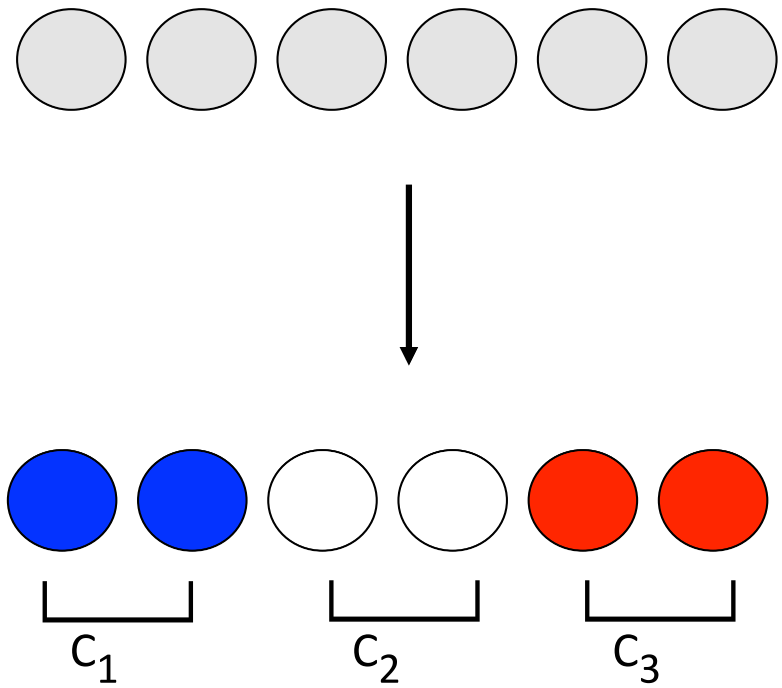
The Problem: Pattern Formation

Homogenous Progenitor Population



Different Differentiated Cell Types

The Problem: Pattern Formation

Different cell types = different gene expression profiles

Therefore problem is:

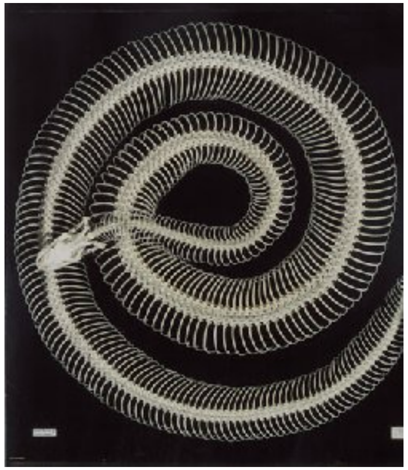
How is differential spatial pattern of gene expression controlled?

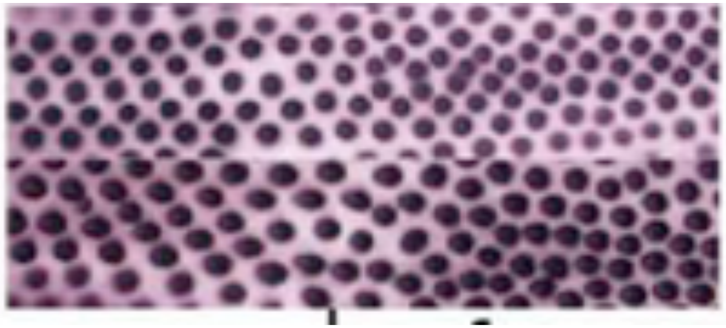
Requirement:

A symmetry breaking event to provide a spatial polarization
A mechanism to convert polarized signal to discrete regions of differential gene expression

Strategies of Pattern Formation

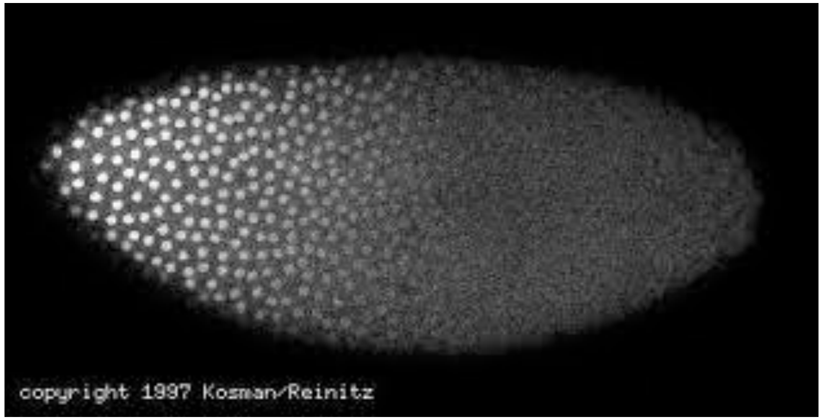
Oscillations –
cell intrinsic
e.g. Somitogenesis



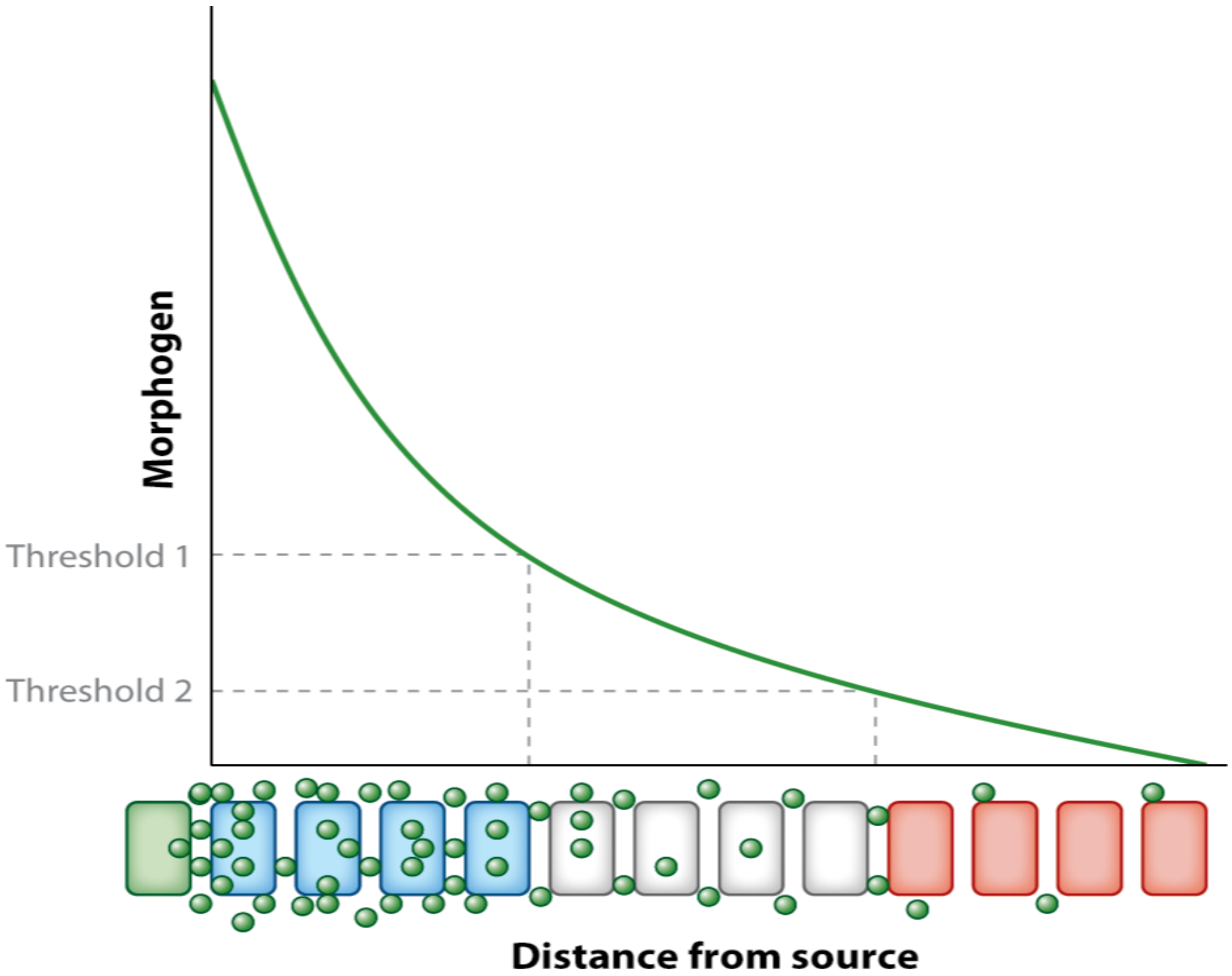


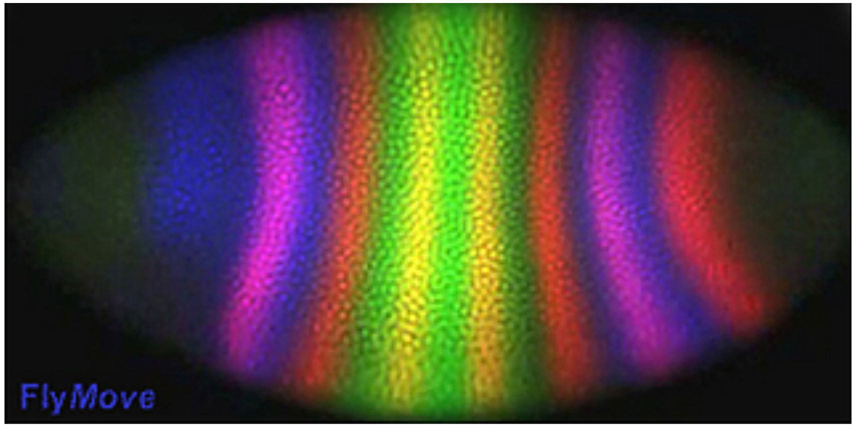
Local Cell-Cell Interactions –
self organizing
e.g. Notch-Delta signaling

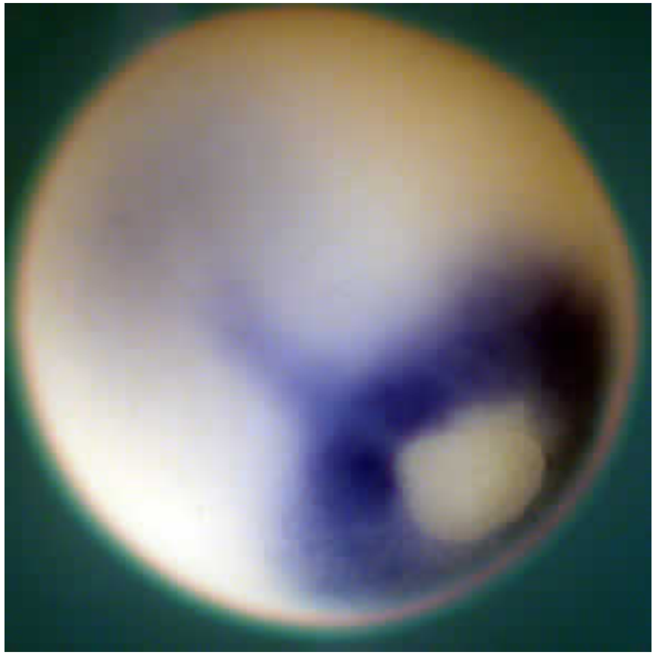
Morphogens –
Isn't self organizing
But long range

