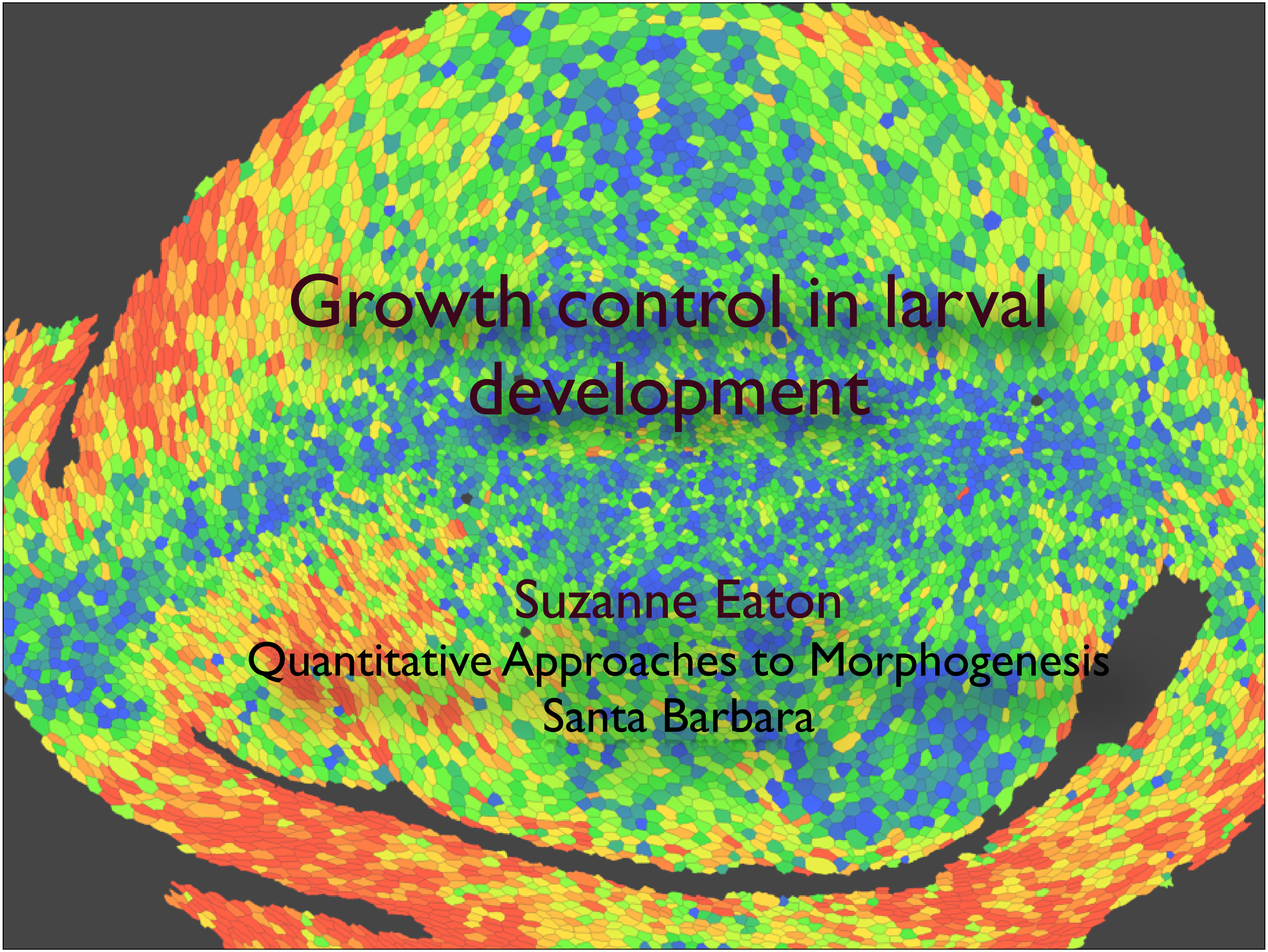
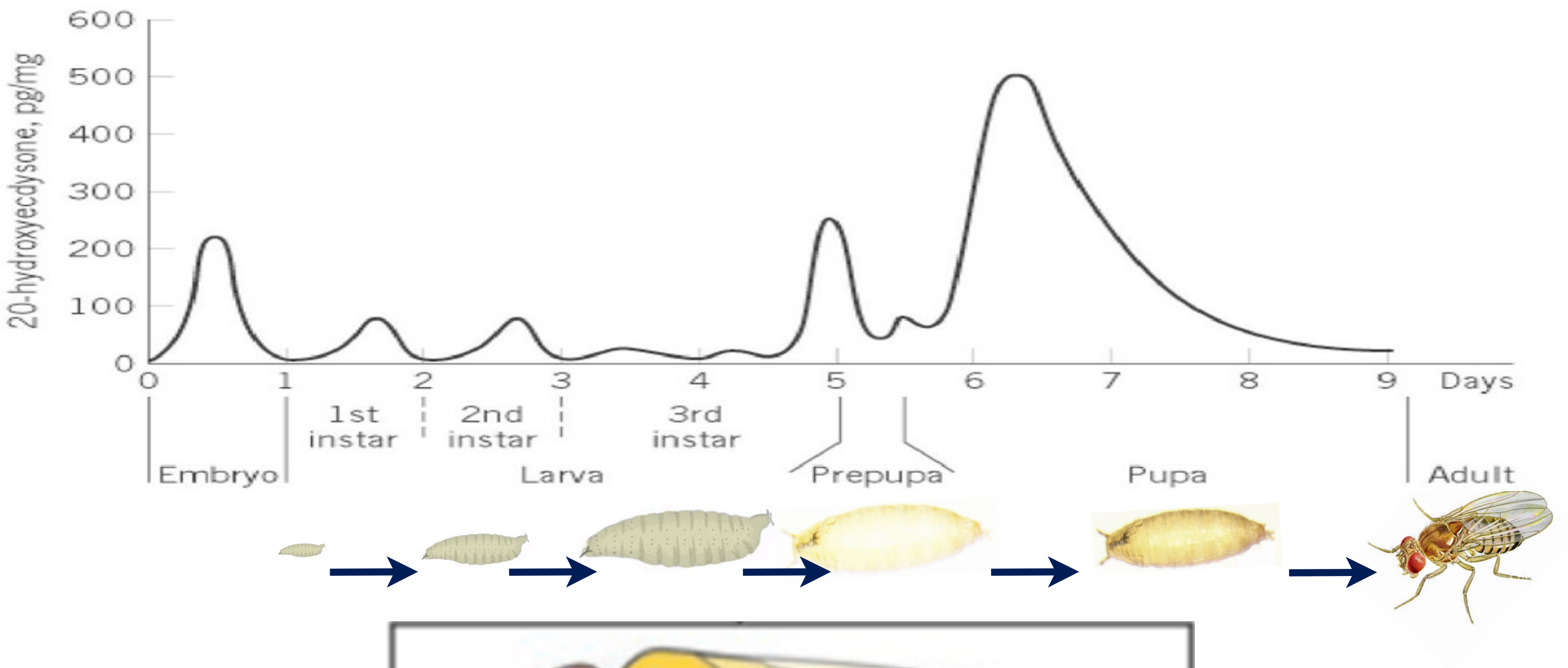


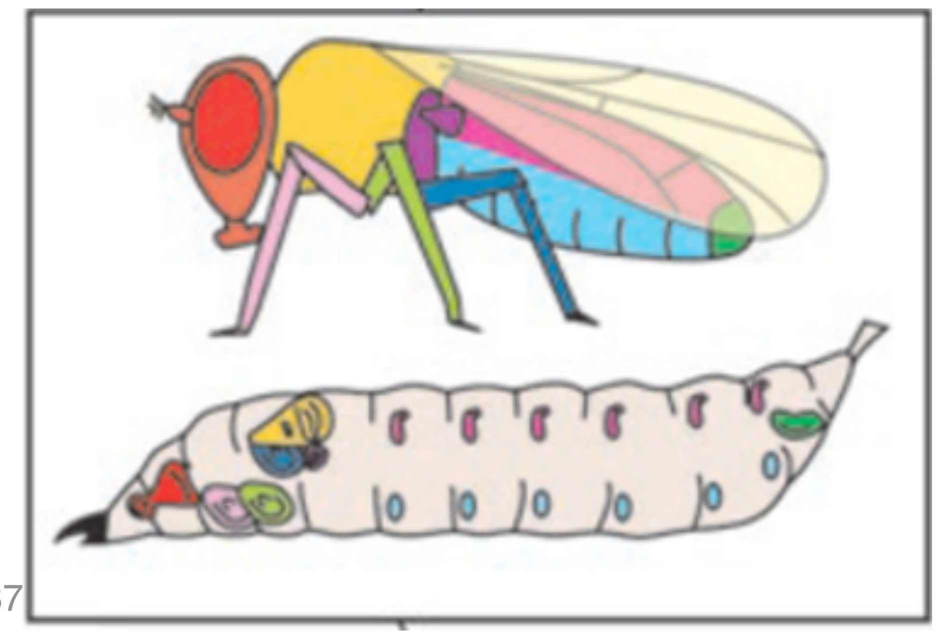
Growth control in larval development

Suzanne Eaton

Quantitative Approaches to Morphogenesis

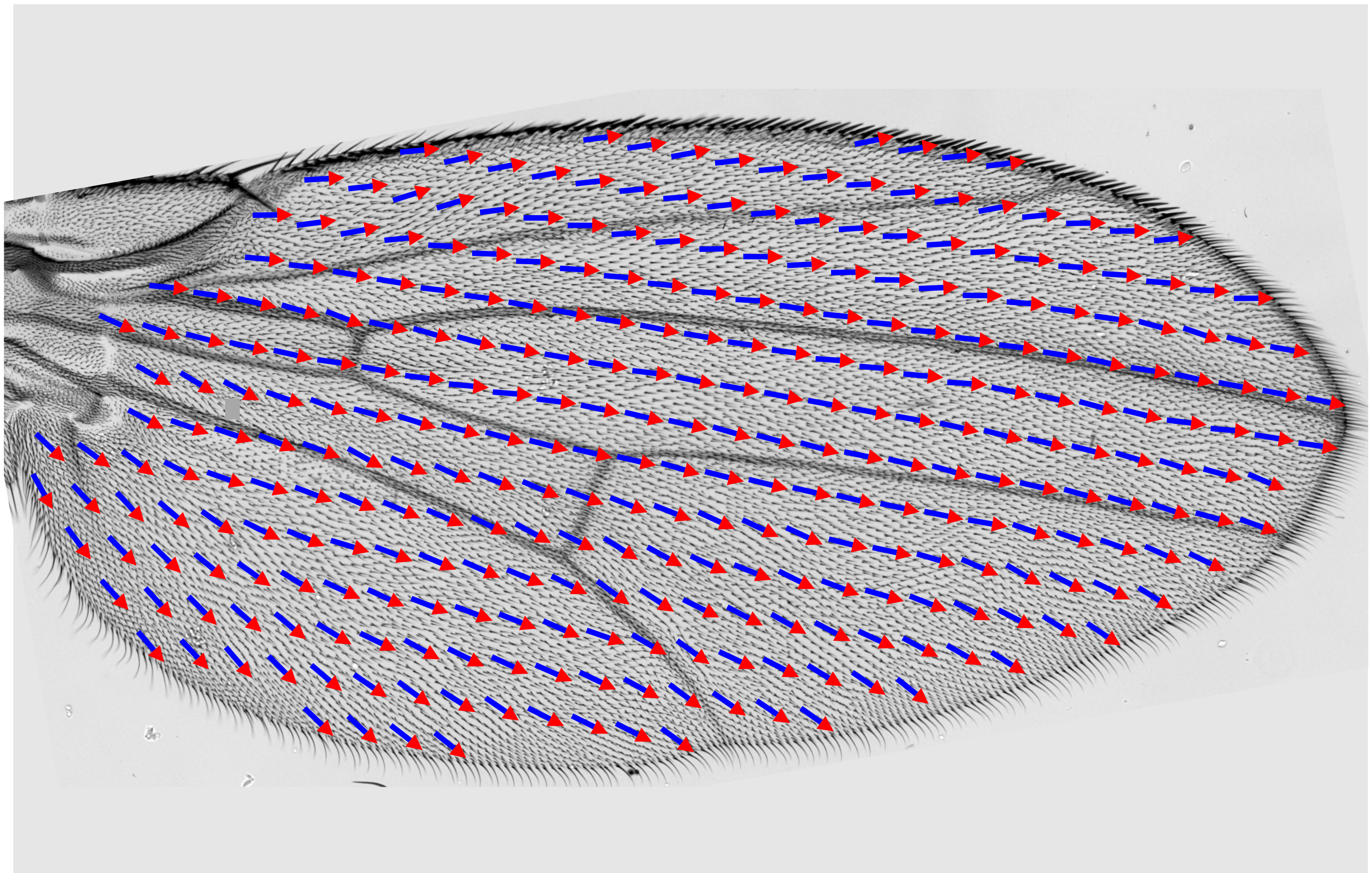
Santa Barbara



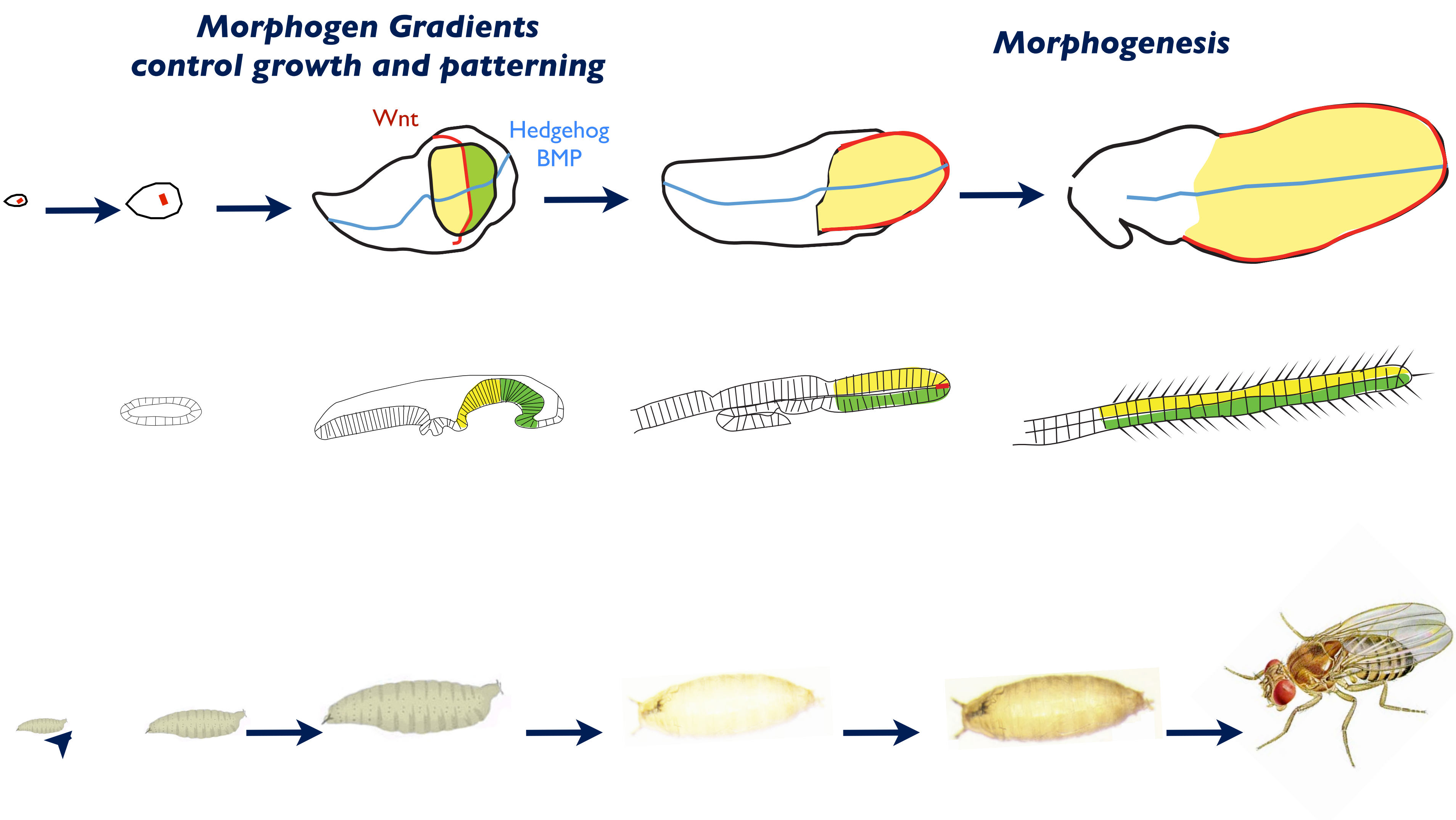


A. Shingleton (2010)
Organogenesis 6:2, 76-87

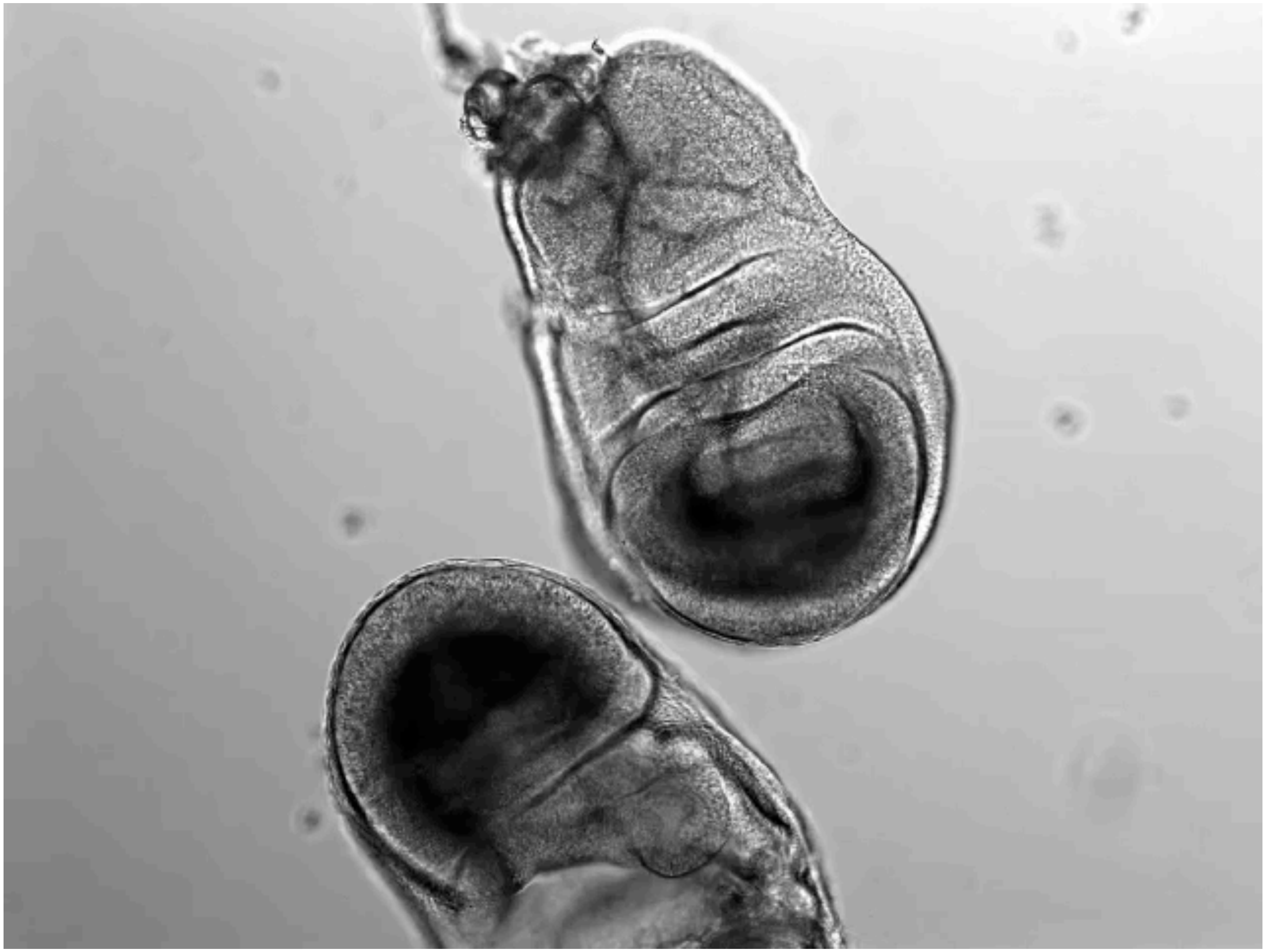
What determines final tissue size shape and pattern?



