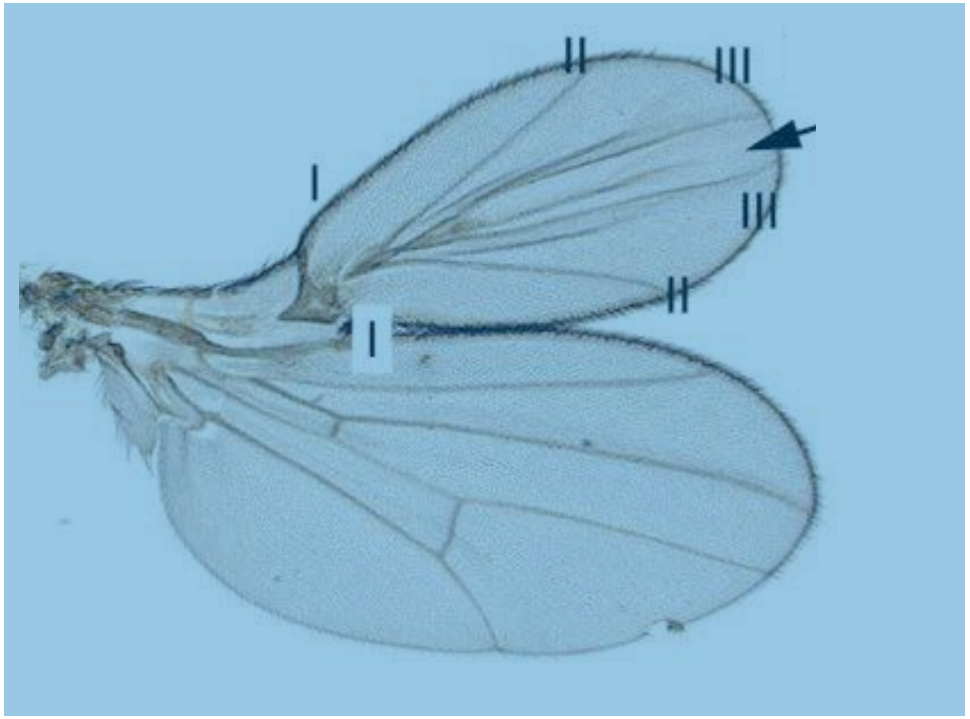
Organism and Tissue Growth



Course 4: Intrinsic control of growth and patterning

Thomas Lecuit
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE
1530

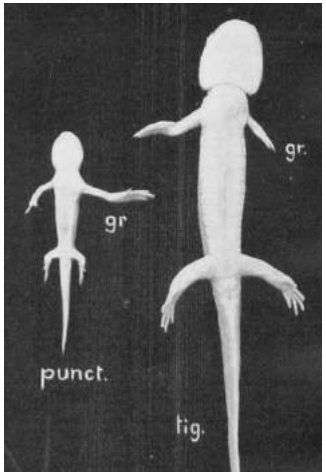
• Summary: intrinsic and organ specific growth control

— In plants and animals tissue growth is driven by both cell division (increase in cell number) and cell growth (increase in cell size).
—The two processes are coordinated but can be independently regulated

- Cell growth is controlled intrinsically (cellular anabolism: Ras, Pi3K, TOR signalling)
- Cell division is controlled by intrinsic cell cycle regulators (Cdk/cyclins, E2F, Rb…)

- Two modalities of growth compensation:
- *Cell Autonomous:* The pattern of cell division does not affect tissue size: cell growth is dominant over and compensates for cell division defects (eg. policy, cdc2, endoreplication).
- *Cell Non-Autonomous:* Perturbations in cell growth (via *Minute*, *Myc* mutations) does not affect tissue size due to compensation dependent on *cell competition.*

- These compensatory mechanisms are specific to each organ
- **Compensation reveals organ specific tissue size sensing/measurement**

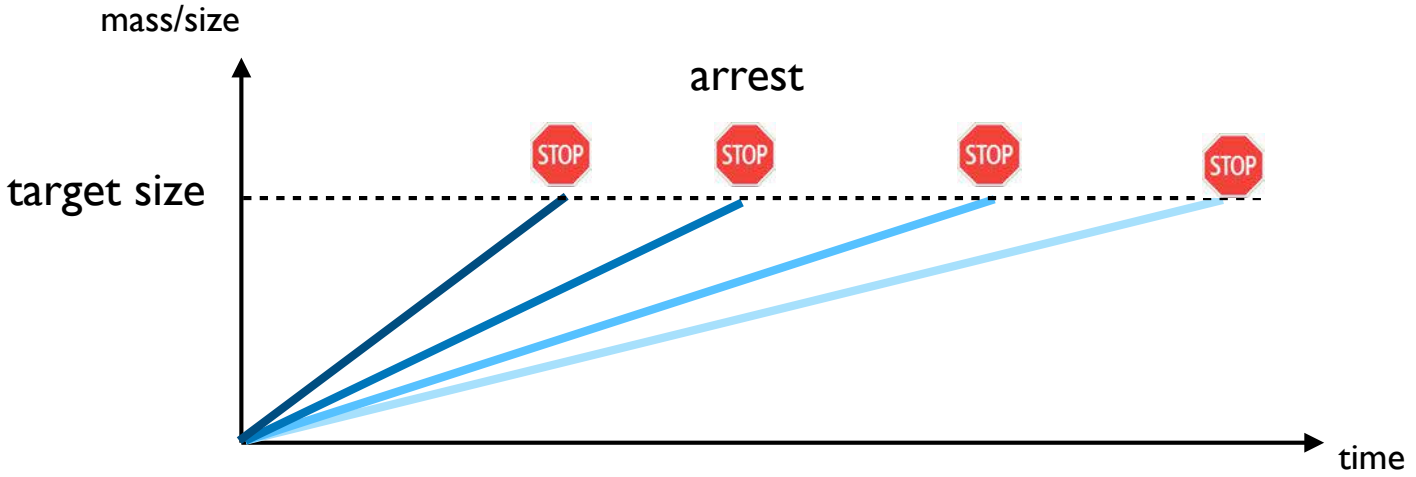


• The Outstanding Question

—How is growth of each organ arrested when the it reaches its appropriate size?

• Control of growth arrest at target size

— Organ and organism size may be regulated by:
growth duration and/or growth rate.
— Need to regulate growth arrest at the appropriate target size:
process vs endpoint regulation.



— What is measured? Intrinsic « ruler » of growth: « size-meter »
Organ intrinsic feedback of target organ size on cell growth/proliferation

—Need to consider scaling between factors that promote growth and size itself

1. Energy and mass/volume
2. Pattern and mass/volume
3. Mechanics and mass/volume

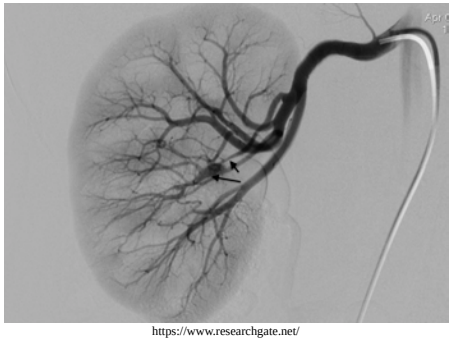
—Need to consider feedback mechanisms operating across scales (organ/tissue to cell)

• Organ growth arrest and energy delivery constraints

— Hypothesis: An asymptotic mass emerges from imbalance between energy supply and energy demand associated with Kleiber law for each organ? (see course 2, 19 Nov)

$$B = B_0 M^{3/4}$$

- Constraints imposed by energy delivery through a fractal branching network with invariant termini
- Organ-level scaling law: the whole organism power law scaling of metabolic power to mass can be explained by organ-level scaling of metabolic power to organ mass.



https://www.researchgate.net/

$$\mathrm{REE}_p = \Sigma\left(a \times M^b \times T_i\right).$$

$$K_i = a \times M^b,$$

for liver: $(K \times T) = 19.56 \times M^{0.6046}$; $r = 0.9694$,

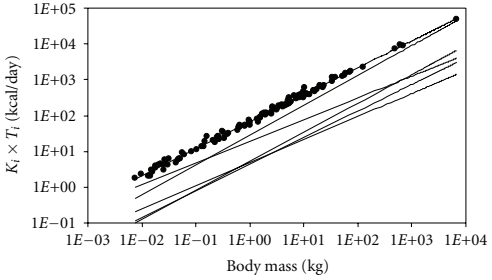
for brain: $(K \times T) = 4.82 \times M^{0.6446}$; $r = 0.9538$,

for heart: $(K \times T) = 5.16 \times M^{0.8137}$; $r = 0.9830$, (9)

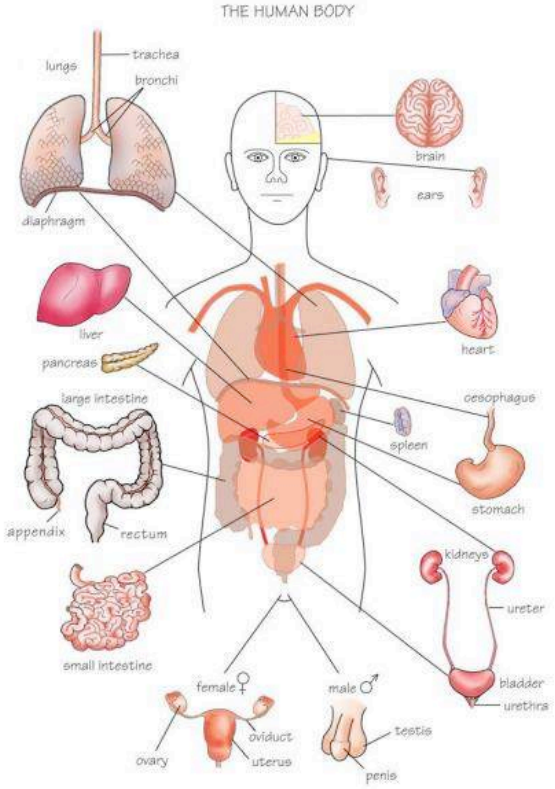
for kidneys: $(K \times T) = 4.35 \times M^{0.7441}$; $r = 0.9825$,

for residual: $(K \times T) = 28.16 \times M^{0.8402}$; $r = 0.9996$.

$\mathrm{REE}_p = 66.33 \times M^{0.754}$; $r = 0.9975$.



Z. Wang, et al and S.B. Heymsfield *ISRN Zoology* (2012)
doi:10.5402/2012/673050



http://clipart-library.com/clipart/pcod9jo5i.htm

• Organ growth arrest and energy delivery constraints

— Hypothesis: An asymptotic organ mass emerges from imbalance between energy supply-side and energy demand given the Kleiber law *for each organ.*

• Growth equation $\frac{dm}{dt} = \left(\frac{m_c}{E_c}\right)B - \left(\frac{B_c}{E_c}\right)m$

and Kleiber's law $B = B_0 m^{3/4}$

give $\frac{dm}{dt} = am^{3/4} - bm$ with $a \equiv B_0 m_c/E_c$, $b \equiv B_c/E_c$.

supply — demand

B_0 constant within taxon (fish, bird, mammal etc)

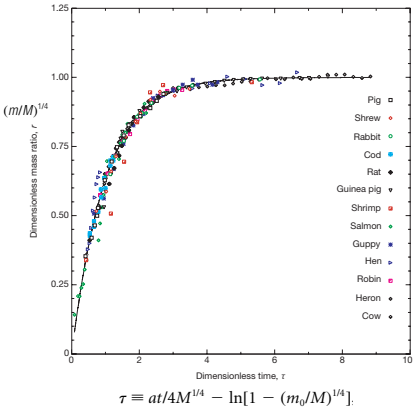
• Asymptotic mass/size for $dm/dt = 0$ $M = (a/b)^4 = (B_0 m_c/B_c)^4$

• Law of growth: $\left(\frac{m}{M}\right)^{1/4} = 1 - \left[1 - \left(\frac{m_0}{M}\right)^{1/4}\right] e^{-at/4M^{1/4}}$

m: mass at time t
m_0: mass at birth
M: final mass

• « Universal » growth $r = 1 - e^{-\tau}$

Fraction of metabolic power allotted to maintenance
The fraction allotted to growth exponentially decreases to 0
(growth arrests)



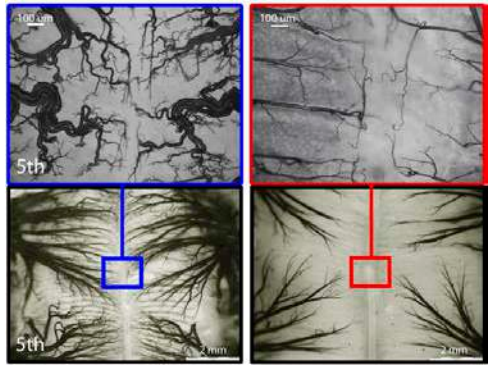
$\tau \equiv at/4M^{1/4} - \ln[1 - (m_0/M)^{1/4}]$

G. West, J. Brown and B. Endquist. (2001) *Nature* 413

• Organ growth arrest and energy delivery constraints

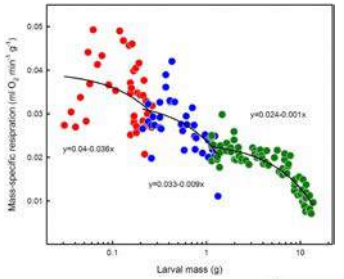
—Supply-side constraints versus metabolic demand in *Manduca sexta*

- The tracheal (ie. respiratory) system is over-built after molting and becomes limiting at the end of each instar
- The demand (which increases) cannot be met by supply
- This size-sensing (critical weight) depends on the limited ability of a fixed tracheal system to sustain the oxygen supply to a growing individual

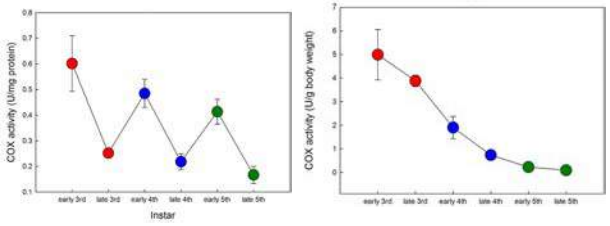


V. Callier and F. Nijhout Proc Natl Acad Sci U S A. 2011 Aug 30;108(35):14664-9. doi: 10.1073/pnas.1106556108

- Yet, the mass-specific metabolic power at the beginning of each instar decreases from molt to molt and supply is no limiting at onset of each instar (respiratory system is « over-built »)
- So supply-side constraints cannot explain everything

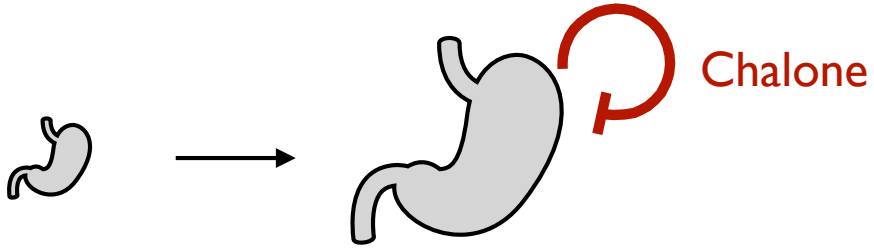


- Demand also decreases over time (mass-specific mitochondrial activity, cytochrome c oxydase COX) due to reduced proportion of highly metabolic tissues



V. Callier and F. Nijhout PLoS One. 2012;7(9):e45455. doi: 10.1371/journal.pone.0045455.

• Chalones: secreted negative feedback inhibitors of growth



Biol. Rev. (1962), 37, *pp.* 307–342

THE CONTROL OF MITOTIC ACTIVITY IN ADULT MAMMALIAN TISSUES

By W. S. BULLOUGH

Department of Zoology, Birkbeck College, University of London

One common theory has been that tissue mitotic activity may be controlled by a tissue mitotic inhibitor in such a way that the greater the tissue mass within the body space, the lower will be the rate of cell production.

It is suggested that the members of this complex of chemical messengers, which may possibly prove to be a family of proteins, should be called *chalones*. The word chalone (χαλάω, to make slack) was originally proposed by Schäfer (1916) to distinguish a chemical messenger which has a depressant action and for which he felt the term hormone (Starling, 1906) was inappropriate. This suggestion was never adopted but the word may be most appropriate in the present case since the chalones seem to act most powerfully in slowing growth as the adult state is reached.

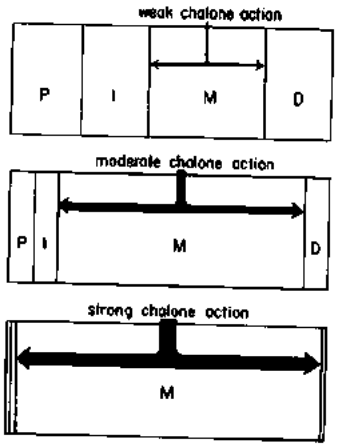
- hormone vs chalone

There also arises a question of terminology. Substances of the kind under discussion do not fall within the usual definition of a hormone as a systemically distributed chemical substance 'produced by one tissue with the primary function of exerting a specific effect of functional value on another tissue' (Huxley, 1935). On the contrary, these are substances each of which is produced by a tissue with the primary function of controlling the growth, and perhaps the differentiation, of that same tissue.

CANCER RESEARCH

VOLUME 25 NOVEMBER 1965 NUMBER 10

Mitotic and Functional Homeostasis: *A Speculative Review*



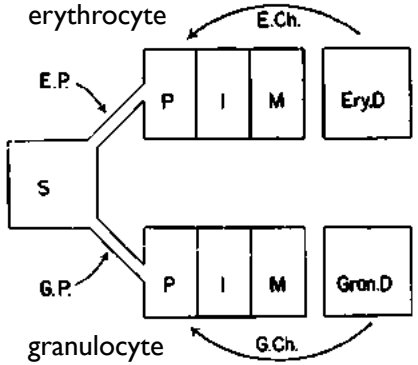
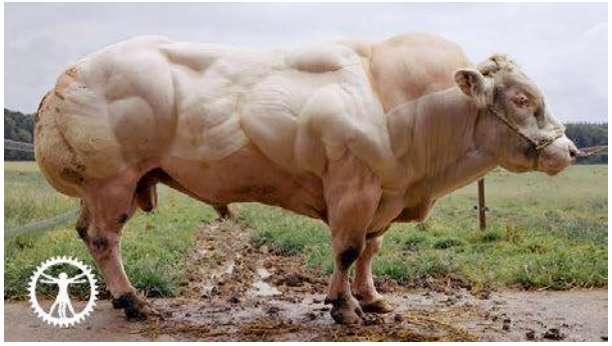


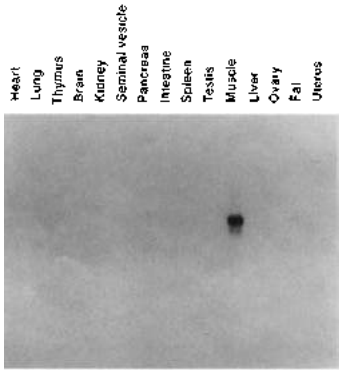
CHART 5.—Diagrams illustrating the different proportions of cell types in different tissues. *P*, progenitor cells; *I*, immature cells; *M*, mature cells; *D*, mature cells moving towards death. *Top*, duodenal mucosa with a high rate of cell production and loss; *middle*, epidermis with a moderate rate of cell production and loss; and *bottom*, liver with a low rate of cell production and loss.

