Embryonic semaphores

Morphogens and contact-mediated signalling in developmental patterning

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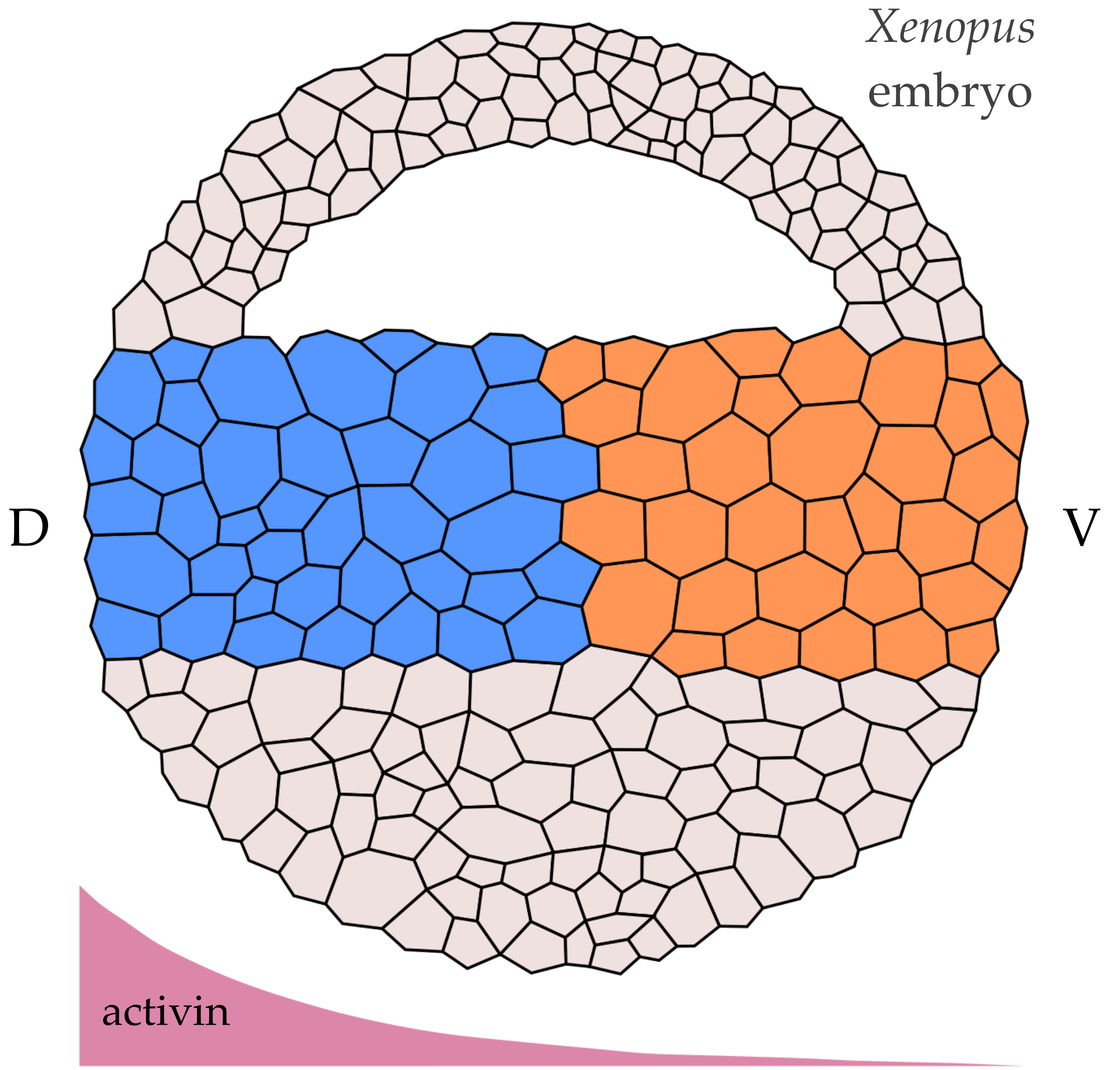
Workshop on "Flags, Landscapes, Signaling" *May 10th, 2024*

Positional information via morphogen gradients

- The concept of "positional information" relates to the idea that cells in developing embryos receive *external cues* to identify their positions within a coordinate system (with respect to boundaries), and then interpret this information to achieve distinct states.
- This can be achieved through the detection of the local concentration of an external gradient of signaling molecules (**morphogens**).
- A morphogen gradient arises from the secretion of molecules from a localized source, which diffuse and degrade over time.



Role of morphogen gradients

The graded distribution of morphogens allows the embryo to establish polarity along different axes.

The presence of genes that are expressed at high/low morphogen concentration aids in mesodermal patterning.

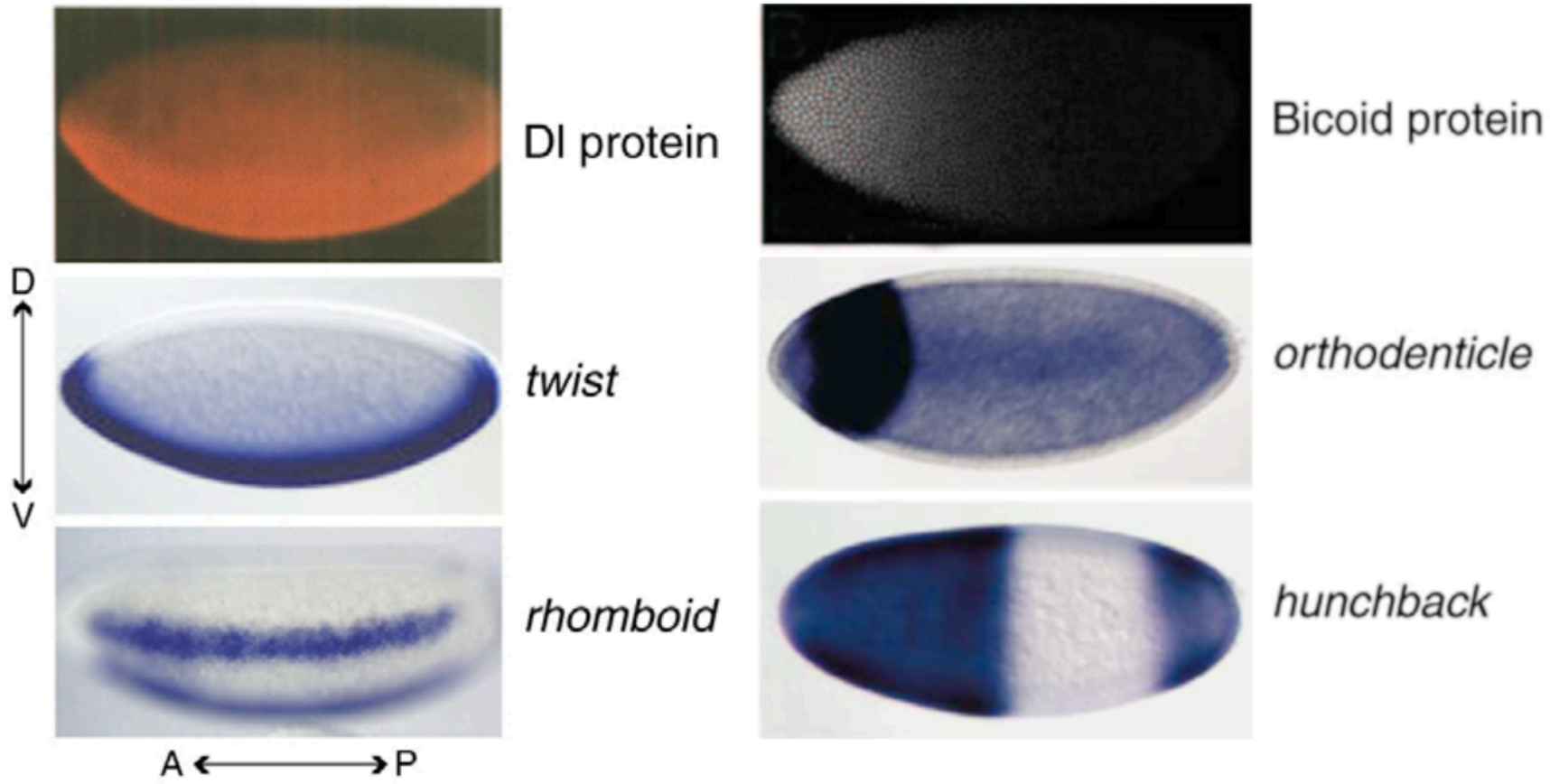
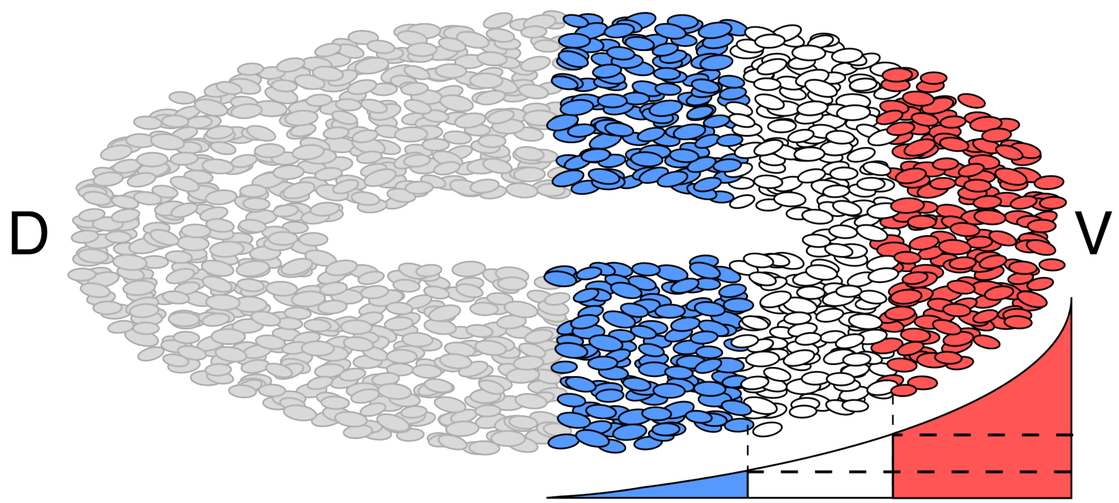
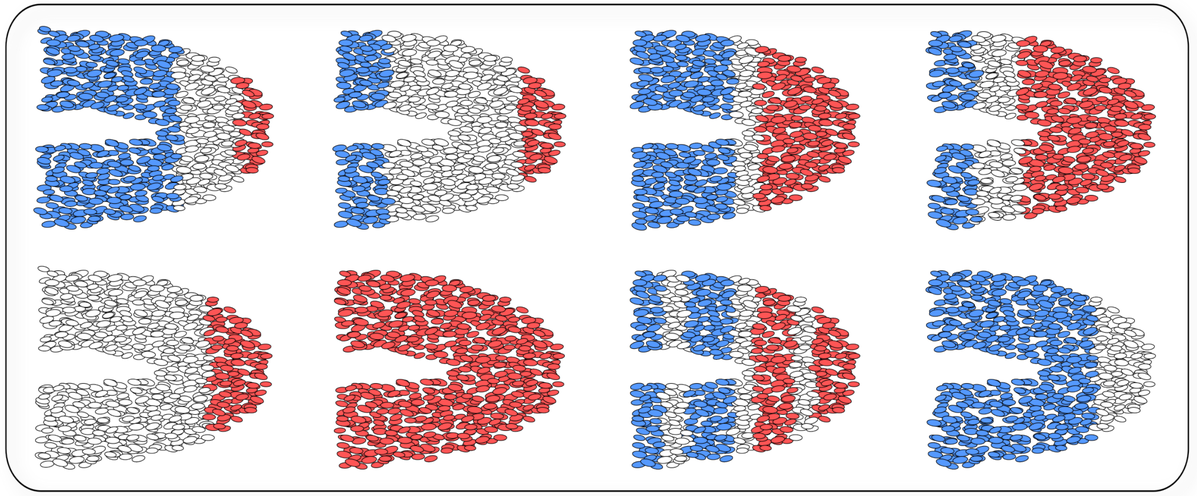


image source: H. L. Ashe & J. Briscoe, *Development* **133**: 385–394 (2006).