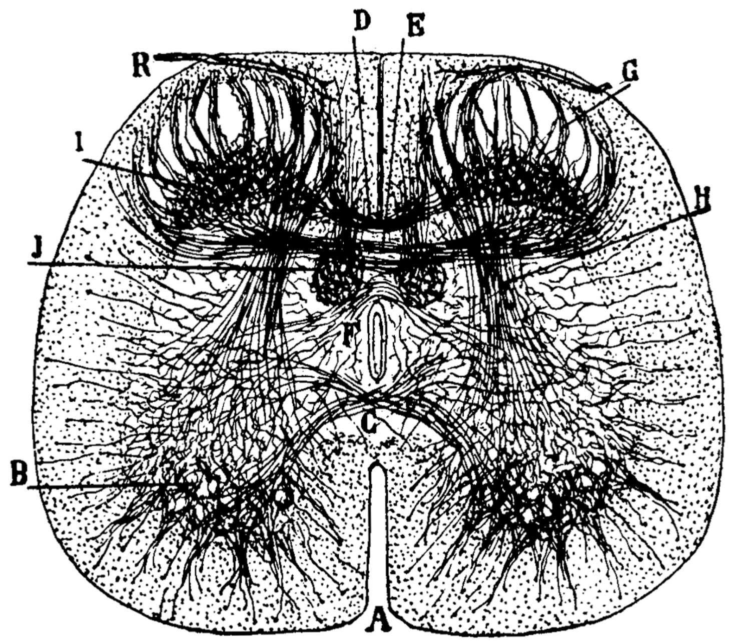


MORPHOGEN GRADIENTS

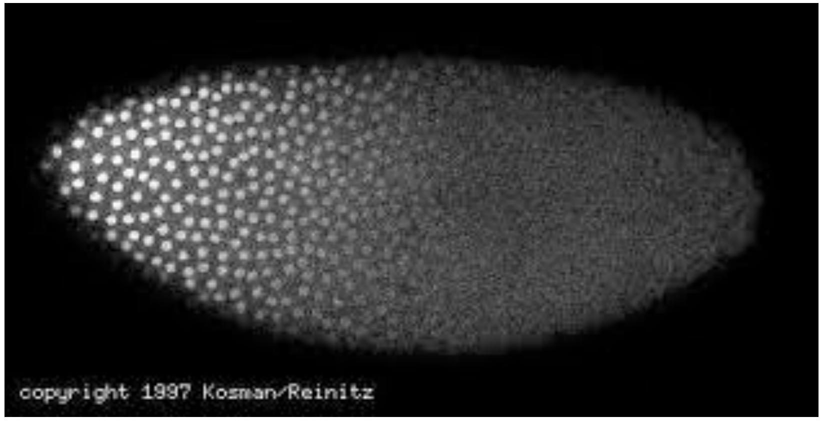


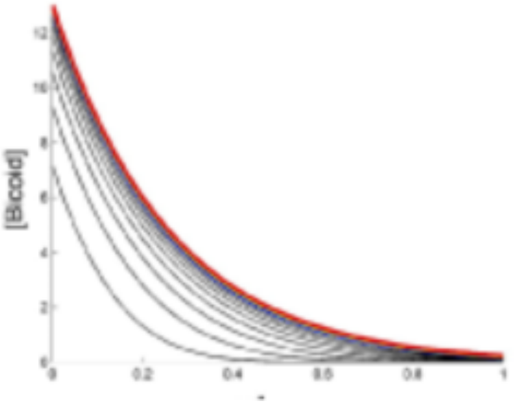

FlyMove



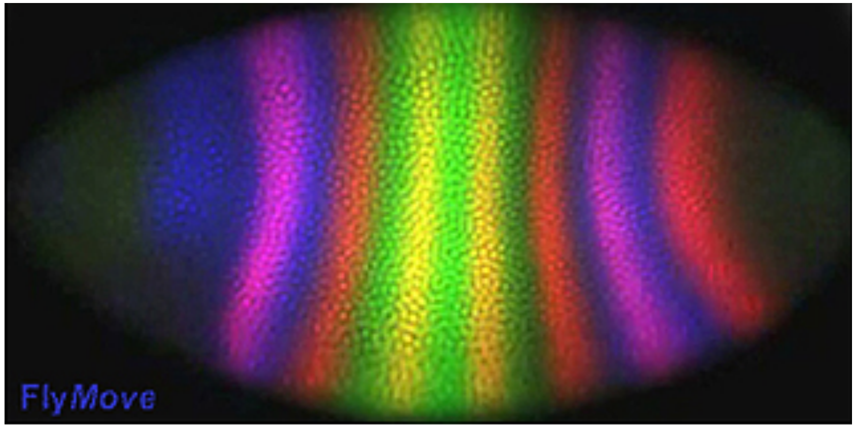

D
E
R
G
I
H
J
F
C
B
A

The early Drosophila embryo is a syncitium

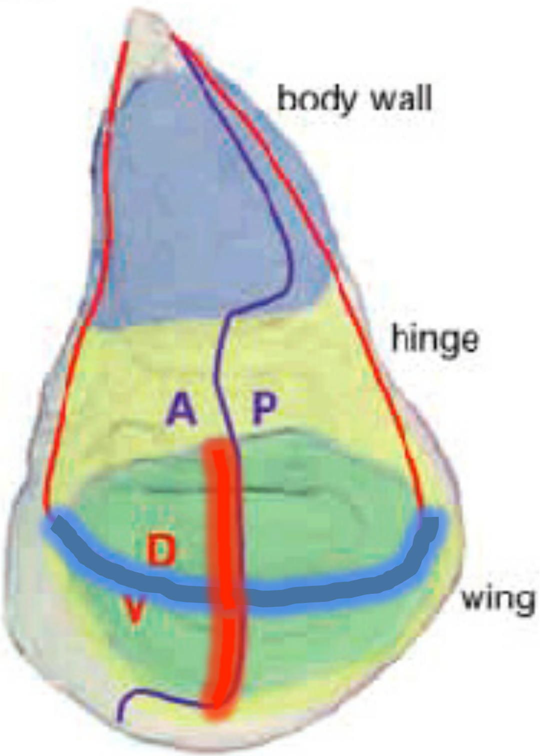


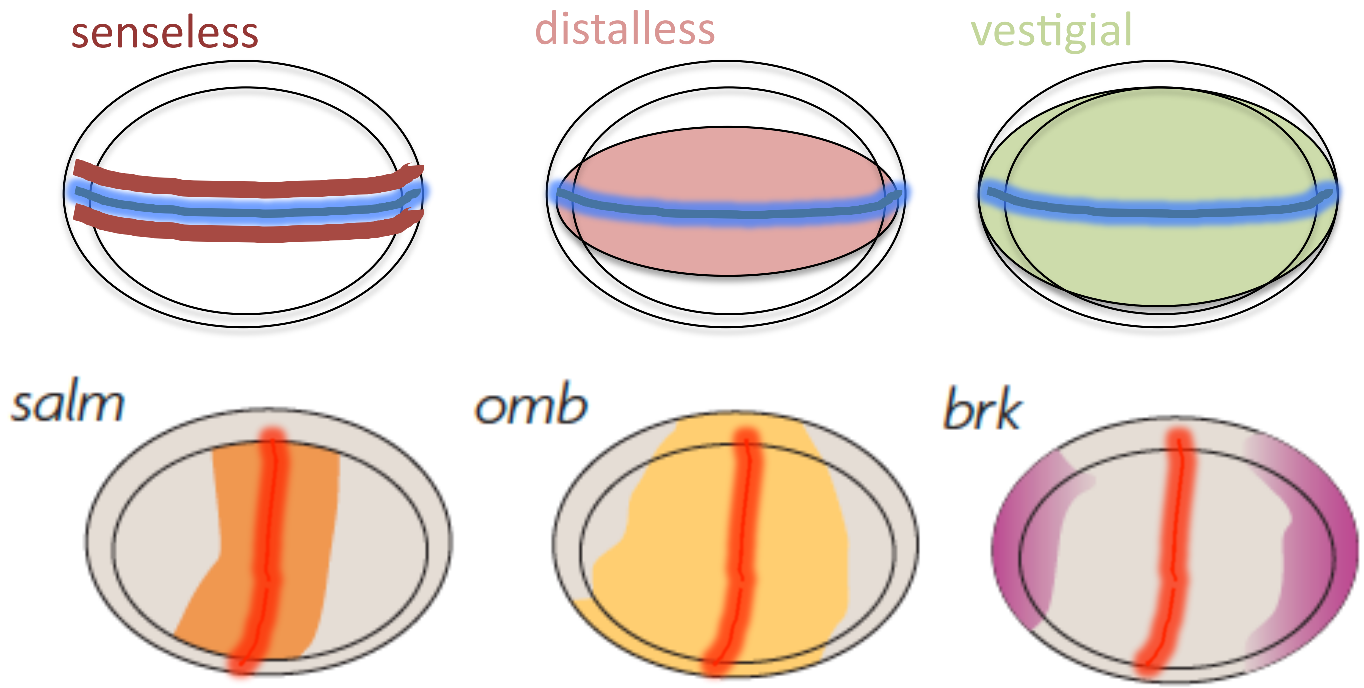


Grimm O et al. Development 2010;137:2253-2264

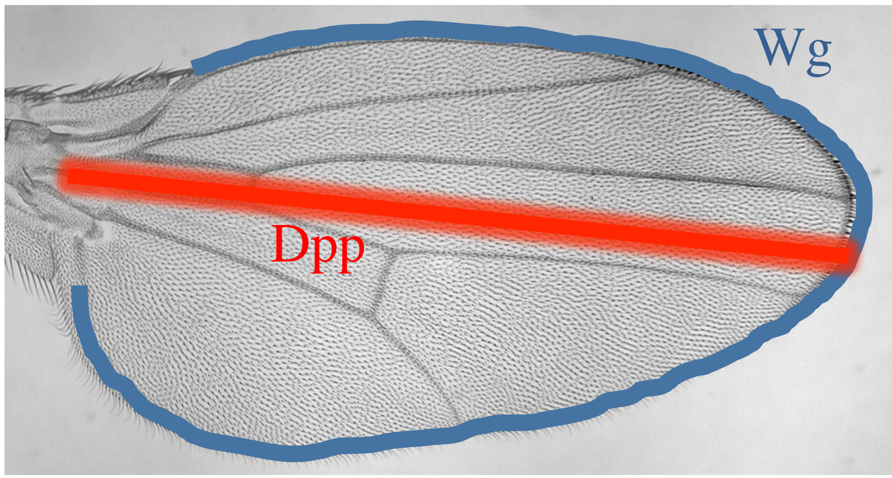


Wingless and Dpp in wing imaginal discs

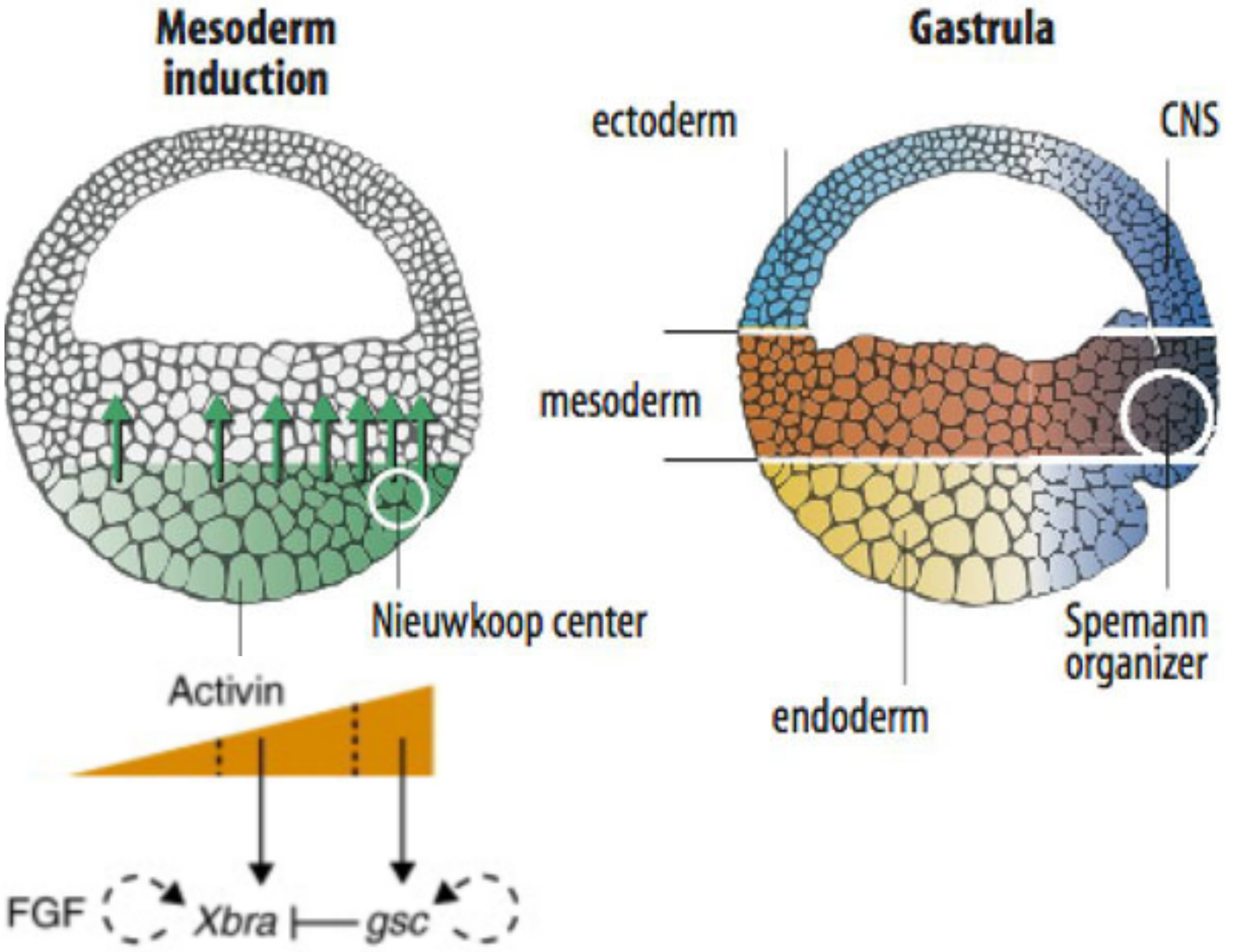




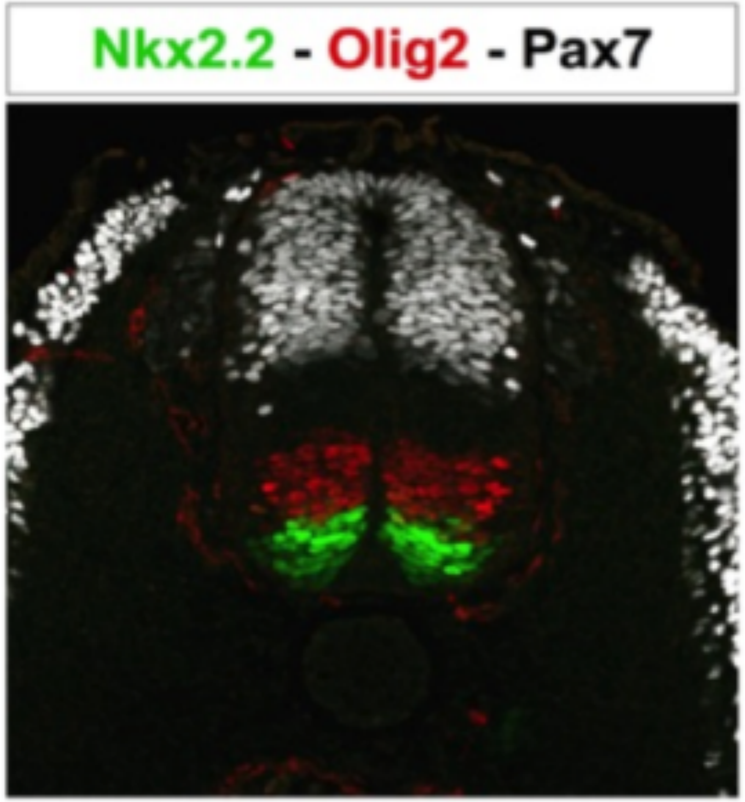
Adapted from Affolter and Basler

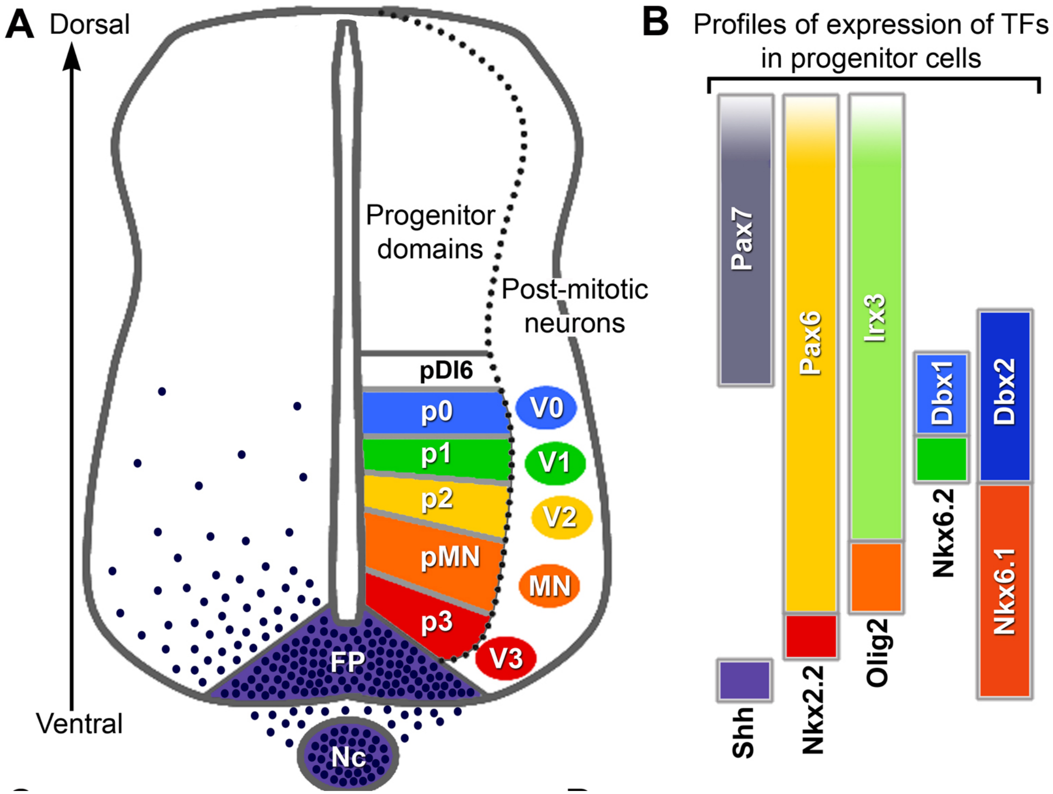


Graded signal transduction: Mesoderm

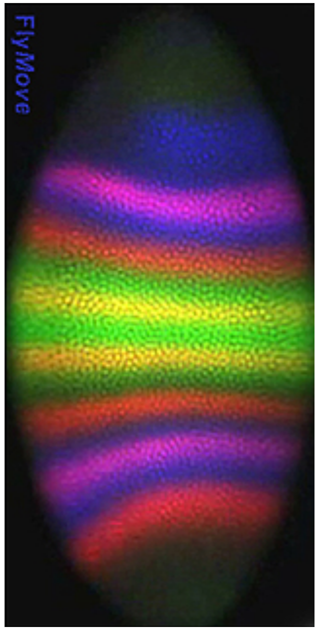


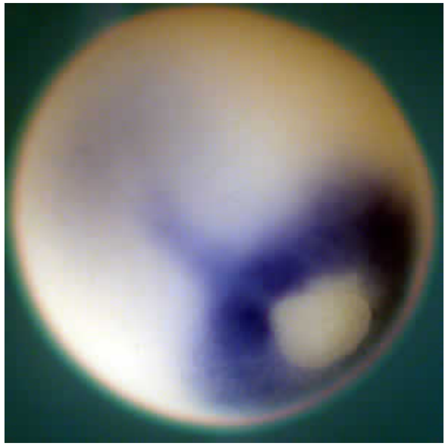
Neural patterning

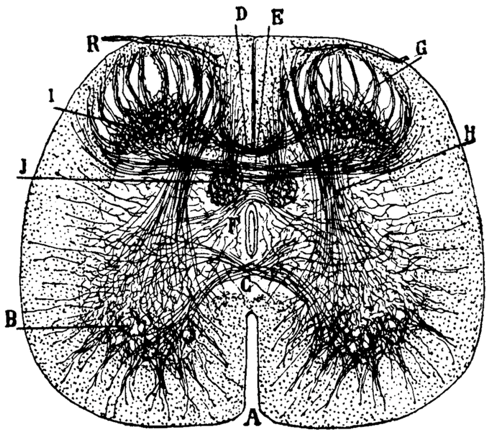


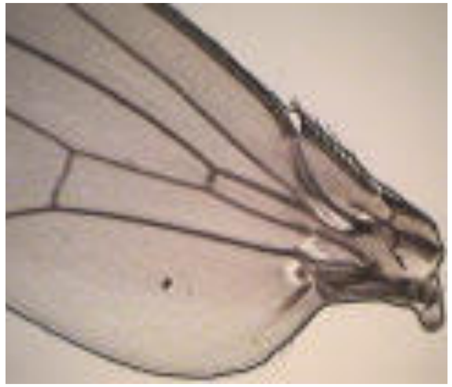


Different time scales of patterning







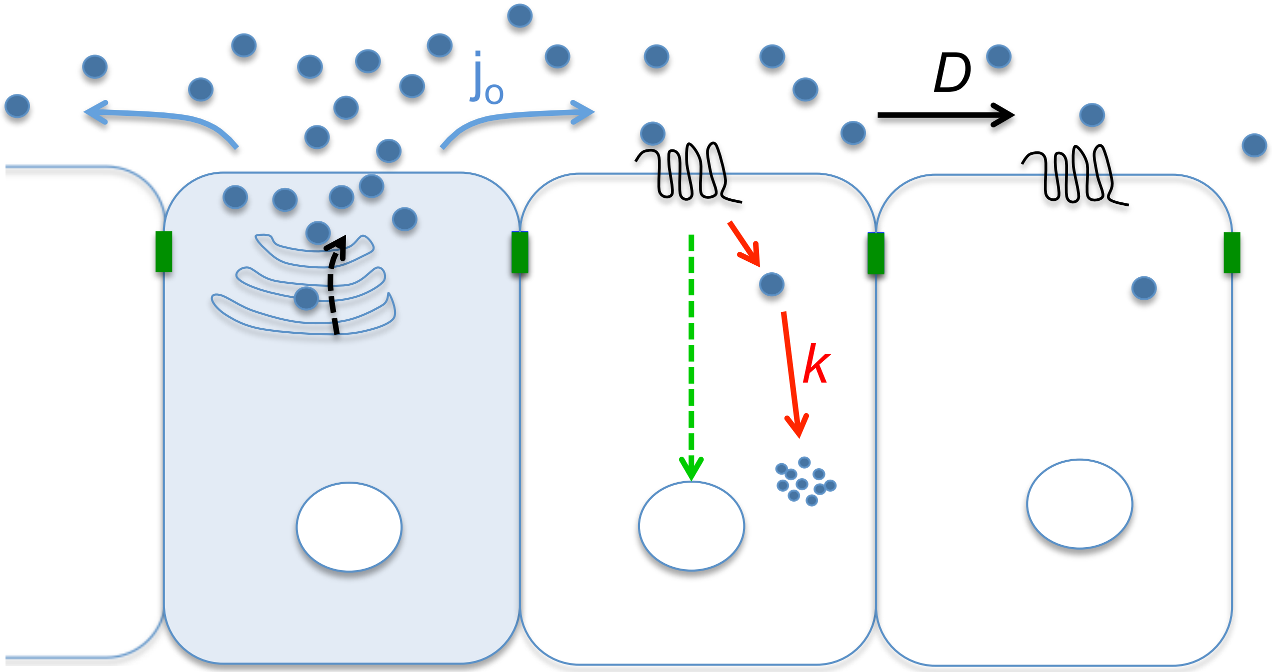


Minutes → Hours → Days

Morphogens:

- Identification – what and where
- Formation - intercellular transmission
- Perception - signal transduction
- Interpretation - convert to discrete responses

Establishing a morphogen gradient



-Current j_0 [molecules/(μm s)] at the source boundary
= Rate of secretion

-Uniform degradation with a rate k (s^{-1})
= Extracellular protease + Lysosomal targeting

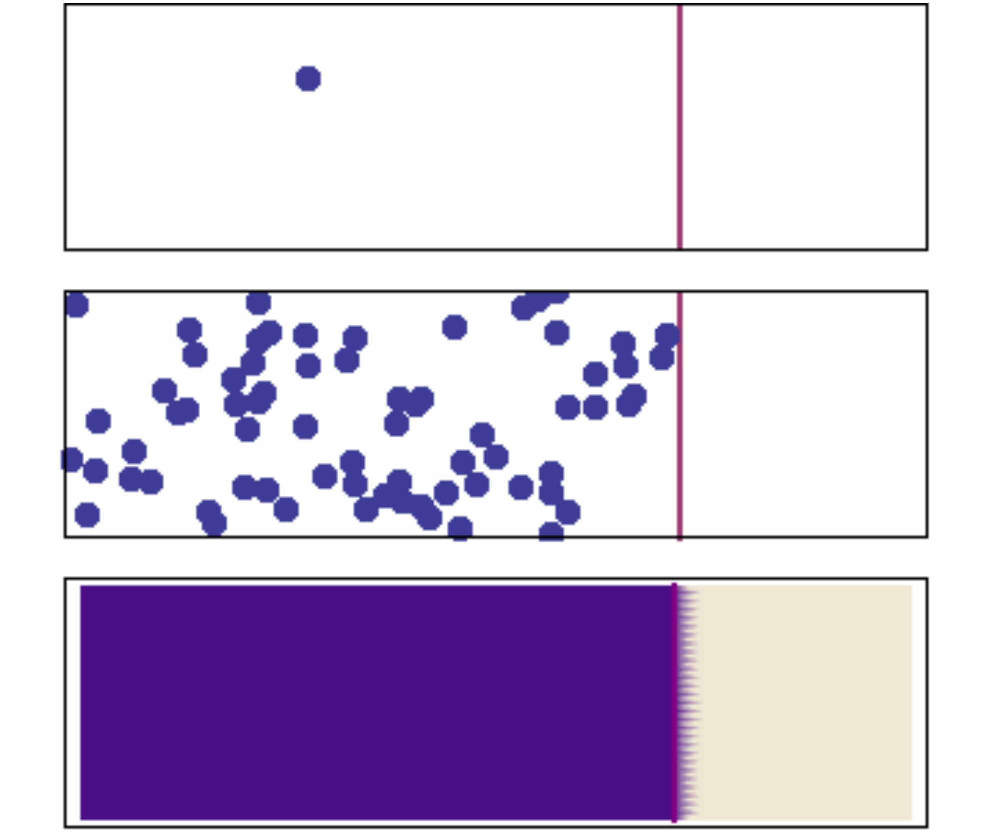
-Effective diffusion coefficient D (mm^2/s).
= Diffusion or any random walk process

$$\frac{\partial C}{\partial t} = j_0(x) + D\frac{\partial^2 C}{\partial x^2} - kC$$

Steady state solution $C = C_0\, e^{-x/\lambda}$

where $c_0 = \frac{j_0}{\sqrt{Dk}}$ and $\lambda = \sqrt{\frac{D}{k}}$

Diffusion: molecules undertake random walk. Move from regions of high concentration to regions of low concentration, with rate proportional to the concentration gradient (spatial derivative). Results in a flux, J.



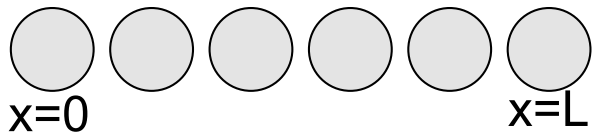
$$J = -D \frac{\partial C}{\partial x}$$

https://upload.wikimedia.org/wikipedia/commons/4/4d/DiffusionMicroMacro.gif

Initial conditions: t=0

C=0 for all x

C(x,0)=0



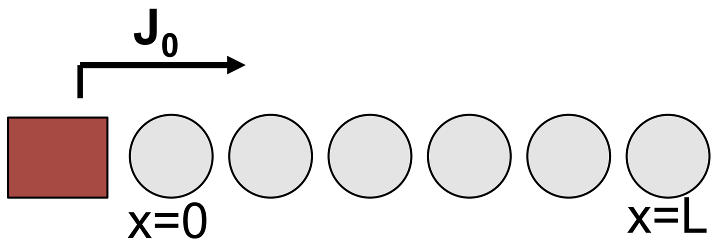
Boundary conditions:

Morphogen produced at x=0 at constant rate $=J_0$ 'Flux'

Flux caused by concentration difference and diffusion counteracts:

$$J = -D \frac{\partial C}{\partial x}$$

$$D \frac{\partial C}{\partial x}\bigg|_{x=0} = -J_0$$

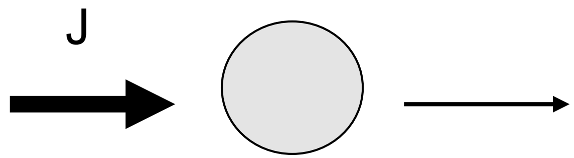


Morphogen is 0 at the end of the tissue (x=L)

$$D \frac{\partial C}{\partial x}\bigg|_{x=L} = 0$$

DIFFUSION

Prior to steady state concentration is changing over **time** at a location. Indicates that there must be a difference in flux in to and out of every **location**



$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial x}$$

Continuity equation: no molecules are lost or gained across the tissue

Flux caused by concentration difference and diffusion counteracts it

$$J = -D\frac{\partial C}{\partial x}$$

Substitute for J
$$\frac{\partial C}{\partial t} = -\frac{\partial}{\partial x}\left(-D\frac{\partial C}{\partial x}\right) = D\frac{\partial^2 C}{\partial x^2}$$

DEGRADATION

Take simple case where morphogen is degraded at a constant rate:

$$\frac{\partial C}{\partial t} = -kC$$

k is rate of degradation

Put diffusion and degradation together

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - kC$$

STEADY STATE

At ss there is no change in concentration at a position

$$\frac{\partial C}{\partial t} = 0$$

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - kC = 0$$

$$D\frac{\partial^2 C}{\partial x^2} = kC$$

Spread of morphogen balanced by degradation

$$\frac{\partial^2 C}{\partial x^2} = \frac{k}{D}C$$

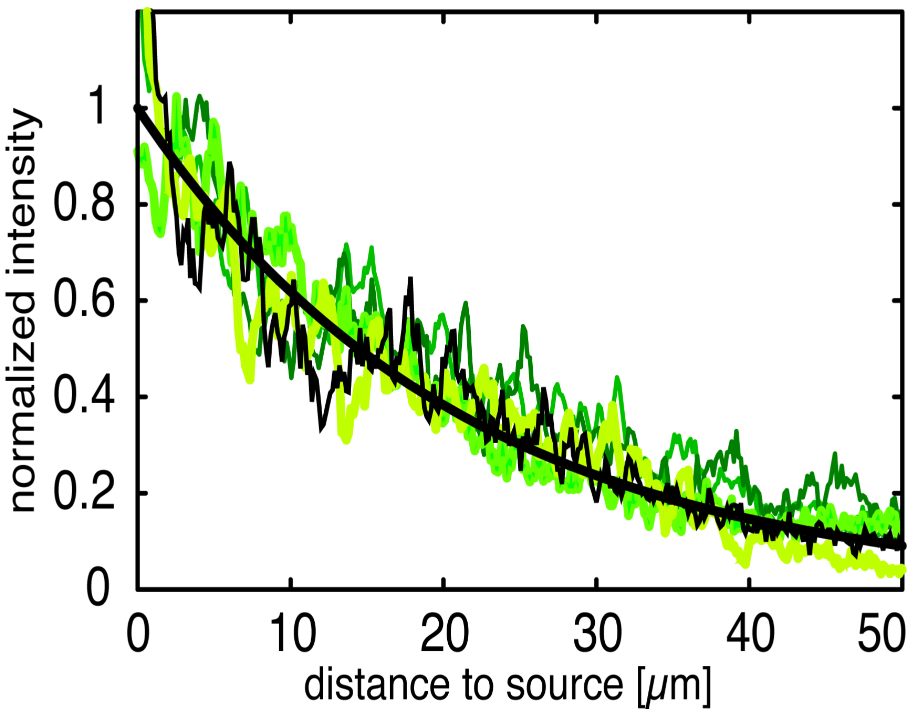
Second order derivative. Solution is proportional to conc. Parameters and concentration are positive therefore look for a solution that gives only positive