Wing development through larval and pupal stages

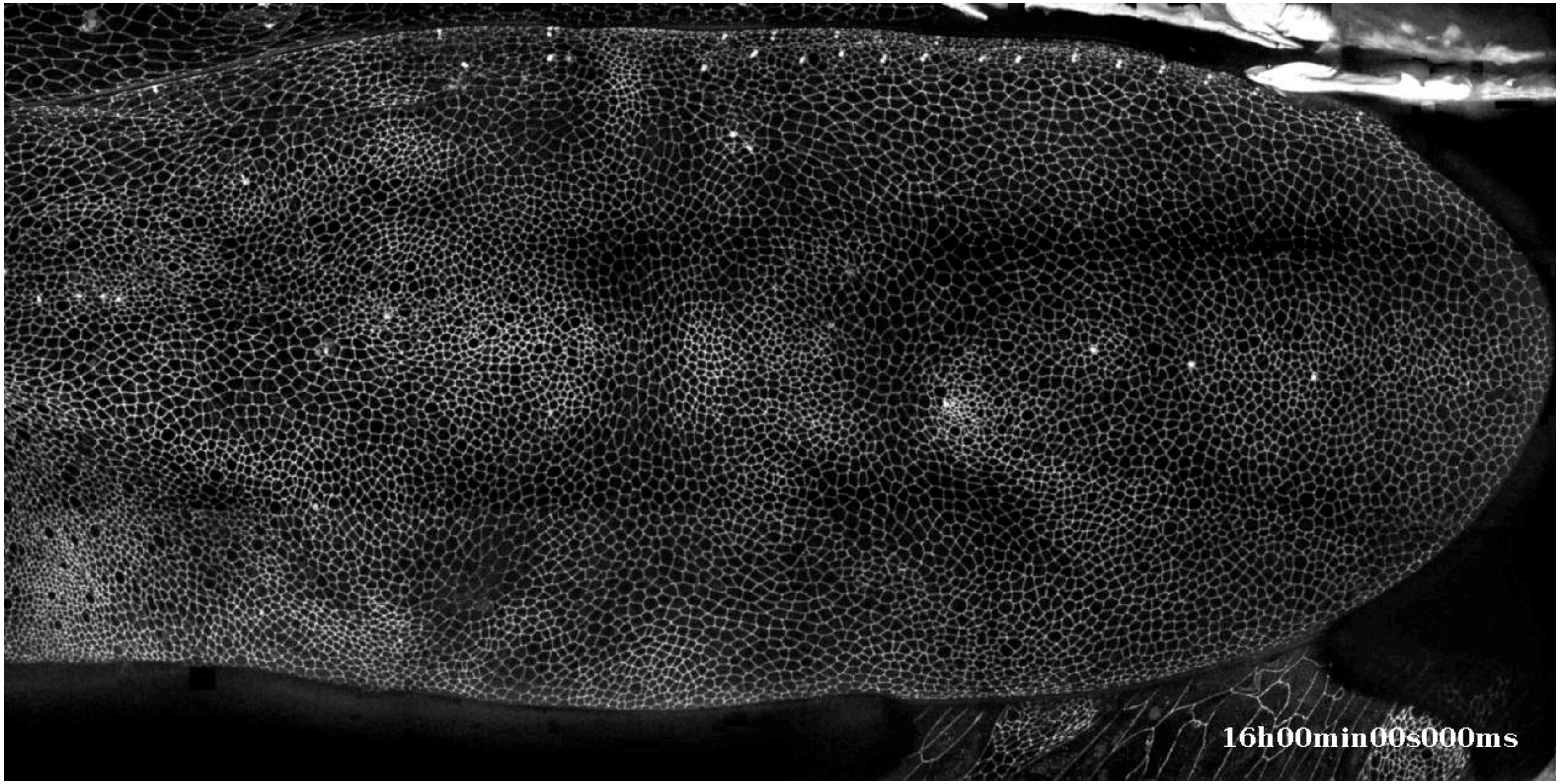


Ecdysone induces wing eversion after pupariation

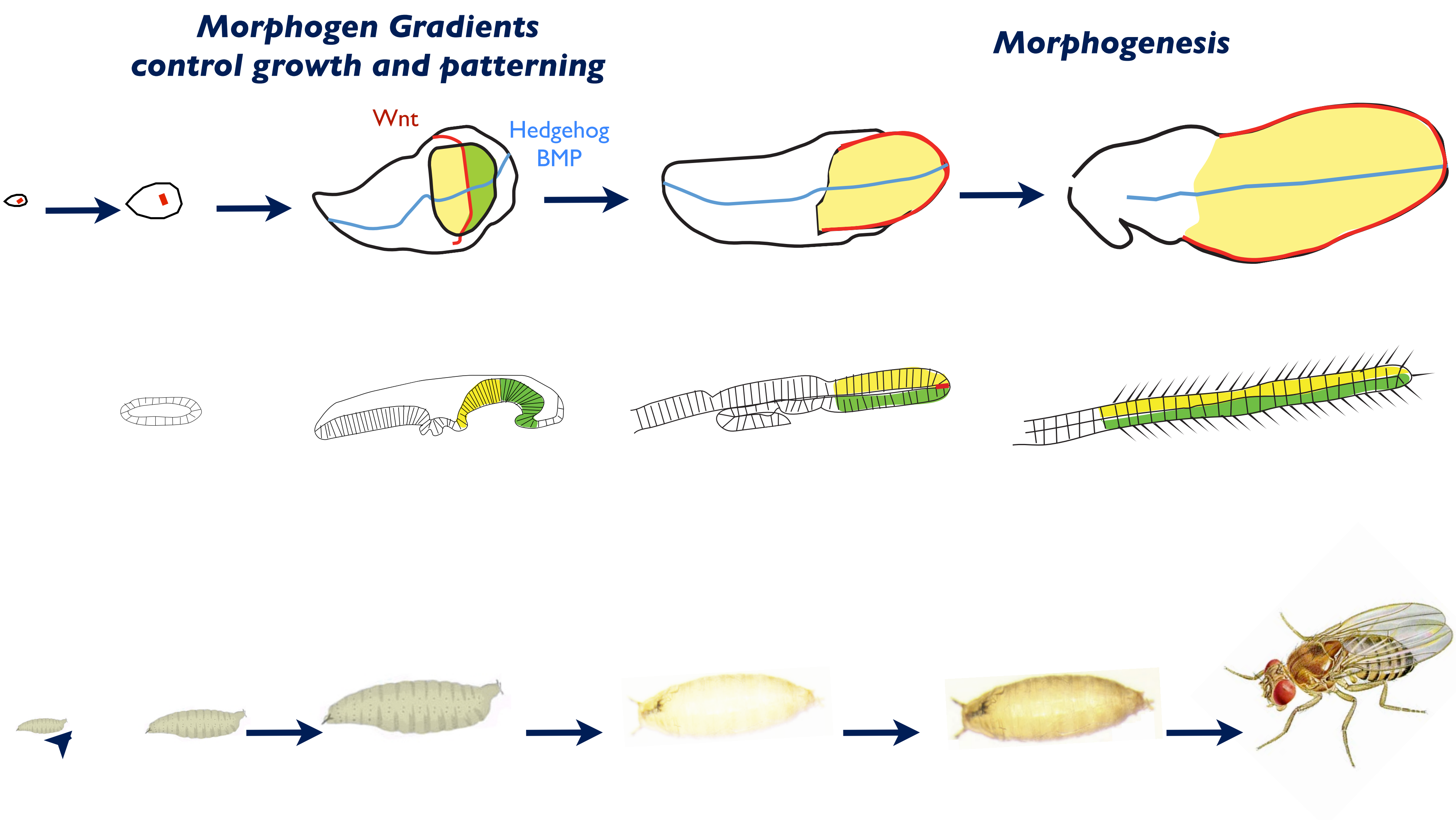


Natalie Dye

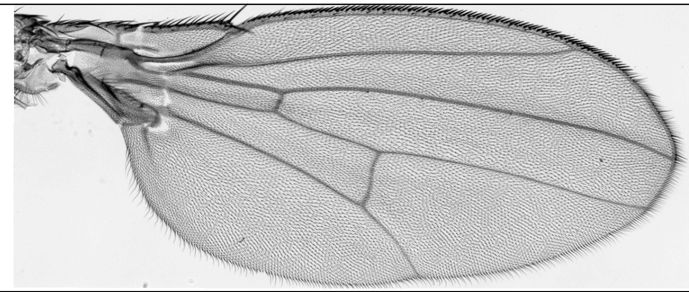
Epithelial remodeling refines wing shape in the pupa



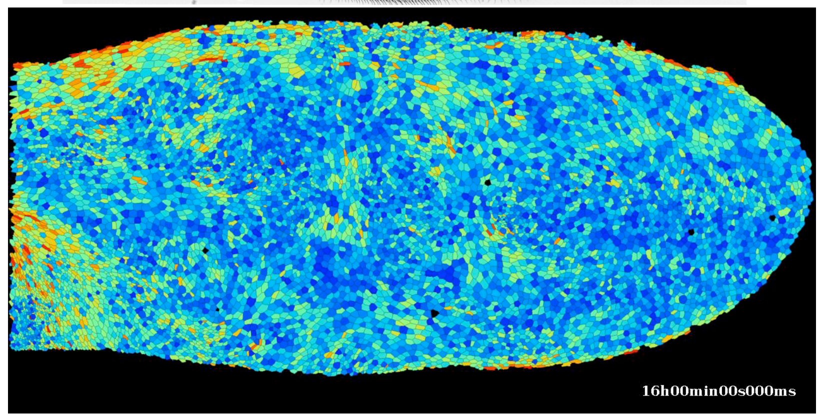
Wing development through larval and pupal stages



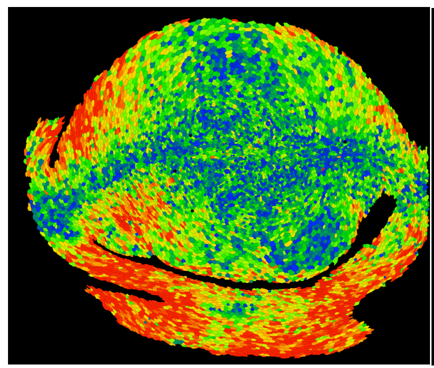
Tissue size and shape

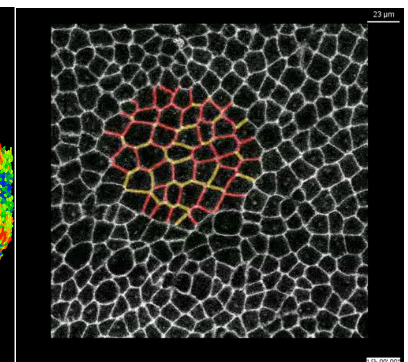


Collective cell behaviour



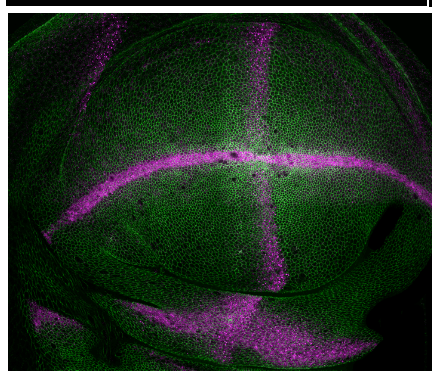
Cell properties

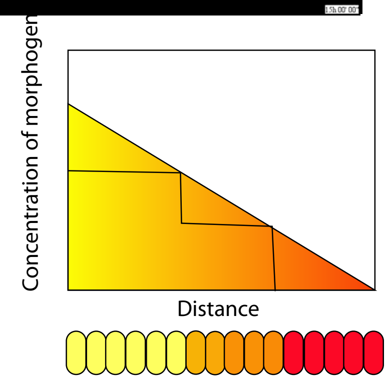




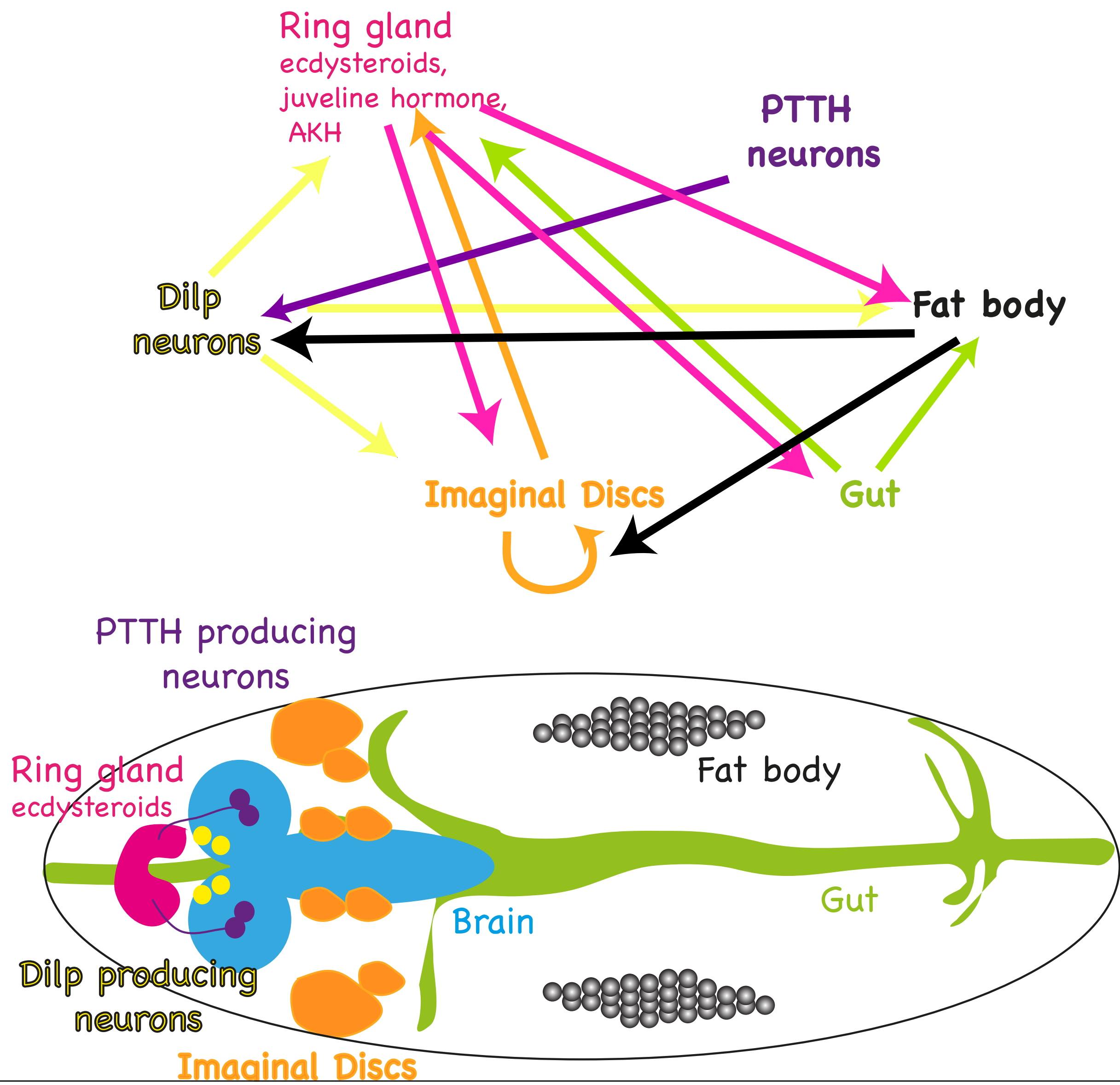
Information

patterns of signaling
and gene expression





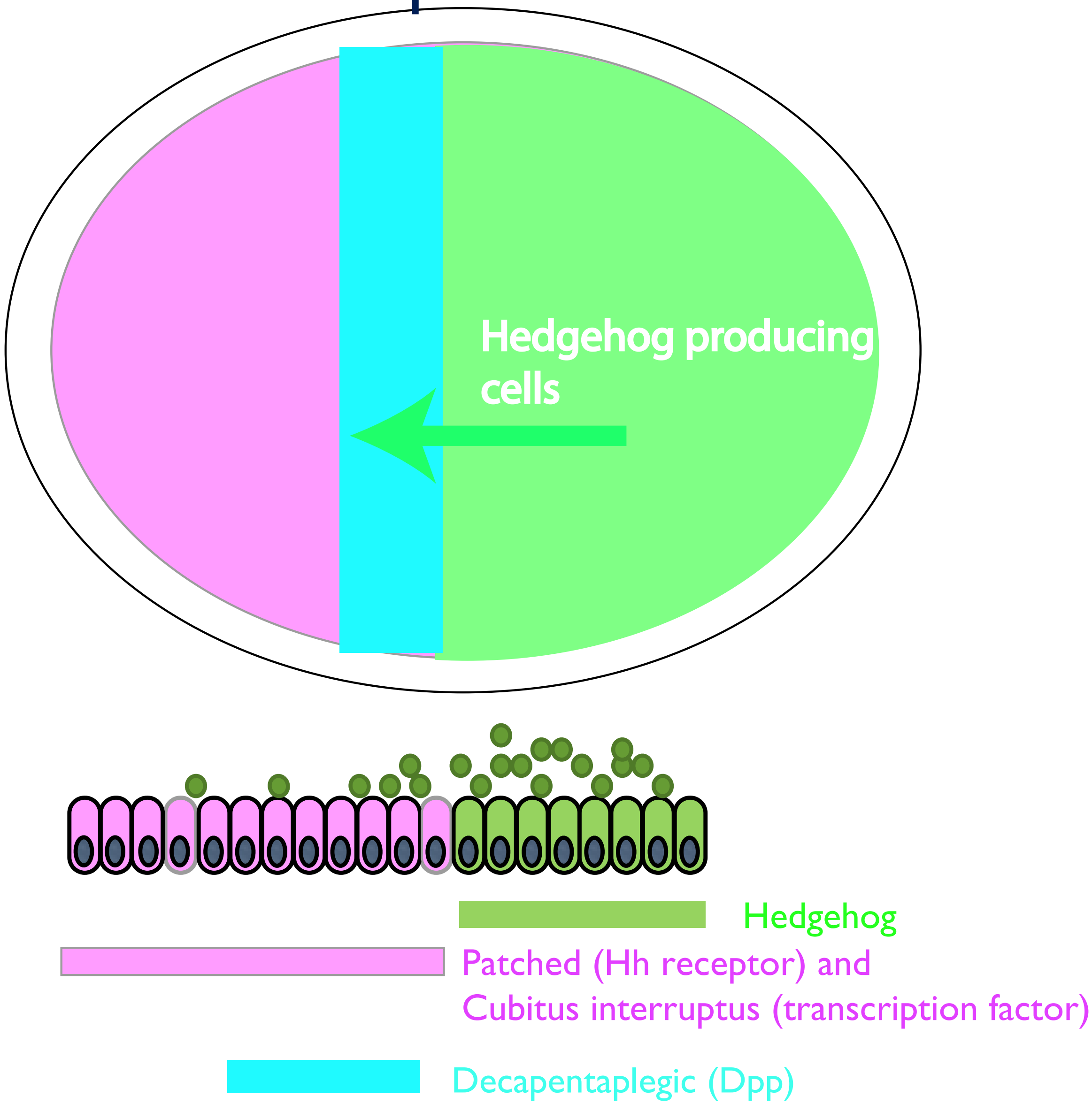
Metabolic networks and size control



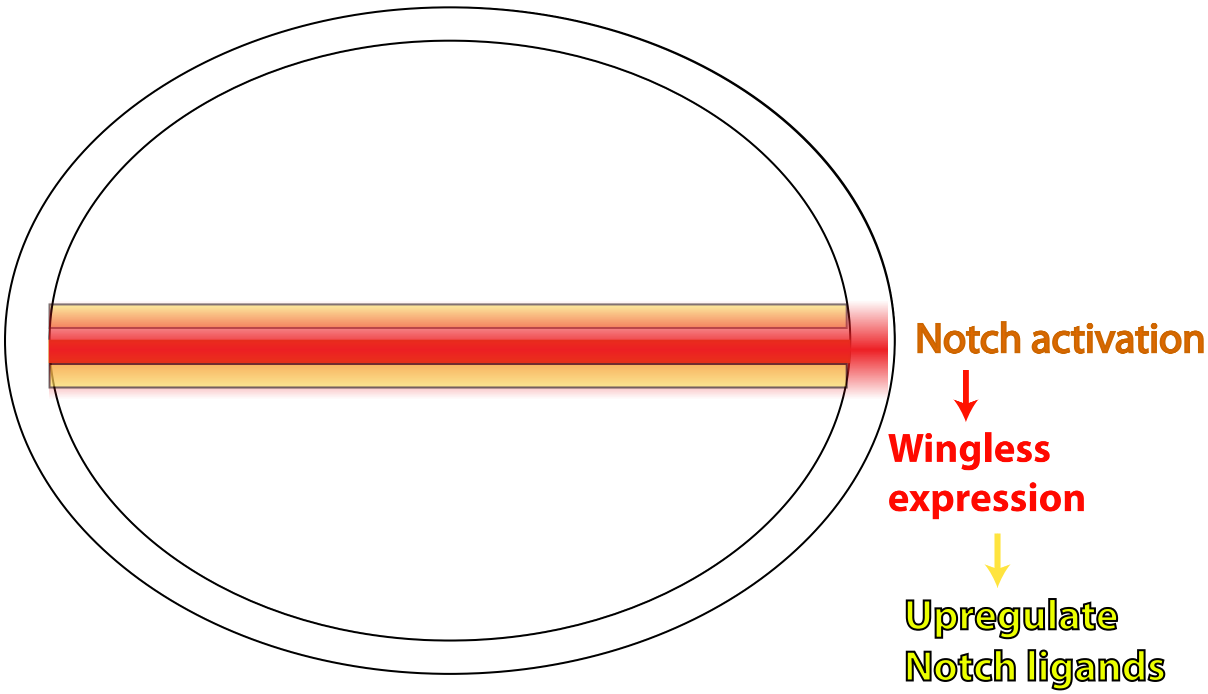
Control of tissue size and shape

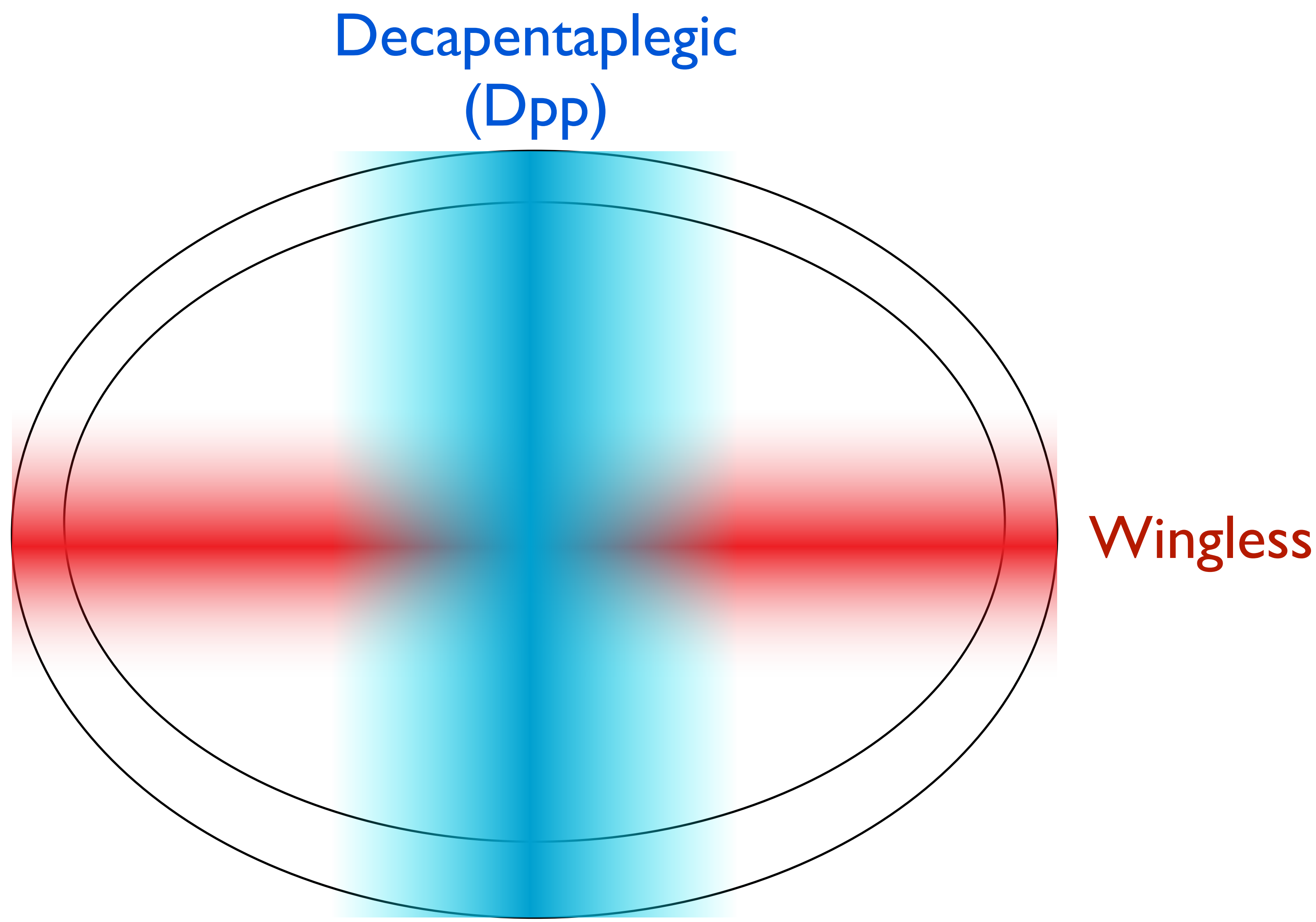
- autonomous (morphogen gradients, tissue mechanics)
- metabolic networks

Hedgehog and Decapentaplegic form gradients in the anterior-posterior axis

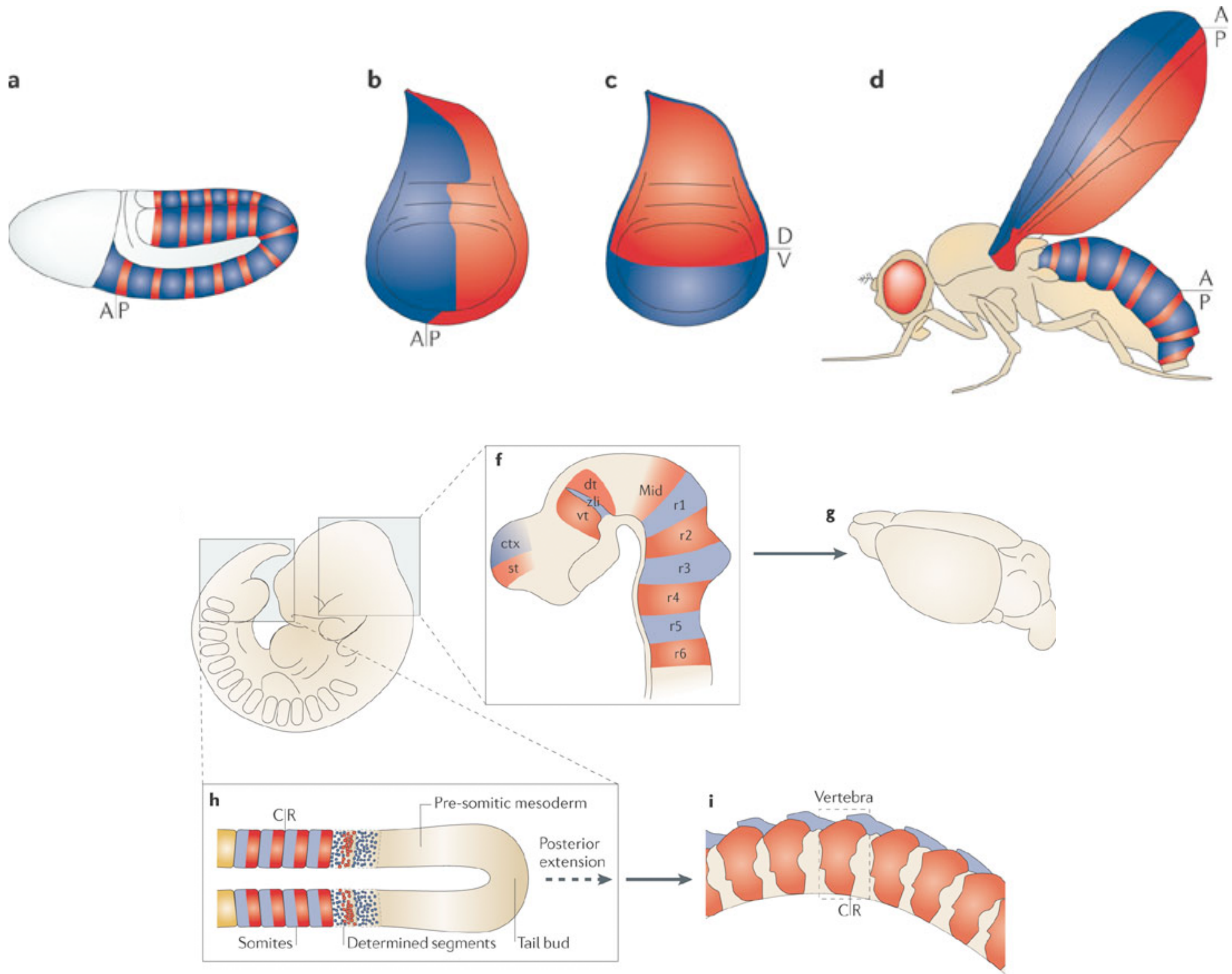


A Wingless/Notch feedback loop at the dorsal-ventral boundary stabilizes Wingless expression