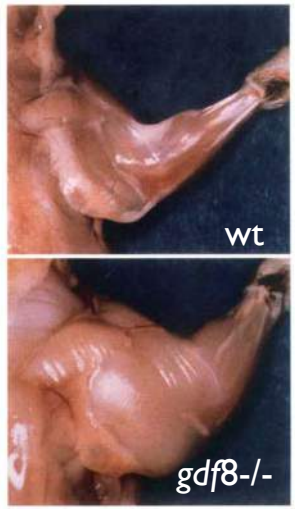
• Chalones: secreted negative feedback inhibitors of growth

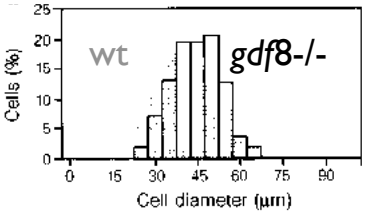
GDF 8/Myostatin:

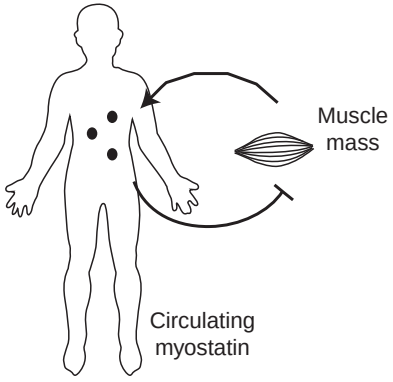
- TGFß member secreted negative feedback inhibitor of muscle differentiation produced by myocytes. Autocrine inhibition
- Induces cell cycle inhibitor p21 (of cdk1) and promotes cell differentiation

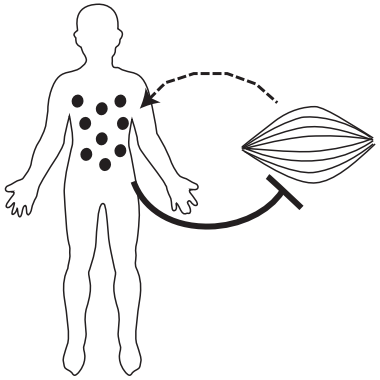








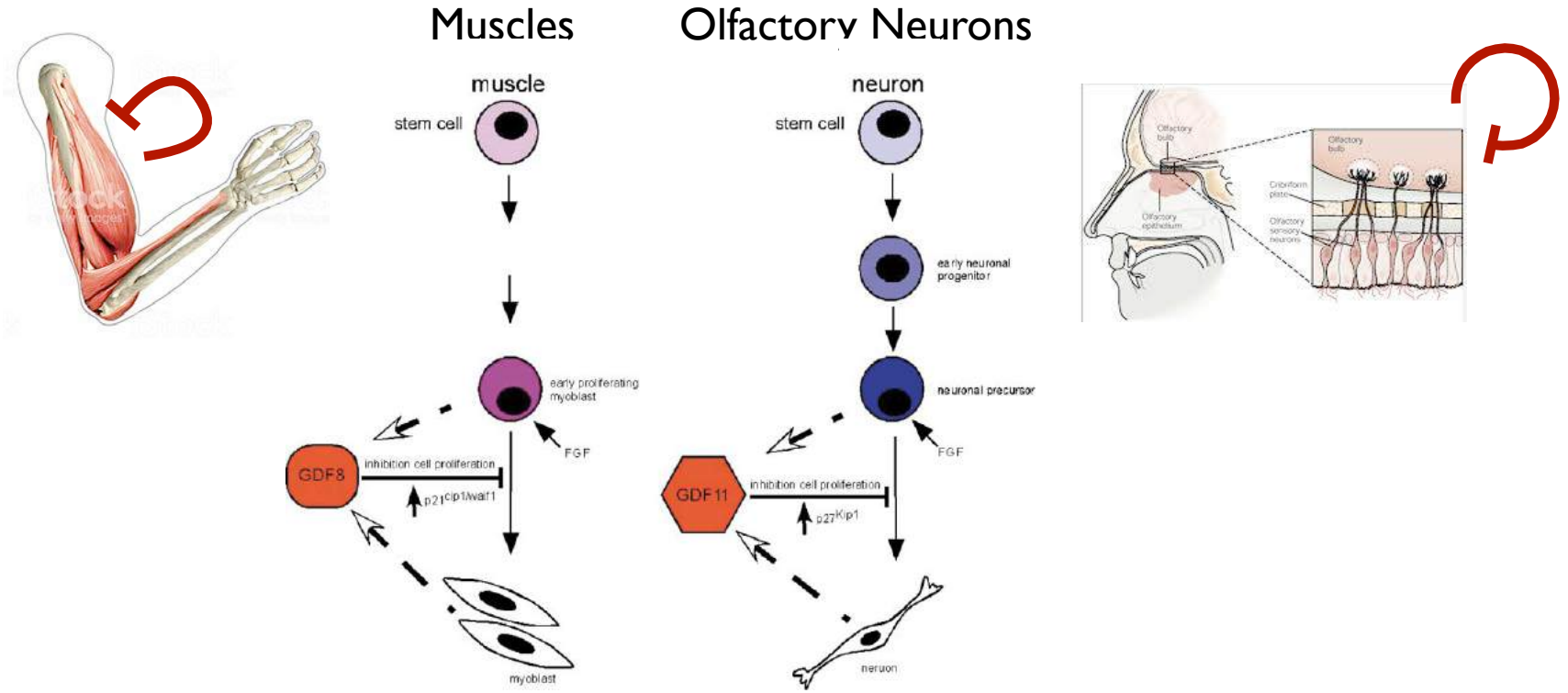




McPherron AC, Lawler AM, Lee SJ. *Nature*. 1997;387:83–90.

• Chalones: secreted negative feedback inhibitors of growth

- Different TGFß family secreted factors are produced by terminally differentiated cells and feedback on progenitor growth



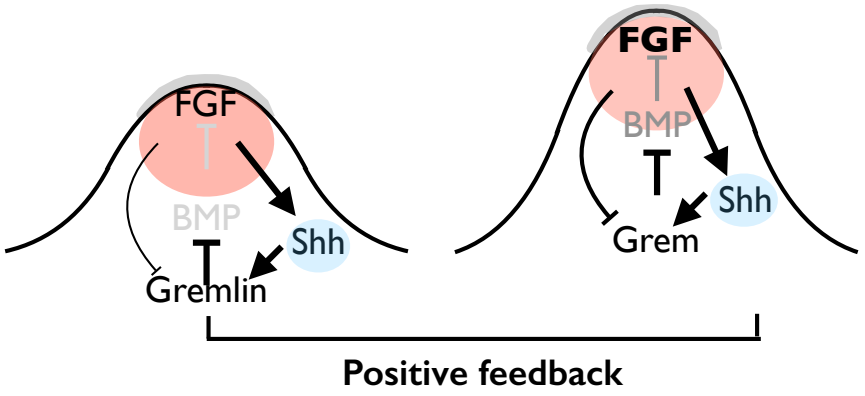
McPherron AC, Lawler AM, Lee SJ. *Nature*. 1997;387:83–90.

Wu, H.H., et al and Calof, A.L. (2003). Neuron *37*, 197–207.

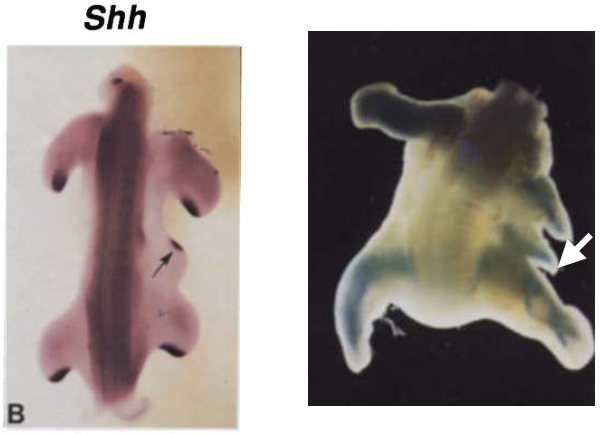
• Organ specific negative feedback circuits of growth

Limbs: FGF/Shh/BMP network

Transition between positive and negative feedback loop
arrests growth of vertebrate limbs



- FGFs promote growth of mesenchymal cells in limb bud
- **Positive feedback:**
  —FGF induces polarising signal Shh
  —Shh induces, and FGF represses Gremlin an inhibitor of BMP which itself antagonises FGF.
  —FGF is maintained through a Positive feedback that promotes growth during limb development



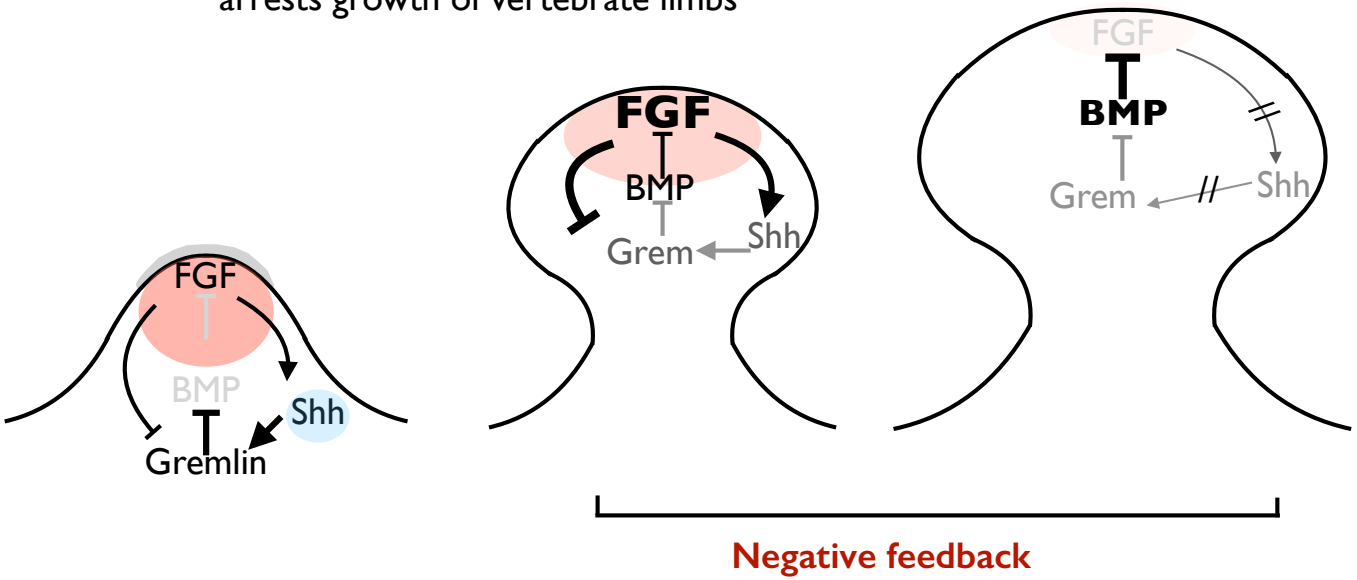
An FGF 2 bead induces a new limb

Cohn MJ, Izpisua-Belmonte JC, Abud H, Heath JK, Tickle C. (1995). *Cell* 80: 739 – 746.

• Organ specific negative feedback circuits of growth

Limbs: FGF/Shh/BMP network

Transition between positive and negative feedback loop arrests growth of vertebrate limbs



- **Negative Feedback:**
  —As the limb grows, FGF levels increase and Gremlin repression increases
  —Limb growth leads to increased distances between Gremlin and FGF (Mouse) and Shh and Gremlin (Chick): break of positive feedback loop and induction of BMP dependent negative feedback

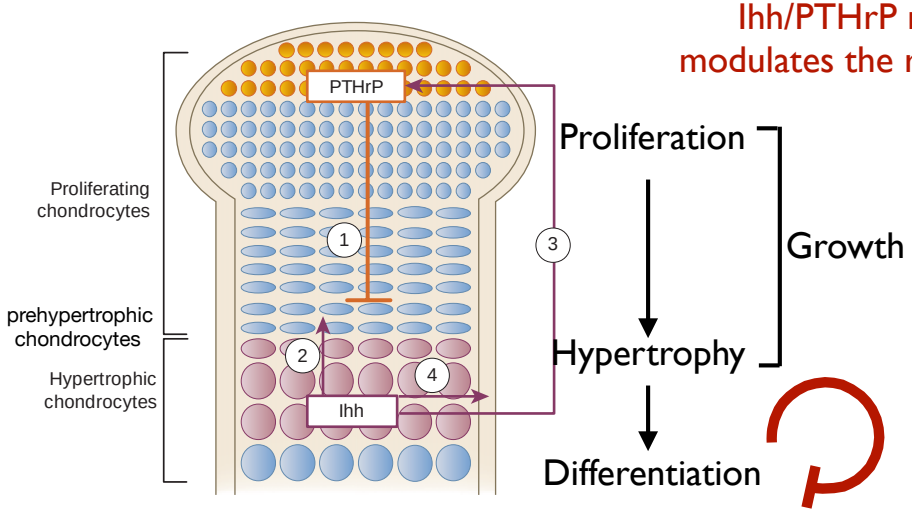
Scherz, P. J., Harfe, B. D., McMahon, A. P. & Tabin, C. J. (2004) *Science* 305, 396–399.

J. Verheyden and X. Sun *Nature. (*2008);454(7204):638-41

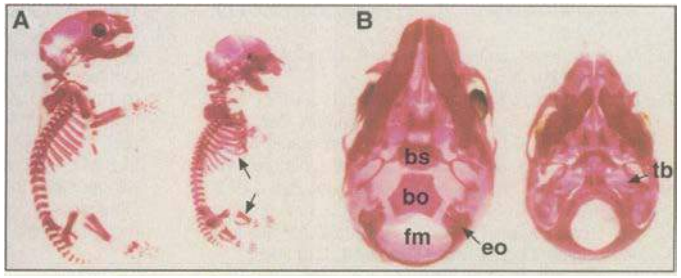
• Organ specific negative feedback circuits of growth

Bones

Ihh/PTHrP negative feedback loop
modulates the rate of bone differentiation



HM. Kronenberg (2003) *Nature* 423:332-336



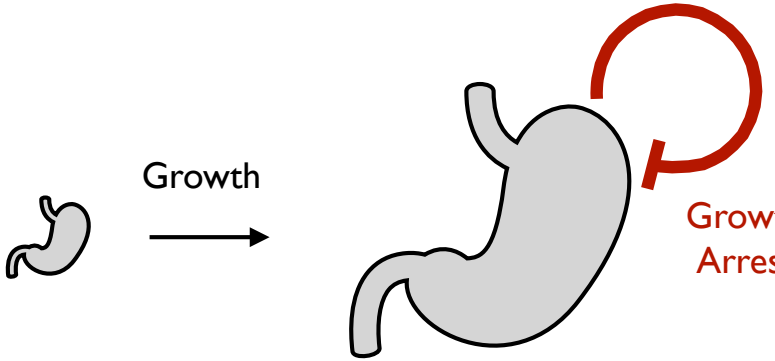
wt pthrpR-/- wt pthrpR-/-

premature growth arrest and differentiation

- Chondrocytes proliferate, then differentiate into hypertrophic chondrocytes and eventually in osteoblasts
- PTHrP sustains proliferation of chondrocytes by blocking Ihh expression.
- Following growth, Ihh is expressed at a distance in hypertrophyic chondrocytes (out of reach from PTHrP)
- IHh promotes hypertrophic chondrocyte differenciation and chondrocyte proliferation
- Differentiated cells induce feedback inhibitor of differentiation (via induction of PTHrP)

Lanske, B. *et al.* Kronenberg HM. *Science* **273,** 663–666 (1996).
Vortkamp, A. *et al.* Tabon C. . *Science* **273,** 613–622 (1996).

• The « size-meter» as an organ-scale negative feedback



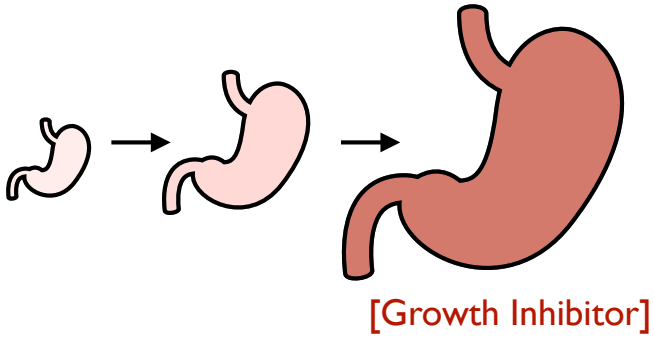
—Measure of tissue size

• Amount of inhibitory feedback signal: which scales with cell number/size

-Myocytes (GDF8/Myostatin) repress proliferation of myoblasts

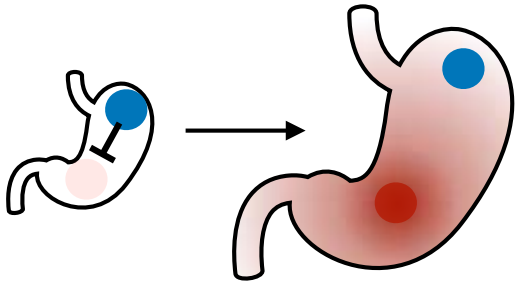
-Olfactory receptor neurons (GDF11) repress proliferation of neuronal precursor

-FGF production during limb growth: inhibition of Gremlin



• Tissue spacing between signal sources

Failure to sustain repression (via Gremlin) of negative feedback loop (BMP)

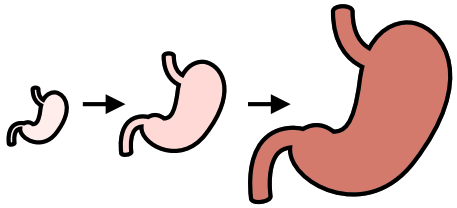


• The « size-meter » as an organ-scale negative feedback

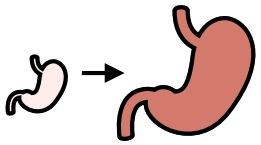
—How robust is such a system of feedback inhibition based on tissue size?

The number of cells does not necessarily scale with the [concentration] of inhibitor
Importance of: Tissue Geometry (size of pool/sink)
Inhibitor production rate, diffusion/transport and stability
What is the sensitivity to geometry (ie. size) given biochemical parameters?
What is the sensitivity to biochemical parameters given geometry?
Implies: fine tuning of growth rate to biochemical parameters of feedback at the cellular scale.
Or interdependency.
Cell growth/division can potentially advect (transport) and dilute inhibitor, or affect stability, etc

Control



Stability of inhibitor ++
production rate =
Cell growth rate =



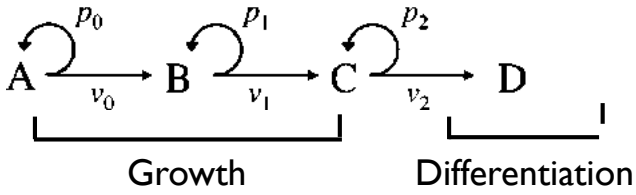
Stability & production of inhibitor =
Cell growth rate ++

Hypothesis: Feedback of cell growth rate on inhibitor stability (positive feedback) could rescue size:: Robustness
Necessity to invoque nested feedbacks: global and local.

Stability of inhibitor ++
Cell growth rate ++

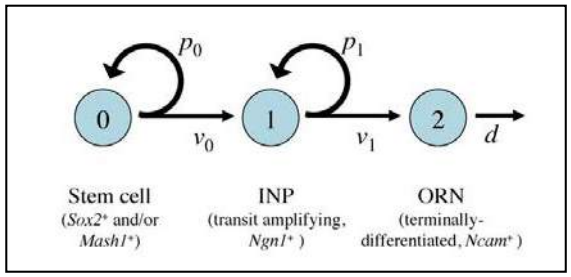
• Stability of Growth: cellular lineage

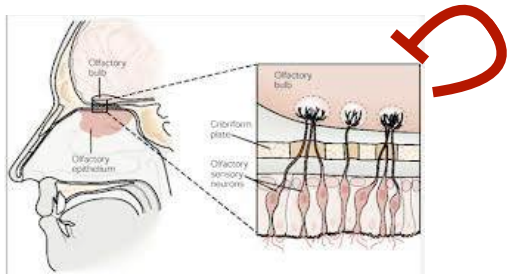
—Cell lineages balance growth (of stem cell pool and intermediates) and terminal differentiation



—What ensures the stability of a cell lineage?

v : rate constant of cell division (1/length of cell cycle)
p: probability of replication (remain in same stage)





Systems sensitivity to p_0 is infinite: unless exactly ½ of cells remain stem cells and ½ differentiate (p_0=0.5), stem cells pool collapses or explodes. But stem cells do not always divide asymmetrically. They may divide also symmetrically, *ie*. produce 2 or 0 stem cells.

Question: How does p_0=0.5 as a *population average* if not absolutely true for all cells?