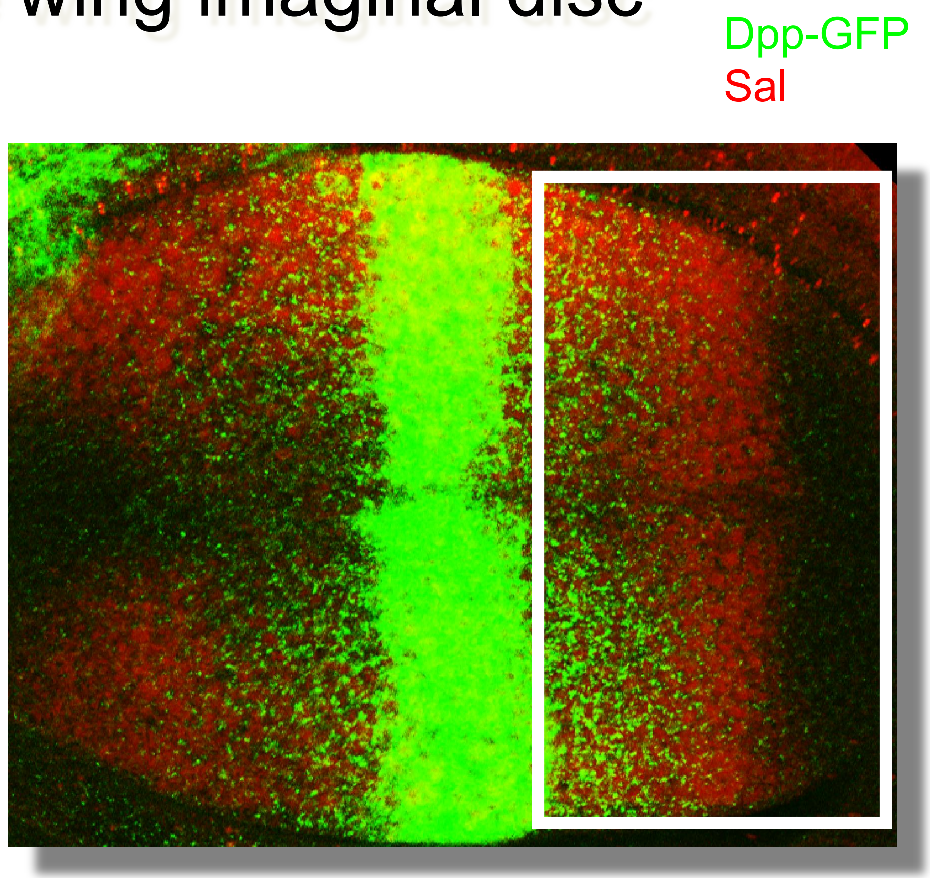
$$C = C_0\, e^{-x/\lambda}$$

$$C_0 = \frac{J_0}{\sqrt{Dk}}$$

$$\lambda = \sqrt{\frac{D}{k}}$$

Dpp gradient in the wing imaginal disc



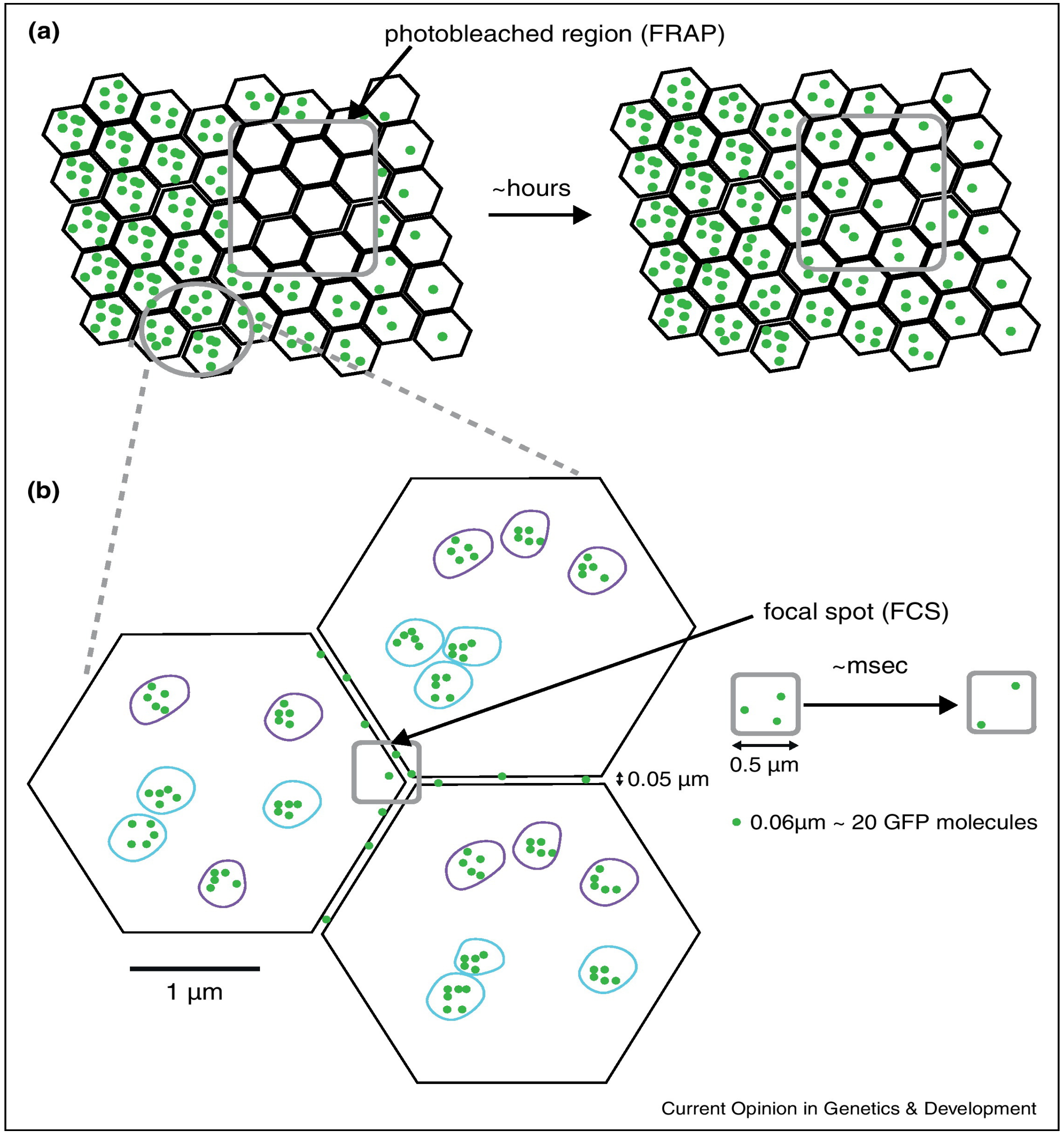


$$C = C_0 \, e^{-x/\lambda}$$

$$c_0 = \frac{j_0}{\sqrt{Dk}} \qquad \lambda = \sqrt{\frac{D}{k}}$$

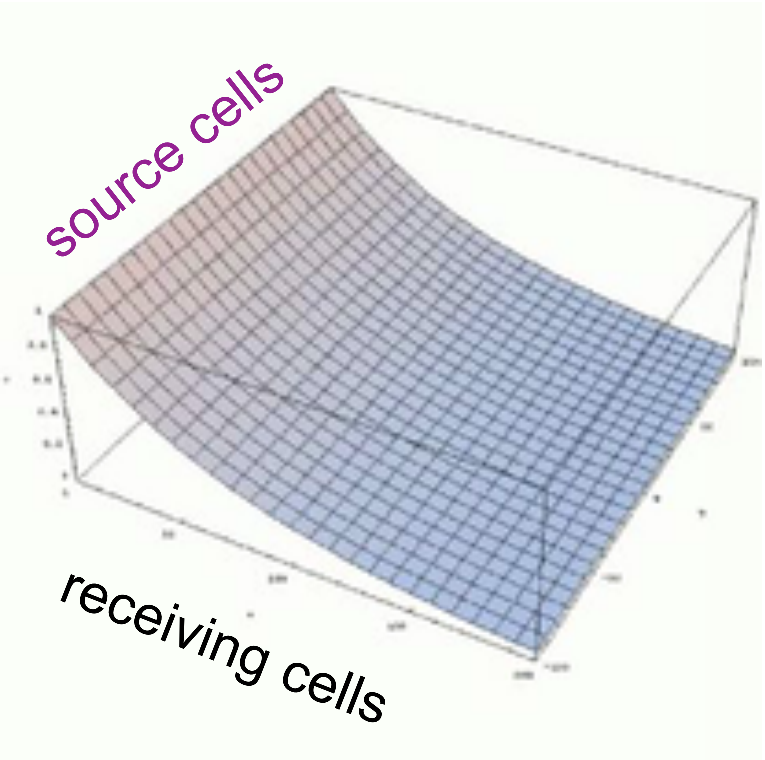
Kicheva et al. (2007)Science 315:521-5

Techniques to measure gradient kinetics



Anna Kicheva , Tobias Bollenbach , Ortrud Wartlick , Frank Jülicher , Marcos Gonzalez-Gaitan
Investigating the principles of morphogen gradient formation: from tissues to cells
Current Opinion in Genetics & Development Volume 22, Issue 6 2012 527 - 532

FRAP
(Fluorescence Recovery After Photobleaching)



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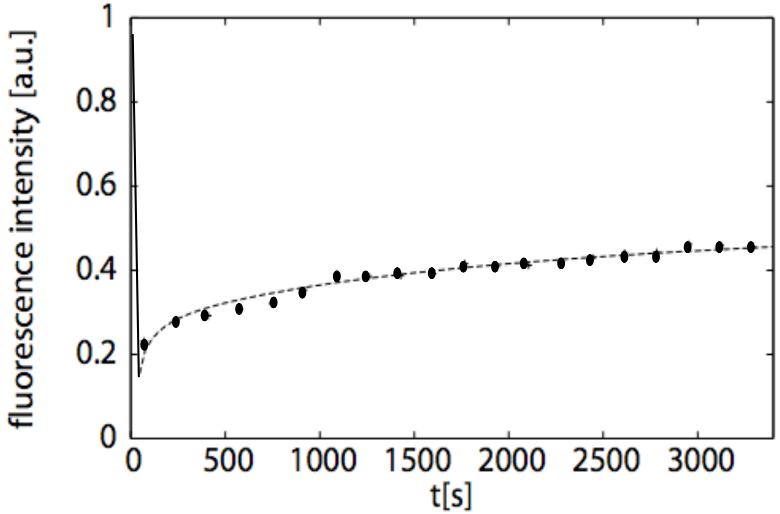
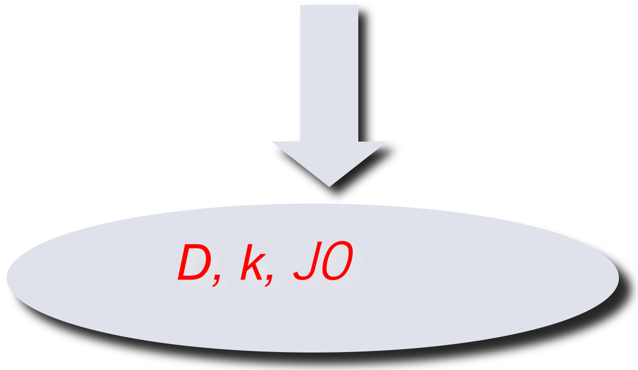
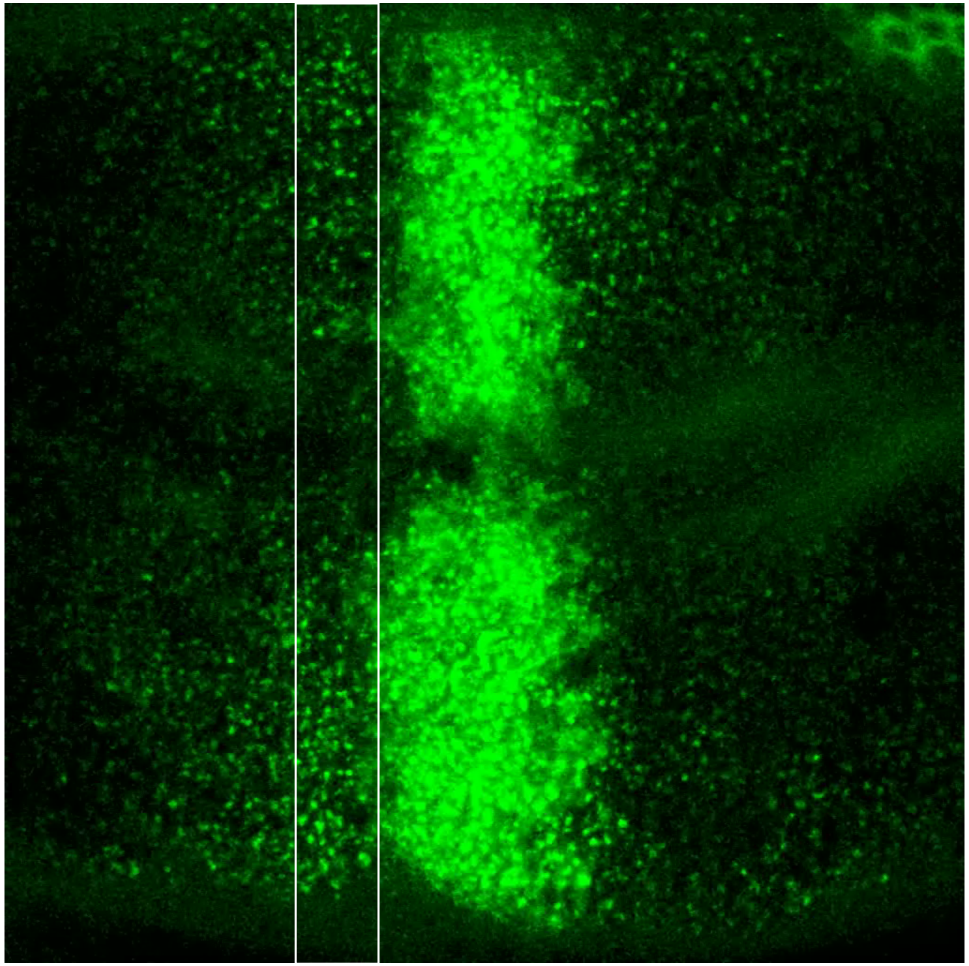
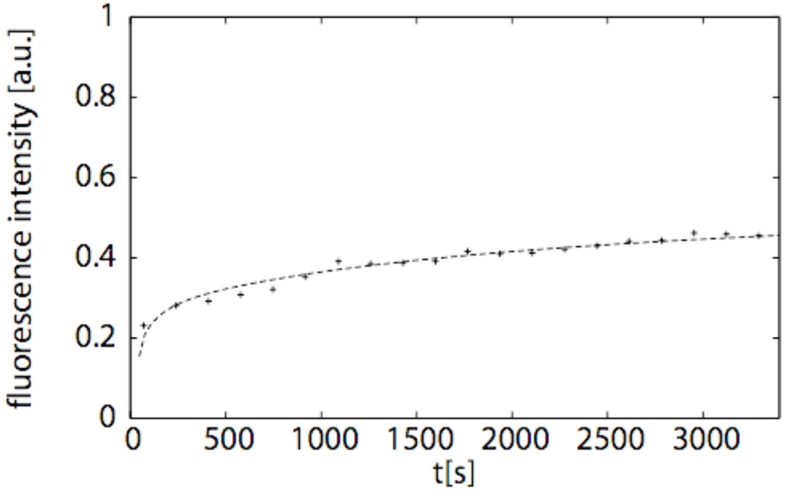