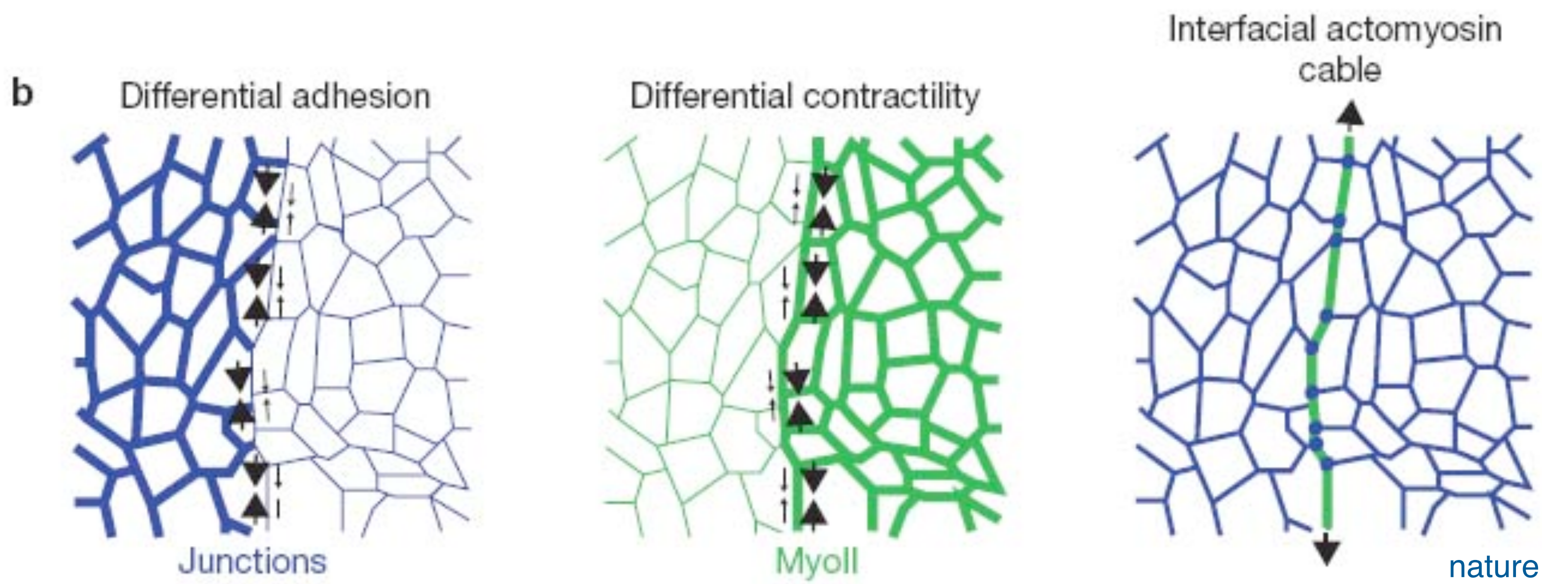

Decapentaplegic
(Dpp)
Wingless

Signaling interfaces set up lineage restriction boundaries between compartments



Christian Dahmann, Andrew C. Oates & Michael Brand, 2011 Nature Reviews | Genetics

Compartments are separated by elevated tension at interfacial cell contacts

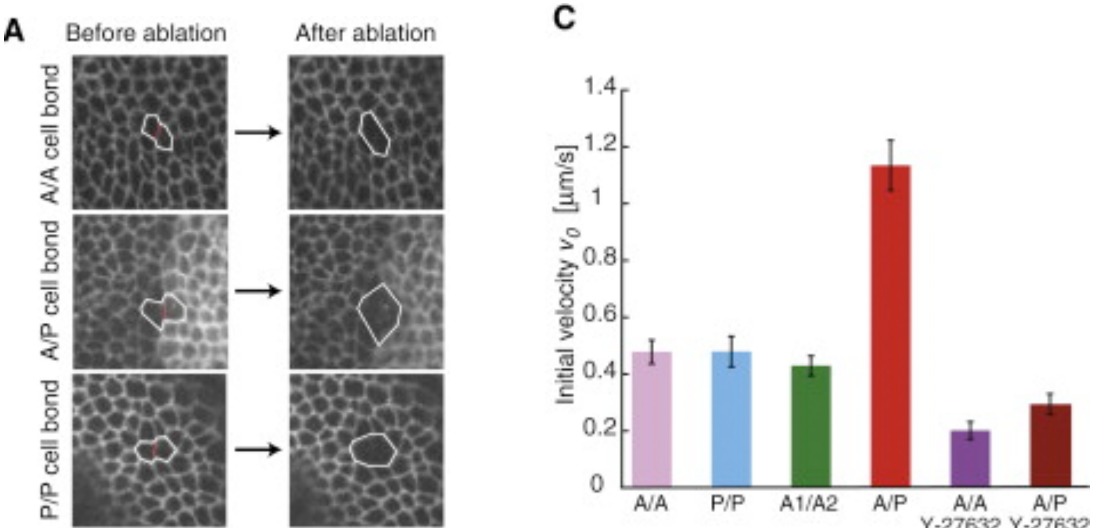


nature cell biology
news and views

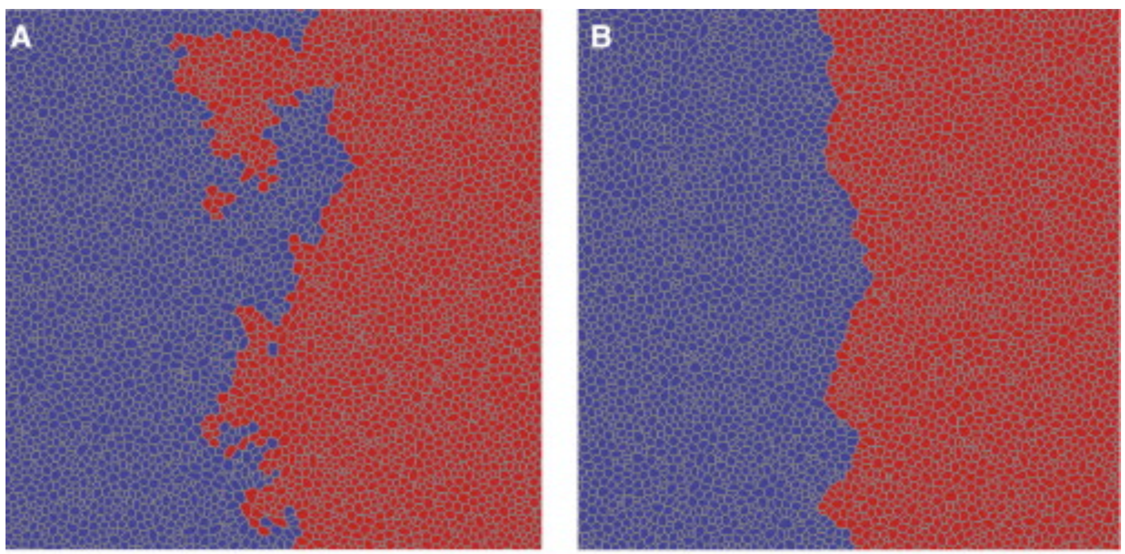
Adam C. Martin and Eric F. Wieschaus 2010

Aliee, M. et al. Current Biology (2012)

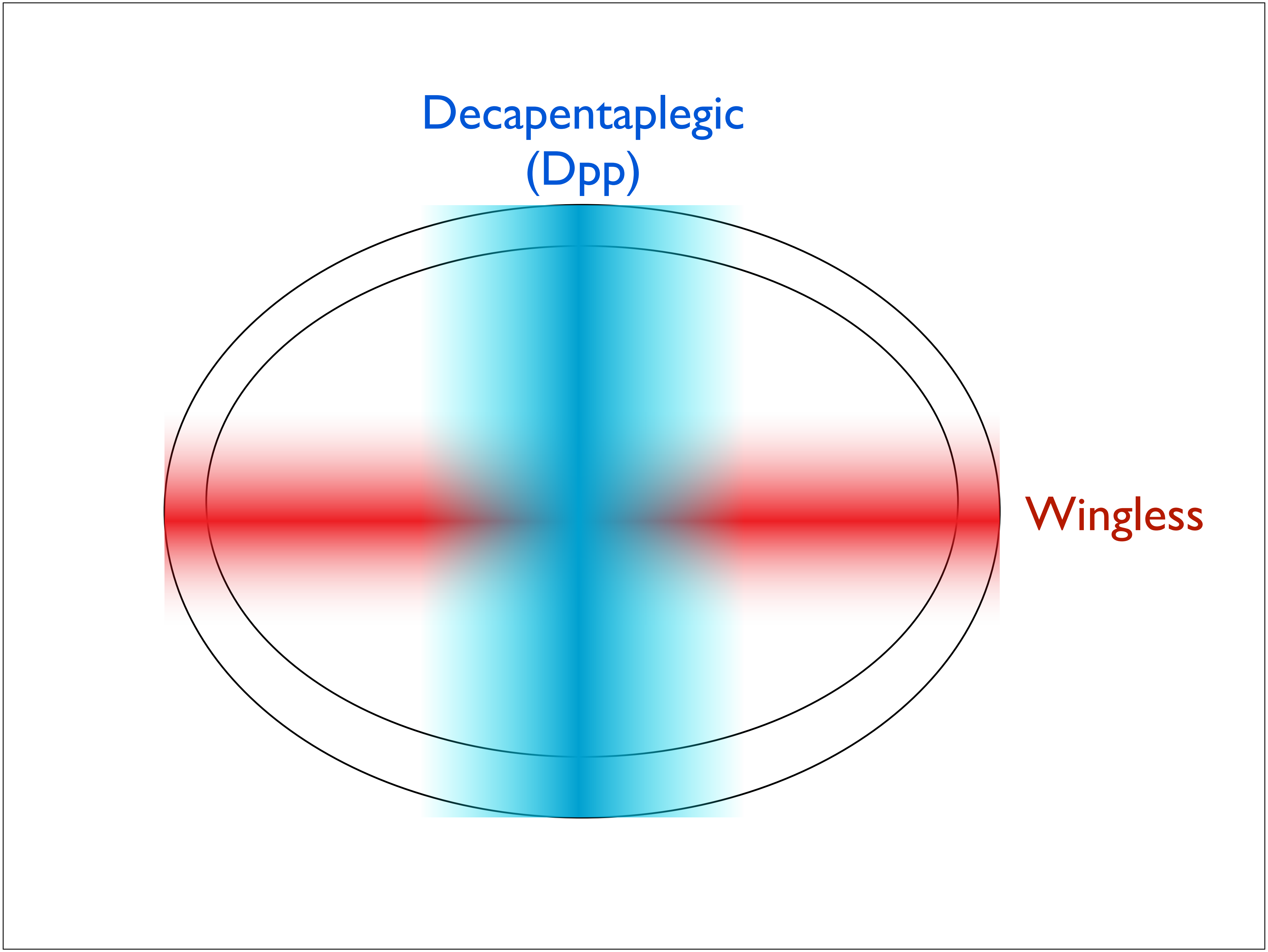
Landsberg, K. P. et al. Current Biology (2009).



Laser ablation shows tension on compartment interface bonds is higher

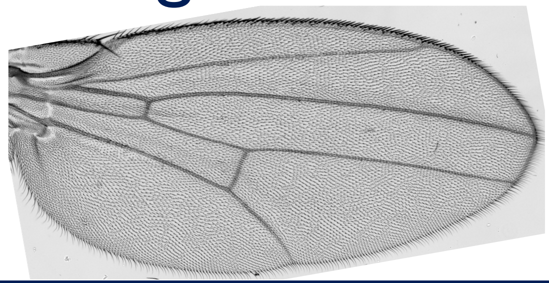


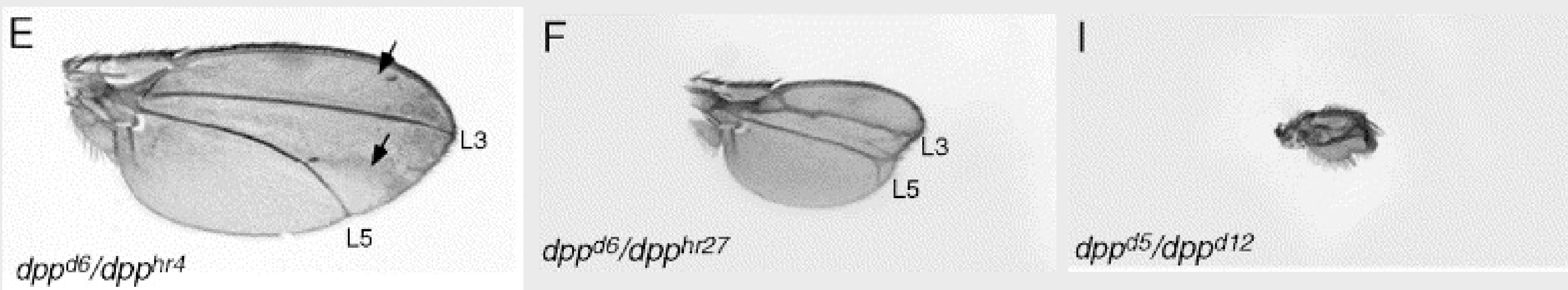
Vertex model simulations show increased tension can account for separation during growth


Decapentaplegic
(Dpp)
Wingless

Wingless/Notch and Dpp signaling couple patterning and growth

wild type



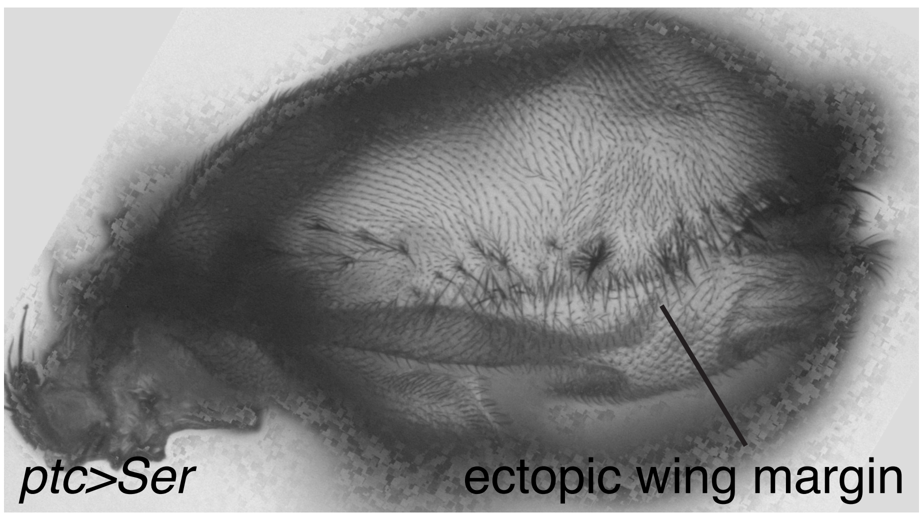


less and less Dpp activity →

less and less Wingless/Notch activity →

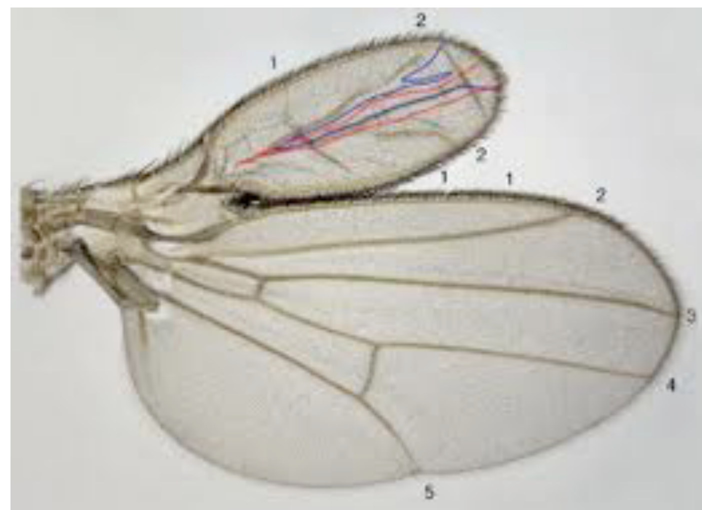
Ectopic morphogen signaling couples extra growth to altered patterning

Ectopic Wg/Notch activity



J.P. Couso[1], E. Knust[2], A. Martinez Arias, 1995, Current biology
Image from Sagner et al., 2012

Ectopic Dpp activity

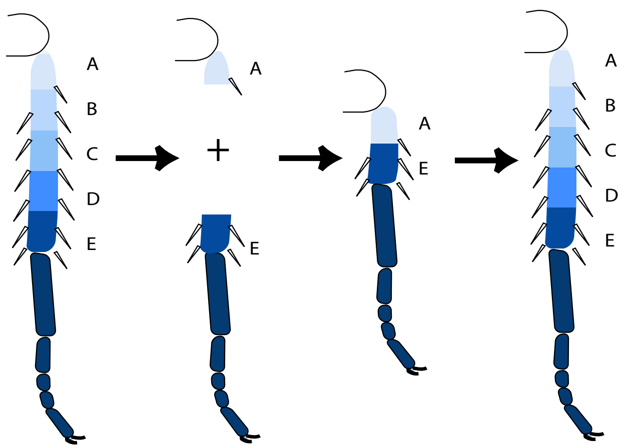


Nuria Serrano[1], Patrick H O'Farrell, 1997 Current Biology[2], How do morphogens control and couple growth and patterning?

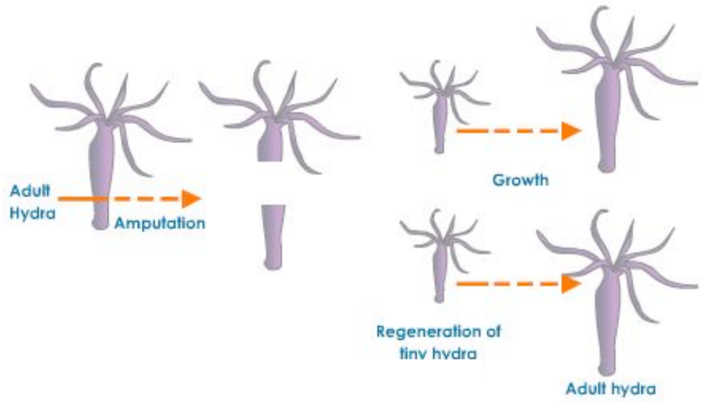
How do tissues know how much to grow?

How do tissues know which direction to grow?

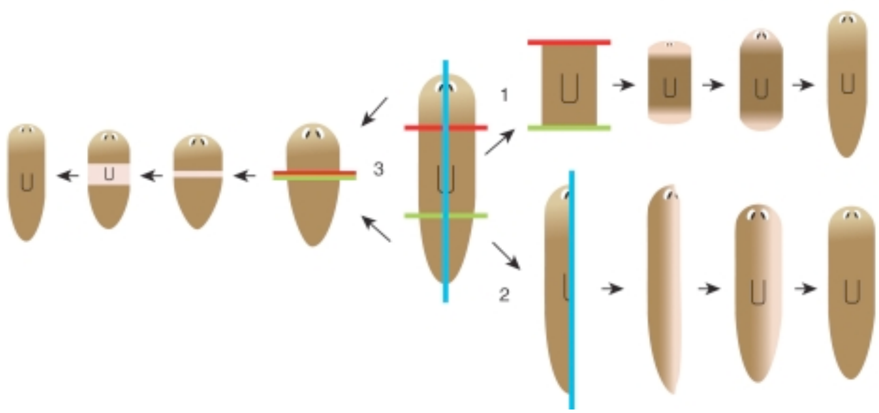
Tissues are shaped by information from morphogen gradients



Regeneration in the cockroach leg



Regeneration in Hydra



Regeneration in Planaria

Tissues are shaped by information from morphogen gradients

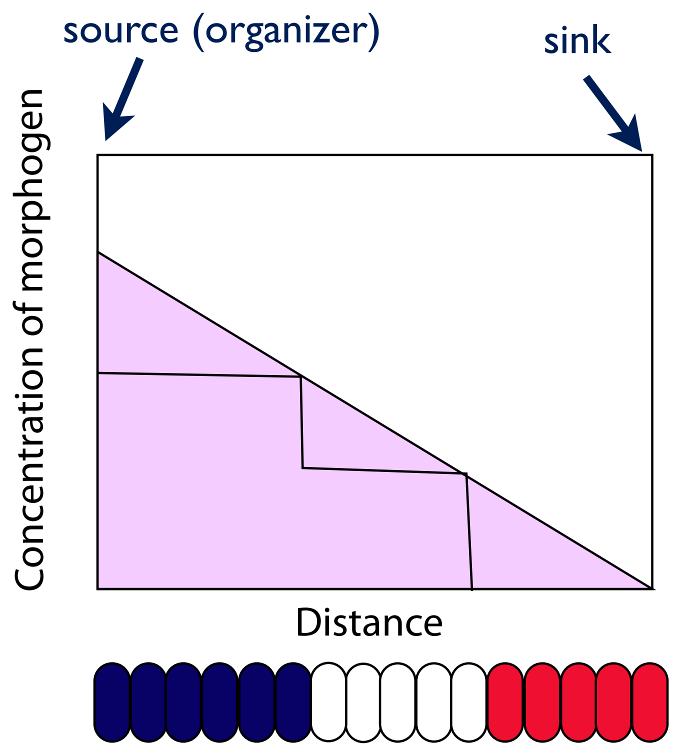
T.H. Morgan C.M. Child Lewis Wolpert Francis Crick Hans Meinhardt
(1901-1979)

The french flag model

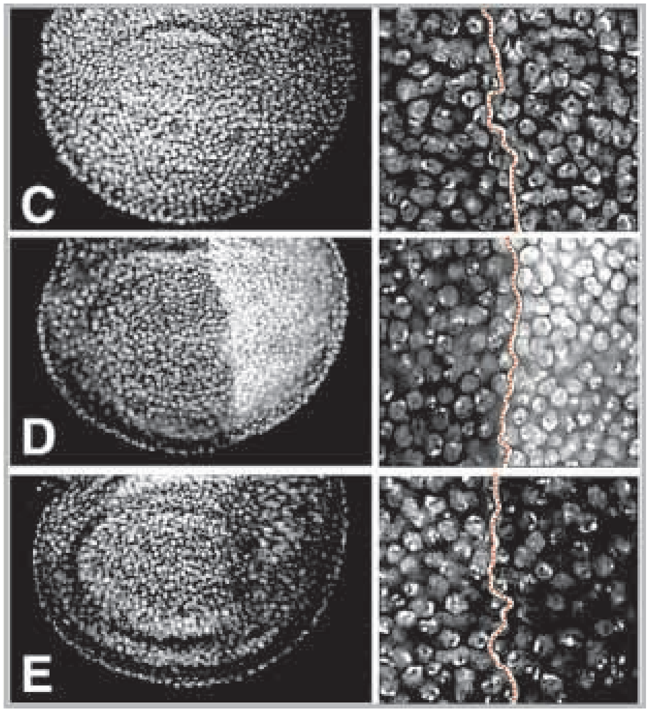
✓ Positional identities are conferred by threshold responses to specific morphogen levels.