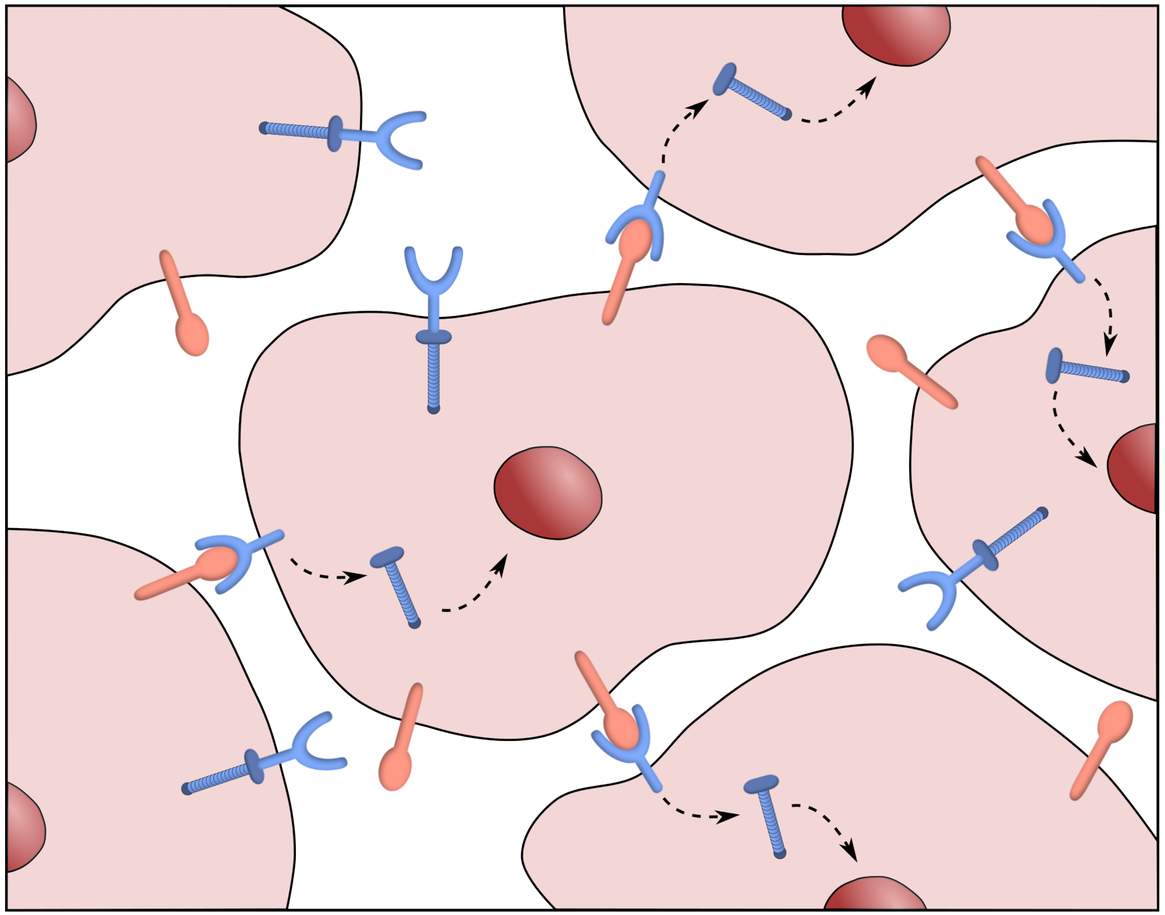
“Tuning” a flag

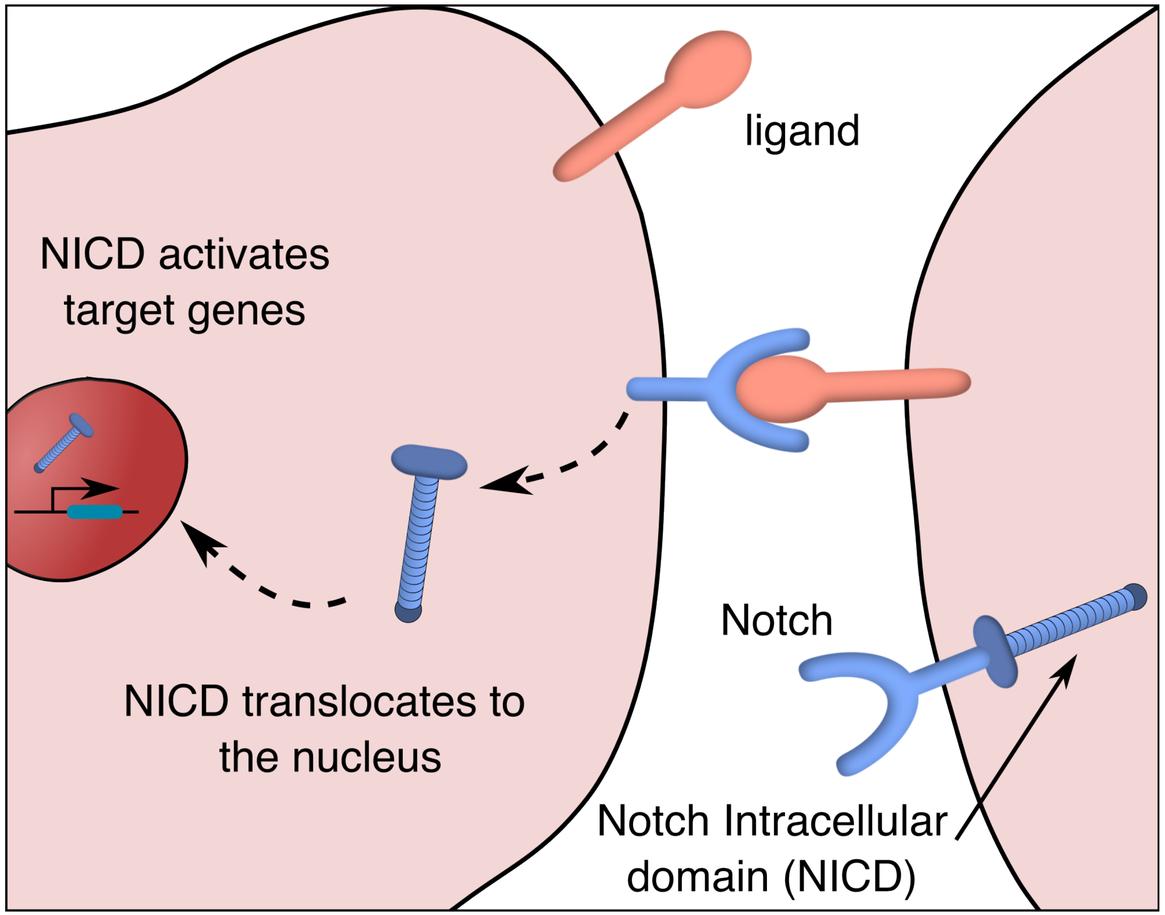




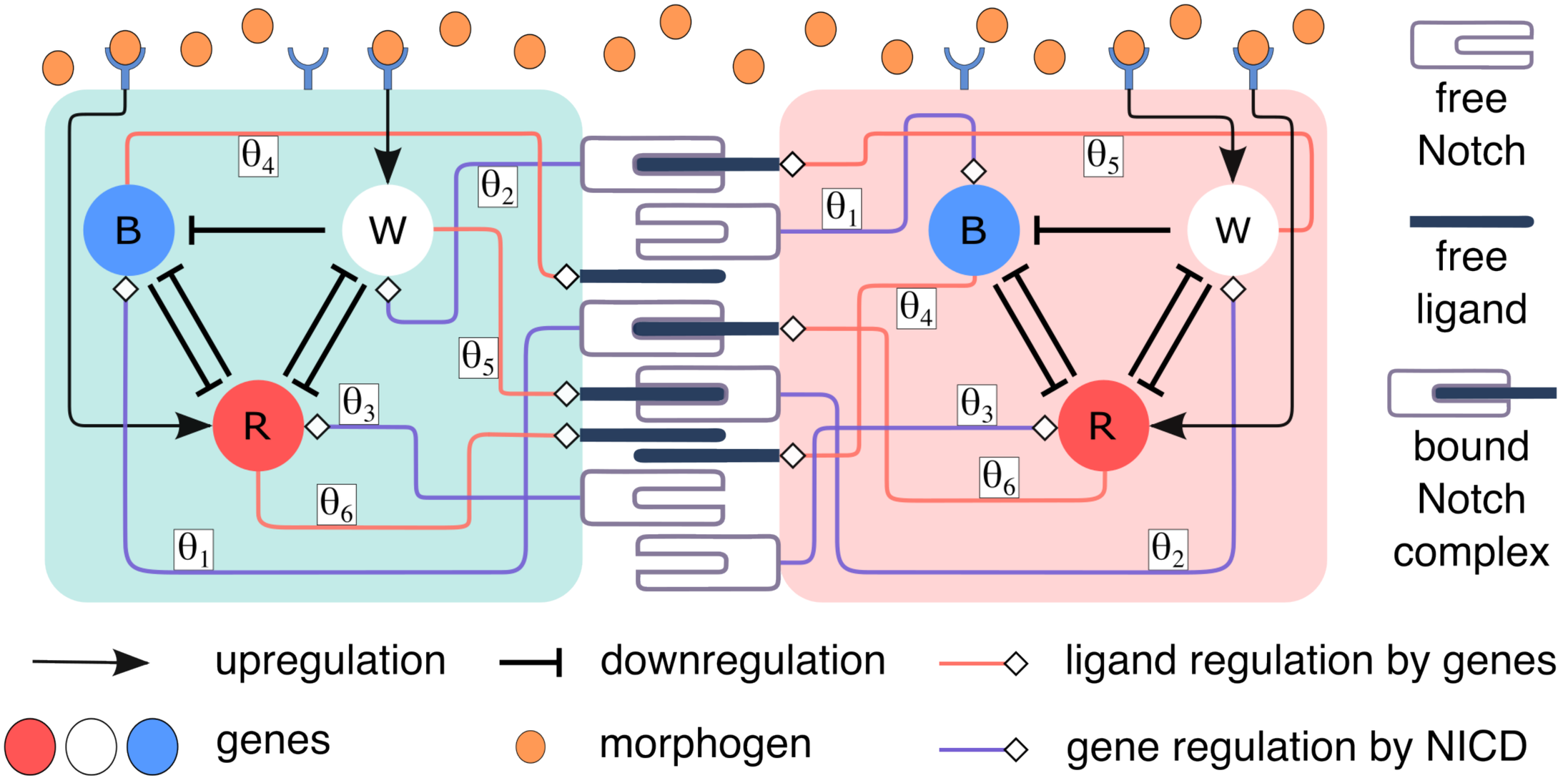
- Is it possible to vary the size of cell-fate domains in a regulated manner?
- Could inter-cellular communication play a role?
- How do we prevent pathological outcomes that do not preserve the number and sequence of these domains?

The Notch signalling pathway



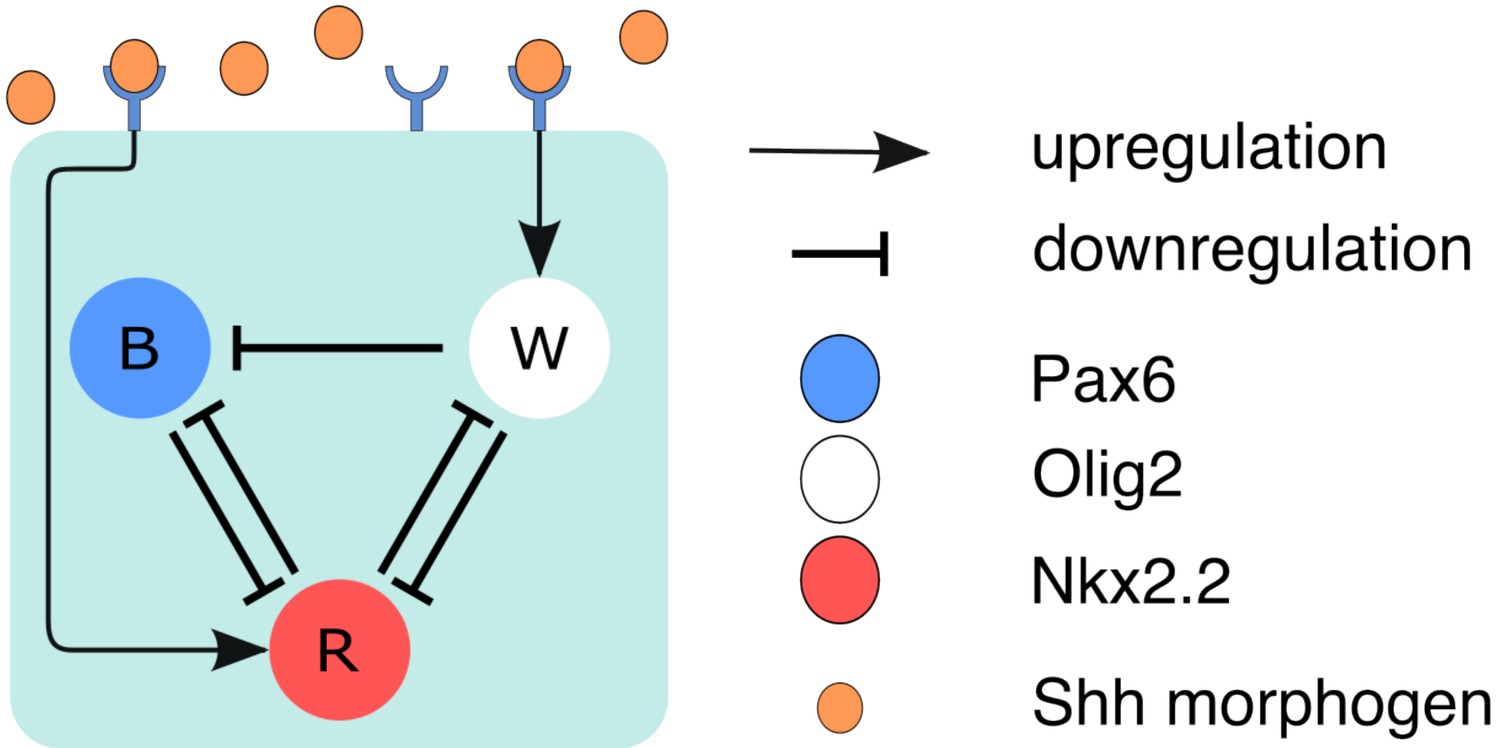


Overview of the model



Three-gene interpretation module

- We use a three-gene interpretation module* associated with the Sonic hedgehog (Shh) morphogen gradient in vertebrate neural tubes.
- Pax6 expression occurs even in the absence of the Shh morphogen.



* N. Balaskas et al., *Cell* **148**:273 (2012).

Model of Balaskas et al. (2012)

The time evolution of the expression levels of the three genes are described by:

$$\frac{dB}{dt} = \frac{\alpha}{1 + \left(\frac{R}{K}\right)^{h_1} + \left(\frac{W}{K}\right)^{h_2}} - k_1 B$$

$$\frac{dW}{dt} = \frac{\beta S_M}{1 + S_M} \frac{1}{1 + \left(\frac{R}{K}\right)^{h_3}} - k_2 W$$

$$\frac{dR}{dt} = \frac{\gamma S_M}{1 + S_M} \frac{1}{1 + \left(\frac{B}{K}\right)^{h_4} + \left(\frac{W}{K}\right)^{h_5}} - k_3 R$$

α, β, γ : maximal growth rates

k_1, k_2, k_3 : decay rates

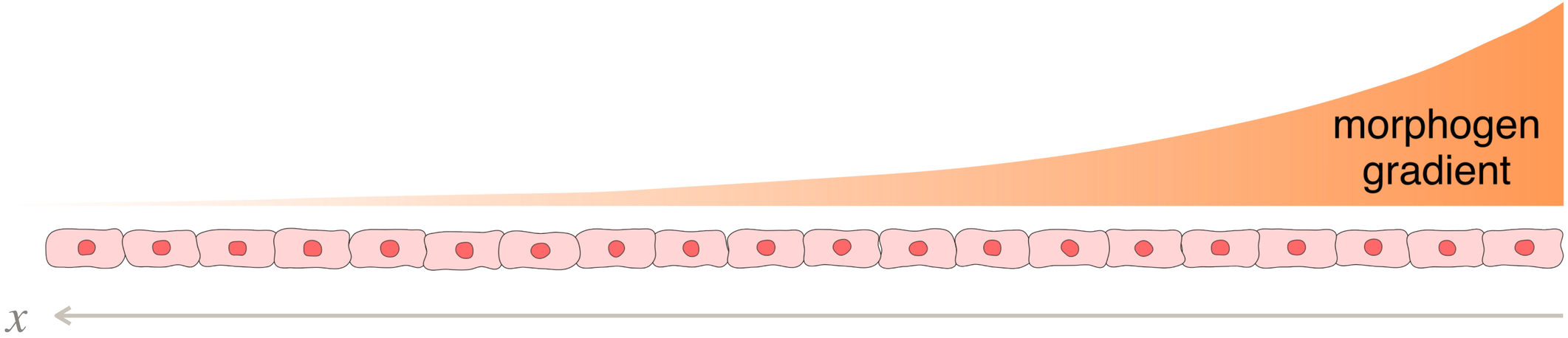
K : threshold of response function

h_1, h_2, h_3, h_4, h_5 : Hill coefficients

S_M : morphogen level

N. Balaskas et al., *Cell* **148**:273 (2012).

Morphogen gradient



- We consider a linear array of cells ($i = 1,2,\ldots,30$) responding to a morphogen (Shh) whose concentration decays exponentially away from the source.
- This spatial profile is mirrored in the concentration of signaling molecules (S_M) released as a result of morphogen molecules binding to receptors on the cell membrane:

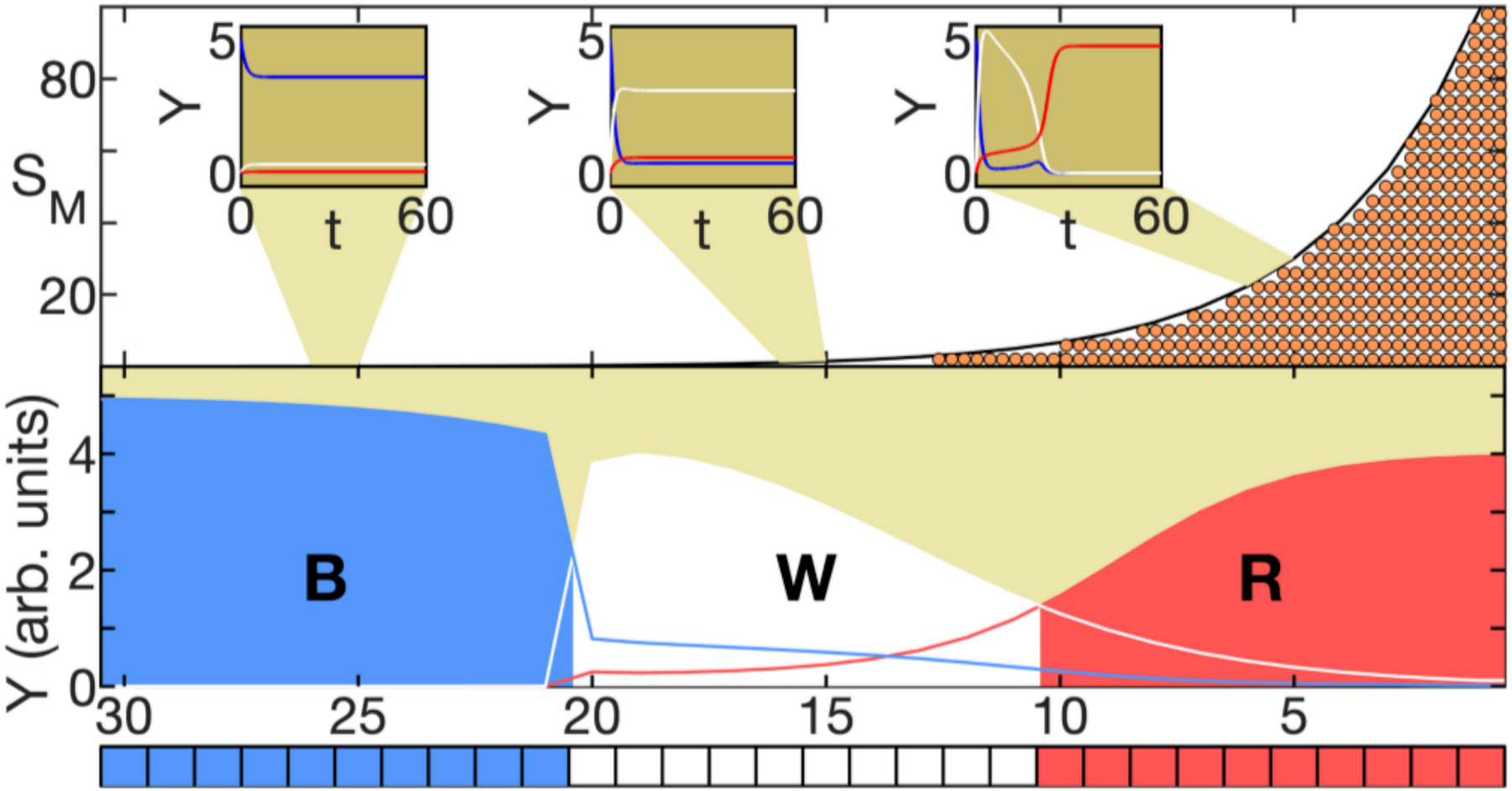
$$S_M(x) = S_M(0)\ \exp(-x/\lambda_M)$$

Behaviour of an uncoupled array of cells

We simulate this model over a linear array.

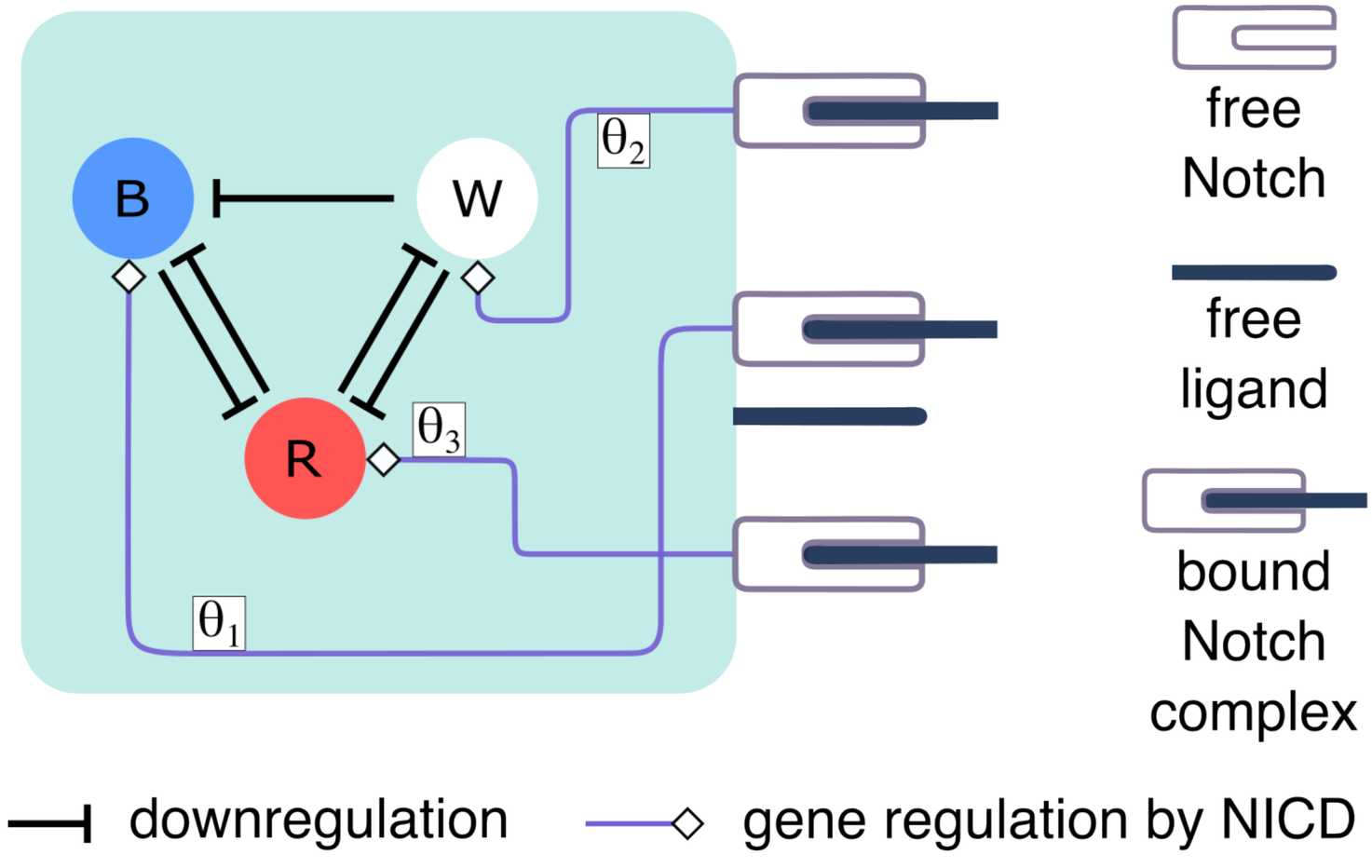
We track the expression levels *Y* of each of the three genes.

After an initial transient period. three cell-fate domains arise.



Incorporating juxtacrine signaling

For simplicity, we assume that NICD either upregulates all of the genes or downregulates all of them.



Incorporating juxtacrine signaling

If the concentrations of NICD and ligand are N^b and L, respectively, then:

$$\frac{dB}{dt} = \frac{\alpha + \varphi_1 \frac{N^b}{K_N}}{1 + \left(\frac{R}{K}\right)^{h_1} + \left(\frac{W}{K}\right)^{h_2} + \xi_1 \frac{N^b}{K_N}} - k_1 B$$

$$\frac{dW}{dt} = \frac{\beta S_M + \varphi_2 \frac{N^b}{K_N}}{1 + S_M + \xi_2 \frac{N^b}{K_N}} \frac{1}{1 + \left(\frac{R}{K}\right)^{h_3}} - k_2 W$$

$$\frac{dR}{dt} = \frac{\gamma S_M + \varphi_3 \frac{N^b}{K_N}}{1 + S_M + \xi_3 \frac{N^b}{K_N}} \frac{1}{1 + \left(\frac{B}{K}\right)^{h_4} + \left(\frac{W}{K}\right)^{h_5}} - k_3 R$$

$\varphi_{1,2,3}$: strength of upregulation

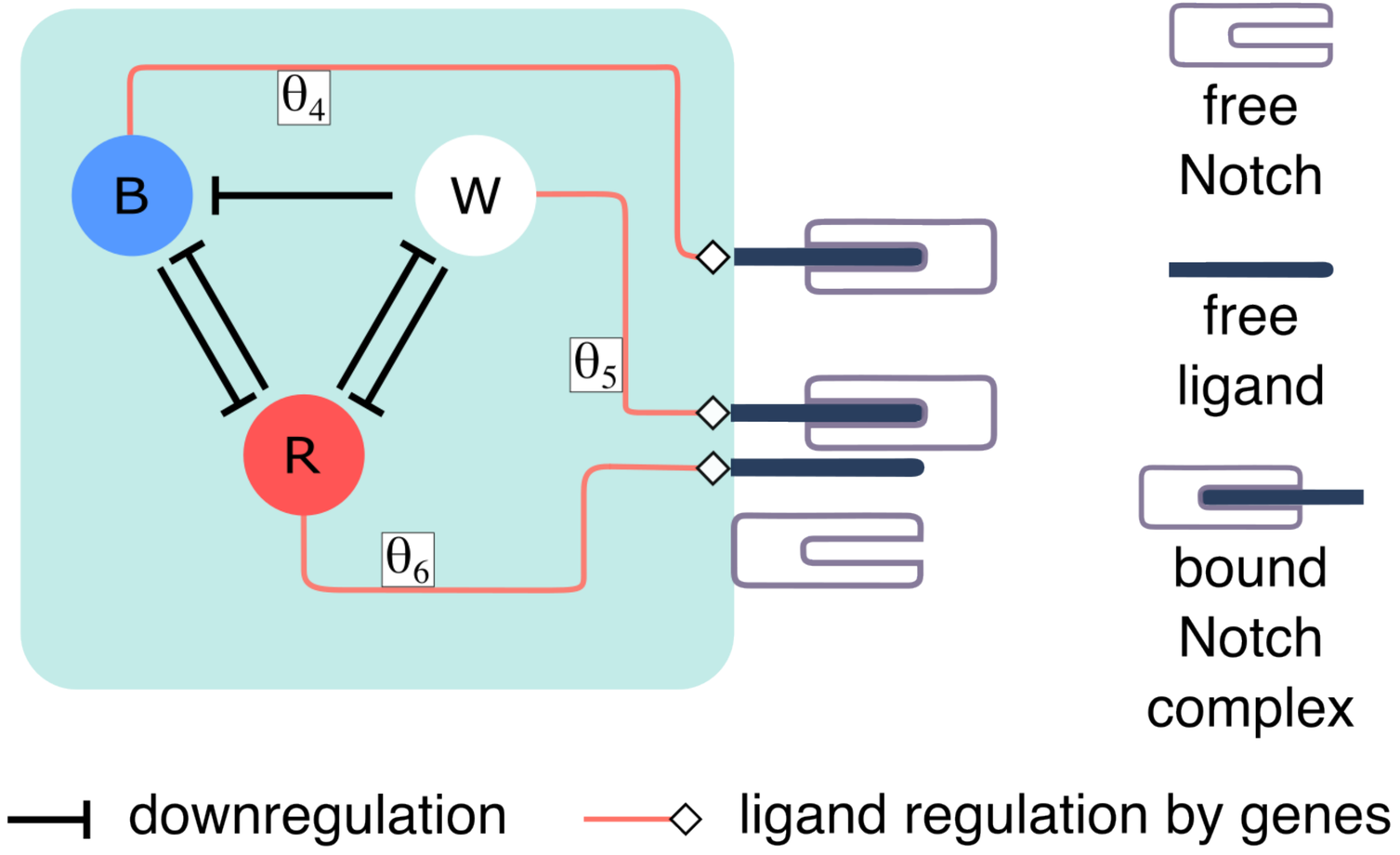
$\xi_{1,2,3}$: strength of downregulation

K_N : threshold of response function

N. Balaskas et al., *Cell* **148**:273 (2012).

Ligand regulation

For simplicity, we assume that the ligand is either activated by all of the genes or inhibited by each of them.



Modelling Notch signaling

The time evolution of the concentrations of ligand (L) and NICD (N^b) is given by:

$$\frac{dL}{dt} = \frac{\beta_L + \phi_4 \frac{B}{K} + \phi_5 \frac{W}{K} + \phi_6 \frac{R}{K}}{1 + \zeta_4 \frac{B}{K} + \zeta_5 \frac{W}{K} + \zeta_6 \frac{R}{K}} - \frac{L}{\tau_L}$$

$$\frac{dN^b}{dt} = \frac{\beta_{N^b} L^{trans}}{K + L^{trans}} - \frac{N^b}{\tau_{N^b}}$$

$\phi_{4,5,6}$: strength of promotion

$\zeta_{4,5,6}$: strength of repression

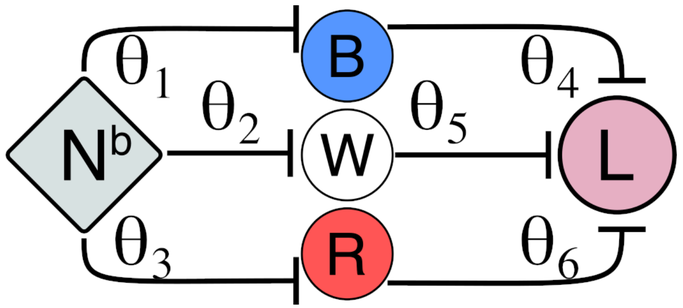
β_{L,N^b} : maximum growth rates

τ_{L,N^b} : mean lifetimes

The trans-binding of Notch receptors to ligands (L^{trans}) of neighbouring cells leads to the release of the receptors intracellular domain (N^b) to be released.

