ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

D-BSSE
Department of Biosystems
Science and Engineering

From Networks to Function – Computational Models of Organogenesis

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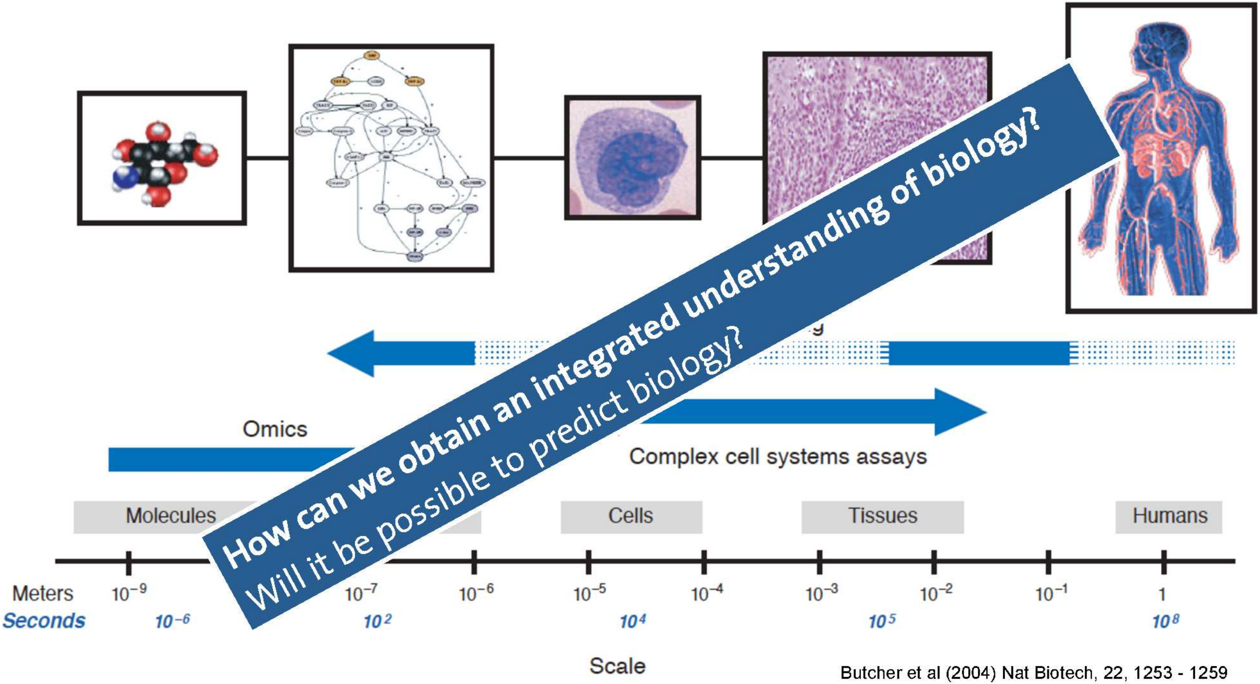
Computational Biology Group (CoBi)
ETH Zurich

Challenge: Integration across scales



How can we obtain an integrated understanding of biology?

Will it be possible to predict biology?

Butcher et al (2004) Nat Biotech, 22, 1253 - 1259

DEVELOPMENTAL SYSTEMS BIOLOGY

©1994
Brad Smith
Elwood Linney
Center for In Vivo Microscopy
Funding: National Center for Research Resources
North Carolina Biotechnology Center

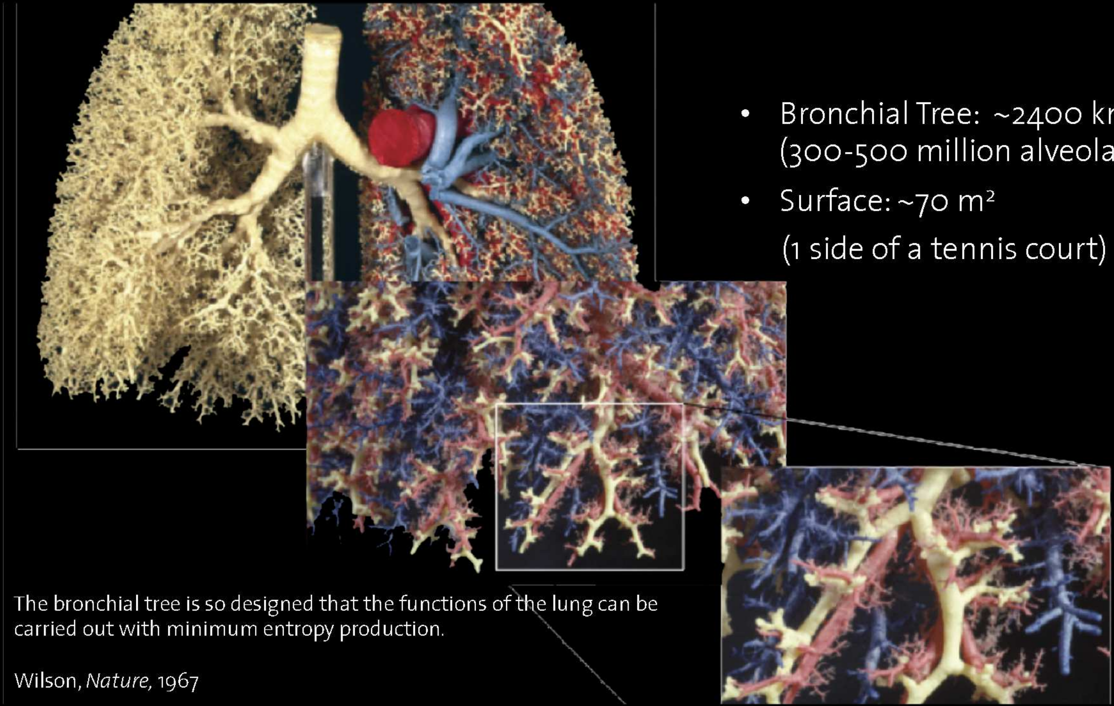
<u>TOPICS</u>

I. **How to break symmetry?**
   Building a lung and a kidney…

II. **How to pattern a growing embryo?**

III. **How to terminate growth?**

BRANCHING MORPHOGENESIS



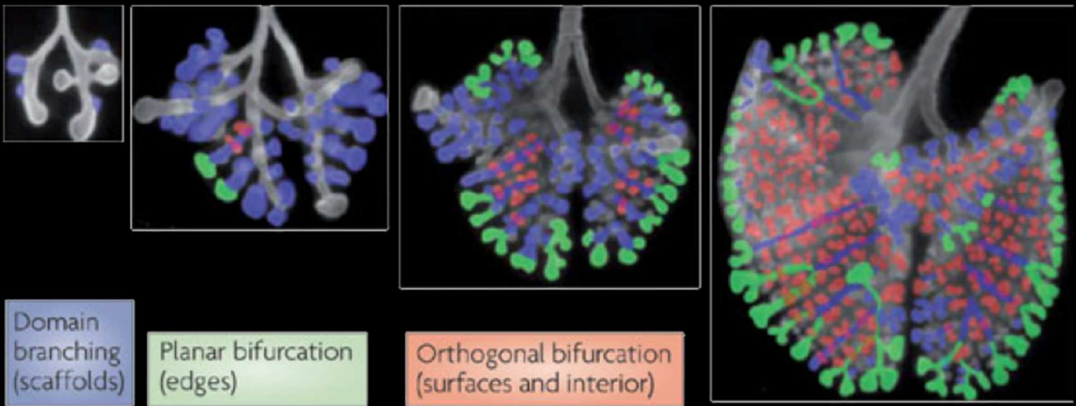
- Bronchial Tree: ~2400 km (300-500 million alveolae)
- Surface: ~70 m²
  (1 side of a tennis court)

The bronchial tree is so designed that the functions of the lung can be carried out with minimum entropy production.

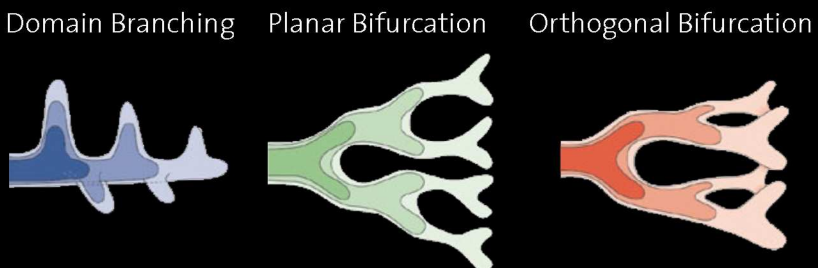
Wilson, *Nature,* 1967

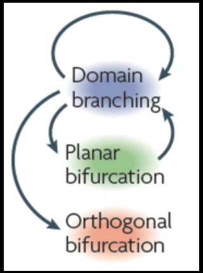
Glenny, *J Appl Physiol*, 2011

LUNG DEVELOPMENT



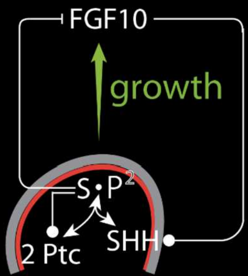
Metzger *et al. Nature*, 2008





Affolter *et al. Nature Reviews* Mol Cell Biology 2009

MODELLING OF THE REGULATORY NETWORK



Denis Menshykau

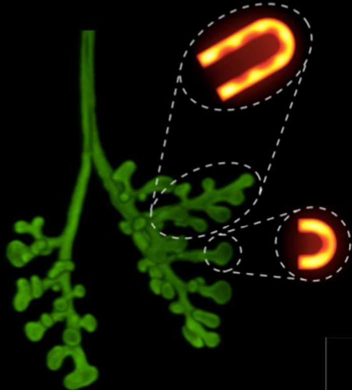
$$\dot{F} = \Delta F + \rho_{\mathrm{F}} \frac{1}{(P^2 S)^n + 1} - \delta_{\mathrm{F}} F$$

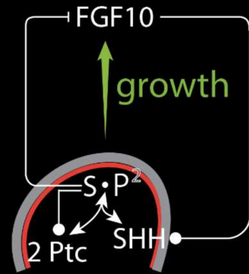
$$\dot{P} = D_{\mathrm{P}} \Delta P + \rho_{\mathrm{P}} - \delta_{\mathrm{P}} P + (\nu - 2\delta_{\mathrm{C}}) P^2 S$$

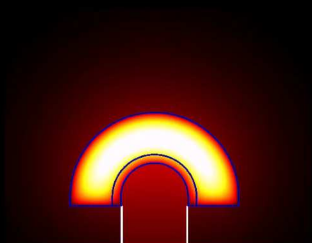
$$\dot{S} = D_{\mathrm{S}} \Delta S + \rho_{\mathrm{S}} \frac{F^n}{F^n + 1} - \delta_{\mathrm{S}} S - \delta_{\mathrm{C}} P^2 S$$

Menshykau et al, (2012) *PLoS Comp Biol*

DETERMINATION OF LUNG BRANCH POINTS

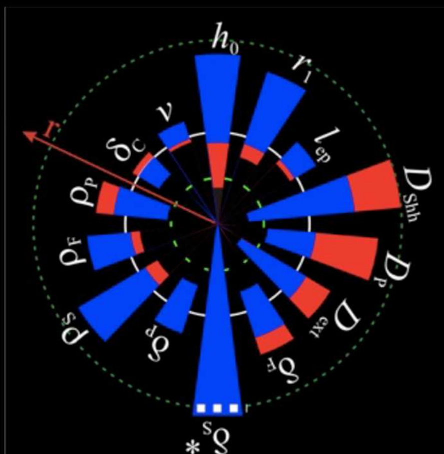


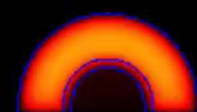




SLOW GROWTH

BIFURCATION





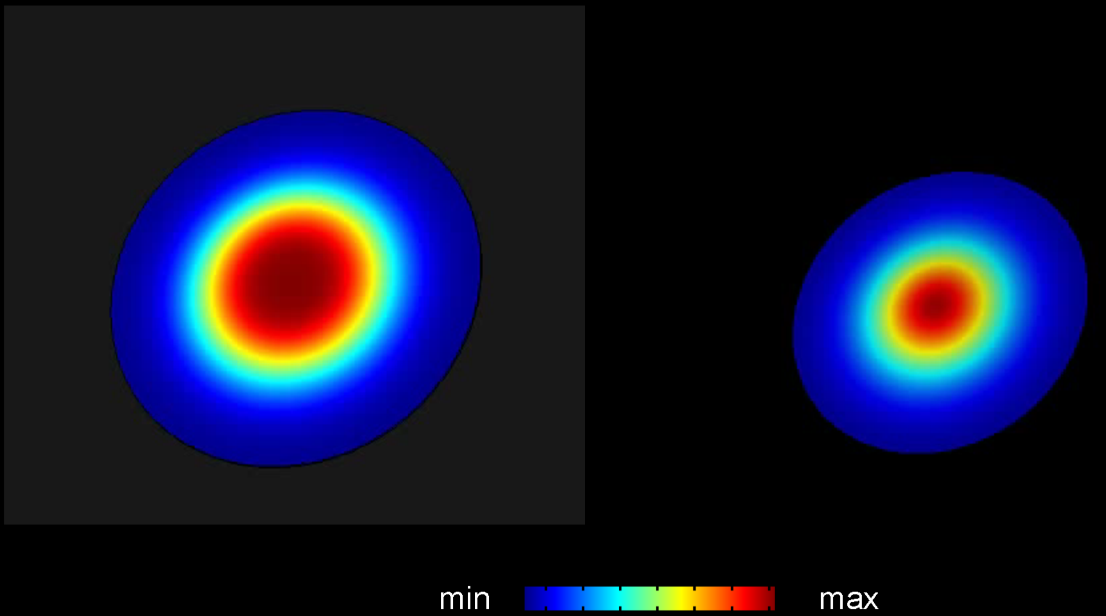
FAST GROWTH

LATERAL BRANCHING

Menshykau et al *PLoS Comp Biol* 2012

PATTERNING & BRANCHING ON A GROWING DOMAIN

Turing mechanism based on a receptor-ligand Interaction permits branching.