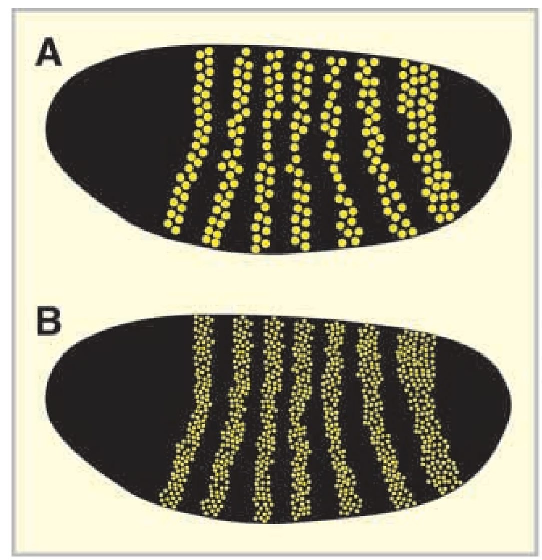
? Cells sense directionality, or polarity, in tissue through the slope of the gradient.

? Cells sense the size of the developmental field through the slope of the gradient



Discs (and other tissues) measure size not cell number





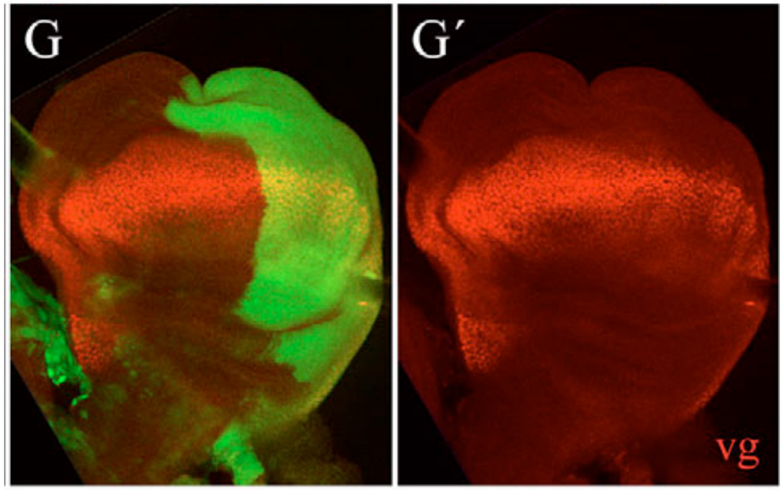
Measuring dimensions: the regulation of size and shape

Stephen J. Day[1] and Peter A. Lawrence[2]

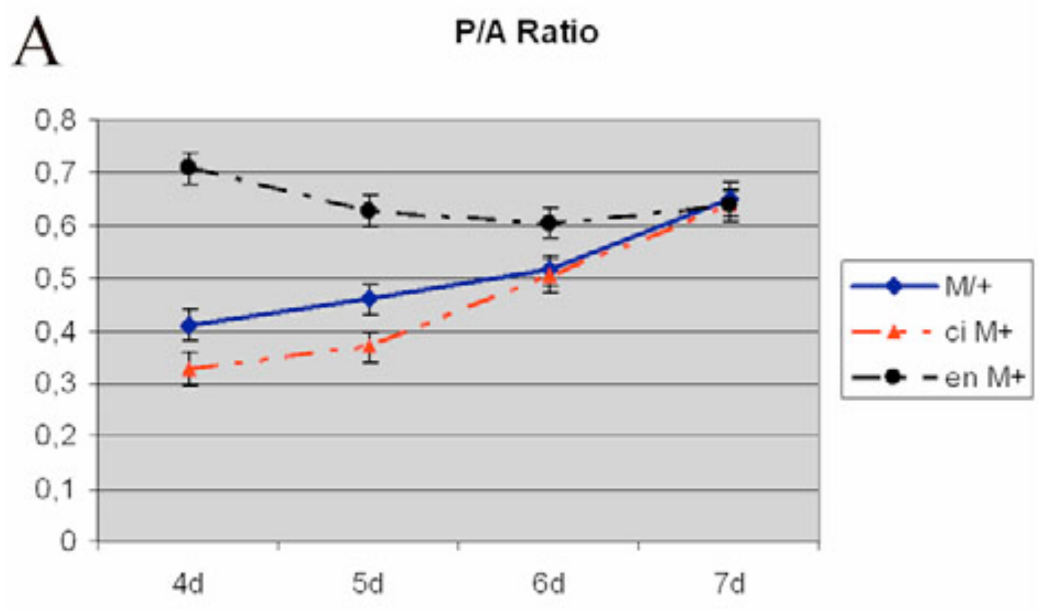
Development 127, 2977-2987 (2000)

DEV2566

Growth is controlled separately in each compartment



green marks posterior cells with a growth disadvantage



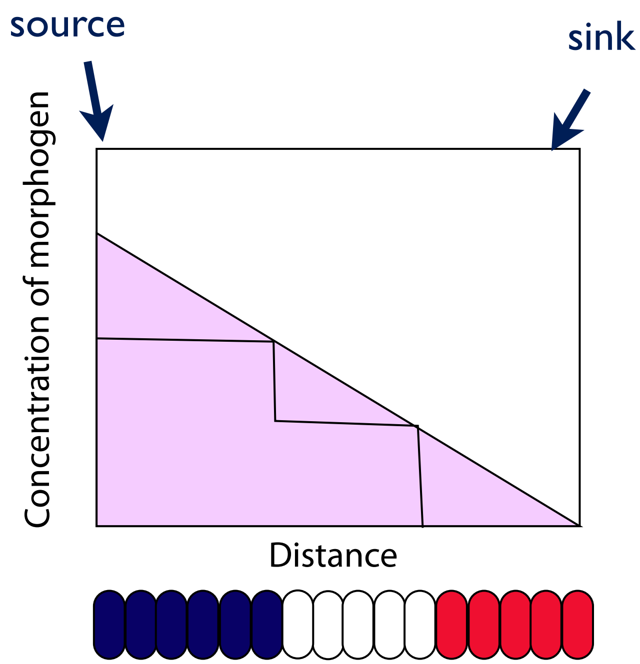
Martin and Morata, 2006
Development

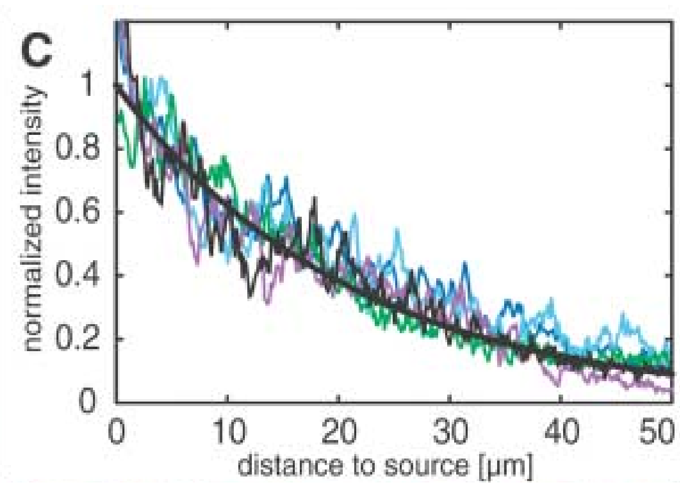
The french flag model

Positional identities are conferred by threshold responses to specific morphogen levels.

Cells sense the size of the developmental field through the slope of the gradient. Growth stops when slope falls below a threshold.

Cells sense directionality, or polarity, in tissue through the slope of the gradient.





$$C(x) = C_0 e^{-\frac{x}{\lambda}} \qquad \lambda = \sqrt{D/k}$$

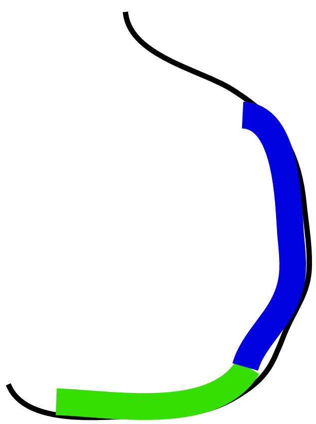
But morphogen concentration decreases exponentially with distance from the source!

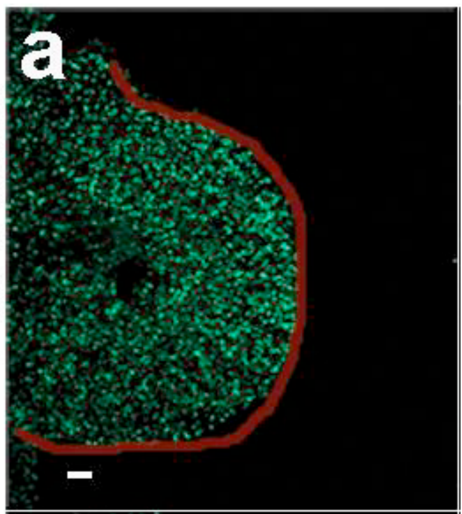
Kicheva et al., 2009

Proliferation rates are not higher near morphogen sources

BrdU incorporation to detect S phase

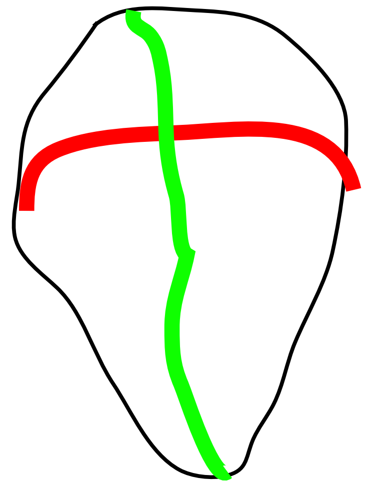
Mouse limb bud

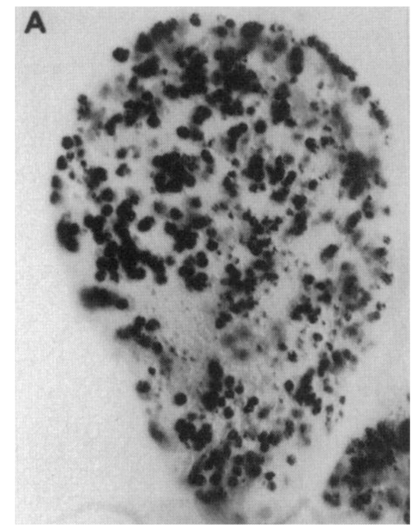




FGF 8/4
Sonic Hedgehog

Drosophila wing disc





Wingless
Dpp, Hedgehog

Integration of growth and specification in chick wing digit-patterning

Matthew Towers[1,2], Ruth Mahood[1], Yili Yin[1] & Cheryll Tickle[1,2]

Nature **452**, 882-886 (17 April 2008)

Cell cycling and patterned cell proliferation in the wing primordium of *Drosophila*

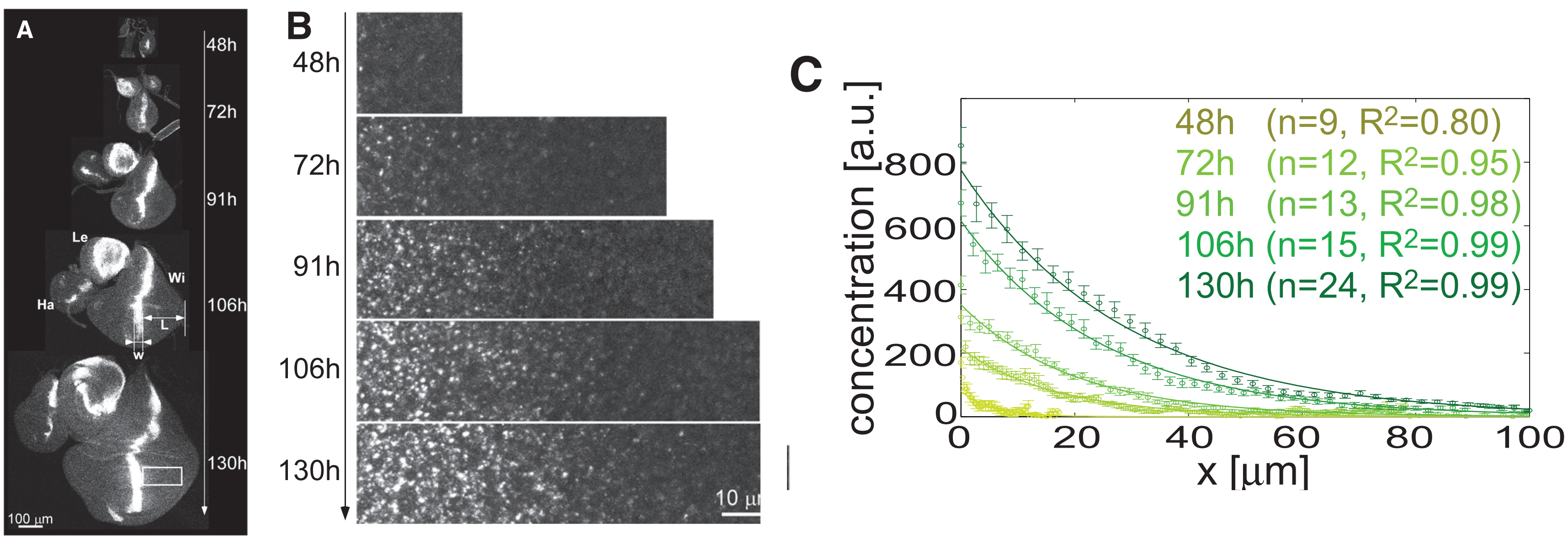
Marco Milán, Sonsoles Campuzano, and Antonio García-Bellido*

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 640–645, January 1996

Clearly proliferation is not proportional to the slope of morphogen gradients

Morphogen dependent growth is not simply concentration-dependent - not like other target genes.

The Dpp gradient scales as the wing disc grows

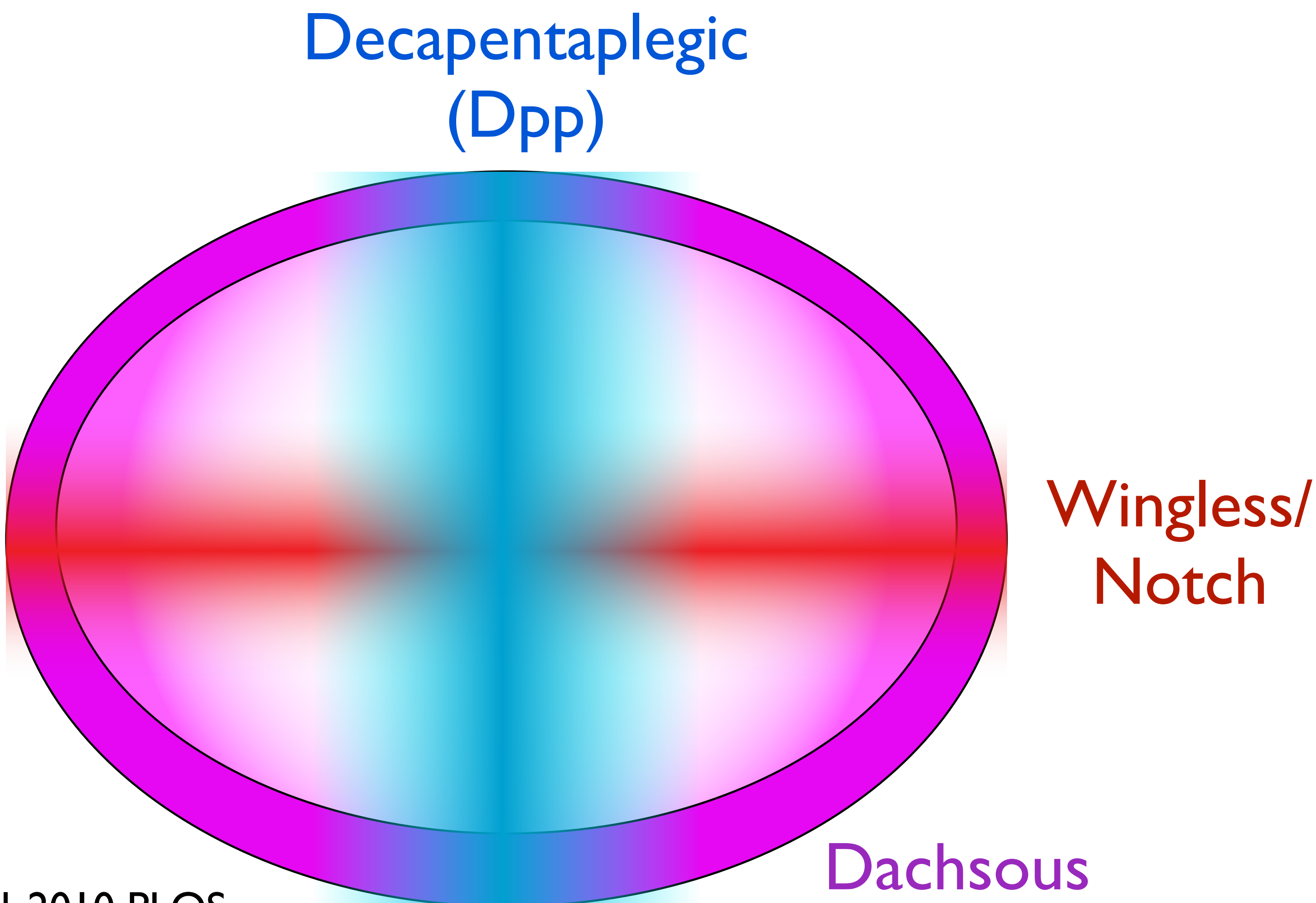


Growth rate correlates with *relative changes* in Dpp activity - cells divide when Dpp activity increases by 50%

Growth arrest occurs as the rate of change in Dpp activity falls below a critical threshold

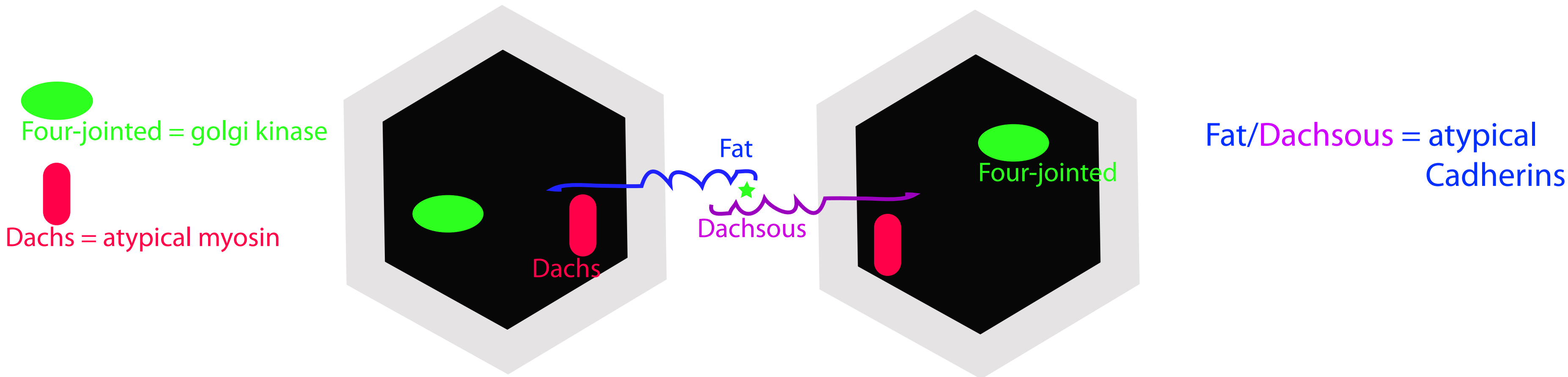
Dynamics of Dpp Signaling and Proliferation Control
O. Wartlick *et al.*
Science **331**, 1154 (2011);

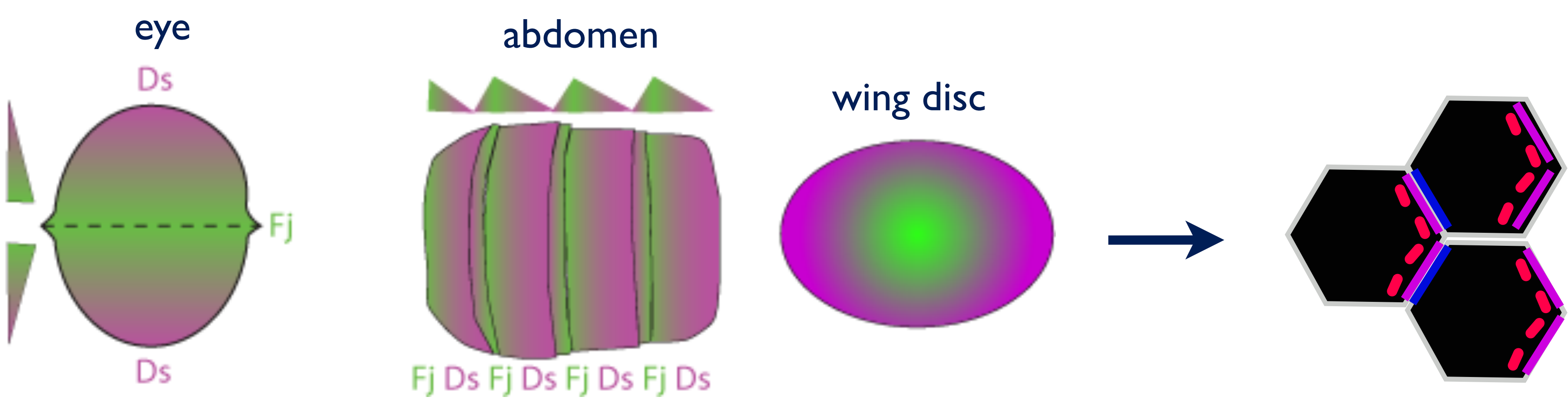
Wingless and Dpp signaling combine to pattern Dachsous/Four-jointed expression



Zecca and Struhl, 2010 PLOS Biology
Rogulja et al., 2008, Dev. Cell

The Fat/Dachsous/Four-jointed system regulates growth and tissue polarity





Planar Polarity Specification through Asymmetric Subcellular Localization of Fat and Dachsous

Amy Brittle,[1,2] Chloe Thomas,[1,2] and David Strutt[1,2]

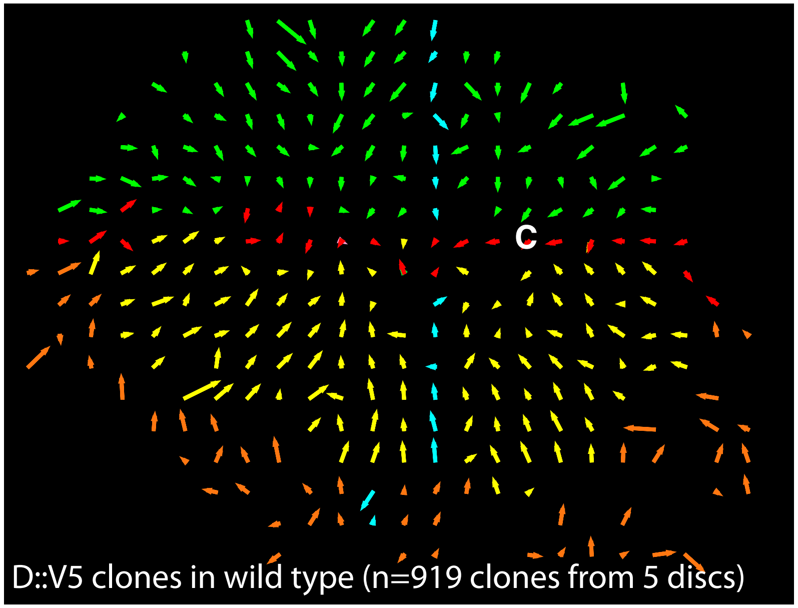
Propagation of Dachsous-Fat Planar Cell Polarity

Abhijit A. Ambegaonkar,[1] Guohui Pan,[1] Madhav Mani,[2] Yongqiang Feng,[1,3] and Kenneth D. Irvine[1,*]

Planar polarization of the atypical myosin Dachs orients cell divisions in *Drosophila*

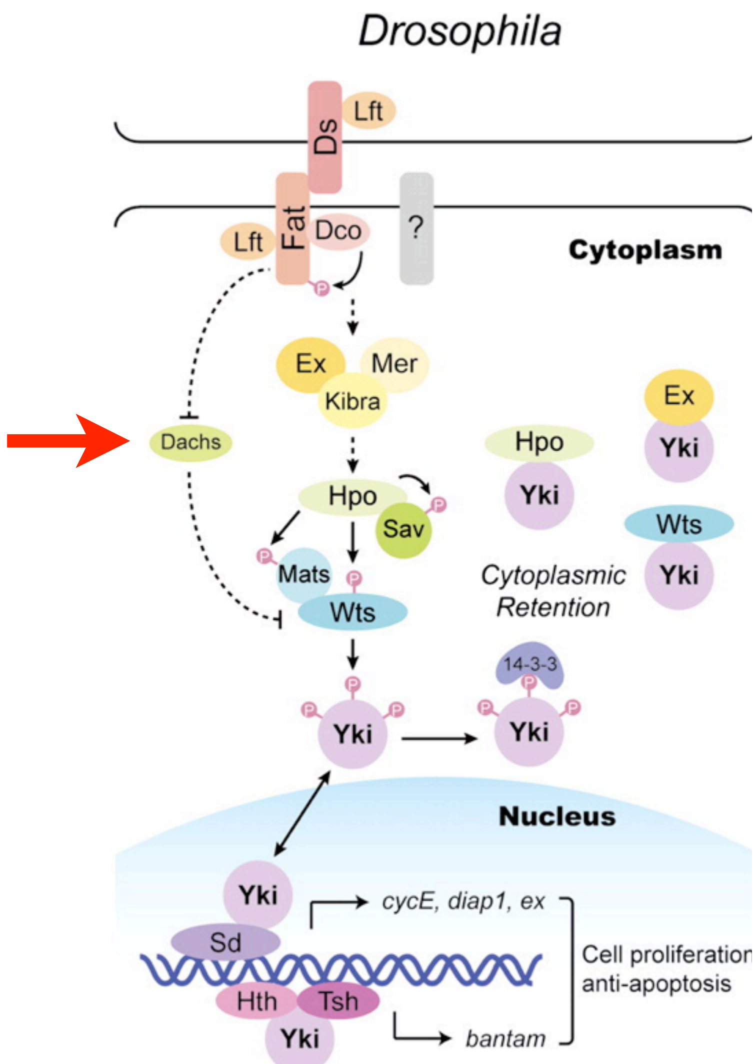
Yanlan Mao, Alexander L. Tournier, Paul A. Bates, et al.