N. Balaskas et al., *Cell* **148**:273 (2012).

Classes of intercellular interactions

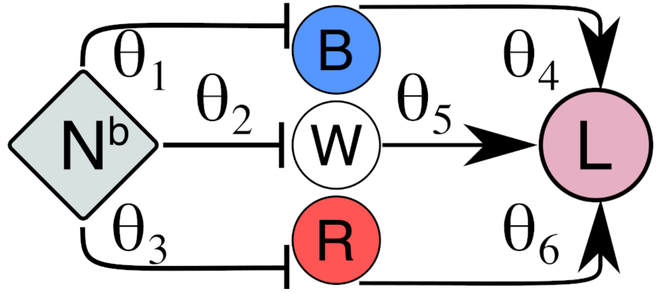


type I : $(0, \theta_i, 0, \theta_j)$

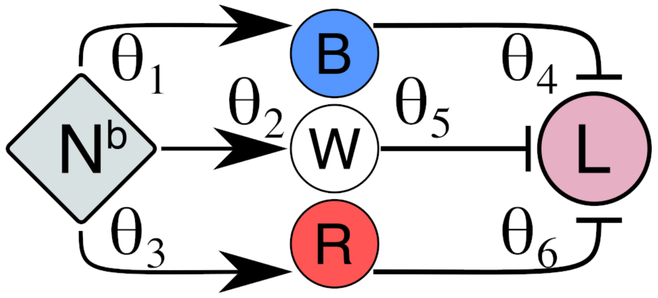
Using the same model, we can describe 4 classes of intercellular interaction, specified by the parameter set $(\varphi_i, \xi_i, \phi_j, \zeta_j)$, $i = 1,2,3$, $j = 4,5,6$.

For downregulation, $\varphi = 0$ (or $\phi = 0$).

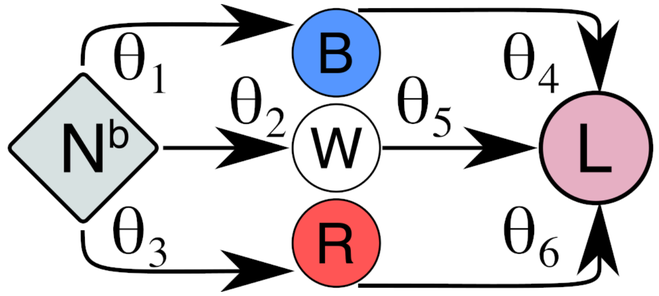
For upregulation $\xi = 1$ (or $\zeta = 1$) while $\varphi \geq 1$ (or $\phi \geq 1$).



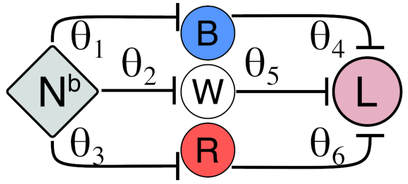
type II : $(0, \theta_i, \theta_j, 1)$

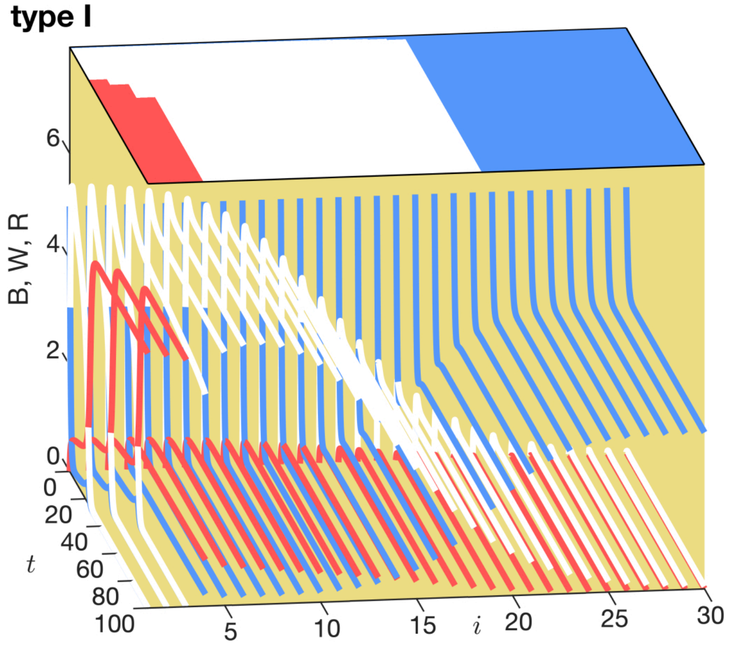


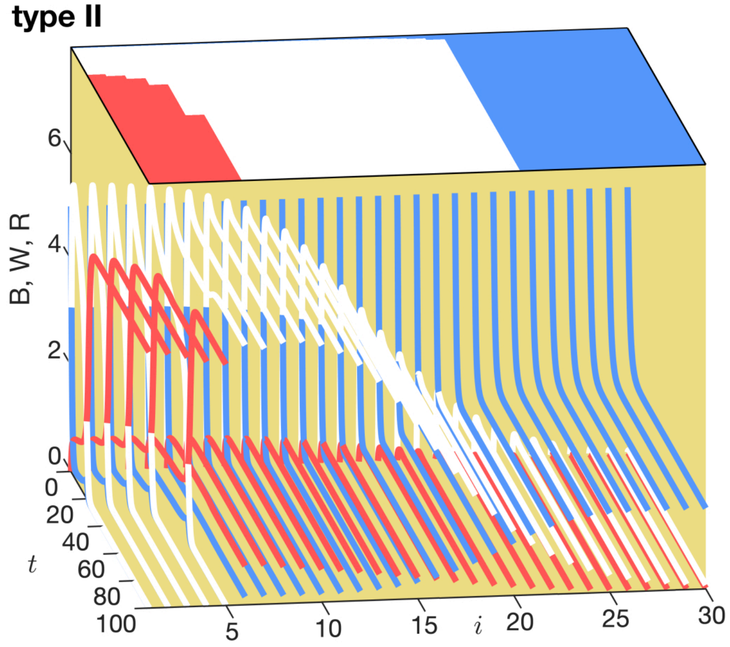
type III : $(\theta_i, 1, 0, \theta_j)$

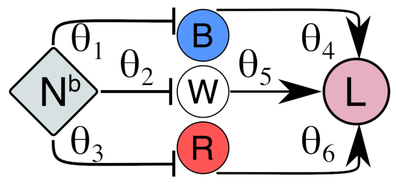


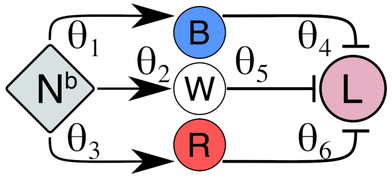
type IV : $(\theta_i, 1, \theta_j, 1)$

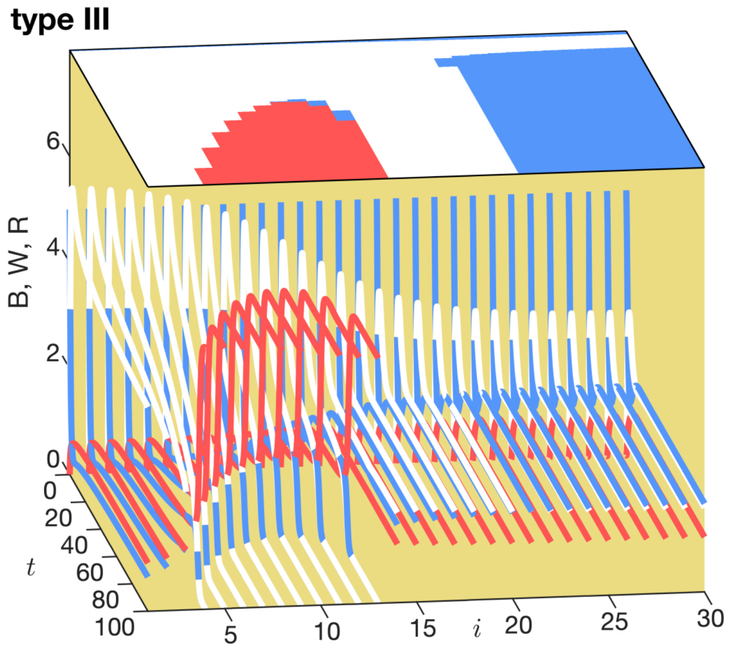


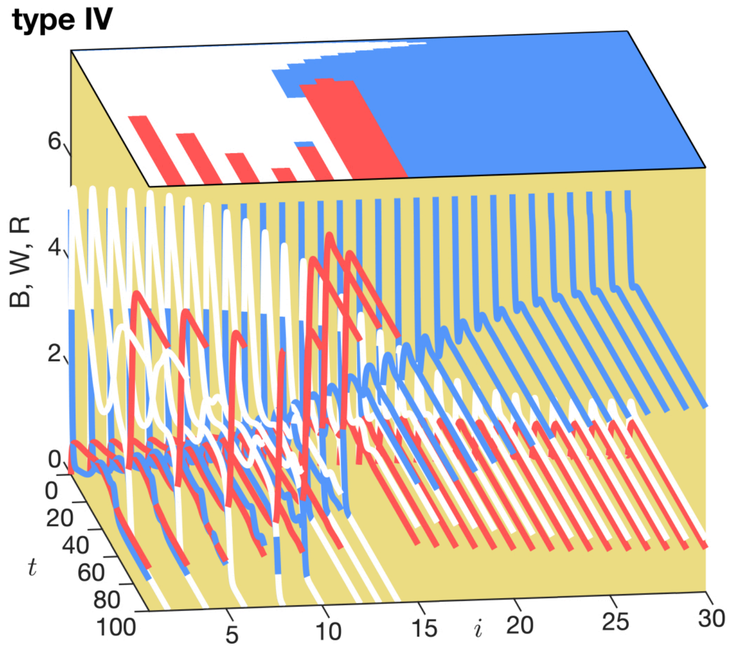


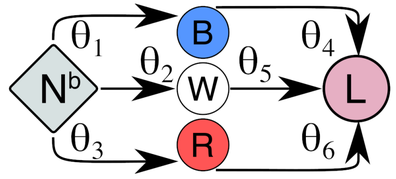




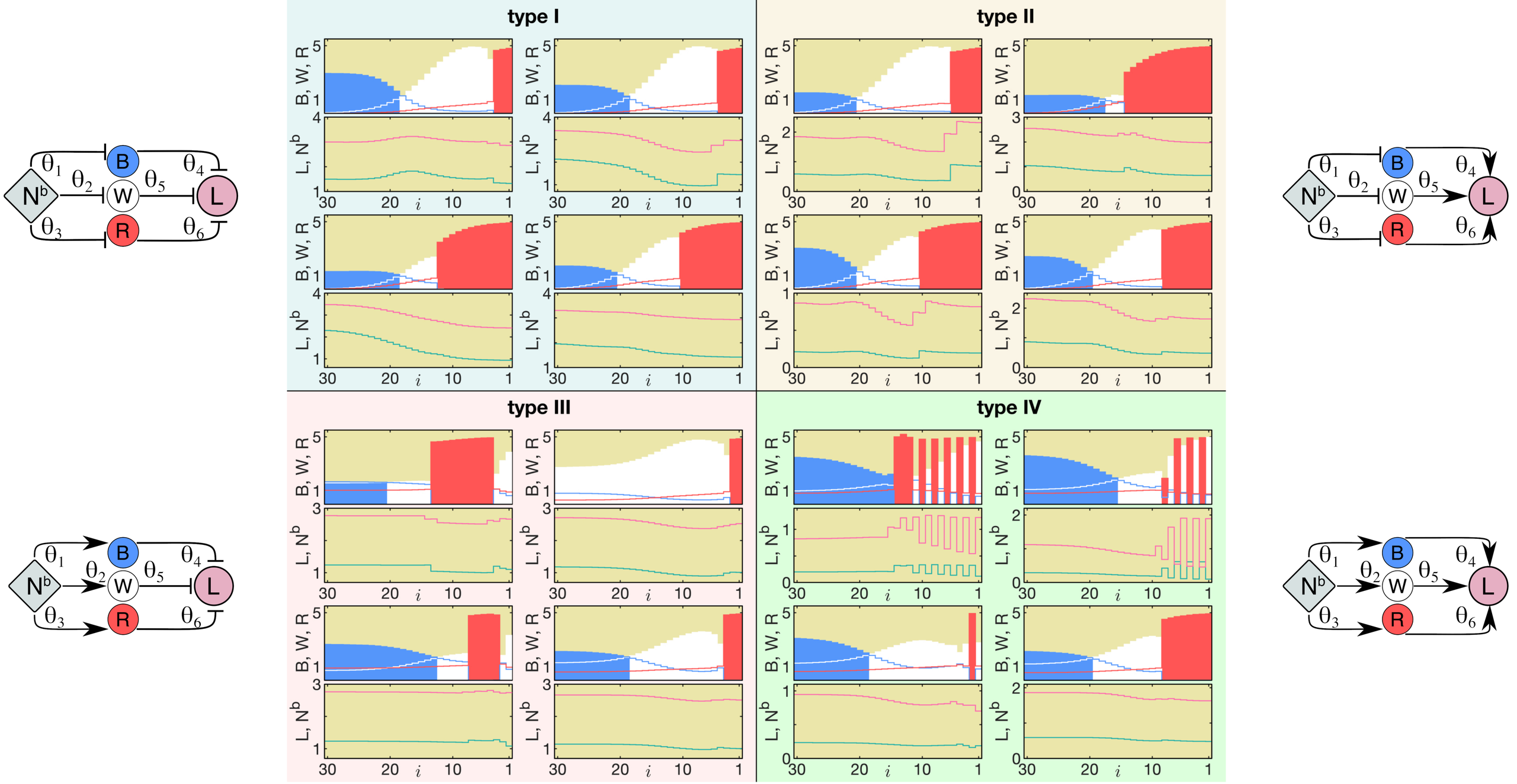






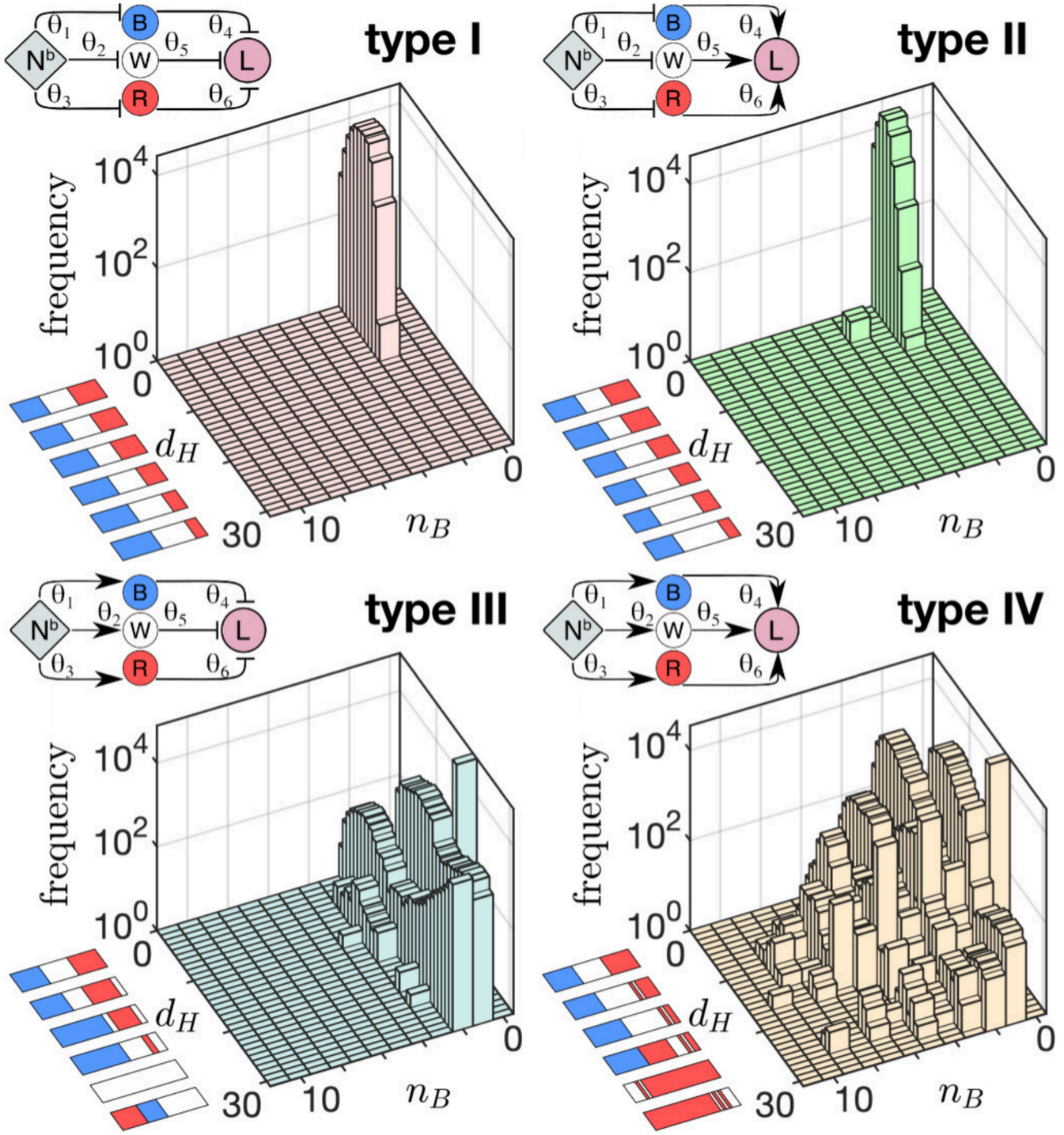


Spatio-temporal evolution of the expression levels of the patterning genes for the four coupling types



