To Send or Receive but not Both:

The role of cis-interactions between Notch and Delta

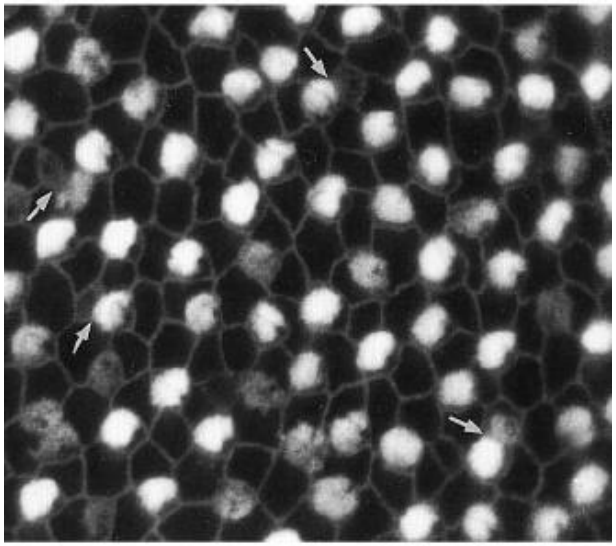
Computational Morphodynamics, KITP 2009

David Sprinzak

Elowitz Lab, California Institute of Technology

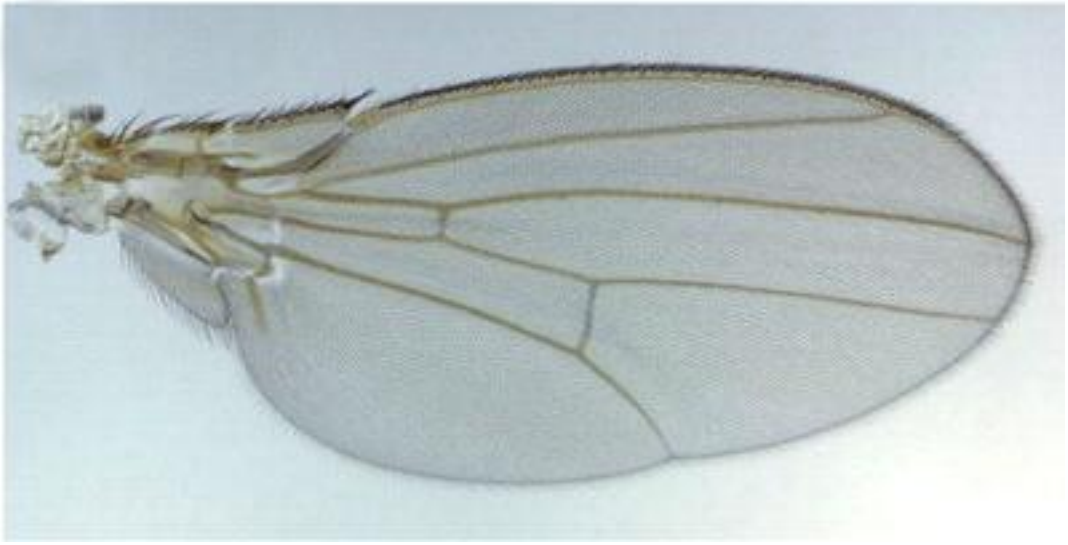
Fine Grained Developmental Pattern Formation

Inner ear development (chick)



Goodyear and Richardson, J. Neurosci 1997

Wing Vein Patterning (Drosophila)



Lai et al, Development 2005

THE THEORY OF THE GENE

BY

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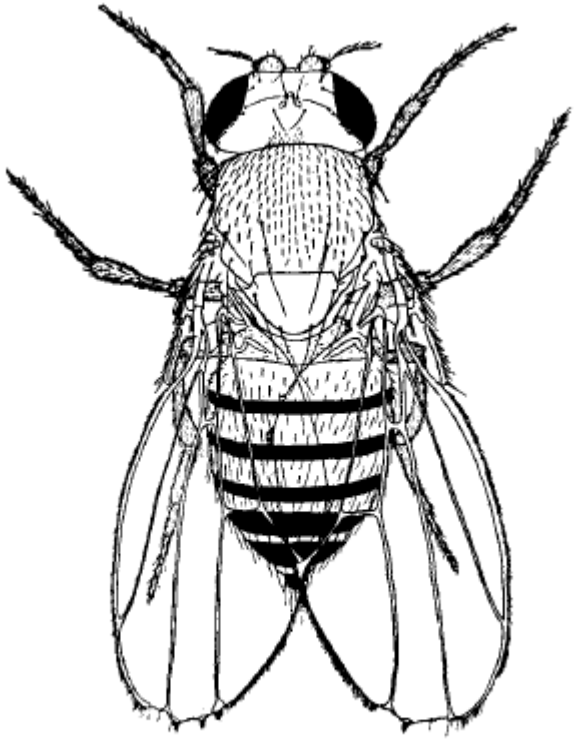


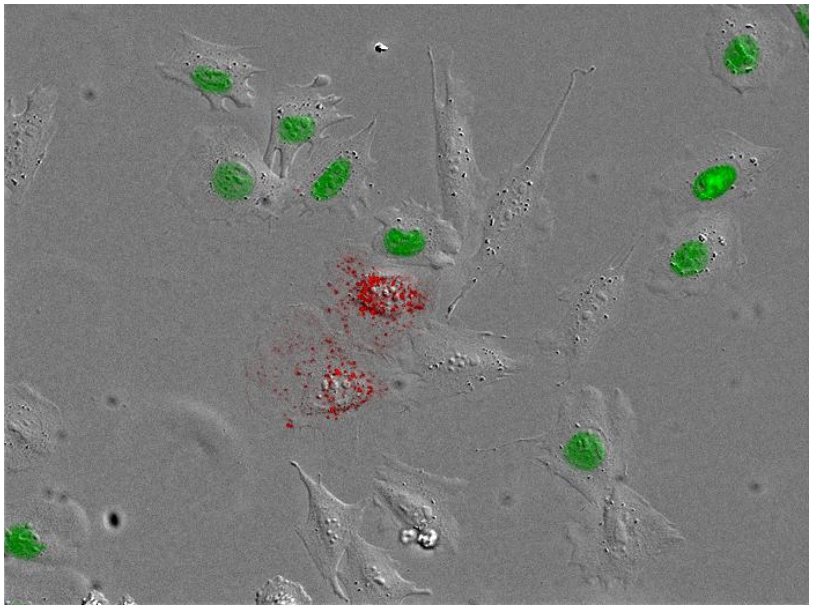
FIG. 44.

Notch-wing, a dominant sex-linked, recessive lethal of Drosophila melanogaster.

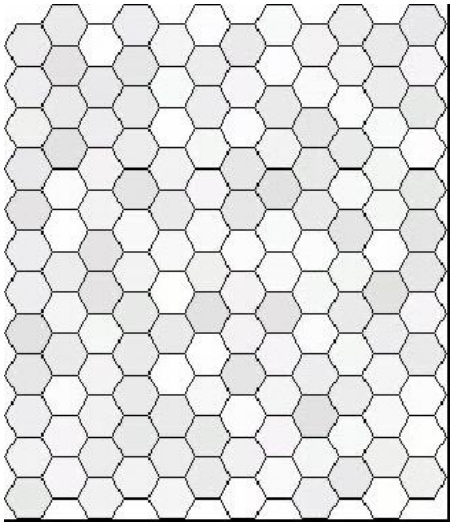
How do the molecular properties
of the Notch-Delta signaling system affect
developmental patterning?

Approach: combine quantitative time lapse microscopy and mathematical modeling

Quantitative time laps microscopy



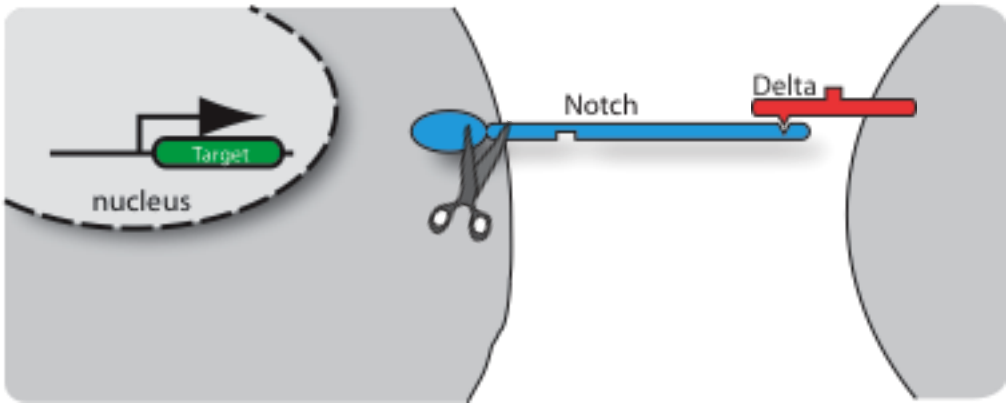
Mathematical modeling



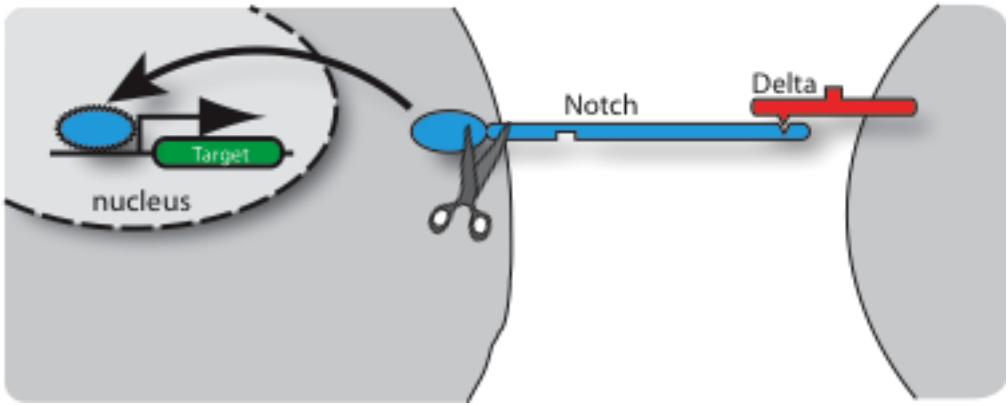
Use synthetic approach:

- Synthetic constructs (Notch-Gal4)
- Study dynamics in mammalian cell culture system