- Negative feedback

A. Lander et al . (2015) *PLoS Biol* 7(1): e1000015. https://doi.org/10.1371/journal.pbio.1000015

• Stability of Growth: cellular lineage

OPEN ACCESS Freely available online PLOS BIOLOGY

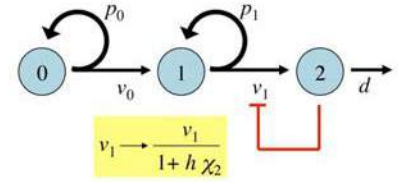
Cell Lineages and the Logic of Proliferative Control

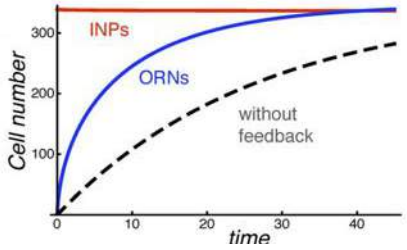
Arthur D. Lander[1,2,3☯*], Kimberly K. Gokoffski[1,4,5☯], Frederic Y. M. Wan[3,5], Qing Nie[2,3,5], Anne L. Calof[1,3,4*]

- Negative feedback

proportional control

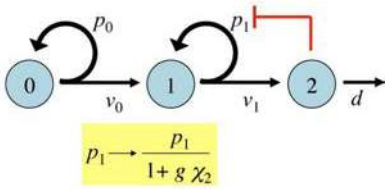
(output proportional to error)
does not compensate for steady deviation from reference
high rate, low gain (amplification from input to output)
requires high progenitor load to achieve high rate

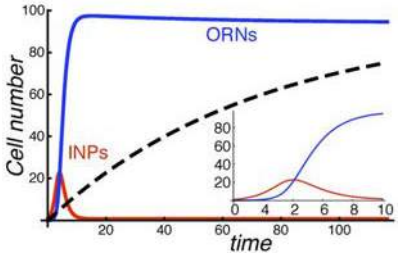




integral control

(output depends on integral of error)
compensates well for steady deviation from reference
resistant to external and internal perturbations, high rate
does not require high progenitor load





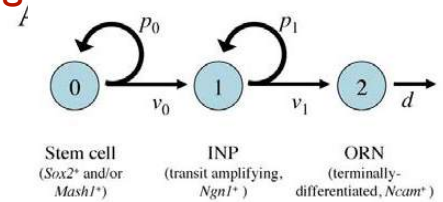
in silico regeneration « experiment »:
simulation of return to steady-state
after ablation of ORNs ($_2$= 0)

A. Lander et al . (2015) PLoS Biol 7(1): e1000015. https://doi.org/10.1371/journal.pbio.1000015

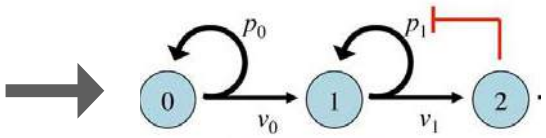
• Stability of Growth: cellular lineage

—GDF11 as a proportional and integral feedback controller of lineage

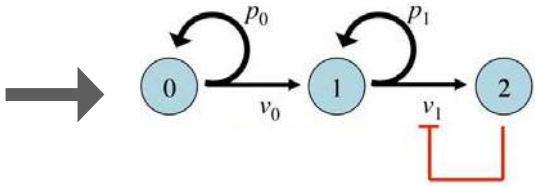
- GDF11 is produced by differentiated olfactory receptor neurons (ORNs)
- BrdU labelling of INPs and assess time course of NCAM immunoreactivity (markers of ORNs).
- INP replication delays differentiation of INPs. Thus, <u>delay in NCAM expression indicative of replication probability p_1 of INPs.</u>



- 18h chase: 60% of BrdU+ cells become ORNs without GDF11.
- Addition of low GDF11 increases ORN production indicative of reduced probability of INP replication (p_1 lowered)



- Addition of higher GDF11 reduces ORNs indicative of lengthening of cell cycle progression (v_1 lowered)
- If so, longer chase should reduce this inhibition, as observed (36h)



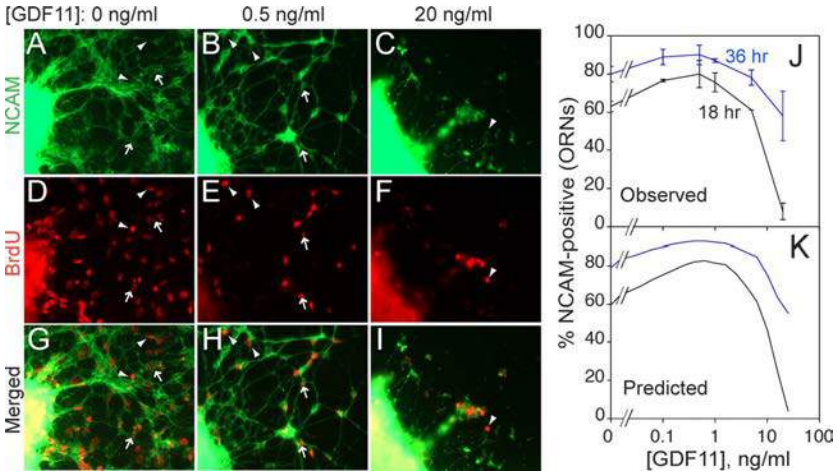


Figure 4. Experimental Demonstration That GDF11 Regulates p_1 and v_1

A. Lander et al . (2015) PLoS Biol 7(1): e1000015. https://doi.org/10.1371/journal.pbio.1000015

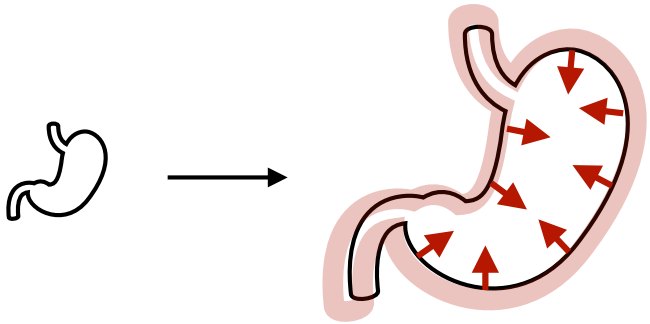
• The « size-meter » as an organ-scale negative feedback

- Mechanical feedback

Synthesis of an external mechanical constraint.
For example: Stiff extracellular matrix, contractile tissue (eg. gut), non-growing tissue boundary.

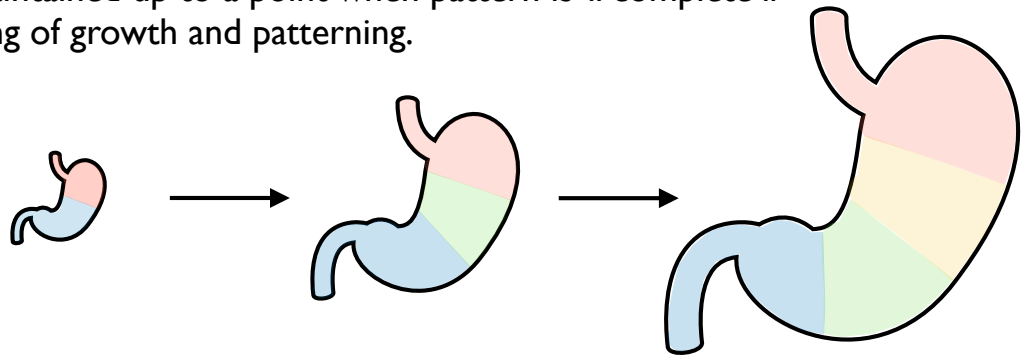
The feedback requires that mechanical constraints are dependent on growth/size of the tissue/organ
And that mechanical feedback blocks cell growth/division and/or induces apoptosis.

(Cours #5 Growth and Mechanics)



- Completion of tissue pattern:

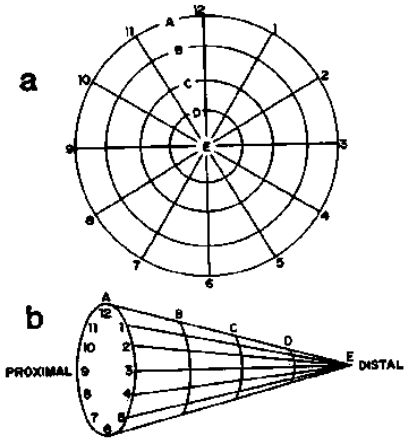
Growth is maintained up to a point when pattern is « complete »
Ensures scaling of growth and patterning.



• Size regulation and patterning

—regeneration and intercalary growth

Positional information
(cf Lewis Wolpert *JTB* 1969, See also Inaugural lecture)
polar coordinate model
(radial and angular positional value)

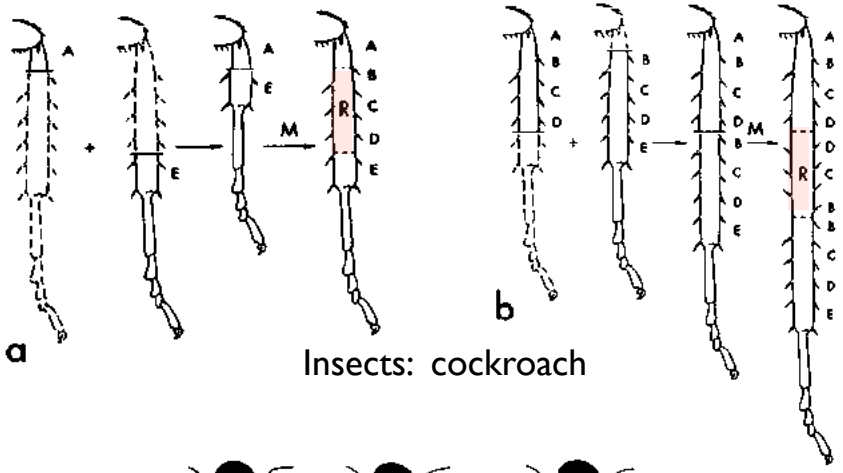


When normally nonadjacent positional values in either the circular or the radial sequence are confronted in a graft combination or as a result of wound healing, growth occurs at the junction until cells with all the intermediate positional values have been intercalated; then growth ceases.

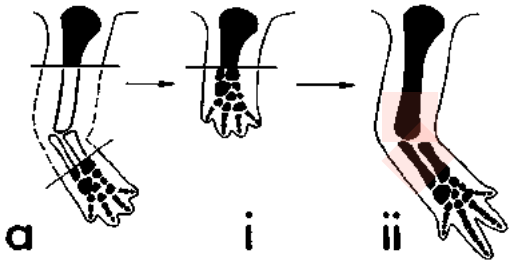
Pattern Regulation in Epimorphic Fields

Cells may make use of a polar coordinate system for assessing their positions in developing organs.

Vernon French, Peter J. Bryant, Susan V. Bryant



Insects: cockroach



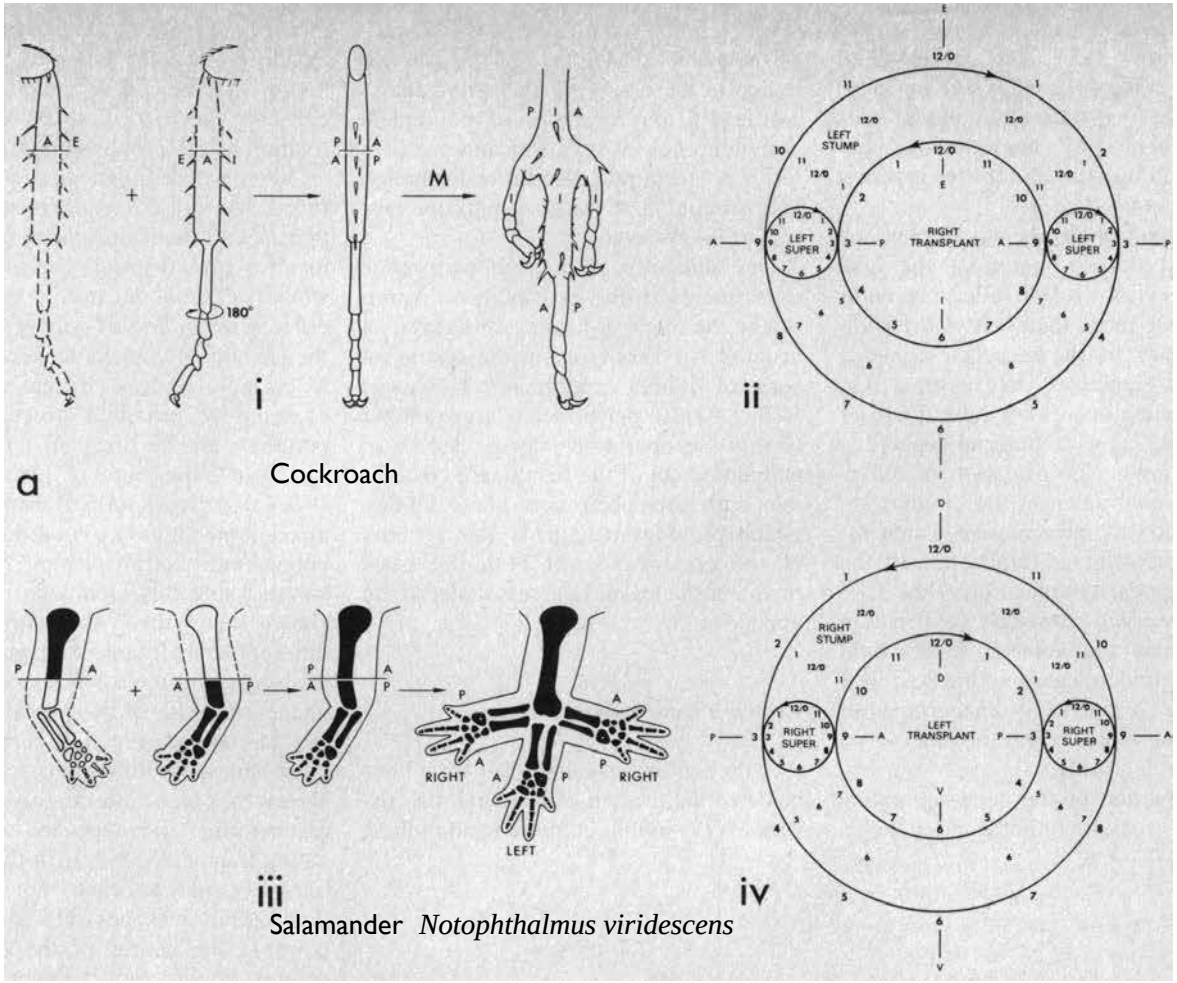
Amphibians: *Notophthalmus viridescens*

Reviews

V. French, P.J. Bryant and S.V. Bryant *Science* (1976). 193::969-981
S.V. Bryant , V. French, and P.J. Bryant *Science* (1981). 212::993-1002

• Size regulation and patterning

—regeneration and intercalary growth



Cockroach V. French, *J. Embryol. Exp. Morphol.* 1976; 35, 267

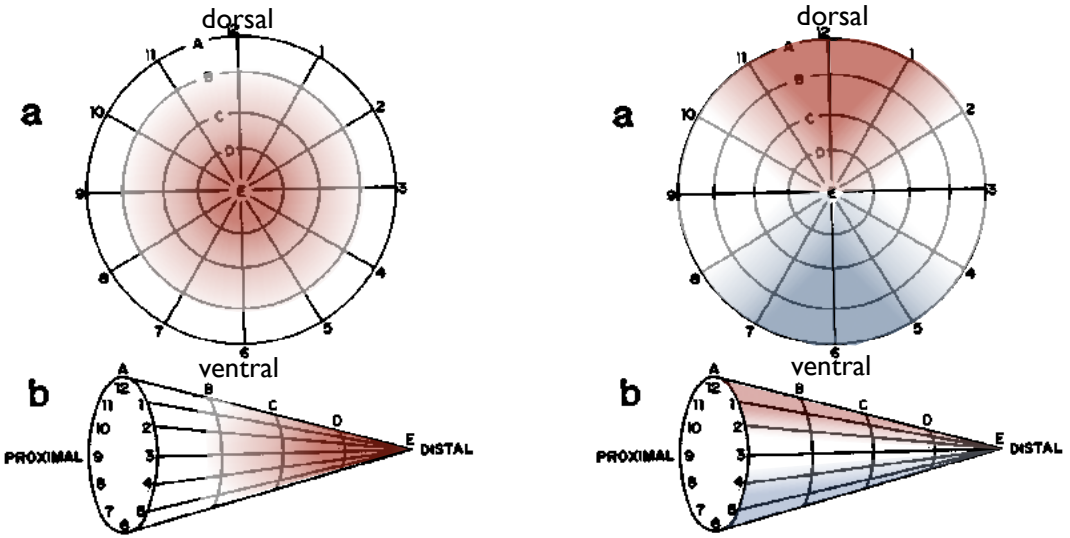
Salamander L.E. Iten and S. Bryant *Dev Biol.* 1975;44(1):119-47.
S. Bryant and L.E. Iten. *Dev Biol.* 1976; 50(1):212-234.

Reviews V. French, P.J. Bryant and S.V. Bryant *Science* (1976). 193::969-981
S.V. Bryant , V. French, and P.J. Bryant *Science* (1981). 212::993-1002

• Size regulation and patterning

—regeneration and intercalary growth: possible mechanisms

- Positional information resulting from molecular morphogen gradients
- Growth is coupled to long range diffusion/transport of morphogen gradients



V. French, P.J. Bryant and S.V. Bryant *Science* (1976). 193::969-981

- Positional information resulting from local cell interactions and cell surface molecules.

S.V. Bryant , V. French, and P.J. Bryant *Science* (1981). 212::993-1002

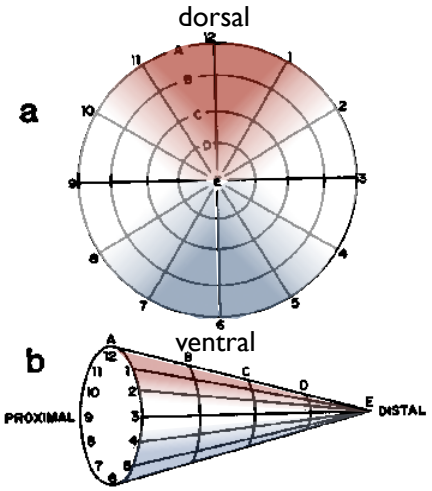
« Entelechia » model

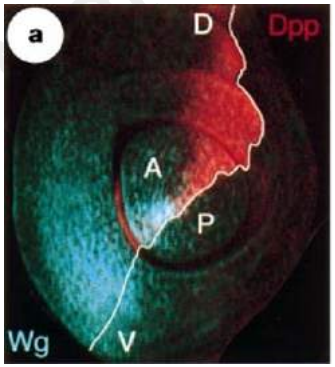
A. C. Garcia-Bellido. and A. Garcia-Bellido. *Int. J. Dev. Biol.* 42: 353-362 (1998)
A. Garcia-Bellido. *Int. J. Dev. Biol.* 53: 1291-1303 (2009) doi: 10.1387/ijdb.072459ag