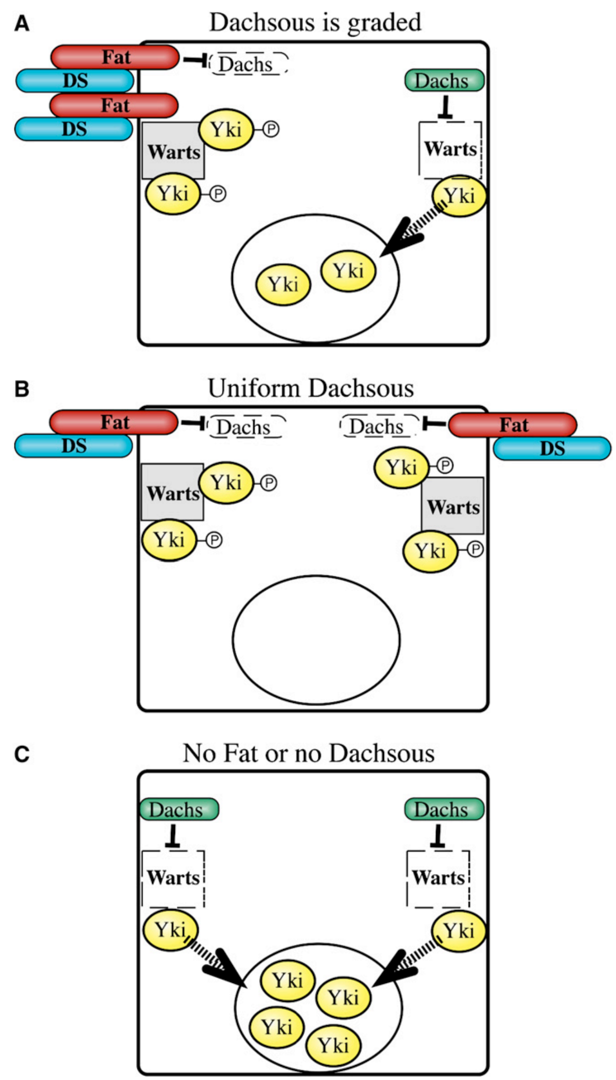
Polarity of Dachsous/Dachs domains in the wing disc orients down the Dachsous gradient



Rogulja et al, Dev Cell 2008
Brittle et al, Current Biology 2012
Sagner et al., Current Biology 2012

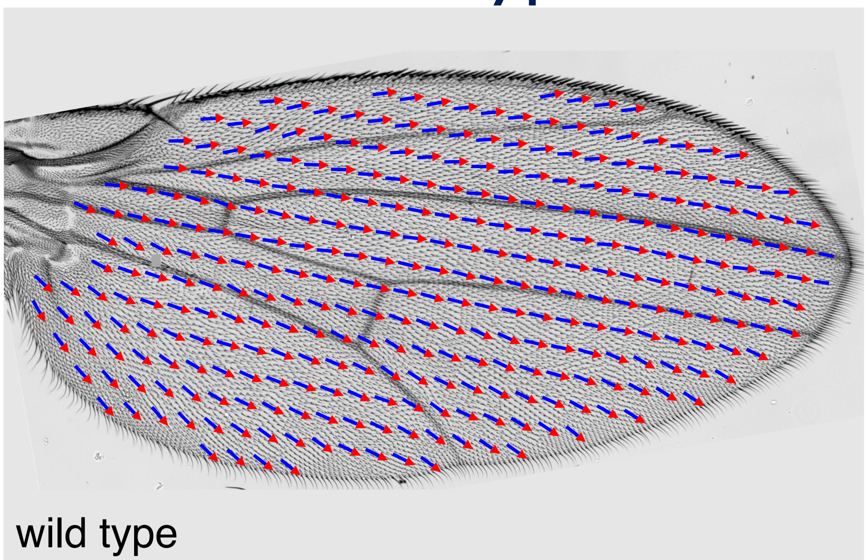
The Fat/Dachsous/Four-jointed system regulates growth through the Hippo pathway



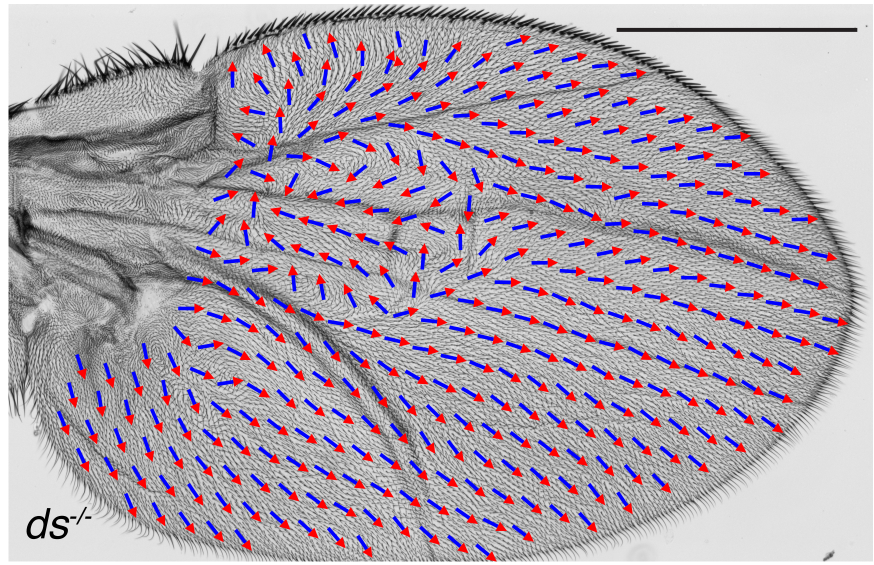


The Fat/Dachsous pathway influences the amount and orientation of growth, and planar polarity

wild type

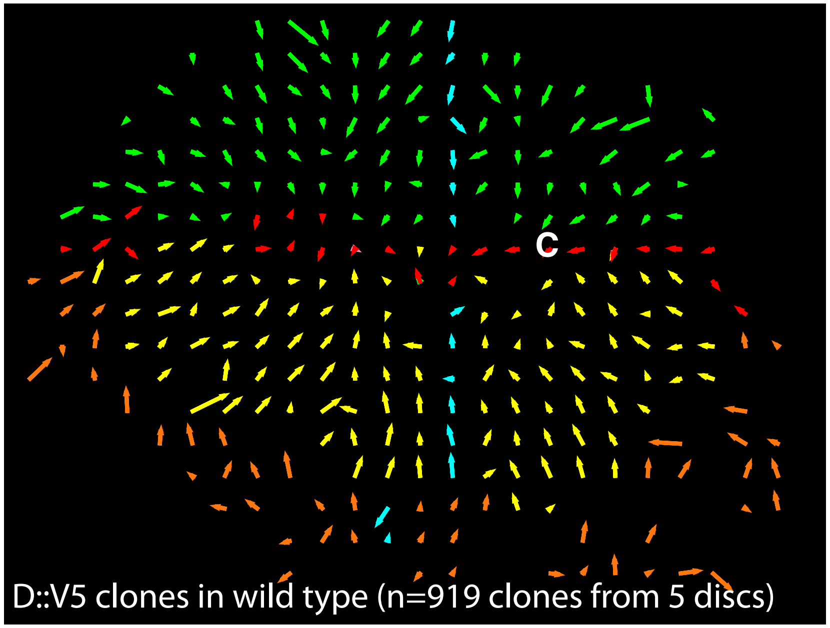


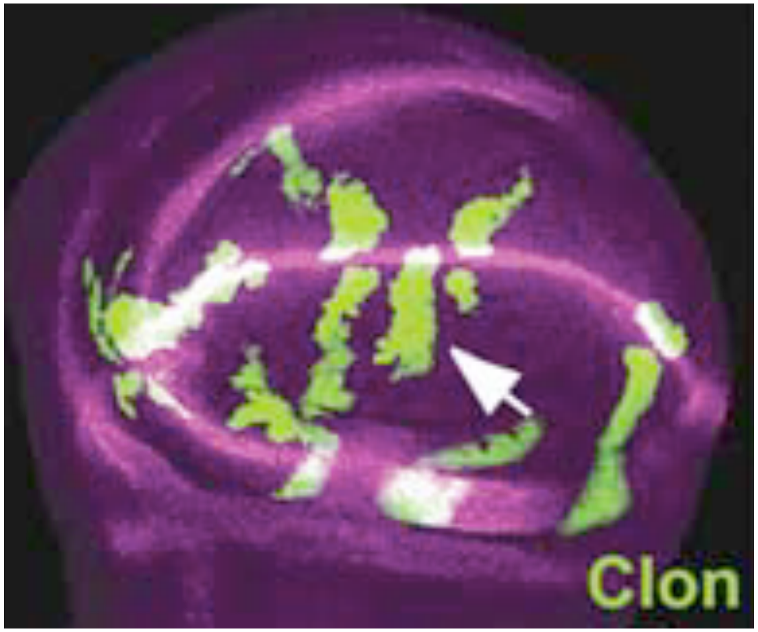
Dachsous mutant



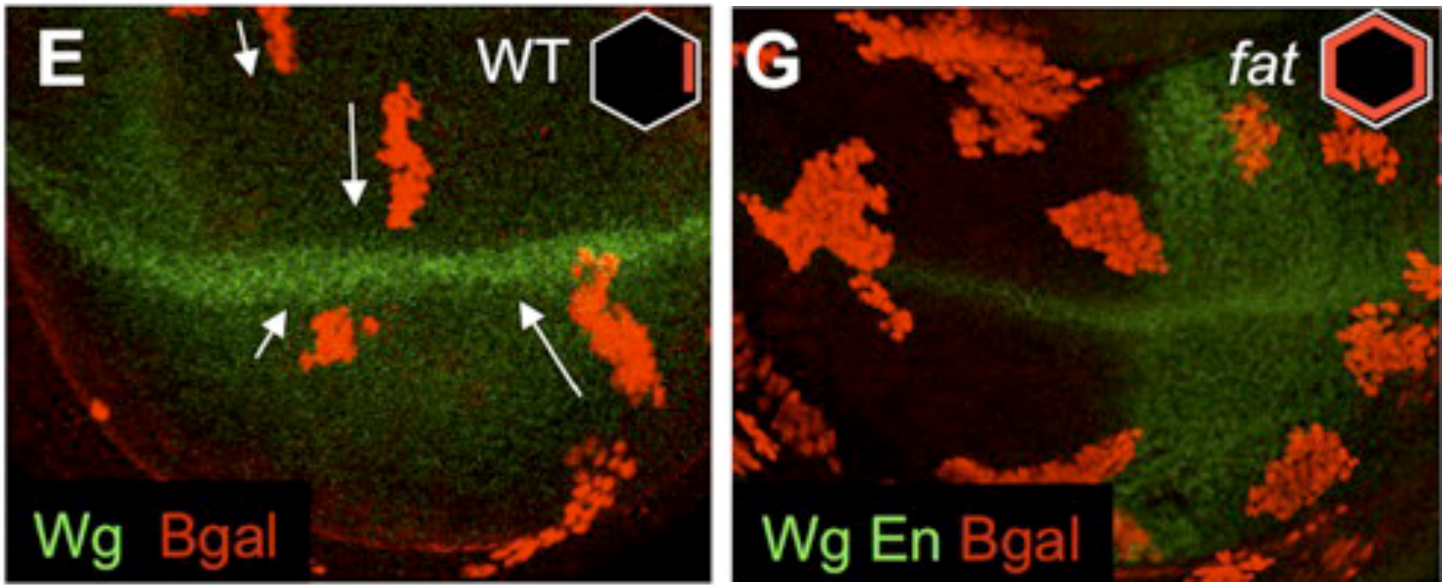
Dachs mutant

Growth orientation in the wing disc is aligned with Ds/ Dachs polarity





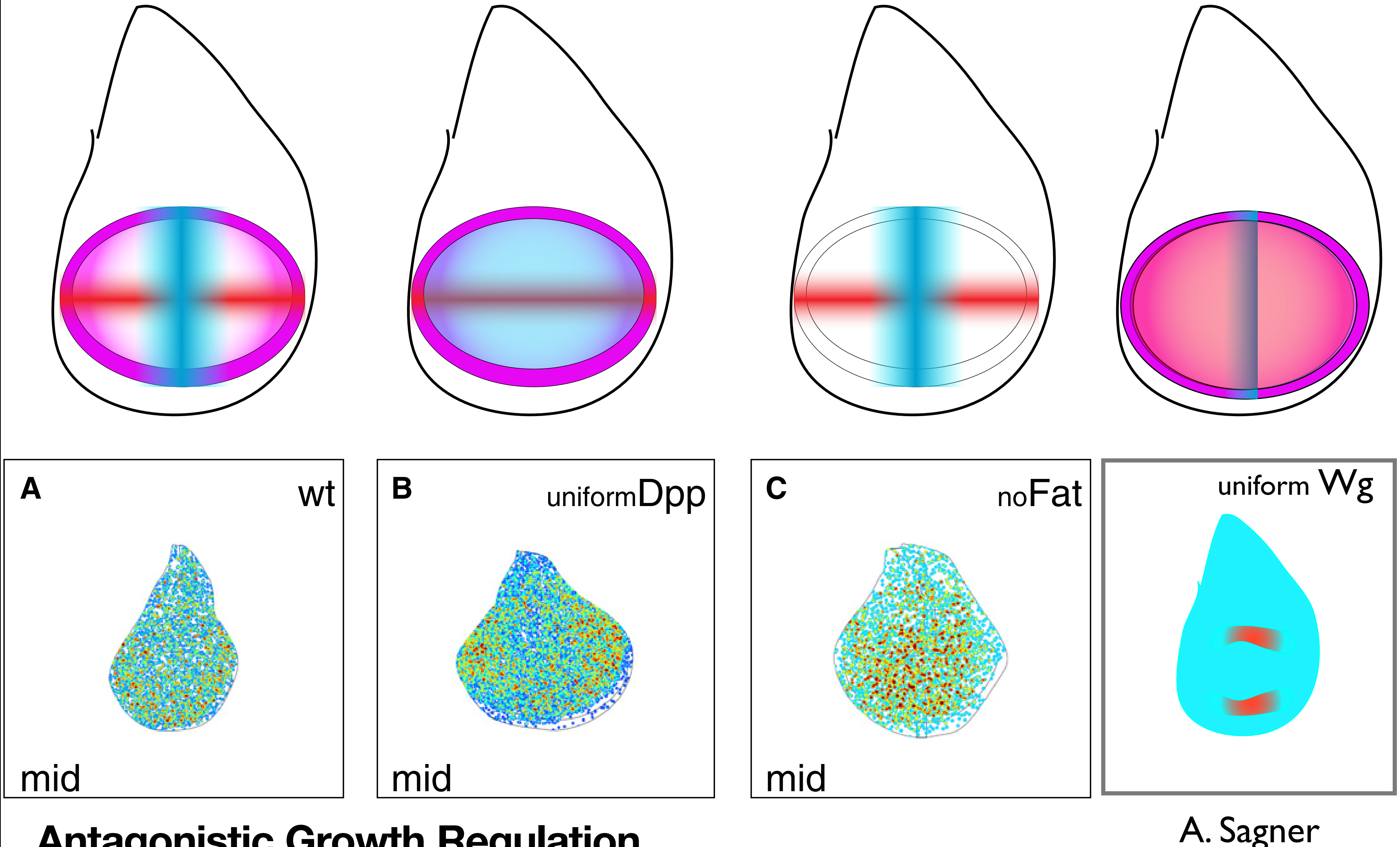
Growth orientation is disturbed by mutations in Fat, Dachsous or Dachs



Baena Lopez et al., 2005, Current biology
Mao et al., 2011, Genes and Dev.

Could Hippo pathway activity account for uniform proliferation in the wing?

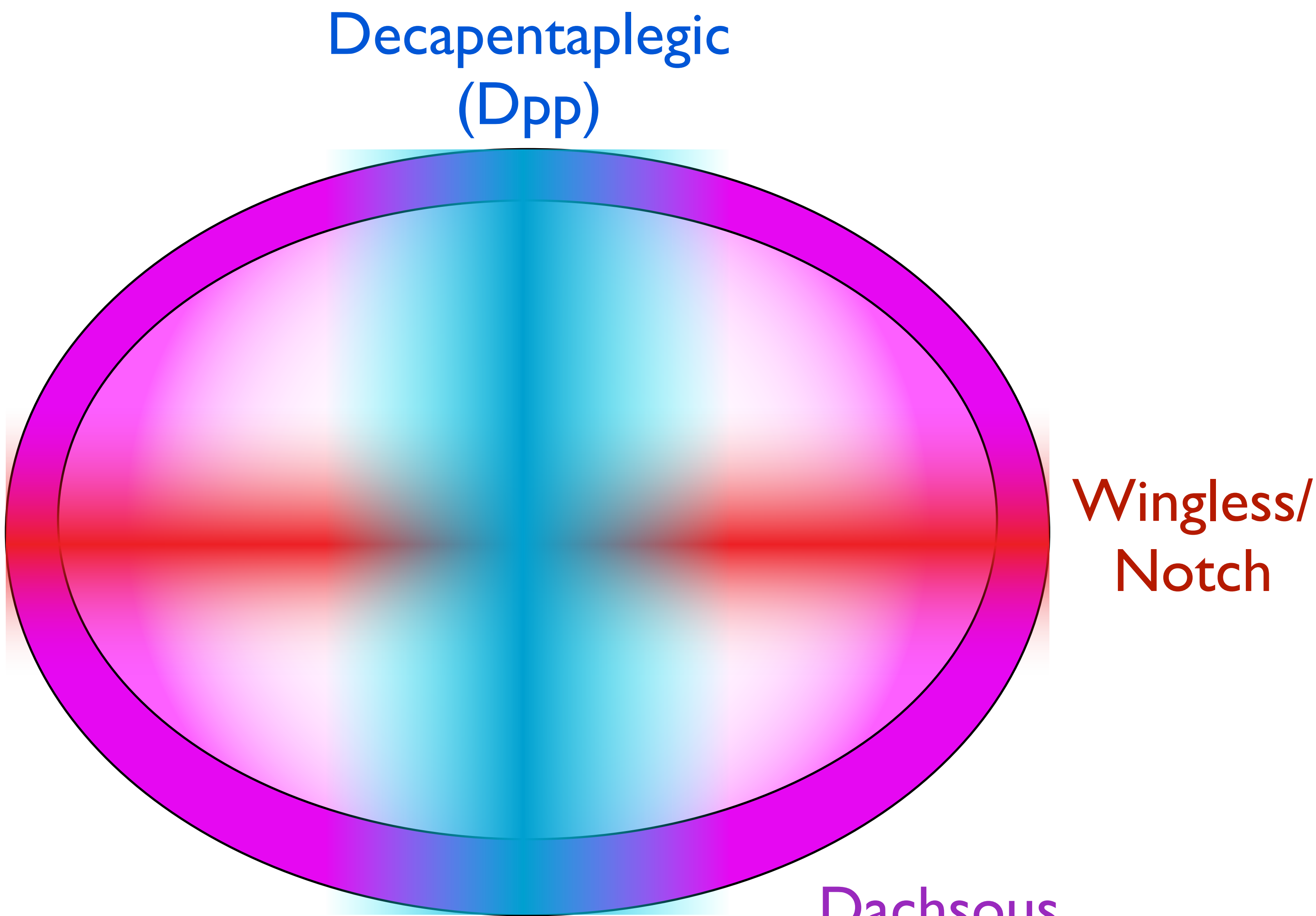
Fat inhibits growth in the middle of the disc



Antagonistic Growth Regulation by Dpp and Fat Drives Uniform Cell Proliferation

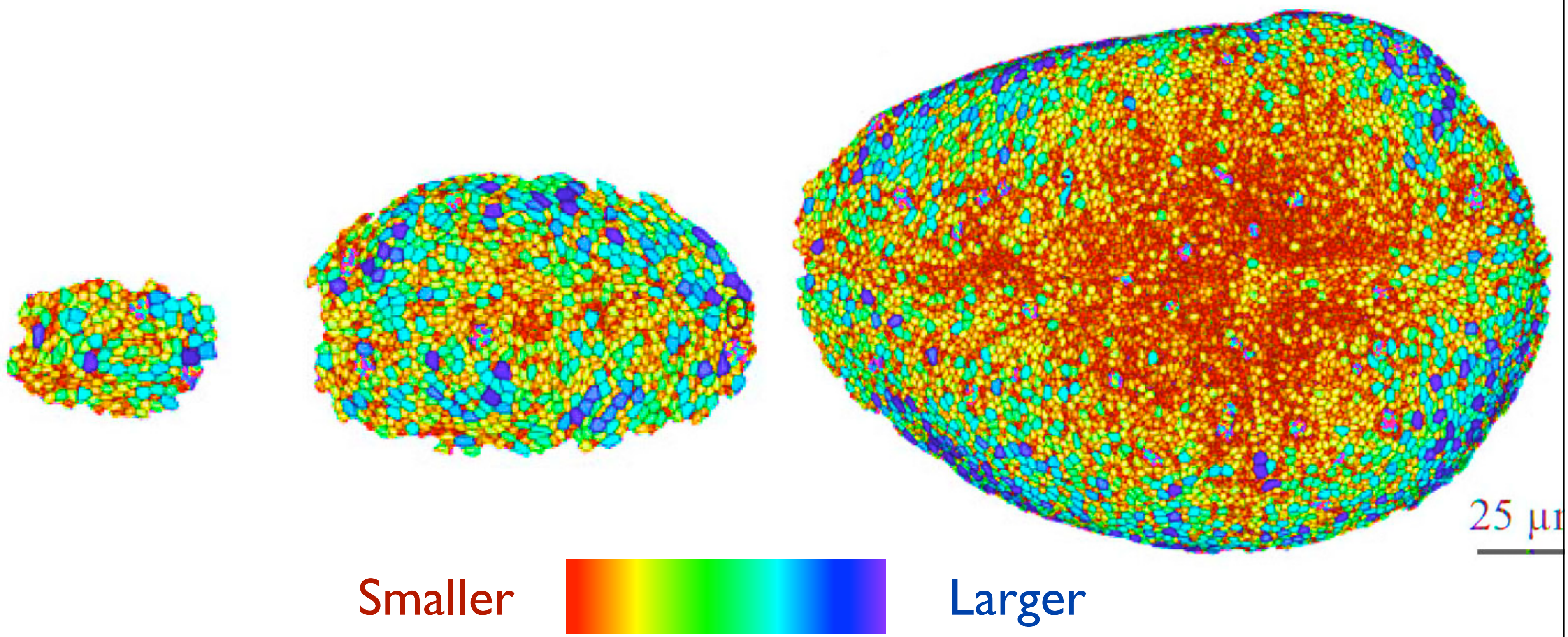
Gerald Schwank,[1] Gerardo Tauriello,[2] Ryohei Yagi,[1] Elizabeth Kranz,[1] Petros Koumoutsakos,[2] and Konrad Basler[1,*]

Uniform proliferation may result from a combination of different signals



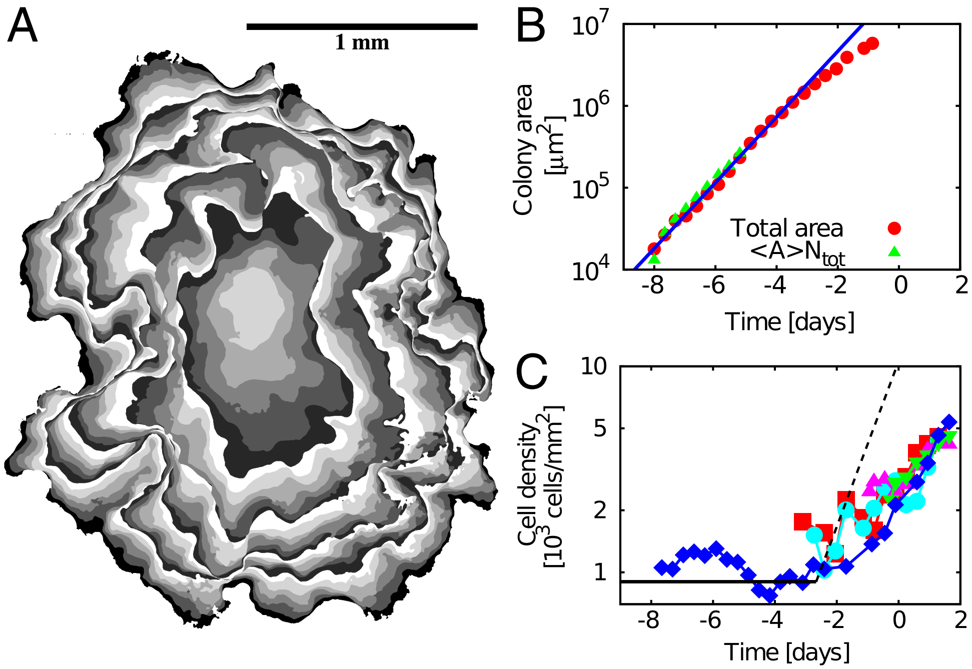
But what sets the growth end-point?