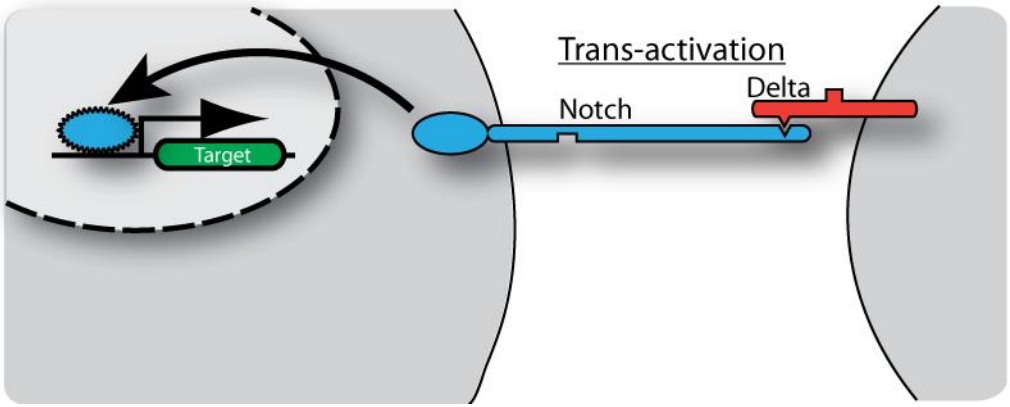
Delta trans-activates Notch



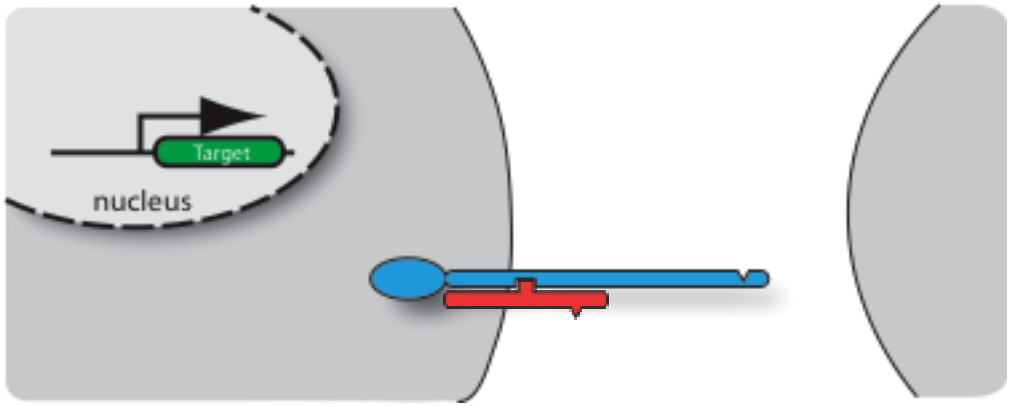
Delta trans-activates Notch



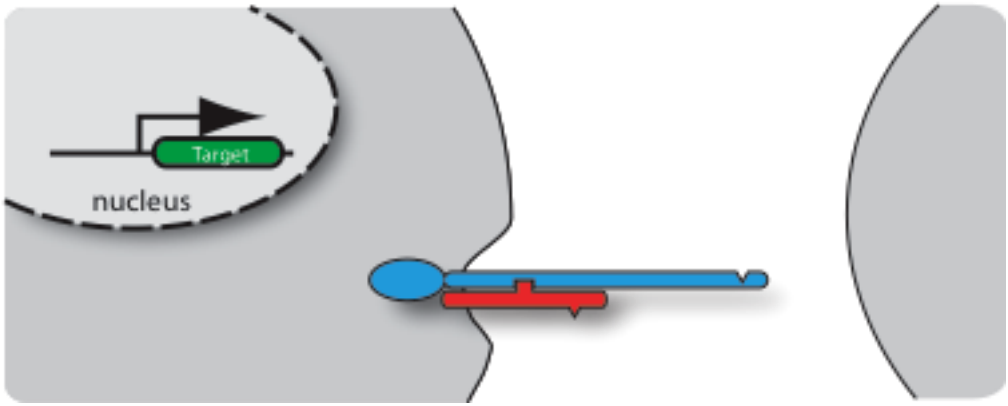
Delta Trans-activates Notch



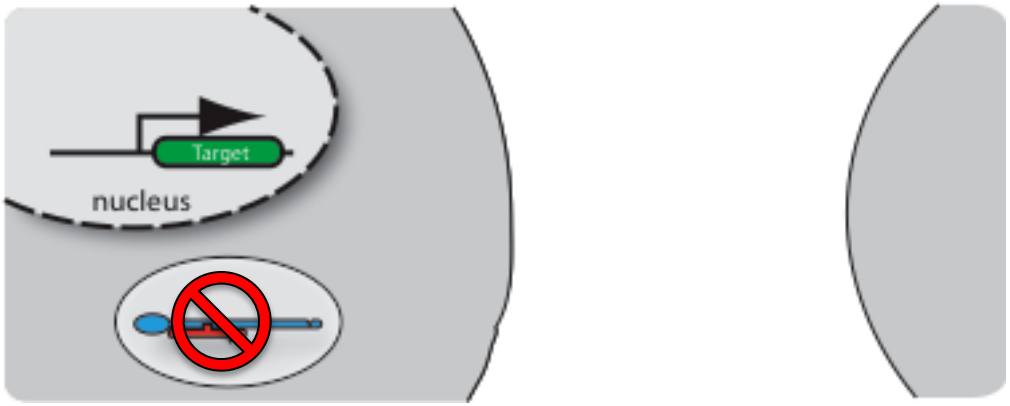
Delta cis-inhibits Notch



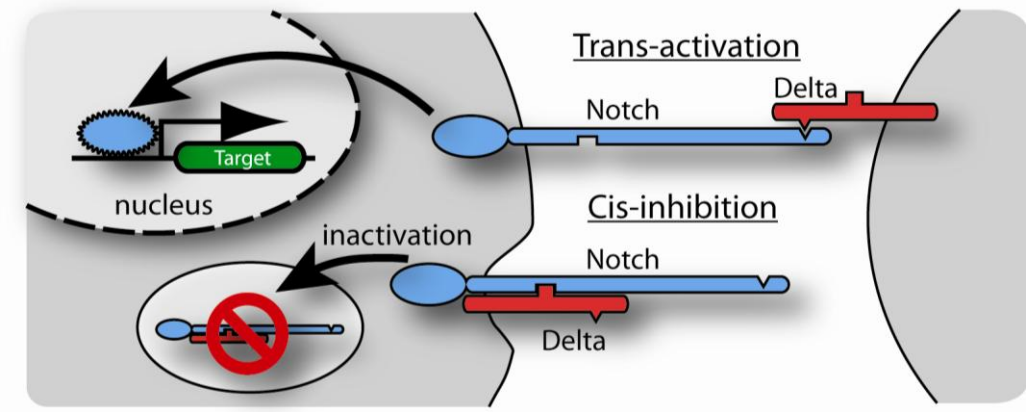
Delta cis-inhibits Notch



Delta cis-inhibits Notch

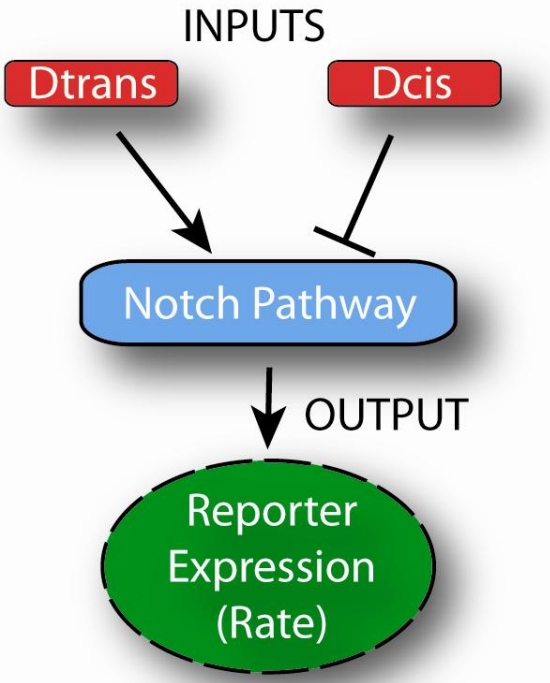


Notch and Delta interact in cis and in trans



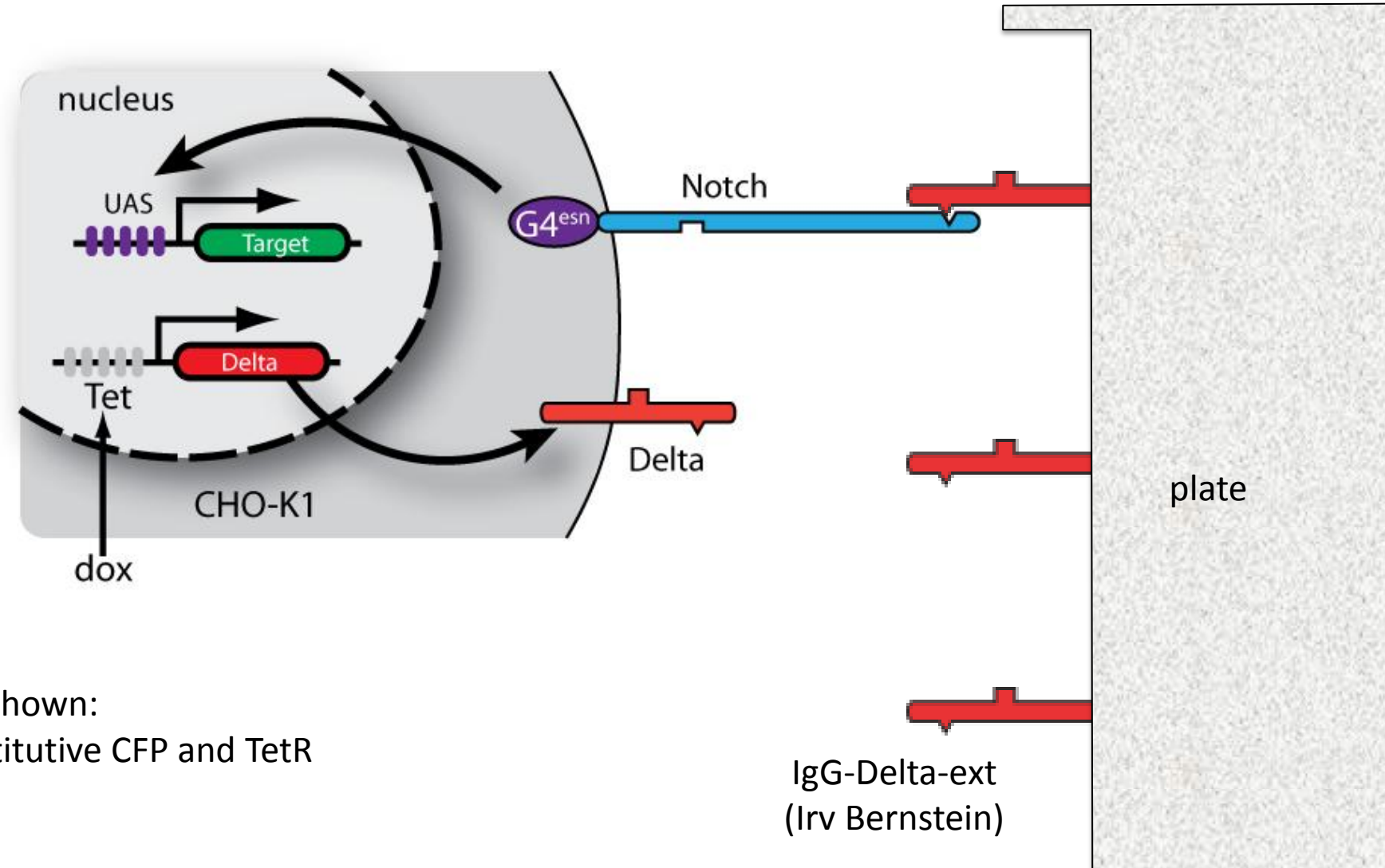
(Diagram is only schematic - cis and trans interaction occur in the same Notch domain)

How do the Notch pathway integrates these two inputs?



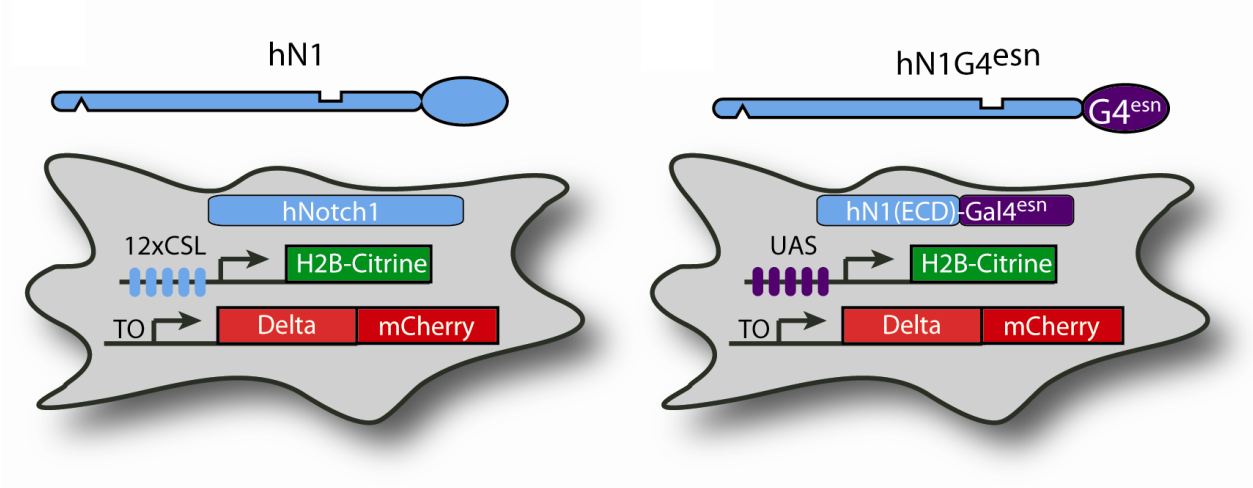
Why do you need such a mechanism?