• Size regulation and patterning

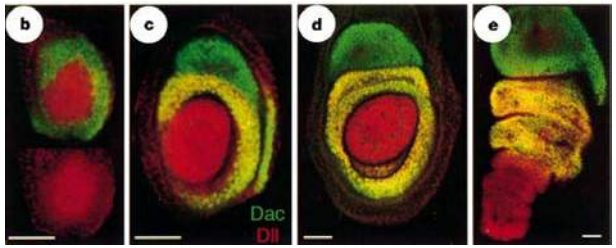
—Morphogen gradients and leg patterning

- Proximal distal patterning occurs in 2D sheets of cells: gene expression in discs of different radii.
- Wg and Dpp are concentration dependent morphogens that pattern the proximo-distal and dorso-ventral axes
- The synergistic and concentration dependent activities of Wg and Dpp control the nested expression of genes in discs of different radii





morphogens



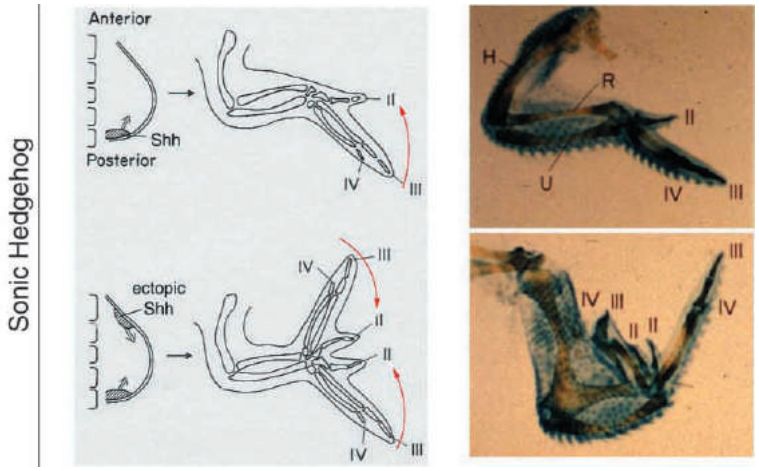
2D pattern: discs 3D pattern

T. Lecuit, S. Cohen. *Nature*. 1997; 388:139-45.

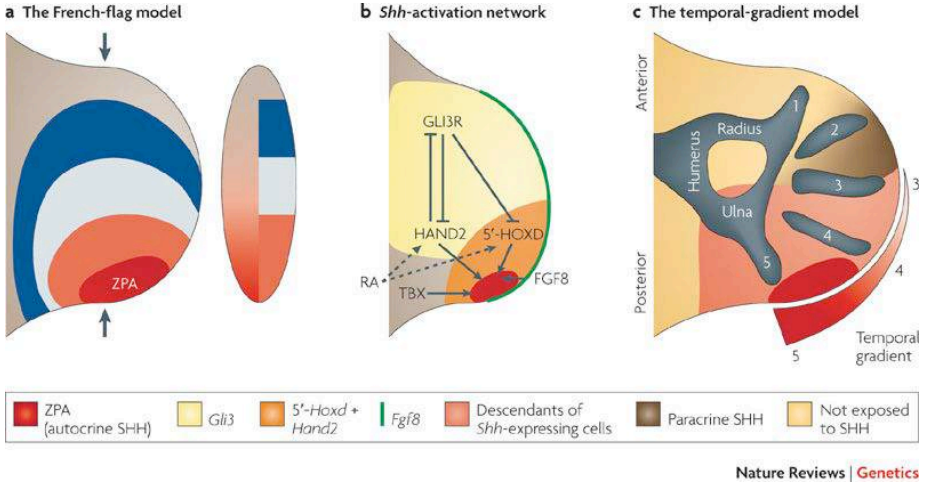
• Size regulation and patterning

—Morphogens: Growth factors and with patterning activity

- Gradient of concentration/activity of a molecule (Shh)
- Activity thresholds



Tetsuya Tabata, Yuki Takei *Development* 2004 131: 703-712

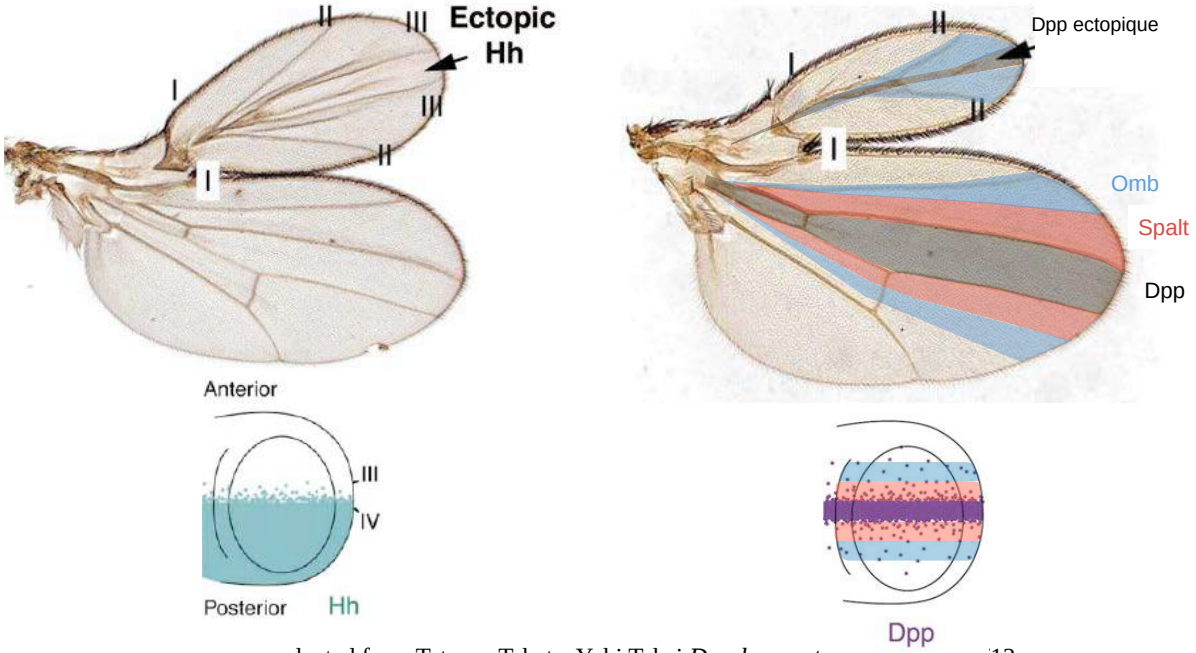


Rolf Zeller et al. *Nature Reviews Genetics* **10**:45–858 (2009)

• Size regulation and patterning

—Morphogens: Growth factors with patterning activity

- Gradient of concentration/activity of a molecule
- Activity thresholds



adapted from Tetsuya Tabata, Yuki Takei *Development* 12

M. Zecca et al. and K. Basler. *Dev* 1995

D. Nellen et al. and K. Basler. *Cell* 1996

T. Lecuit et al. and S. Cohen. *Nature* 1996

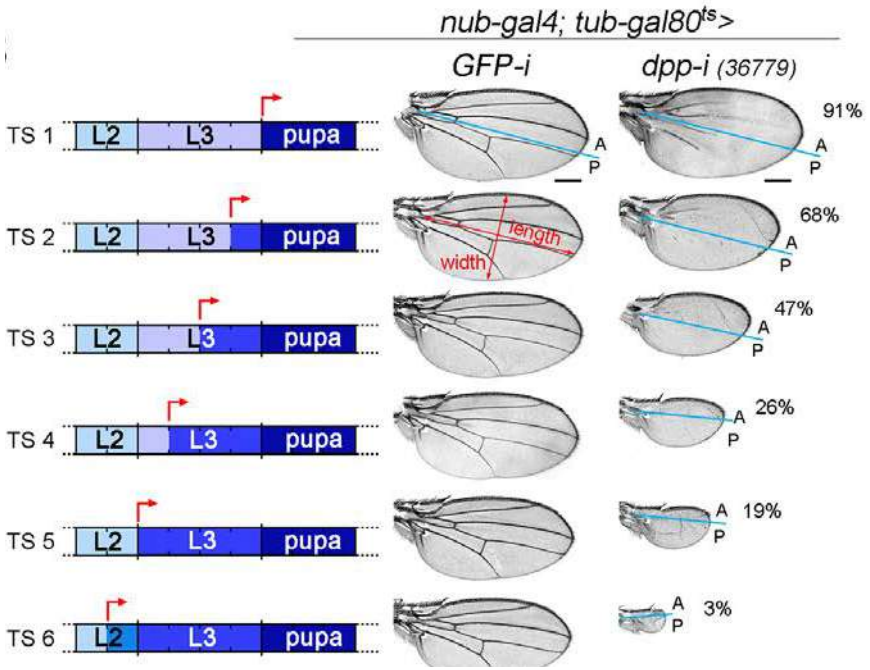
T. Tabata and T. Kornberg. *Development* 121, 3359-3369 (1995)

• Morphogen: Organ-size dependent growth inducer

—Morphogens: Growth factors with patterning activity

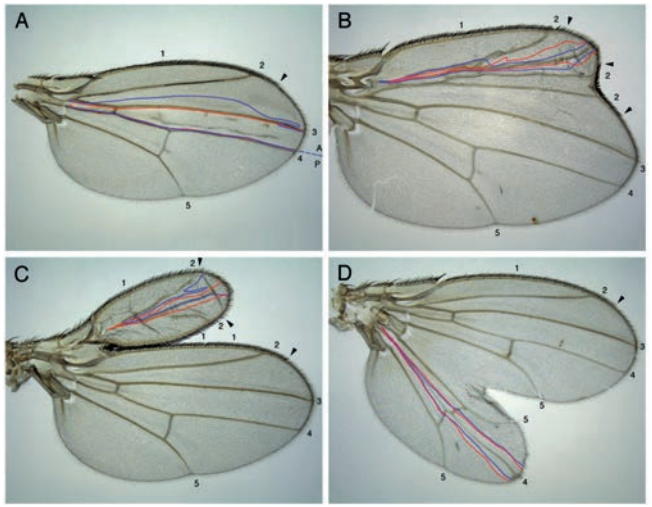
- A source of expression of the morphogen Dpp induces axis duplication: ie. duplication of patterns AND extra tissue growth (intercalary growth)

Dpp inhibition



L. Barrio and M. Milan. *eLife* (2017);6:e22013. DOI: 10.7554/eLife.22013

Dpp ectopic expression

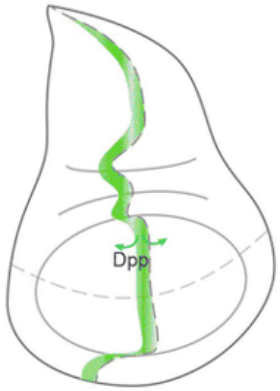


M. Zecca K. Basler and G. Struhl. *Development* 121, 2265-2278 (1995)

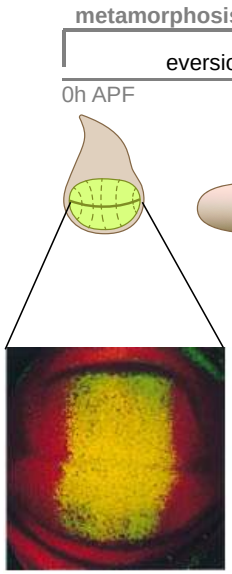
• Morphogen: Organ-size dependent growth inducer

—Morphogens: Growth factors with patterning activity

- Dpp acts directly at a distance from its source
- Dose/concentration dependent effect of target gene activation
- Existence of activity thresholds

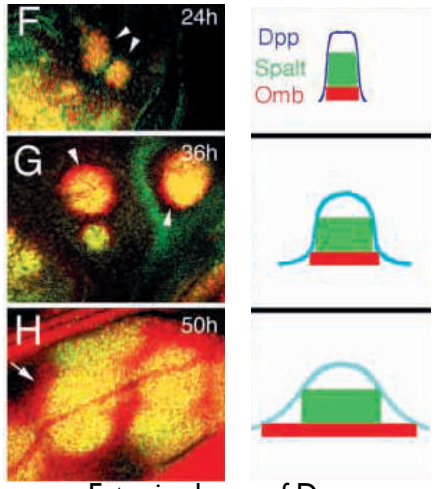


Dpp



Spalt
Omb

Overexpression of Dpp



Ectopic clones of Dpp

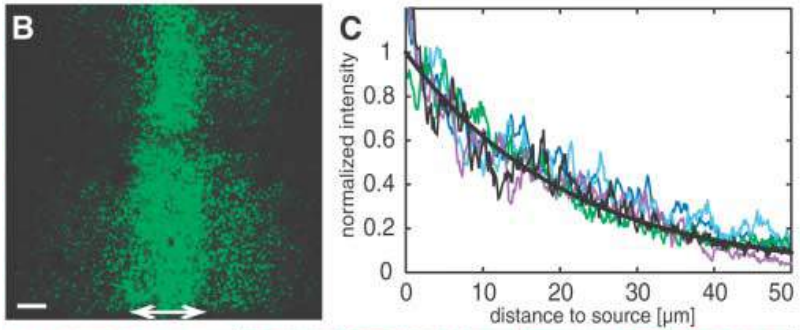
D. Nellen et al. and K. Basler. *Cell* 1996; 85(3):357-68.
T. Lecuit et al. and S. Cohen. *Nature* 1996;381(6581):387-93

T. Lecuit and S. Cohen *Development.* 1998 Dec;125(24):4901-7.

• Morphogen: Organ-size dependent growth inducer

—Morphogens: Growth factors with patterning activity

- Dpp::GFP forms an exponential gradient from its source



Dpp::GFP morphogen gradient profile

$$\partial_t C = \underbrace{D\nabla^2 C}_{\text{diffusion}} - \underbrace{kC}_{\text{degradation}} + \underbrace{2j_0\delta(x)}_{\text{production}} \qquad (1)$$ Mass conservation

$$C(x) = C_0 e^{-\frac{x}{\lambda}} \qquad (2)$$ Steady state solution

$$\lambda = \sqrt{D/k} \qquad (3)$$ Decay length

$$C_0 = j_0/\sqrt{Dk} \qquad (4)$$ Source concentration

$D = 0.10 \pm 0.05\ \mu m^2/s$

$k = 2.52 \times 10^{-4} \pm 1.29 \times 10^{-4}\ s^{-1}$

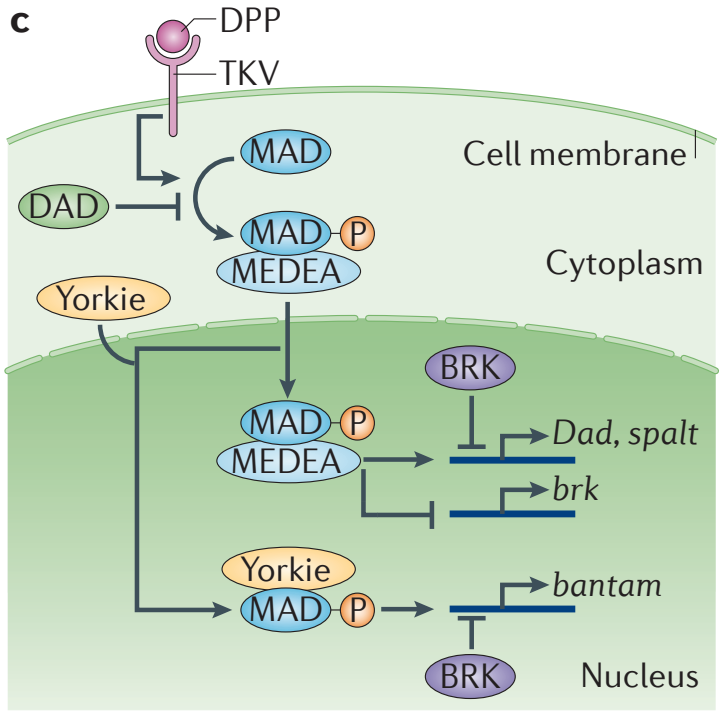
$j_0 = 3.98 \pm 2.34$ molecules/(μm × s)

Anna Kicheva et al. M. Gonzalez-Gaitan *Science* 315, 521 (2007); doi: 10.1126/science.1135774

• Morphogen: Organ-size dependent growth inducer

— Dpp Signalling pathway and growth promoting activity

- Dpp controls growth via the Hippo/Yorkie signalling pathway



• Patterning

mRNA that blocks apoptosis and induces cell proliferation

B. Thompson and S. Cohen . (2006) *Cell* 126, 767–774

• Growth

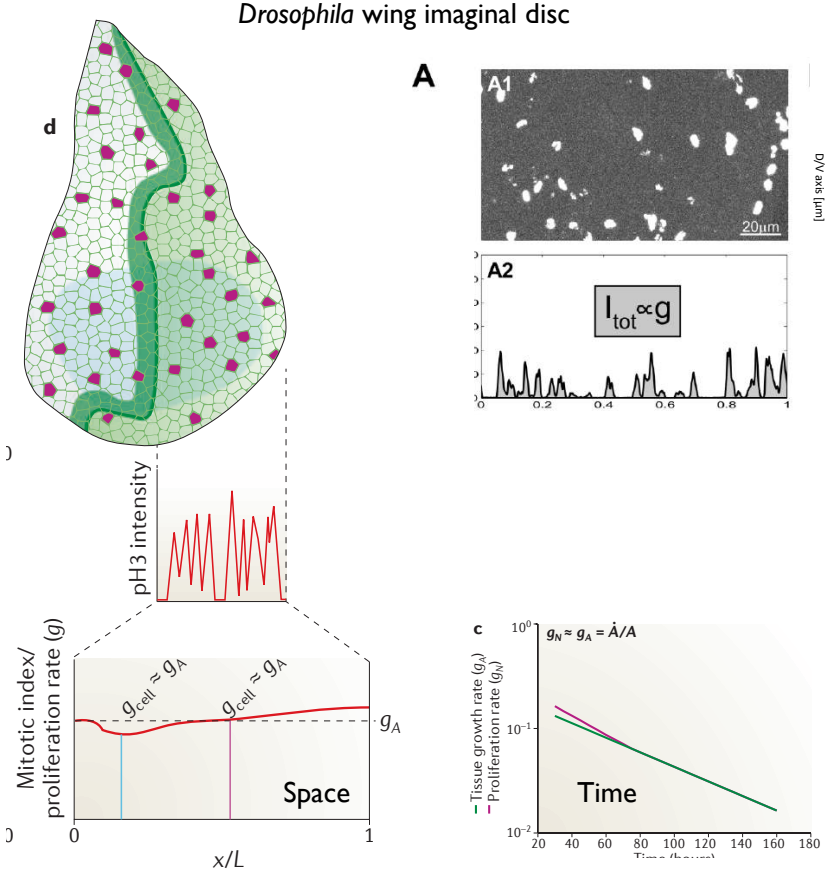
O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Nature Reviews Mol. Cell Biol.* 12: 594-604

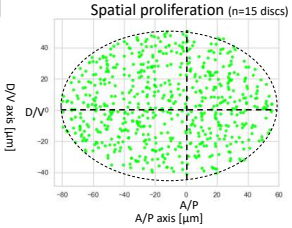
• Morphogen: Organ-size dependent growth inducer

— Spatial and temporal patterns of growth rate

- Growth rate decays over time and spatial heterogeneities are limited

Drosophila wing imaginal disc



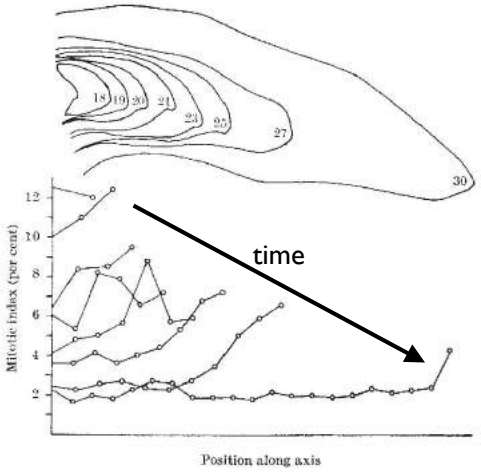


Stefan Harmansa IBDM

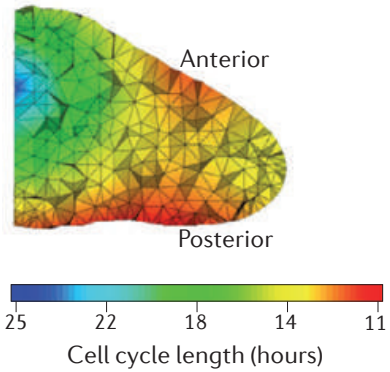
O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011)
Science 331:: 1154; doi: 10.1126/science.1200037

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011)
Nature Reviews Mol. Cell Biol. 12: 594-604

Chick limb bud



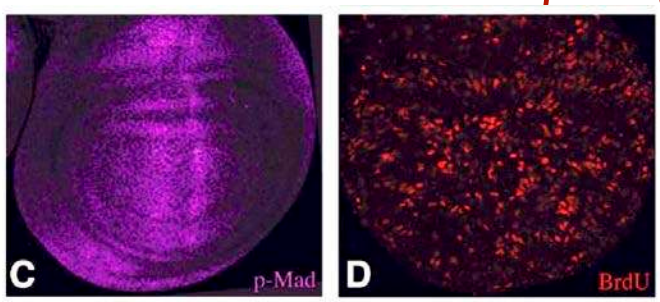
Hornbruch, A. & Wolpert, L. Cell division in the early growth and morphogenesis of the chick limb. *Nature* 226, 764–766 (1970).



Boehm, B. *et al.* and J. Sharpe *PLoS Biol.* **8**, e1000420 (2010).

