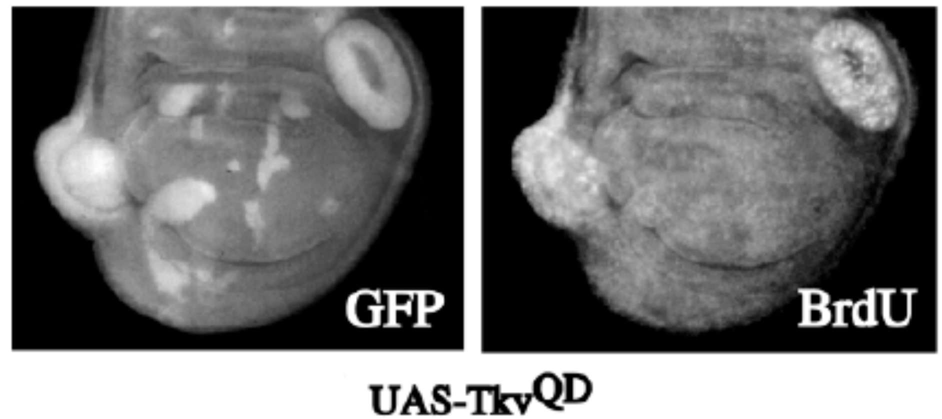
From Rogulja, Rauskolb, and Irvine 2008

Dpp drives proliferation and autonomous growth



Tkv^{QD} = Constitutively activated Dpp receptor

Martin-Castellanos and Edgar (2002)



Add autonomous growth term G_m (Hill fcn of [morphogen])

$$\frac{R_{n+1}}{R_n} = \left(1 + \frac{G_0}{(1+F)(1+D)(1+U)} + G_m\right)$$

G_0 = Base growth rate of cell

G_m = Growth effect of morphogen

$F \propto$ (Minimum fraction of bound Ft among neighbors) $\times$ (number of neighbors)

$D \propto$ (Minimum fraction of bound Ds among neighbors) $\times$ (number of neighbors)

$U \propto$ Number of unbound Ft and Ds

What stops growth?
How is homeostasis achieved?

Putting in penalty P in order to stop growth does not work.

$$\frac{R_{n+1}}{R_n} = \left(1 + \frac{G_0}{(1+F)(1+D)(1+U)} + G_m - P\right)$$

$$P = \text{Growth penalty}$$

Simulation:

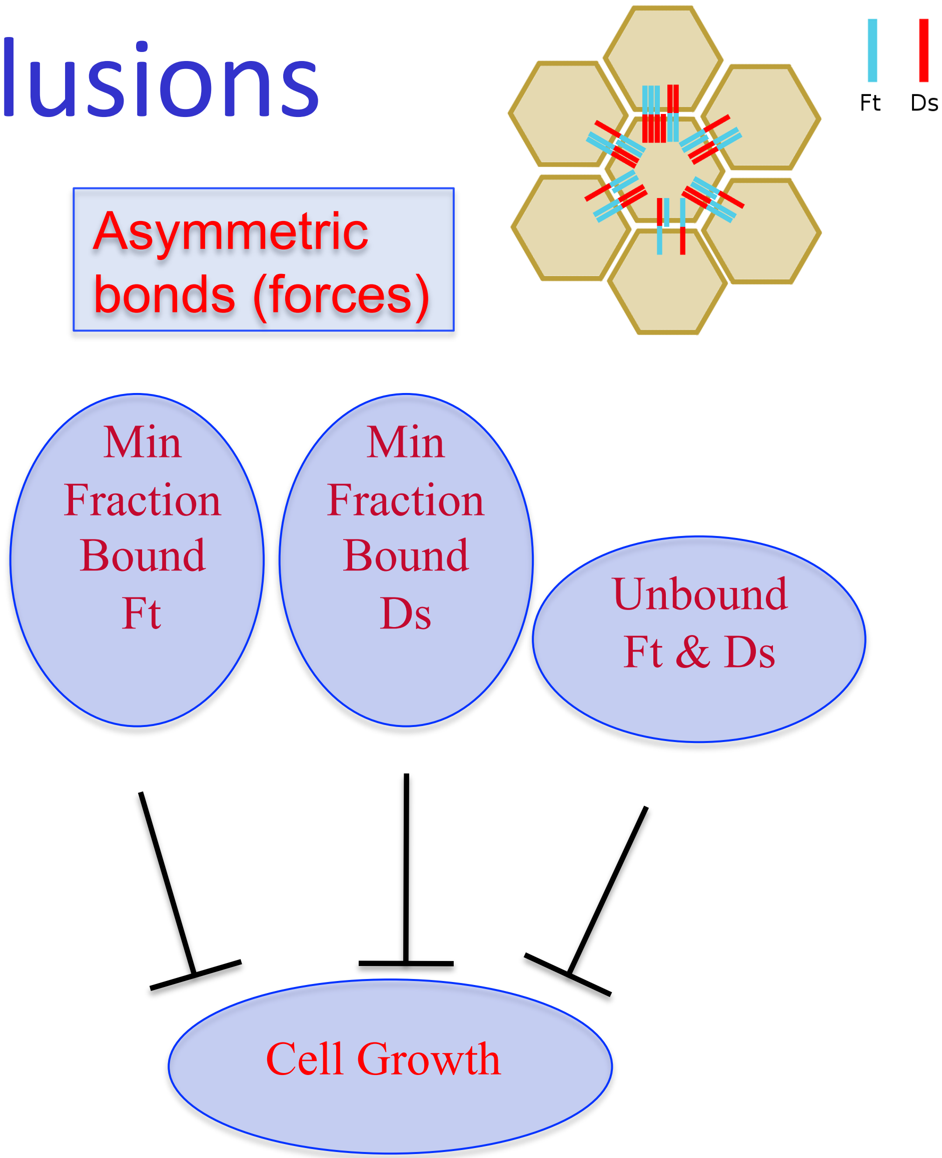
•Disagreement with experiment:

- Penalty so big there is no growth.
- Or, if P is smaller, discs never stop growing in simulations due to morphogen growth rate G_m.
- Even if G_m-P = 0, discs mutant for Ft and/or Ds never stop growing in simulations.

Some other mechanisms must be involved in homeostasis.

Conclusions

- Our hypothesis: Asymmetric protocadherin Ft/Ds bonds regulate cell growth in the *Drosophila* wing disc.
- Simulations of cell growth in agree well with experiments.
- But what stops growth?



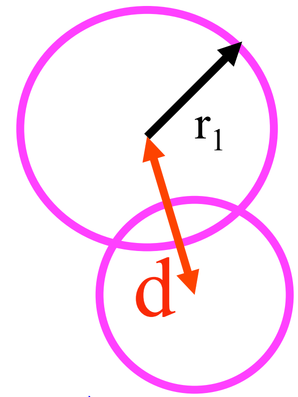
Acknowledgements

Arthur Lander

Funded by NIH through UCI Center for Complex Systems Biology

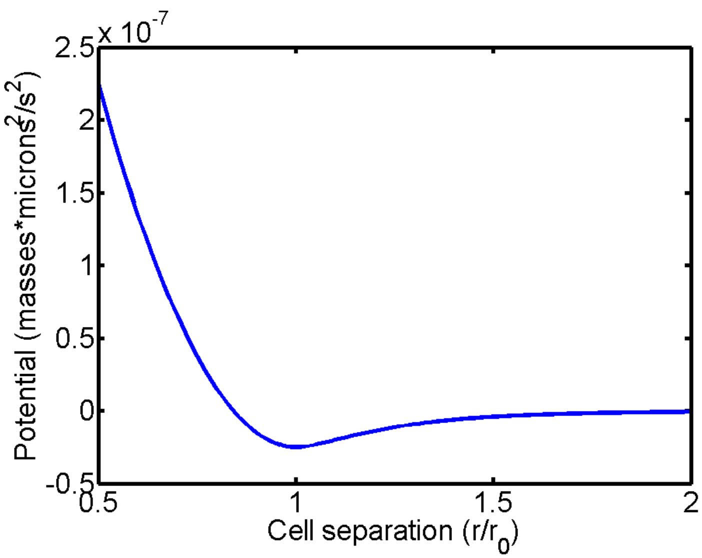
THE END

Elastic Interaction Between Adjacent Cells



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

$$V(d) = \begin{cases} \frac{k}{2}\left[d_o - d\right]^2 - \frac{C_{gap}}{12} & \text{if } d < d_o = (r_1 + r_2) \\ \frac{C_{gap}}{6}\left[\frac{1}{2}\left(\frac{d_o}{d}\right)^{12} - \left(\frac{d_o}{d}\right)^{6}\right] & \text{if } d > d_o \end{cases}$$



Compression or tension from neighbors keeps cells from flying apart and from overlapping too much. It does not affect a cell's growth.