Platform for analyzing Notch response to cis and trans Delta



Not shown:
constitutive CFP and TetR

System for analyzing Notch response to cis and trans Delta

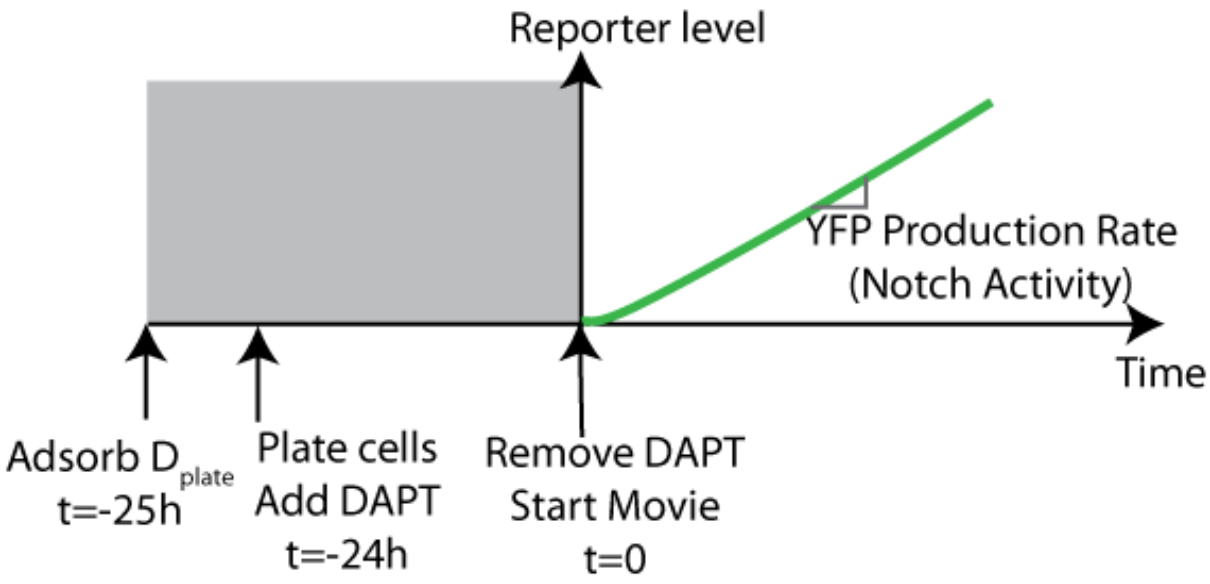


Base cell line: CHO-K1

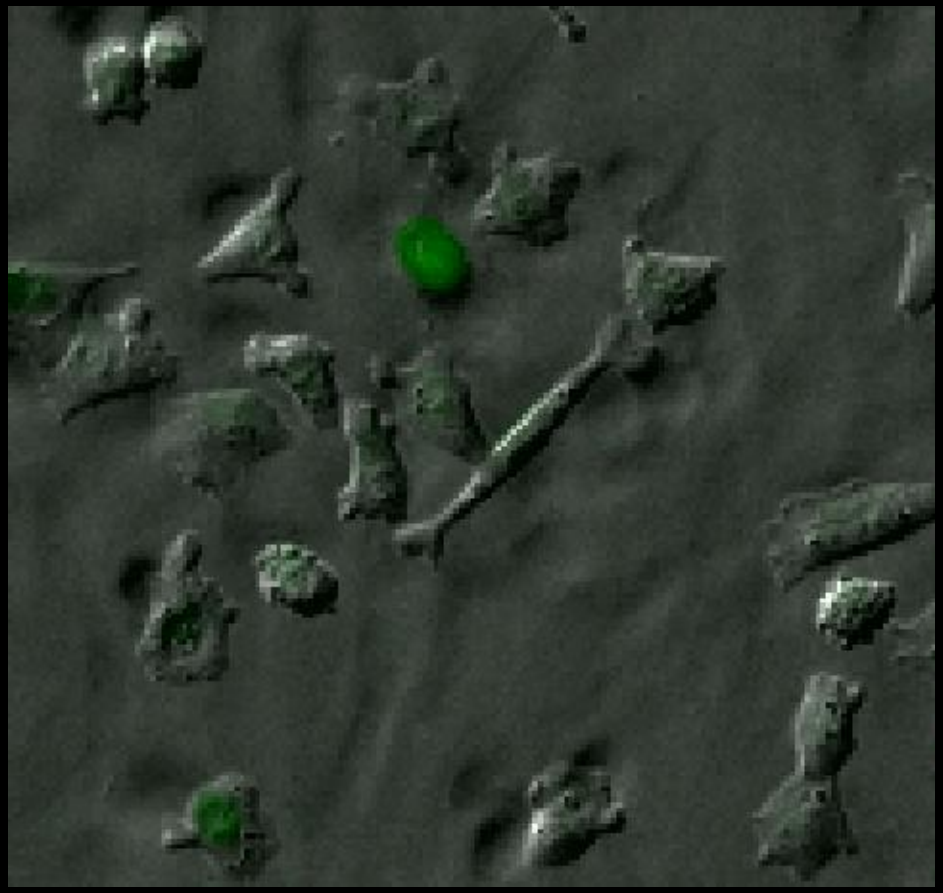
Not shown: TetR, CMV-H2B-Cerulean

rDll1 (from Gerry Weinmaster)
12xCSL (from Urban Lendahl)
hN1 (from Jon Aster)

Notch response to trans-activation

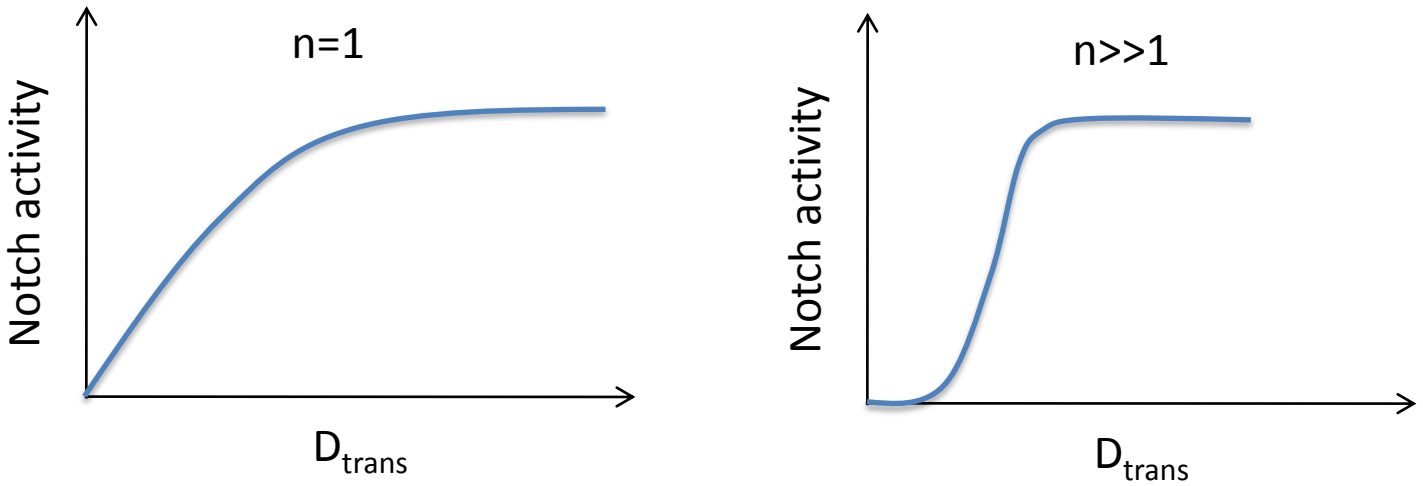


Notch response to trans-activation



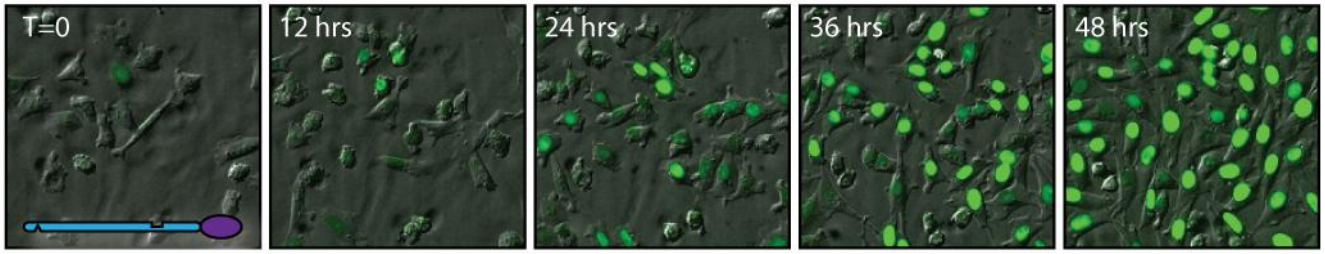
Green – Notch response

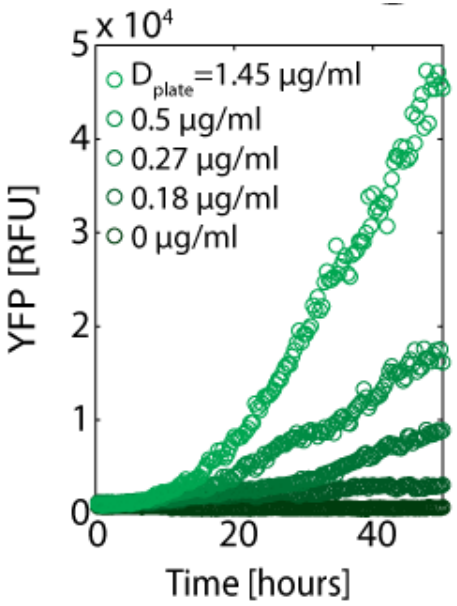
What is the cooperativity of Notch response to trans-Delta?

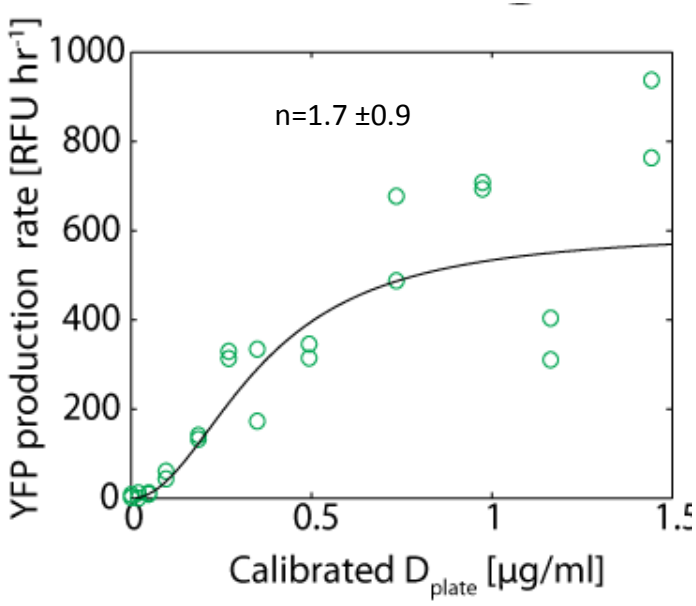


From modeling of Lateral Inhibition expect >2

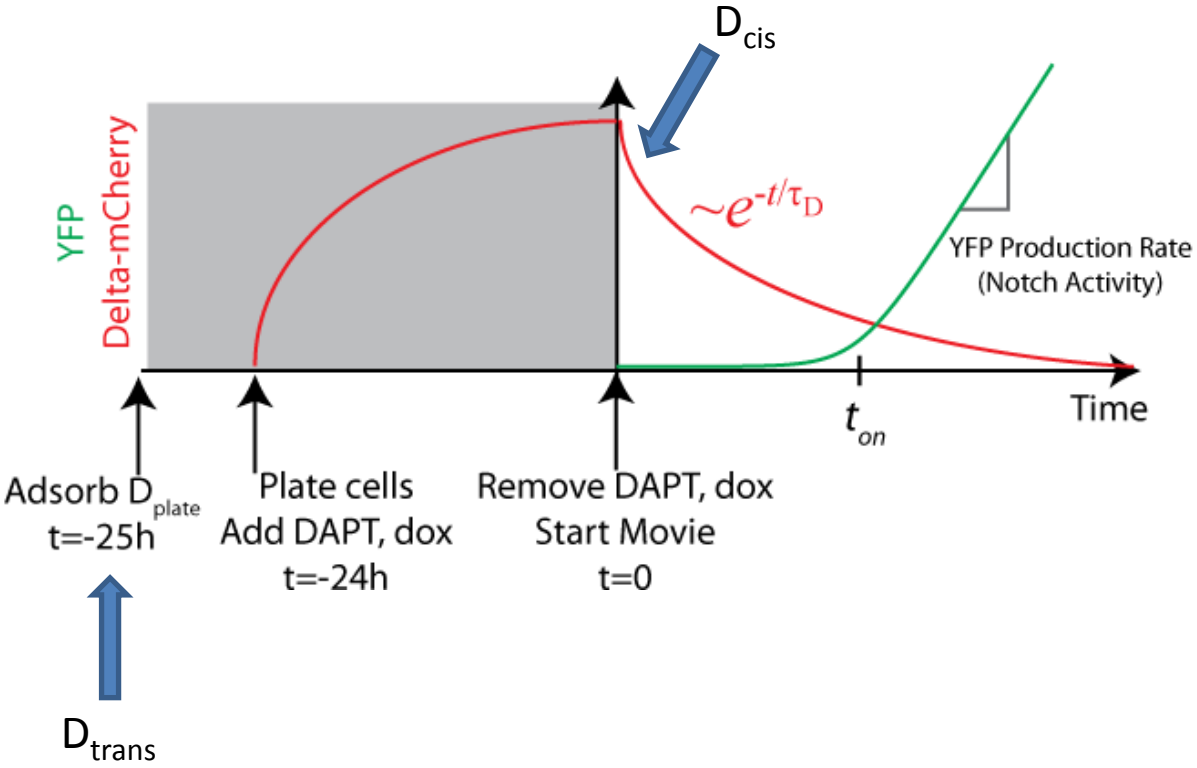
Notch response to trans-activation is graded