• Morphogen: Organ-size dependent growth inducer

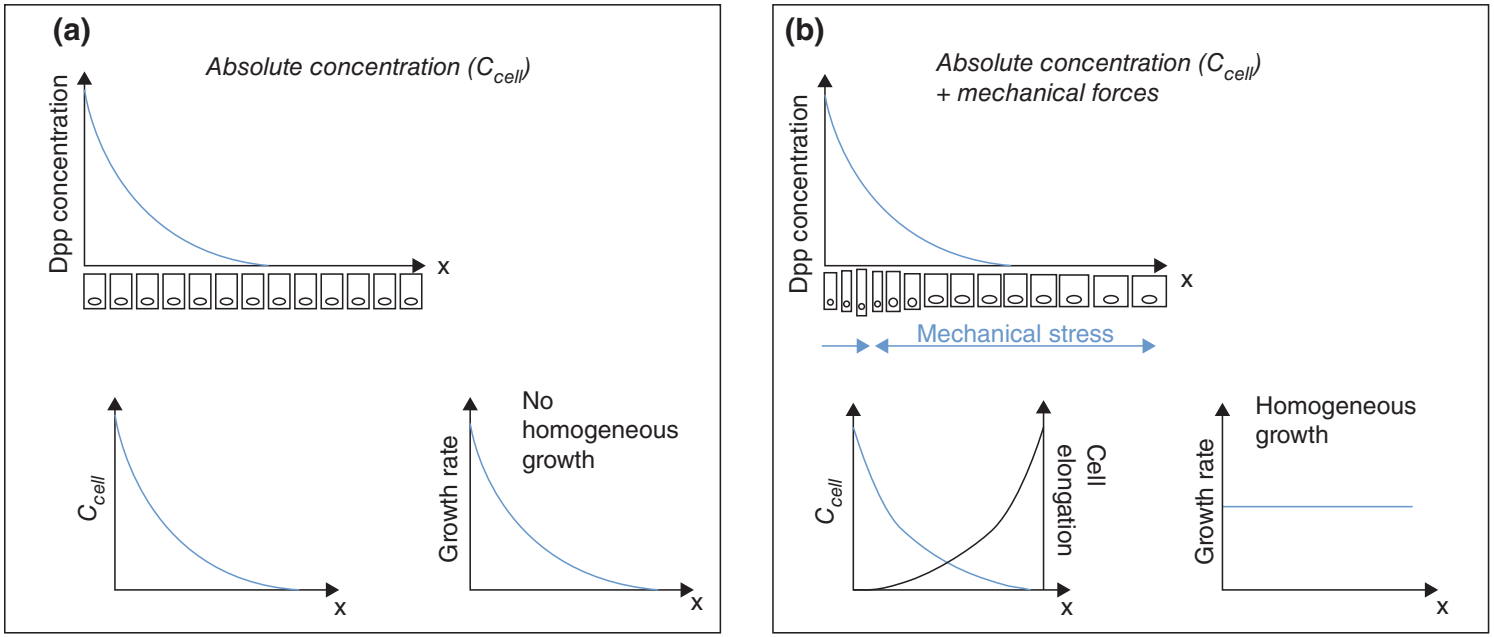
— How can a gradient of a growth factor induce *uniform* growth? « instructive model »



D. Rogulja & K. Irvine. (2005) *Cell*, Vol. 123, 449–461

- Hypothesis: Combined effect of [concentration] and mechanical forces (see course #5)

$$C(x) = C_0 e^{-\frac{x}{\lambda}}$$



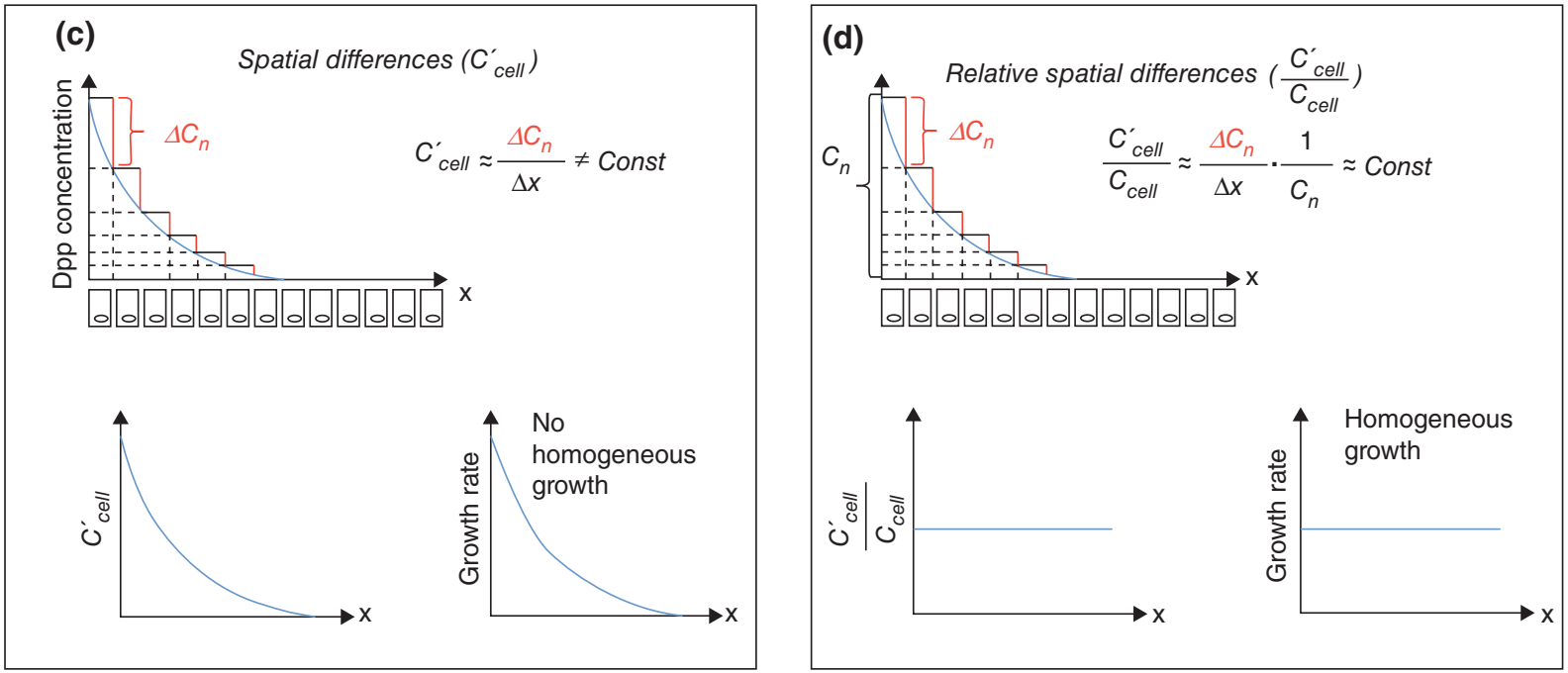
M. Romanova-Michaelides et al. and F. Jülicher and M. Gonzalez-Gaitan. *WIREs Dev Biol* 2015, 4:591–608. doi: 10.1002/wdev.195

• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform* growth?

- The relative (ie. normalised) spatial difference in [concentration] is spatially uniform with an exponential gradient
- **C'/C = cte**

$$C(x) = C_0 e^{-\frac{x}{\lambda}}$$



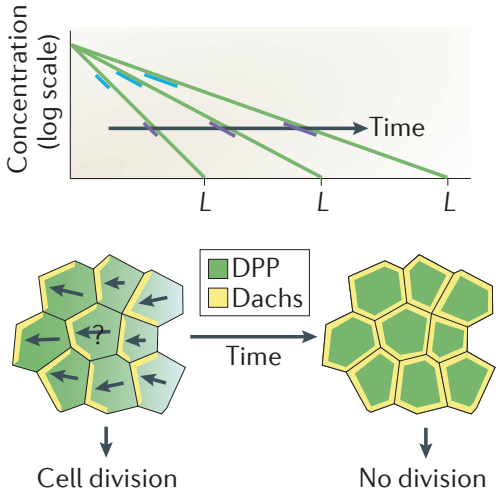
M. Romanova-Michaelides et al. and F. Jülicher and M. Gonzalez-Gaitan. *WIREs Dev Biol* 2015, 4:591–608. doi: 10.1002/wdev.195

• Organ-size dependent growth inducer

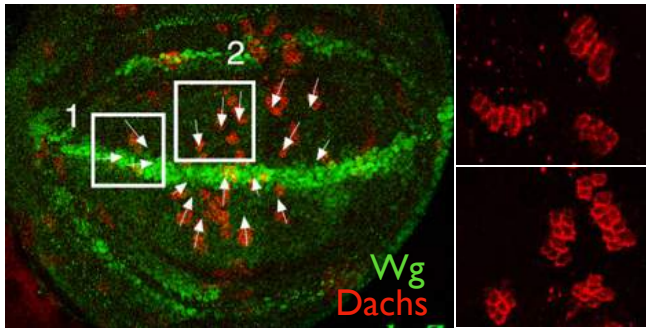
— How can a gradient of a growth factor induce *uniform* growth?

- Spatial model: slope of the gradient ***C'/C* = cte**

$$C(x) = C_0 e^{-\frac{x}{\lambda}}$$



—Requires gradient scaling so the gradient covers the whole field ✓
—Scalar gradient causes vectorial/nematic gradient: ✓
eg. Planar polarity and connection to growth
— A local discontinuity in Dpp signalling induces growth
—Growth is blocked if Dpp is uniform at all time



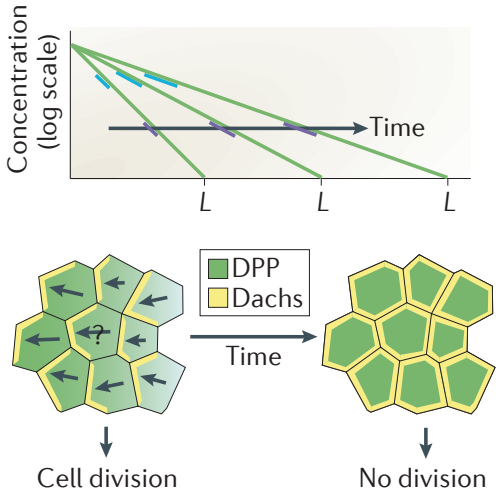
Rogulja, D., Rauskolb, C. & Irvine, K. D. Morphogen control of wing growth through the Fat signaling pathway. *Dev. Cell* **15**, 309–321 (2008).

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Nature Reviews Mol. Cell Biol.* 12: 594-604

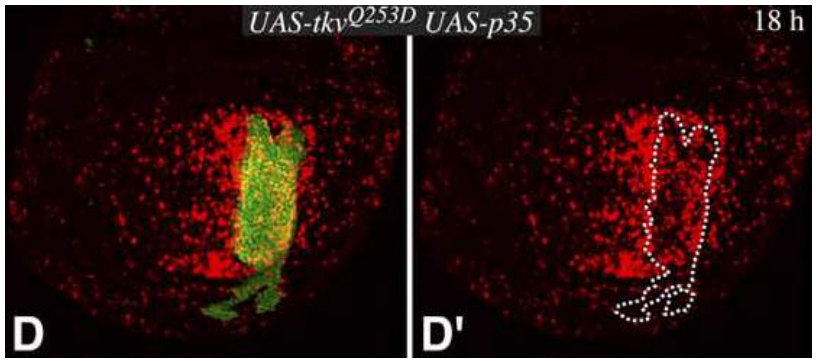
• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform* growth?

- Spatial model: slope of the gradient ***C'/C* = cte** $C(x) = C_0 e^{-\frac{x}{\lambda}}$



—Requires gradient scaling so the gradient covers the whole field ✓
—Scalar gradient causes vectorial/nematic gradient:
eg. Planar polarity and connection to growth ✓
— A local discontinuity in Dpp signalling induces intercalary growth ✓
—Growth is blocked if Dpp is uniform at all time



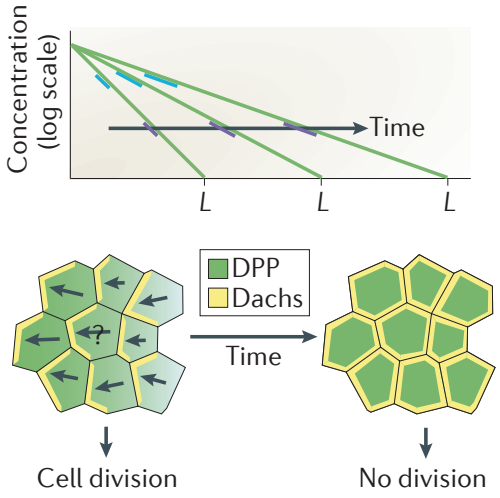
Tkv253QD: constitutive activation of Dpp signalling causes non autonomous cell proliferation (BrdU)

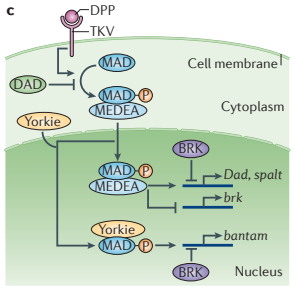
D. Rogulja & K. Irvine. (2005) *Cell*, Vol. 123, 449–461
[see also: A. C. Garcia-Bellido. and A. Garcia-Bellido. *Int. J. Dev. Biol.* 42: 353-362 (1998)]

• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform* growth?

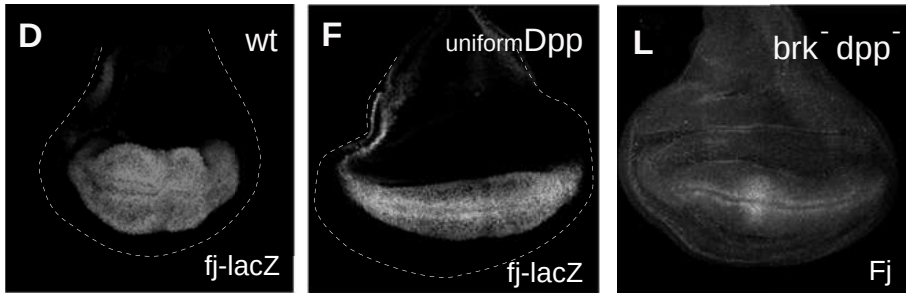
- Spatial model: slope of the gradient ***C'/C* = cte** $C(x) = C_0 e^{-\frac{x}{\lambda}}$





—Requires gradient scaling so the gradient covers the whole field
—Scalar gradient causes vectorial/nematic gradient:
eg. Planar polarity and connection to growth
— A local discontinuity in Dpp signalling induces growth
—Growth is blocked if Dpp is uniform at all time

Caution: Is Dpp perfectly uniform and is this at all time?

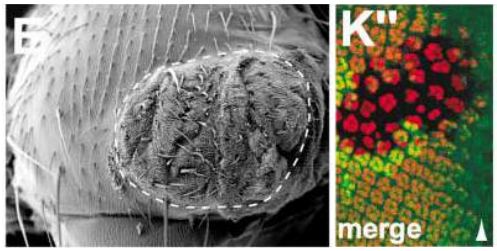


G. Schwank et al. and K. Basler (2011) *Developmental Cell 20*, 123–130

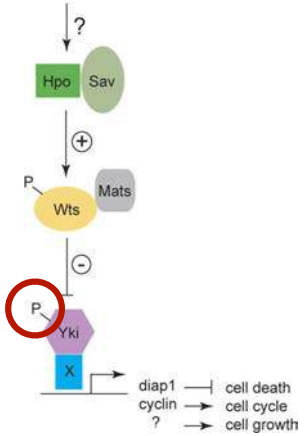
• Size regulation by the Hippo pathway

—A universal cell and tissue growth promoting pathway

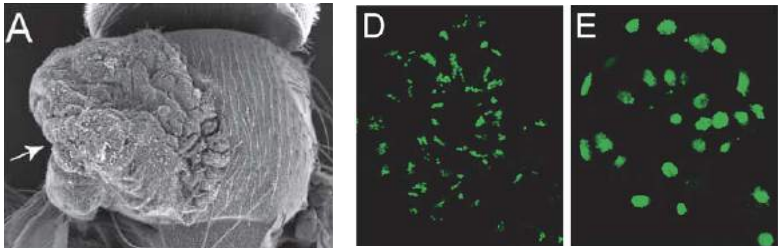
- Identification of Hippo, a Ste20Kinase that represses cell and tissue growth



S. Wu et al. and D. Pan (2003) *Cell*, Vol. 114, 445–456
K Harvey et al . I. Hariharan. (2003) *Cell*, Vol. 114, 457–467

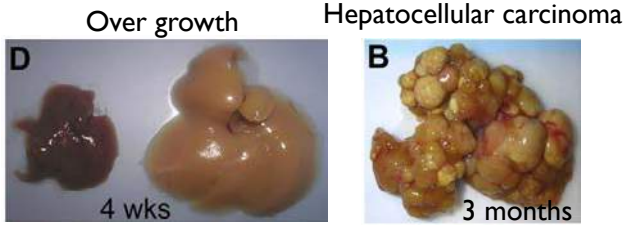


- Hippo represses the transcription factor Yorkie
- Yorkie promotes growth via expression of Cyclin E and inhibition of apoptosis

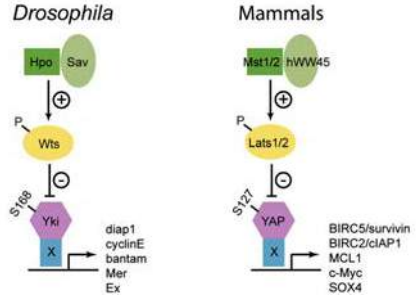


J. Huang et al. and D. Pan. (2005) *Cell*, Vol. 122, 421–434
J. Dong et al. and D. Pan. (2007) *Cell* 130, 1120–1133

- Phosphorylation of Yorkie by the kinase Warts blocks nuclear translocation and Yorkie activity
- Mammalian orthologue YAP and Lats control tissue growth in the liver, and tumorogenesis



Overexpression of YAP

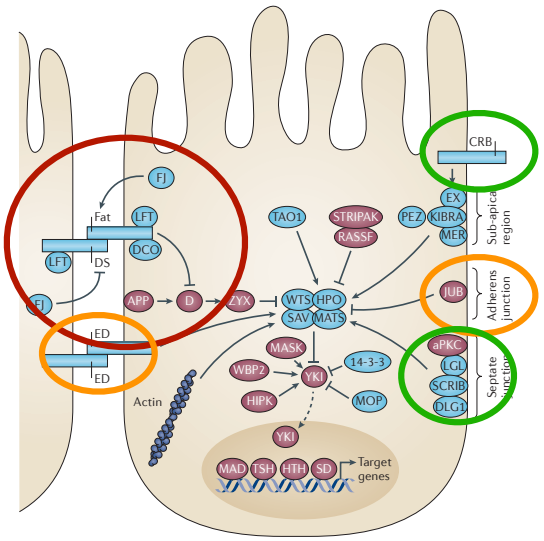


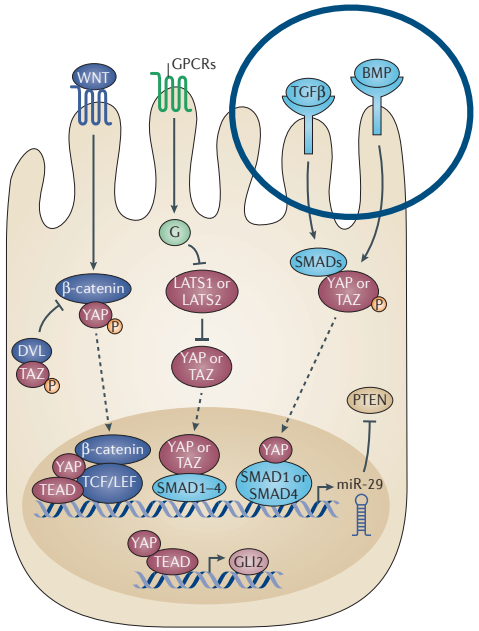
J. Dong et al. and D. Pan. (2007) *Cell* 130, 1120–1133

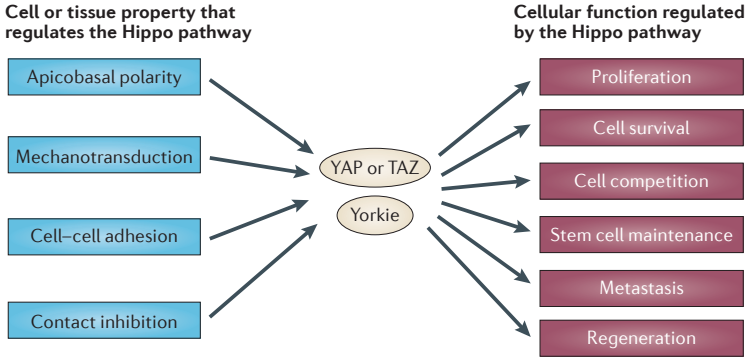
• Size regulation by the Hippo pathway

—A universal cell and tissue growth promoting pathway

- The hippo pathway integrates many cellular inputs:
  - — **polarity** cues: epithelial integrity
  - — cell contacts: **adhesion** molecules
  - — mechanical stimuli (see cours #5)
  - — growth factor signalling: **TFGß/Dpp** and **Fat**