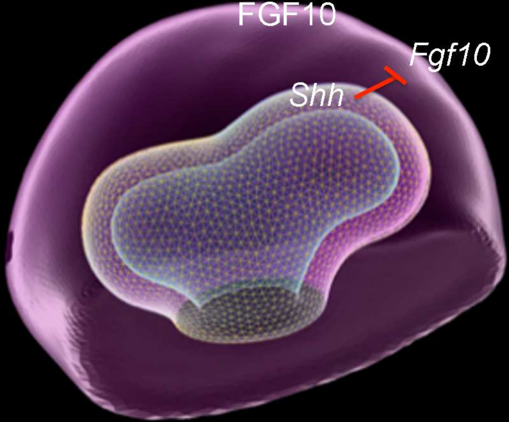


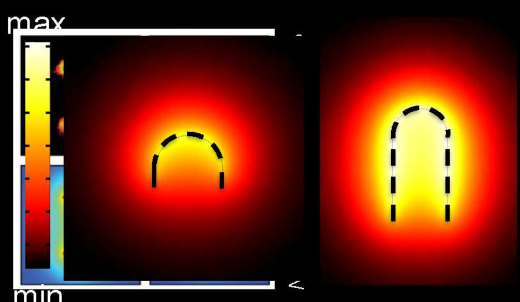
Menshykau et al. (2014) Development

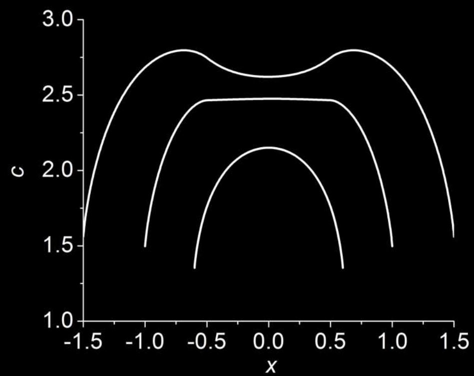
Alternative Mechanisms

1. EPITHELIUM – MESENCHYME DISTANCE-DEPENDENT REGULATION
   i. SHH -| Fgf10 results in bifurcating profile at the tips
   ii. Steeper Gradients at shorter distance

2. GEOMETRY-INDUCED GRADIENTS



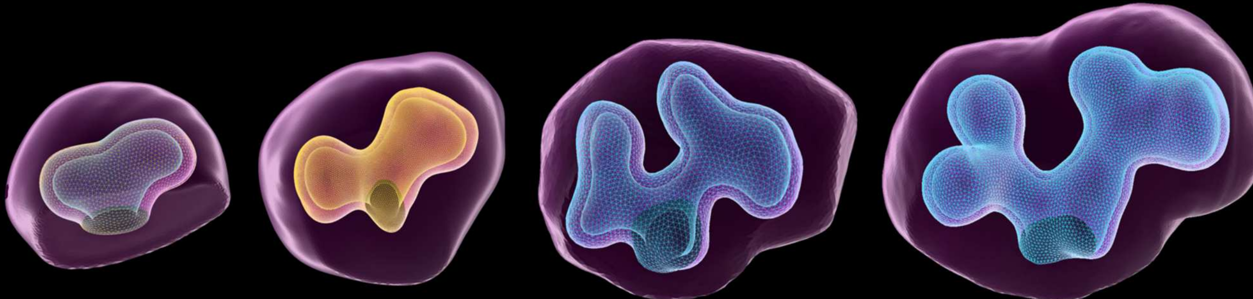




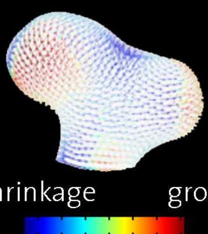
MODEL TESTING with EMBRYONIC DATASETS

A sequence of segmented lung bud geometries (from Blanc *et al* PLoS One 2012)

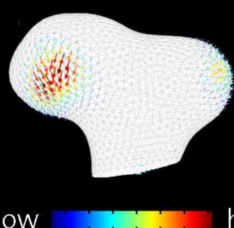
Stage 1 Stage 2 Stage 3 Stage 4



Displacement field

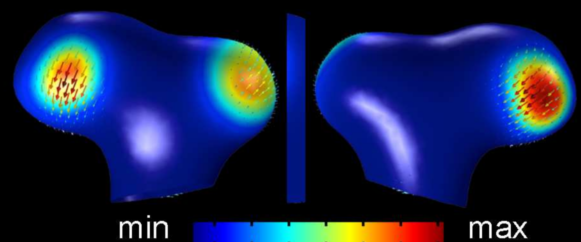


Growth field

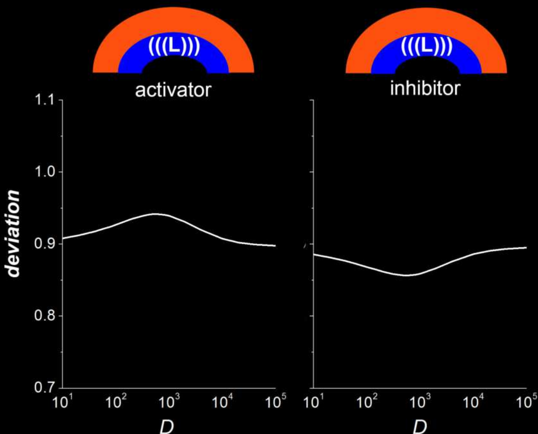


Computed Signalling field

Signalling (solid) & Growth (arrows) fields

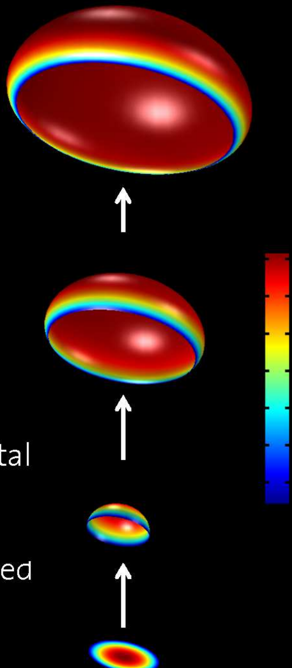


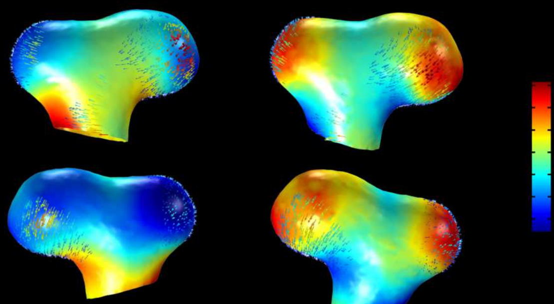
Geometry-based Mechanisms – Stage 1->2



Deviation between computed and measured growth fields:

$$\Delta = \sqrt{\sum_{\mathrm{EM}} (|\overline{v}_{\mathrm{n}}| - S$$





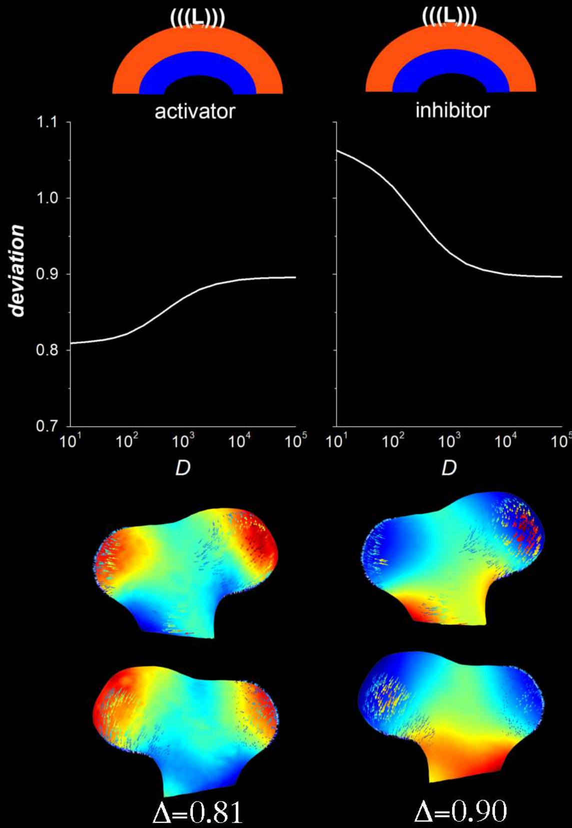
Δ=0.90 Δ=0.86

arrows – experimental growth field

solid color – computed growth field

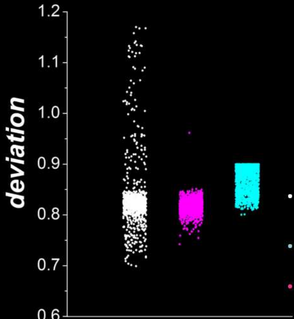
Menshykau et al, Development, 2014

Distance & Gradient Based Mechanisms – Stage 1->2



TURING-BASED MECHANISMS

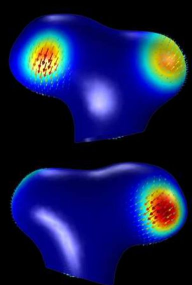
FGF10



· Turing space

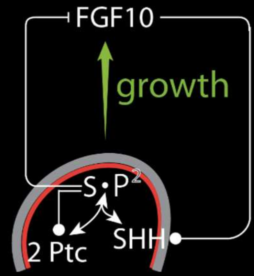
· out of Turing space

· unclassified



Δ=0.62

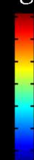
SHH



Deviation between computed and measured growth fields:

$$\Delta = \sqrt{\sum_{\mathrm{EM}} (|\overline{v}_{\mathrm{n}}| - S_{\mathrm{n}})^2}$$

high

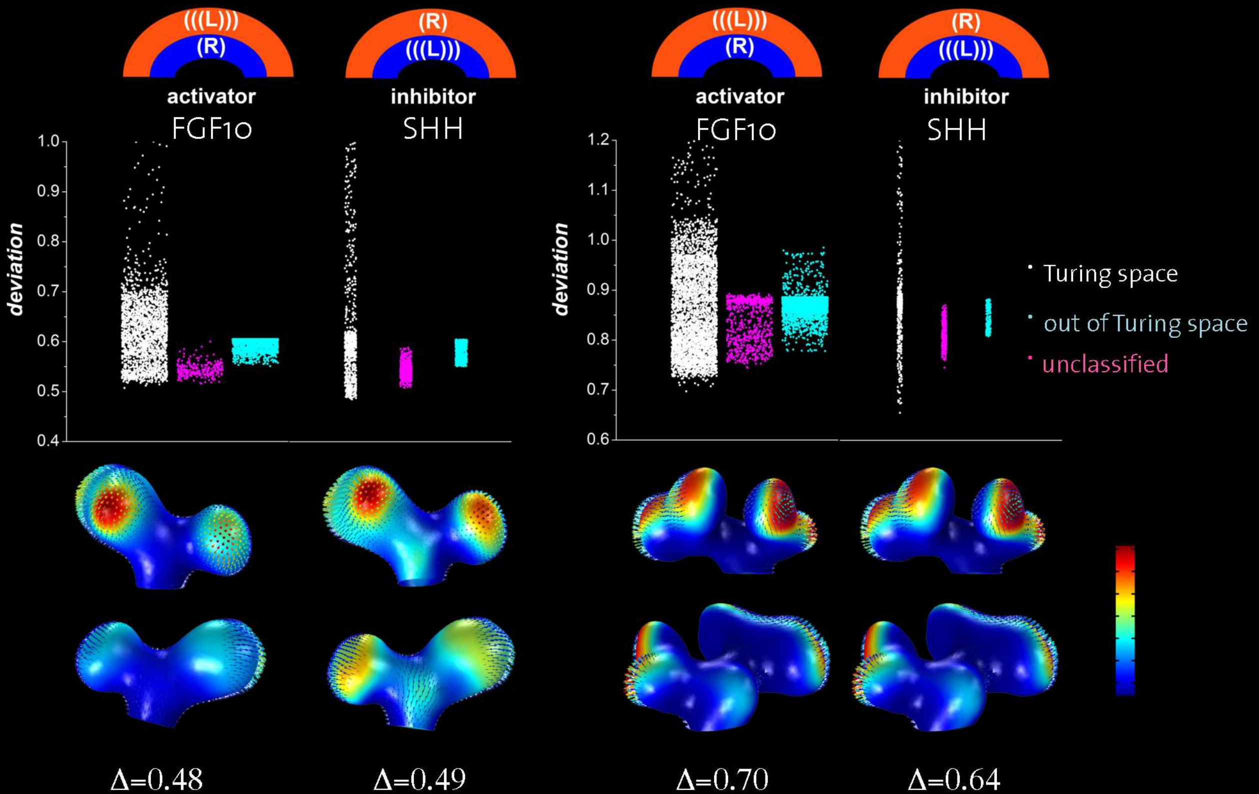


low

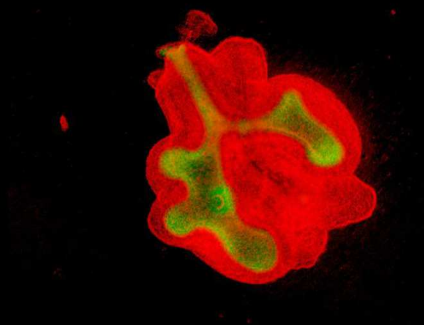
growth field:
arrows – experimental
solid color –computed