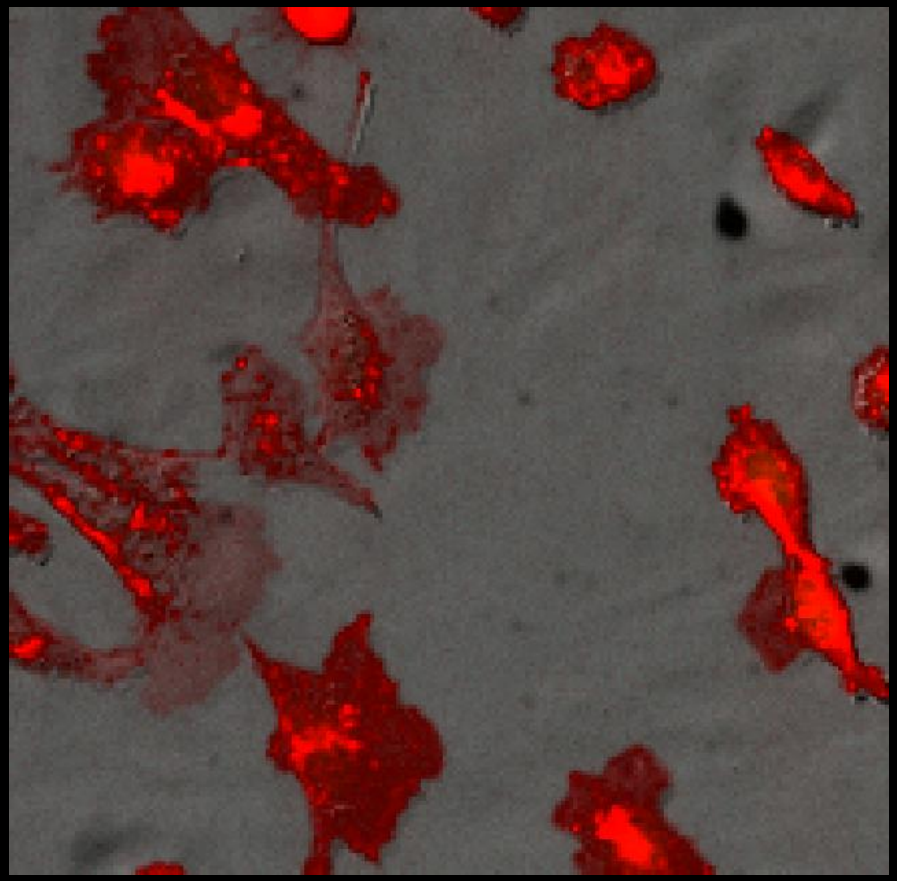




Measuring Notch response to cis and trans Delta

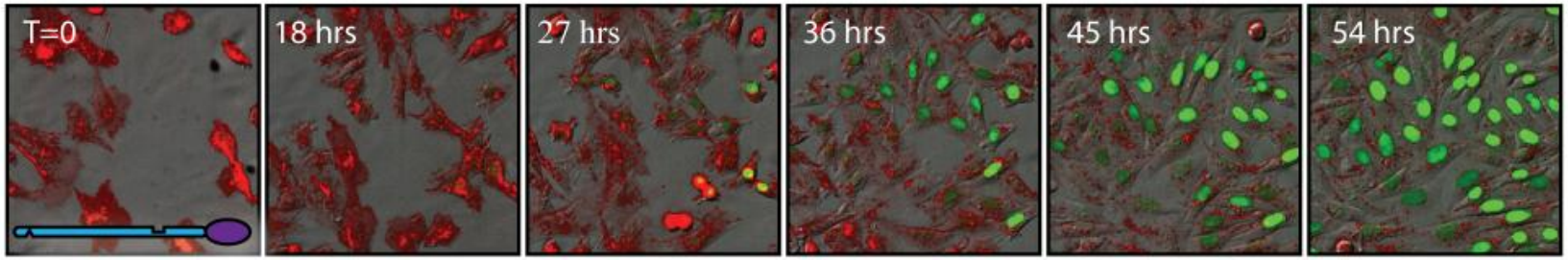


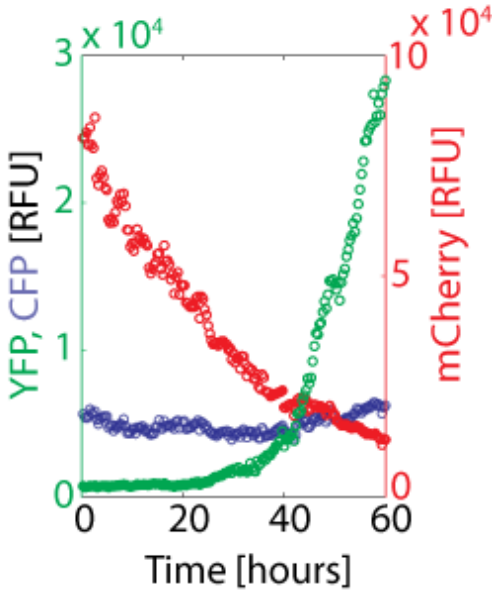
Notch activity in response to cis-Delta



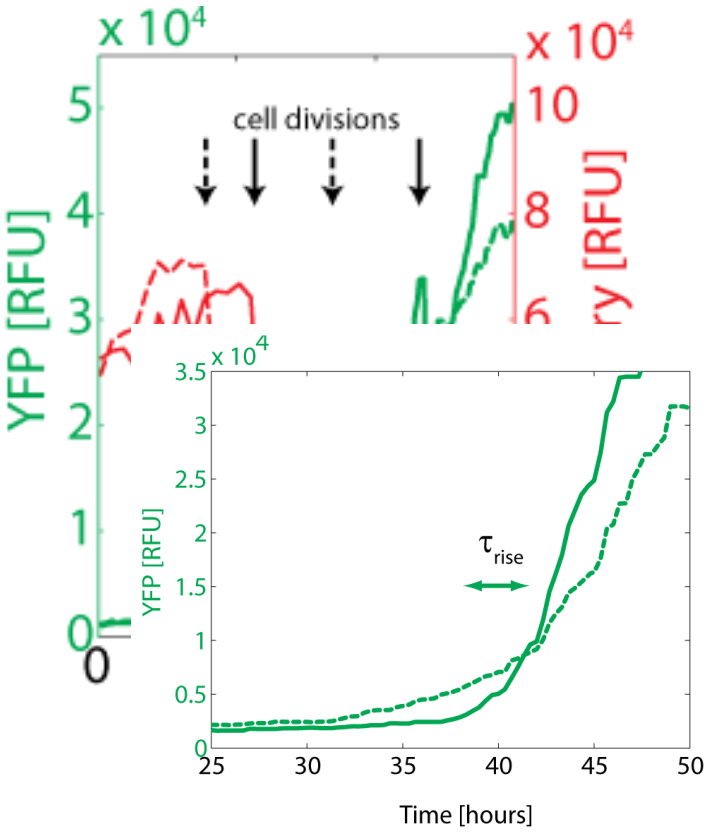
Red – Delta
Green – Notch response

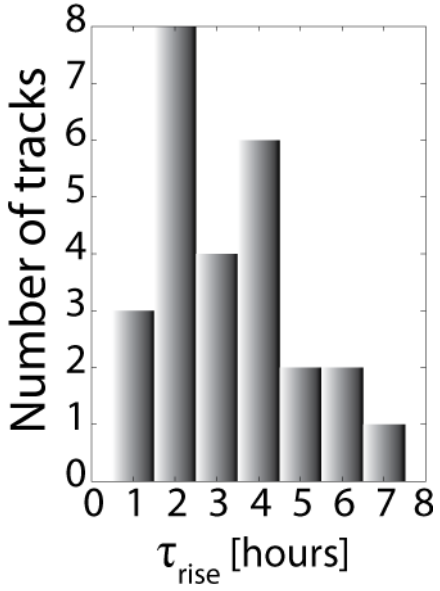
Cis-Delta exhibits sharp response





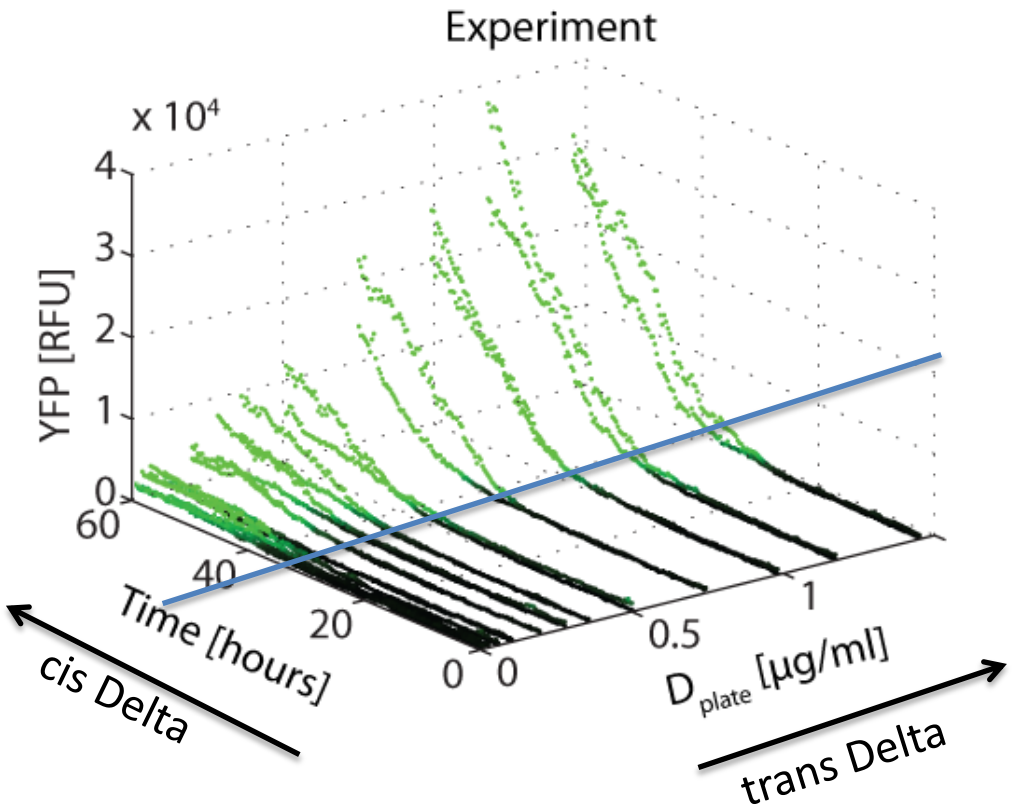
And even sharper in single cells…





Effective cooperativity ≈ $\tau_{\text{Delta decay}} / \tau_{\text{rise}} \approx 12$

Response of Notch signaling to both cis and trans Delta



What have we got?

- Graded response to trans-Delta
- Sharp response to cis-Delta
- Threshold for cis-Delta independent of trans-Delta

Mathematical model

Trans-activation reaction:

Notch ICD

$$N + D_{trans} \rightleftharpoons [ND_{trans}] \rightarrow S$$

Cis-inhibition (mutual inactivation) reaction:

$$N + D_{cis} \rightleftharpoons [ND_{cis}] \rightarrow \phi$$

N and D_{cis} are also produced and degraded at constant rate

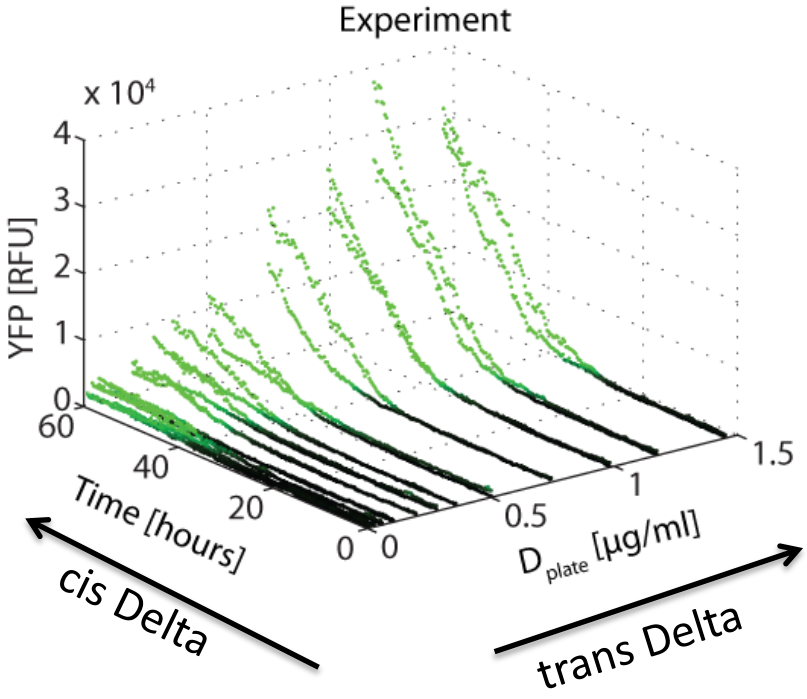
Mathematical model

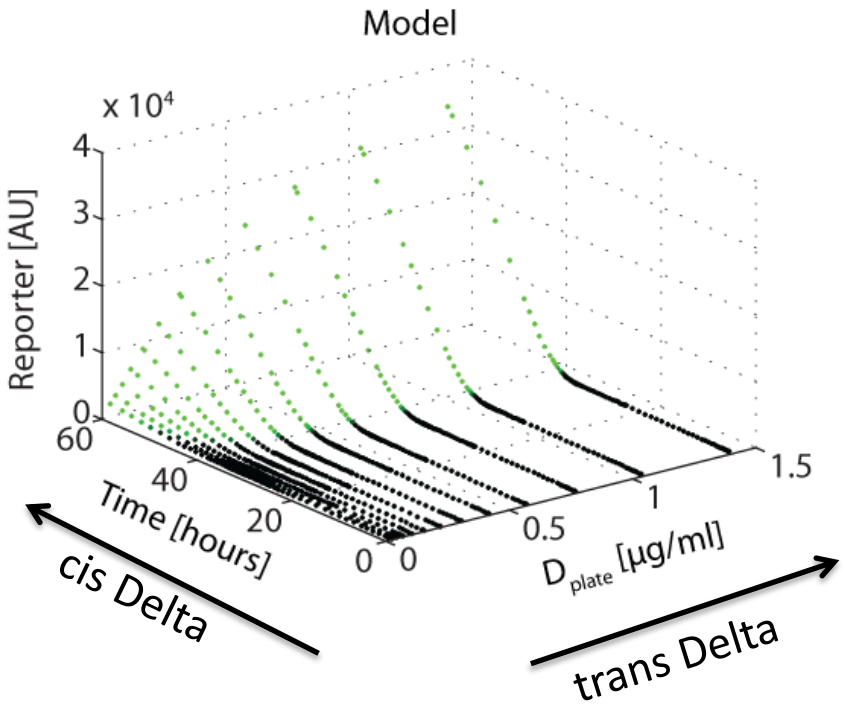
$$\frac{dN}{dt} = \beta_N - \gamma N - \frac{ND_{cis}}{k_C} - \frac{ND_{trans}}{k_t}$$