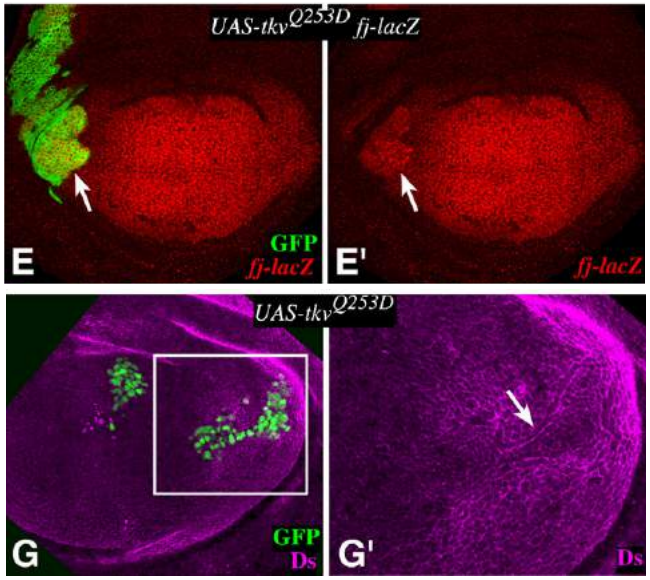
K. Harvey et al. *Nat Rev Cancer.* 2013 Apr;13(4):246-57

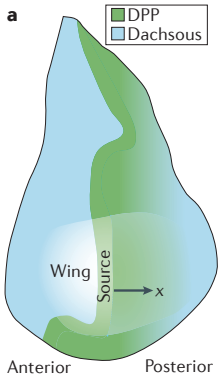
• Morphogen: Organ-size dependent growth inducer

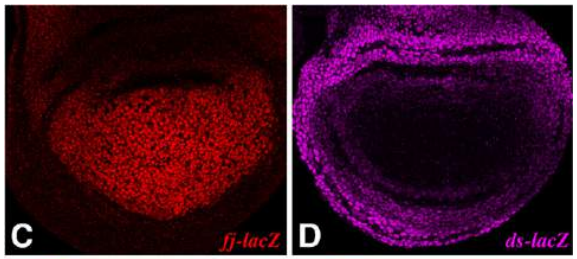
— How can a gradient of a growth factor induce *uniform growth?*

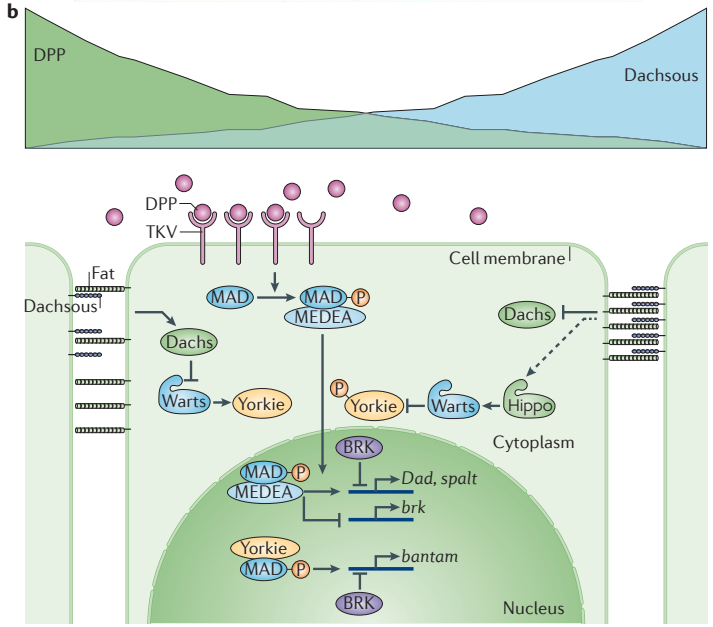
- Spatial model: slope of the gradient ***C'/C* = cte** $C(x) = C_0 e^{-\frac{x}{\lambda}}$
- Dpp controls growth via the Fat/Dachsous signalling pathway

- High Dpp signalling affects Fat signalling









Rogulja, D., Rauskolb, C. & Irvine, K. D. Morphogen control of wing growth through the Fat signaling pathway. *Dev. Cell* **15**, 309–321 (2008).

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Nature Reviews Mol. Cell Biol.* 12: 594-604

• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform growth?*

- Spatial model: slope of the gradient $C'/C = cte$ $C(x) = C_0 e^{-\frac{x}{\lambda}}$
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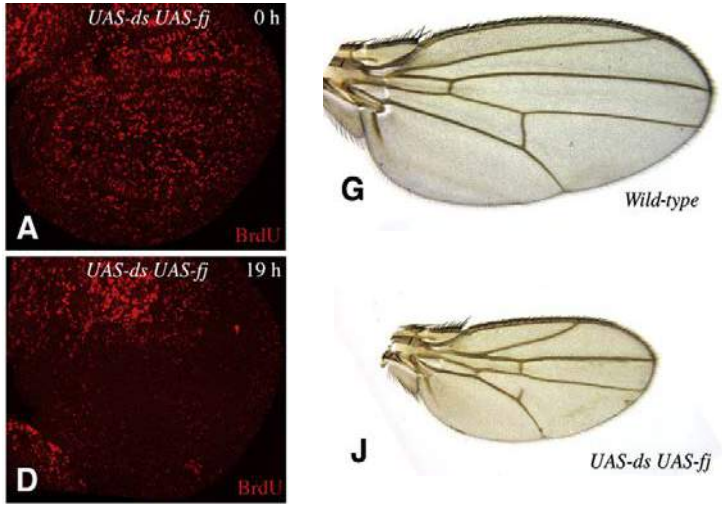
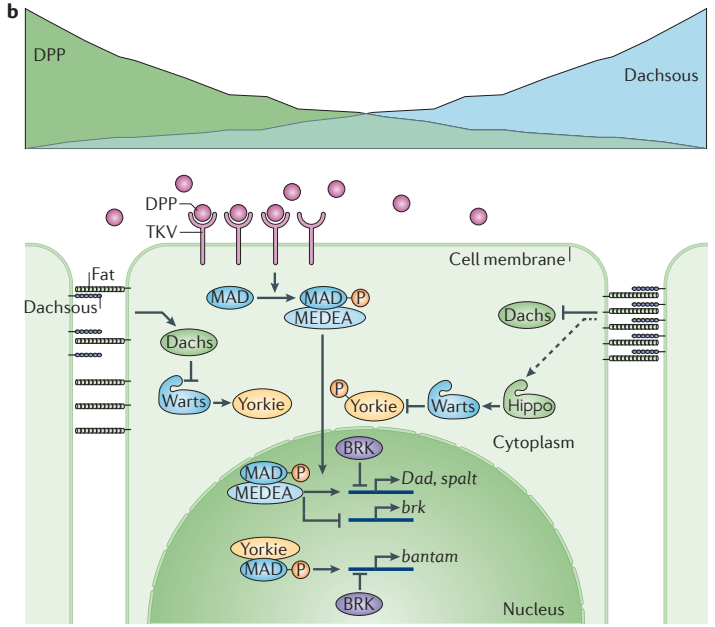


high uniform Fat signalling



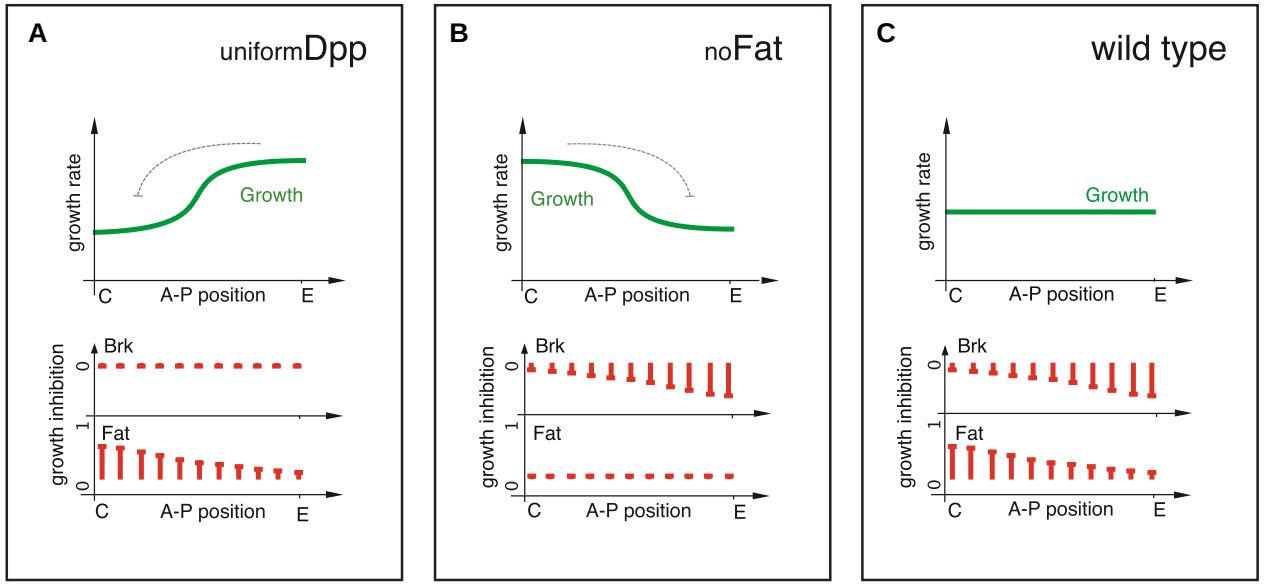
Rogulja, D., Rauskolb, C. & Irvine, K. D. Morphogen control of wing growth through the Fat signaling pathway. *Dev. Cell* **15**, 309–321 (2008).

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Nature Reviews Mol. Cell Biol.* 12: 594-604

• Morphogen: Organ-size dependent growth inducer

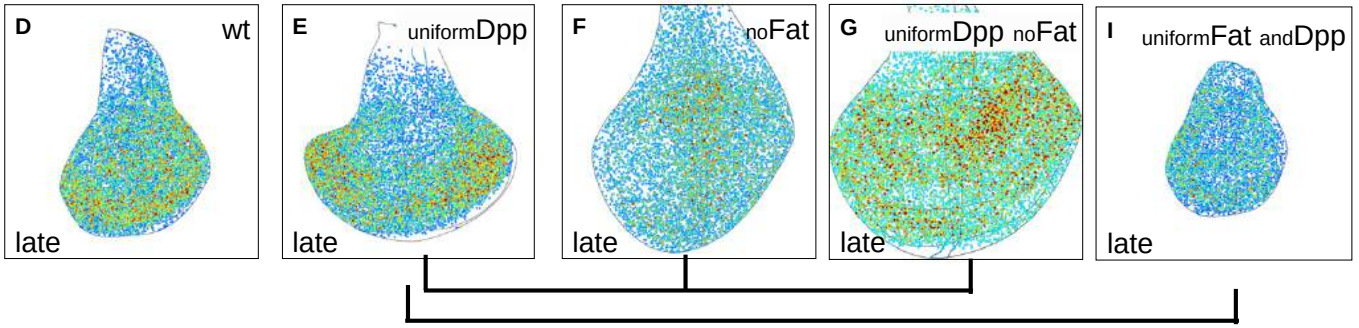
— How can a gradient of a growth factor induce *uniform growth?*

- Complementary roles of the counter gradients of Dpp and Fat



Growth constant *k:*

$A(t) = A_0 e^{kt}$



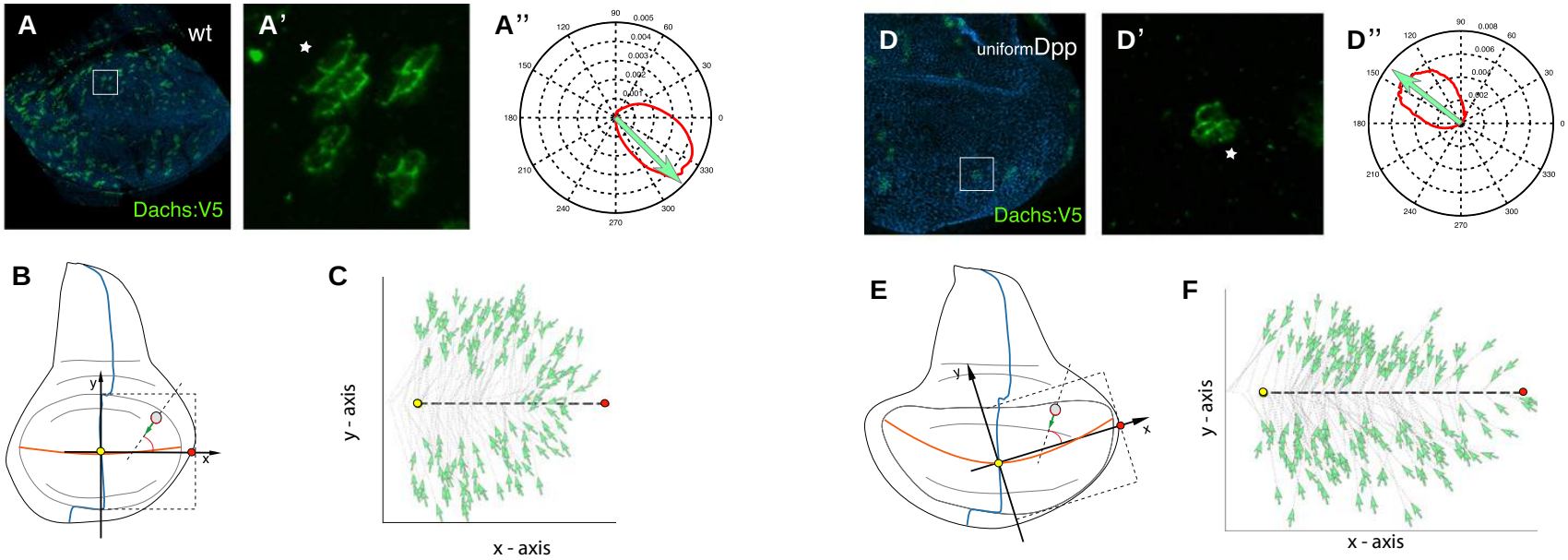
G. Schwank et al. and K. Basler (2011) *Developmental Cell 20*, 123–130

• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform growth* and *growth arrest?*

- Complementary roles of the counter gradients Dpp and Fat
- Dpp may not determine the gradient of Fat activity

- How could such a system confer robustness? The two gradients are supposed to be independent and yet have perfectly complementary shapes



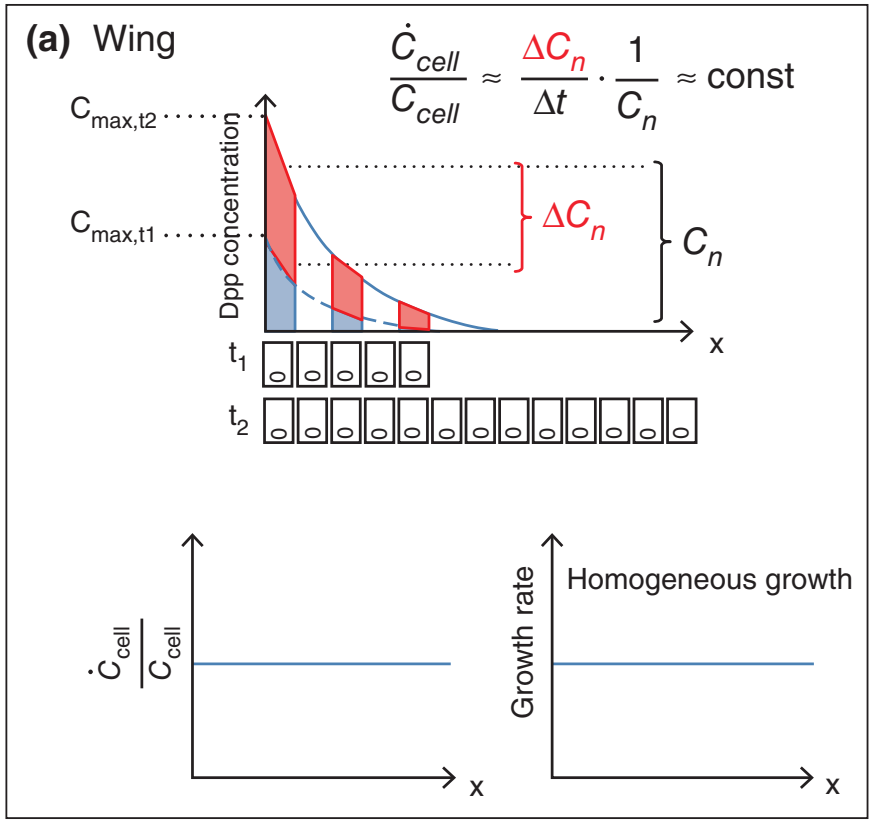
G. Schwank et al. and K. Basler *Developmental Cell 20*, 123–130

Rogulja, D., Rauskolb, C. & Irvine, K. D. Morphogen control of wing growth through the Fat signaling pathway. *Dev. Cell* **15**, 309–321 (2008).

• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *uniform* growth?

- Temporal Model: Consider the dynamical properties of the morphogen gradient $C(x) = C_0 e^{-\frac{x}{\lambda}}$
- In some conditions, the normalised temporal variation of C is a constant:

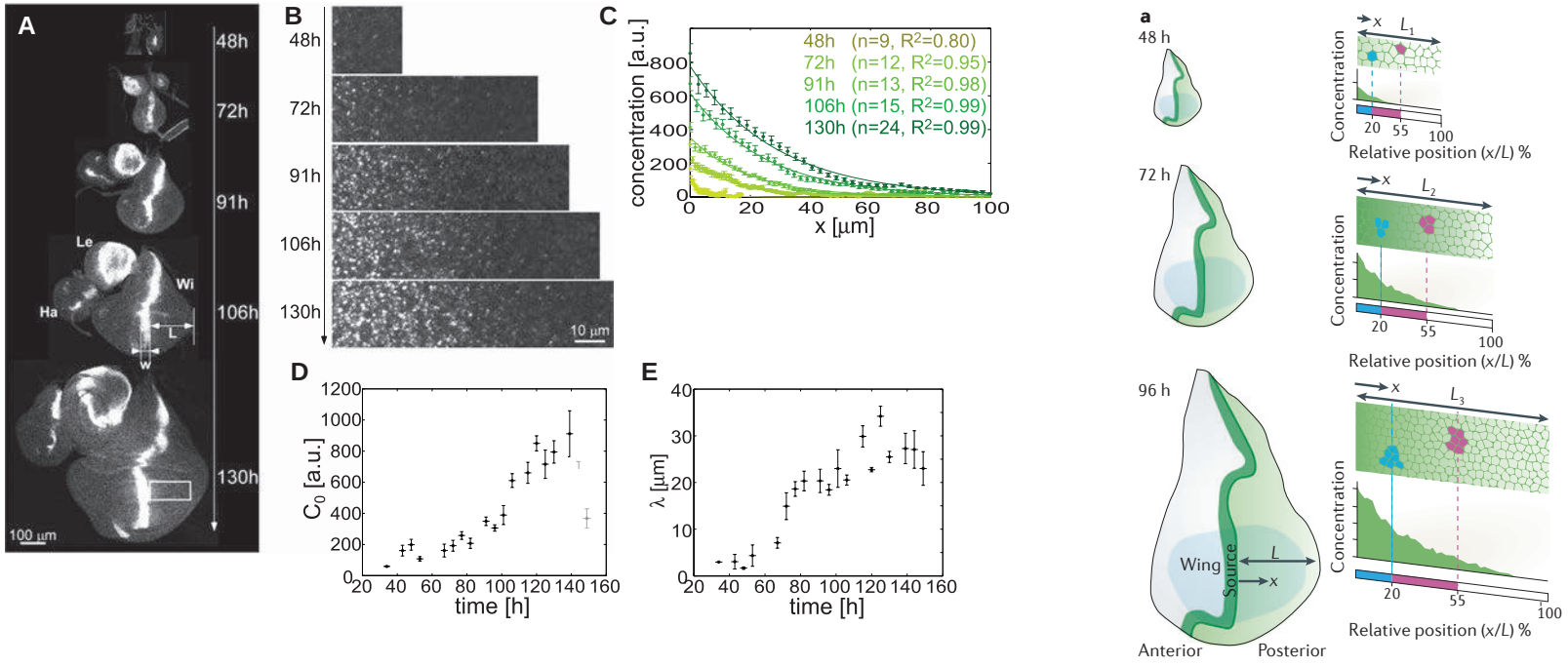


M. Romanova-Michaelides et al. and F. Jülicher and M. Gonzalez-Gaitan. *WIREs Dev Biol* 2015, 4:591–608. doi: 10.1002/wdev.195