Final expression levels of the patterning genes for the four coupling types

- We consider 10^5 parameter sets $\Theta = \{\theta_1, \ldots, \theta_6\}$, where each θ_k is sampled from [1,10] for activation parameters and [0.1,1] for inhibition parameters.
- We quantify the variation in observed flags in terms of n_B, the number of *fate boundaries*, and d_H, the Hamming distance to the idealized flag (obtained in the absence of coupling).
- Repression of B, W, R almost always results in flags having two boundaries.
- Coupling types I & II are much closer to the idealized flag in terms of d_H.

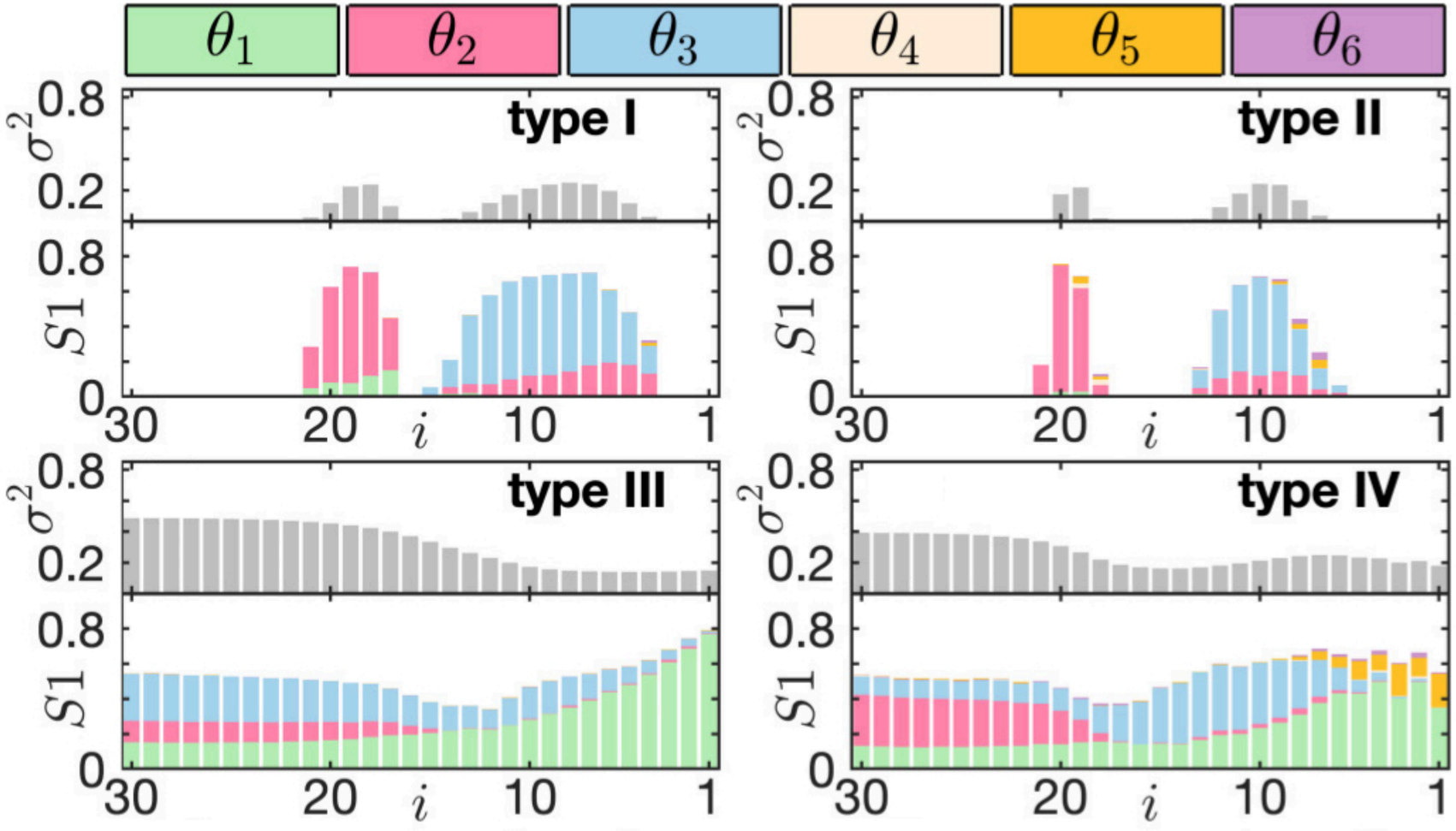


The Sobol' method

- This is a variance-based sensitivity analysis technique that we can use to quantify the contribution of each of the parameters $\Theta = \{\theta_1, \ldots, \theta_6\}$ in determining the cell fates.
- We consider the final state of each cell i to be represented by $F_i \in \{0,1,2\}$ corresponding to blue, white and red.
- At each site i we compute the variance σ^2 in fate F_i across realizations.
- We measure the first-order sensitivity indices $S1$ at each site i by computing the variance of $\langle F_i | \theta_j \rangle_{\theta_{k(\neq j)}}$ and normalising by the corresponding σ^2.

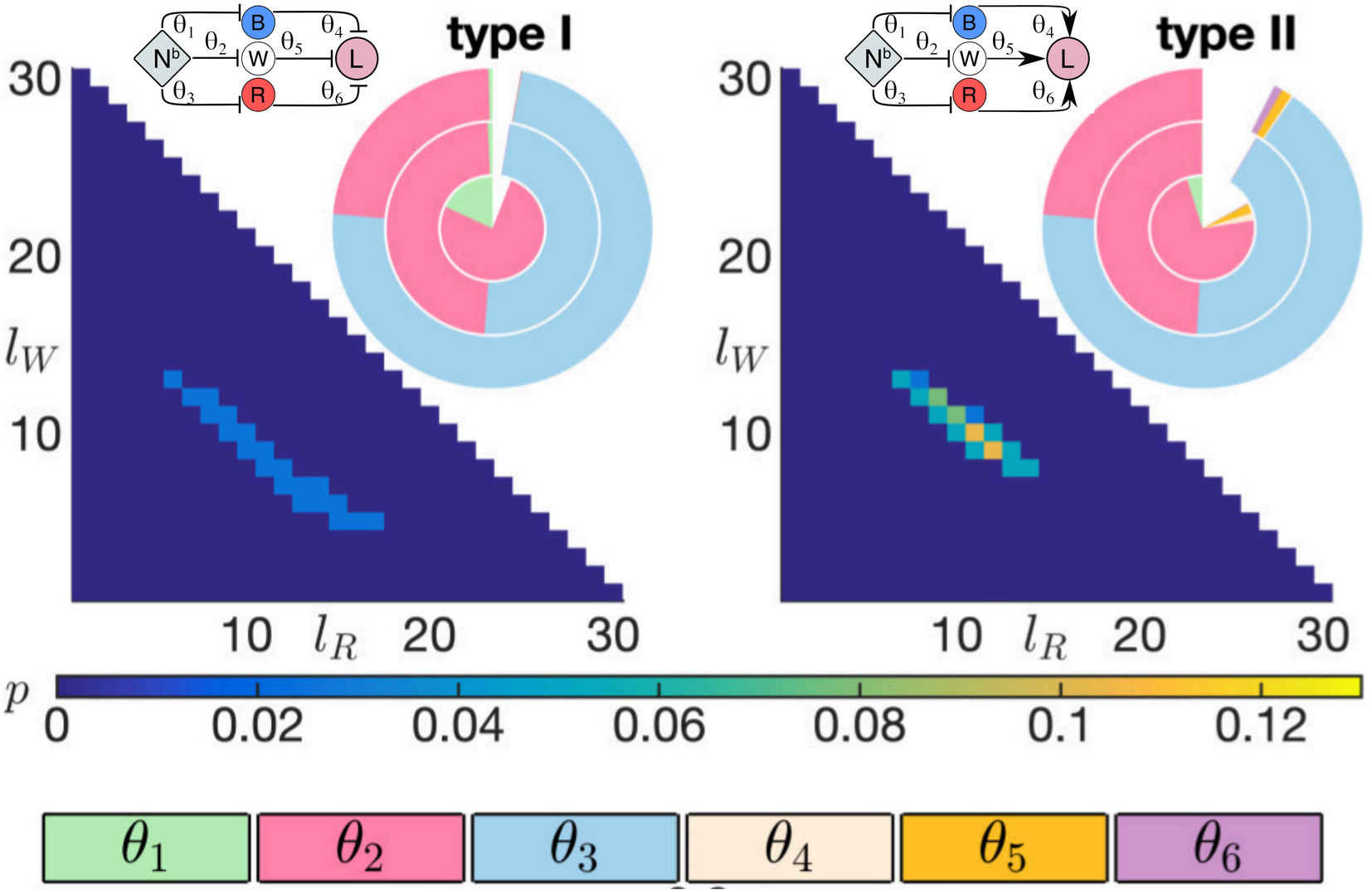
Dependence of cell fate variation on location

- The variance σ^2 is mostly negligible in types I & II.
- Only θ_2 and θ_3 contribute significantly to all coupling types.
- The bulk of variation in F_i can be explained from $S1$ alone.

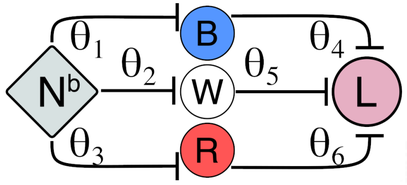


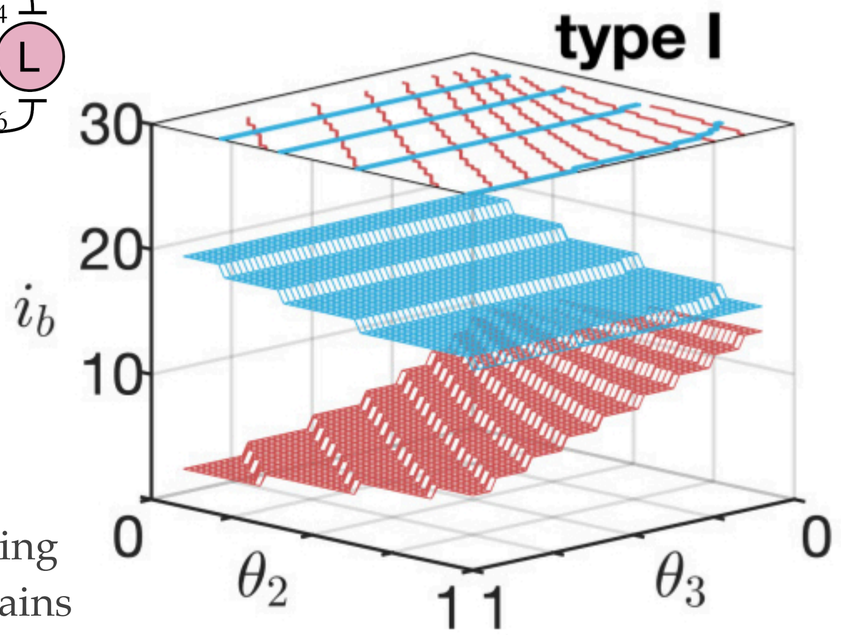
Sensitivity of segment lengths

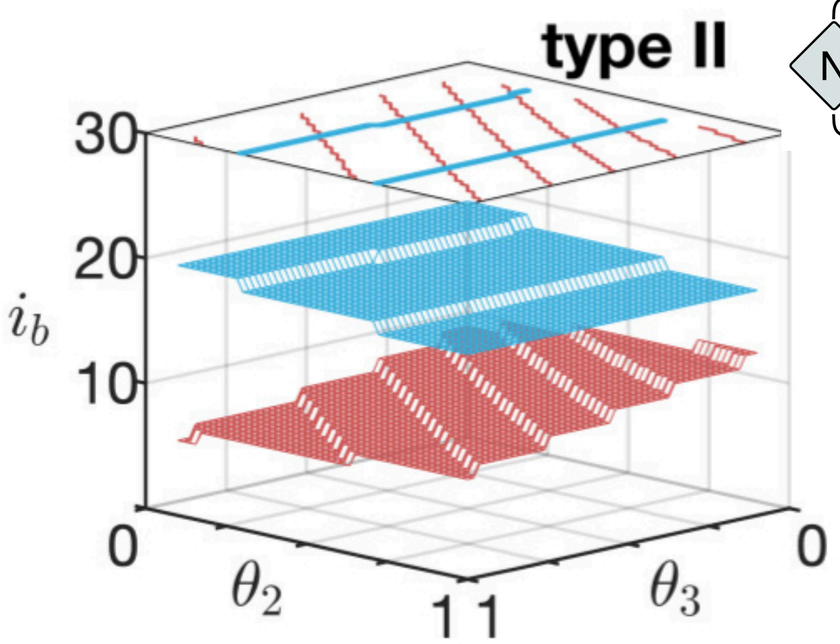
- For types I & II, the lengths of the white and red segments seen from the ensemble simulations are narrowly distributed around the values of the uncoupled case ($l_R^* = 10, l_W^* = 10$).
- Pie charts display results of a sensitivity analysis in terms of l_R (outer), l_W (middle) and l_B (inner).