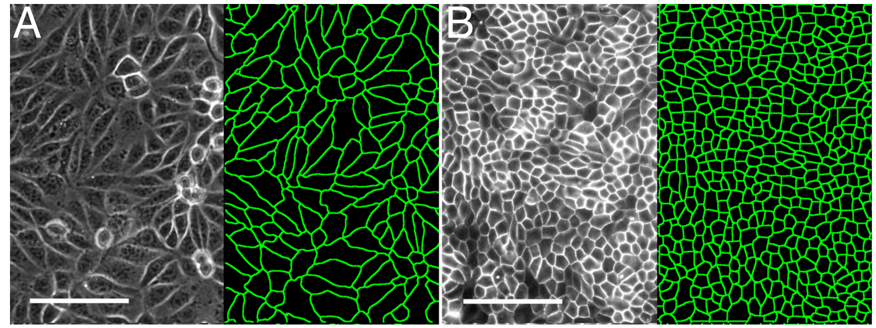
Wing disc cells reduce their cross-sectional area as the wing grows



Epithelial compression blocks growth and promotes delamination

Compression inhibits growth





Colony size increase is limited by movement of cells at the periphery - cells become increasingly compressed.

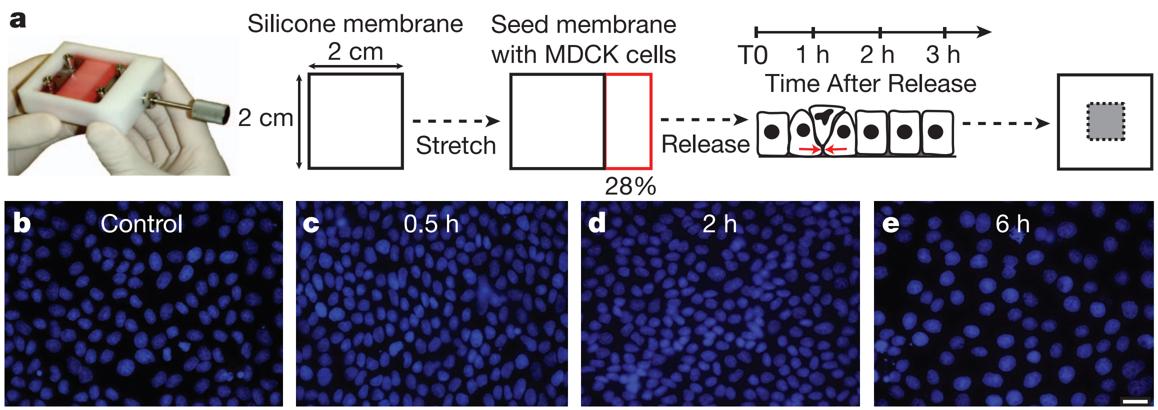
Proliferation stops at a critical colony size when cells are sufficiently compressed

Collective and single cell behavior in epithelial contact inhibition

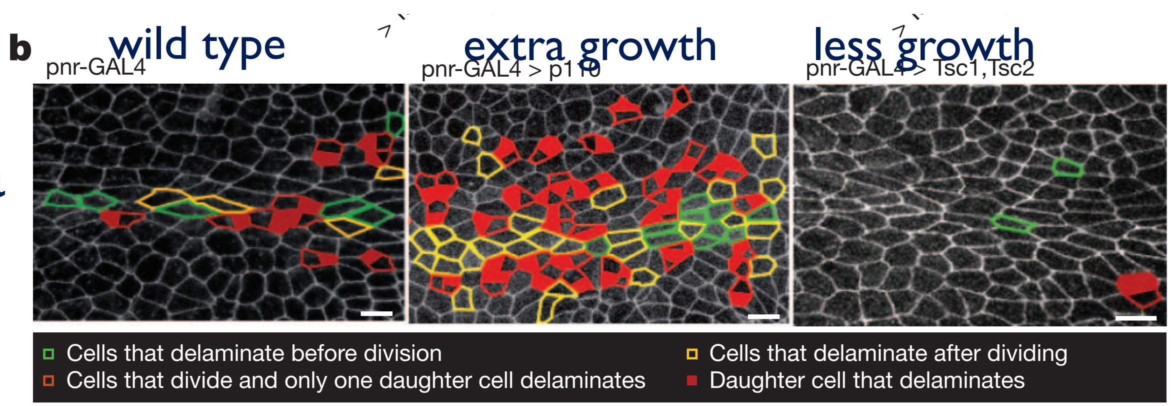
Alberto Puliafito[a,1], Lars Hufnagel[a,b,1], Pierre Neveu[a], Sebastian Streichan[b], Alex Sigal[c], D. Kuchnir Fygenson[d], and Boris I. Shraiman[a,d,2]

Compression induces delamination

MDCK



Drosophila thorax



Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding

Eliana Marinari[1]*, Aida Mehonic[2]*, Scott Curran[1], Jonathan Gale[3], Thomas Duke[2] & Buzz Baum[1]

Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia

George T. Eisenhoffer[1]*, Patrick D. Loftus[1]*, Masaaki Yoshigi[2], Hideo Otsuna[3], Chi-Bin Chien[3], Paul A. Morcos[4] & Jody Rosenblatt[1]

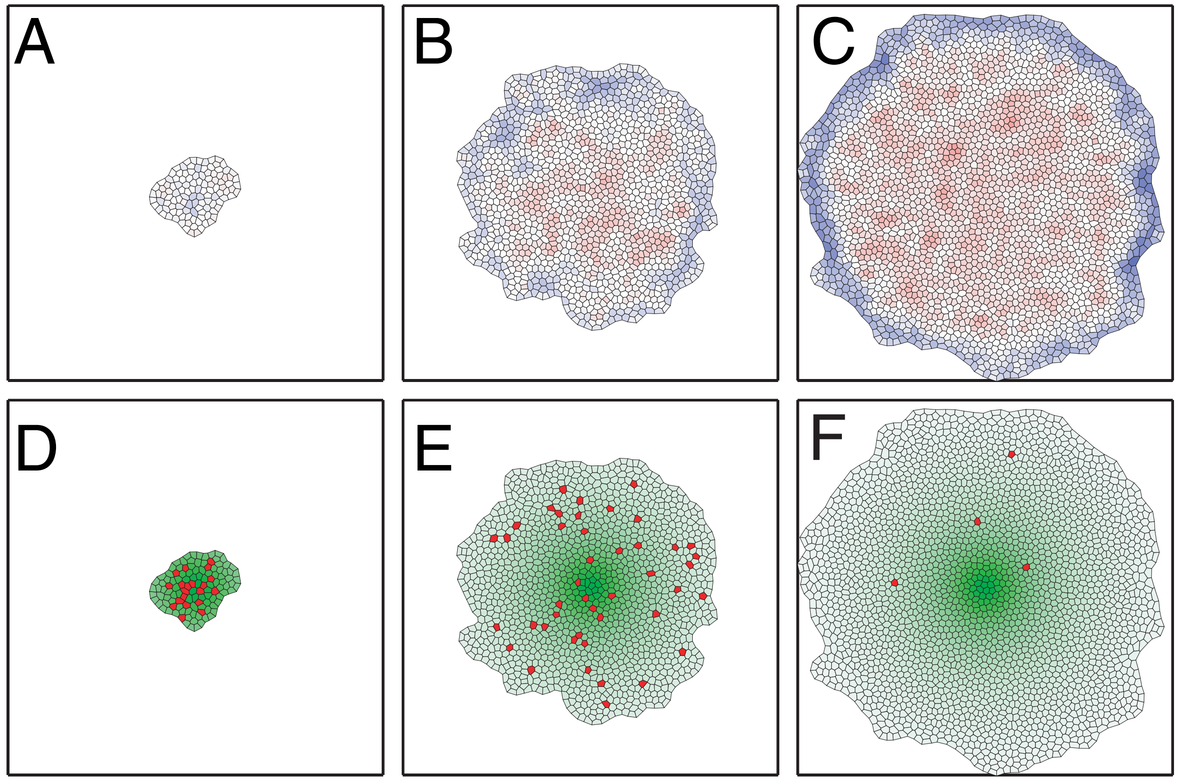
| NATURE | VOL 484 | 26 APRIL 2012

On the mechanism of wing size determination in fly development

Lars Hufnagel*, Aurelio A. Teleman†, Hervé Rouault‡, Stephen M. Cohen†, and Boris I. Shraiman*§

PNAS | March 6, 2007 | vol. 104 | no. 10 | 3835–3840

$$E(r_i, \xi_\alpha) = \sum_\alpha \left[\rho_\alpha + a(V_\alpha - V_0)^2 + b \sum_{\beta = \nu(\alpha)} (\xi_\alpha - \xi_\beta)^2 + c(\xi_\alpha - 1)^2 \right],$$

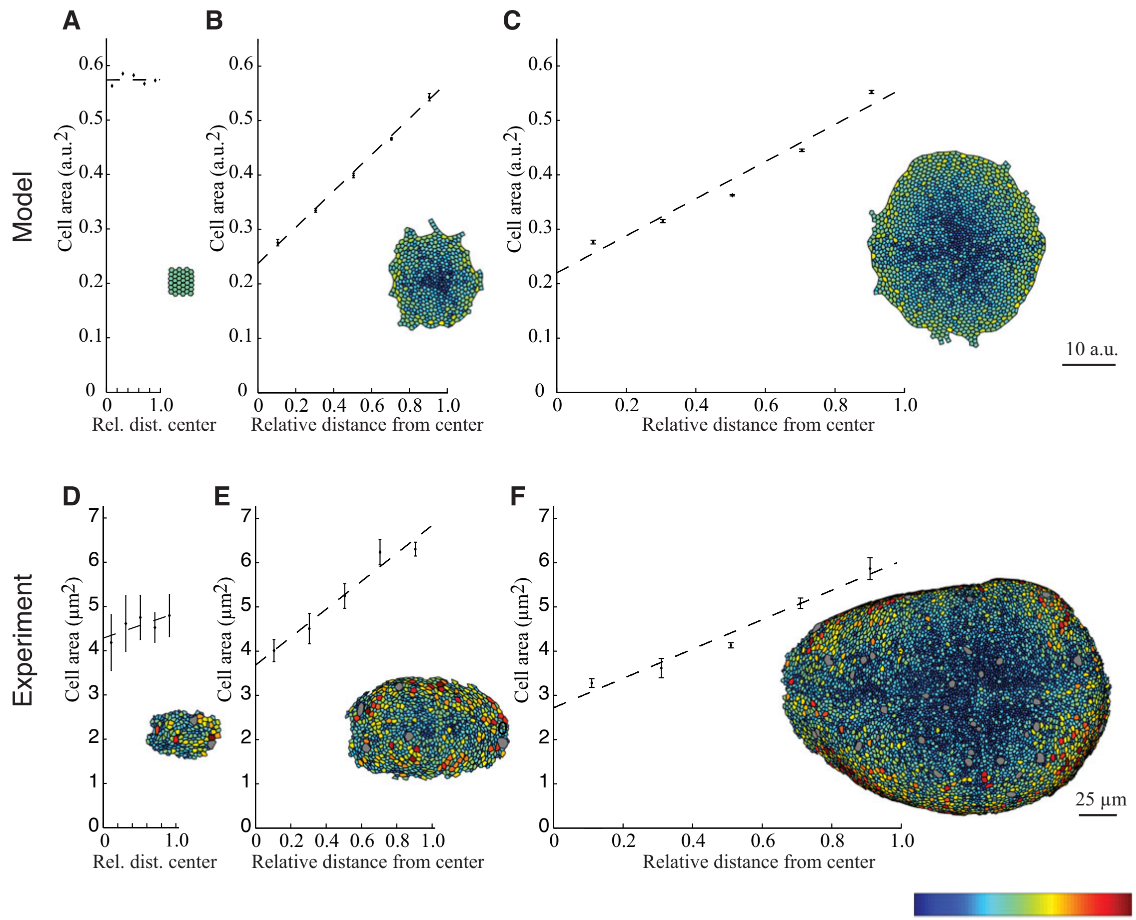


Proliferation rate depends on morphogen levels and on local stress

Growth stops in the disc center as a result of compression, and at the edges as a result of low morphogen levels

Integrating force-sensing and signaling pathways in a model for the regulation of wing imaginal disc size

Tinri Aegerter-Wilmsen[1], Maria B. Heimlicher[1], Alister C. Smith[1], Pierre Barbier de Reuille[2], Richard S. Smith[2], Christof M. Aegerter[3] and Konrad Basler[1,*]



Growth stops in the disc center as a result of compression, and at the edges as a result of a compression gradient sensed by Fat/Ds

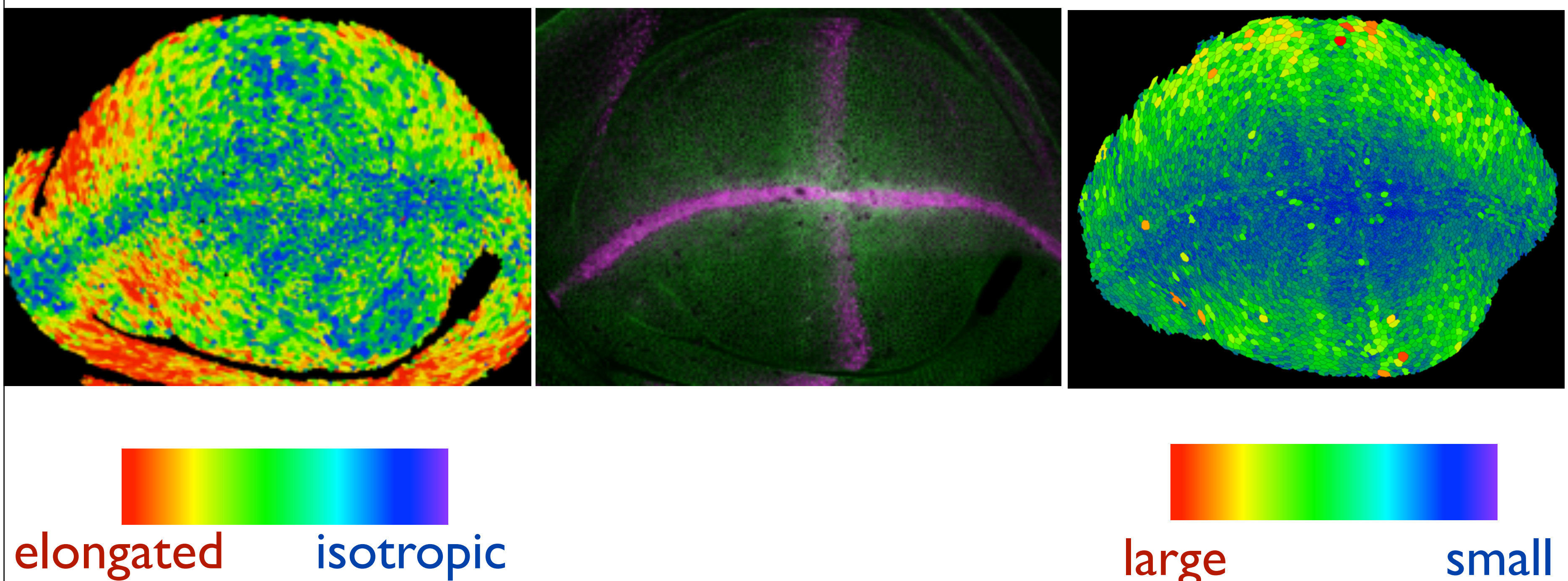
Some questions about growth control....

What are the molecular readouts of compression - does the Fat pathway mediate compression-induced growth inhibition?

Dachs, Merlin and Expanded at cytoskeletally associated.
Yorkie is homologous to YAP-TAZ, which mediates mechanotransductive signals in mammals

Some questions about growth control....

How do local variations in mechanical properties of cells influence global growth patterns?



Patterns of anisotropic cell shapes in the wing disc

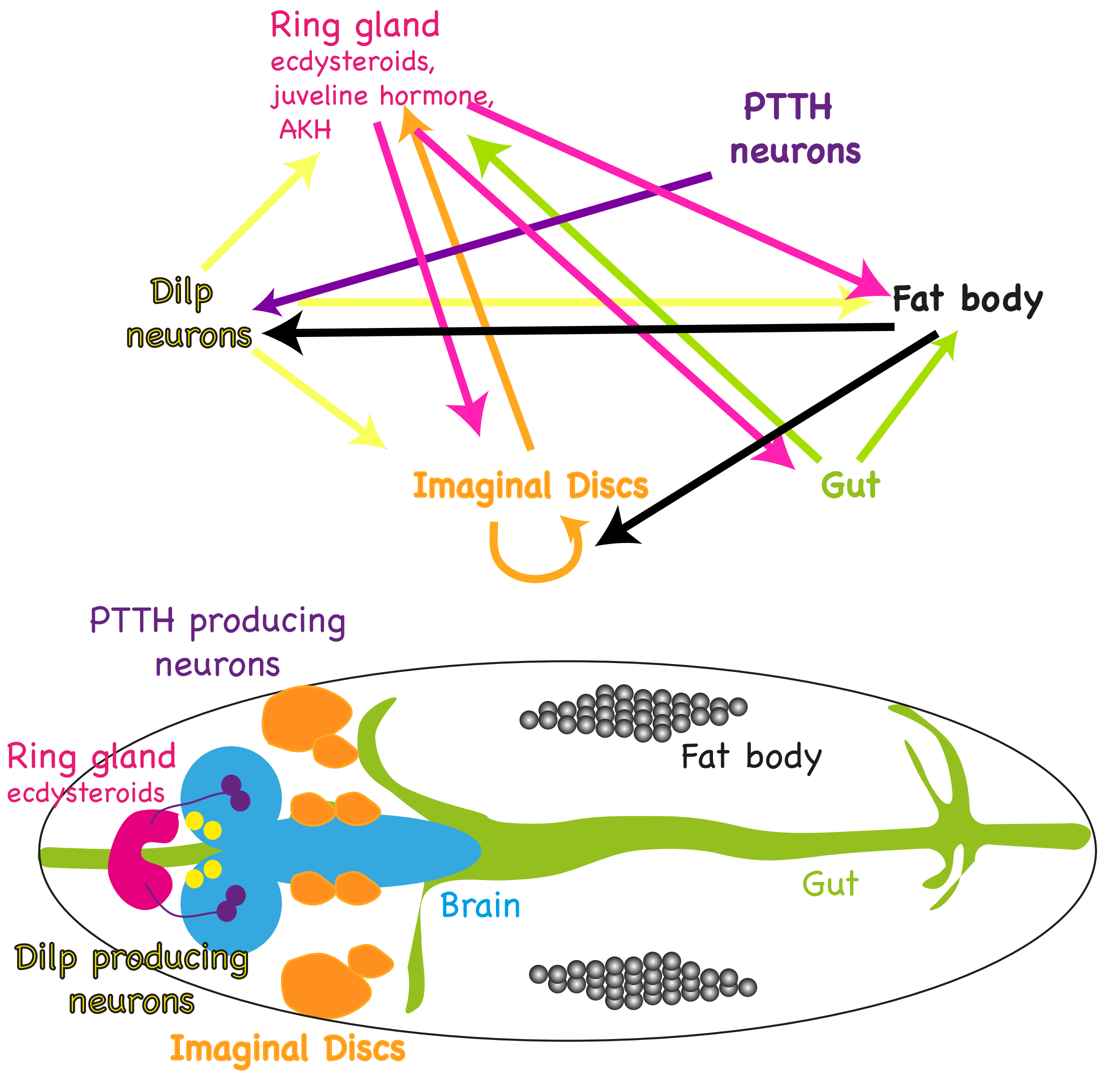
Some questions about growth control....

How would mechanical growth control mechanisms scale in different organs?