• Morphogen: Organ-size dependent growth inducer

— Dynamics of morphogen gradient with growth

- At any time, steady state description of an exponential morphogen: $C(x) = C_0 e^{-\frac{x}{\lambda}}$

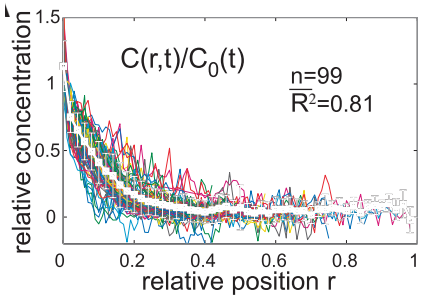


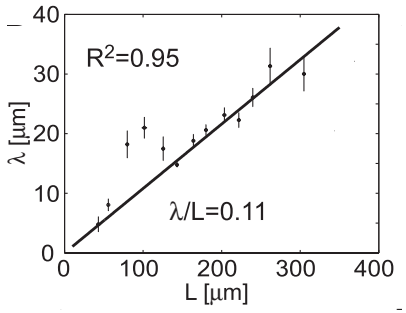
O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan
Science **331**, 1154 (2011); doi: 10.1126/science.1200037

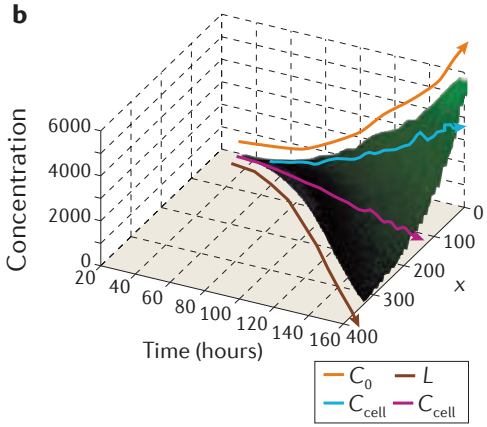
• Morphogen: Organ-size dependent growth inducer

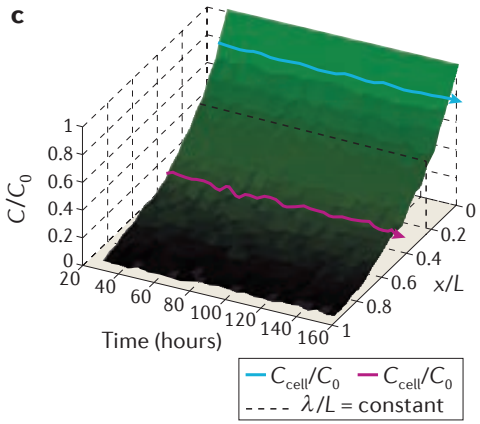
— Dynamics of morphogen gradient with growth: scaling

- Renormalisation shows gradient scaling (decay length increases over time)







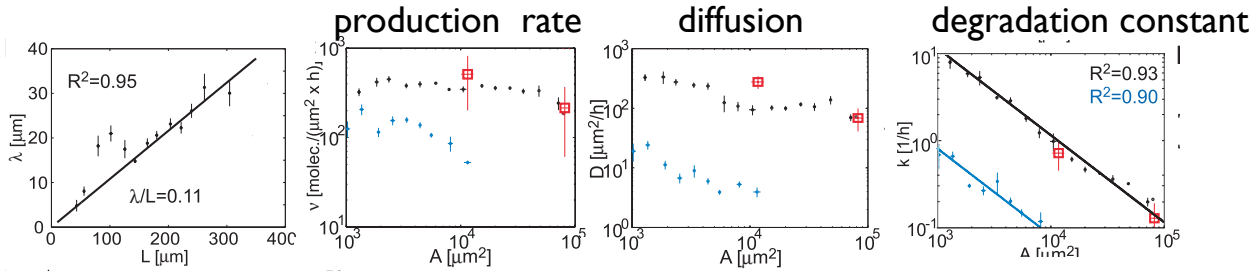


O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan *Science* **331**, 1154 (2011); doi: 10.1126/science.1200037

• Morphogen: Organ-size dependent growth inducer

— Dynamics of morphogen gradient with growth: scaling

- Scaling is not due to change in diffusion or production rate, but to reducing degradation constant

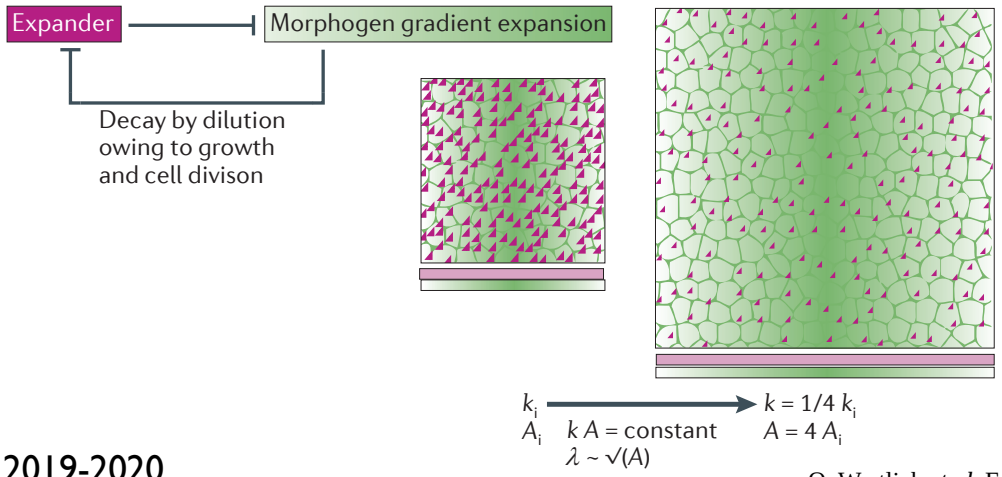


$\lambda = \sqrt{D/k}$ and $k \sim 1/A$ gives λ/L =cte

$A \sim L^2$

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011)
Science 331:: 1154; doi: 10.1126/science.1200037

- Expander **dilution** model of gradient scaling:
  —degradation is reduced over time as inhibitor of morphogen stability is diluted



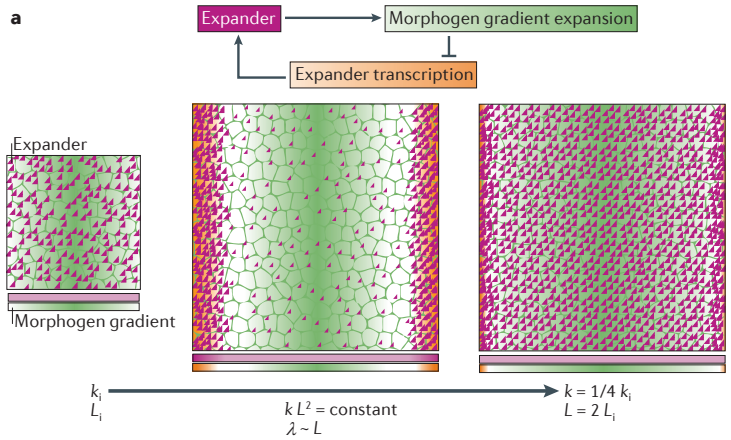
O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011)
Nature Reviews Mol. Cell Biol. 12: 594-604

• Morphogen: Organ-size dependent growth inducer

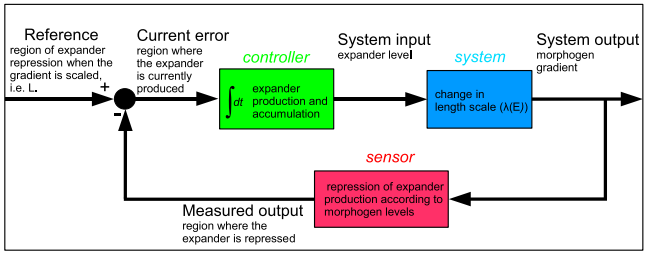
— Dynamics of morphogen gradient with growth: scaling

- Expander **repression** model of gradient scaling:

- Morphogen represses a factor that diffuses and promotes M stability (antagonises degradation)



integral feedback controller



O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Nature Reviews Mol. Cell Biol.* 12: 594-604

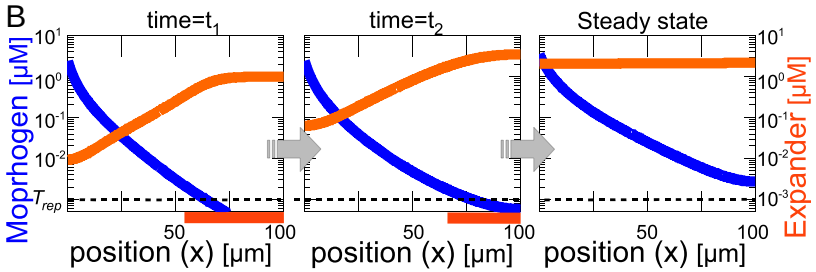
Morphogen (M) $$\frac{\partial [M]}{\partial t} = D_M \nabla^2 [M] - \alpha_M [M], \qquad \textbf{[1]}$$

Expander (E) $$\frac{\partial [E]}{\partial t} = D_E \nabla^2 [E] - \alpha_E [E] + \beta_E \frac{T_{\text{rep}}^h}{T_{\text{rep}}^h + [M]^h}, \qquad \textbf{[3]}$$

non-linear repression

Diffusion of M is enhanced by E or degradation of M is reduced by E

$$D_M = D_M\ ([E];[M]); \quad \alpha_M = \alpha_M\ ([E];[M])$$



E is produced in distal regions of field
E diffuses and enables M expansion which represses E further away
Steady state is reached when M reaches the edge of the field and $[M] > T_{rep.}$

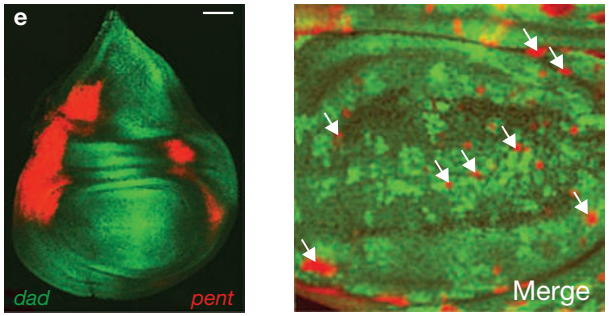
Ben-Zvi, D. & Barkai, N. *Proc. Natl Acad. Sci. USA* **107**, 6924–6929 (2010)

• Morphogen: Organ-size dependent growth inducer

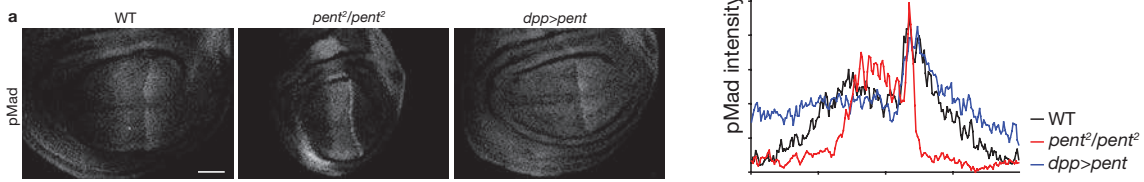
— Dynamics of morphogen gradient with growth: scaling

- Experimental evidence of a negative feedback inhibitor of the Dpp morphogen gradient

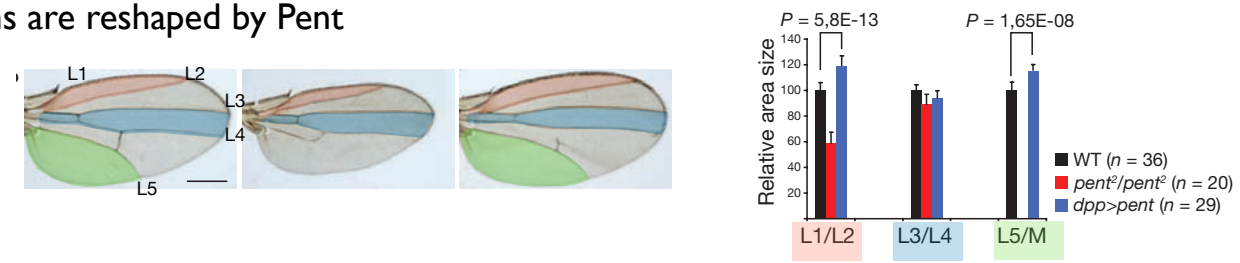
The expander Pentagon is repressed by Dpp
(clones mutant for Dpp signalling express *pent)*



Dpp activity gradient is shaped by Pentagon: expansion when Pent is over expressed, reduced in mutant



Wing patterns are reshaped by Pent



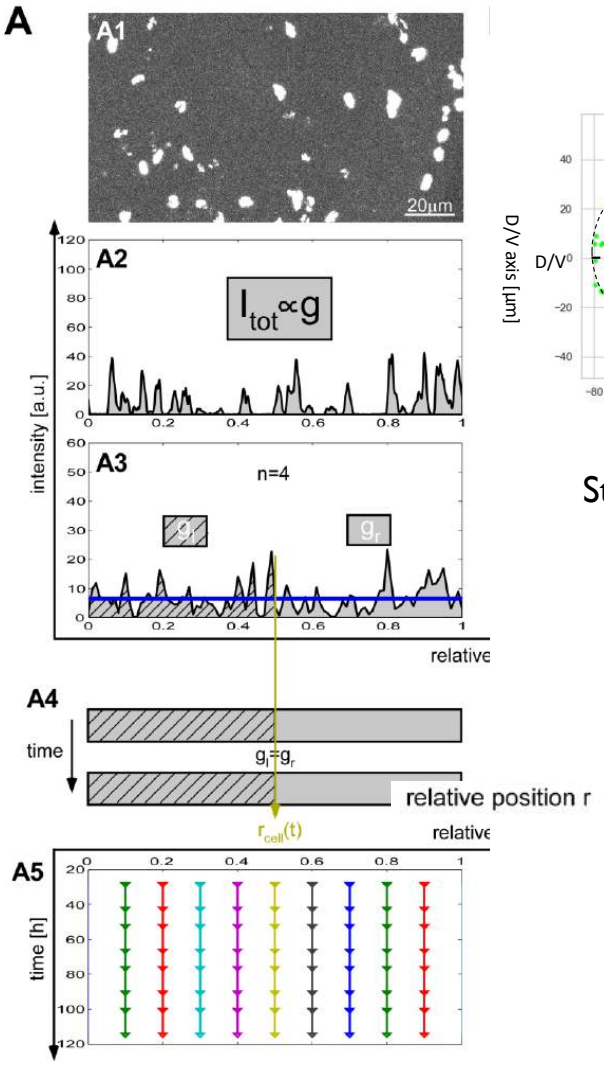
Vuilleumier, R. *et al.* M. Affolter and G. Pyrowolakis. *Nature Cell Biol.* **12**, 611–617 (2010).

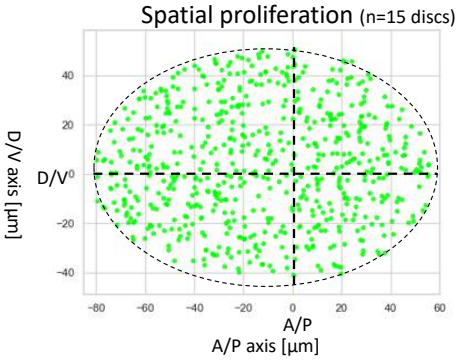
• Morphogen: Organ-size dependent growth inducer

— Dynamics of morphogen gradient with growth: growth homogeneity

Growth is uniform so the normalised position of a cell down the gradient is invariant

$$r_{\text{cell}} = x_{\text{cell}}(t)/L(t)$$





Stefan Harmansa IBDM

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Science* 331:: 1154; doi: 10.1126/science.1200037

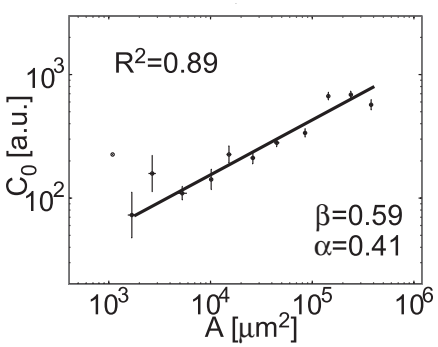
• Morphogen: Organ-size dependent growth inducer

—Tissue specific, cellular growth rule:

- Power law relates Dpp at the source and tissue size: Implications

O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011)
Science 331:: 1154; doi: 10.1126/science.1200037

- **Theory:**

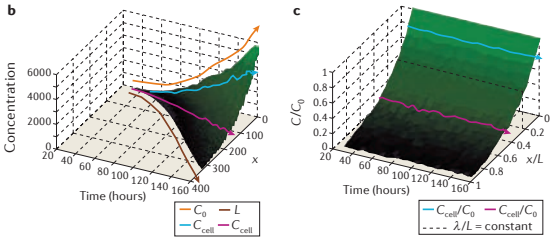


$$C_0(t) \sim A(t)^{\beta} \qquad (1)$$

$$C_{cell}(t) \sim A(t)^{\beta}$$ since $C(r_{cell},t)/C_0(t)$ is a constant

- Therefore: $\dot{C}_{cell}/C_{cell} = \dot{C}_0/C_0 = \beta(\dot{A}/A) = \beta g$

where tissue growth rate is $g = \dot{A}/A$



And the cell proliferation rate $g_{cell} \approx \frac{1}{\beta}\frac{\dot{C}_{cell}}{C_{cell}}$ (2)

is proportional to the relative temporal change in Dpp concentration

$\alpha = \Delta C_{cell}/C_{cell}$ $\quad C_{cell}/C_{cell} \approx (\Delta C_{cell}/\theta)/C_{cell}$ where the cell cycle time $\theta \approx \ln 2/g_{cell}$

$$\alpha = \frac{\Delta C_{cell}}{C_{cell}} \approx \beta \ln 2 = 0.41$$

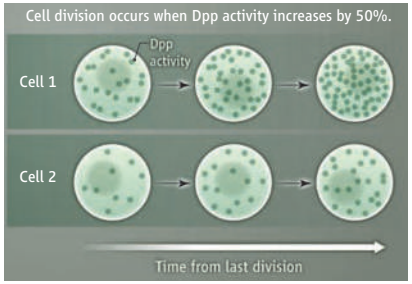
- Thus, cells divide when they experience a 40% increase in Dpp concentration or 50% of intracellular signalling *S* increase

$\alpha_s = 48\% \pm 2\%$ ($\quad g_{cell} \approx \frac{\ln 2}{\alpha_s}\frac{\dot{S}_{cell}}{S_{cell}}$

• Morphogen: Organ-size dependent growth inducer

—Tissue specific, cellular growth rule:

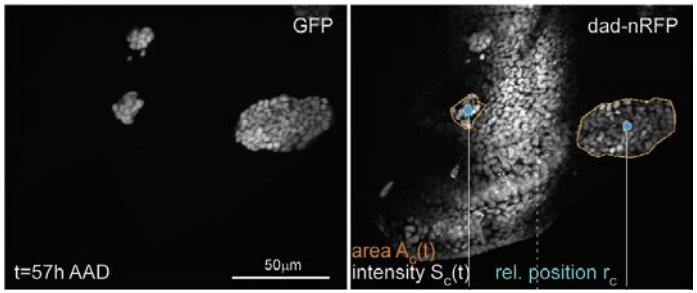
Cell cycle length is set by the timescale of 50% Dpp signalling increase

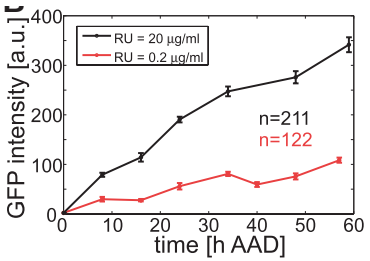


- **Experimental test:** Exogenous system of signalling increase using a constitutive activation of the Dpp receptor Tkv (*S* in the experiment)

$\dot{S}_{\text{cell}}$ depends on [RU]