$$\frac{dD_{cis}}{dt} = \beta_D - \gamma D_{cis} - \frac{ND_{cis}}{k_C}$$

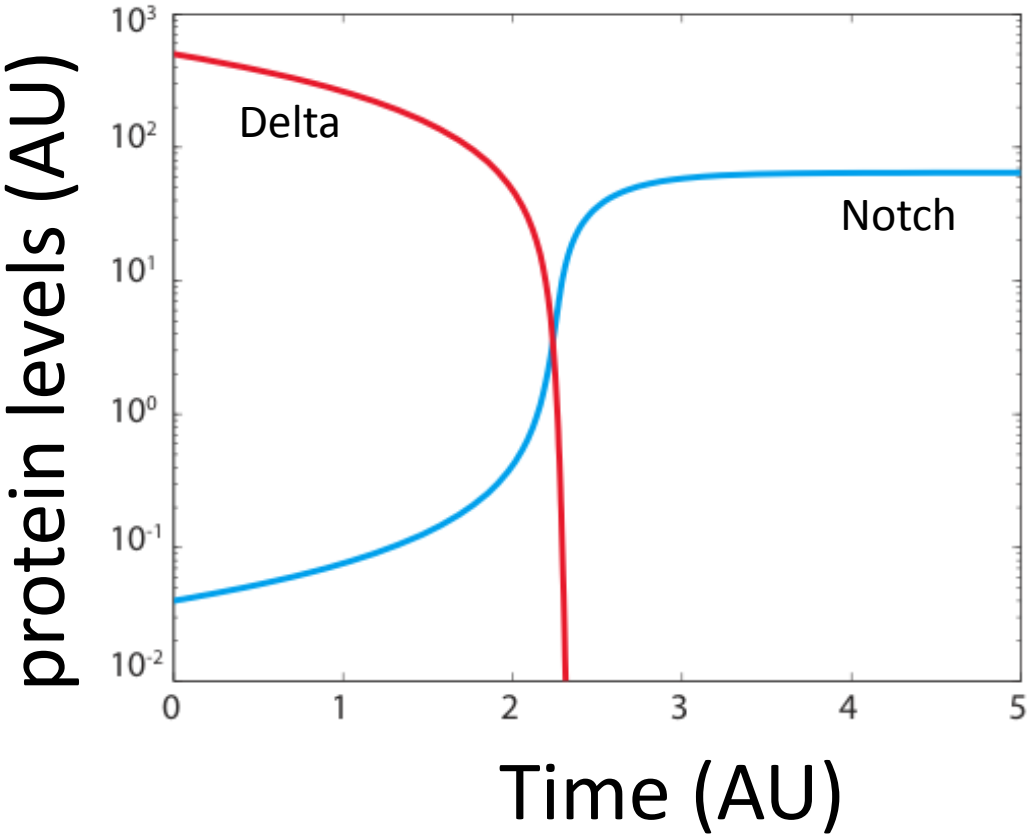
$$\frac{dS}{dt} = \frac{ND_{trans}}{k_t} - \gamma_S S$$

Model captures main experimental observations

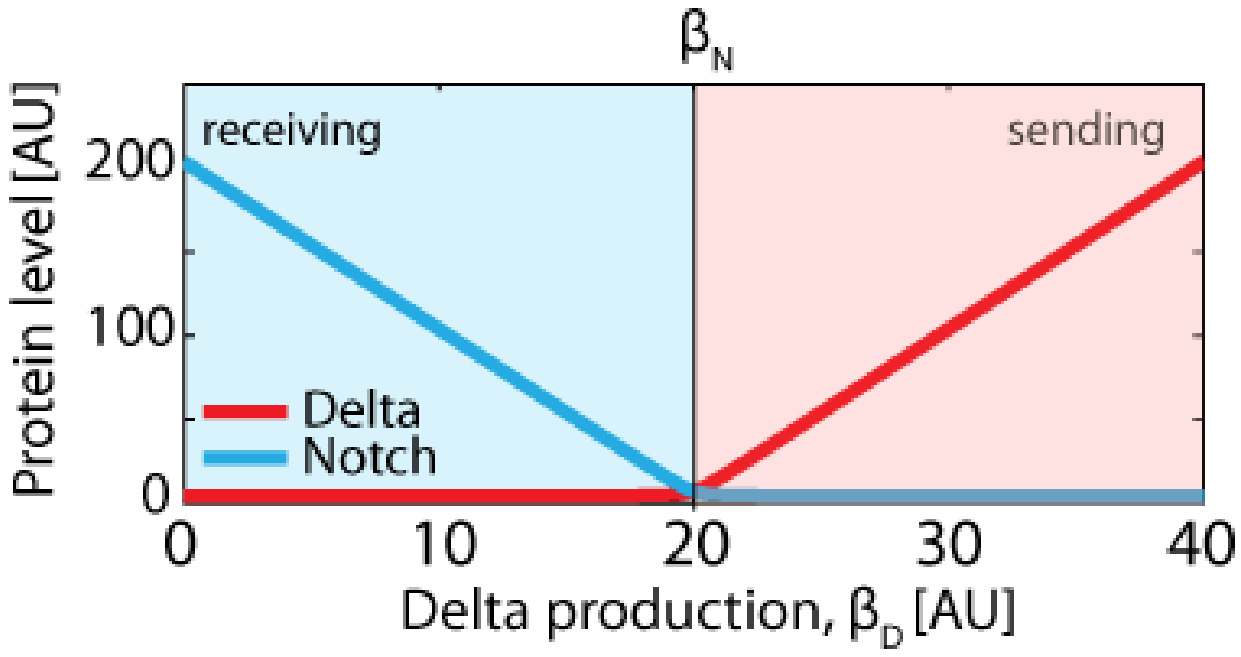




Mutual inactivation of Notch and Delta leads to an ultrasensitive response

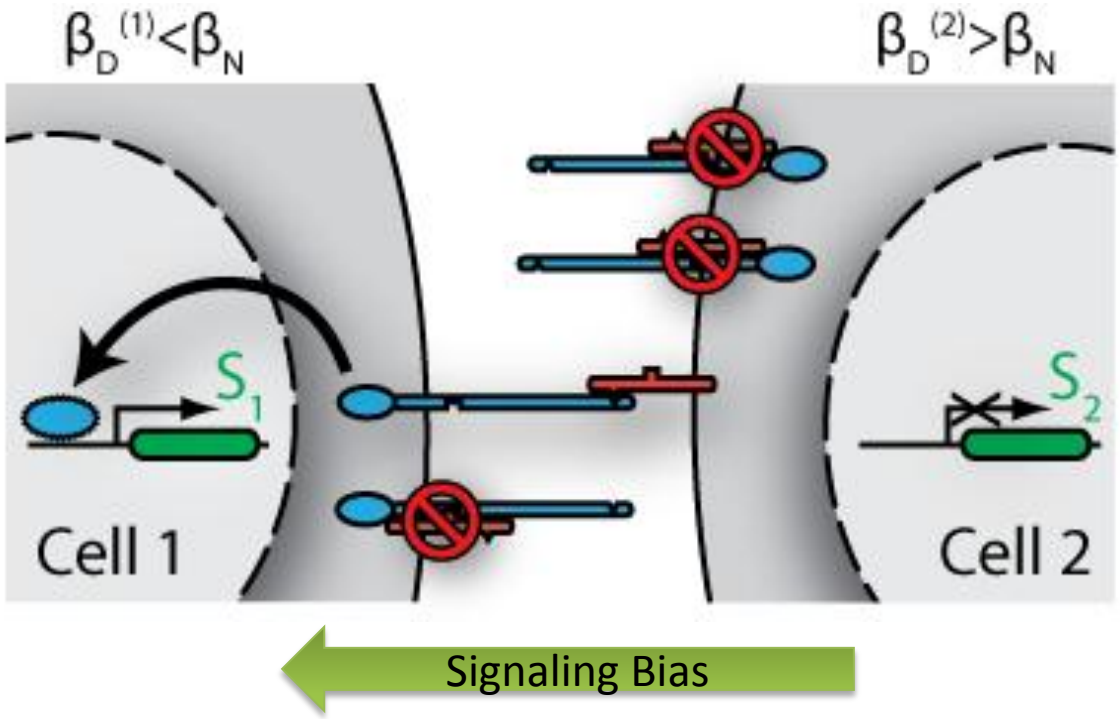


A signaling switch
‘Sender’ or a ‘receiver’ but not both

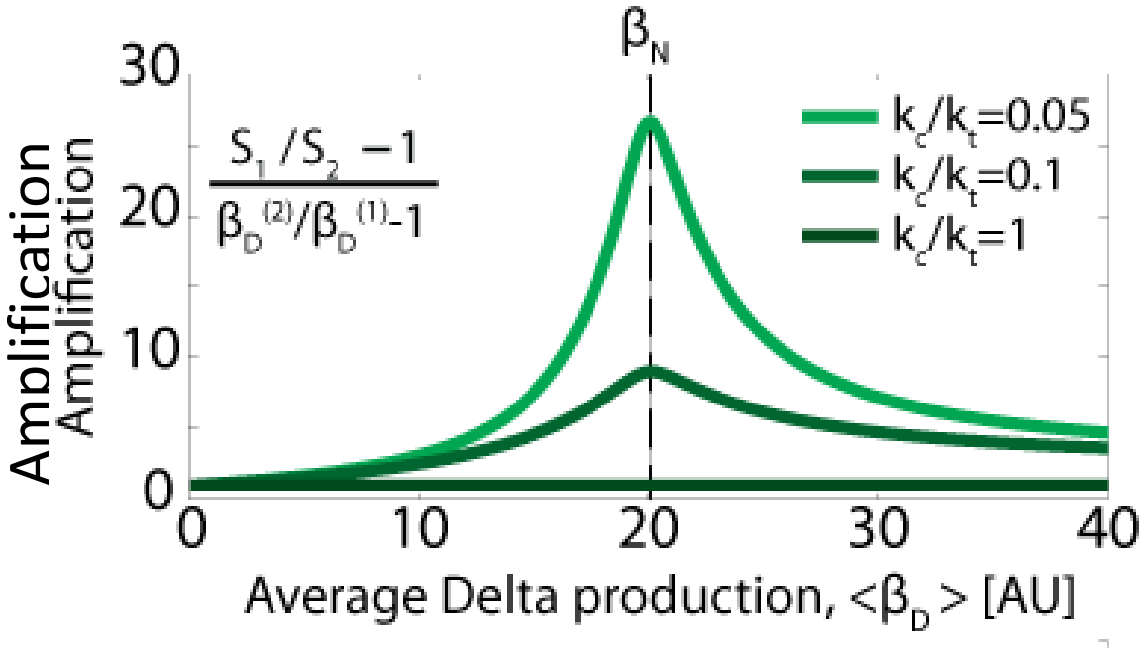


An inherent property of the Notch-Delta signaling
Without any transcriptional feedback !

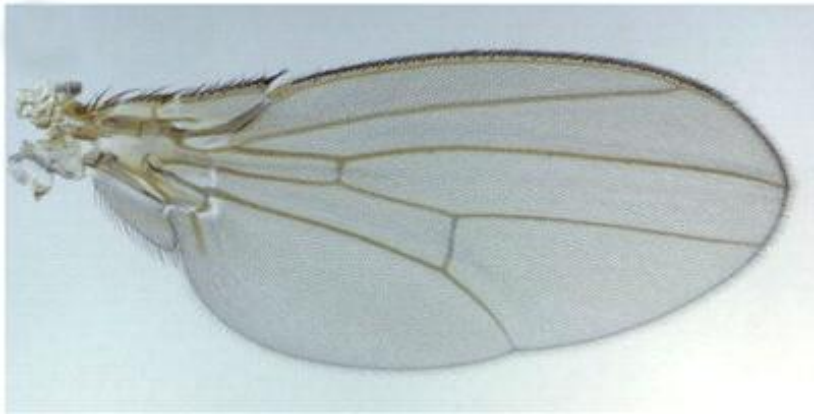
Signaling bias in two neighboring cells



Mutual inactivation amplifies differences between cells

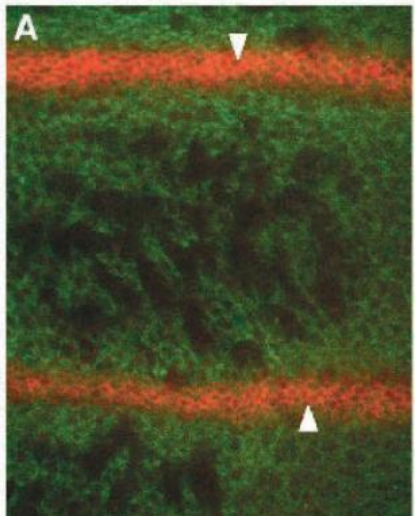


Implications for Pattern formation: Sharp boundary in the wing vein



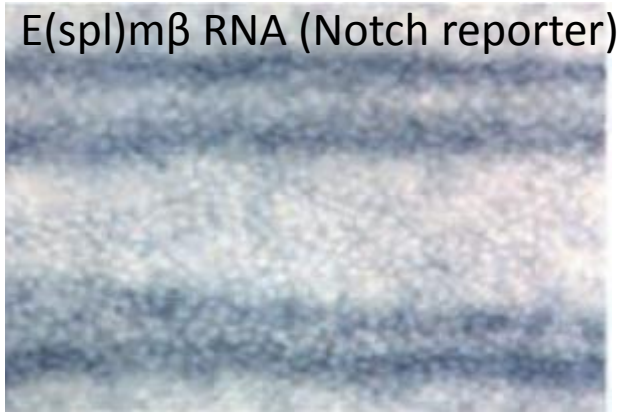
Notch (green)