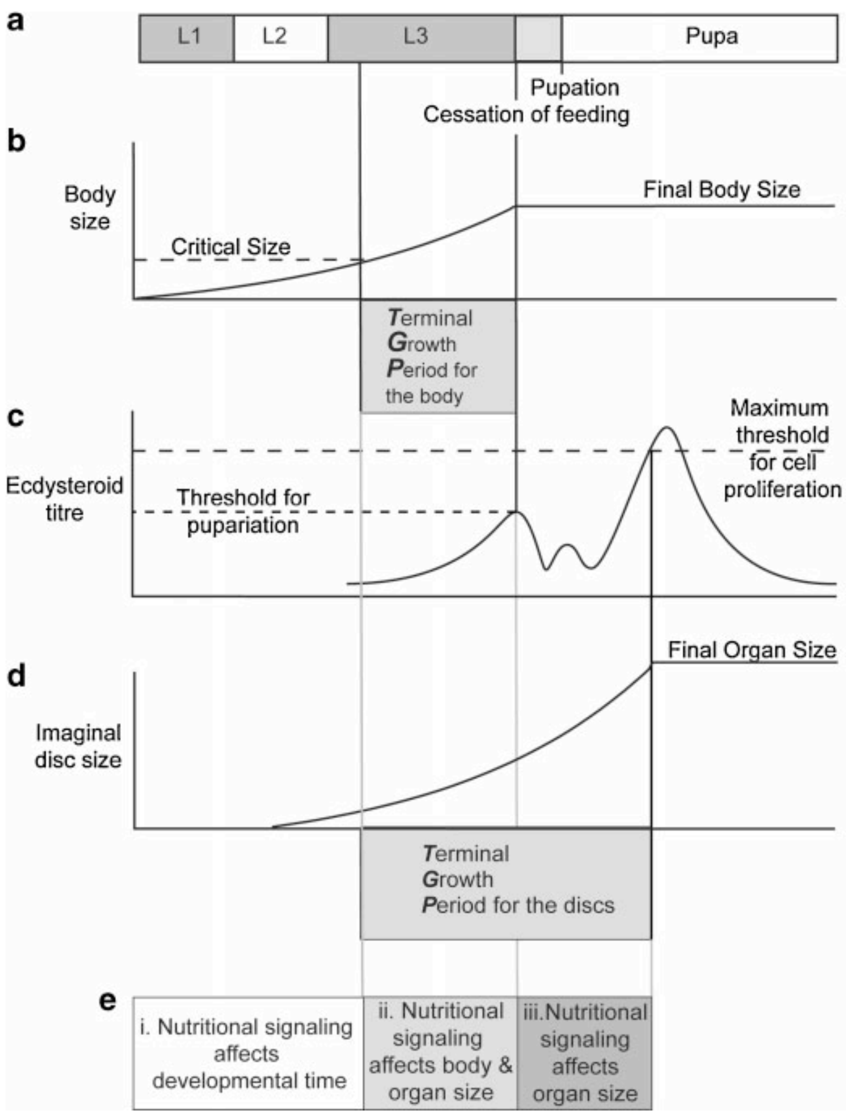
or to give different wing sizes under different temperatures and nutritional conditions?

well fed	poorly fed
low temperature	high temperature
high oxygen	low oxygen

Disc growth and growth termination are controlled by a network of inter-organ signaling



Adult body size depends on the length of the terminal growth phase

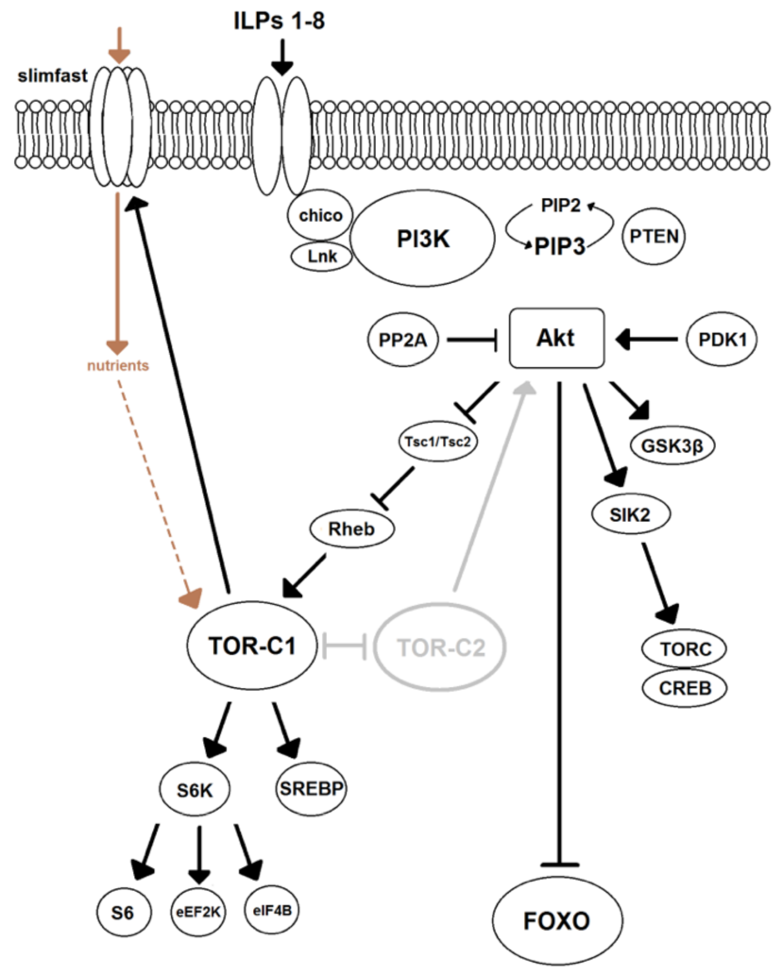


When larvae reach critical size, they become committed to pupariate (before that starvation arrests development)

The larval growth phase is terminated by a pulse of ecdysone

Nutritional state after critical weight regulates the amount of disc growth through insulin signaling

Drosophila have 8 Insulin/IGF-like peptides and one Insulin receptor



Different Dilps regulate disc growth under different conditions

An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control

Walter Brogiolo*, Hugo Stocker*, Tomoatsu Ikeya*, Felix Rintelen*, Rafael Fernandez† and Ernst Hafen*

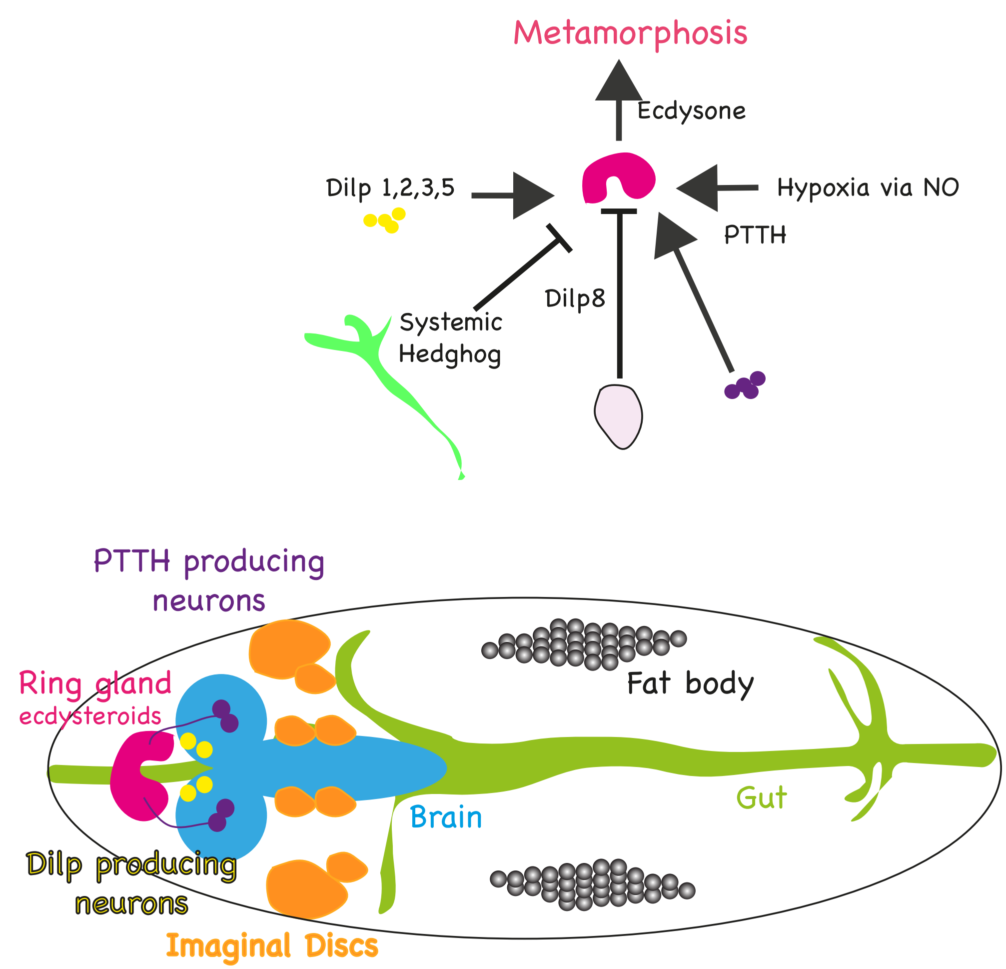
A Fat Body-Derived IGF-like Peptide Regulates Postfeeding Growth in Drosophila

Naoki Okamoto,[1,4] Naoki Yamanaka,[2,4] Yoshimasa Yagi,[1] Yasuyoshi Nishida,[1] Hiroshi Kataoka,[3] Michael B. O'Connor,[2] and Akira Mizoguchi[1,*]

A Drosophila Insulin-like Peptide Promotes Growth during Nonfeeding States

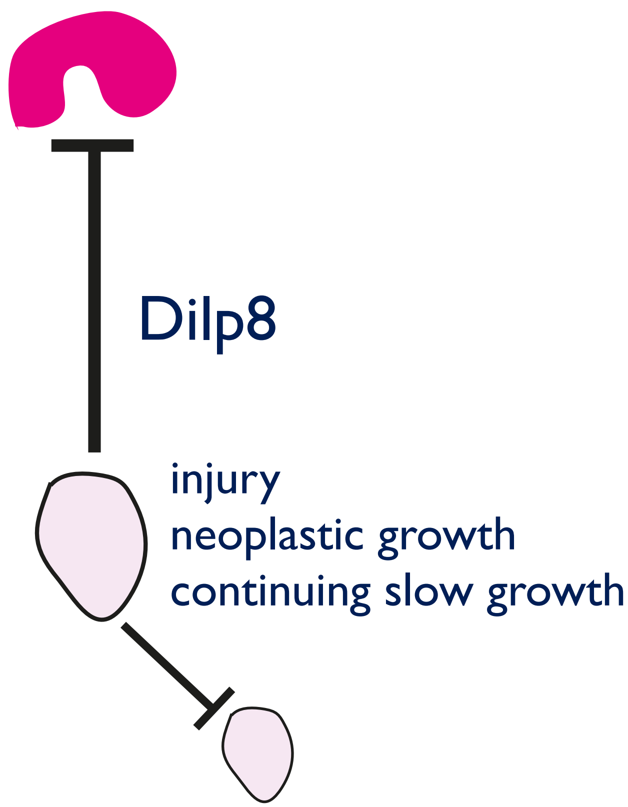
Maija Slaidina,[1] Re´ nald Delanoue,[1] Sebastian Gronke,[2] Linda Partridge,[2] and Pierre Le´ opold[1,*]

Ecdysone production by the ring gland terminates growth and initiates metamorphosis



Multiple signals regulate ecdysone production

Injured or slow-growing discs block ecdysone production and delay pupariation



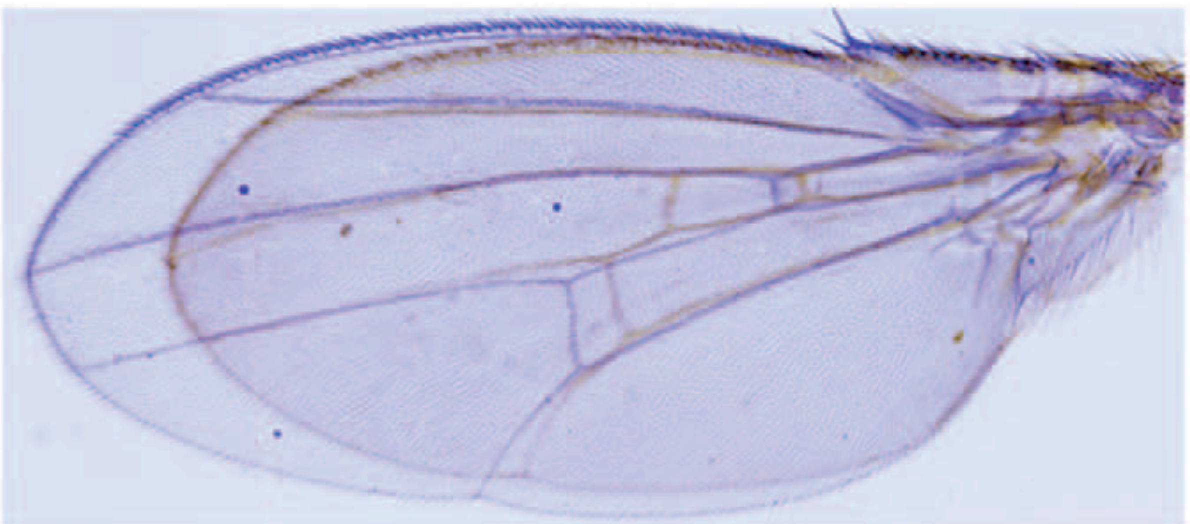
Dilp8 mutants can't delay pupariation in response to disc injury

Bradley C. Stieper, Mania Kupershtok, Michael V. Driscoll, Alexander W. Shingleton *
2008, Developmental Biology

Andres Garelli,* Alisson M. Gontijo,* Veronica Miguela, Esther Caparros, Maria Dominguez†
2012, SCIENCE

Julien Colombani,* Ditte S. Andersen,*† Pierre Léopold†
2012, SCIENCE

Dilp8 coordinates disc growth to harmonize organ size



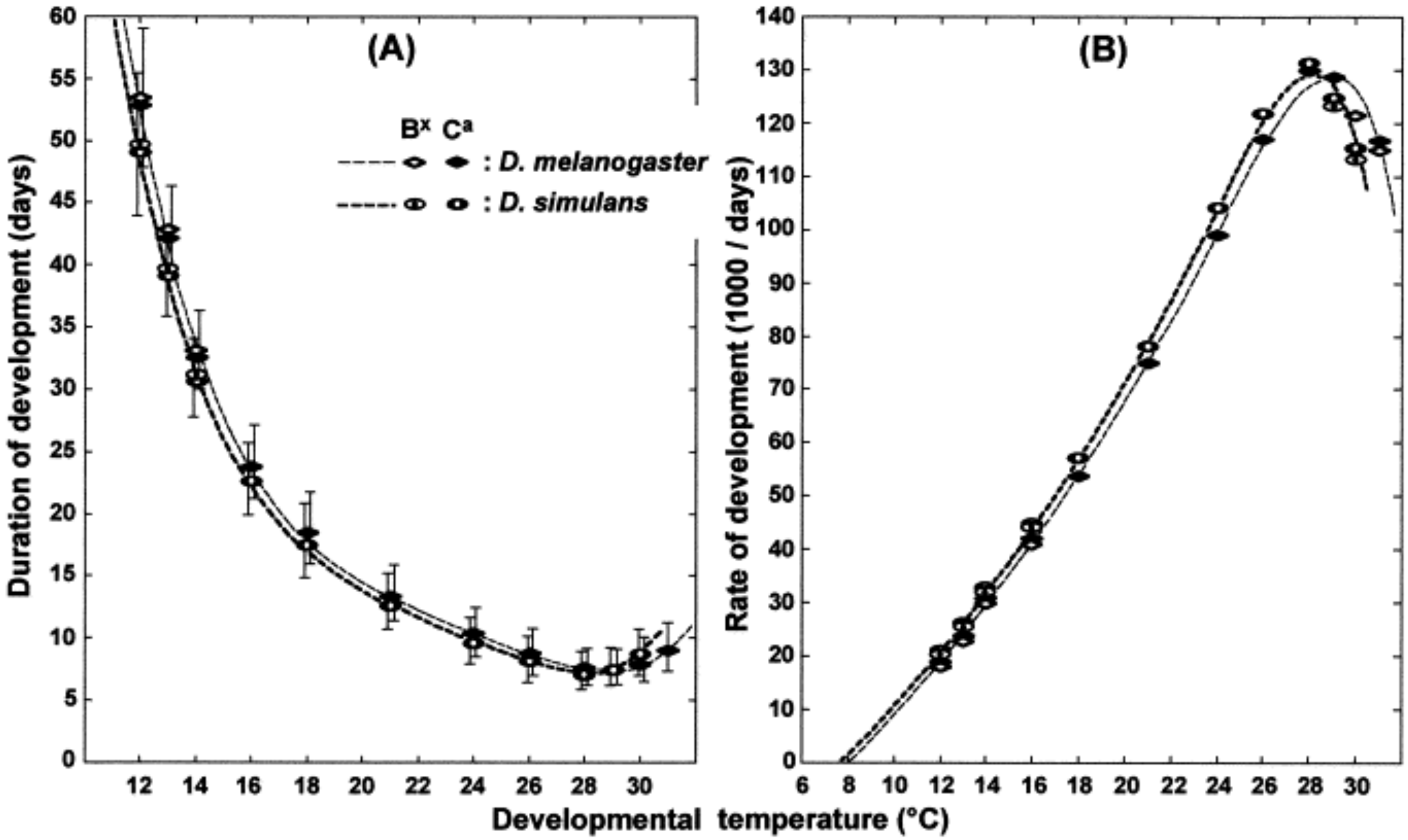
Dilp mutants don’t maintain the correct proportional size of different organs

left wing area (63.171 pixels)
right wing area (76.437 pixels)

Andres Garelli,* Alisson M. Gontijo,* Veronica Miguela, Esther Caparros, Maria Dominguez†

2012, SCIENCE

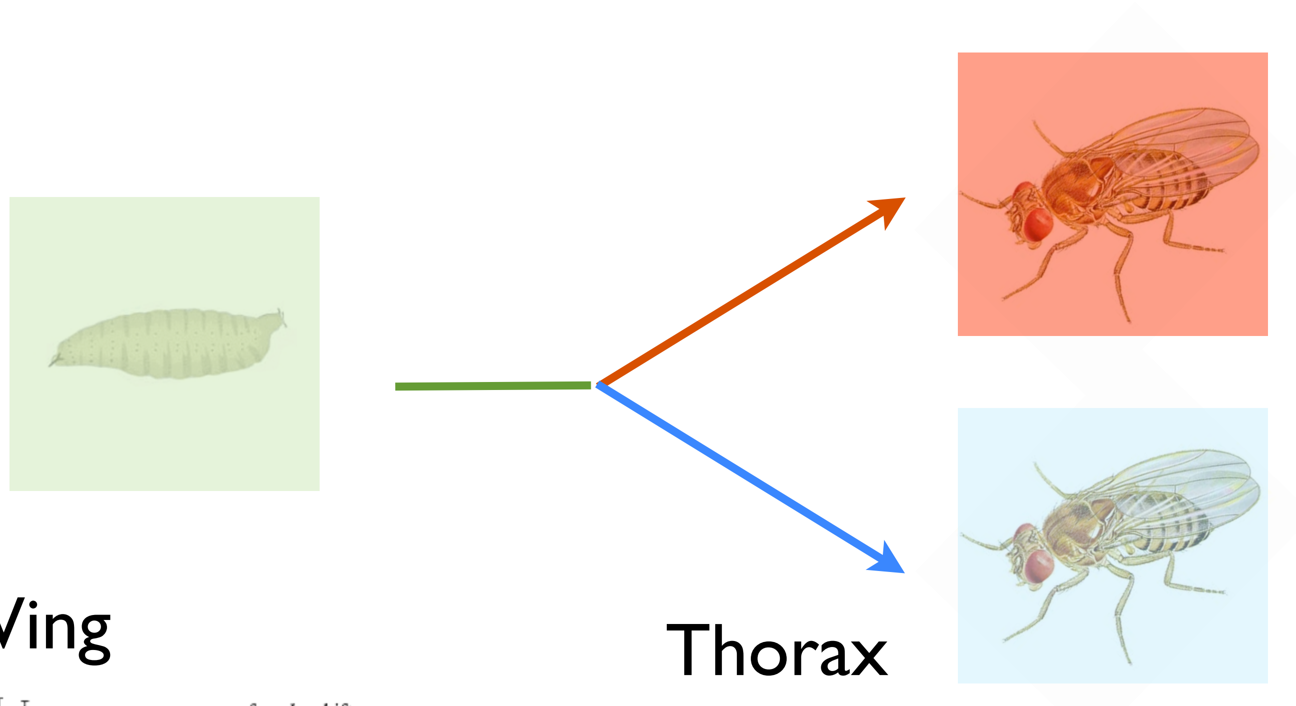
Developmental rate increases with temperature



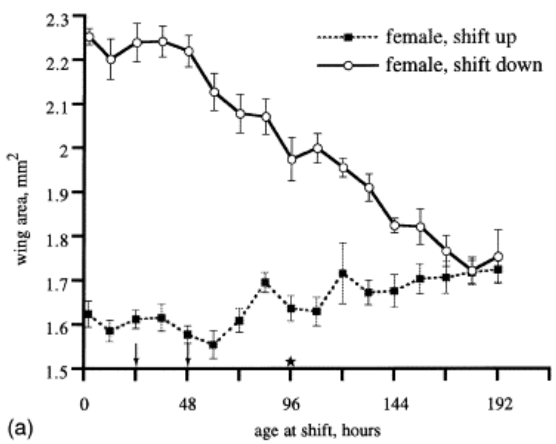
...but body size decreases!

Body size and cell size in *Drosophila*: the developmental response to temperature

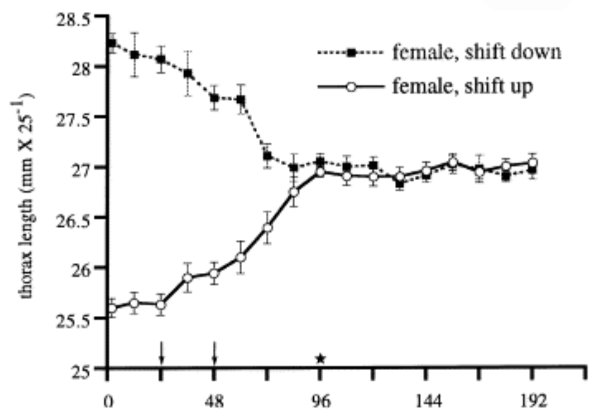
1 Vernon French[a], Marieke Feast[a, b], Linda Partridge[c]