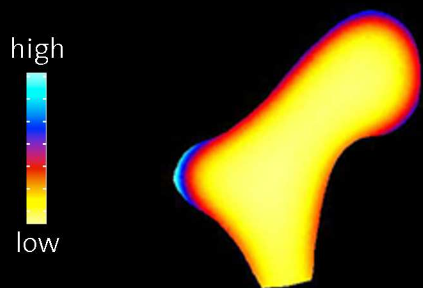
Menshykau et al. (2014) Development

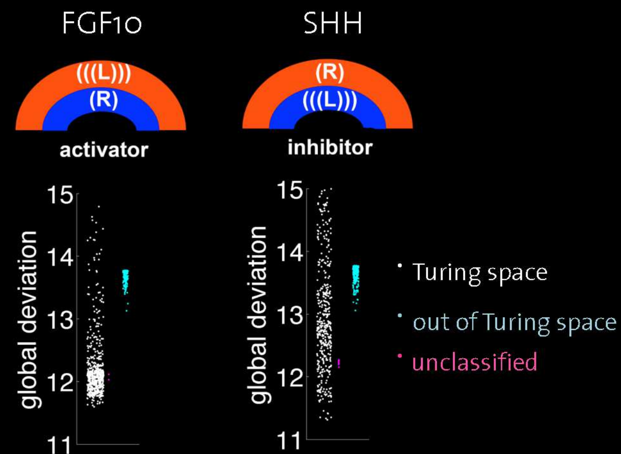
TURING-BASED MECHANISMS– LATER STAGES



DYNAMIC DATASET SUPPORTS TURING MECHANISM

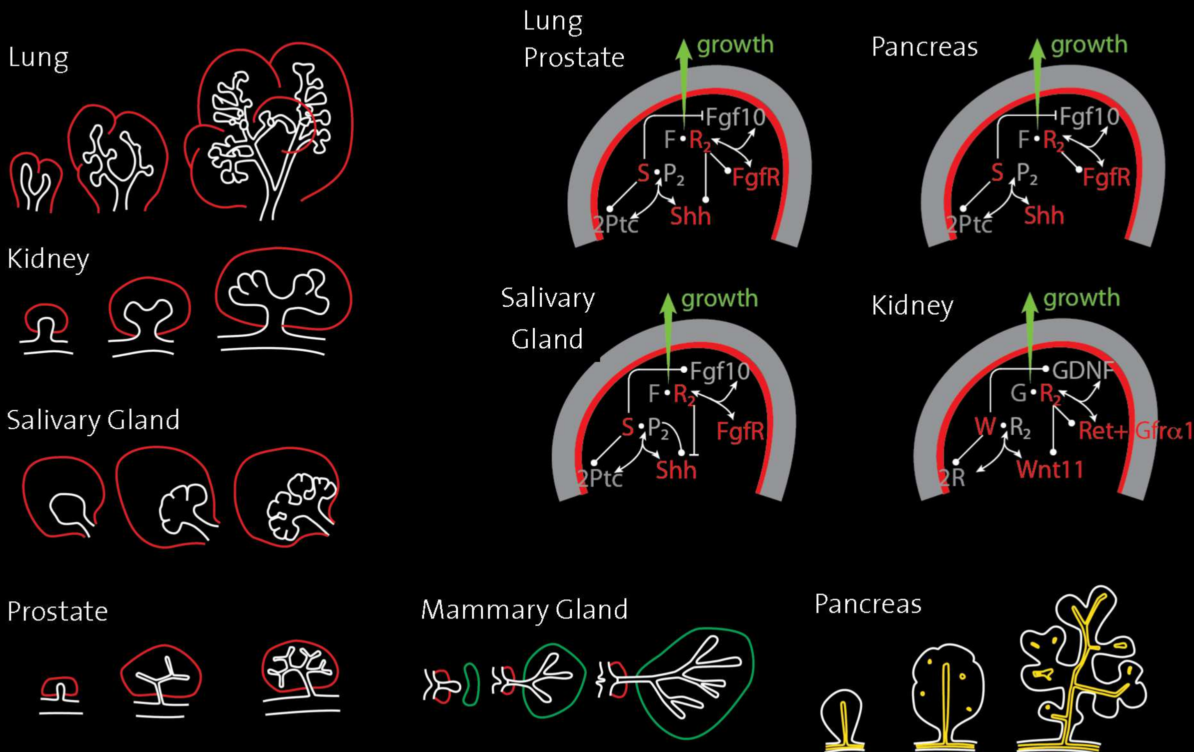






Menshykau et al. (2014) Development

BRANCHING IN DIFFERENT ORGANS



Iber & Menshykau, Open Biology (Royal Soc), 2013

CONCLUSION on BRANCHING MORPHOGENESIS

- PERIODIC PATTERNING: Ligand-receptor based Turing models (but none of the other proposed models) predict growth fields during branching morphogenesis
- DELAYED BRANCHING: The mesenchyme appears to locally delay the branching program
- SPACE-FILLING TREE: The Turing Mechanism recognizes open mesenchymal space
- GENERALITY: The Mechanism applies to Lung & Kidney Branching Morphogenesis despite different molecular networks

400 μm

TURING PATTERNS

Turing Pattern
Alan Turing 1952

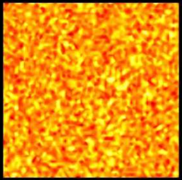
OPEN ISSUES

① What are the molecular Turing Components?

② Size of Turing Space

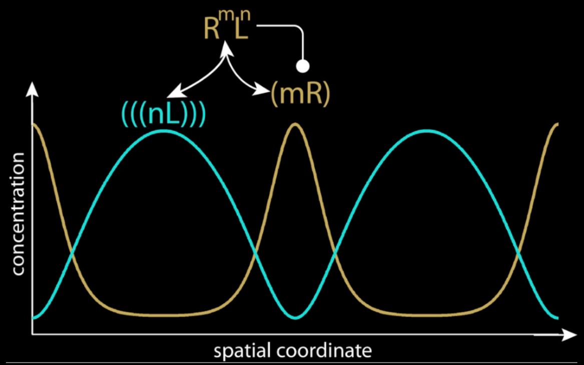
③ Pattern Robustness to Noisy Initial Conditions

Stable System - without Diffusion



Instable System - with Diffusion

1. LIGAND-RECEPTOR BASED TURING PATTERNS



$$\dot{R} = \Delta R + \gamma(a - R + R^2 L)$$

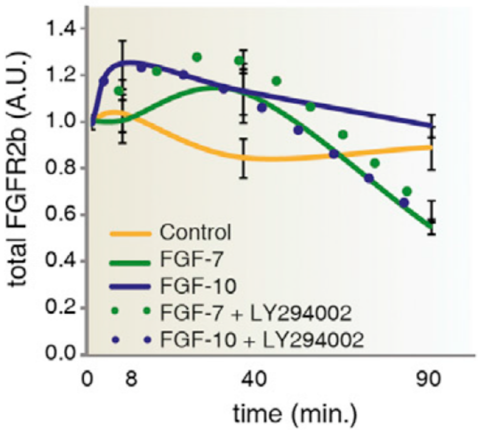
$$\dot{L} = D\Delta L + \gamma(b - R^2 L)$$

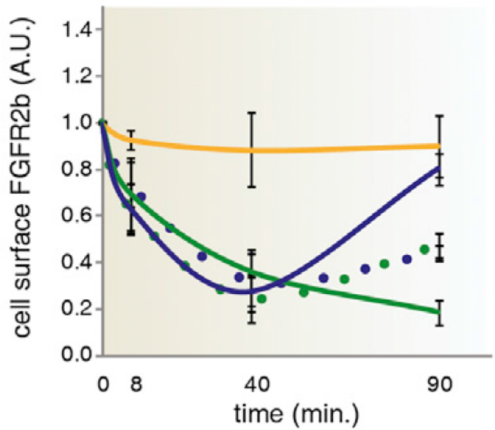
REQUIREMENTS:

1. Faster diffusion of ligands relative to receptors
2. Cooperative Binding
3. Positive Feedback of ligand-receptor signaling on receptor abundance

These conditions are met by many ligand-receptor systems.

FGF10, but not FGF7 meets the Turing conditions



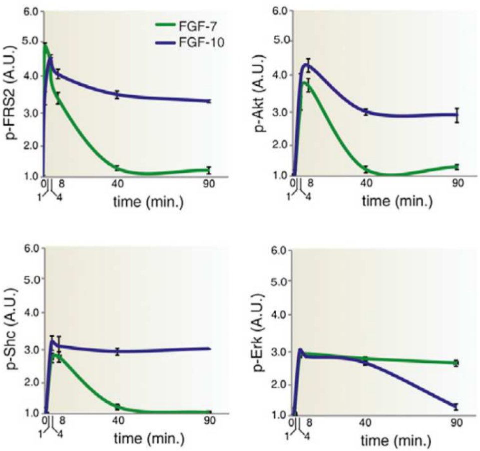


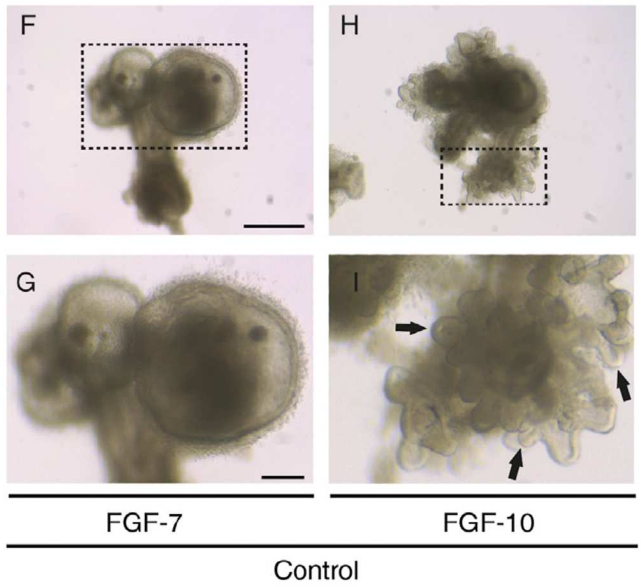
Recycling of FGFR2b to the membrane in response to FGF10, but not FGF7 in FGFR2b-transfected HeLa cells.

Francavilla, C., et al. (2013). Mol Cell **51**(6): 707-722.

Bellusci lab

FGF10, but not FGF7 enhances budding





Francavilla, C., et al. (2013). Mol Cell **51**(6): 707-722.

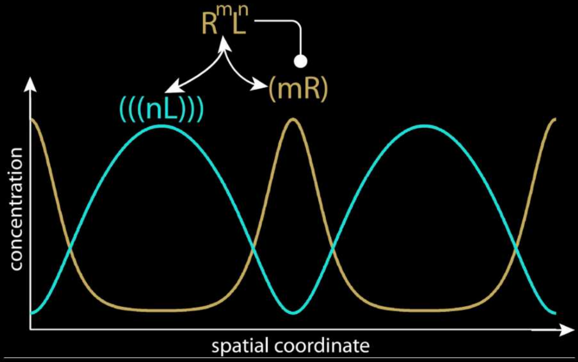
Bellusci lab

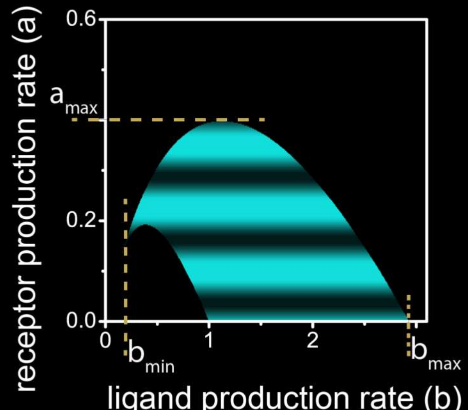
2. MECHANISMS TO ENLARGE TURING SPACE

$$\dot{R} = \Delta R + \gamma(a - R + R^2 L)$$
$$\dot{L} = D\Delta L + \gamma(b - R^2 L)$$