Delta (red)



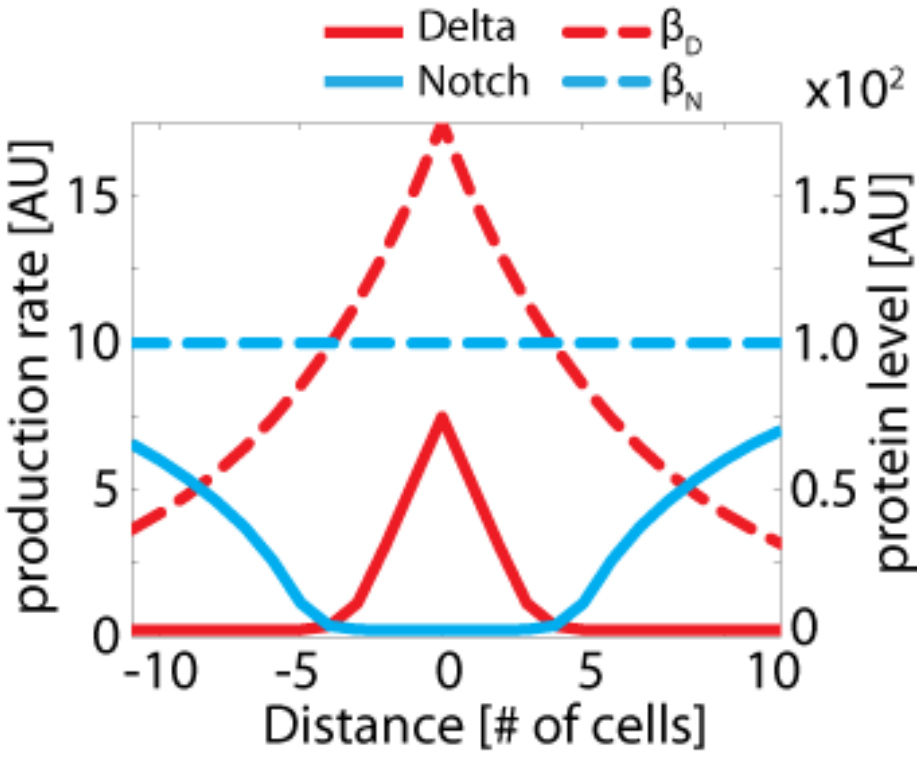
Huppert et al. (1997)

E(spl)mβ RNA (Notch reporter)

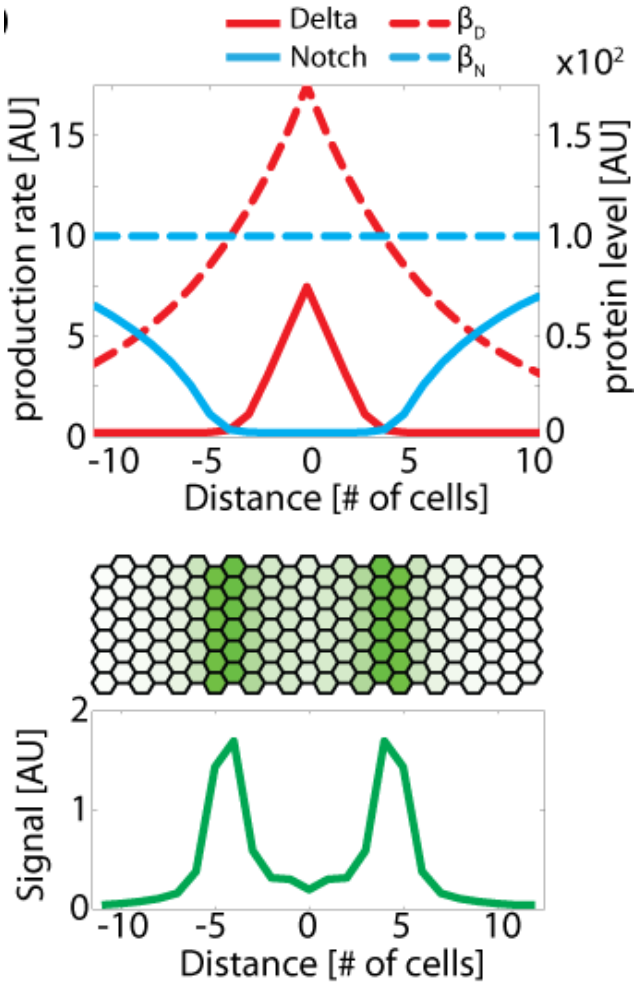


de Celis et al. (1997)

Sharp boundary in the wing vein



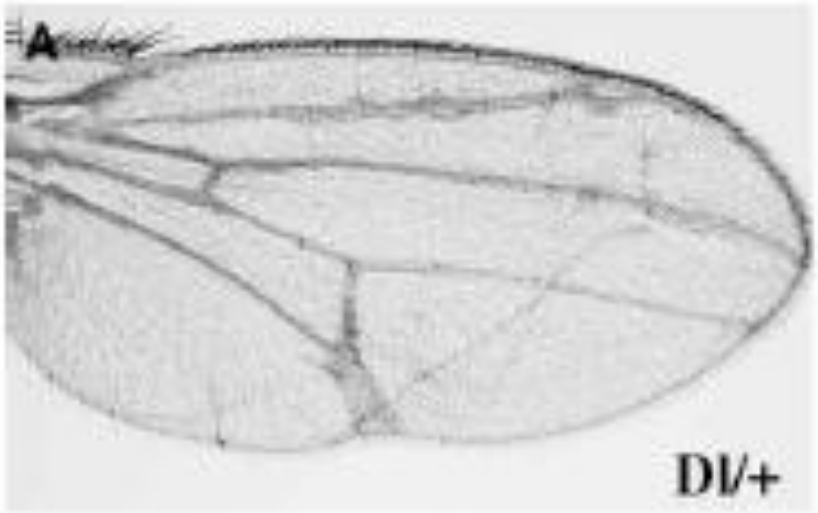
Sharp boundary in the wing vein

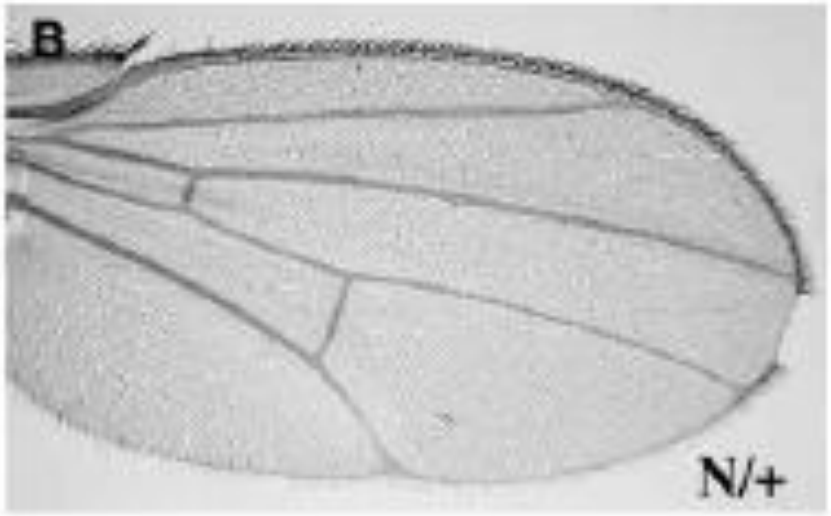


Model explains gene dosage phenotypes

Wt

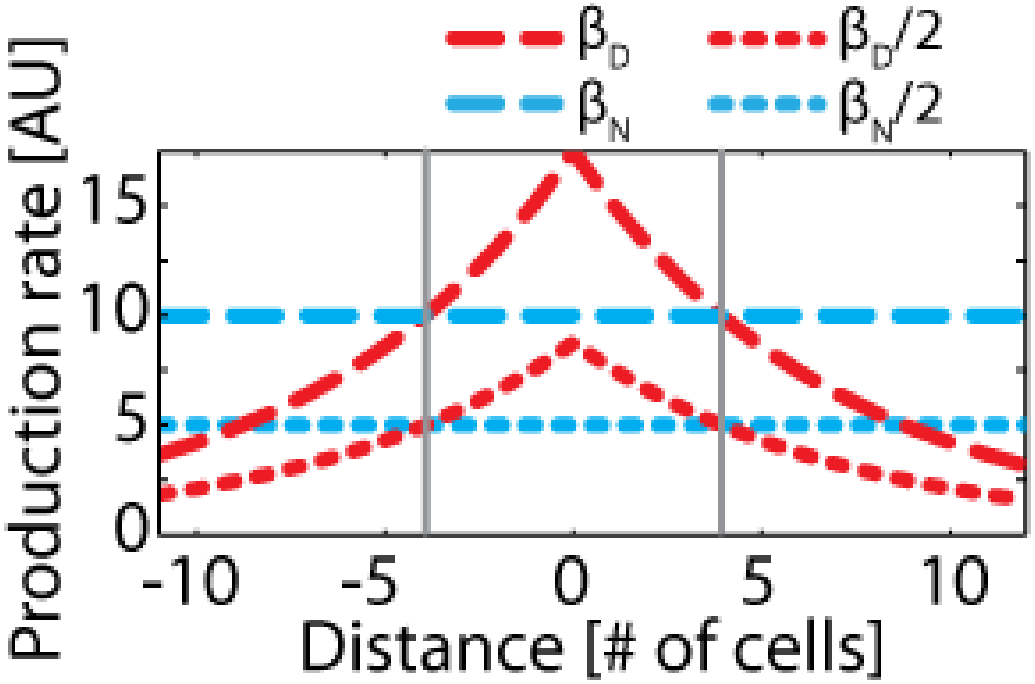
N/+ and Dl/+





de Celis and Bray (2000)

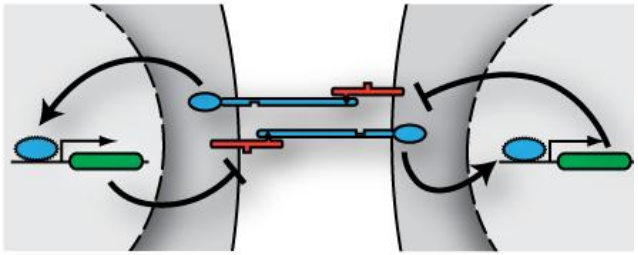
Model explains gene dosage phenotypes



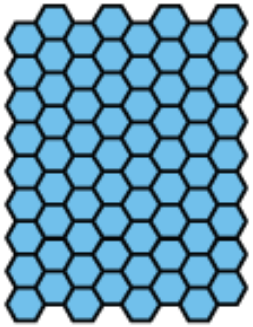
Boundary formed when:

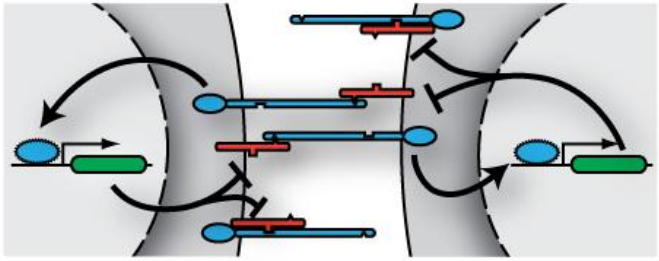
$$\beta_D(x) = \beta_N$$

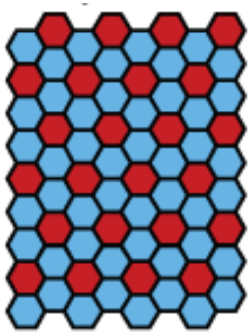
Lateral Inhibition with Mutual Inactivation mechanism circumvent the need for cooperativity



Requires n>2

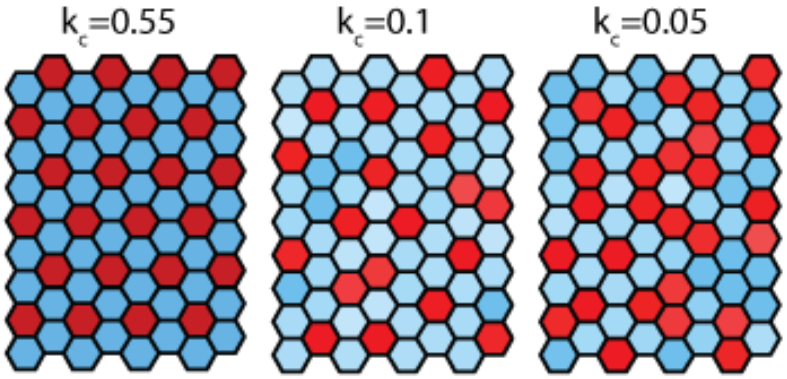






Work with n=1!