Kicheva et al. (2007)Science 315:521-5

FRAP of GFP-Dpp





D = 0.10 µm²/s ±0.05

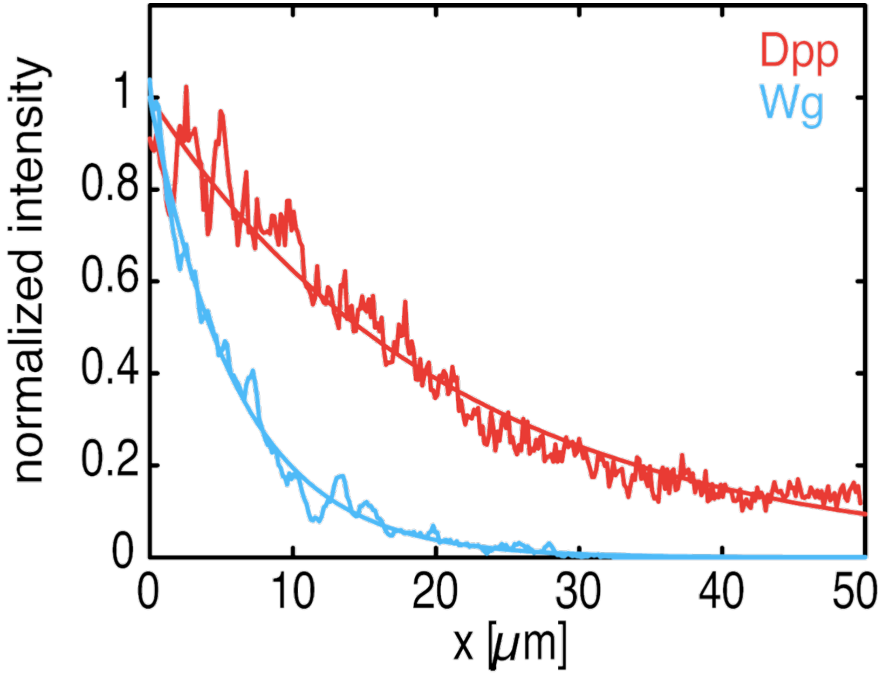
k = 0.000252 s^{-1} ± 0.0001

ν = 2.69 molecules/s x cell ±1.58

Kicheva et al. (2007)Science 315:521-5

gradient profile results from specific kinetic behavior

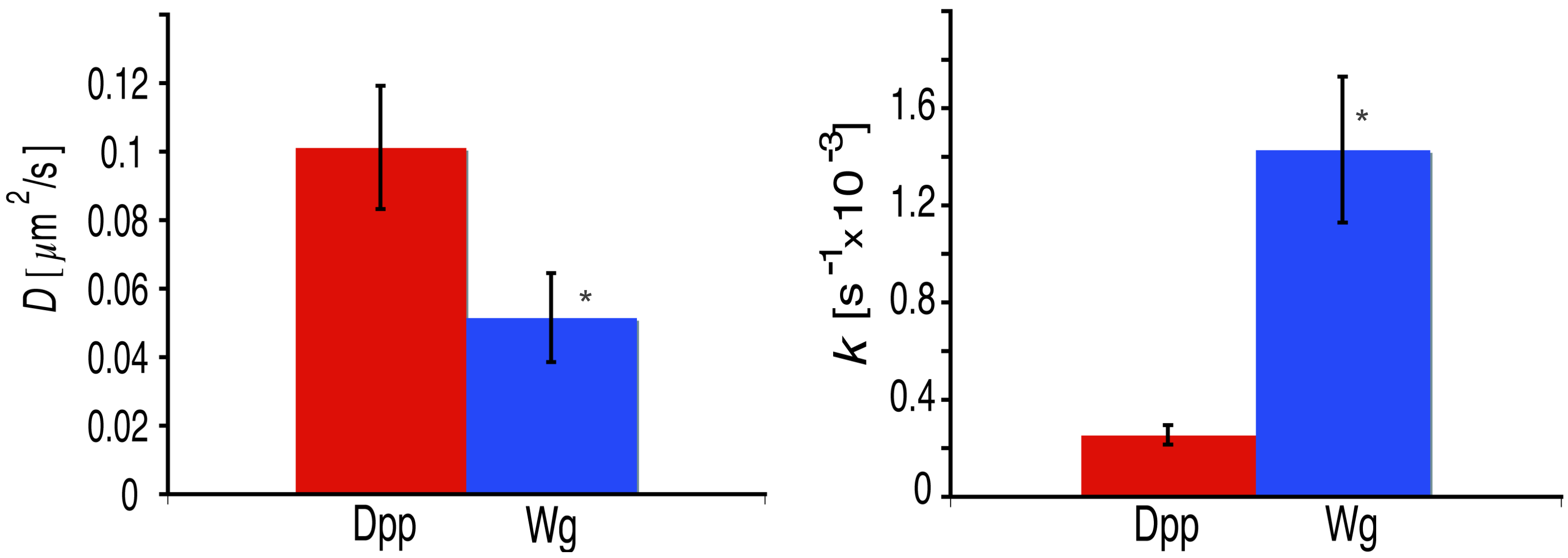
D, k, ν, ψ



decay length $\lambda = \sqrt{\frac{D}{k}}$

Dpp 20.2 μm ± 5.7 (SD), n = 26
Wg 5.8 μm ± 2.04 (SD), n=12

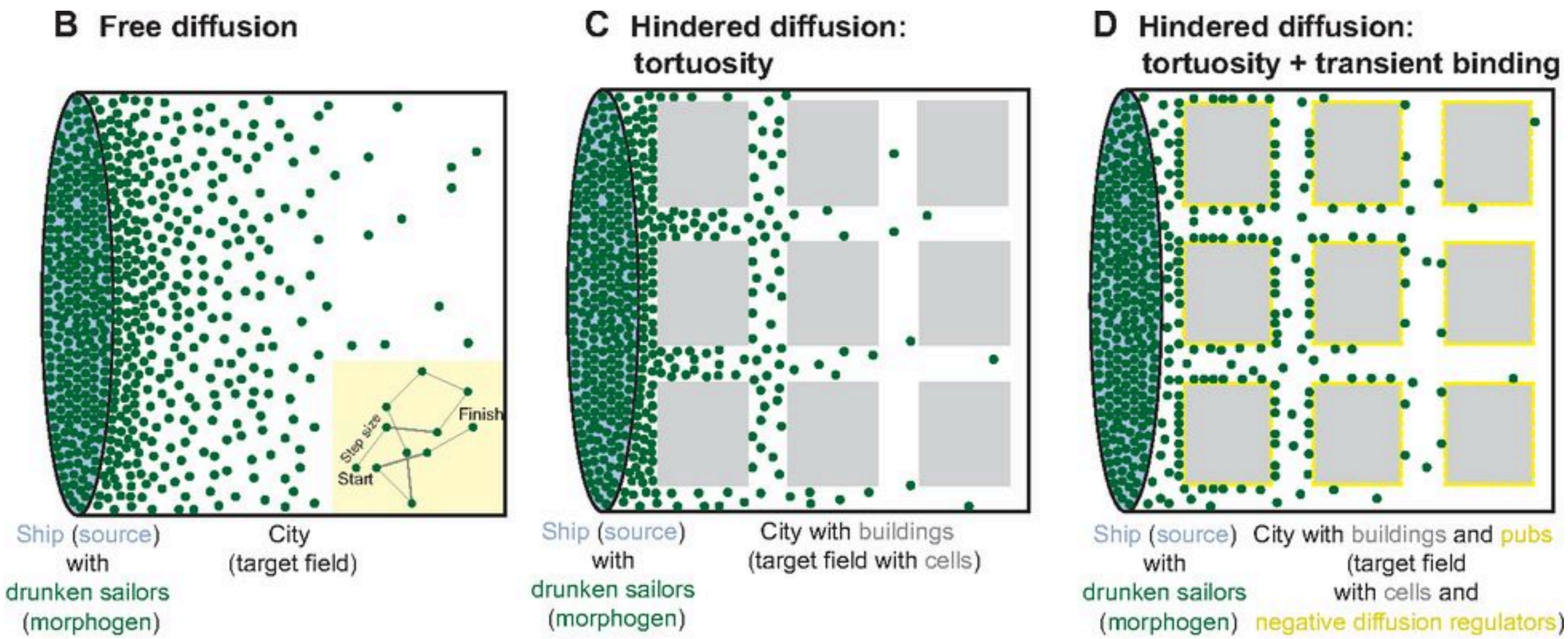
kinetics of the Dpp vs the Wg gradient



$$\lambda = \sqrt{\frac{D}{k}}$$

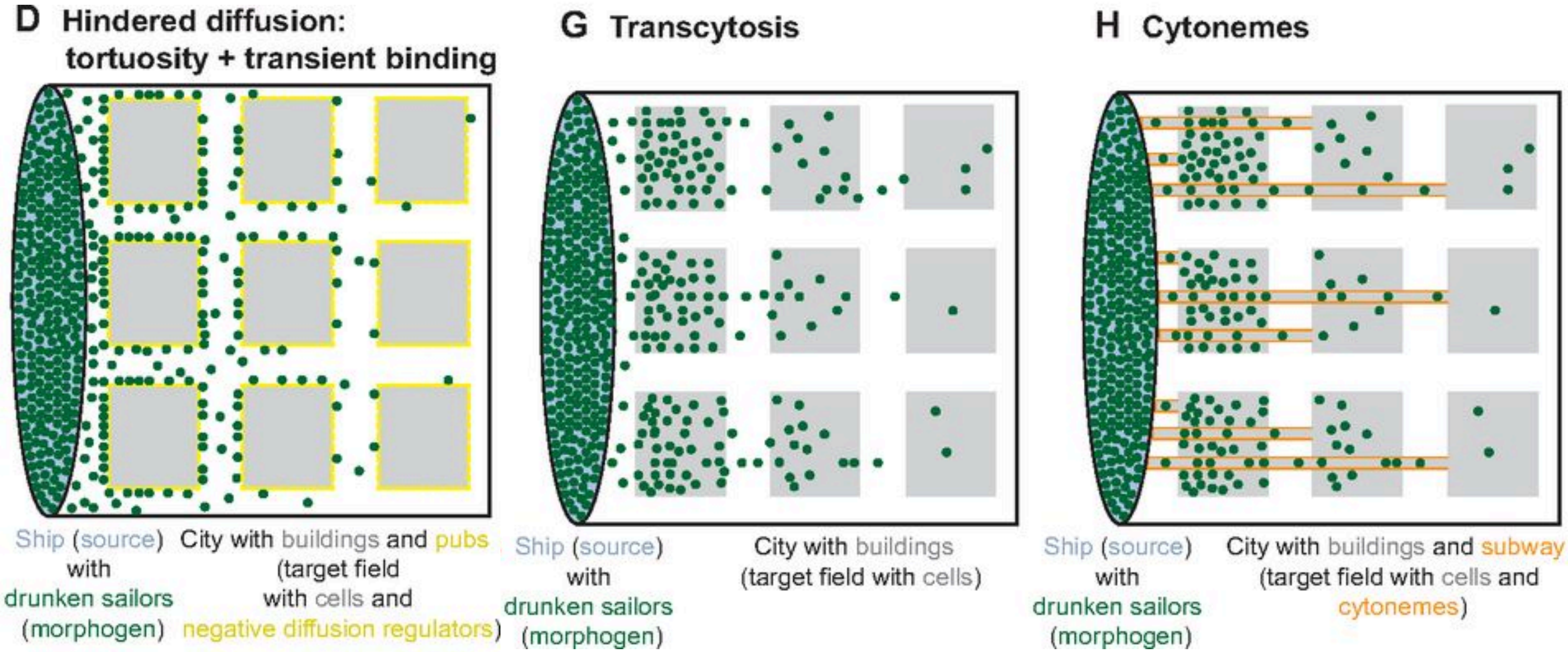
Kicheva et al. (2007)Science 315:521-5

DIFFUSION + DEGRADATION



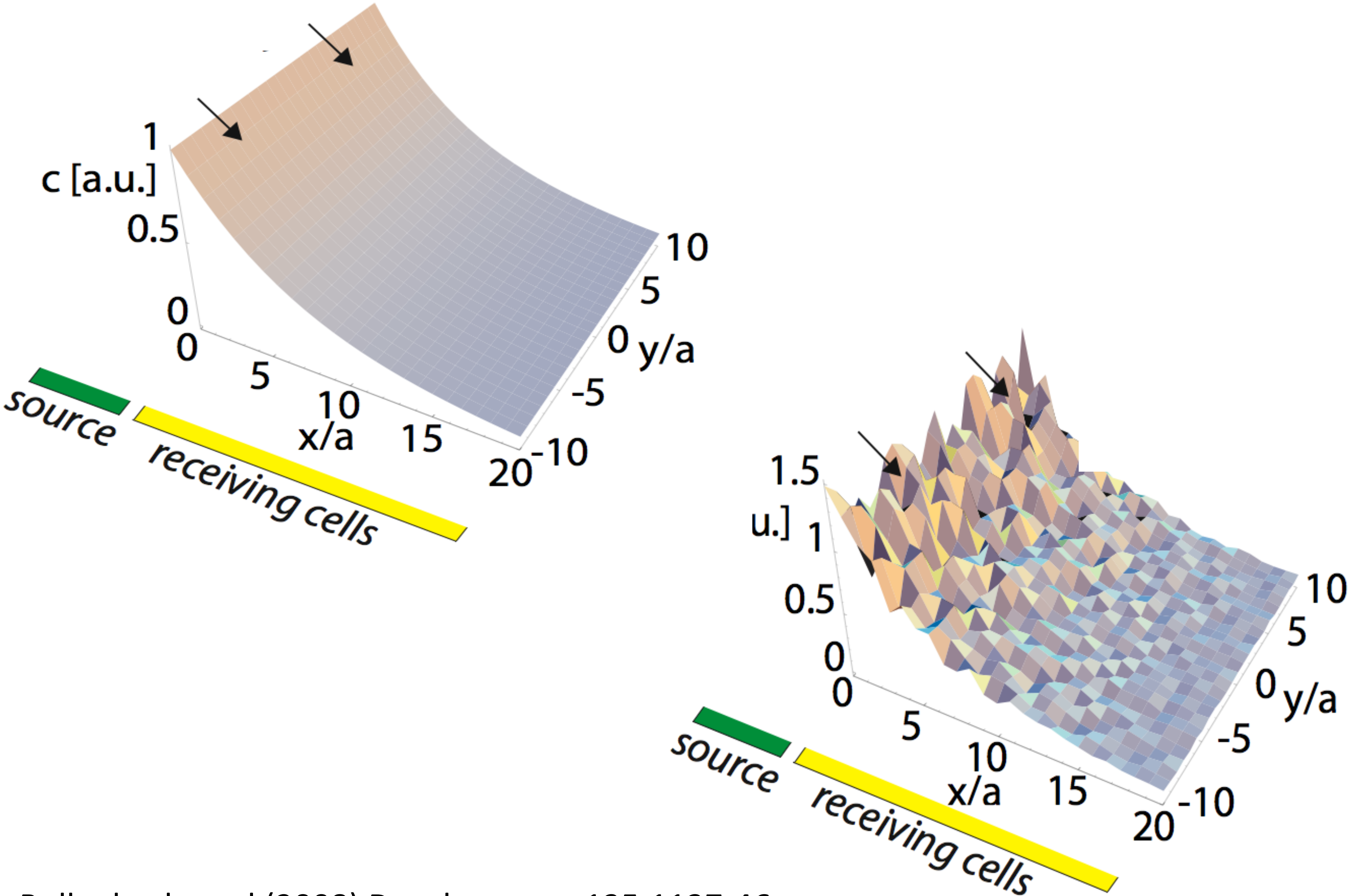
Müller P et al. Development 2013;140:1621-1638

Route of movement



Müller P et al. Development 2013;140:1621-1638

Noise



Bollenbach et al (2008) Development 135:1137-46

Morphogens:

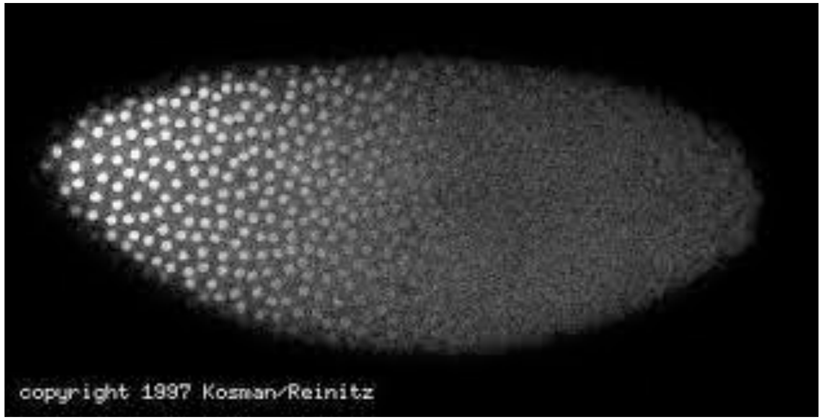
- Identification – what and where
- Formation - intercellular transmission
- Perception - signal transduction
- Interpretation - convert to discrete responses

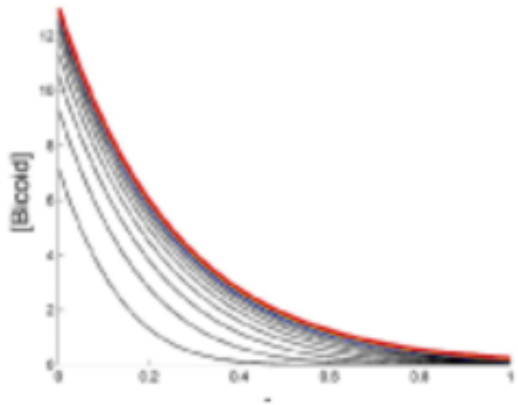
Morphogen Interpretation

- How does the signaling pathway transform the extracellular ligand?
- How is differential gene expression encoded in cis-regulatory elements?
- What role does the transcriptional network play in producing discrete spatial patterns of gene expression?
- How does cell proliferation and tissue growth affect gradient interpretation?

‘Direct’ Morphogens

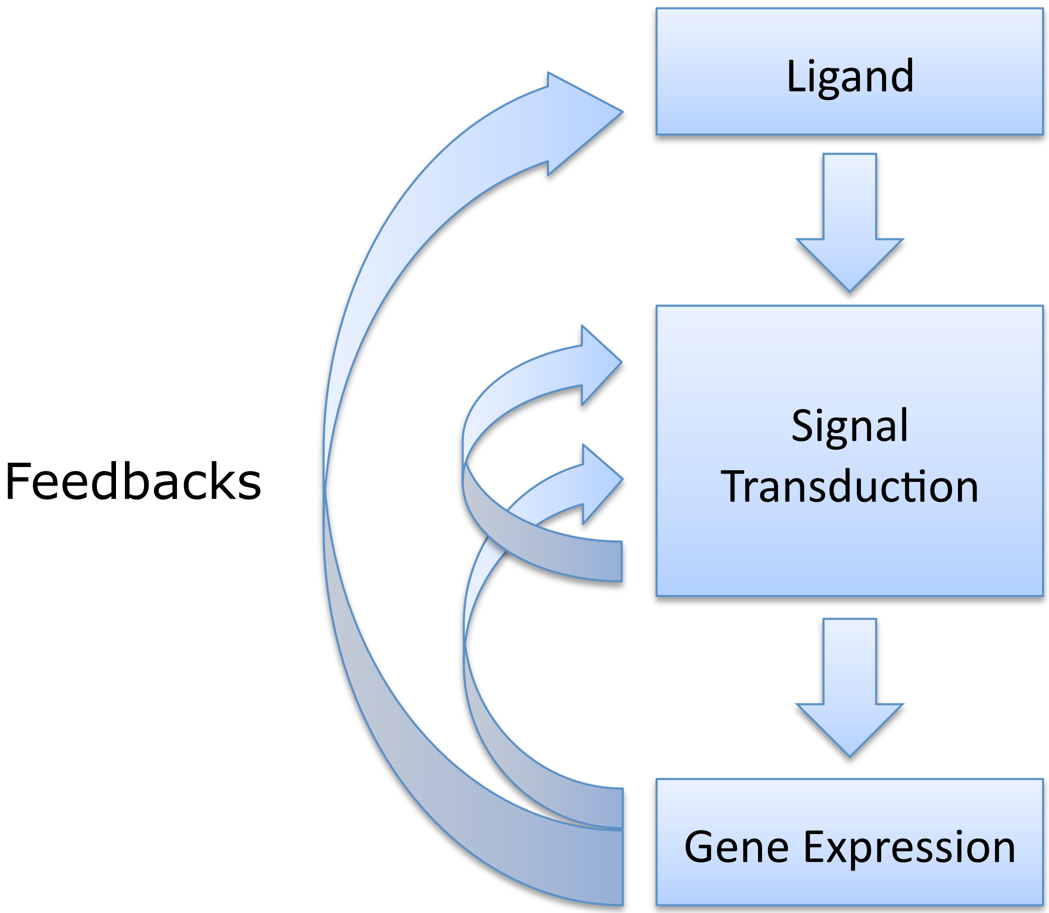
BICOID





Grimm O et al. Development 2010;137:2253-2264

Signal Transduction



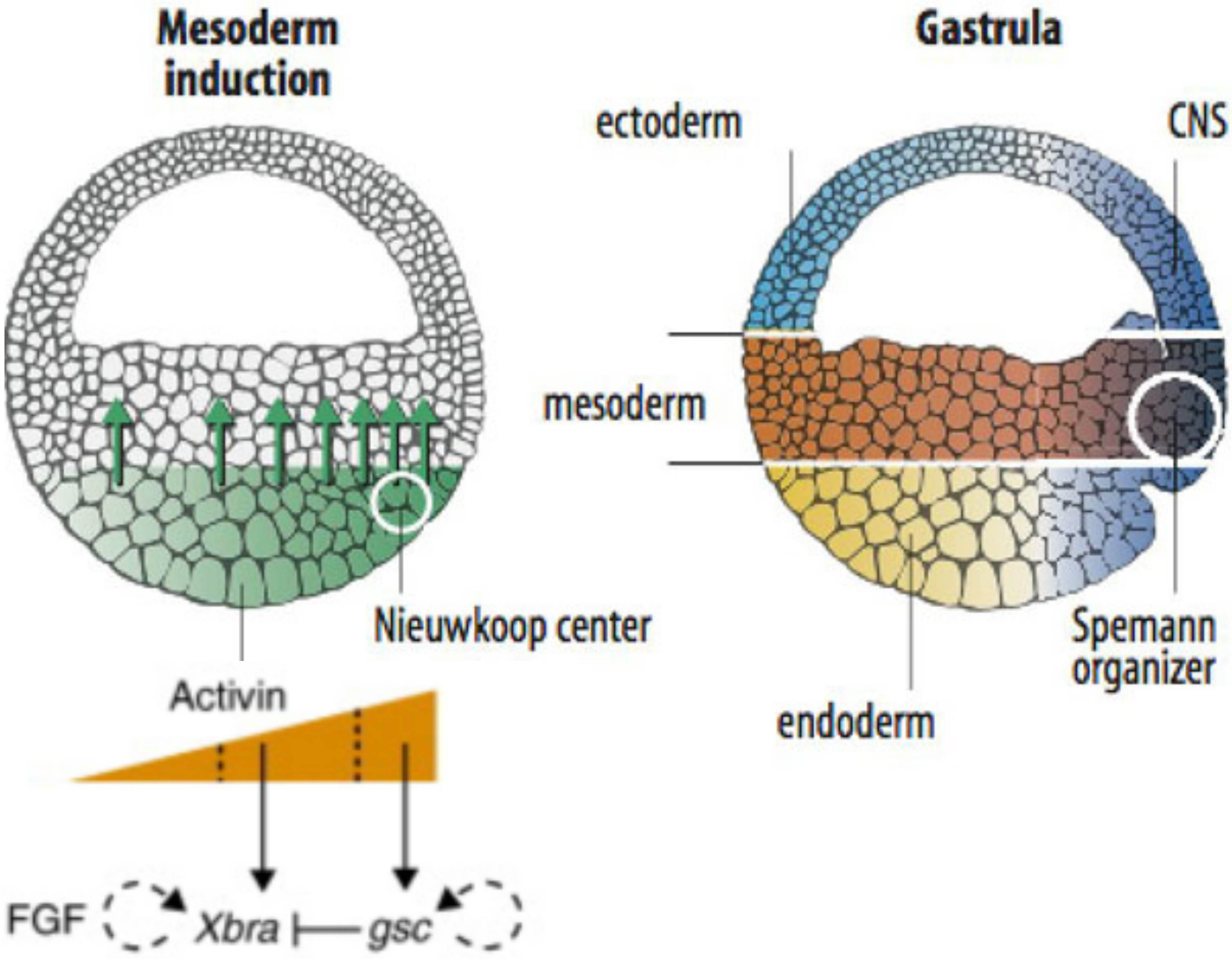
Dynamics

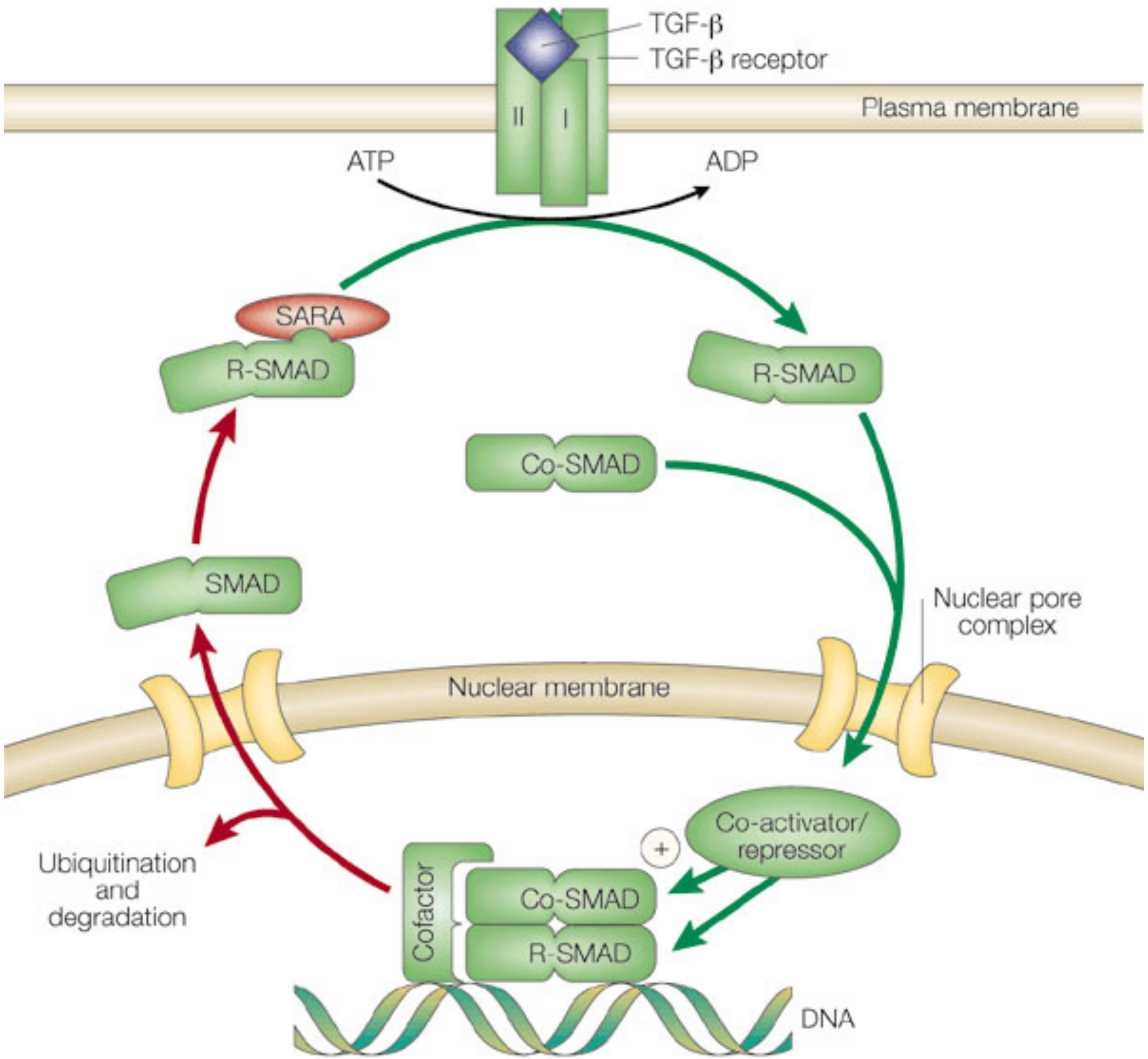
Non-linearities

Time Scales

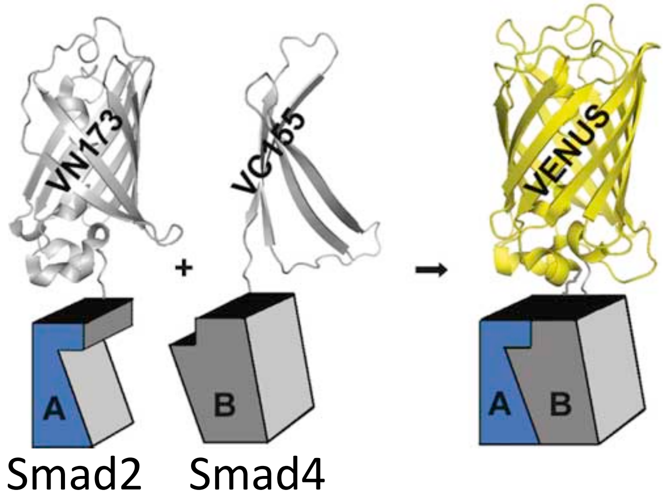
- Sensors and reporters – single cell, time resolved data
- Mechanistic models – interpret experimental observations