Tamas Kurics

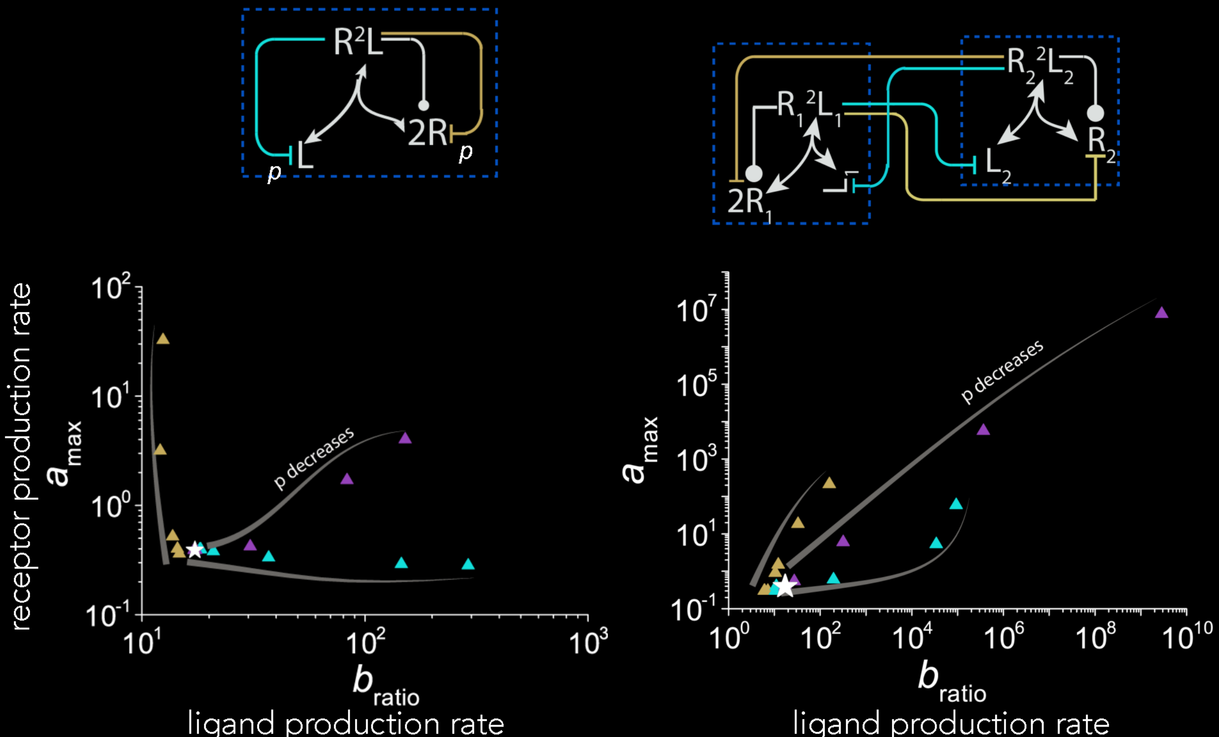

Denis Menshykau



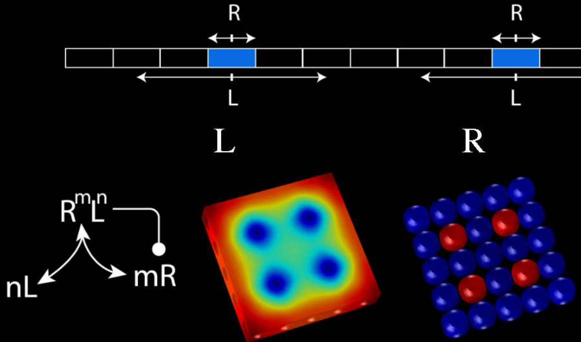


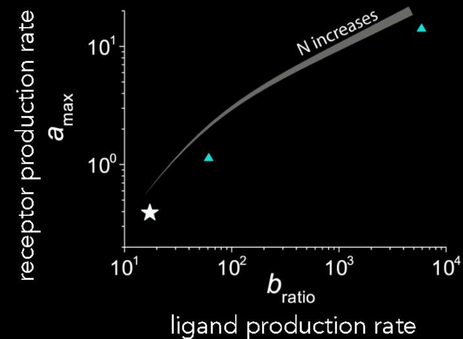
Kurics, Menshykau, Iber, Phys Rev E, 2014

NEGATIVE FEEDBACKS & COUPLING OF TURING MODULES ENLARGE TURING SPACE

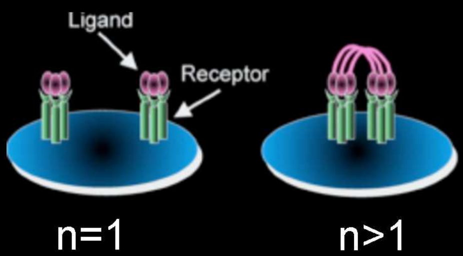


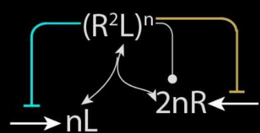
TURING PATTERNS EXIST ON CELLULAR DOMAINS & LARGER TURING SPACE AT HIGHER CELL DENSITY

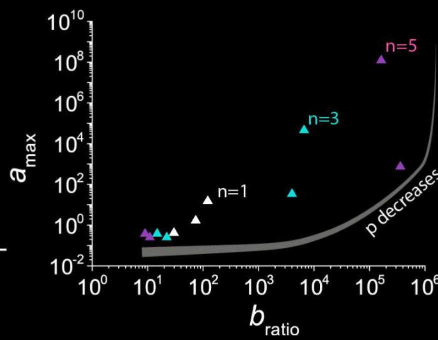




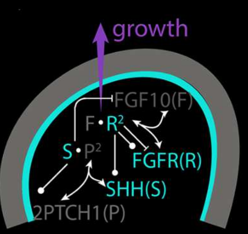
RECEPTOR CLUSTERING ENLARGES TURING SPACE

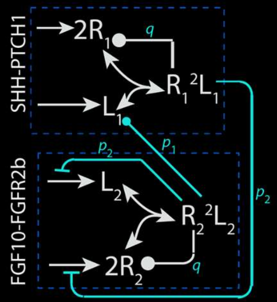


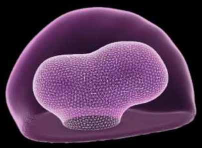


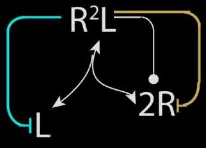


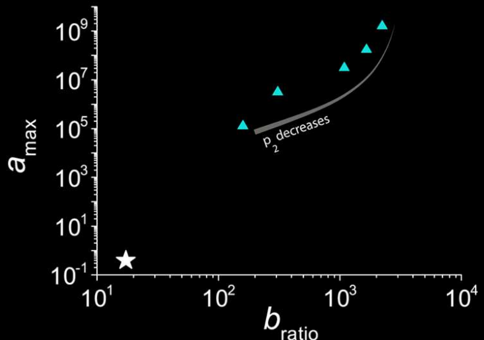
2. TURING SPACE FOR MODELS OF BRANCHING MORPHOGENESIS







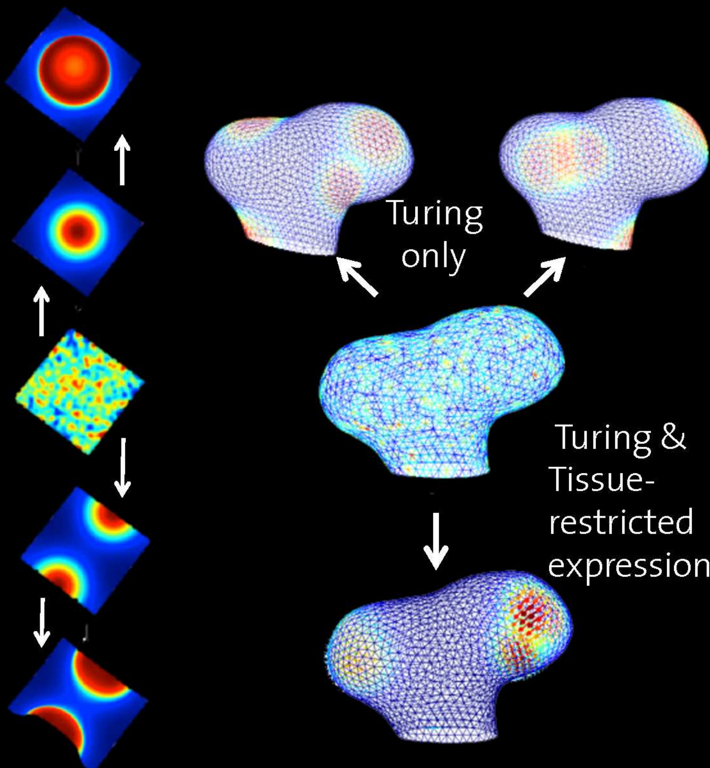




Physiological Models of Branching Morphogenesis have huge Turing Space.

Menshykau et al. (2014) Development

3. ROBUST BRANCHING MORPHOGENESIS

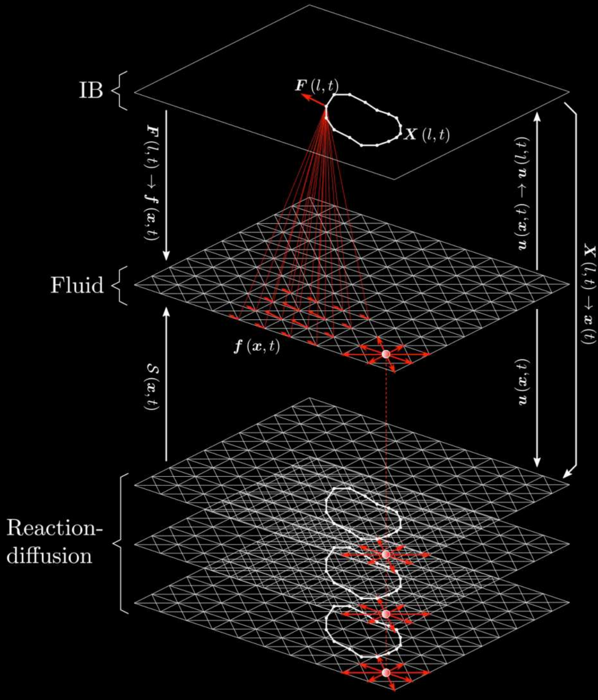


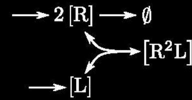
Separation of ligands and receptors in different tissues ensures that the same pattern emerges, in spite of noisy initial conditions.

Menshykau et al. (2014) Development

LBIBCell: A SIMULATION FRAMEWORK FOR TISSUE MORPHOGENESIS

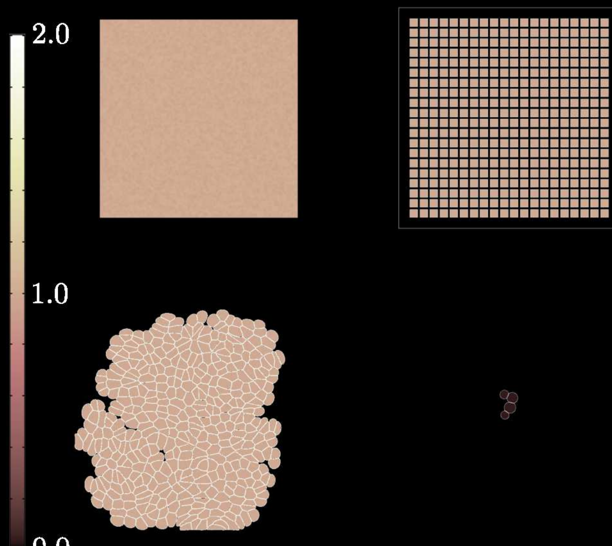
Simon Tanaka





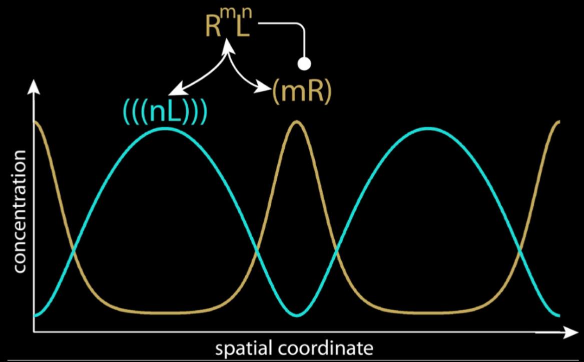
$$\partial_t\,[\mathrm{R}] = \Delta\,[\mathrm{R}] + \gamma\left(a - [\mathrm{R}] + [\mathrm{R}]^2\,[\mathrm{L}]\right)$$

$$\partial_t\,[\mathrm{L}] = d\Delta\,[\mathrm{L}] + \gamma\left(b + [\mathrm{R}]^2\,[\mathrm{L}]\right)$$



Tanaka et al, Bioinformatics, 2015

CONCLUSION

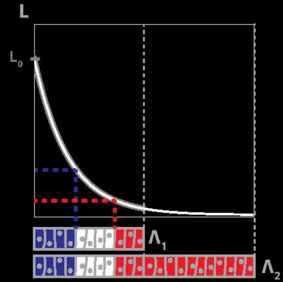


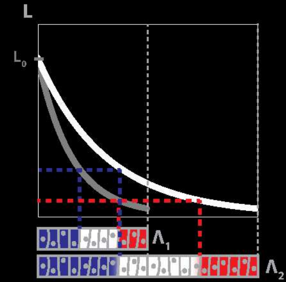
- PARAMETER SPACE: Negative Feedbacks, Coupling, and Receptor clustering and restriction to single cells increases size of feasible parameter space
- ROBUSTNESS: Separation of ligand and receptors in different tissue layers makes Turing mechanisms robust to noisy initial conditions
- TISSUE GROWTH: Cell-based simulations of growing tissue shows that different patterns can emerge than on continuous static domains