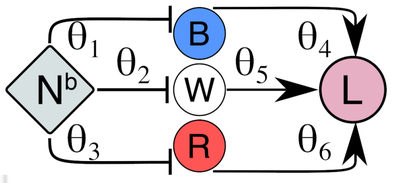


Dependence of boundary location on significant parameters







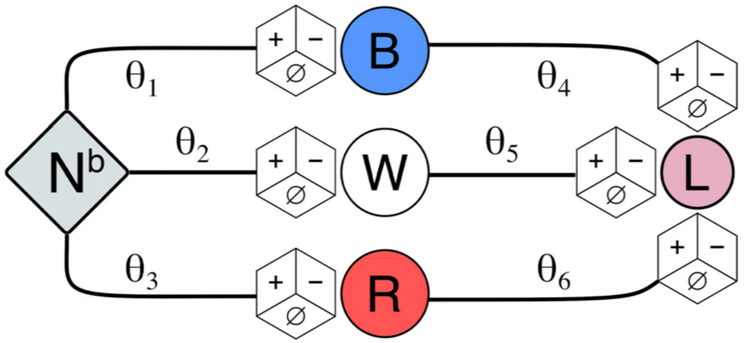


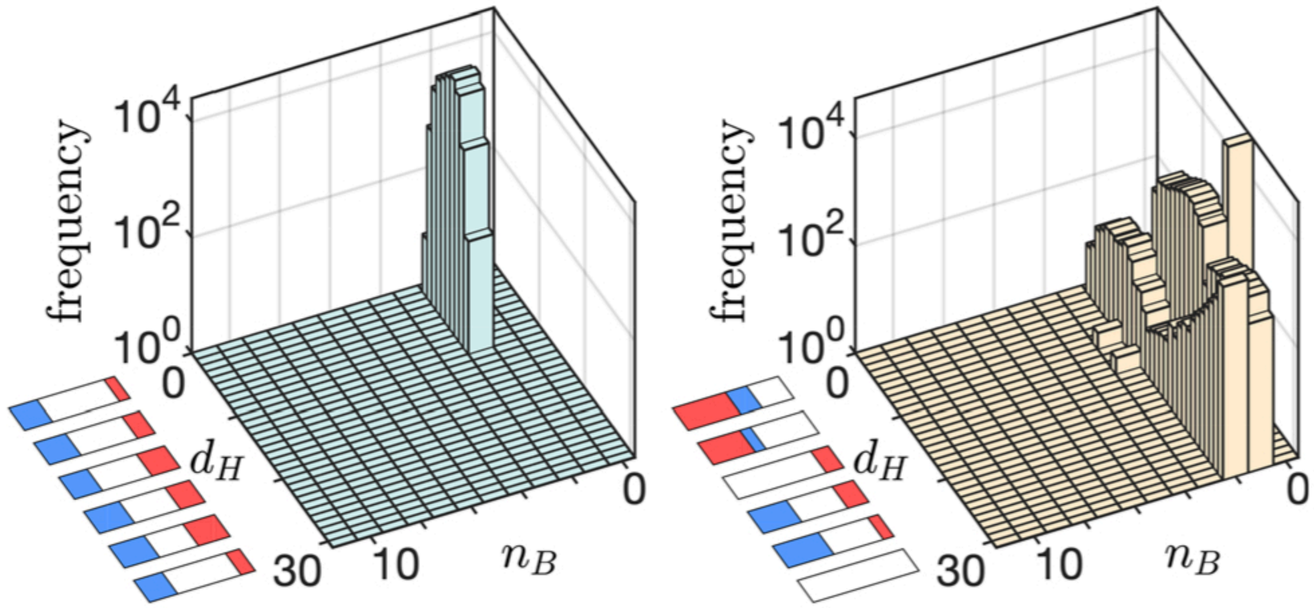
i_b: location of the boundary separating the R and W domains (red), and the B and W domains (blue).

- Increasing θ_2 expands both the R & B regions at the expense of W.
- Increasing θ_3 expands both the R region with virtually no change to the B region.

Using a generalized architecture

- The modelling framework can be easily generalized to consider scenarios where each of the 6 interactions can correspond to either upregulation (+), downregulation (−) or no effect (∅).
- We can thus investigate $3^6 = 729$ different classes of intercellular coupling, and in each case we simulate the system dynamics with 10^4 combinations of coupling strengths $\theta_1 \ldots, \theta_6$.

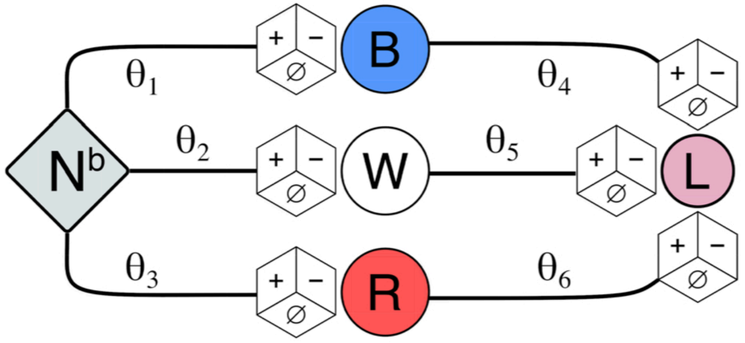


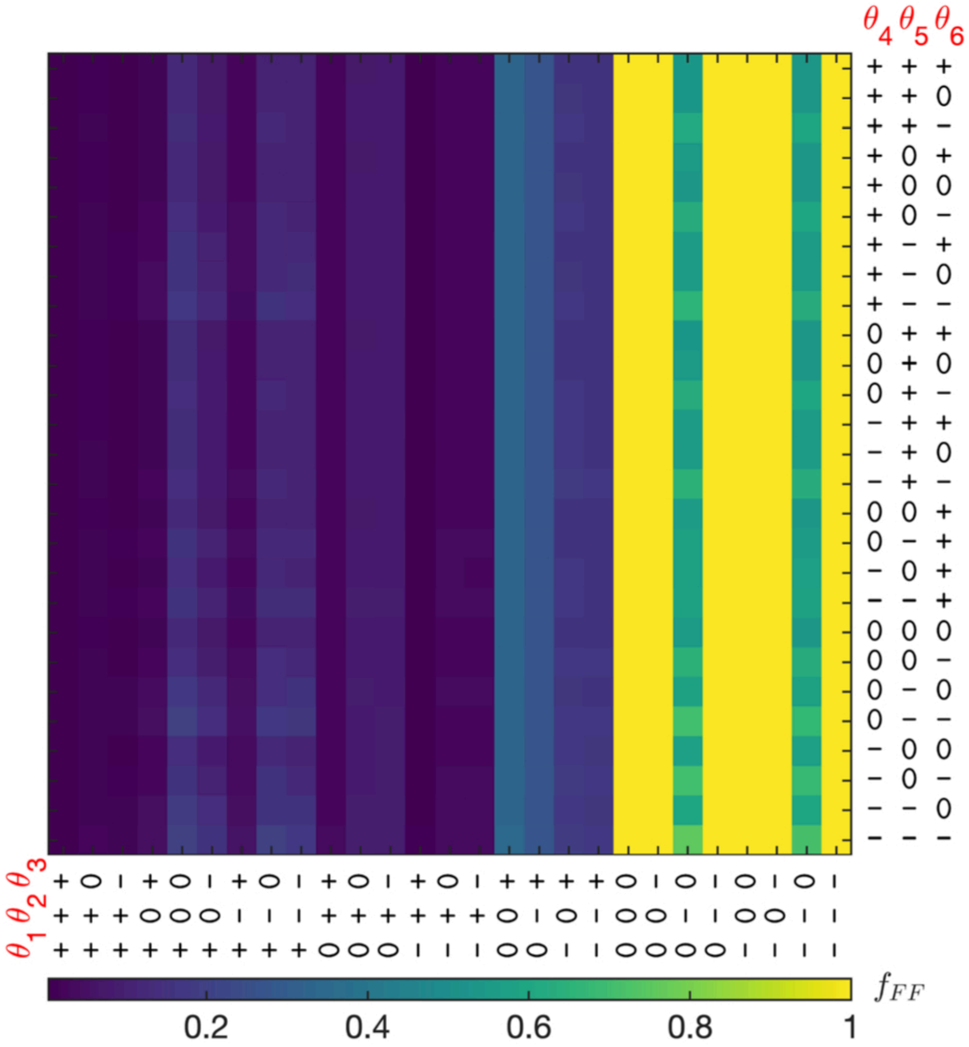


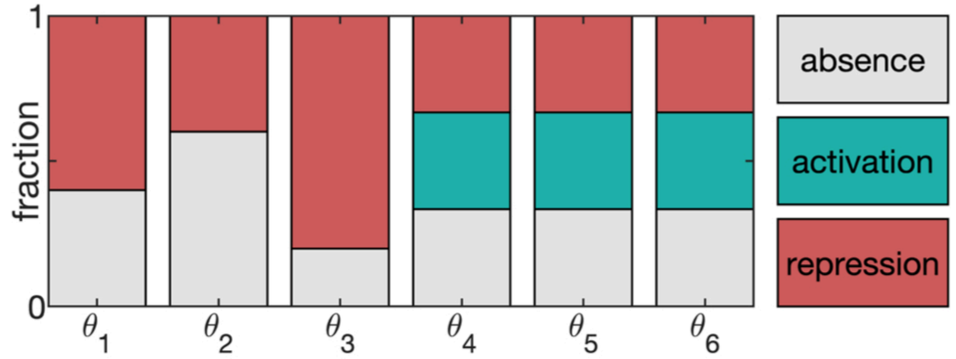
$(-, \varnothing, -, -, -, +)$

$(+, +, +, +, \varnothing, -)$

- For each of the 729 classes, we find the frequency (f_{FF}) that one observes “French flags”, having exactly 3 distinct regions, occurring in the expected chromatic order.
- The nature of regulation of ligand by patterning genes (governed by θ_{4-6}) plays an extremely minor role in regulating cell fate pattern.
- We find that desired flags are almost never obtained if θ_{1-3} is in the “+” class, i.e. if NICD upregulates one of the genes.







Conclusion (Part I)

- Juxtacrine signaling can in principle play a key role adaptively regulating cellular differentiation in developing tissues.
- This could potentially increase the robustness of the system in generating the desired flag by compensating for mutations affecting the production and/or interpretation of the morphogen.
- This framework integrates two paradigms for investigating biological pattern formation:
  - A **boundary-organized** mechanism involving a prepattern, via a morphogen gradient
  - A **self-organised** mechanism involving interactions between constituent species.

Publications:

C. Kuyyamudi, S. N. Menon, and S. Sinha, *Phys. Rev. E* **103**, 062409 (2021).

C. Kuyyamudi, S. N. Menon, and S. Sinha, *Indian J. Phys* **96**, 2657 (2022).

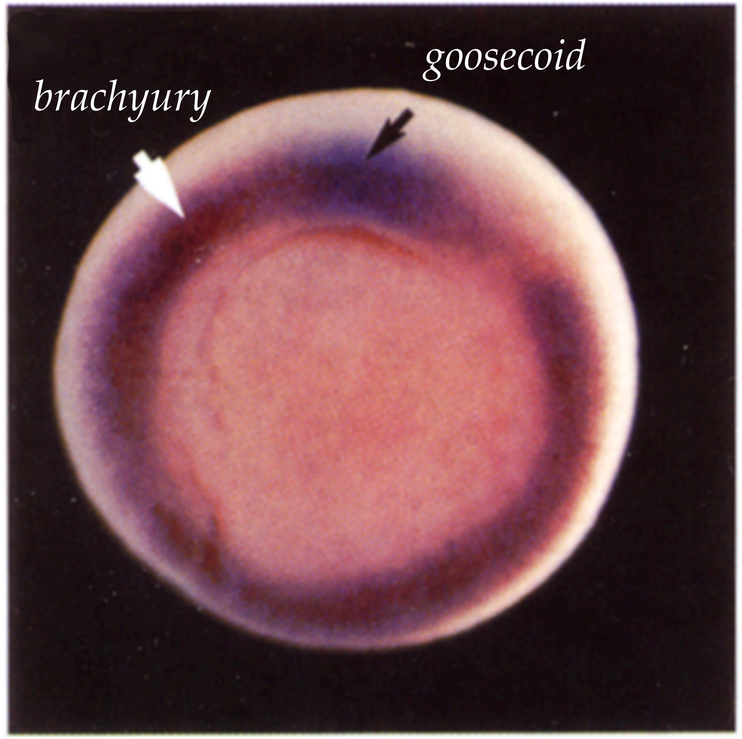
Associated code:

https://github.com/boyonpointe/Notch-a-French-flag

Development of cell-fate boundaries

As the embryo develops, sharp and robust boundaries are established between cells expressing different patterning genes. Their locations are invariant for a given species.

Development of a boundary between the expression domains of the genes *goosecoid* and *brachyury* in the Xenopus embryo.



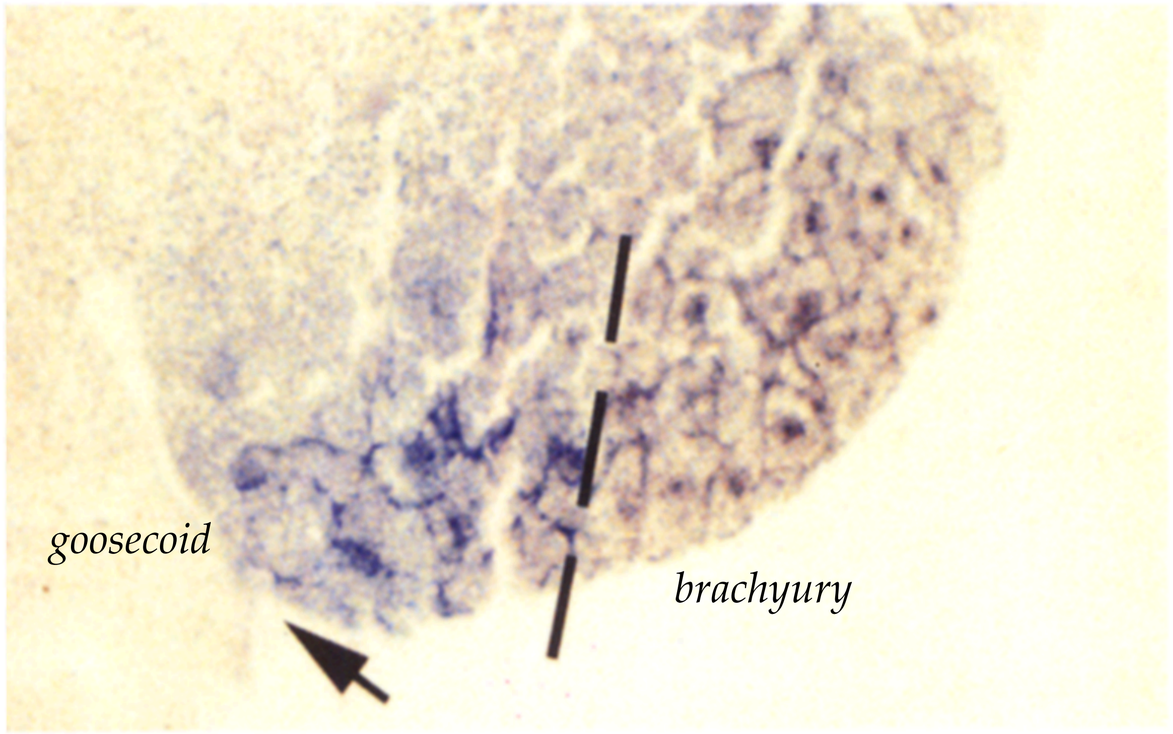
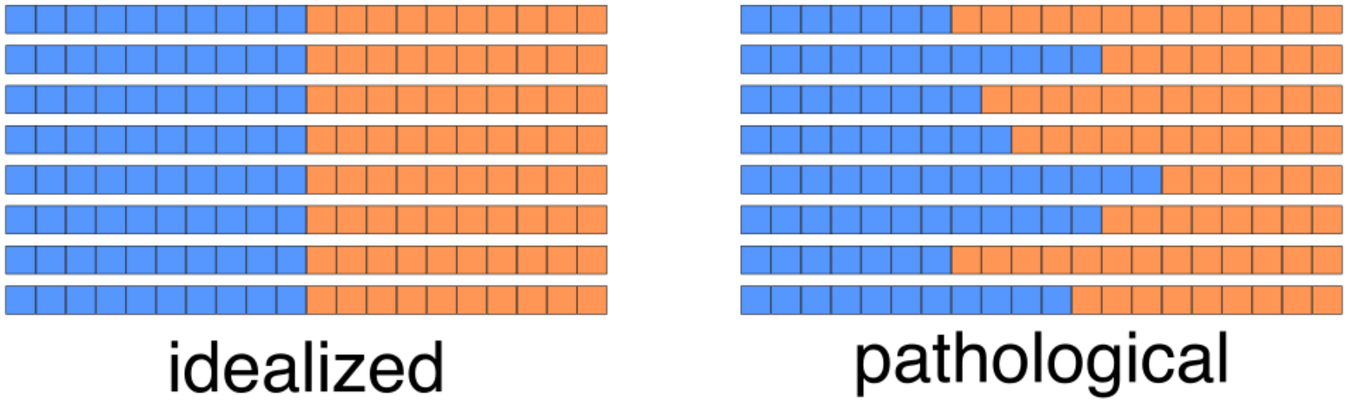