$\dot{S}_{\text{cell}} / S_{\text{cell}}$ is greater at tissue edge where *S* is lower





O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Science* 331:: 1154; doi: 10.1126/science.1200037

• Morphogen: Organ-size dependent growth inducer

—Tissue specific, cellular growth rule:

Cell cycle length is set by the timescale of 50% Dpp signalling increase

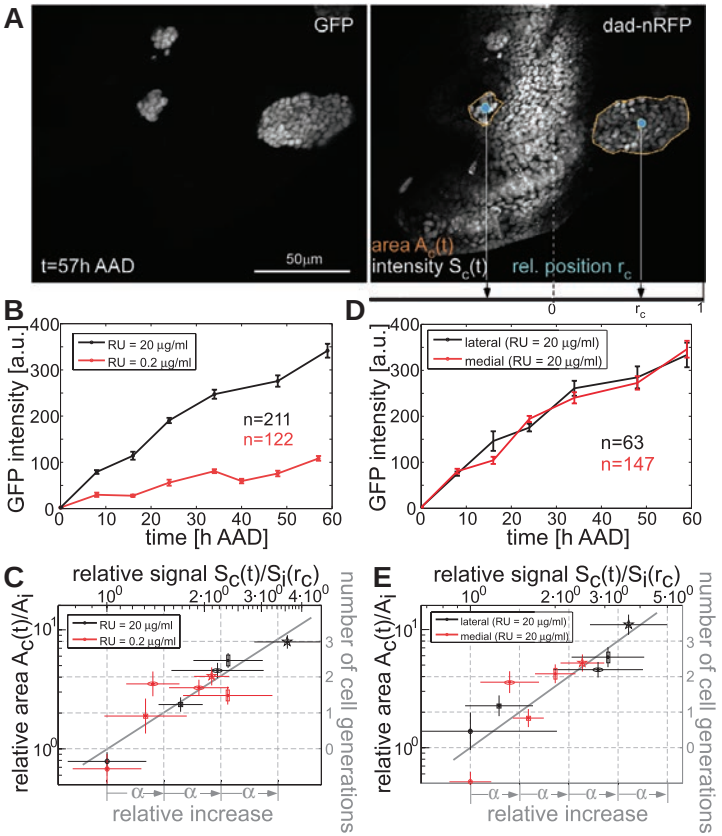
- **Experimental test:** Exogenous system of signalling increase using a constitutive activation of the Dpp receptor Tkv (S in the experiment)

$\dot{S}_{cell}$ depends on [RU]

$\dot{S}_{cell}/S_{cell}$ is greater at tissue edge where S is lower

Prediction 1: clones grow larger at the periphery as observed

Prediction 2: $\dot{S}_{cell}/S_{cell} = \beta(\dot{A}/A) = \beta g$ as observed



O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Science* 331:: 1154; doi: 10.1126/science.1200037

• Morphogen: Organ-size dependent growth inducer

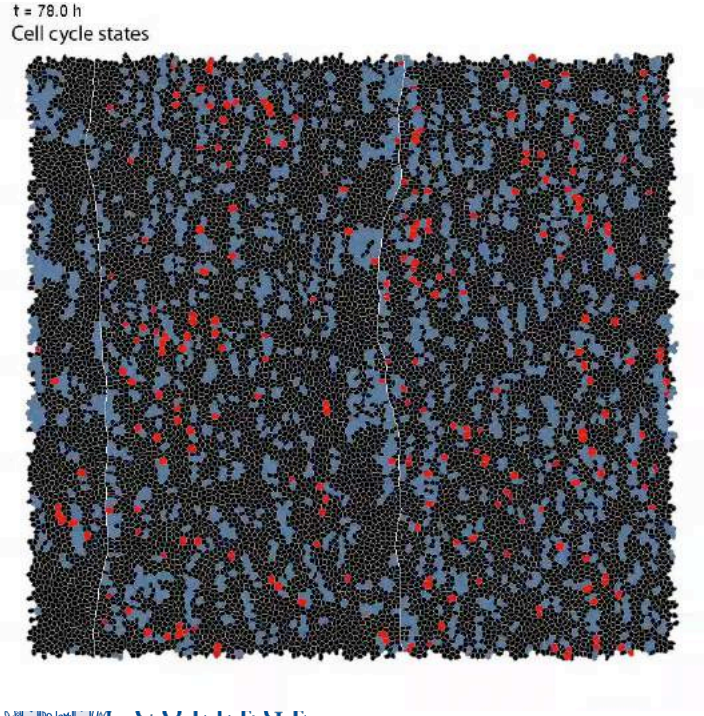
—Tissue specific, cellular growth rule:

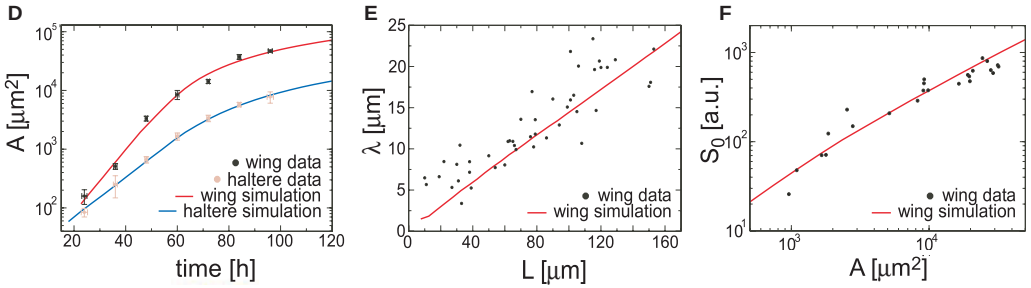
Cell cycle length is set by the timescale of 50% Dpp signalling increase

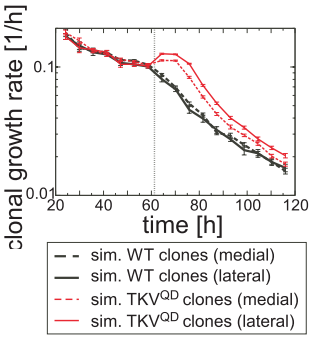
- **Simulations:** Dpp production and diffusion are constant as observed.

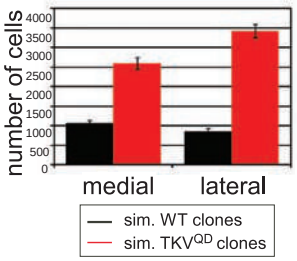
  Dpp degradation rate decays such that its average value becomes inversely proportional to the cell number (expander model).

  A cell divides when the relative increase of the local Dpp level reaches a threshold value 0.5 (« growth rule »).









O. Wartlick *et al.* F. Jülicher and M. Gonzalez-Gaitan (2011) *Science* 331:: 1154; doi: 10.1126/science.1200037

• Morphogen: Organ-size dependent growth inducer

—Tissue specific, cellular growth rule:

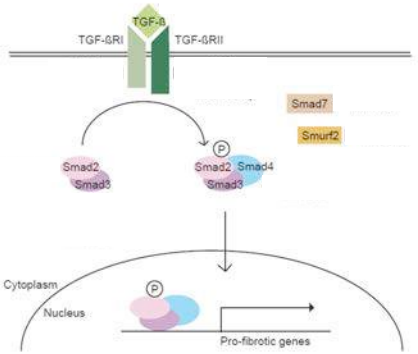
Possible mechanisms for temporal growth rule: measuring the signal increase rate

Dynamics of TGF-β signaling reveal adaptive and pulsatile behaviors reflected in the nuclear localization of transcription factor Smad4

Aryeh Warmflash[a,b], Qixiang Zhang[b], Benoit Sorre[a,b], Alin Vonica[b,1], Eric D. Siggia[a,2], and Ali H. Brivanlou[b,2]

[a]Center for Studies in Physics and Biology and [b]Laboratory for Molecular Vertebrate Embryology, The Rockefeller University, New York, NY 10065

A. Warmflash et al. and E. Siggia and A. Brivanlou. (2012) www.pnas.org/cgi/doi/10.1073/pnas.1207607109



- R-Smads (Smad1, 2, 3, 5 and 8) phosphorylation quantitatively follows receptor activation
- But co-activator Smad4, which relays R-Smad activation shows transient activation (nuclear translocation)

—Evidence of pathway adaptation by negative feedback

Encoding of Temporal Signals by the TGF-β Pathway and Implications for Embryonic Patterning

Benoit Sorre,[1,2,3] Aryeh Warmflash,[1,2,3] Ali H. Brivanlou,[1,*] and Eric D. Siggia[2,*]
[1]Laboratory of Molecular Vertebrate Embryology, The Rockefeller University, New York, NY 10065, USA
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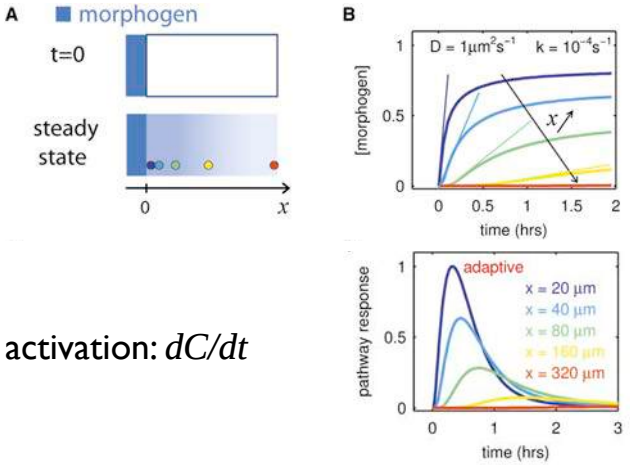
http://dx.doi.org/10.1016/j.devcel.2014.05.022

B. Sorre, A. Warmflash et al, and E. Siggia (2014) *Developmental Cell 30*, 334–342



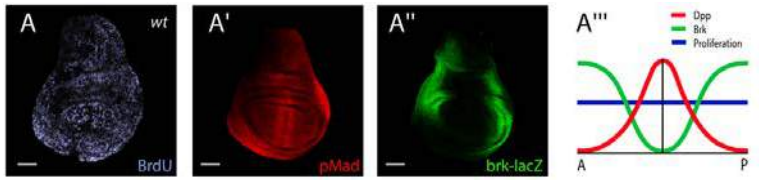
—The rate of pathway activation decays with space.

—In an adaptive system, pathway activity is dependent on speed of activation: *dC/dt*

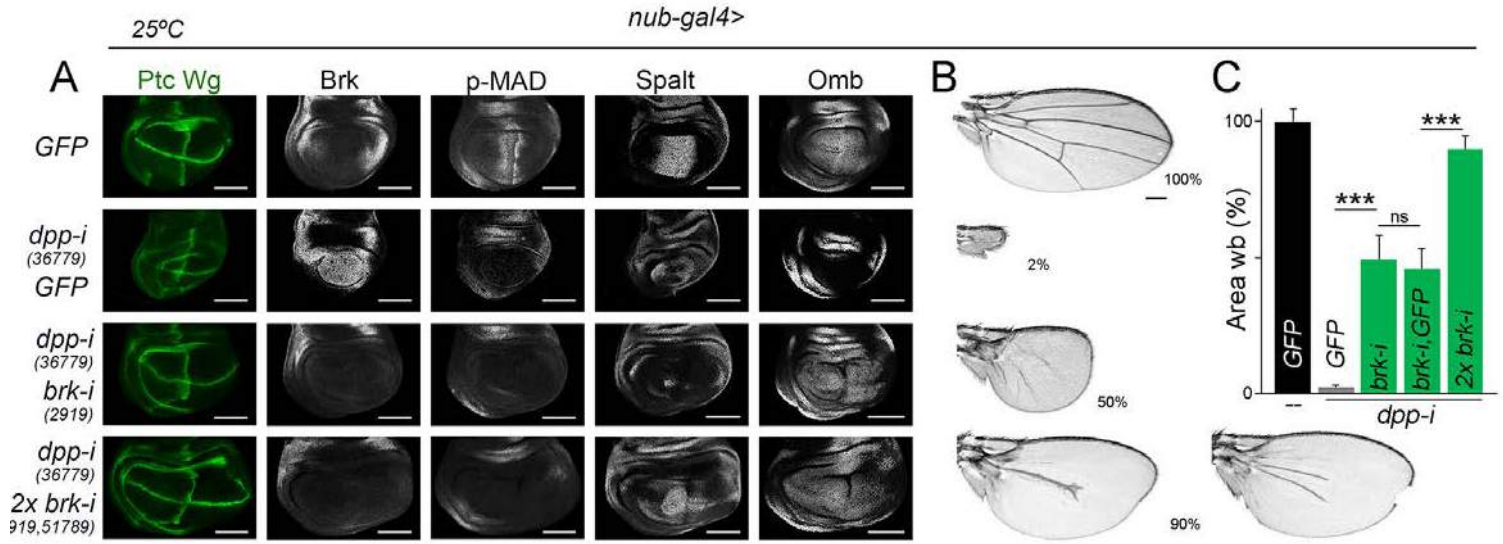
• Morphogen: Organ-size dependent growth inducer

Is the Dpp gradient required for tissue growth?

- Dpp controls growth by inhibiting its target Brk
- Tissue grows in the absence of both Dpp signalling and Brk but growth is no longer uniform and does not normally arrest
- So Dpp is regulating growth



- Assumes complete absence of Dpp in disc center (incorrect)
- Yet Dpp signalling is most likely changing over time.



Barrio and Milan. *eLife* 2017;6:e22013. DOI: 10.7554/eLife.22013

Bosch et al. *eLife* 2017;6:e22546. DOI: 10.7554/eLife.22546

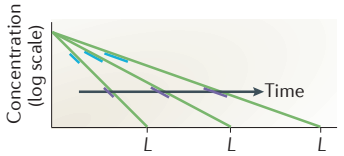
Schwank G, Restrepo S, Basler K. 2008. *Development* 135:4003–4013. doi: 10.1242/dev.025635, PMID: 1902 9041

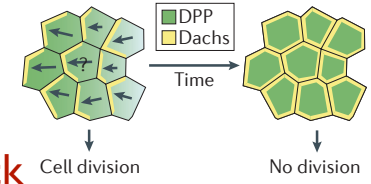
• Morphogen: Organ-size dependent growth inducer

— How can a gradient of a growth factor induce *growth arrest?*

- Spatial model: Flattening of a scaling gradient

—Requires that synthesis rate inversely scales with tissue size





- Temporal model: Degradation rate and Growth Feedback

A gradient of chemical species regulates growth and growth changes the graded chemical pattern by diffusion and advection

— A theoretical model that incorporates:

(1) Production/degradation/diffusion and advection of the morphogen *C*,
(2) the growth rule,
predicts:

— The interdependency of growth homogeneity and gradient scaling depending on critical feedback strength $\beta_c = 2/(1+\varepsilon)$=1 if isotropic growth

— Growth arrest (bounded growth) depending on degradation rate *k* and growth feedback parameter β

For $\beta = \beta_c$, growth is homogeneous but unbounded (scale as $1/t$)
Away from critical point, growth is not perfectly uniform, but becomes bounded if *k* >0, depending on β

degradation
growth
diffusion
synthesis

$$(\partial_t + \mathbf{u}\cdot\nabla)\, C = \nabla\cdot(D\nabla C) - (k+g)C + s, \qquad (1)$$

$$g = \frac{1}{\beta}\frac{D_t C}{C}, \qquad (2)$$

$$0 = \nabla\cdot(D\nabla C) - [k + (1+\beta)g]C + s,$$

final size when growth arrests:

$$\ell^* = \ell(0)[1 + g_0(0)(1+\beta_c)/k_0]^{1/2}.$$

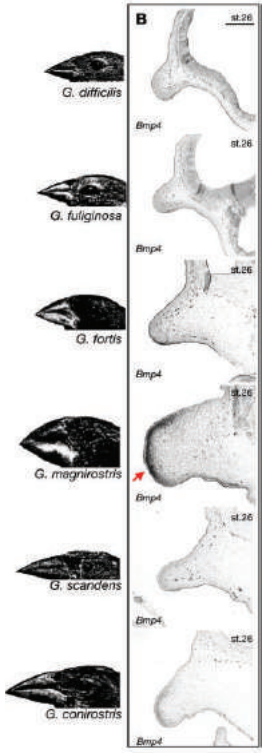
	$\beta < \beta_c$	$\beta > \beta_c$
$k = 0$	$g \sim t^{-1}$	$g \sim t^{-1}$
$k = k_0$	$g \sim e^{-t/\tau}$	$g \sim e^{-t/\tau}$
$k \sim \ell^{-2}$	$g \sim t^{-1}$	$g \sim e^{-t/\tau}$

Aguilar-Hidalgo D, et al and Jülicher F.
*Phys Rev Lett. (*2018); 120(19):198102. doi: 10.1103/PhysRevLett.120.198102.

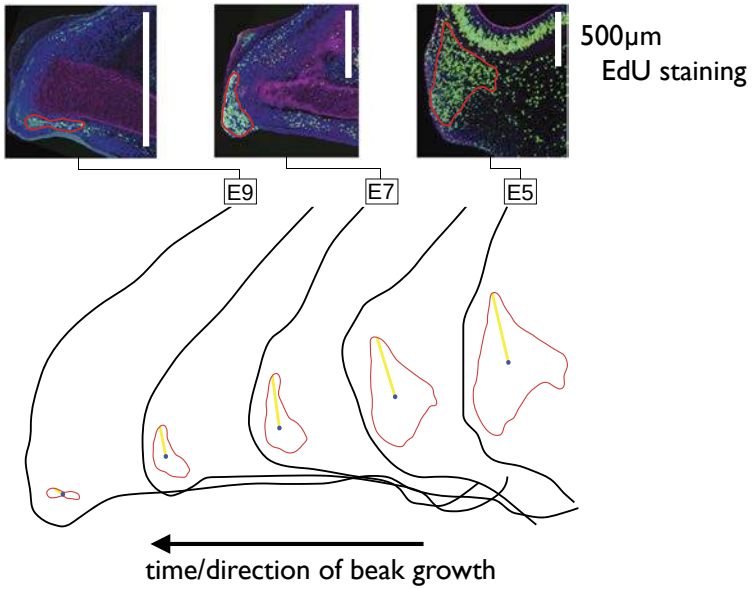
• Morphogen: Organ-size dependent growth inducer

— Spatial temporal regulation of a growth zone by morphogens and beak shape

- BMP4 expression in the mesenchyme induces its growth



The growth zone shrinks at a constant rate over time until it disappears



A. Abzhanov et al, C. Tabin, *Science* (2004) *Nature*. (2006)

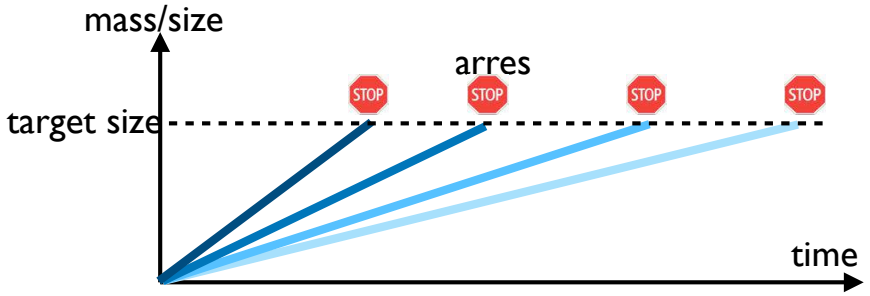
J. Fritz et al, A. Abzhanov & M. Brenner. Nature Communications | 5:3700 | DOI: 10.1038/ncomms4700

• Conclusions

— Cell growth is controlled intrinsically

— What are the mechanisms of organ specific tissue size sensing/measurement?

— What is measured? Intrinsic « ruler » of growth: « size-meter », scaling.



- Energy delivery/demand unbalance.
- Organ-size dependent negative feedback on cell growth/proliferation (chalone)
- « Size-meter »: scaling of chalone concentration and tissue size
  distance between activator and feedback inhibitor
  mechanical signal that scales with size
  patterning cue: morphogen gradient

- Organ-size dependent growth inducer: Morphogen and growth arrest:
  Spatial model: gradient scaling and gradient slope.
  Temporal model: gradient scaling and rate of signal increase

- Importance of considering robustness of mechanisms: e.g. integral feedback

Programmed vs Self-organised regulation of Growth

- hierarchical
- modular
- deterministic rules (ie. genetically encoded)

- no hierarchy
- **feedbacks**
- statistical rules