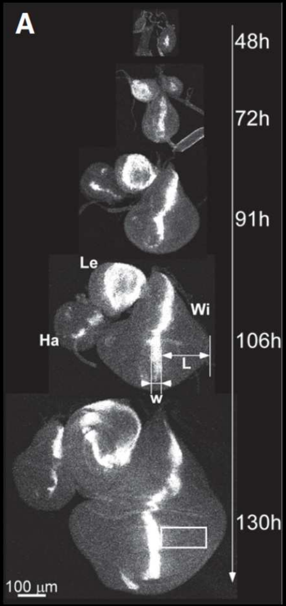
PART II: Scaled Morphogen Read-Out on Growing Domains

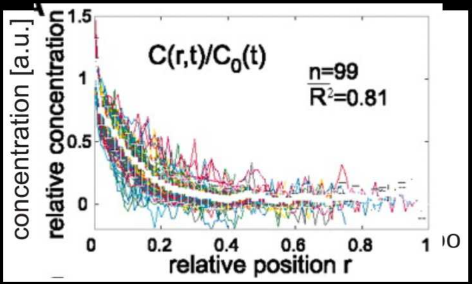
HOW DO PATTERNS SCALE WITH DOMAIN SIZE?

$$\mathbf{c(x)} = \mathbf{c(0)}\exp\left(\frac{-\mathbf{x}}{\lambda}\right)$$

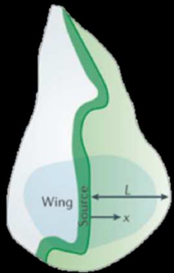




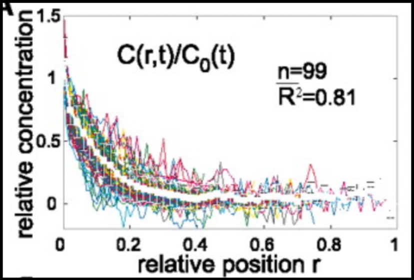




Data from Wartlick et al., *Science*, 2011



HOW DO PATTERNS SCALE WITH DOMAIN SIZE?



The length of the Dpp gradient scales with the length of the growing domain:

How does the Dpp gradient “know” the size of the domain?

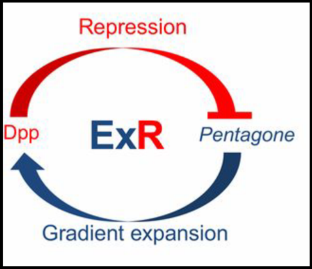
Steady-state model:

$$\mathbf{c}(\mathbf{x}) = \mathbf{c}(\mathbf{0}) \exp\left(\frac{-\mathbf{x}}{\lambda}\right)$$

$$\lambda = \sqrt{\frac{D}{k_{\deg}}}$$

Wartlick et al., *Science*, 2011.

Expansion-repression model:



Averbukh et al., *Development*, 2014.

Advection-Diffusion system scales almost perfectly

Diffusion equation on a 1D growing domain:

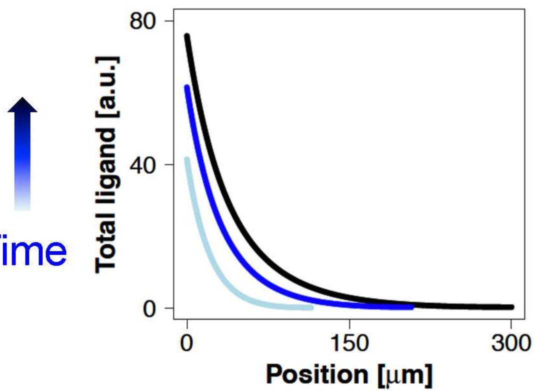
$$\frac{\partial c}{\partial t} = \underbrace{D\frac{\partial^2 c}{\partial x^2}}_{\text{diffusion}} \underbrace{-u\frac{\partial c}{\partial x}}_{\text{advection}} \underbrace{-c\frac{\partial u}{\partial x}}_{\text{dilution}}$$

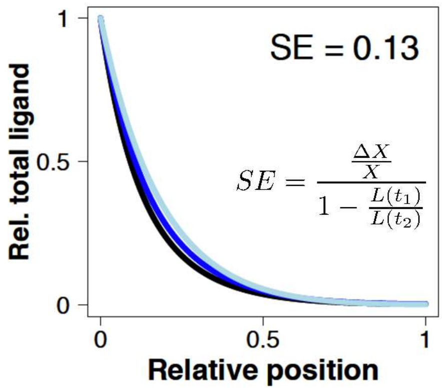
- Transport by Diffusion & Advection
- Dilution of Dpp because of growth
- Constant influx of Dpp from LHS

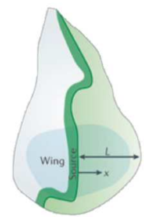
Patrick Fried

$$L(t) = L(0) + v \cdot t$$

Linear uniform growth (speed *v* determined by Wartlick et. al.)

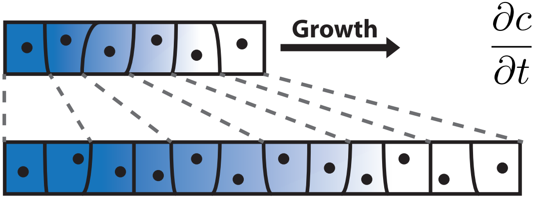






Fried and Iber, *Nat. Commun.*, 2014.

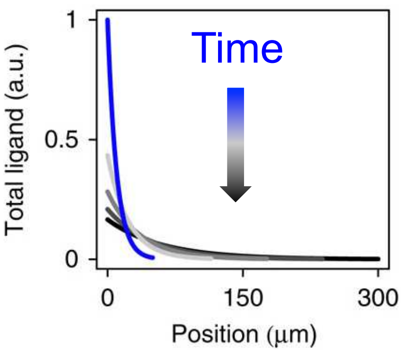
Pefect Scaling by Advection & Dilution

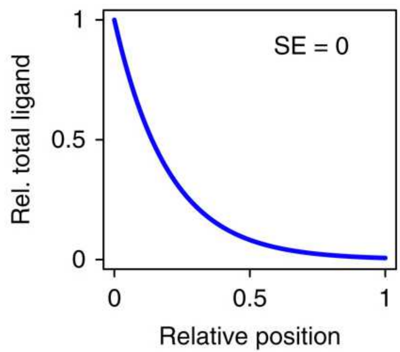


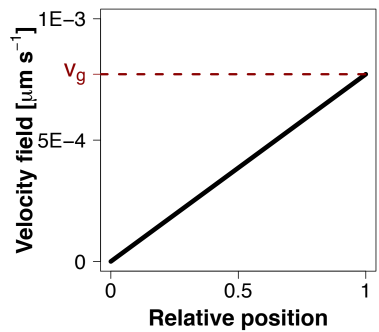
$$\frac{\partial c}{\partial t} = \underbrace{-u\frac{\partial c}{\partial x}}_{\text{advection}} \underbrace{-c\frac{\partial u}{\partial x}}_{\text{dilution}}$$

VELOCITY FIELD for linear uniform growth
(speed *v* determined by Wartlick et. al.)

$$L(t) = L(0) + v \cdot t$$



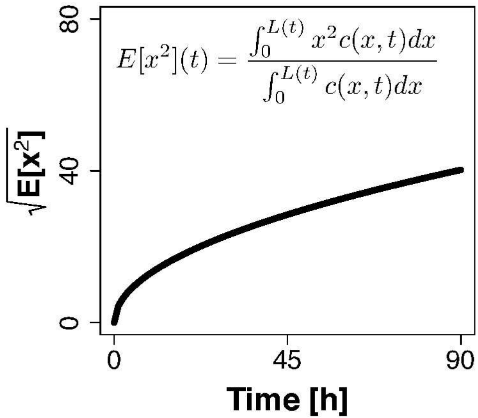




Fried and Iber, *Nat. Commun.*, 2014.

Diffusion expands gradient with square-root of time

$$\frac{\partial c}{\partial t} = D\frac{\partial^2}{\partial x^2}$$



Dirac Delta function as IC:

$$\mathbf{c}(\mathbf{x},\mathbf{t}) = \frac{\mathbf{1}}{\sqrt{\mathbf{4}\pi\mathbf{Dt}}}\exp\left(-\frac{\mathbf{x^2}}{\mathbf{4Dt}}\right)$$

Flux Boundary condition:

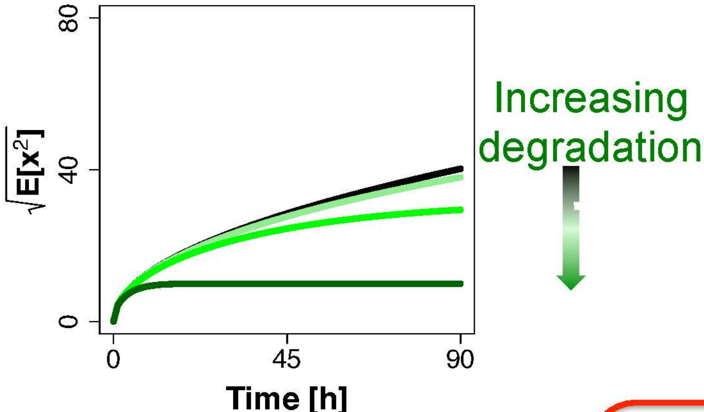
$$\sigma^2 = Dt$$

Diffusion: $\lambda \sim \sqrt{t} = \sqrt{\frac{L(t)}{v} - \frac{L_0}{v}}$

Perfect scaling: $\lambda \sim L(t)$

Fried and Iber, *Nat. Commun.*, 2014.

Degradation limits diffusion-driven gradient expansion



Pre-steady state effect

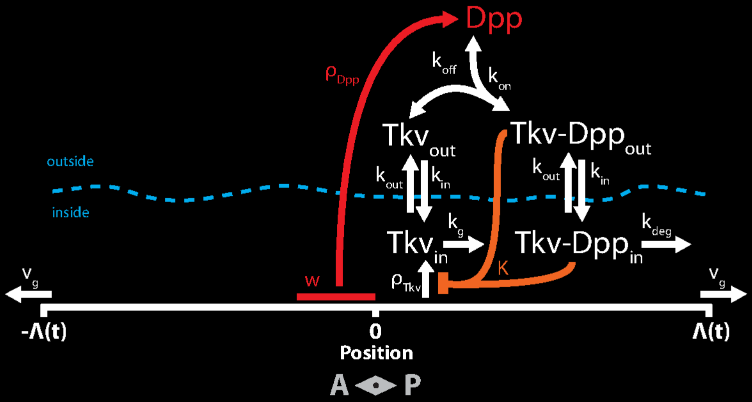
Degradation limits diffusion-driven gradient expansion

Diffusion: $\lambda \sim \sqrt{t} = \sqrt{\frac{L(t)}{v} - \frac{L_0}{v}}$

Perfect scaling: $\lambda \sim L(t)$

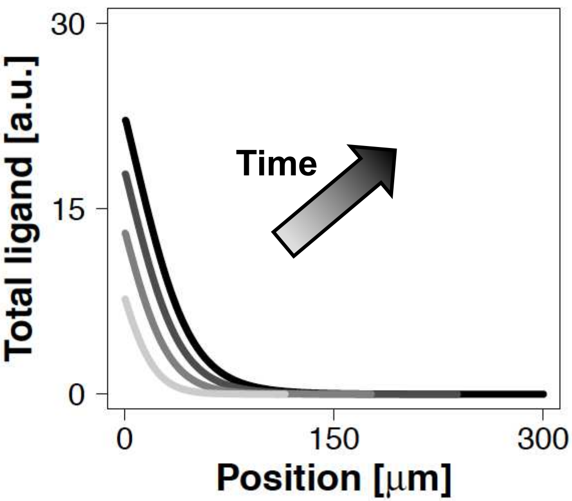
Fried and Iber, *Nat. Commun.*, 2014.

THE DPP SIGNALLING MODEL



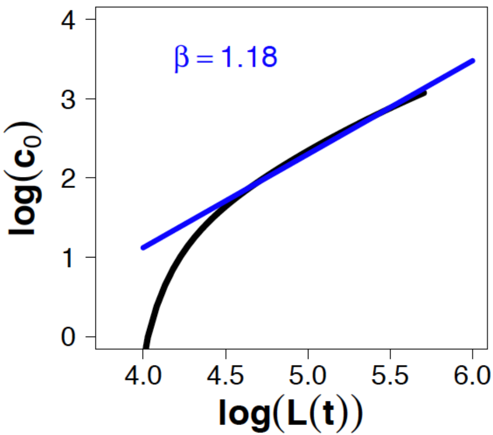
Fried and Iber, Nat Commun (2014)

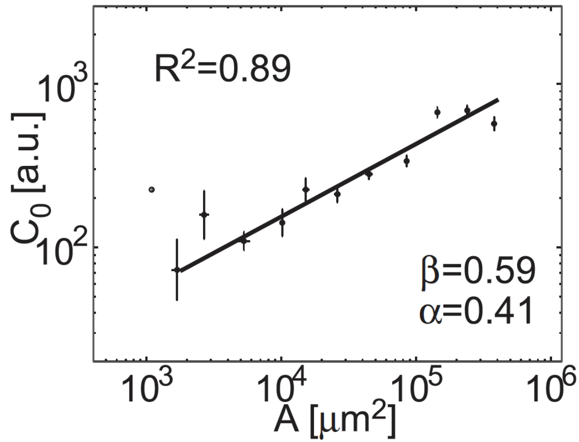
Model shows temporal increase of concentration



Fried and Iber, *Nat. Commun.*, 2014.