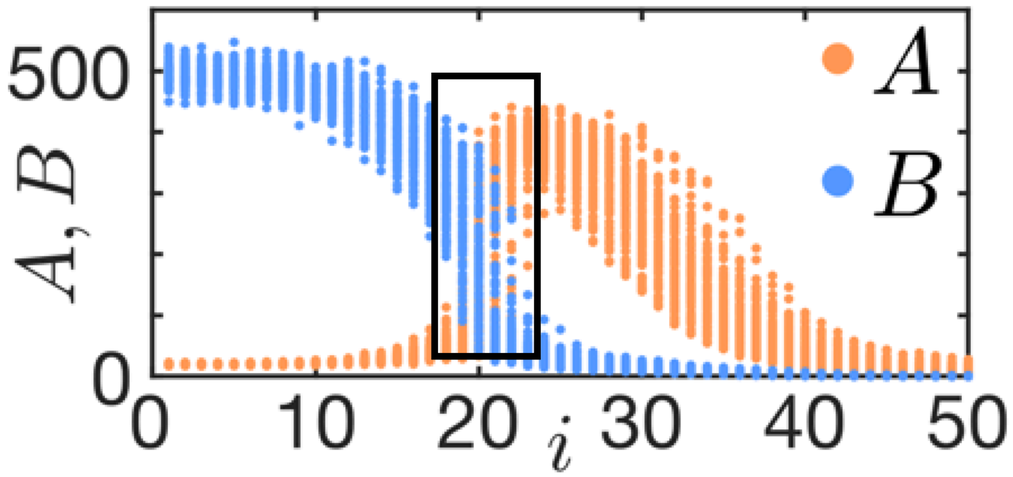
image source: M. Artinger et al., *Mech. Dev.* **65**:187-196 (1997).

Robustness and precision of cell-fate boundaries

The location of the boundary separating fate domains should ideally be invariant and robust.

If fate determination occurs on the basis of the (noisy) morphogen gradient alone, the boundary location will vary.





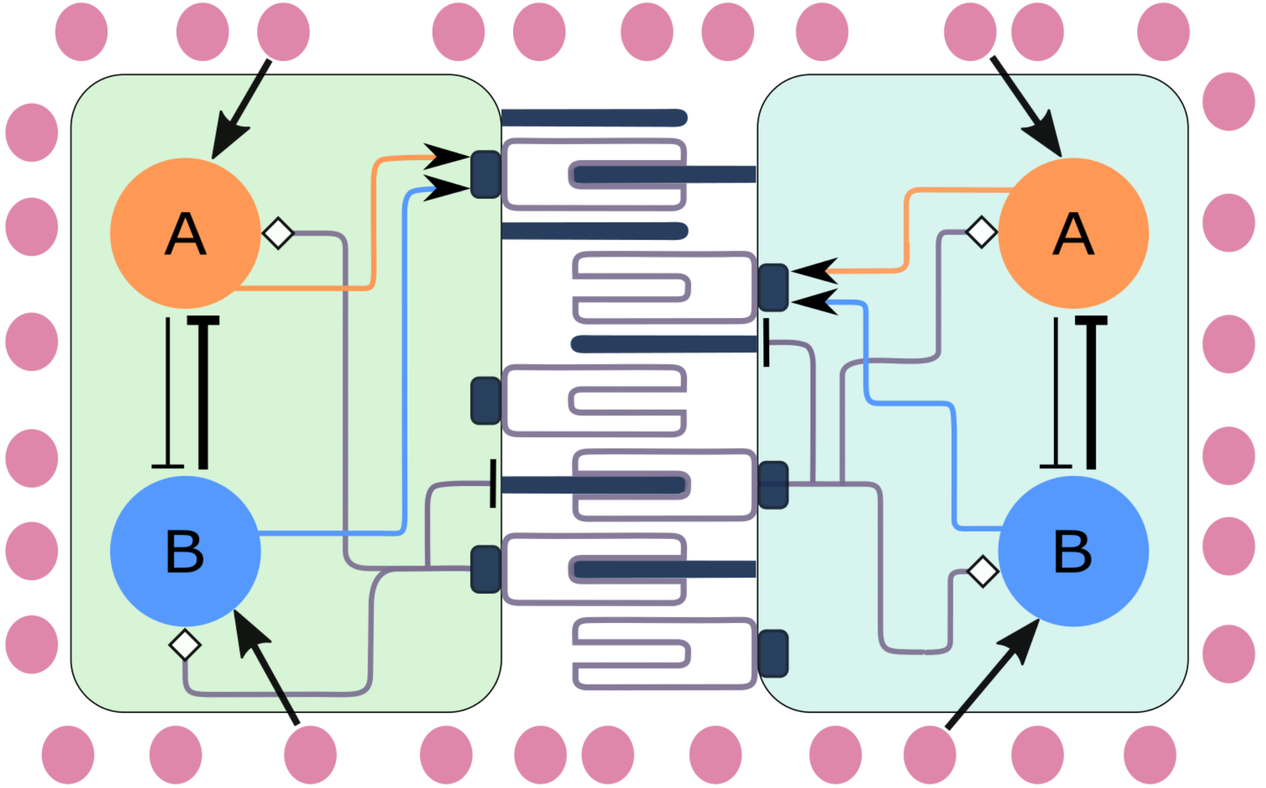
If we have two mutually inhibiting patterning genes expressed at low and high concentrations of morphogen, respectively, the fate of a cell at the interface of the two domains will be ambiguous.

Overview of conceptual framework

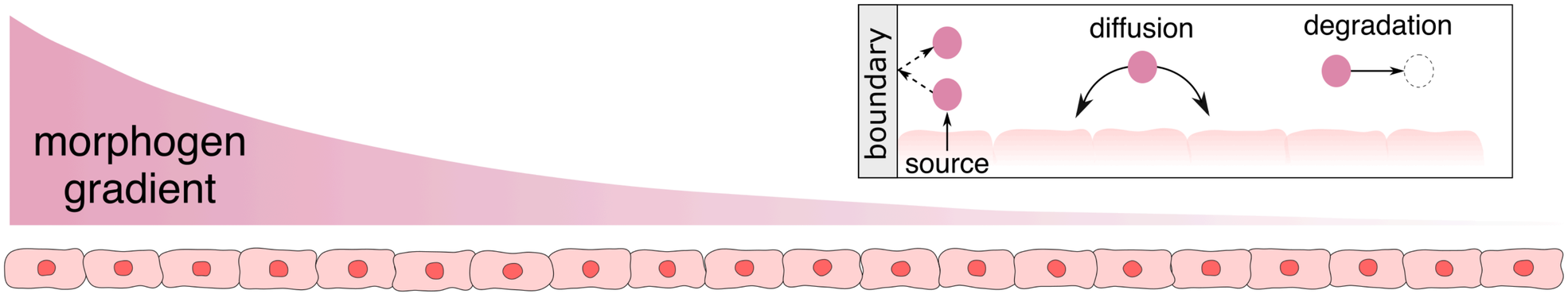
- We assume that the fates of each cell in a tissue subject to a morphogen gradient are governed by two patterning genes A and B.
- We model the stochastic dynamics of all variables $\mathbf{X}$ through a set of coupled equations of the form:

$$d\mathbf{X} = \mathcal{F}_X + \mathcal{G}_X\, dW$$

  where $\mathcal{F}_X$ are the deterministic components and the noise term $\mathcal{G}_X = \eta_X \mathbf{X}$.
- We consider a 1D array comprising $N(=50)$ cells, with 6 species associated with each cell. Each species X a mean lifetime τ_X.



Deterministic components of the dynamics

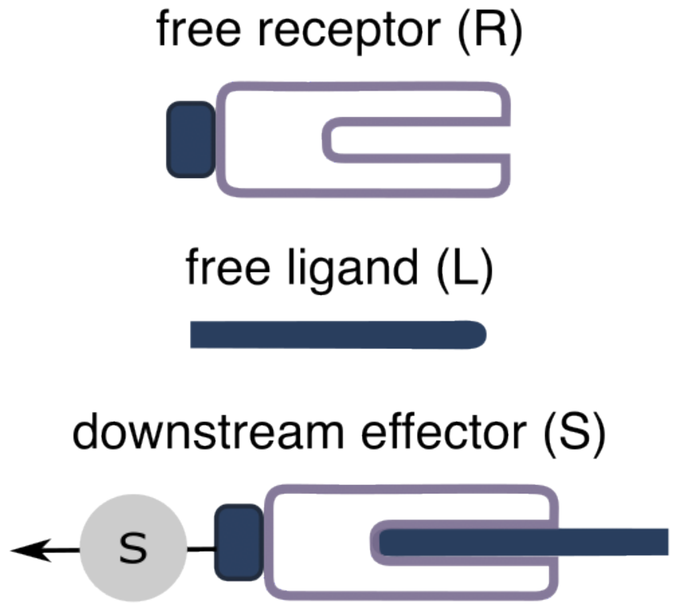


- **morphogen** (M): Produced at a constant rate from a source located at the leftmost cell (site $i = 1$) and diffuses with strength D_M.

$$\mathcal{F}_M = \alpha_M \delta_{i,1} - D_M \nabla^2 M - M/\tau_M$$

- **downstream effector** (S): Produced as a result of *trans*-activation of receptors (R) that bind with ligands of neighbouring cells (L_{tr}).

$$\mathcal{F}_S = k_{tr} R L_{tr} - S/\tau_S$$

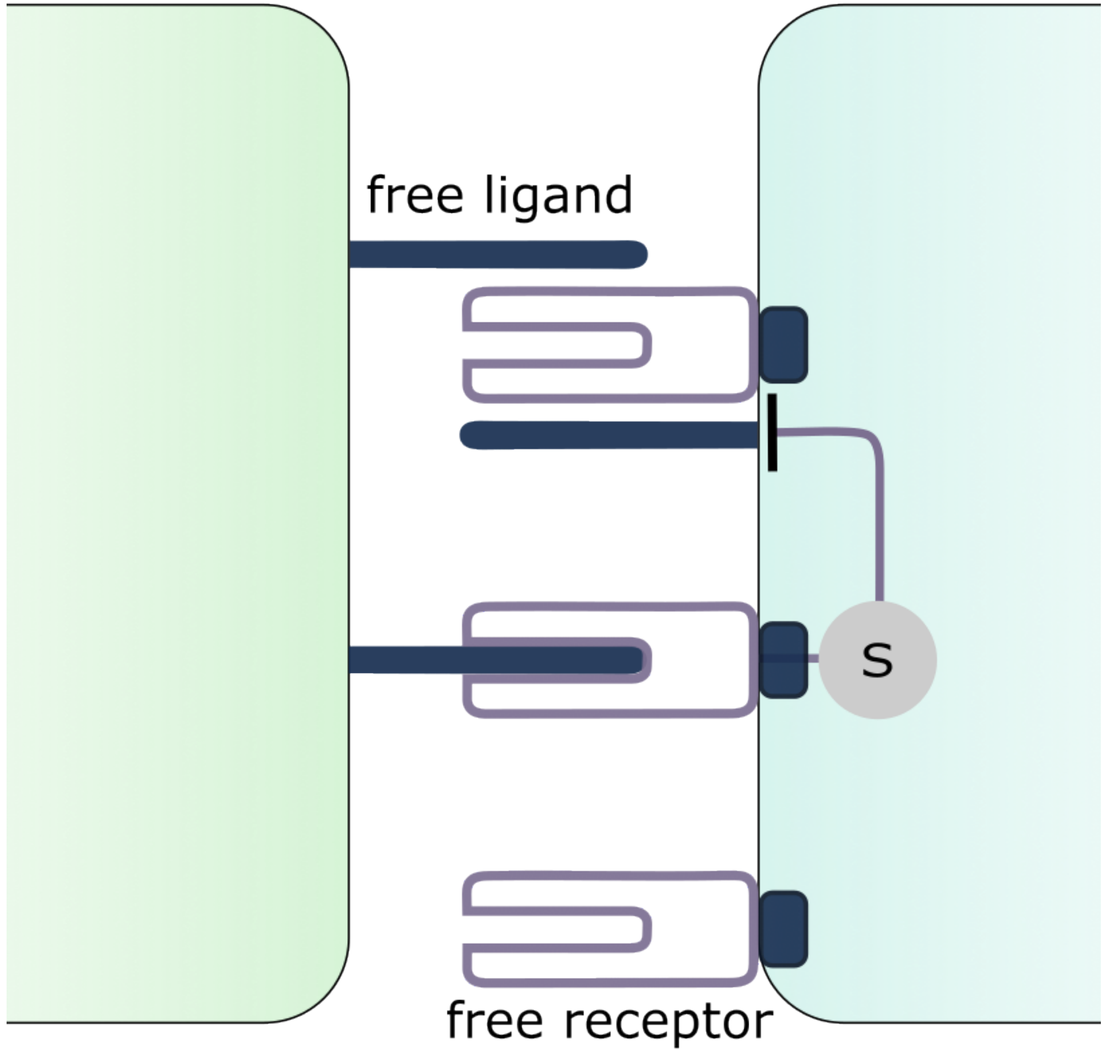


Deterministic components of the dynamics

- **free ligands** (L): Produced at a constant rate in the absence of coupling, and production is *suppressed* when the concentration of downstream effector (S) increases sufficiently (viz. $S > K_5$).