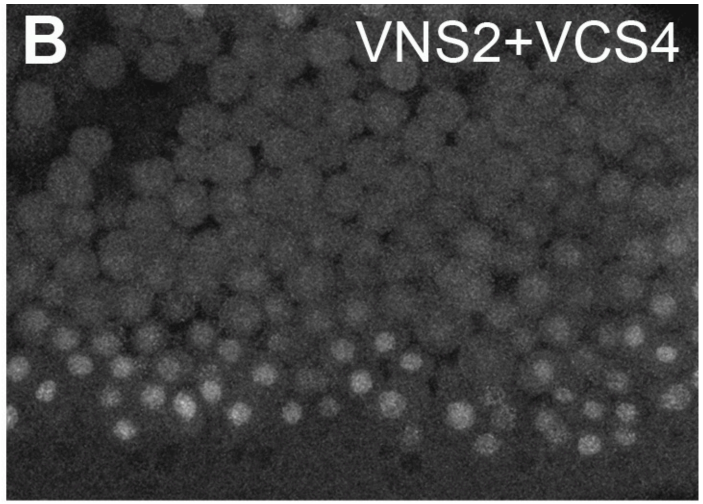
Graded signal transduction: Mesoderm

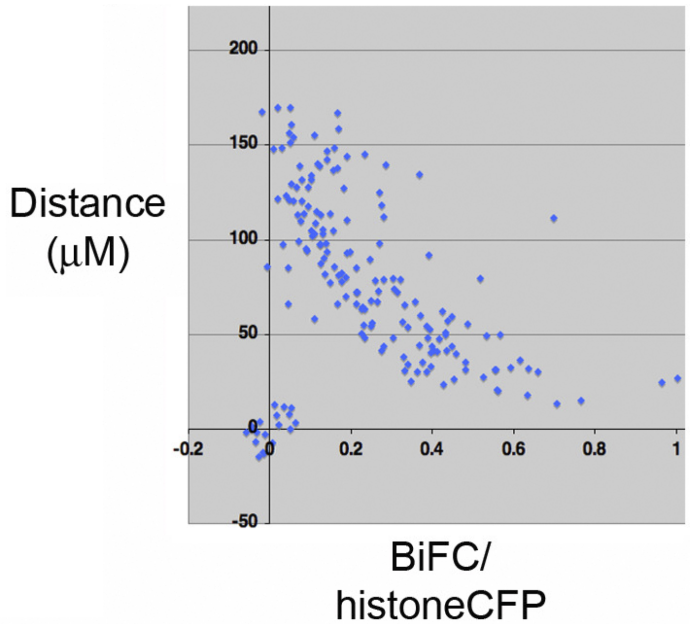



TGF-β
TGF-β receptor
Plasma membrane
II
I
ATP
ADP
SARA
R-SMAD
R-SMAD
Co-SMAD
SMAD
Nuclear pore
complex
Nuclear membrane
Co-activator/
repressor
+
Ubiquitination
and
degradation
Cofactor
Co-SMAD
R-SMAD
DNA

SMAD activity sensors

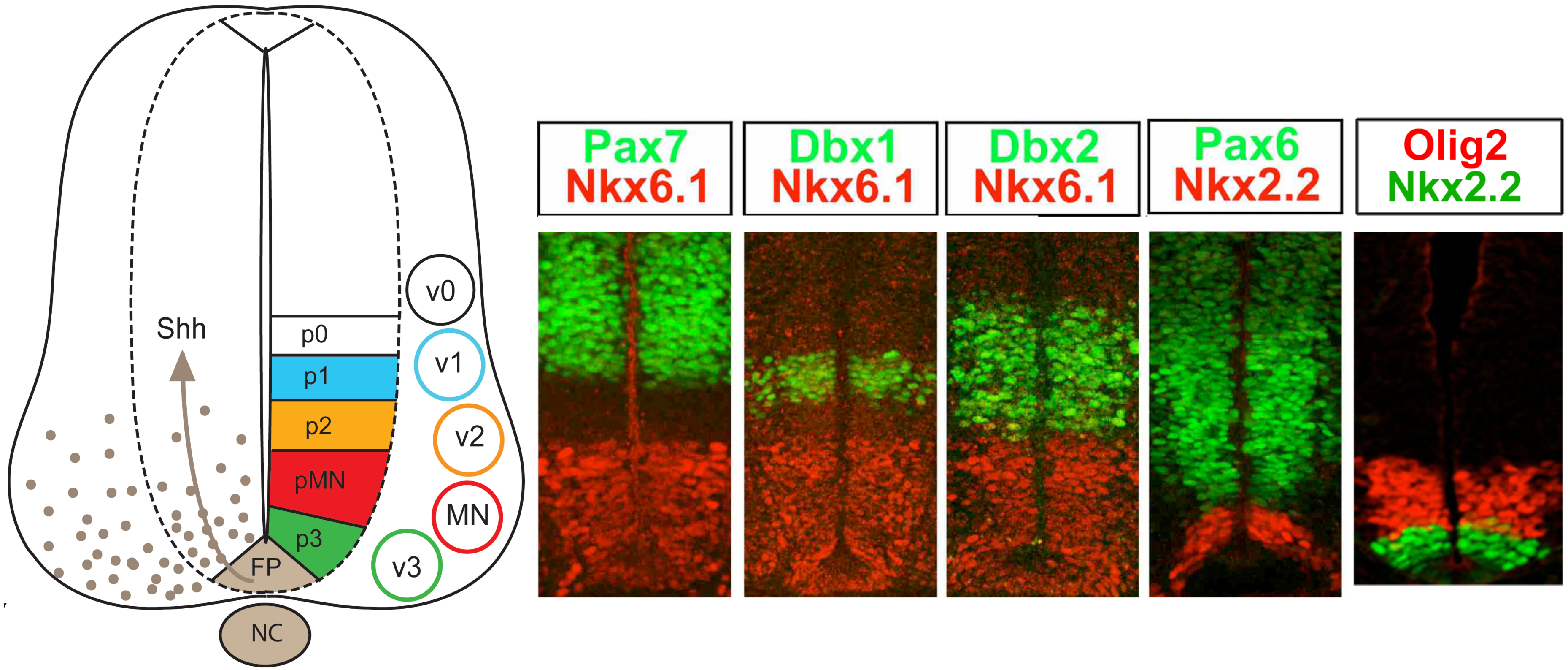




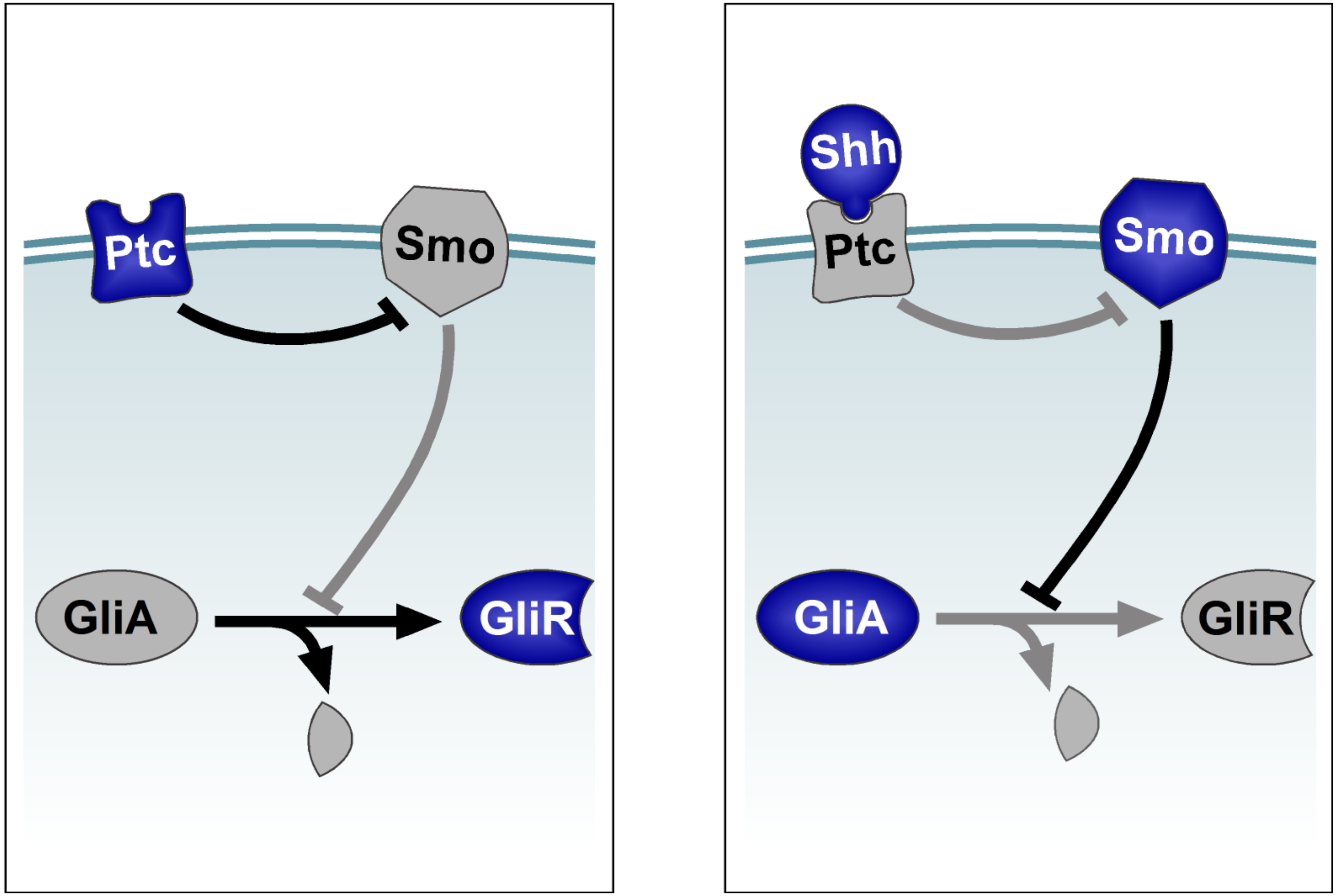


Harvey SA, Smith JC (2009) PLoS Biol 7(5): e1000101. doi:10.1371/journal.pbio.1000101

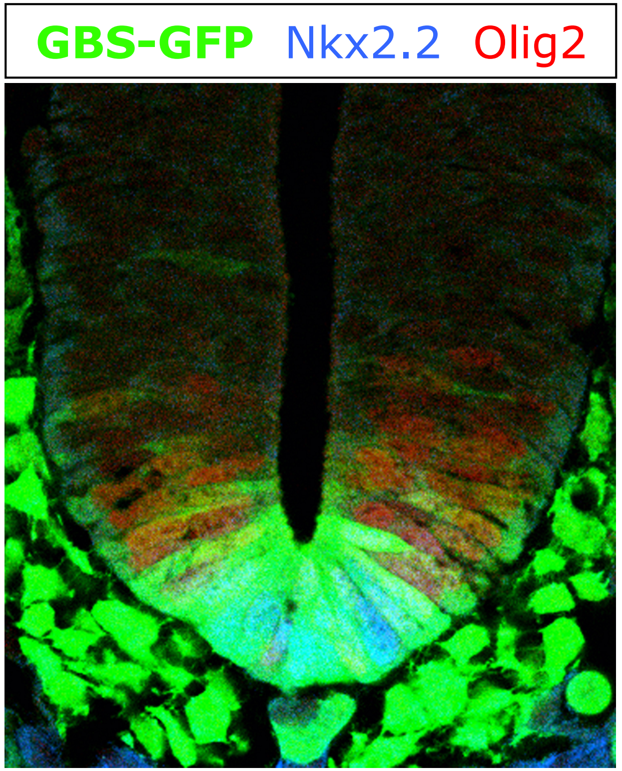
Shh signalling patterns neural progenitors

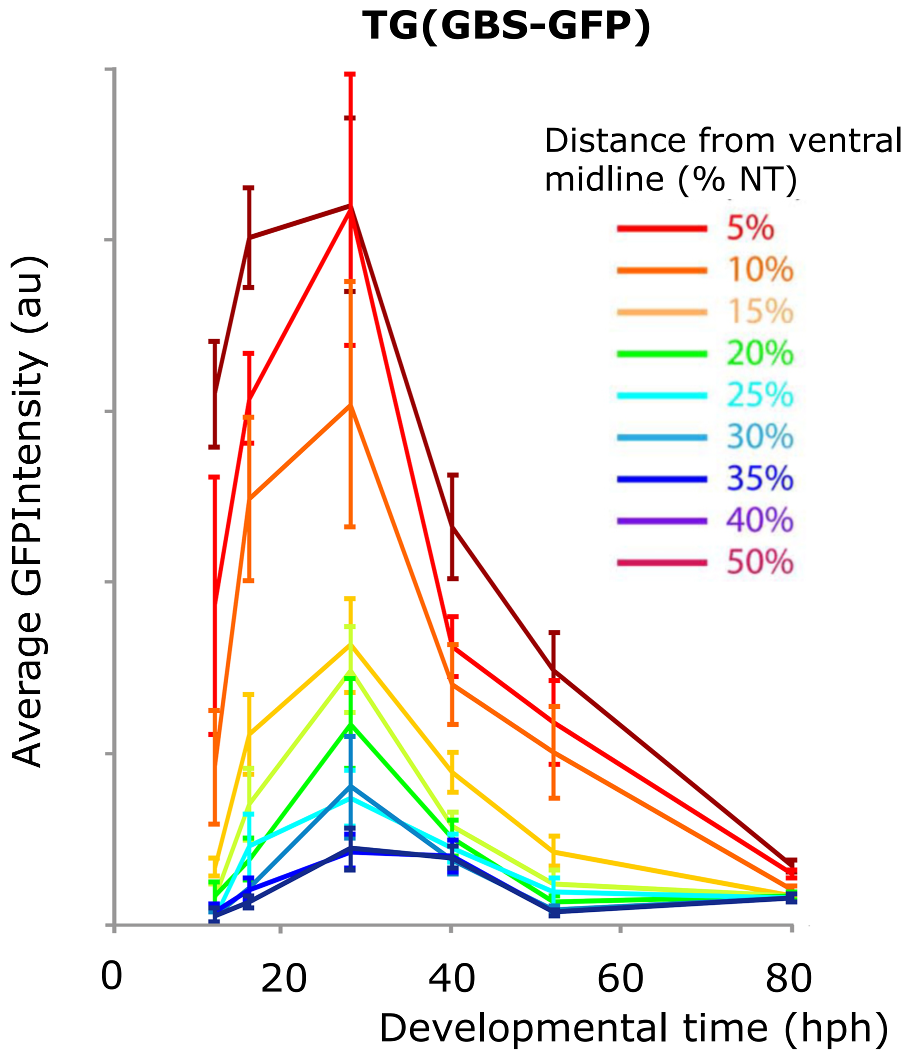


Intracellular communication



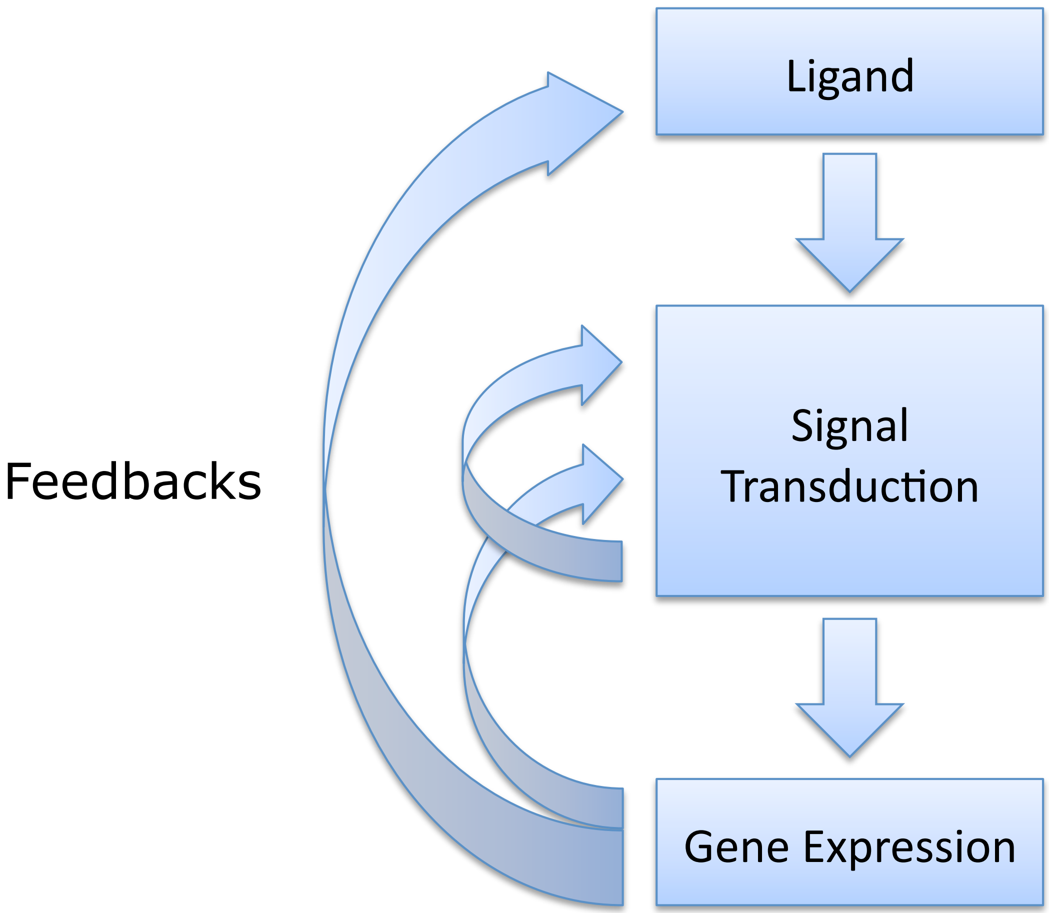
Gli activity is dynamic





Vanessa Ribes

Signal Transduction



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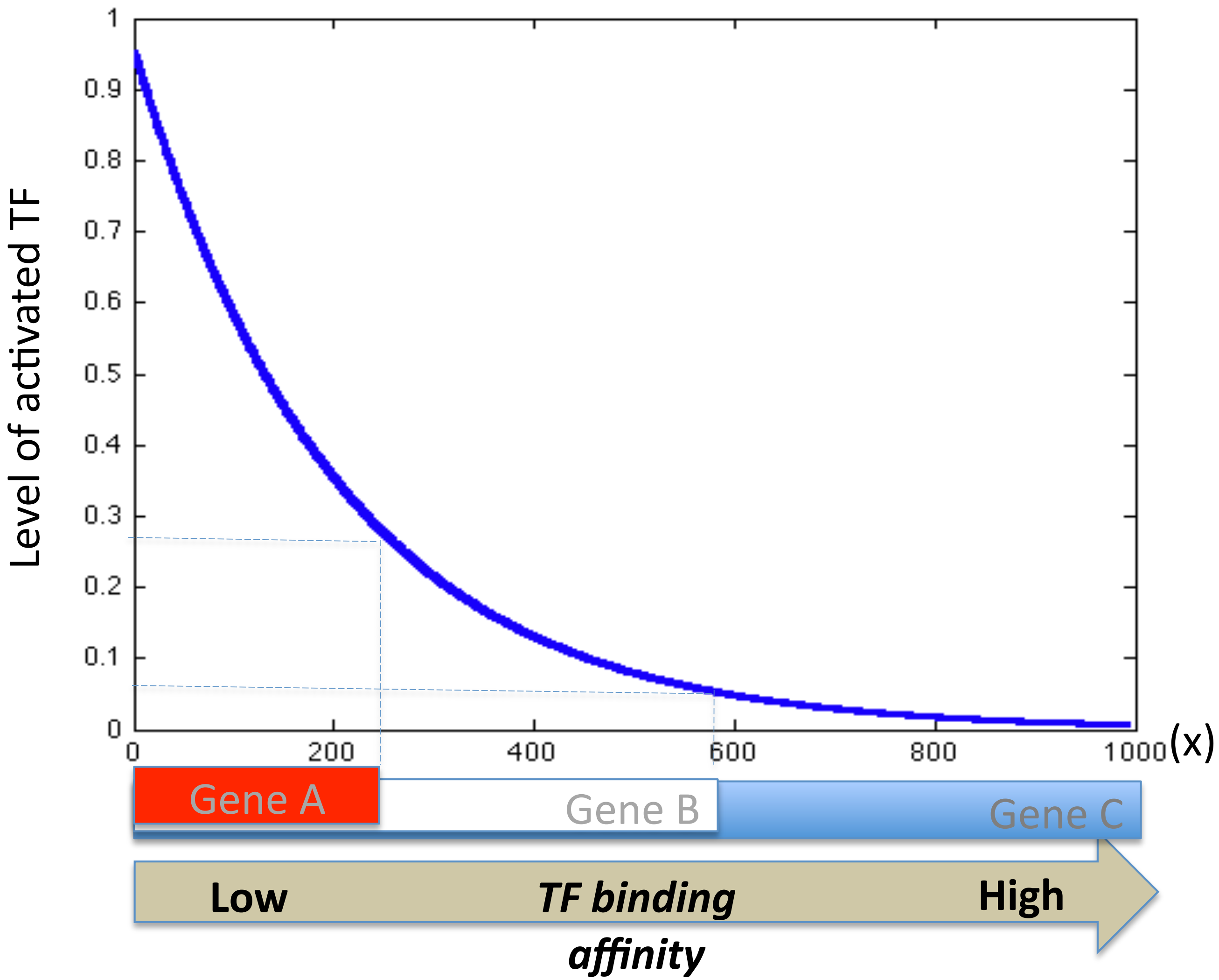
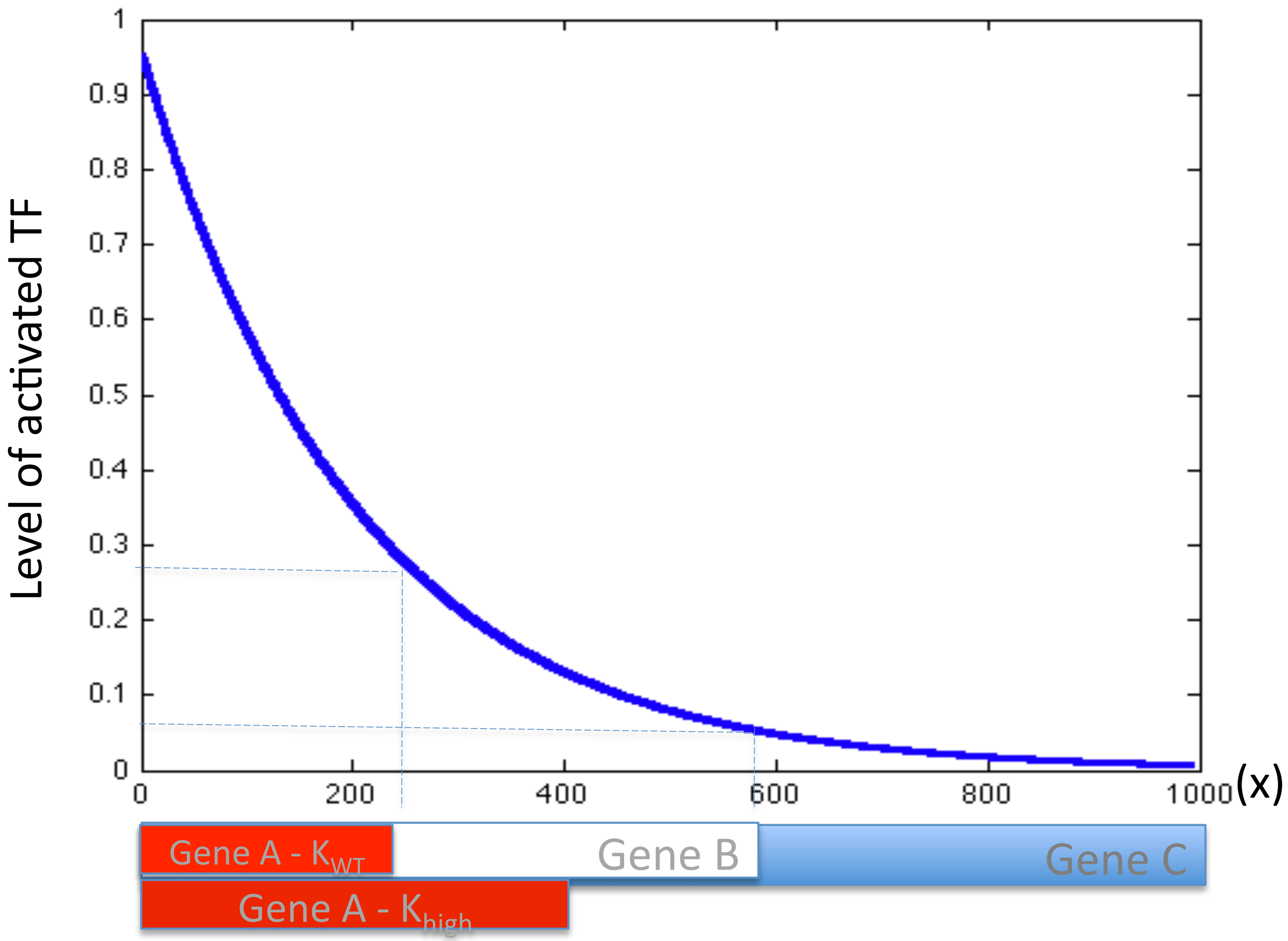
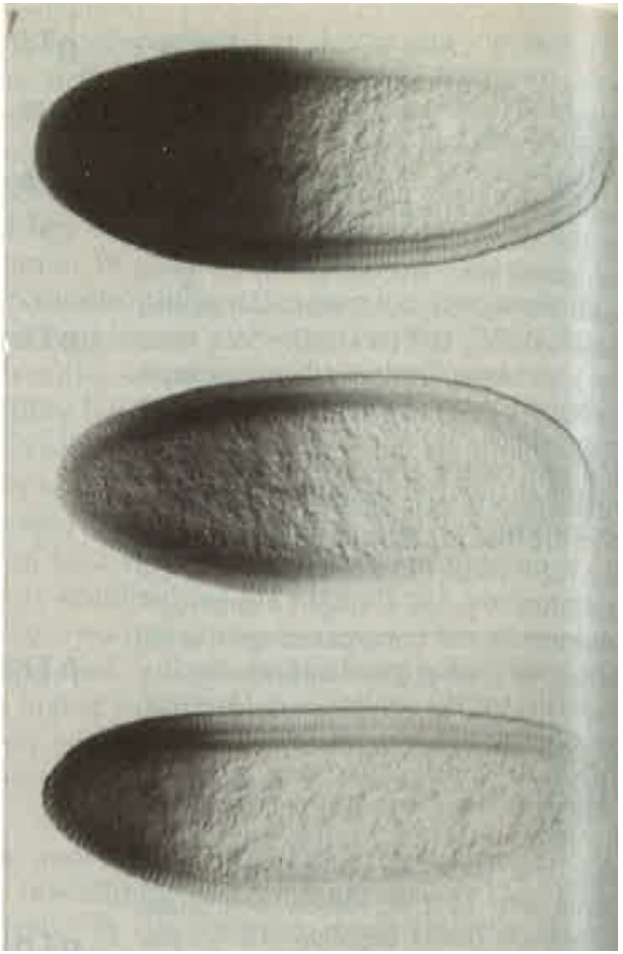
Affinity threshold model