Power-law relation fits temporal Dpp increase



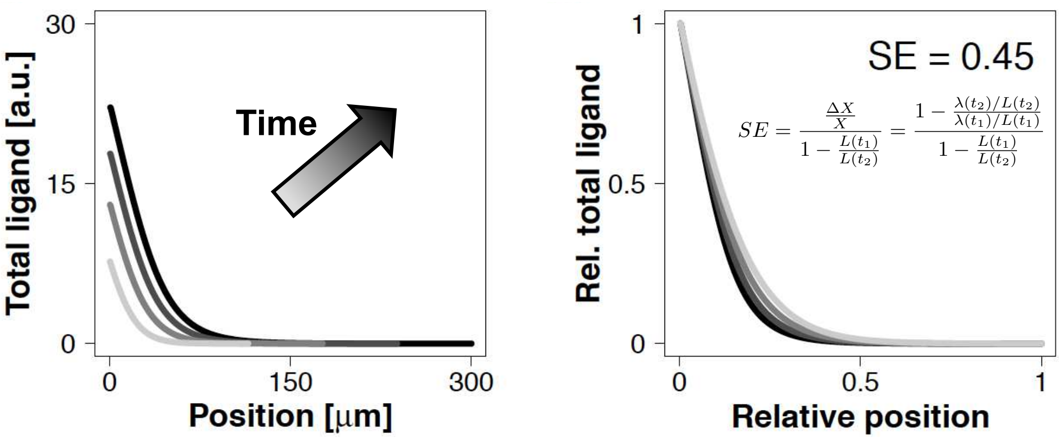


Power-law exponents are the same if A ~ L^2

Fried and Iber, *Nat. Commun.*, 2014.

Wartlick et al., *Science*, 2011.

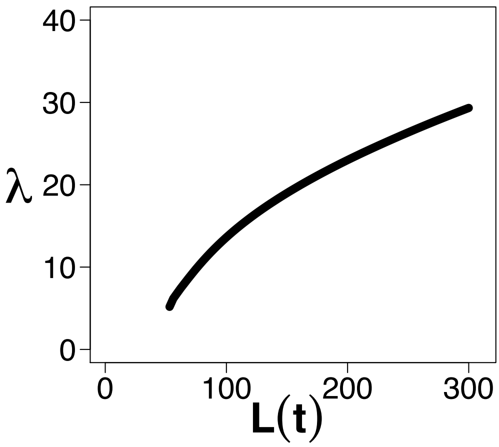
Imperfect scaling is observed in the wing disc model



The gradients scale to some extent but clearly not perfectly.

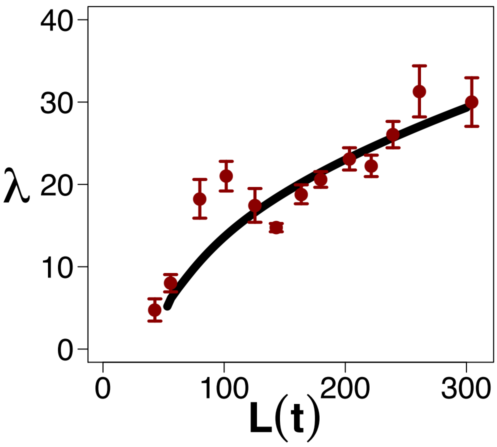
Fried and Iber, *Nat. Commun.*, 2014.

Imperfect scaling explains gradient expansion data

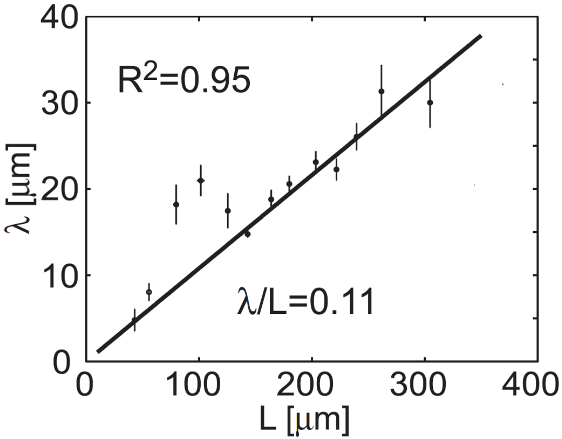


Fried and Iber, *Nat. Commun.*, 2014.

Imperfect scaling explains gradient expansion data

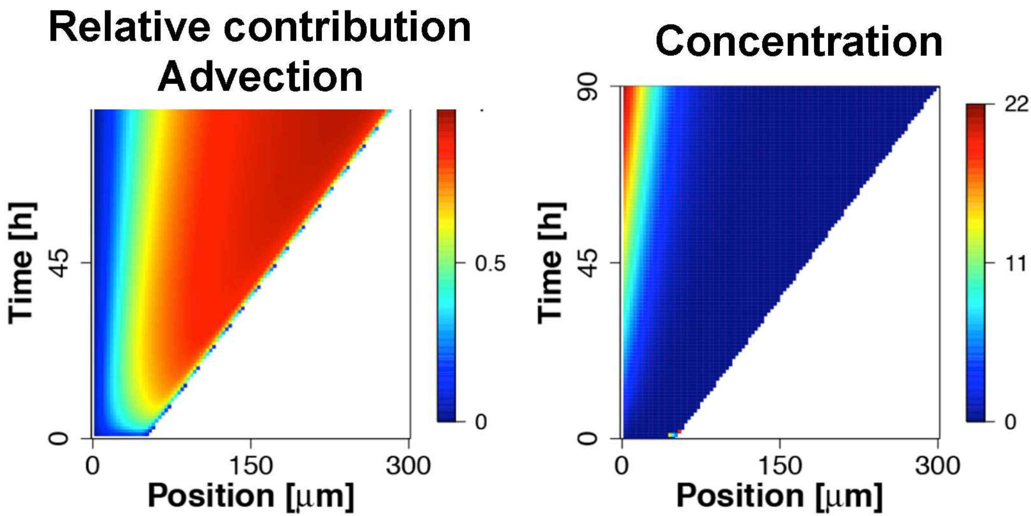


Fried and Iber, *Nat. Commun.*, 2014.



Wartlick et al., *Science*, 2011.

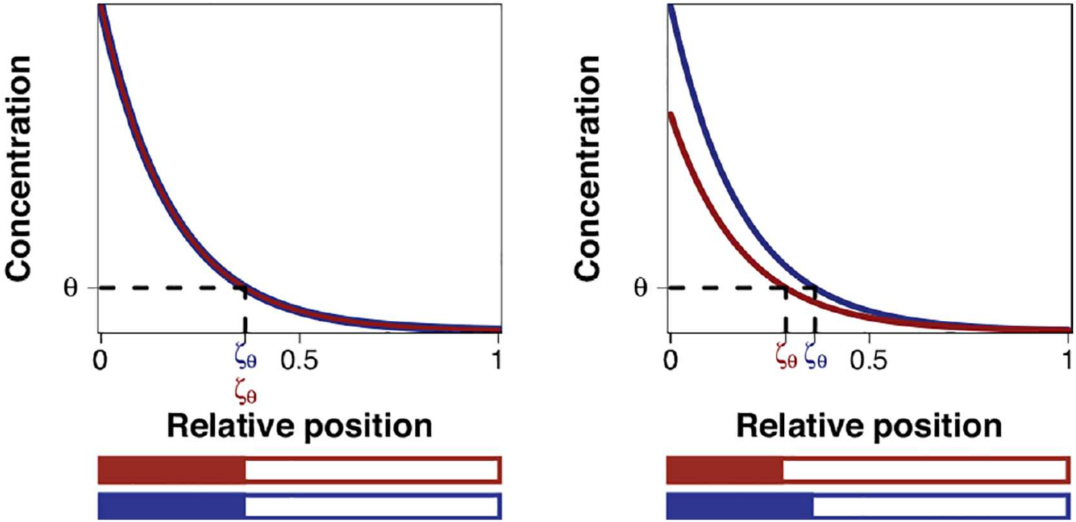
Imperfect scaling is dominated by diffusion



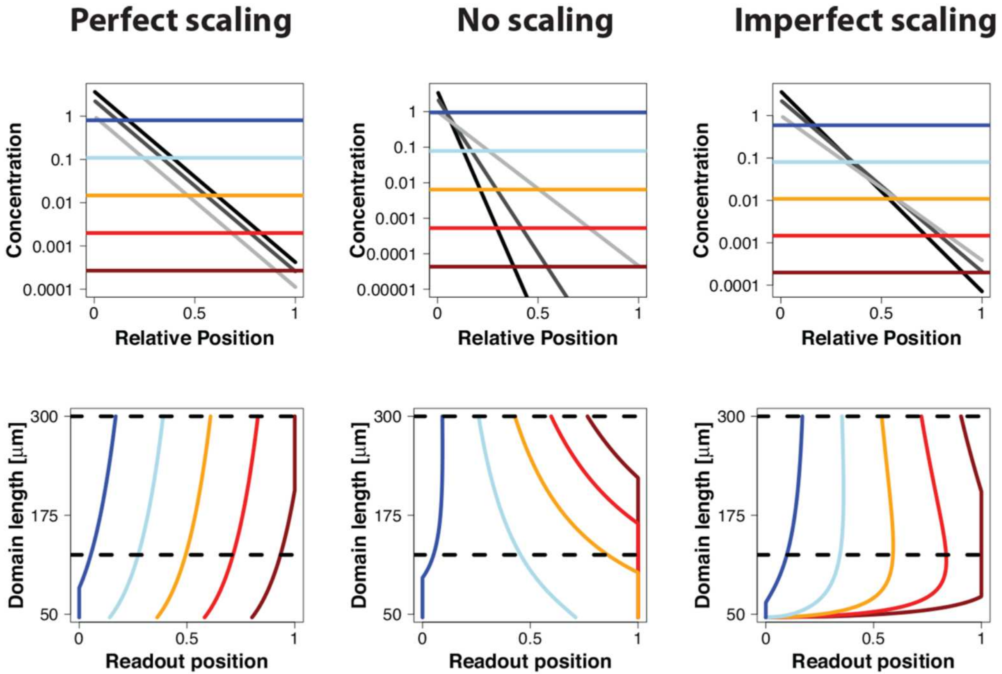
- Diffusion dominates in the front of the tissue
- Advection dominates at the end of the tissue

Fried and Iber, *Nat. Commun.*, 2014.

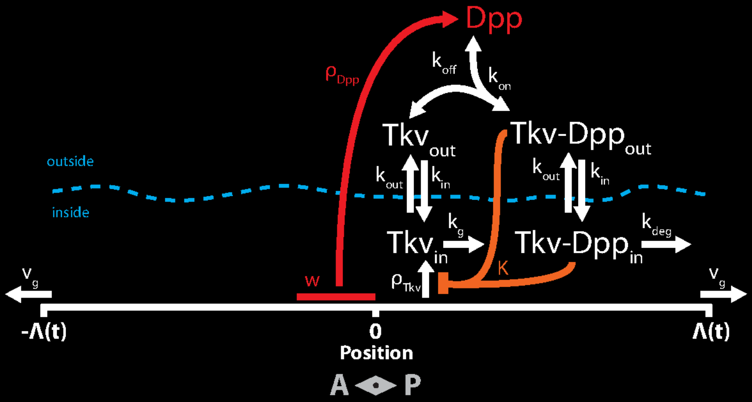
How to read out an increasing, scaled gradient?