And generates a new spectrum of patterns



Summary

• Mutual inactivation of Notch and Delta results in an ultrasensitive signaing switch: A cell is either a ‘sender’ or ‘reciever’

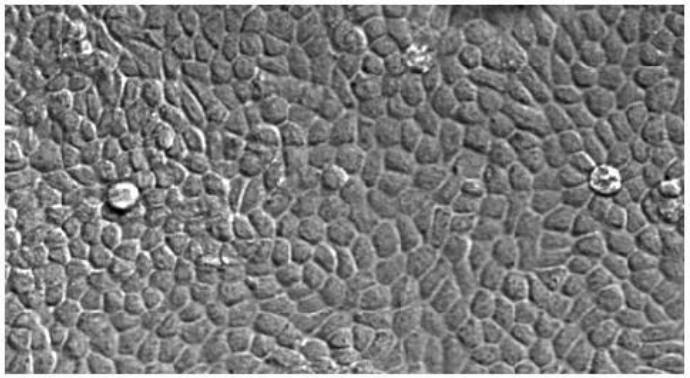
• This intrinsic property of the Notch pathway amplifies differences between neighboring cells *without additional feedback*.

• Major implication in patterning processes

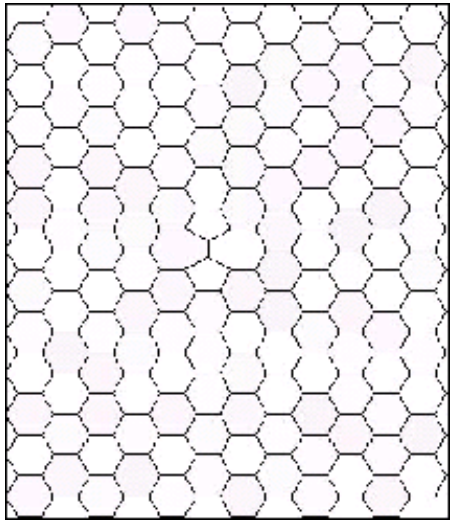
• Mutual inactivation mechanism?

Interaction between morphology and signaling

Role of Disorder



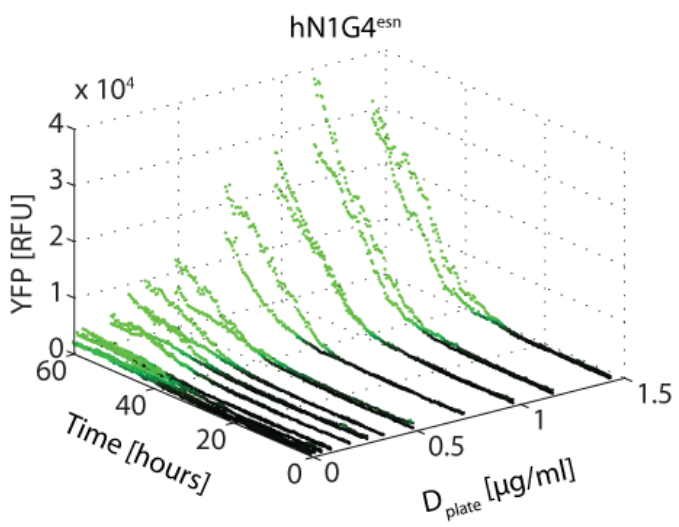
Confluent MDCK cells

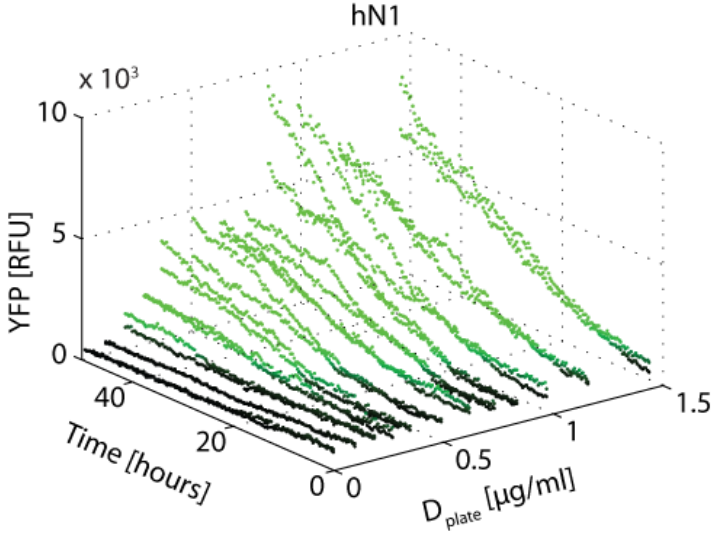


Examples of active interaction between signaling and morphology

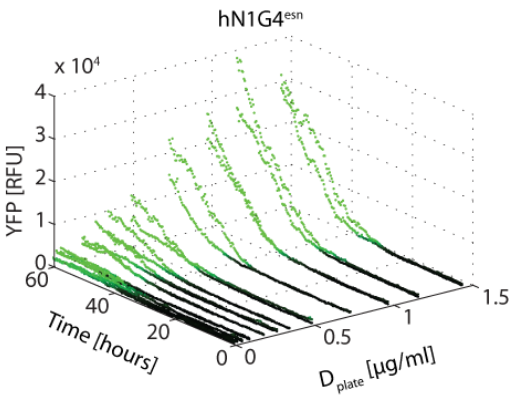
Response of Notch signaling to both cis and trans Delta

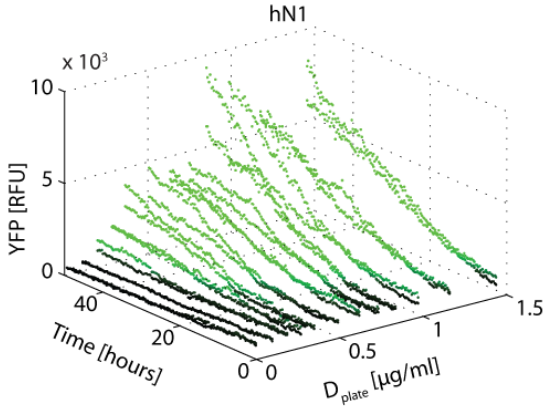
hN1 vs hN1G4^{esn}



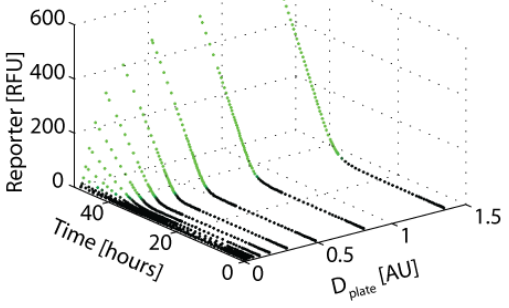


Model explains qualitative difference between measurements

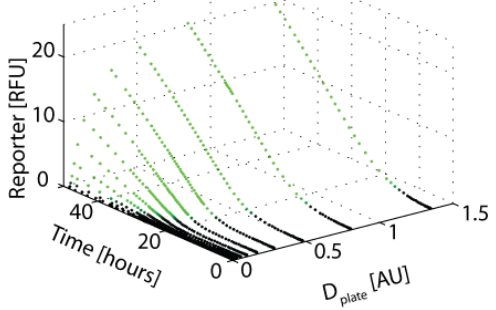


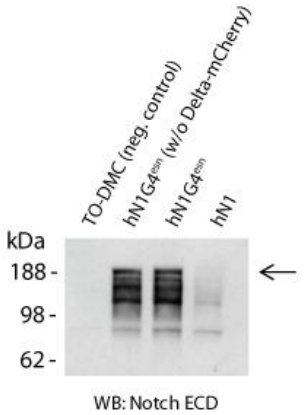


Model: ultrasensitive regime
D_0=400, β_N=20

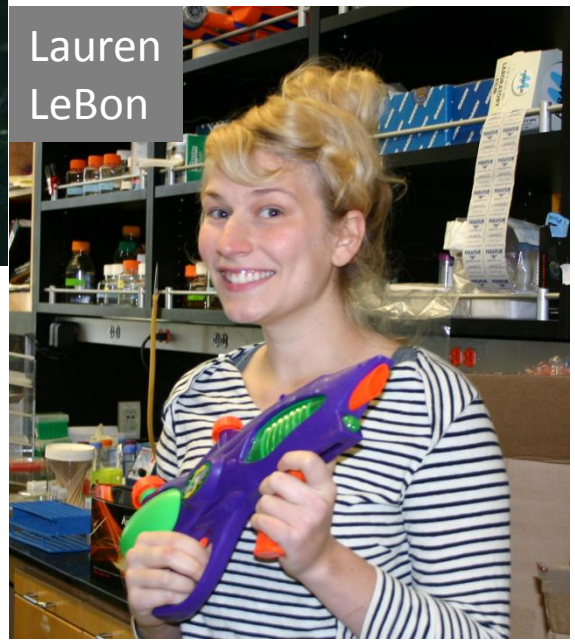


Model: non-ultrasensitive regime
D_0=10, β_N=3





Acknowledgments



Special thanks

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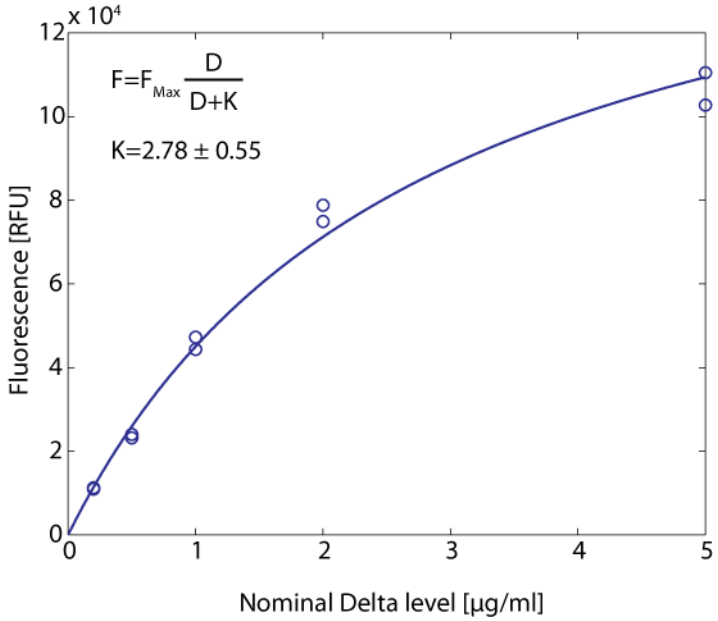
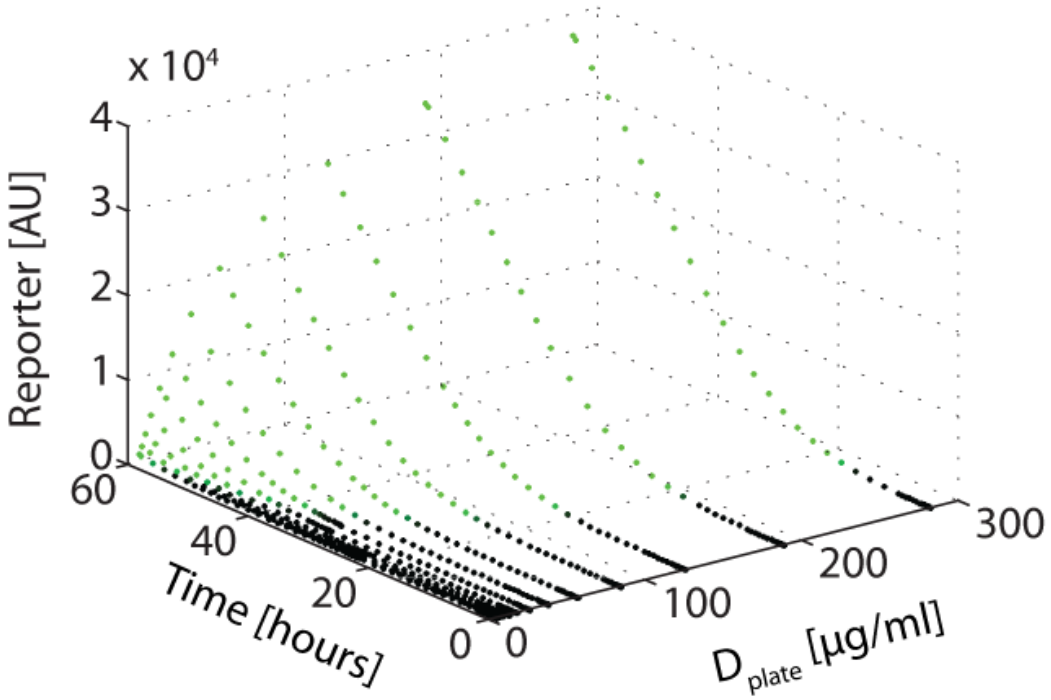
Irv Bernstein