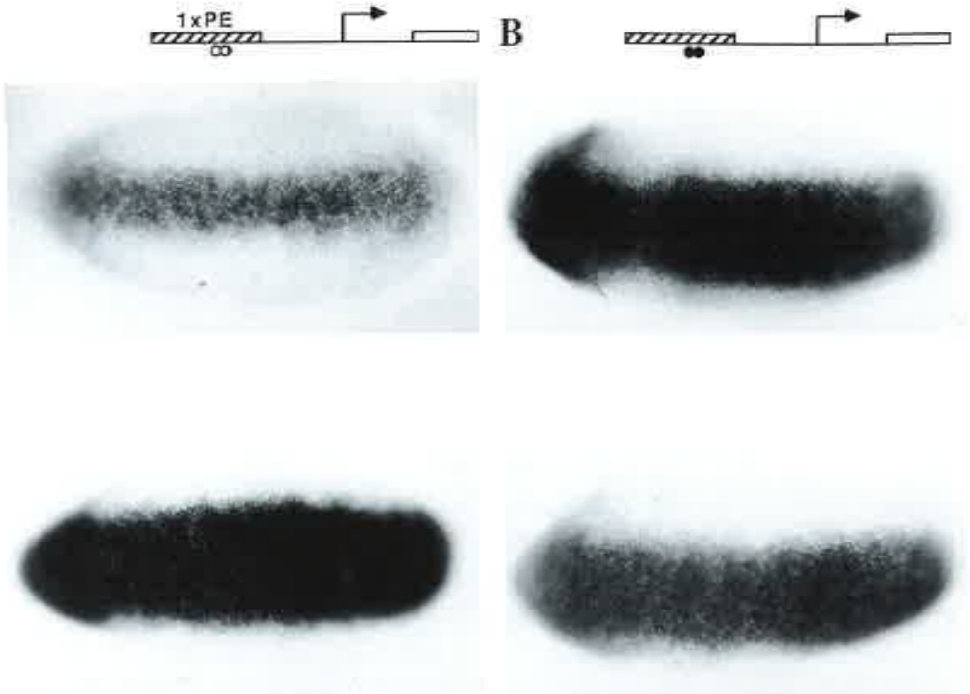
Affinity threshold model



Support for A-T model



Nature (1989) **340**, 363 – 367 W
WOLFGANG DRIEVER,
GUDRUN THOMA & CHRISTIANE NÜSSLEIN-VOLHARD



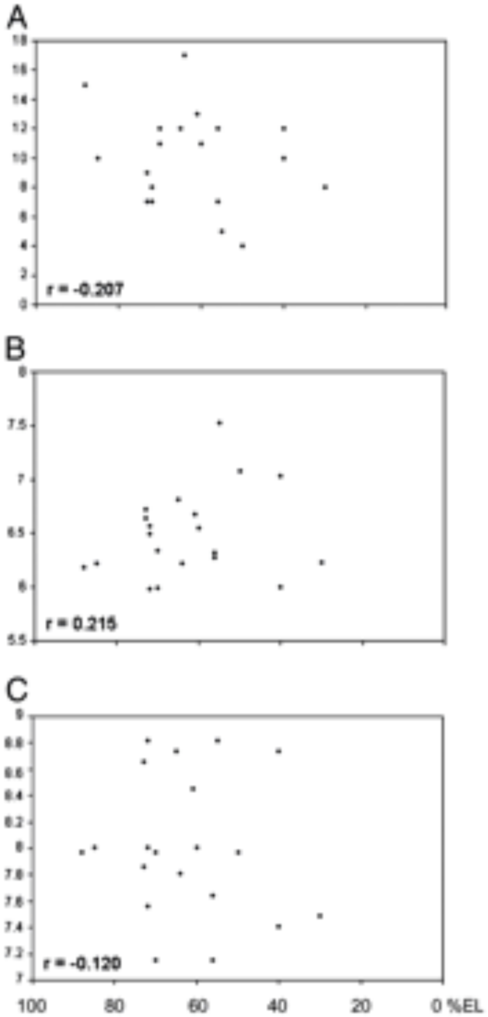
Cell (1993) 72:741–752
Jin Jiang and Michael Levine

Challenges to A-T model

Site Number

Average
PWM Score

Highest
PWM Score



A -> P

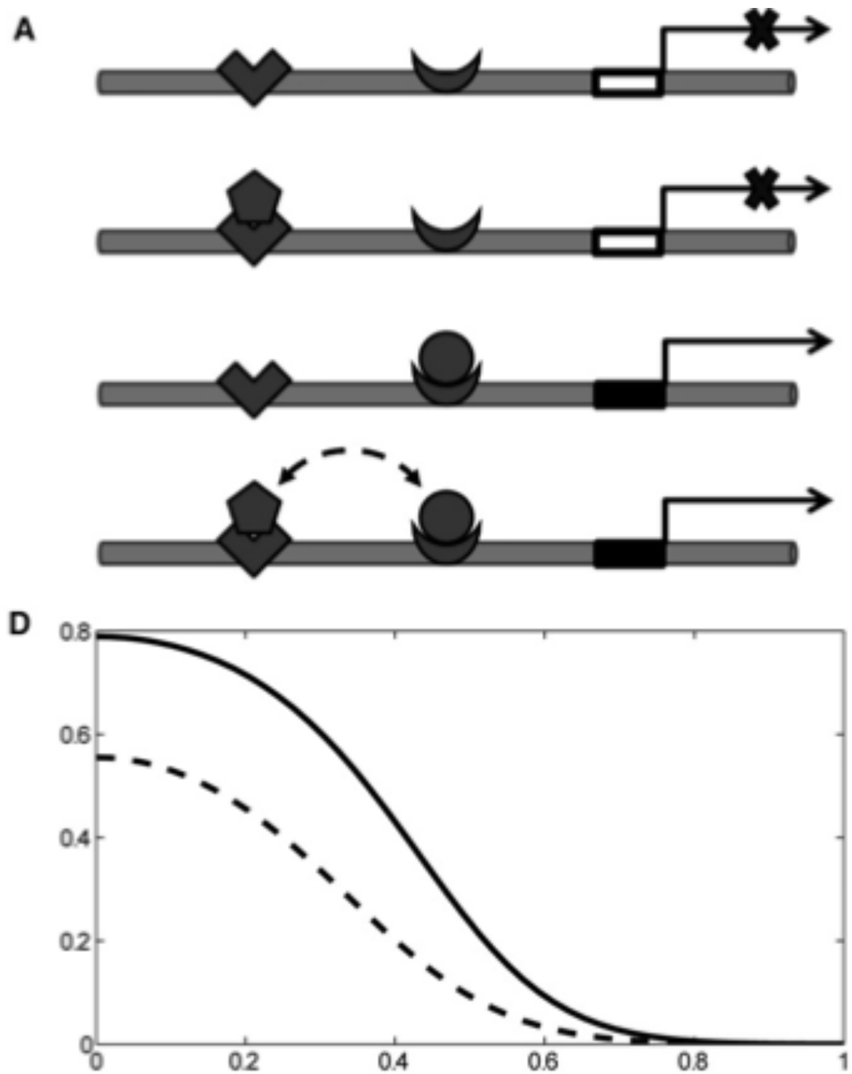
AP limit of expression

Comparison of the expression profile and Bcd binding sites of 21 genes

No correlation

Ochoa-Espinosa et al (2005) *Proceedings of the National Academy of Sciences* 102: 4960–4965

Uniformly expressed factors influence gene expression

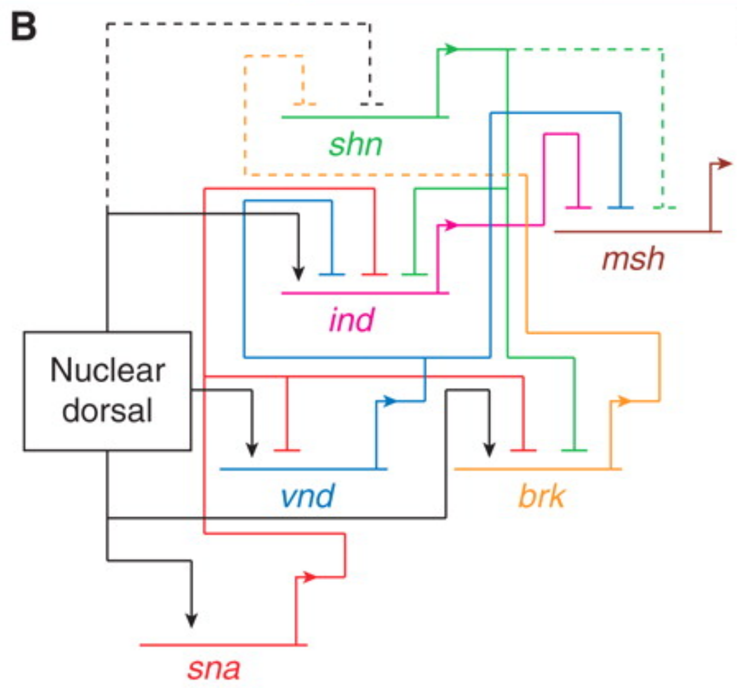


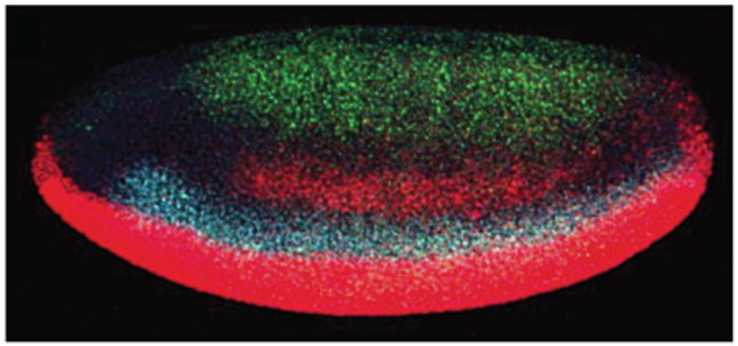
A spatially uniformly expressed TF (zelda) can coop with Morphogen TF (dorsal) to promote gene expression.

Decorrelates morphogen TF binding affinity from response

Kanodia et al (2012) *Biophysical Journal* 102: 427–433.

Morphogen target genes are also TFs



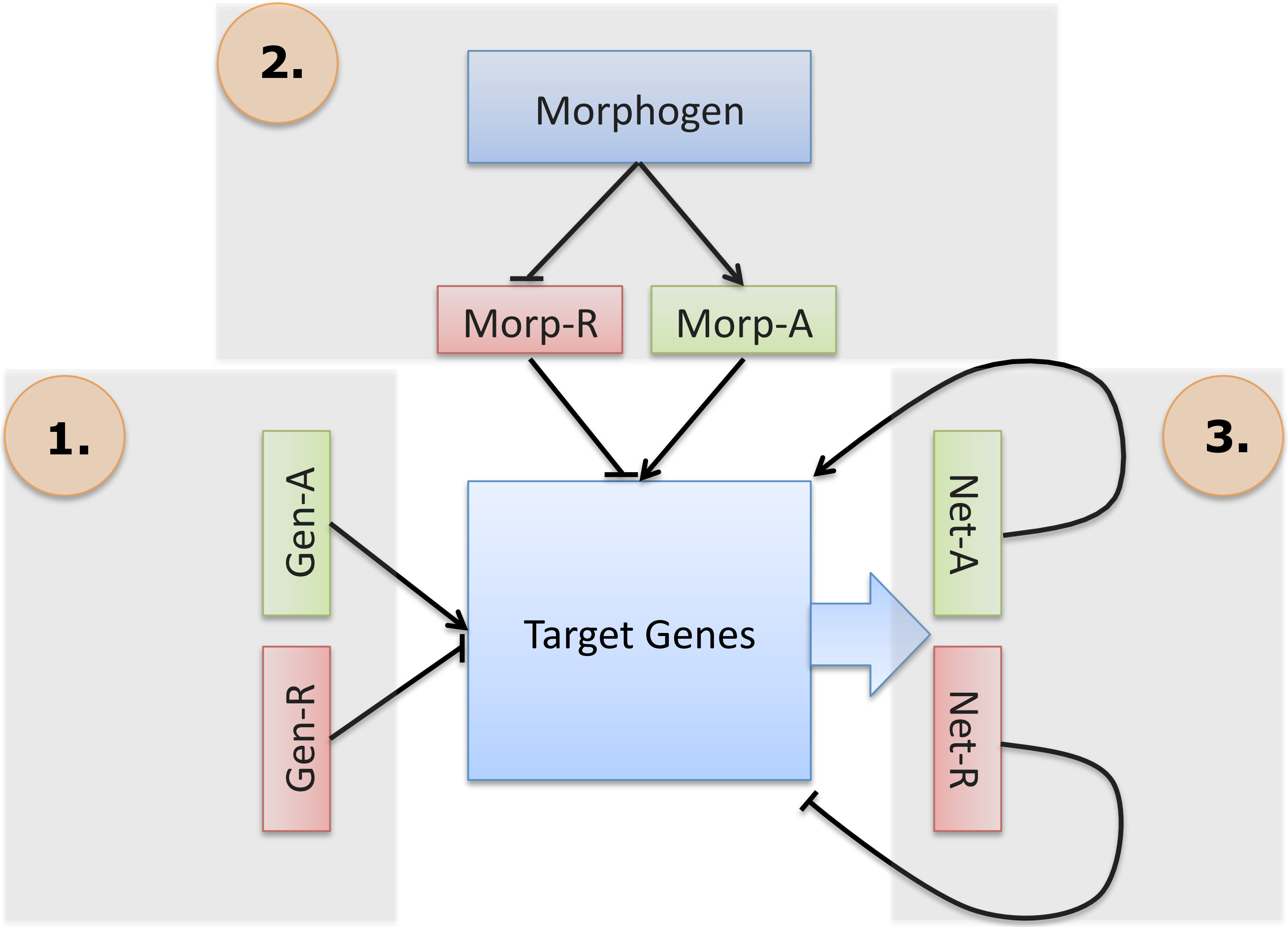


Reeves and Stathopoulos (2009) Cold Spring Harb Perspect Biol a000836

Regulation of Dorsal target genes involves positive and negative interactions between targets

Levine, Stathopoulos and many others

Three Components to Gene Regulation



Morphogen Interpretation

- How does the signaling pathway transform the extracellular ligand?
- How is differential gene expression encoded in cis-regulatory elements?
- **What role does the transcriptional network play in producing discrete spatial patterns of gene expression?**
- How does cell proliferation and tissue growth affect gradient interpretation?

Theoretical Analyses

Development 137, 2385-2395 (2010) doi:10.1242/dev.048033

Predicting embryonic patterning using mutual entropy fitness and in silico evolution

Paul François* and Eric D. Siggia

Molecular Systems Biology 6; Article number 425; doi:10.1038/msb.2010.74
Citation: ***Molecular Systems Biology*** **6:425**

www.molecularsystemsbiology.com

An atlas of gene regulatory networks reveals multiple three-gene mechanisms for interpreting morphogen gradients

James Cotterell[1,2] and James Sharpe[1,3,*]

Development 137, 2385-2395 (2010) doi:10.1242/dev.048033

Predicting embryonic patterning using mutual entropy fitness and in silico evolution

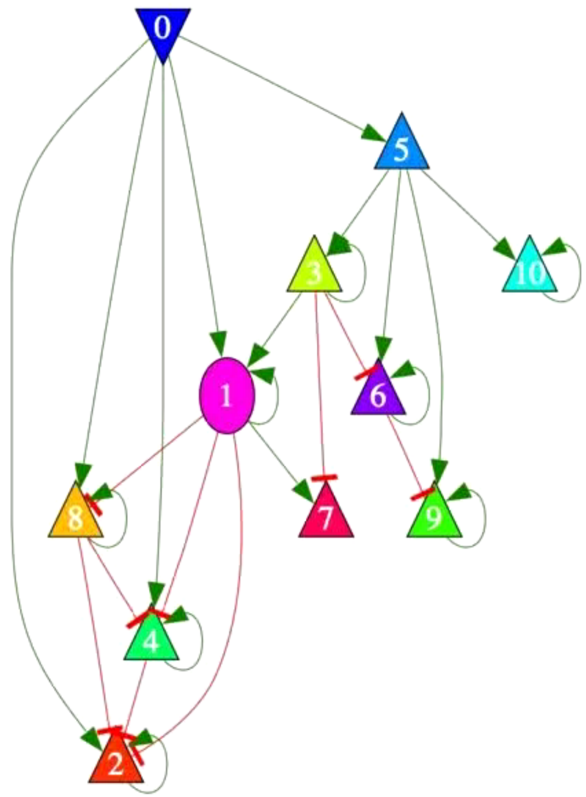
Paul François* and Eric D. Siggia

- In silico evolution of a txn network that patterns (in 1D) a tissue exposed to:
  - A static gradient that disappears before the end of development
  - A gradient that slides across the tissue

Dynamics of the network topology displayed on Fig. 3C

Network is under control of
a morphogen gradient (blue dotted),
time is developmental time