Threshold-based Read-Out

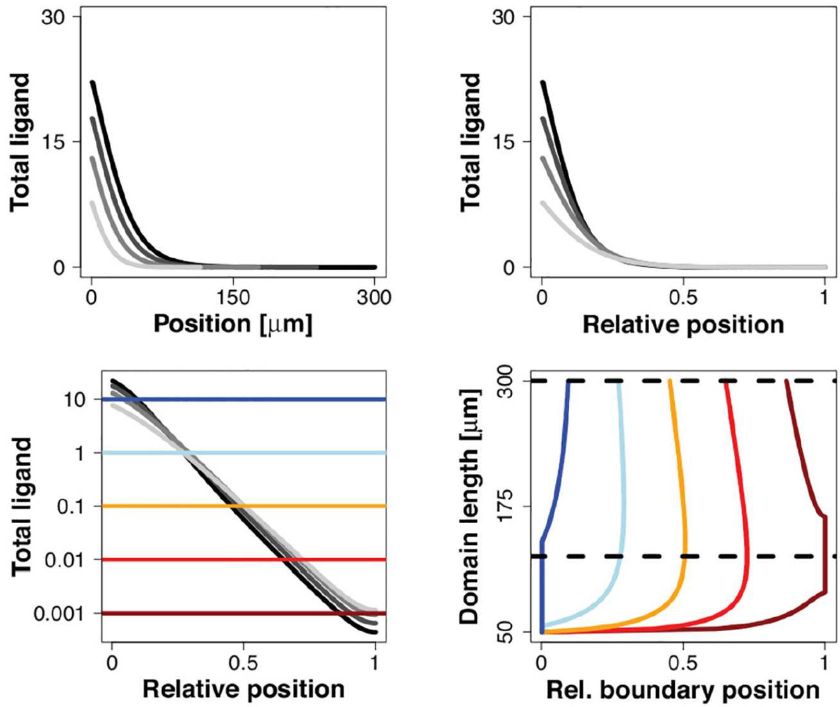


THE DPP SIGNALLING MODEL



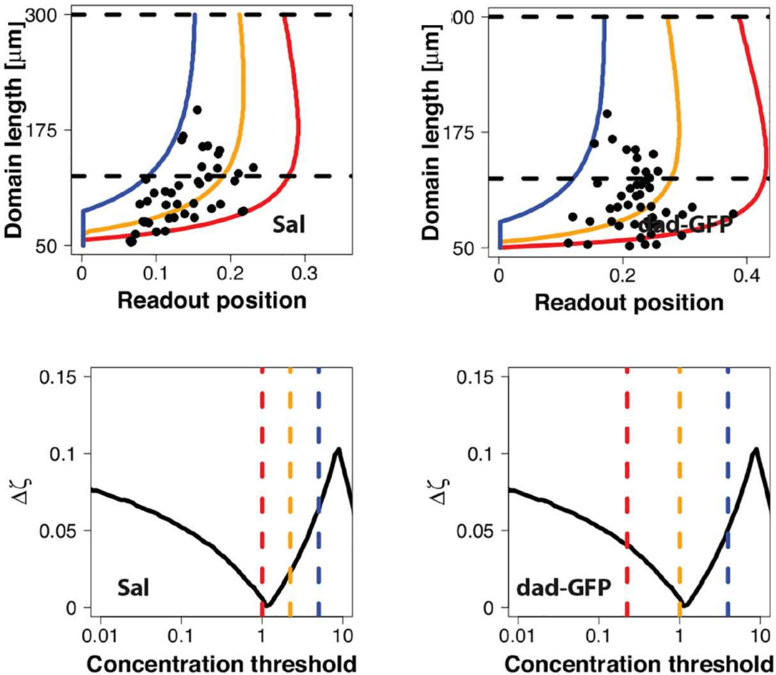
Fried and Iber, Nat Commun (2014)

Threshold-based Read-Out of Dpp

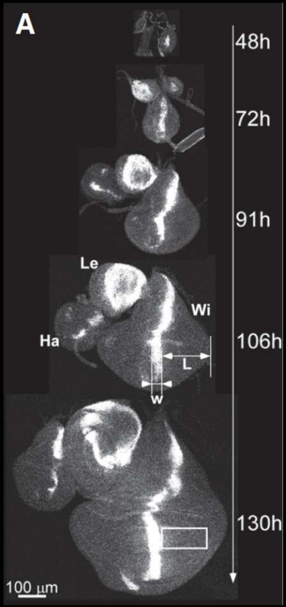


Dpp Gradient Read-Out

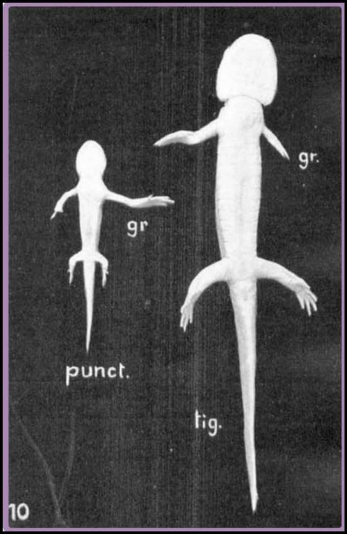
Data points from
Hamaratoglu et al.
PLoS Biol. 2011



Conclusion on Scaling Mechanism



- A general mechanism for **imperfect scaling**
- This mechanism requires pre-steady state kinetics
  -> **low degradation rate of internalized Dpp**
- ***Drosophila* wing disc**:
  - Imperfect scaling is consistent with biological data
  - Threshold-based read-out of Dpp enables precise definition of gene expression domains despite continuous increase of Dpp concentration
- In the wing disc transport is dominated by diffusion. In **other tissues** it could be dominated by **advection**.



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THE JOURNAL OF EXPERIMENTAL ZOOLOGY
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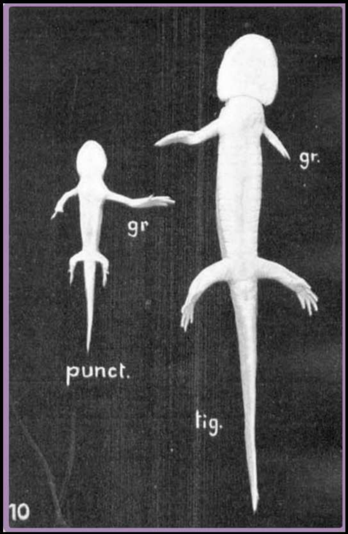
PART III: What determines size?

GROWTH TERMINATION

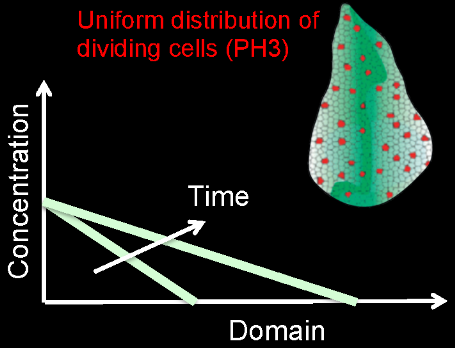


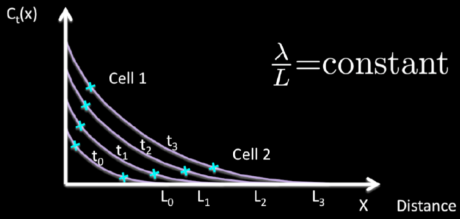
CREDIT: V. C. TWITTY AND J. L. SCHWIND, *THE JOURNAL OF EXPERIMENTAL ZOOLOGY* **59**, 1 (FEBRUARY 1931) © 1931 WILEY-LISS INC., A WILEY COMPANY

I. GENERAL FACTORS

- **surrounding tissue / larvae:** same size if discs develop outside larvae
- **developmental time:** developmental delays do not matter for final size
- **counting of cell divisions:** enhancing or blocking cell divisions does not alter final wing disc size

GROWTH CONTROL

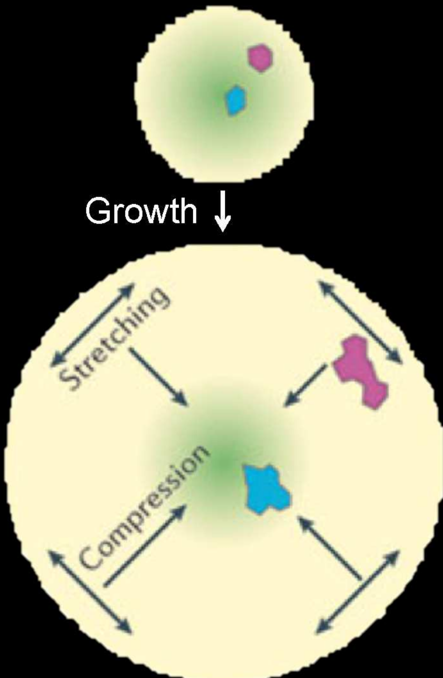




II. MORPHOGENS

- **Proliferation proportional to steepness of (linear) morphogen gradient:** Dpp gradient is of exponential shape
- **Proliferation rate dependent on relative change of Dpp amplitude:** Dpp signalling not necessary for normal growth; imperfect scaling of the Dpp gradient

GROWTH CONTROL



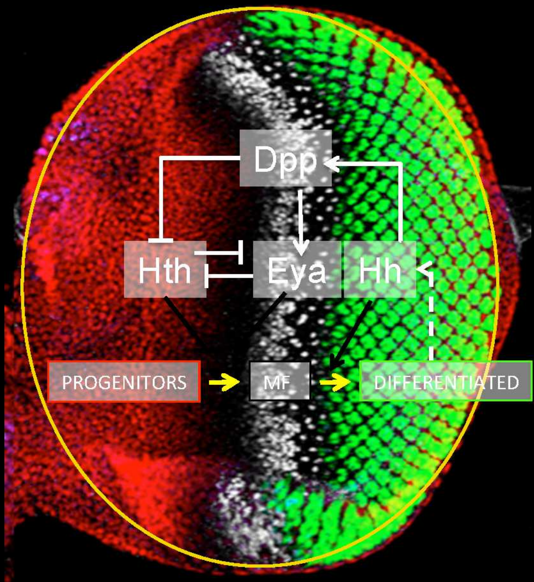
III. TISSUE MECHANICS

- **based on combined Dpp morphogen signalling and mechanical feedback:** Dpp signalling not necessary for normal growth.

IV. CELL DIFFERENTIATION

- Example: loss of growth plate terminates long bone growth

GROWTH CONTROL IN DROSOPHILA EYE



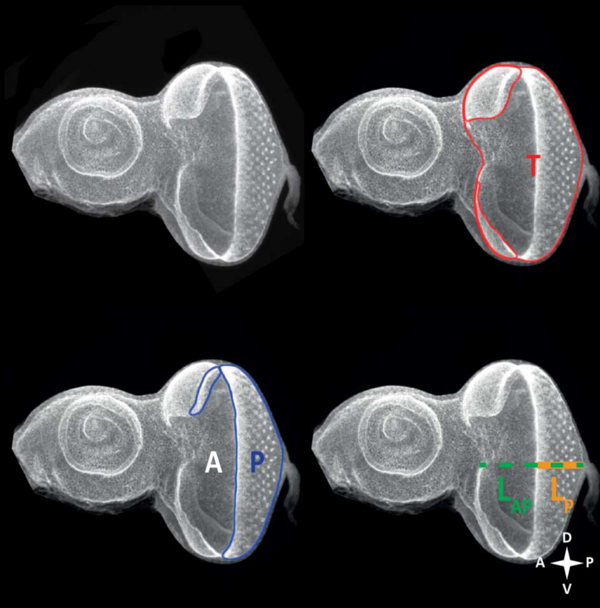
Callomyia sp.

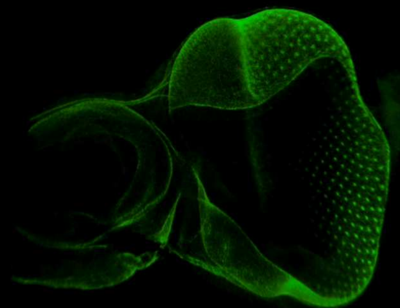
pictures courtesy of Gunilla Ståhls-Mäkelä, Helsinki

CABD (Sevilla): Max Sanchez, Daniel Aguilar, Fernando Casares

ETH Zürich (CH): Patrick Fried, Jannik Vollmer

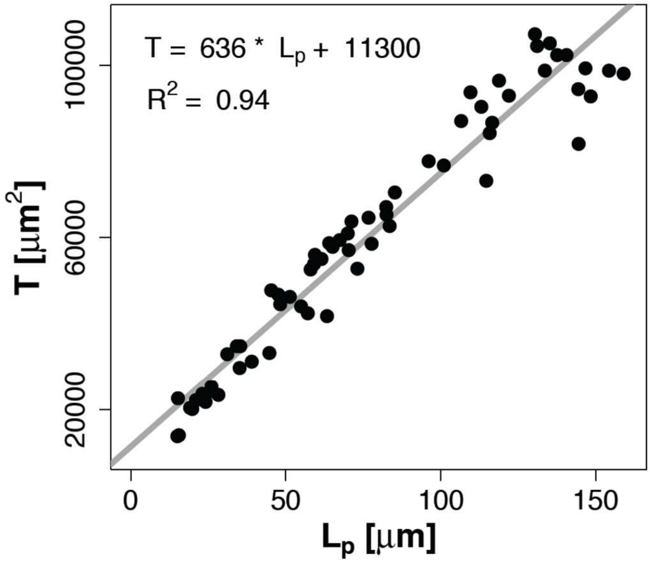
3D Quantitative Growth Measurements





Vollmer et al, *Development.*, 2016

The Eye Disc Area expands linearly with time



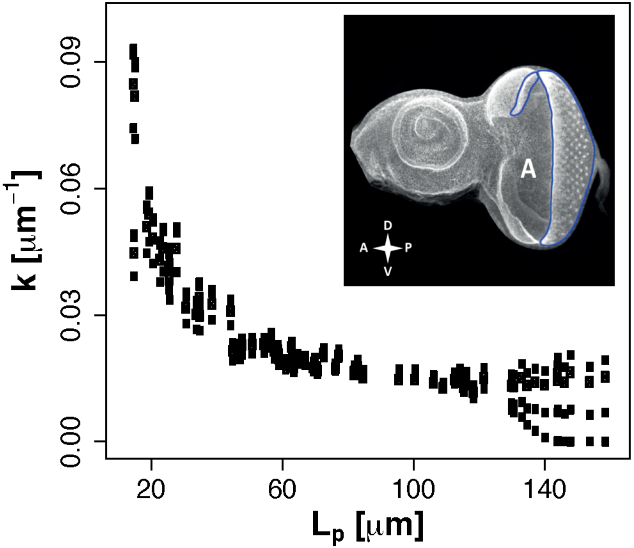
Jannik Vollmer

Patrick Fried

Vollmer et al, *Development.*, 2016

The Area Growth Rate Declines over dev. time

$$\frac{dT}{dL_P} = \frac{dA}{dL_P} + \frac{dP}{dL_P} = k(L_P) \bullet A + L_{MF}(L_P) \bullet (\alpha - 1)$$



The area growth rate on the anterior side declines continuously.