Wing

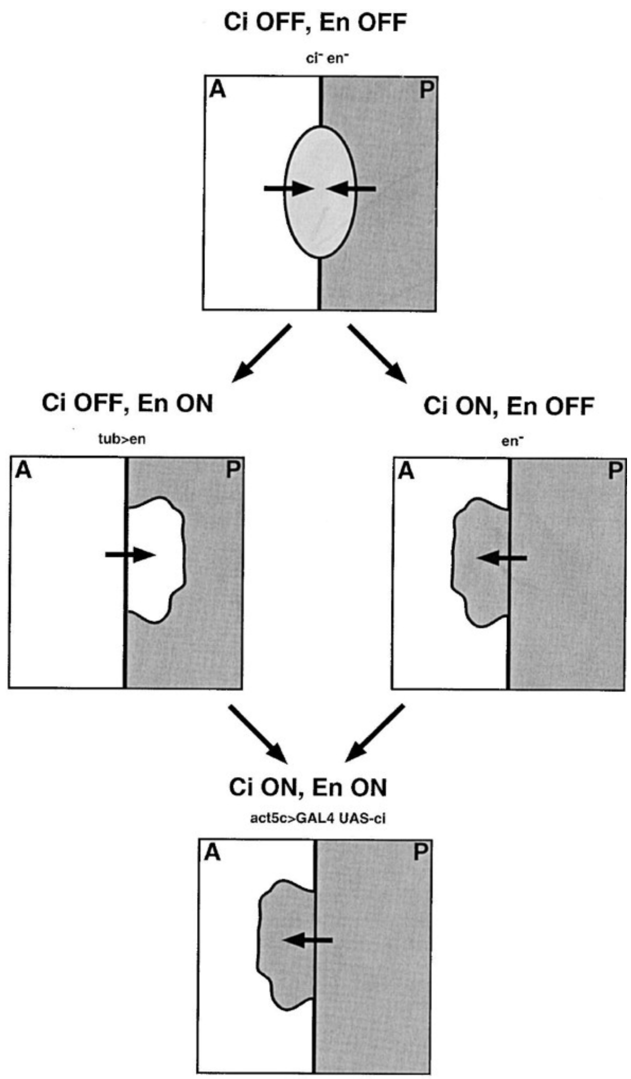


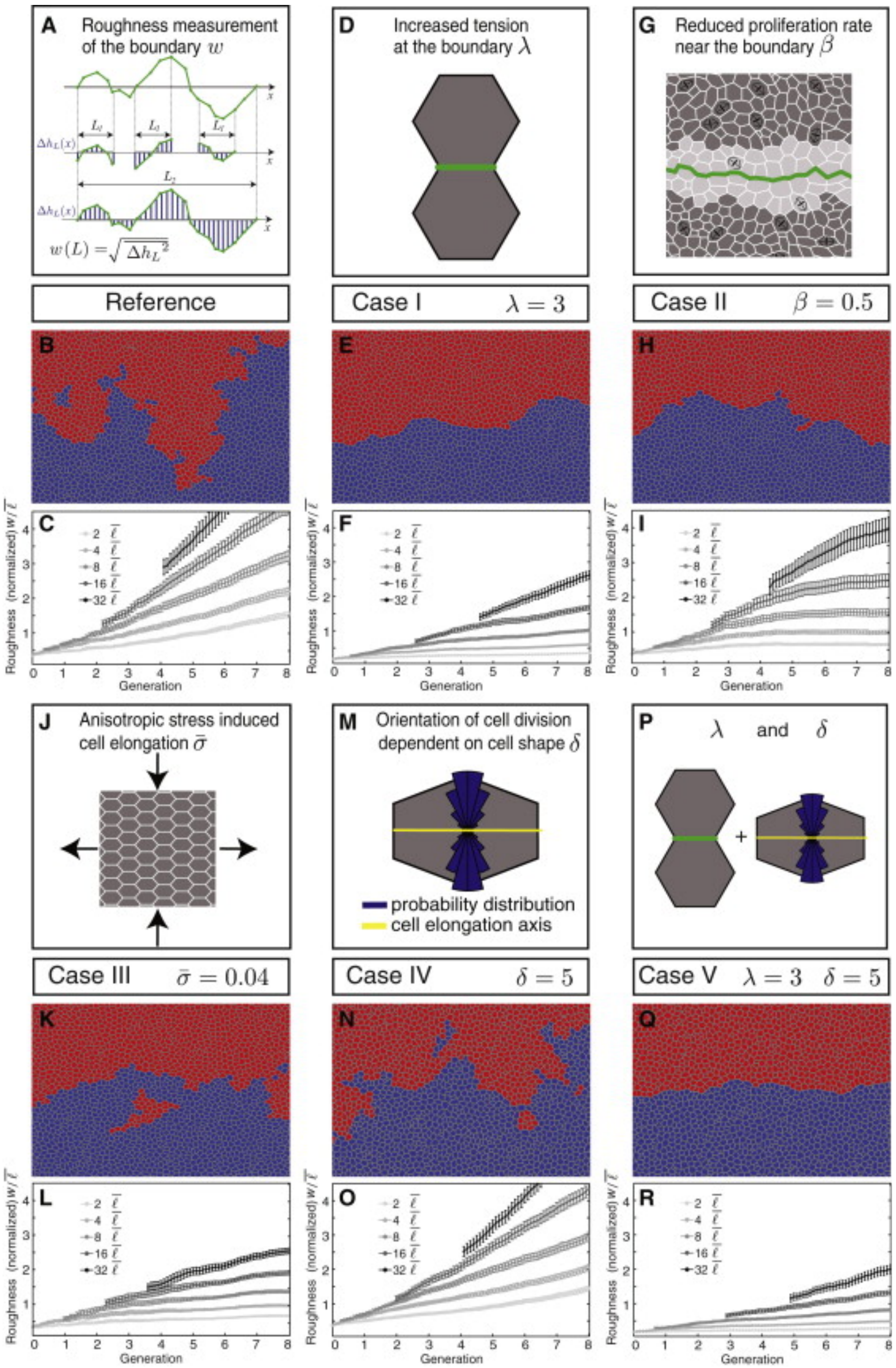
Thorax



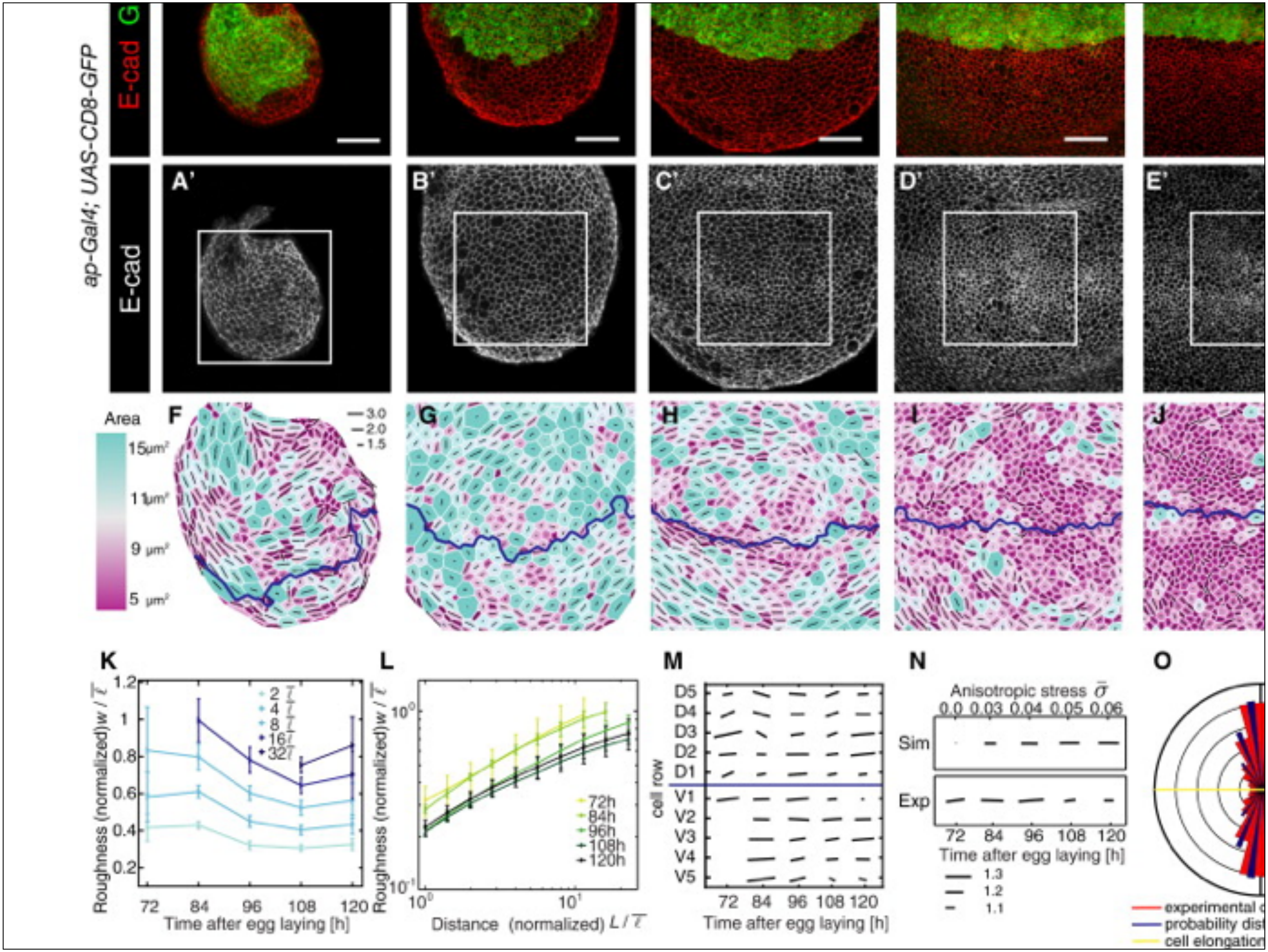
Temperature primarily affects cell size

Hedgehog signaling interfaces separate anterior and posterior cells

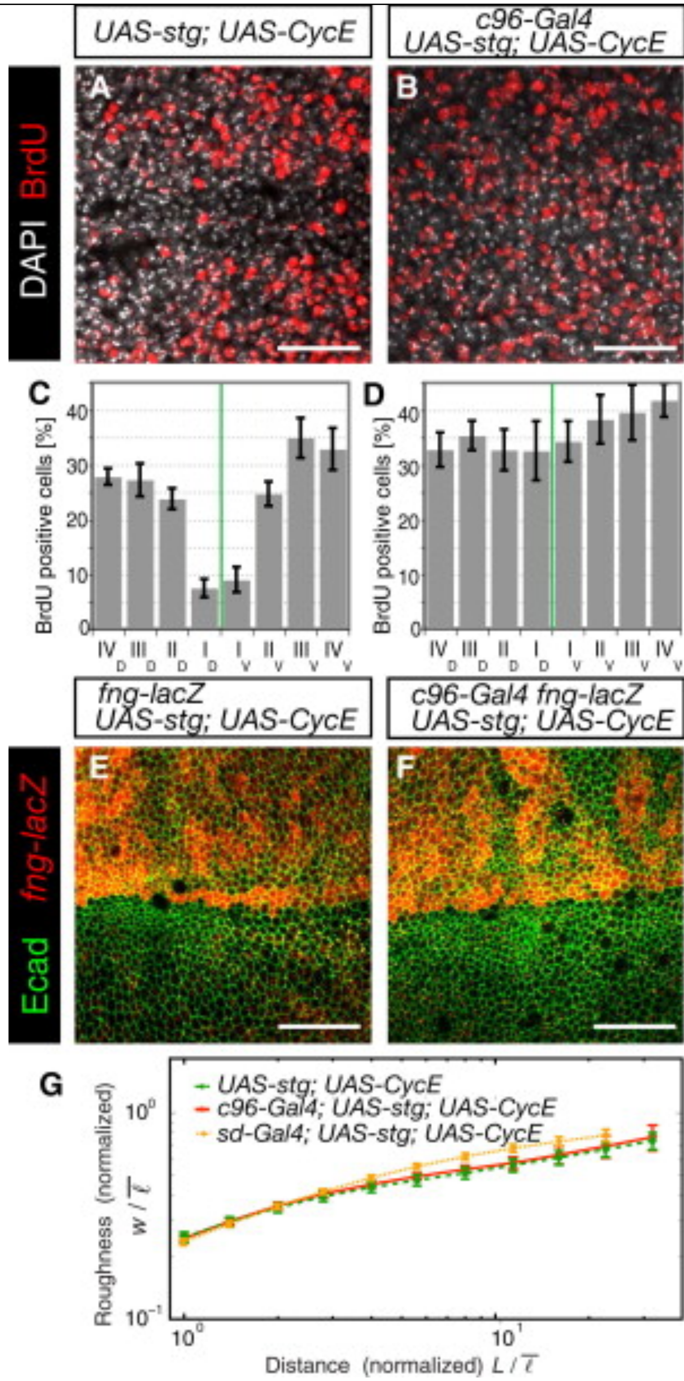


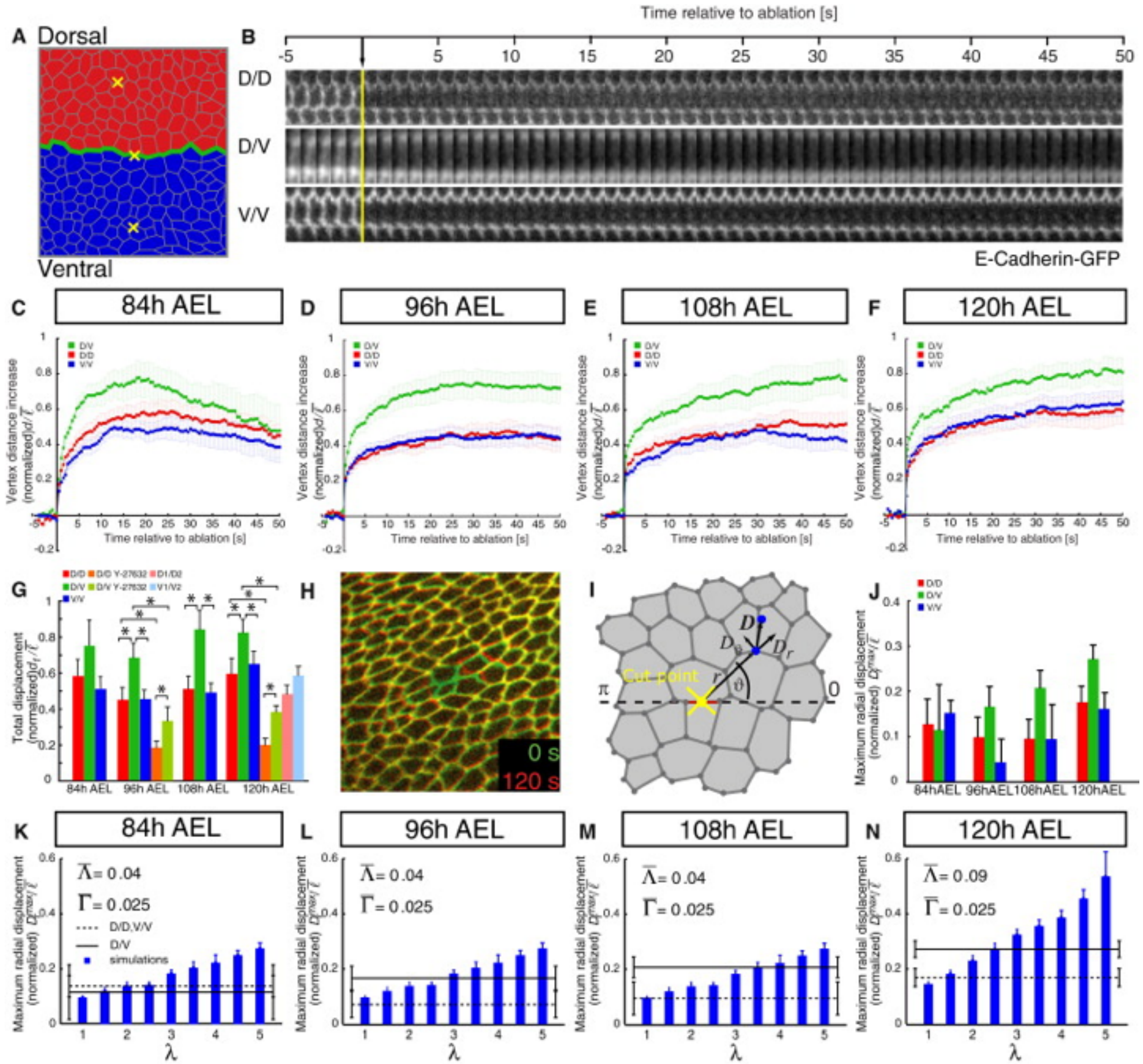


A Roughness measurement of the boundary w
$w(L)=\sqrt{\overline{\Delta h_L}^2}$
D Increased tension at the boundary λ
G Reduced proliferation rate near the boundary β
Reference
Case I $\lambda = 3$
Case II $\beta = 0.5$
Roughness (normalized) $w/\bar{\ell}$
Generation
J Anisotropic stress induced cell elongation $\bar{\sigma}$
M Orientation of cell division dependent on cell shape δ
probability distribution
cell elongation axis
P λ and δ
Case III $\bar{\sigma} = 0.04$
Case IV $\delta = 5$
Case V $\lambda = 3$ $\delta = 5$

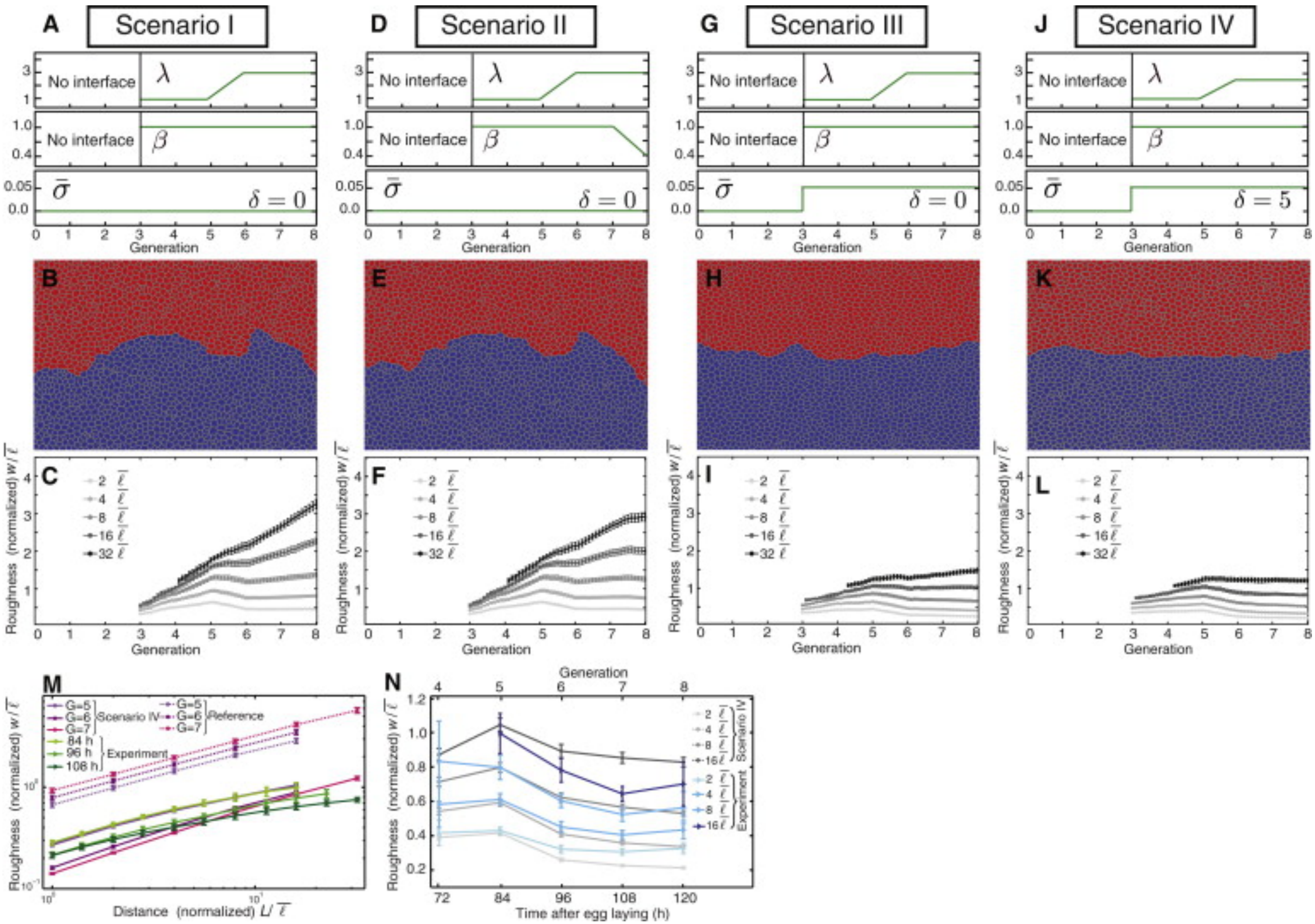


ap-Gal4; UAS-CD8-GFP
E-cad G
E-cad
A'
B'
C'
D'
E'
Area
15µm²
11µm²
9 µm²
5 µm²
F
G
H
I
J
3.0
2.0
1.5
K
Roughness (normalized) $w / \bar{\ell}$
Time after egg laying [h]
$2\bar{\ell}$
$4\bar{\ell}$
$8\bar{\ell}$
$16\bar{\ell}$
$32\bar{\ell}$
L
Roughness (normalized) $w / \bar{\ell}$
Distance (normalized) $L / \bar{\ell}$
72h
84h
96h
108h
120h
M
cell row
D5
D4
D3
D2
D1
V1
V2
V3
V4
V5
Time after egg laying [h]
N
Anisotropic stress $\bar{\sigma}$
0.0 0.03 0.04 0.05 0.06
Sim
Exp
72 84 96 108 120
Time after egg laying [h]
1.3
1.2
1.1
O
experimental
probability dist
cell elongation

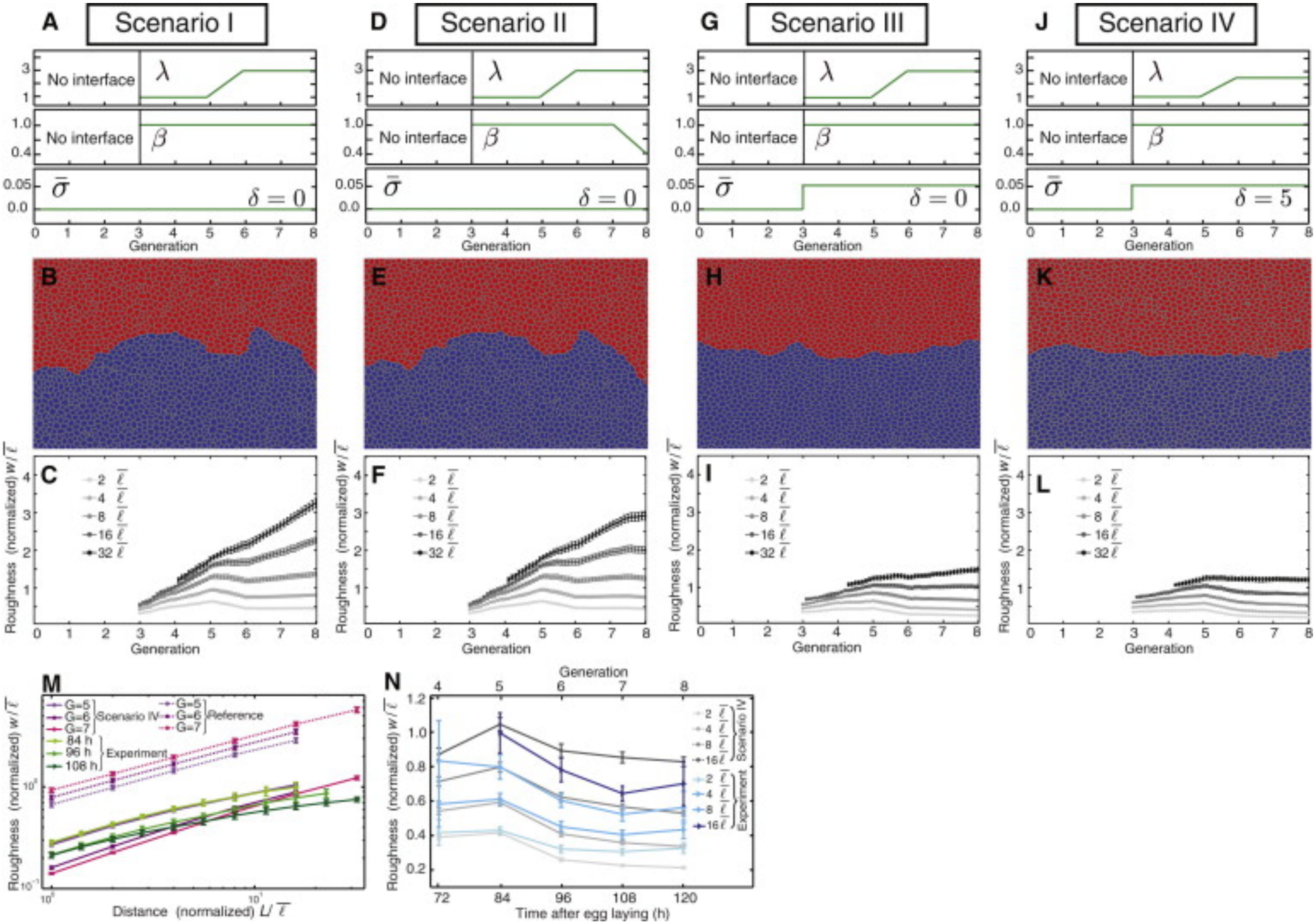

UAS-stg; UAS-CycE
c96-Gal4
UAS-stg; UAS-CycE
DAPI BrdU
A
B
C
D
BrdU positive cells [%]
IV D III D II D I D I V II V III V IV V
fng-lacZ
UAS-stg; UAS-CycE
c96-Gal4 fng-lacZ
UAS-stg; UAS-CycE
Ecad fng-lacZ
E
F
G
UAS-stg; UAS-CycE
c96-Gal4; UAS-stg; UAS-CycE
sd-Gal4; UAS-stg; UAS-CycE
Roughness (normalized) $w / \overline{\ell}$
Distance (normalized) $L / \overline{\ell}$



A Dorsal
Ventral
B
Time relative to ablation [s]
D/D
D/V
V/V
E-Cadherin-GFP
C 84h AEL
D 96h AEL
E 108h AEL
F 120h AEL
Vertex distance increase (normalized) $d_t/\overline{\ell}$
Time relative to ablation [s]
D/V
D/D
V/V
G
D/D
D/D Y-27632
D1/D2
D/V
D/V Y-27632
V1/V2
V/V
Total displacement (normalized) $d_t/\overline{\ell}$
84h AEL
96h AEL
108h AEL
120h AEL
H
0 s
120 s
I
Cut point
π
0
J
Maximum radial displacement (normalized) $D_r^{max}/\overline{\ell}$
84hAEL
96hAEL
108hAEL
120hAEL
K 84h AEL
$\overline{\Lambda}$= 0.04
$\overline{\Gamma}$= 0.025
D/D,V/V
D/V
simulations
L 96h AEL
$\overline{\Lambda}$= 0.04
$\overline{\Gamma}$= 0.025
M 108h AEL
$\overline{\Lambda}$= 0.04
$\overline{\Gamma}$= 0.025
N 120h AEL
$\overline{\Lambda}$= 0.09
$\overline{\Gamma}$= 0.025
λ



A
Scenario I
D
Scenario II
G
Scenario III
J
Scenario IV
No interface
λ
β
σ̄
δ = 0
δ = 5
Generation
B
E
H
K
C
F
I
L
Roughness (normalized) w / ℓ̄
2 ℓ̄
4 ℓ̄
8 ℓ̄
16 ℓ̄
32 ℓ̄
M
G=5
G=6
G=7
Scenario IV
Reference
84 h
96 h
108 h
Experiment
Distance (normalized) L/ ℓ̄
N
Scenario IV
Time after egg laying (h)



A
Scenario I
D
Scenario II
G
Scenario III
J
Scenario IV
No interface
λ
β
σ̄
δ = 0
δ = 5
Generation
B
E
H
K
C
F
I
L
Roughness (normalized) w / ℓ̄
M
G=5
G=6
G=7
Scenario IV
Reference
84 h
96 h
108 h
Experiment
Distance (normalized) L / ℓ̄
N
Time after egg laying (h)

Some more questions about growth control....

How do autonomous disc size control mechanisms integrate with systemic ones?