P. François, E. Siggia
pfrancois@rockefeller.edu

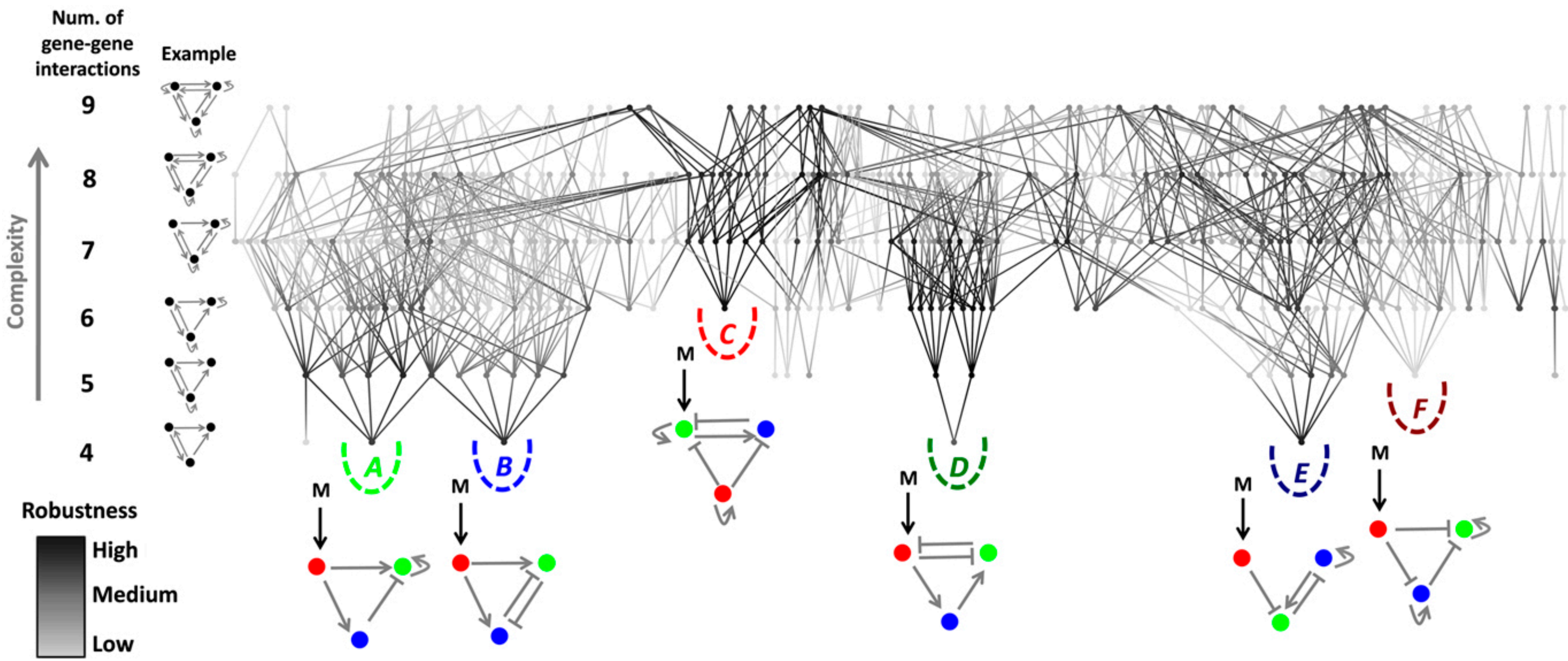


www.molecularsystemsbiology.com

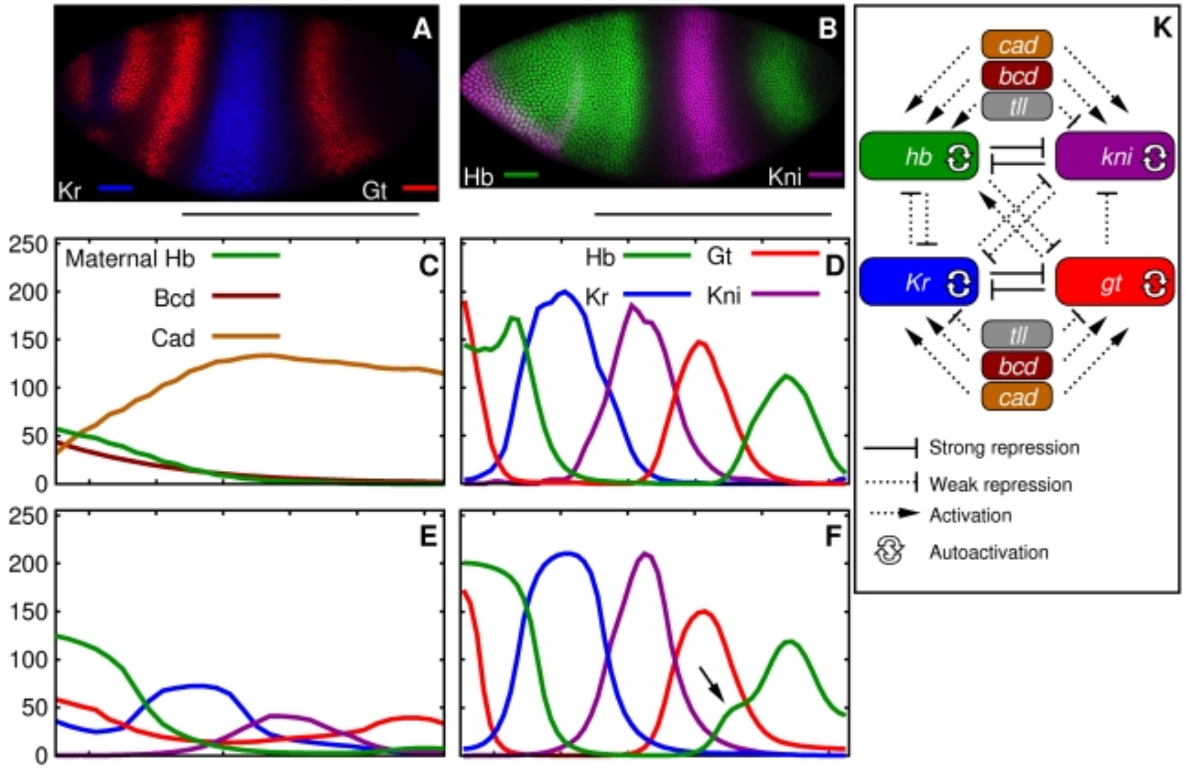
An atlas of gene regulatory networks reveals multiple three-gene mechanisms for interpreting morphogen gradients

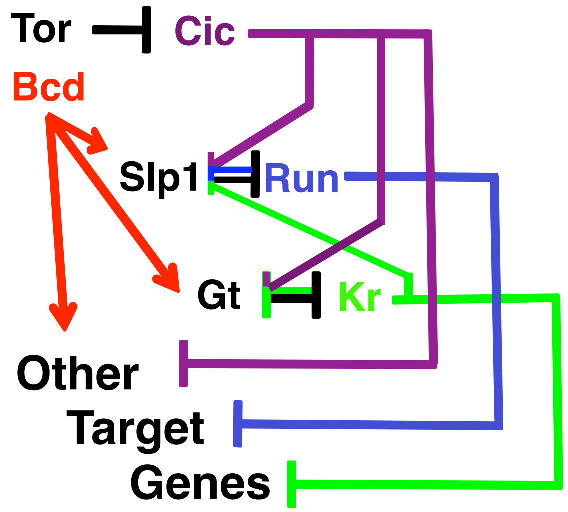
James Cotterell[1,2] and James Sharpe[1,3,*]

- Simulated an 'atlas' of 3 gene networks regulated by a graded input.
- Identified all networks that gave a 'morphogen' – stripe – output
- Classified these networks into ~6 distinct mechanisms



Real World Examples

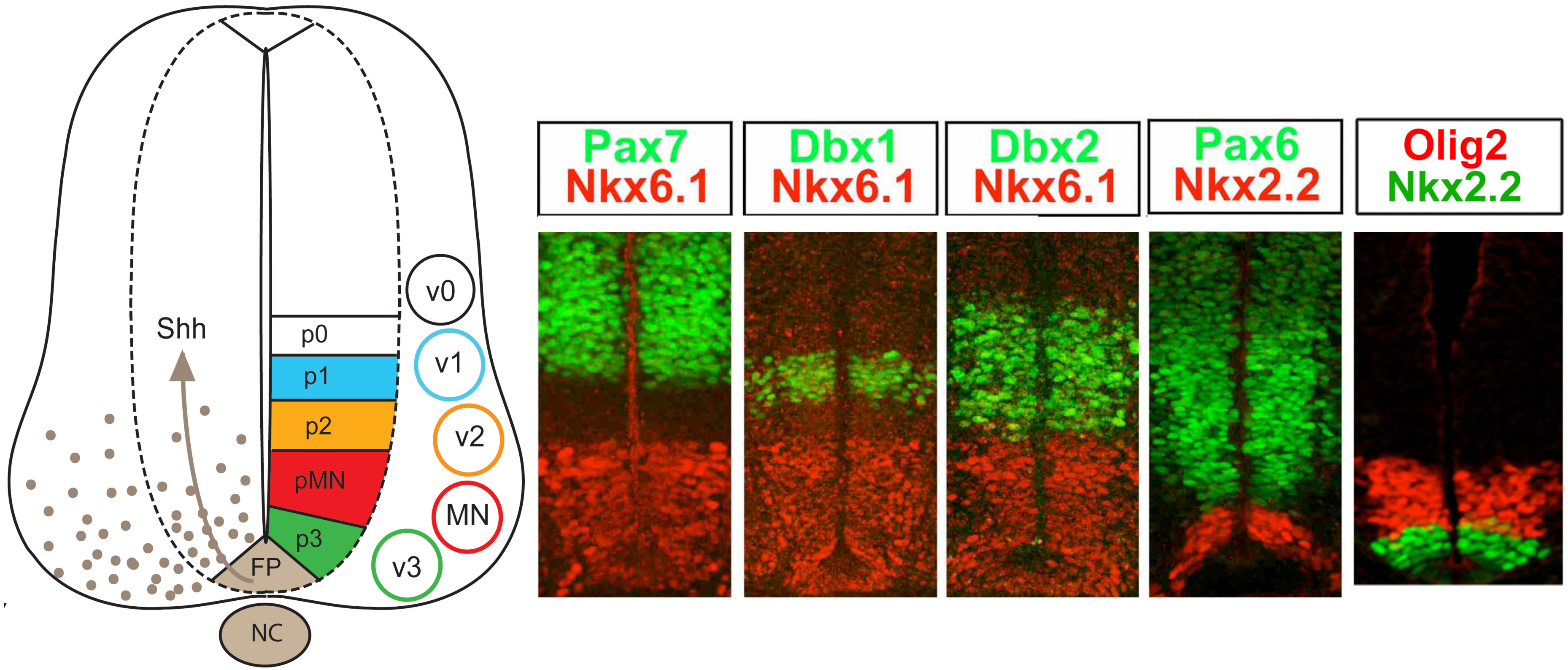




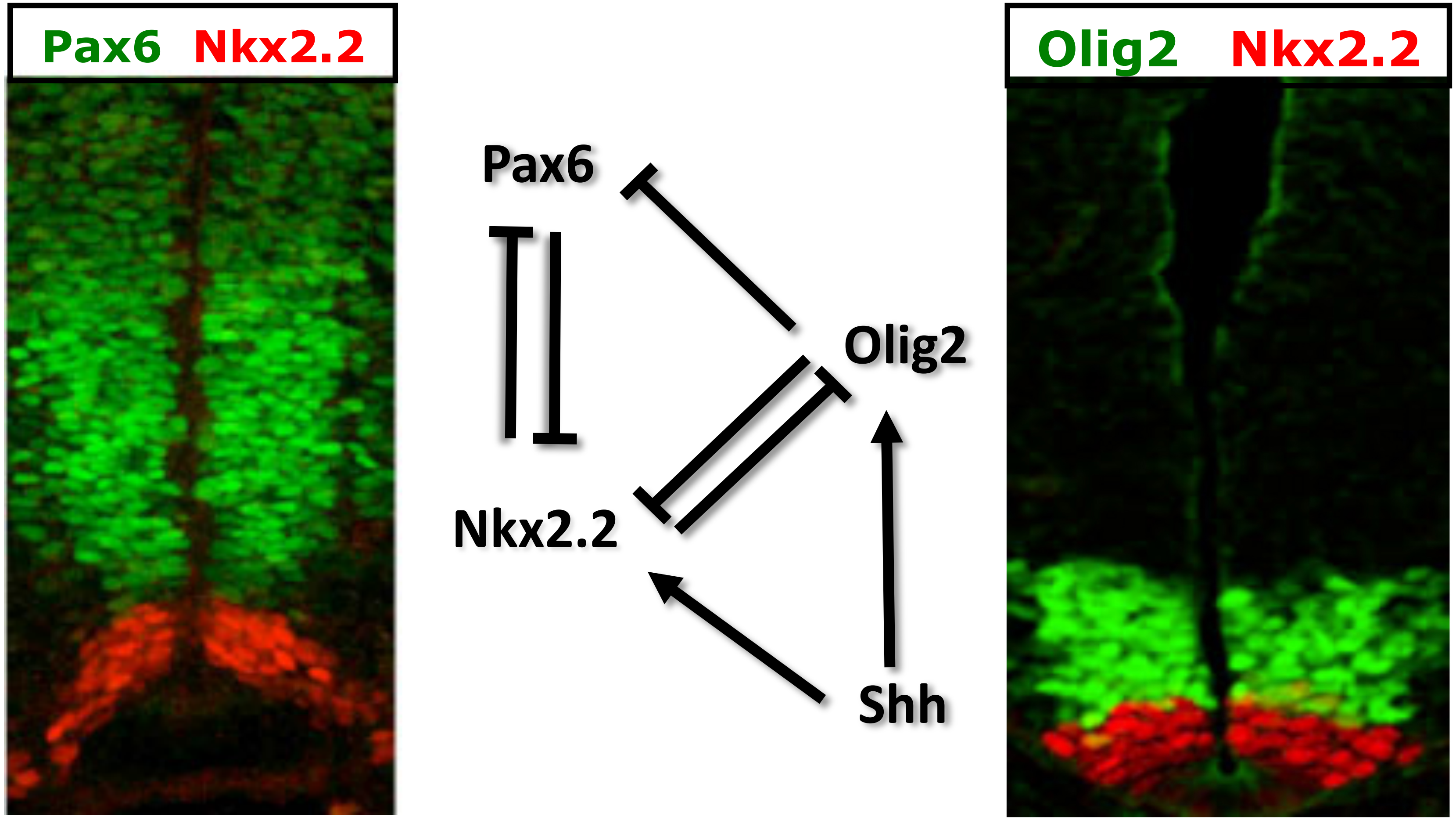
Reinitz and colleagues
Jaeger et al (2004) *Nature* 430: 368–371
Manu et al. *PLoS Biology* 7: e1000049

Small and colleagues
Chen et al (2012) *Cell* 149: 618–629

Shh signalling patterns progenitors

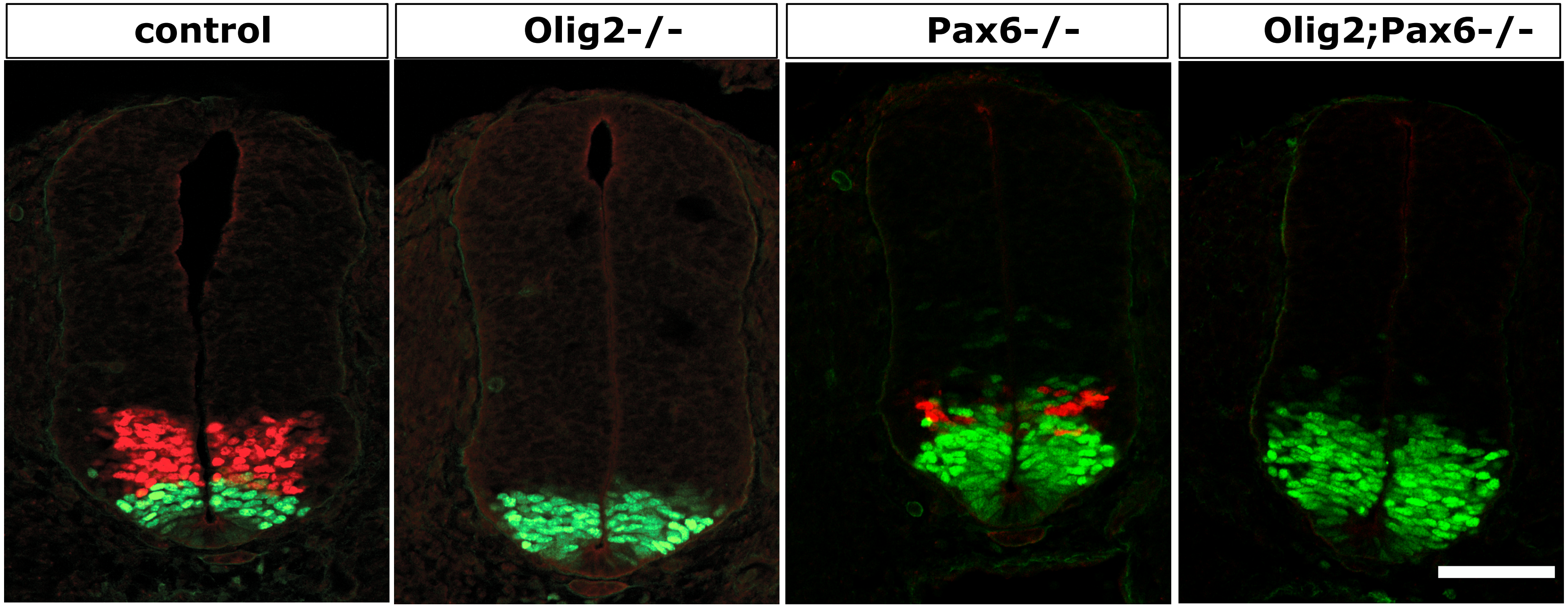


A transcriptional circuit for morphogen interpretation



Pax6 and Olig2 repress Nkx2.2

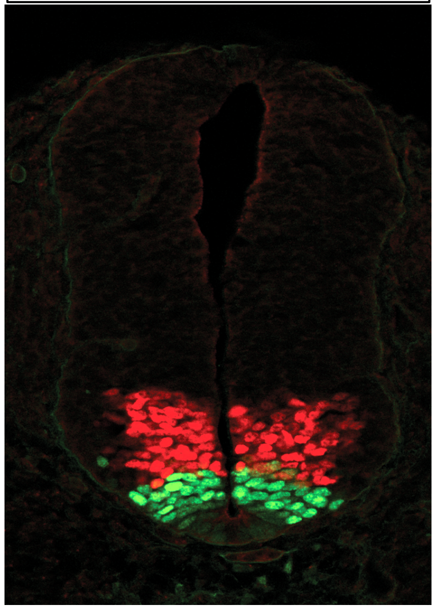
Olig2 Nkx2.2

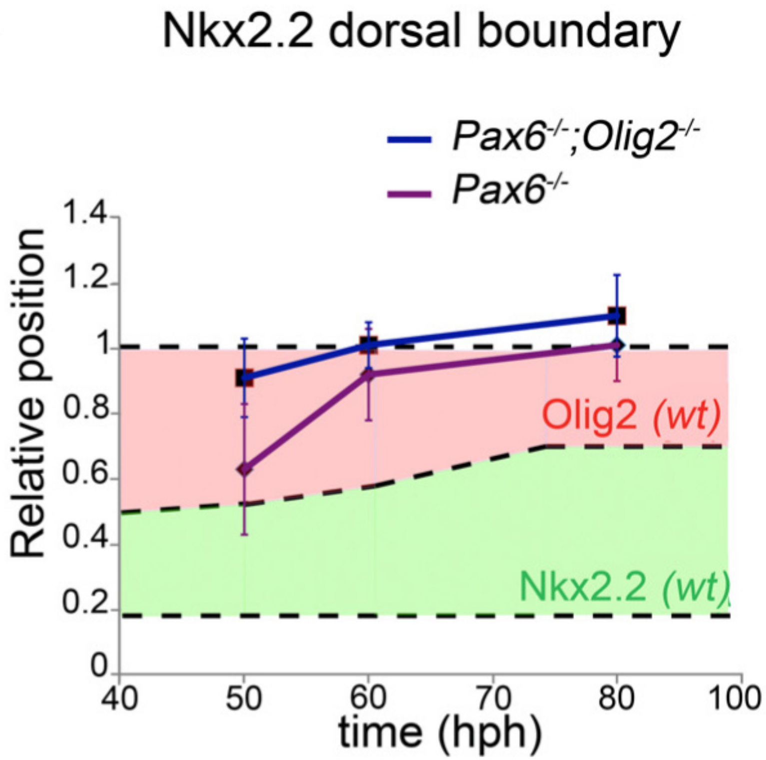


Nikos Balaskas

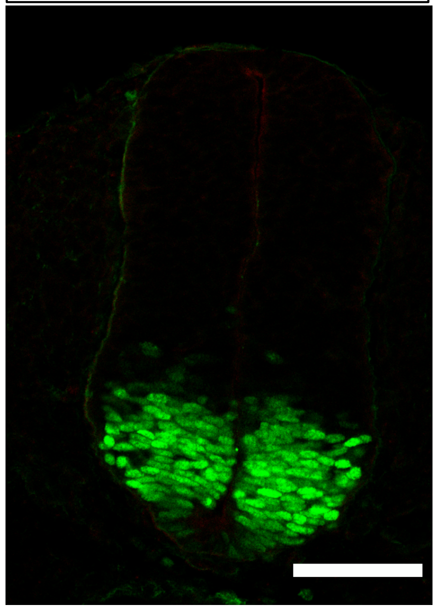
Network generates morphogen response

control



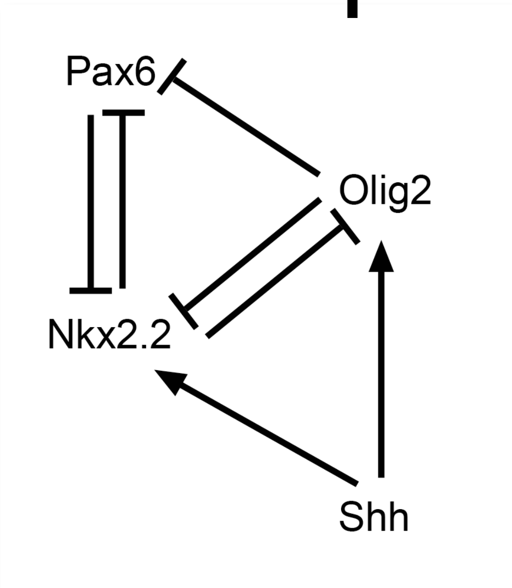


Olig2;Pax6-/-



Nikos Balaskas

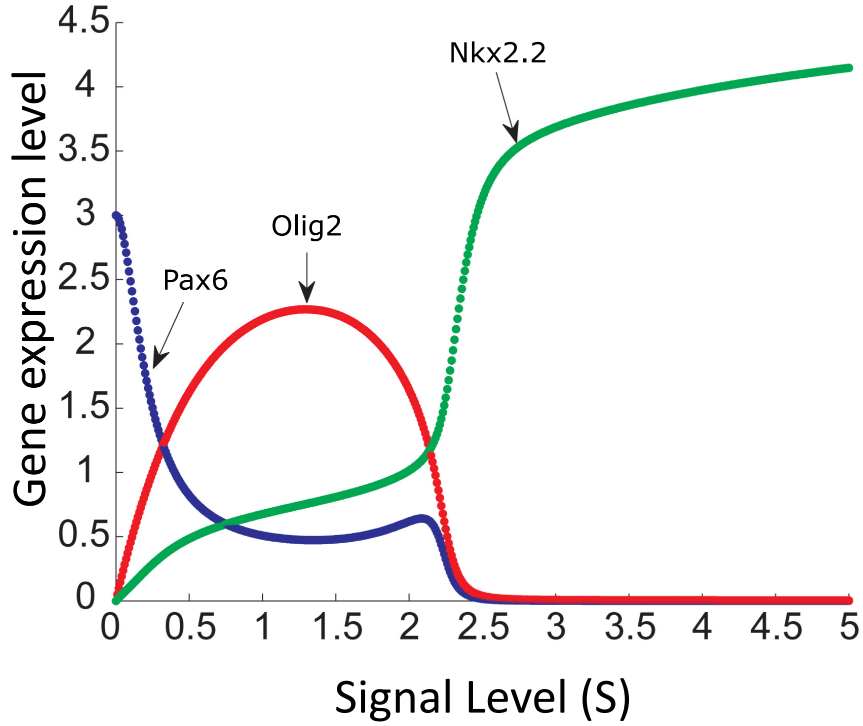
Network generates morphogen response



$$\frac{dP}{dt} = \frac{\alpha}{1 + \left(\frac{N}{N_{critP}}\right)^{h1} + \left(\frac{O}{O_{critP}}\right)^{h2}} - k_1 P$$ Pax6 (P)