Morphogen Concentration in Simulations

- Morphogen (Dpp + Wg) concentration

$$M(r,t) = M_C \exp\left[\frac{H(t) - r}{\lambda}\right]$$

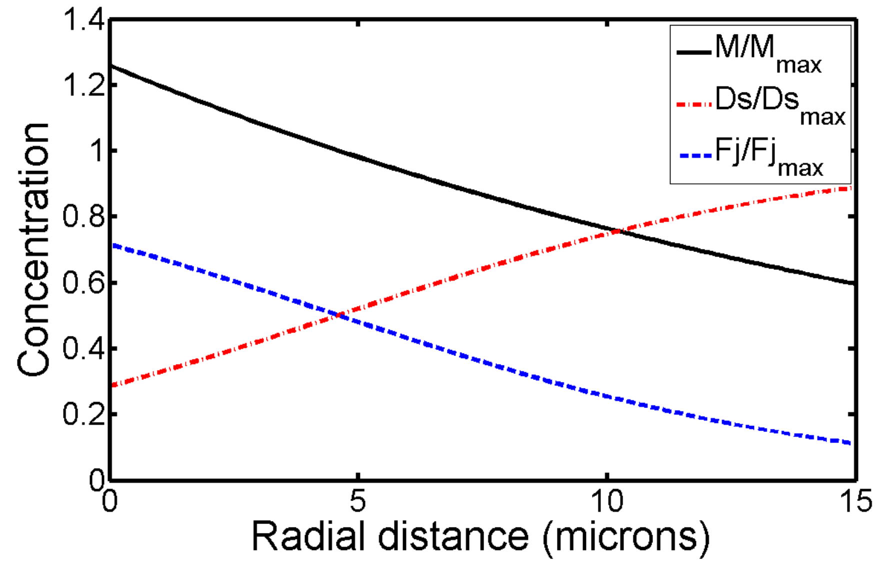
where H(t) is proportional to cell height in disc center and decay length $\lambda = 20\ \mu\text{m}$

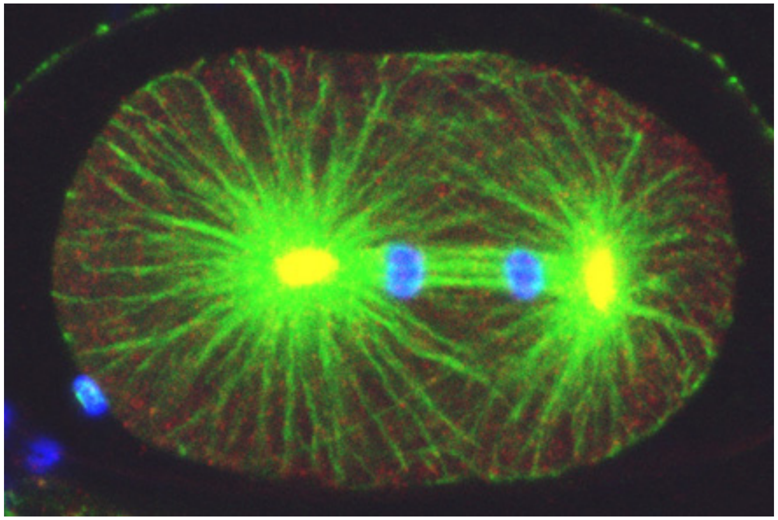
- Dachsous (Ds) concentration

$$[Ds](r,t) = [Ds]_{\max} \frac{1}{1 + \left(\frac{M(r,t)}{M_C}\right)^n} \quad \text{where n = 4}$$

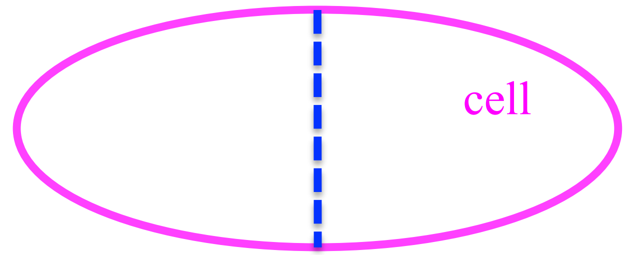
- Four-jointed (Fj) concentration

$$[Fj](r,t) = [Fj]_{\max} \left(1 - \frac{1}{1 + \left(\frac{M(r,t)}{M_C}\right)^n}\right)$$





Cell Division



A Cell's Probability to Divide Increases with Size

$$P = 1 - \frac{1}{1 + \left(\frac{A}{A_0}\right)^n}$$

A = area of a cell
n = 100