Jon Aster

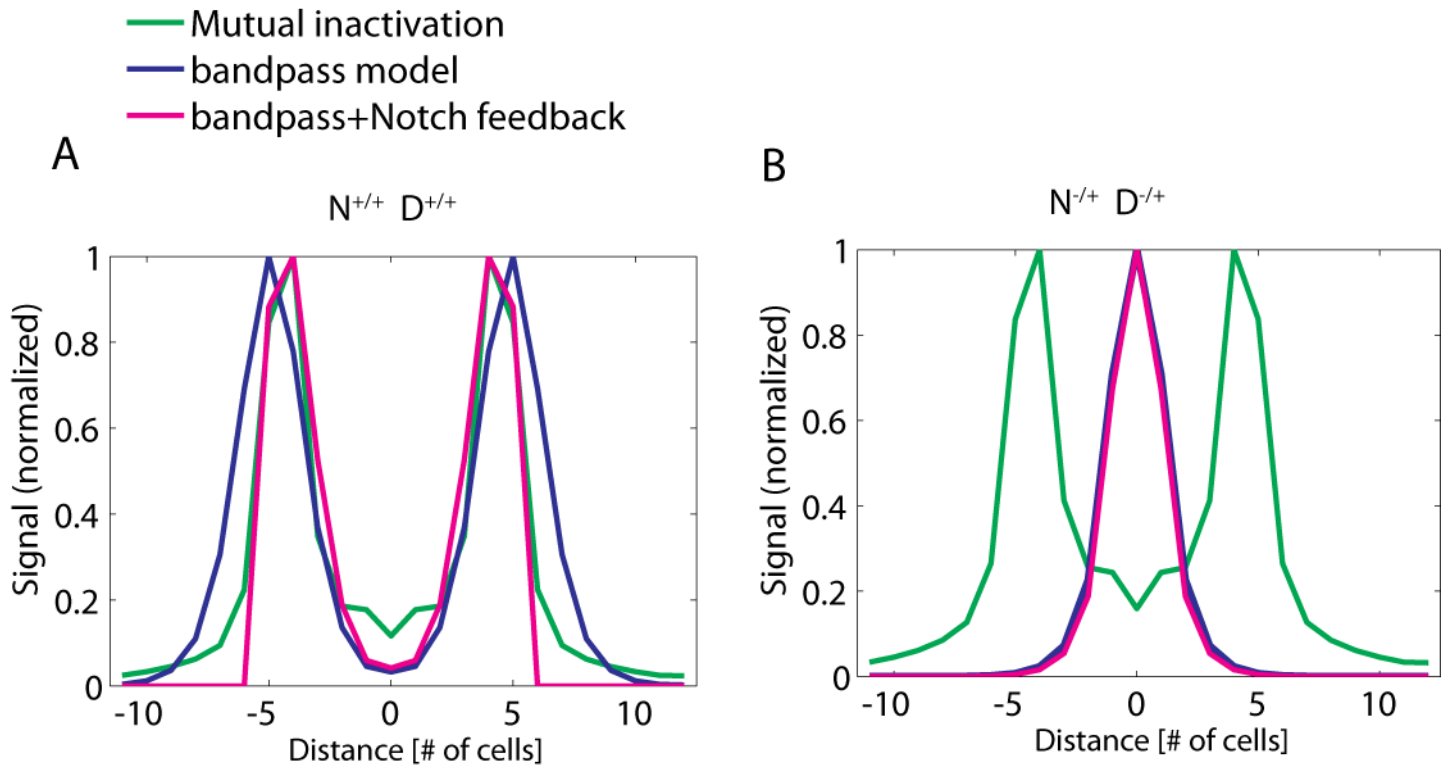
Plate bound Delta calibration



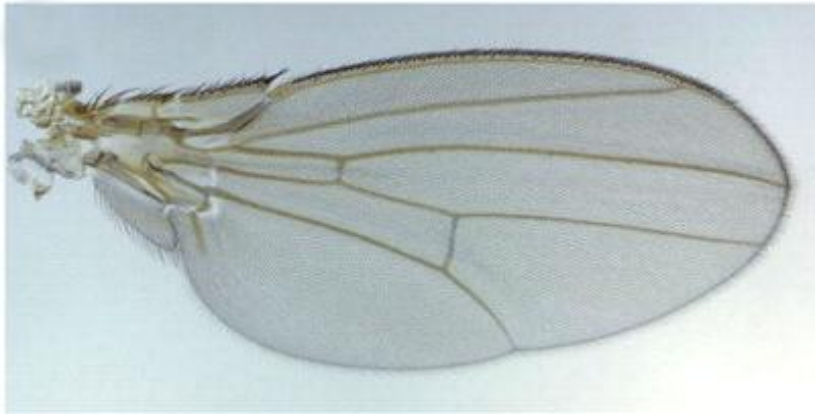
Catalytic model



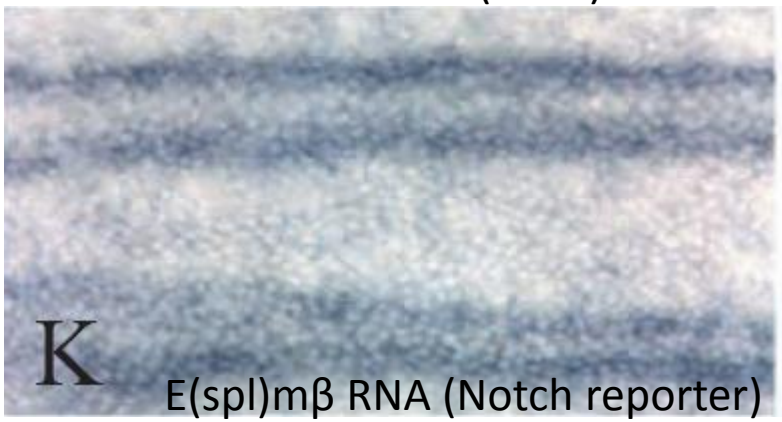
Alternative models for boundary formation

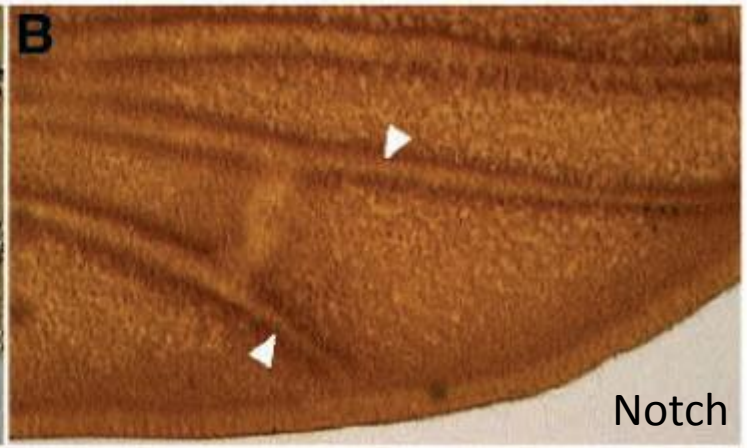


Implications in Pattern formation 1: Sharp boundary in the wing vein



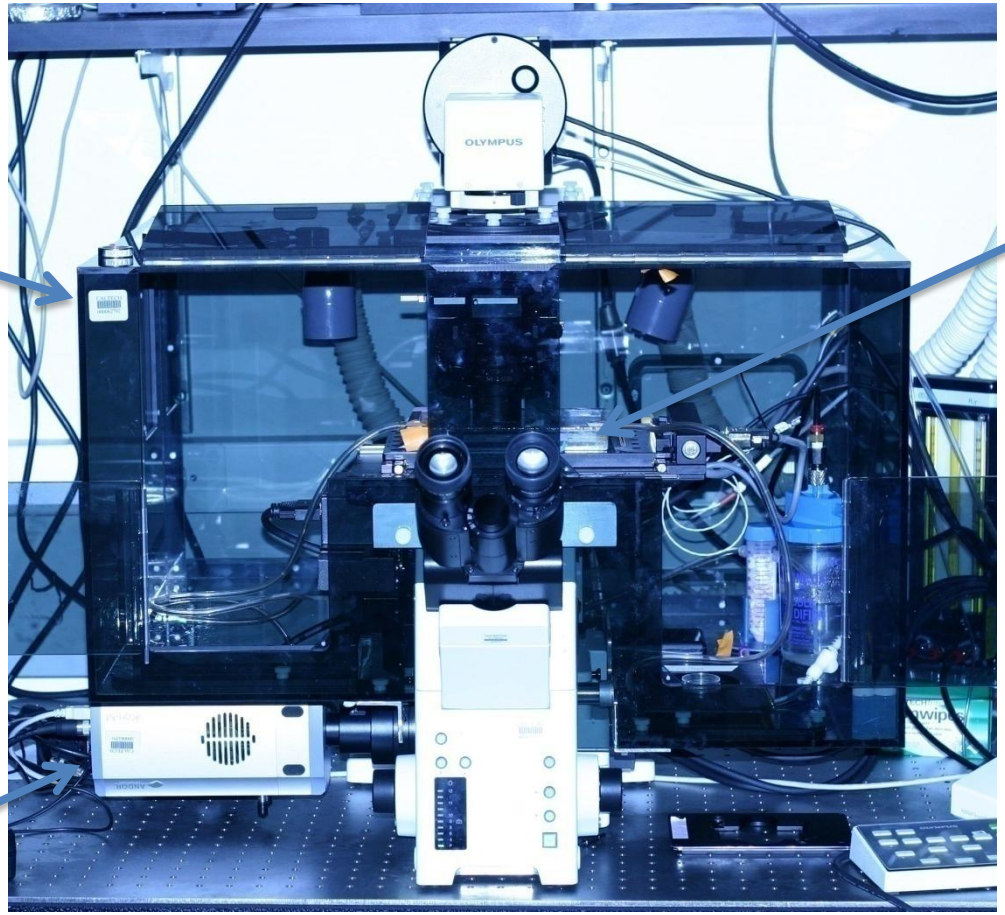
de Celis et al. (1997)





Huppert et al. 1997

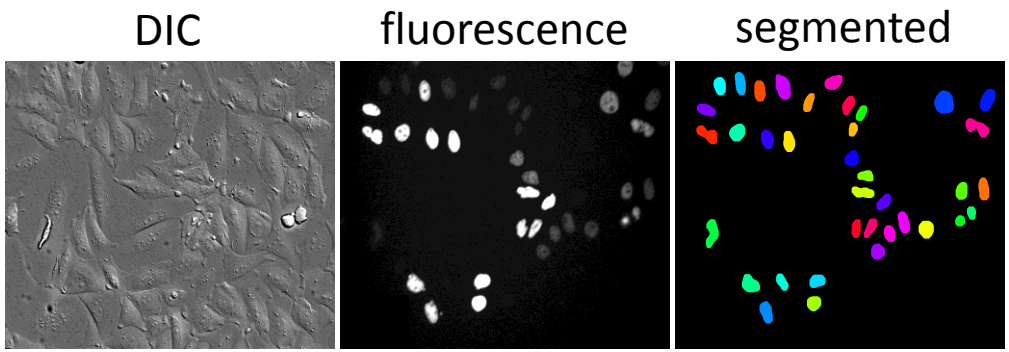
Quantitative time lapse microscopy using fluorescent protein reporters



Fully automated setup :

Allows taking time lapse microscopy movies over extended times (several days)

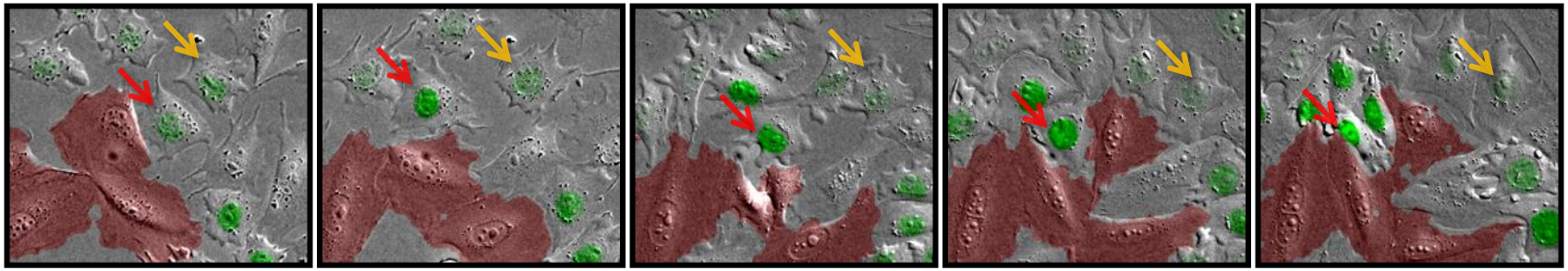
Automated segmentation and tracking

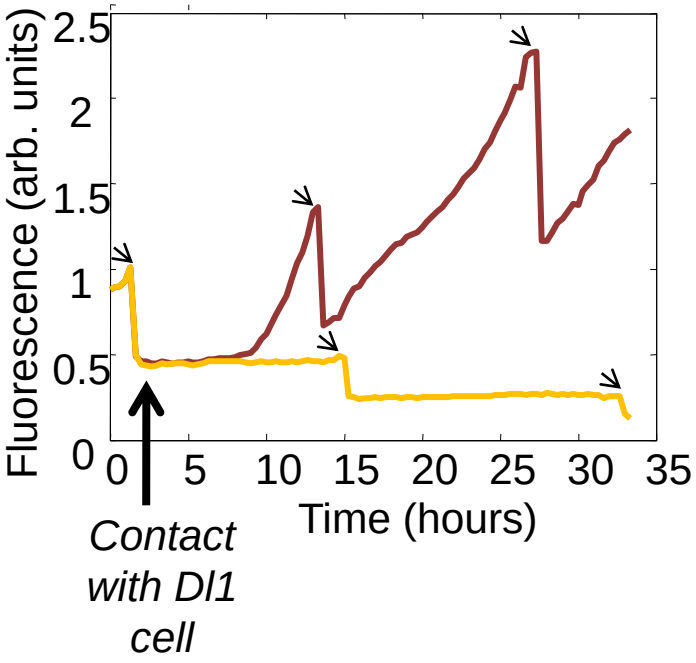


Developed automated image analysis routine:

- Identify (segments) single nuclei.
- Track nuclei through time lapse movie.
- Identify cell division and keeps track of lineage relations.

Quantitative analysis of Notch-Delta Signaling





Conclusions:

- We can track the dynamics of activation.
- We measure a time delay of a few hours between contact and transcriptional response.