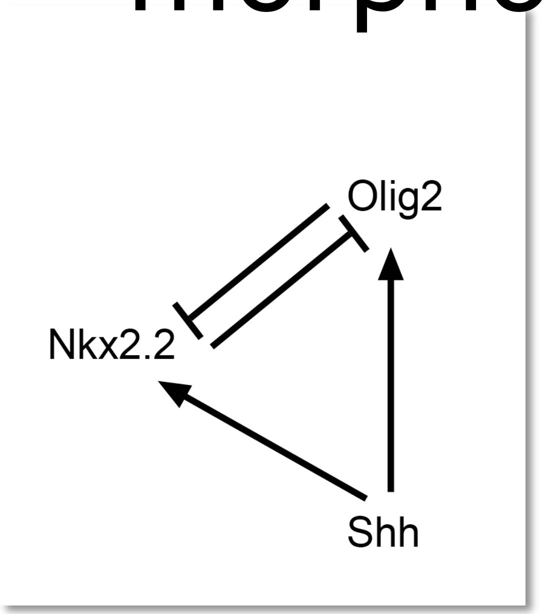
$$\frac{dO}{dt} = \frac{\beta S}{1+S} \times \frac{1}{1 + \left(\frac{N}{N_{critO}}\right)^{h3}} - k_2 O$$ Olig2 (0)

$$\frac{dN}{dt} = \frac{\gamma S}{1+S} \times \frac{1}{1 + \left(\frac{O}{O_{critN}}\right)^{h4} + \left(\frac{P}{P_{critN}}\right)^{h5}} - k_3 N$$ Nkx2.2 (N)



Karen Page and Jasmina Panovska

Network generates morphogen response



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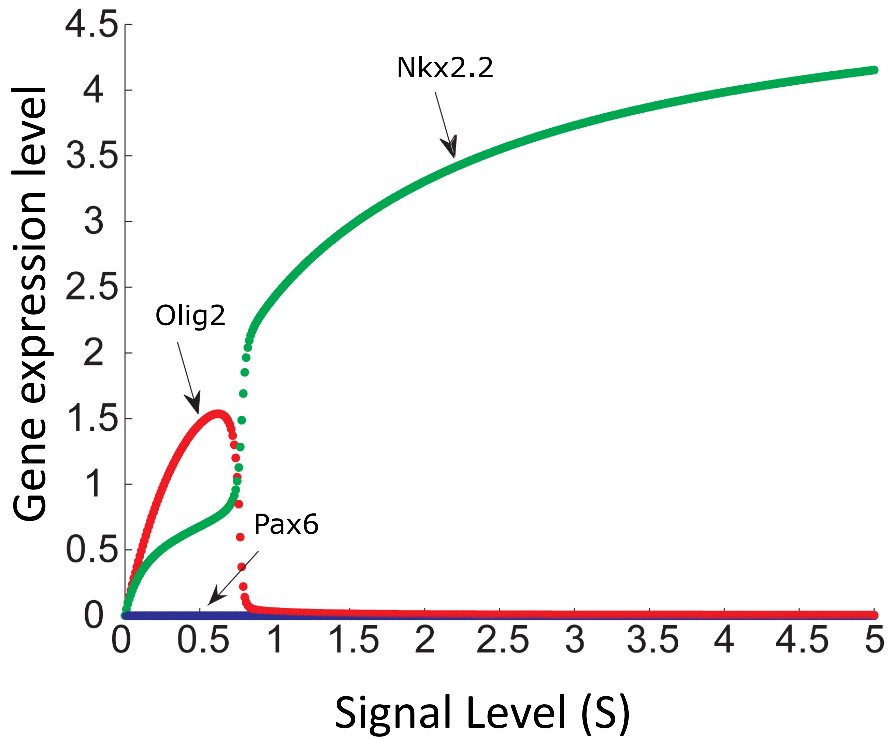
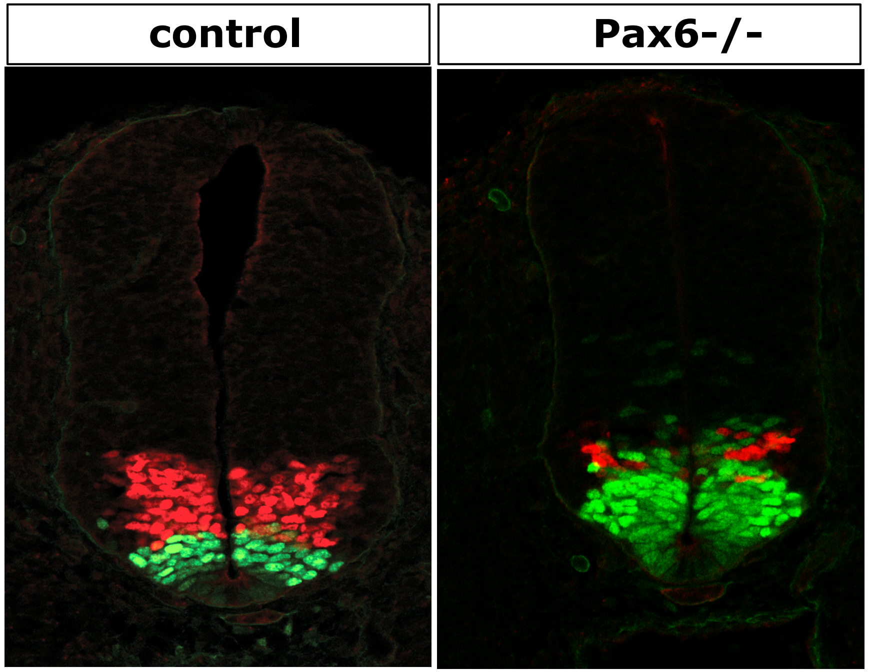
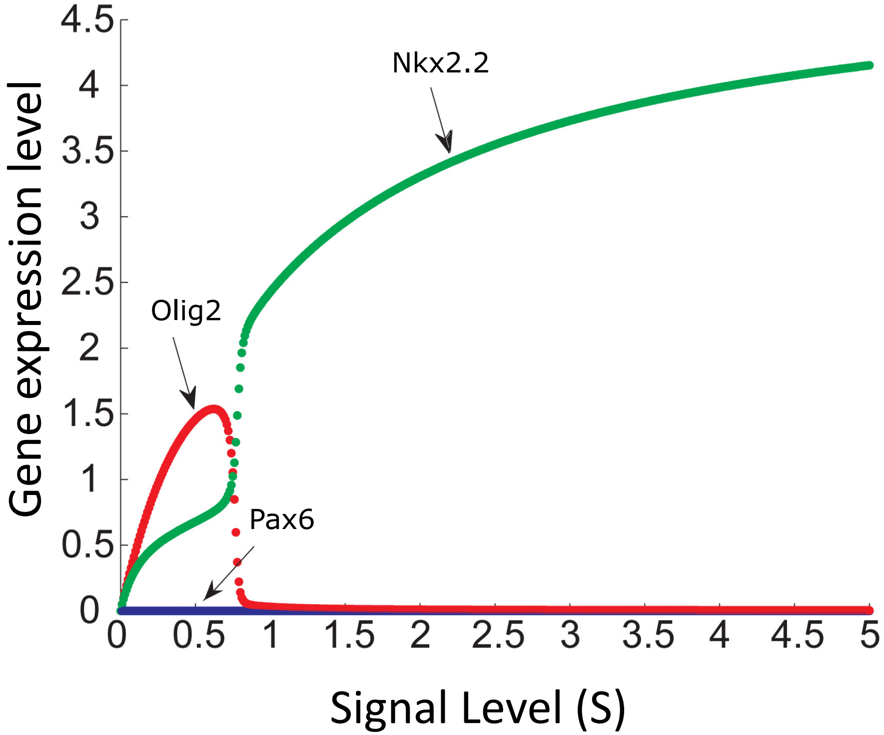


Jasmina Panovska

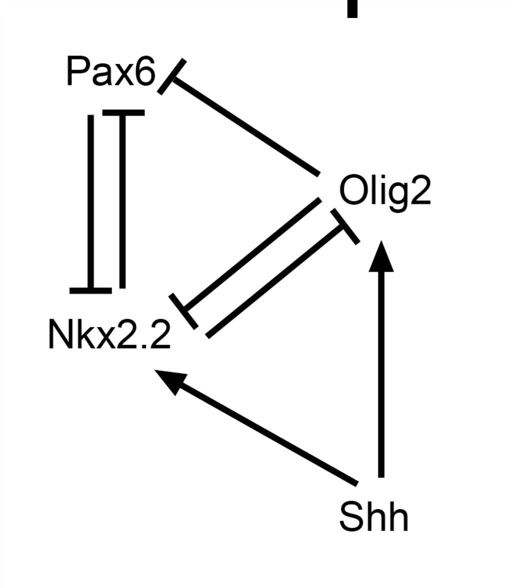
Network generates morphogen response





Nikos Balaskas

Network generates morphogen response



$$\frac{dP}{dt} = \frac{\alpha}{1 + \left(\frac{N}{N_{critP}}\right)^{h1} + \left(\frac{O}{O_{critP}}\right)^{h2}} - k_1 P$$

Pax6 (P)

$$\frac{dO}{dt} = \frac{\beta S}{1 + S} \times \frac{1}{1 + \left(\frac{N}{N_{critO}}\right)^{h3}} - k_2 O$$

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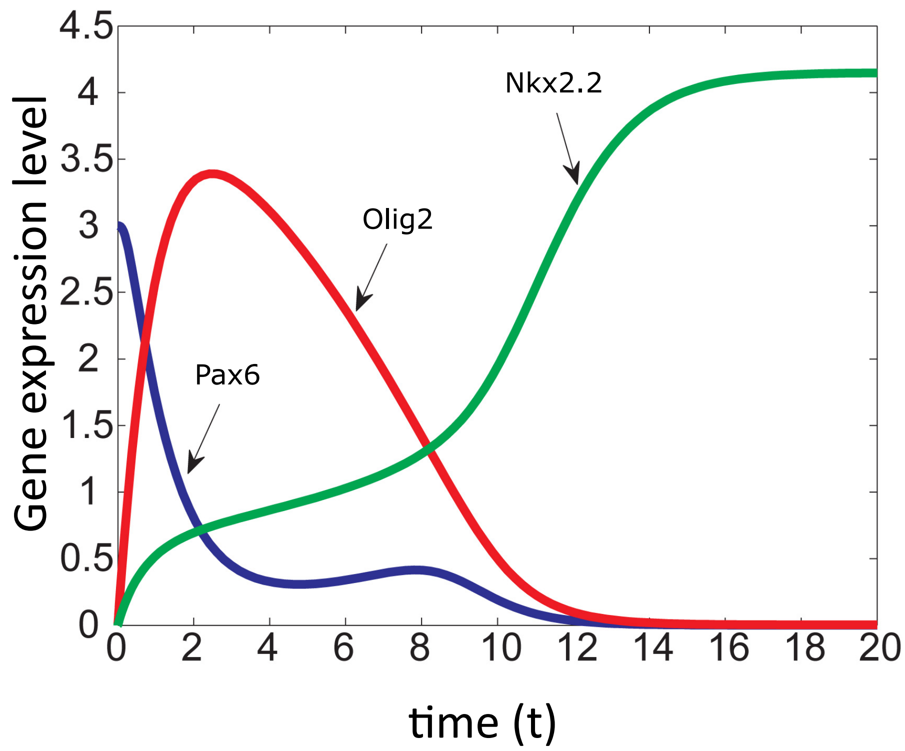
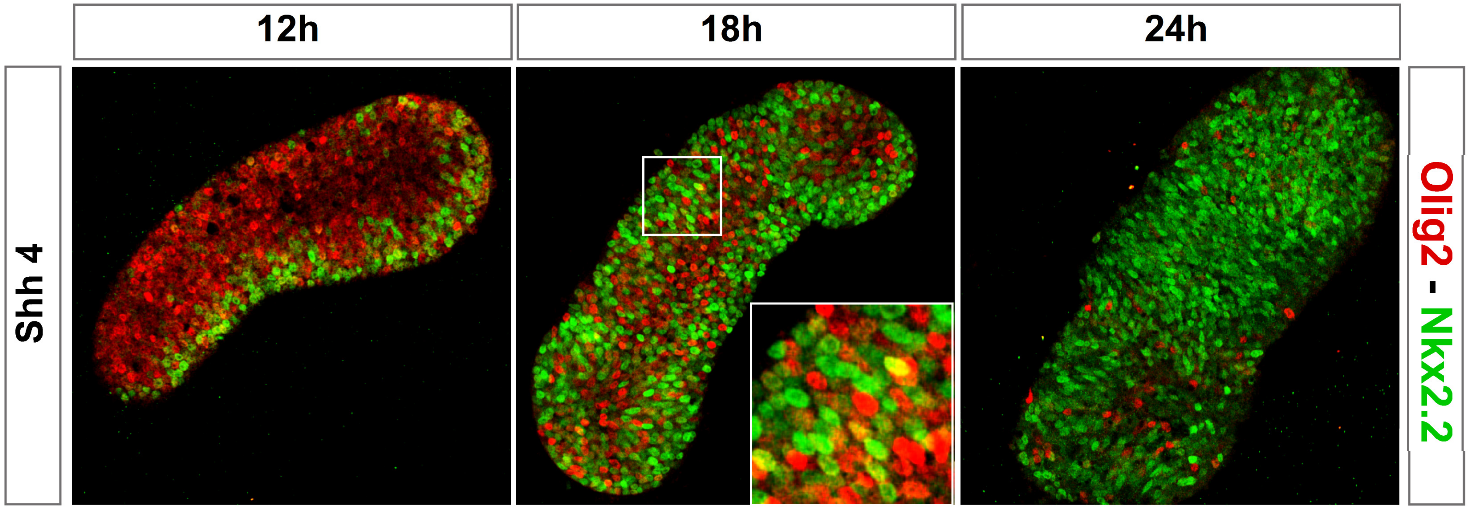
Nkx2.2 (N)



Jasmina Panovska

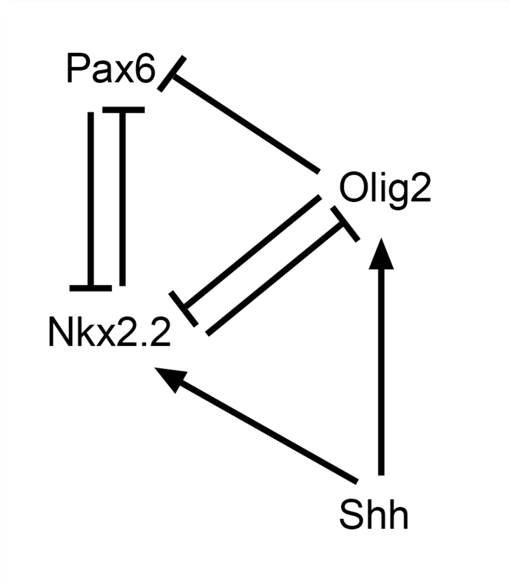
Duration of signalling influences pattern



number of cells

Eric Dessaud

Network confers hysteresis on Nkx2.2



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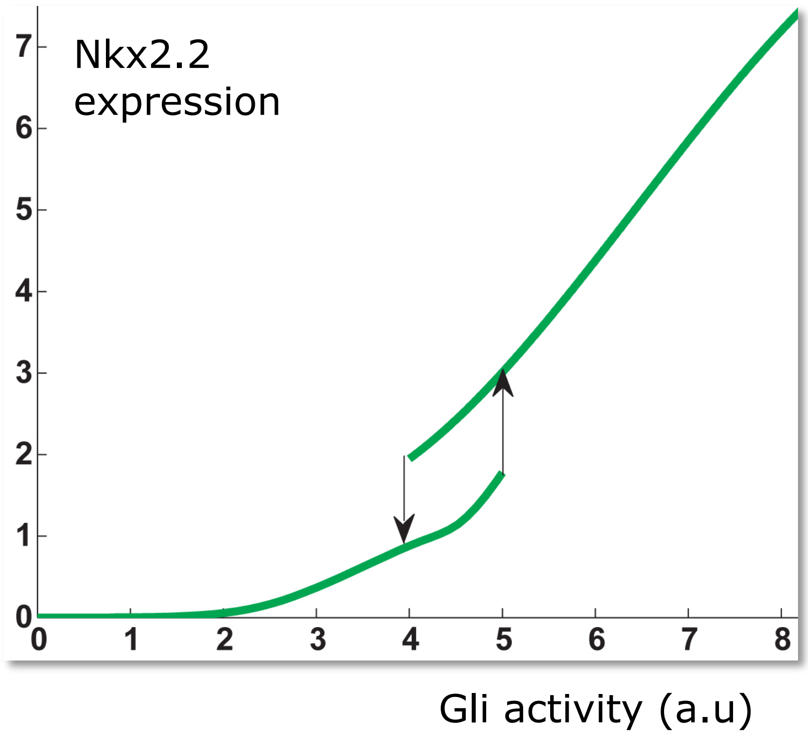
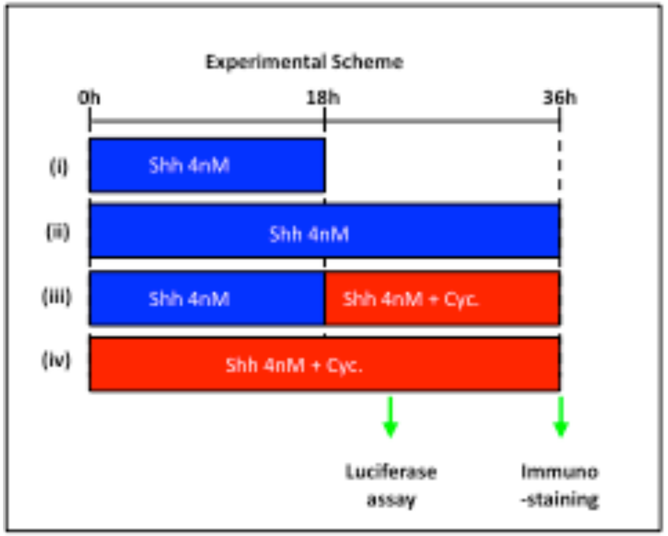
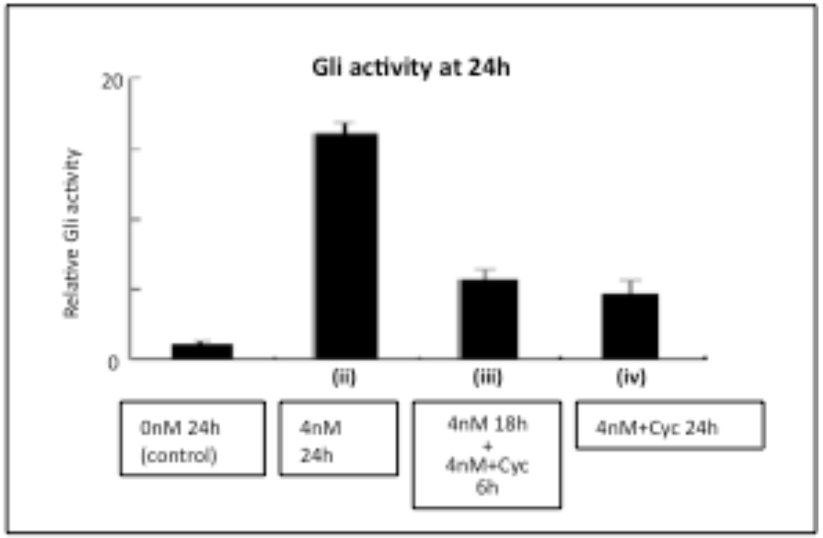
Nkx2.2 (N)

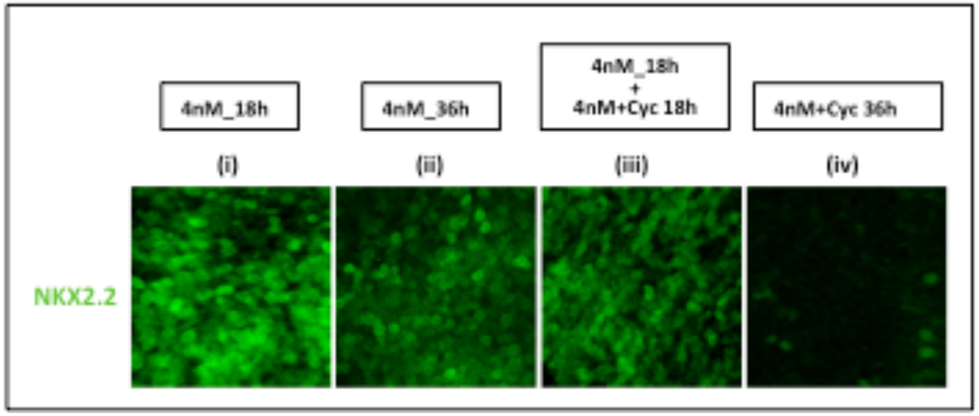


Jasmina Panovska

Network confers hysteresis on Nkx2.2







Noriaki Sasai

A transcriptional circuit for morphogen interpretation