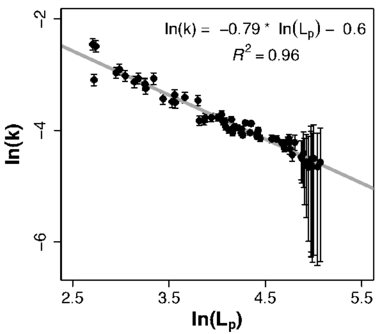
The declining growth rate is not the result of cell differentiation.

Vollmer et al, *Development.*, 2016

What is the Underlying Mechanism?

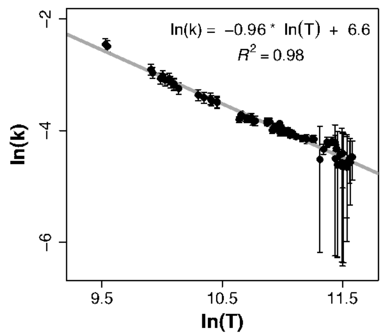
Power law

$$k = k_0 \left(\frac{L_P(0)}{L_P} \right)^{\delta}$$



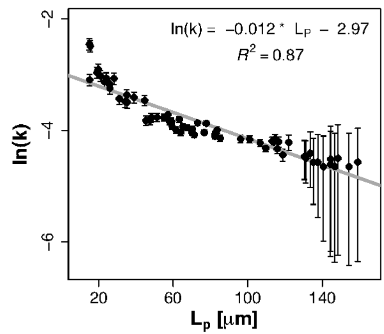
Area-dependent growth law

$$k = k_0 \left(\frac{T(0)}{T(L_P)} \right)$$

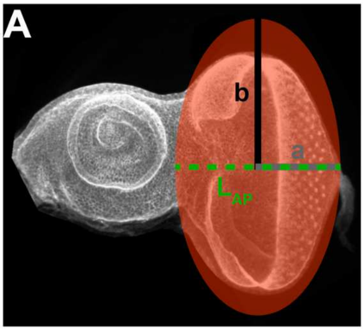


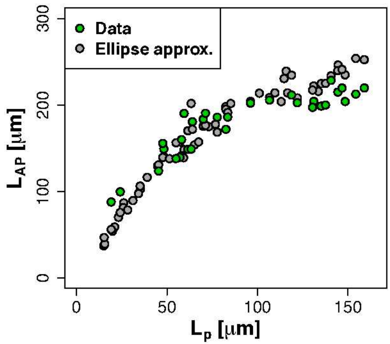
Exponential growth law

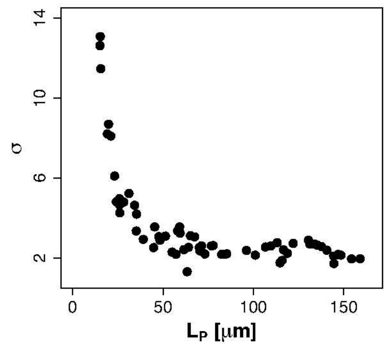
$$k = k_0 e^{-\delta \cdot \Delta L_P} + k_1$$



Ellipse Approximation of the Eye Disc







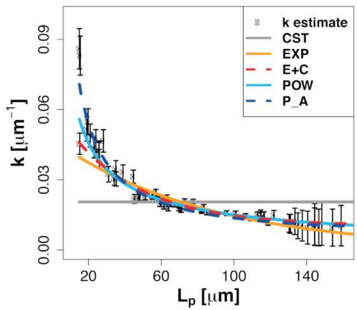
$$T = \pi\, \sigma a^2$$

$$P = \frac{T}{\pi}\left(\cos^{-1}\left(1 - \frac{2L_P}{L_{AP}}\right) - 2\left(1 - \frac{2L_P}{L_{AP}}\right)\sqrt{\frac{L_P}{L_{AP}}\left(1 - \frac{L_P}{L_{AP}}\right)}\right)$$

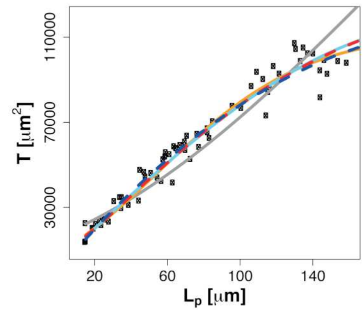
Vollmer et al, *Development.*, 2016

AREA-DEPENDENT GROWTH RATE FITS BEST

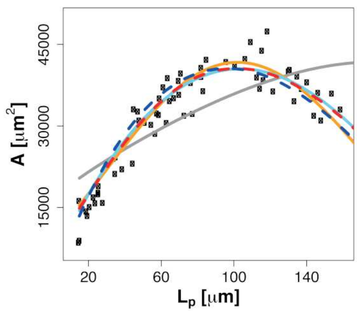
Growth Rate



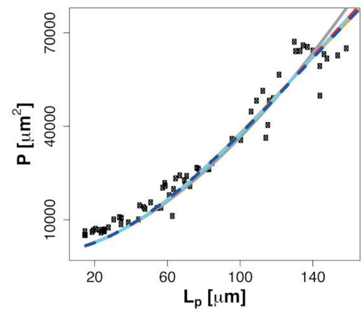
Total Area



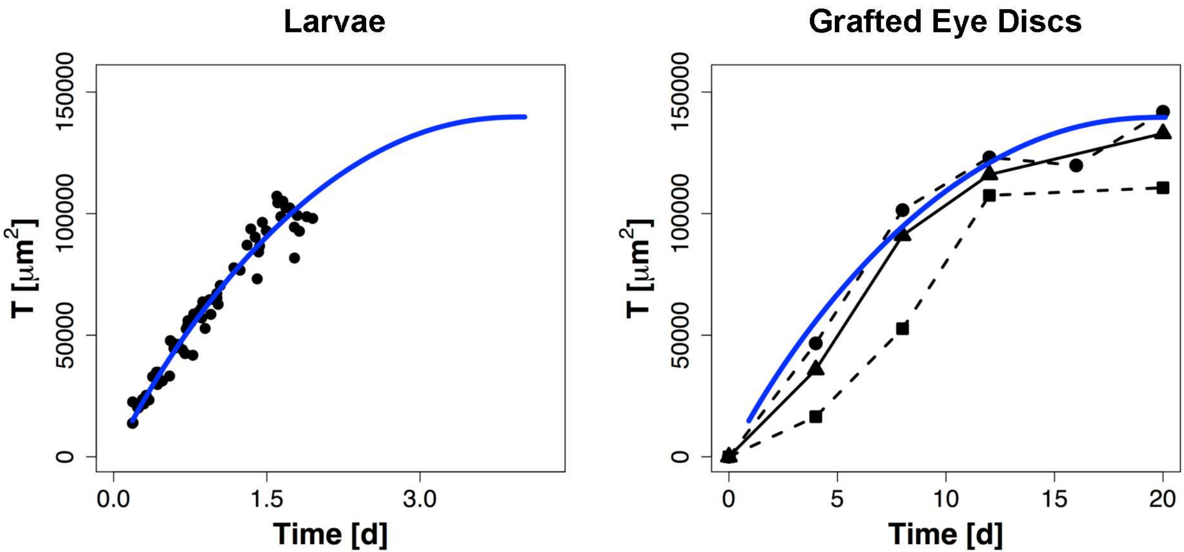
Anterior Area



Posterior Area



Size Preservation At Lower Dev. Speeds

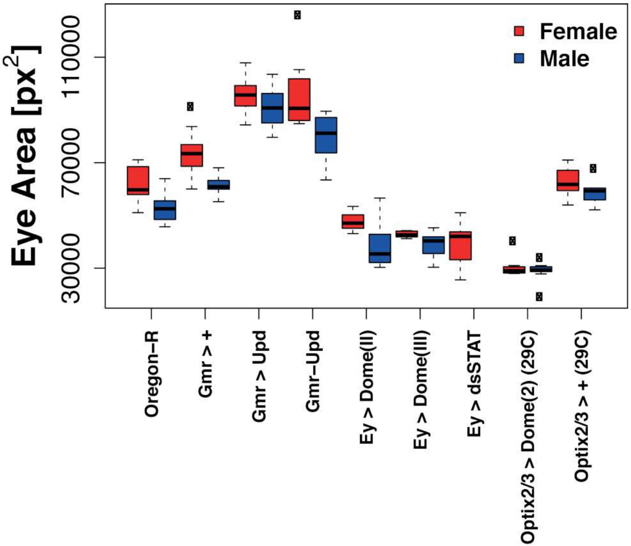


Area-Dependent growth rate naturally preserves final size at lower developmental speeds.

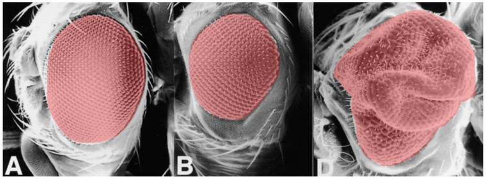
Vollmer et al, *Development*, 2016

CANDIDATE: Cytokine Unpaired (Upd)

Production restricted to initial stages of eye development (before MF starts)



WT -Upd +Upd



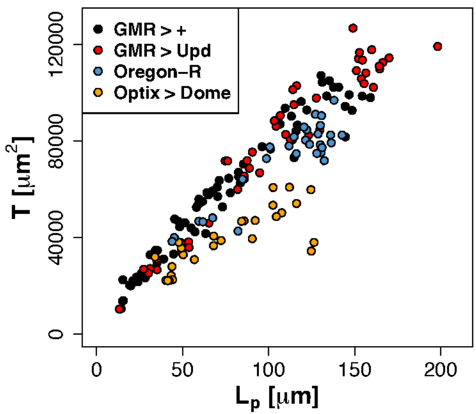
Bach et al., 2003

LARGEST mutant (130% of wt):
Gmr > Upd

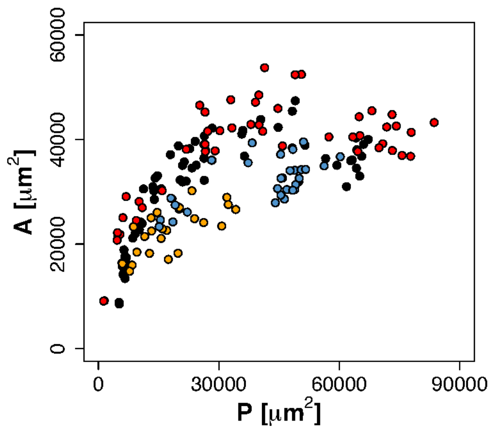
SMALLEST mutant (40% of wt):
Optix > Dome

GROWTH KINETICS IN MUTANTS DIFFER

TOTAL AREA



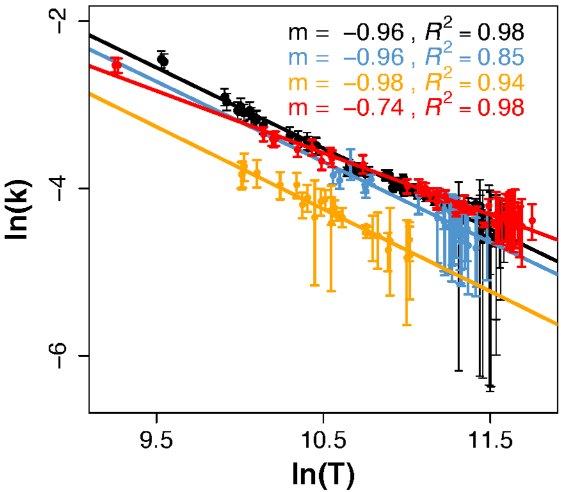
ANTERIOR AREA



Growth Kinetics depend on Upd Production Rate

Area-dependent

$$k = k_0 \left(\frac{T(0)}{T(L_P)} \right)$$



- Control strains have slope -1
- Optix > Dome has substantially lower growth rate, but slope -1
- Continuous expression of Upd in Gmr > Upd leads to slower decay of the growth rate

Conclusion: Eye Disc Growth Control

- Area growth rate declines inversely proportional to area-growth
- Area-dependent growth rate explains size preservation at lower developmental rate
- Upd as candidate for growth control by dilution

Callomyia sp.

pictures courtesy of Gunilla Ståhls-Mäkelä, Helsinki

THANKS!!

Computational Biology Group (CoBi)

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

FNSNF
Swiss National Science Foundation

SIB
Swiss Institute of Bioinformatics

SystemsX.ch
The Swiss Initiative in Systems Biology

CISD

COMSOL Engineers

Everything should be made as simple as possible, but no simpler.
Albert Einstein

Biological Collaborators

BODY AXIS FORMATION

- Technau group (Vienna)

BRAIN DEVELOPMENT

- Taylor Group (Basel)

BRANCHING MORPHOGENESIS

- McMahon group (USC, US)
- Sapin / Blanc (France)

DROSOPHILA EYE DISC

- Casares lab (Sevilla, Spain)

LIMB & BONE DEVELOPMENT

- Mariani group (USC, US)
- Zeller group (Basel)

OVARIAN FOLLICLES & IVF

- De Geyter (Basel)