  Depleted when they bind to receptors of neighbouring cells (R_{tr}).

$$\mathcal{F}_L = \beta_{L_0} + \beta_L \frac{(K_5)^g}{(K_5)^g + S^g} - k_{tr} R_{tr} L - L/\tau_L$$

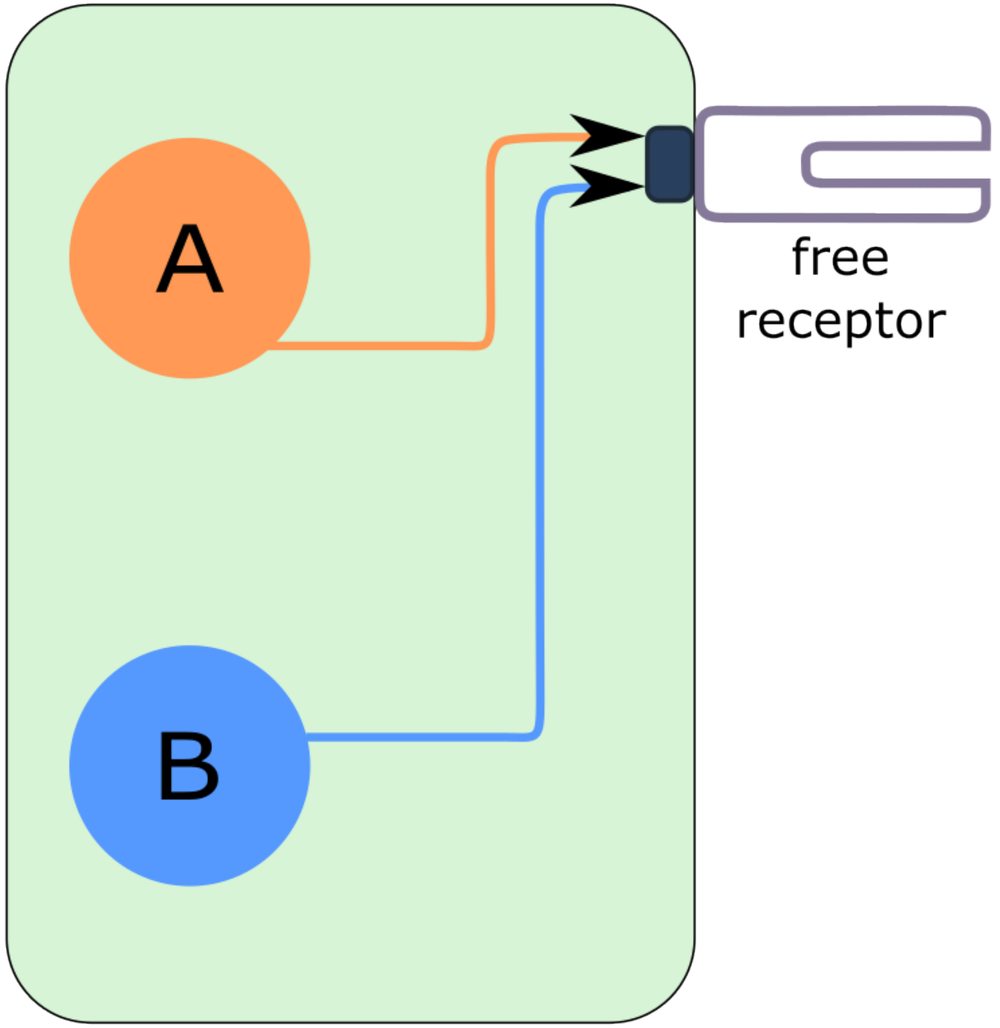


Deterministic components of the dynamics

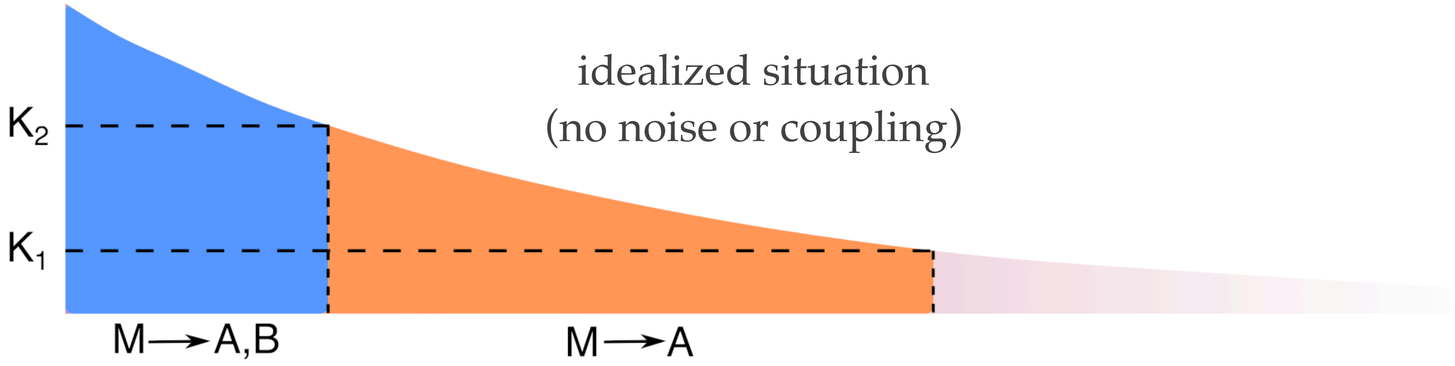
- **free receptors** (R): Produced at a constant rate in the absence of coupling, and production is *enhanced* when A & B are both expressed at sufficiently high levels (viz. $A, B > J$).

  Depleted when they bind to ligands of neighbouring cells (L_{tr}).

$$\mathcal{F}_R = \beta_{R_0} + \beta_R \frac{A^g}{J^g + A^g} \frac{B^g}{J^g + B^g} - k_{tr} R L_{tr} - R/\tau_R$$



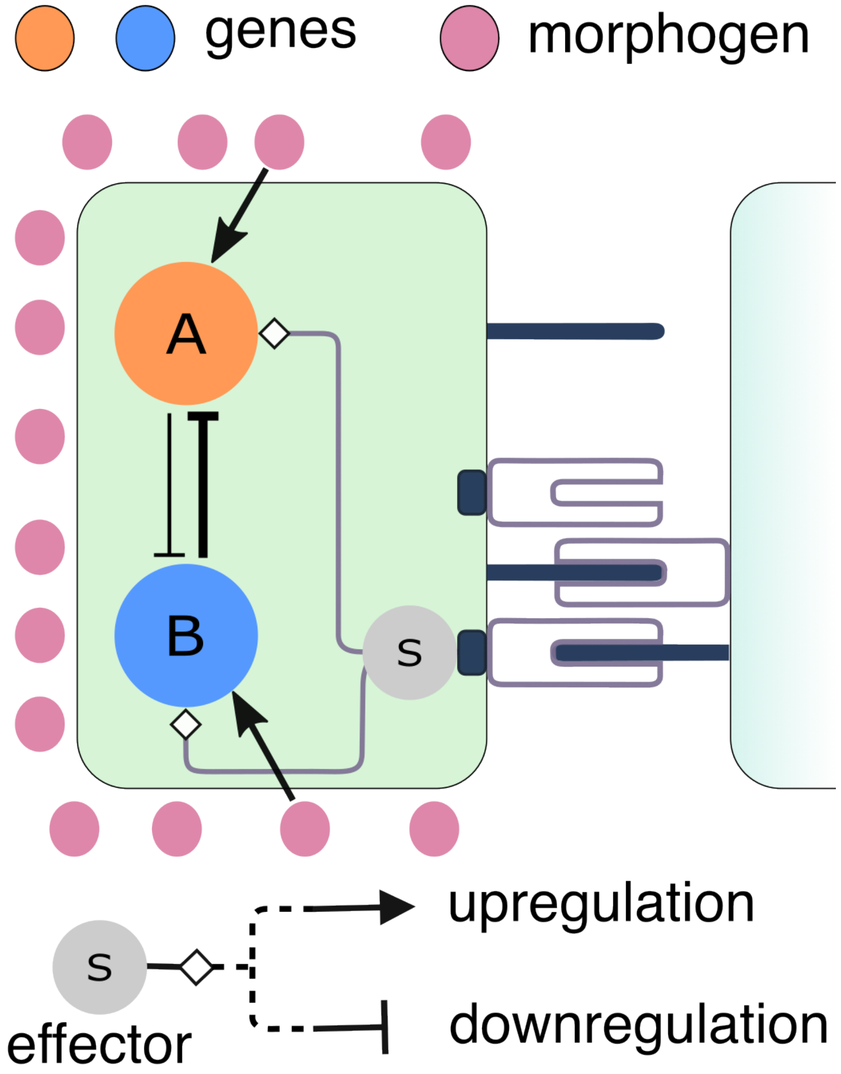
Deterministic components of the dynamics



- **genes** (A & B): We assume that each cell has two mutually repressing genes that are expressed at different levels of morphogen concentration (for A: $M > K_1$, for B: $M > K_2$).

  B is assumed to inhibit A more strongly than the reverse.

  The downstream effector S released upon successful *trans*-activation can either upregulate or downregulate either A or B.

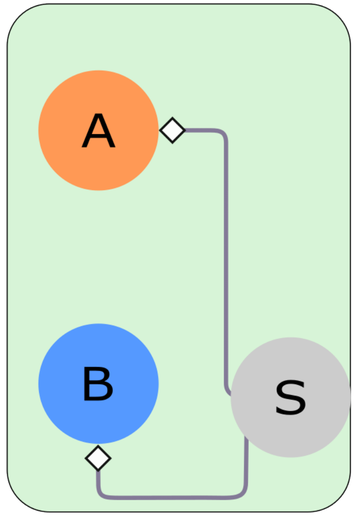


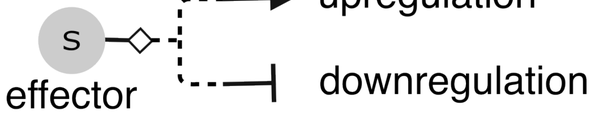
Deterministic components of the dynamics

The deterministic components of the dynamics of A and B :

$$\mathcal{F}_A = \alpha_A \frac{M^h}{M^h + (K_1)^h} \frac{(K_3)^h}{B^h + (K_3)^h} \Phi_A + \gamma_A \frac{S^g}{S^g + Q^g} - A/\tau_A$$

$$\mathcal{F}_B = \alpha_B \frac{M^h}{M^h + (K_2)^h} \frac{(K_4)^h}{A^h + (K_4)^h} \Phi_B + \gamma_B \frac{S^g}{S^g + Q^g} - B/\tau_B$$





possible roles of the downstream effector:

$\Phi_A = Q^g/(Q^g + S^g)$

$\Phi_B = 1$

$\gamma_A = 0$

$\gamma_B = 0$

$\Phi_A = 1$

$\Phi_B = 1$

$\gamma_A > 0$

$\gamma_B = 0$

$\Phi_A = 1$

$\Phi_B = Q^g/(Q^g + S^g)$

$\gamma_A = 0$

$\gamma_B = 0$

$\Phi_A = 1$

$\Phi_B = 1$

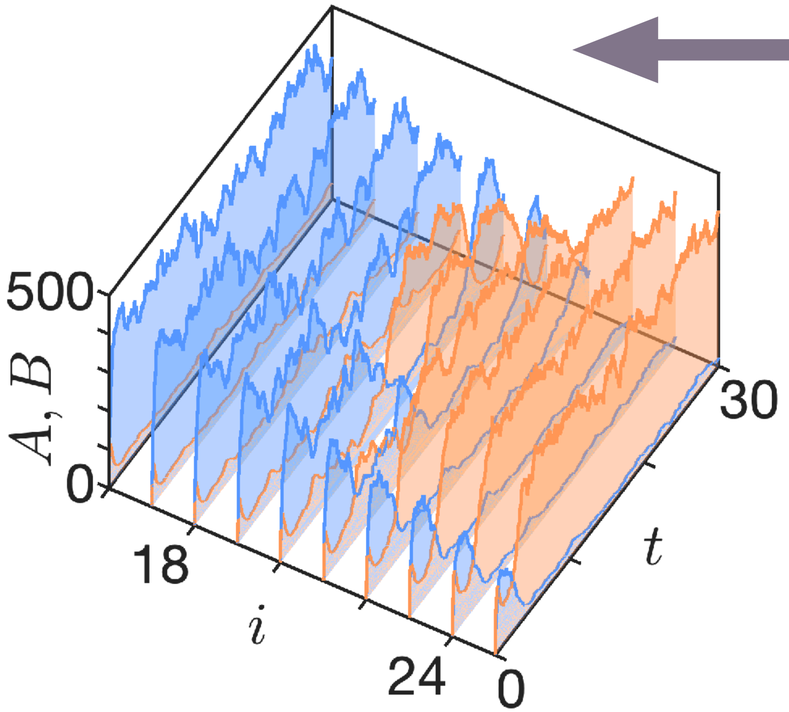
$\gamma_A = 0$

$\gamma_B > 0$

Dynamics with and without intercellular interactions

Large fluctuations in the gene expression levels.

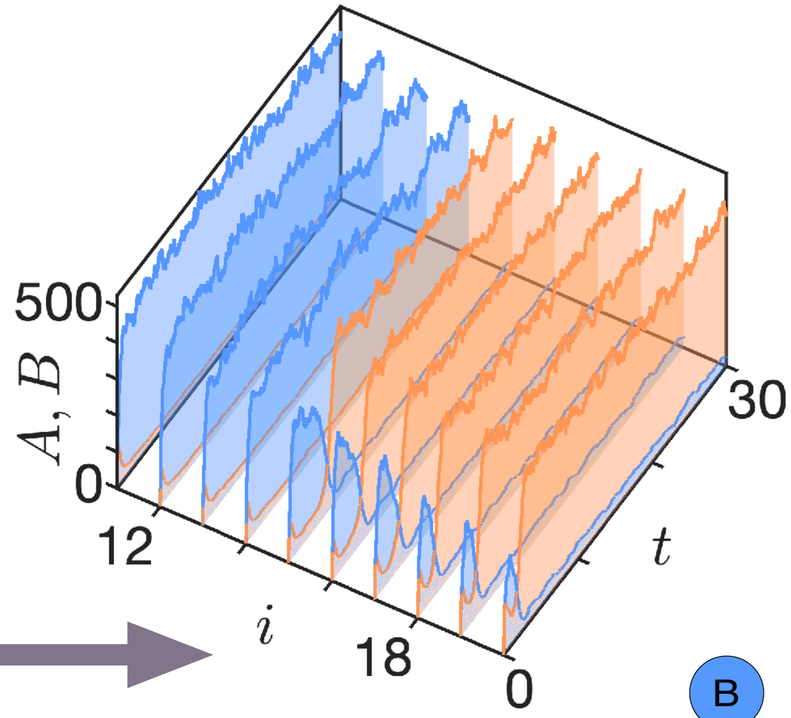
Sustained ambiguity in cell fates at the interface of the domains.



Uncoupled

Cells rapidly converge to their eventual fates.

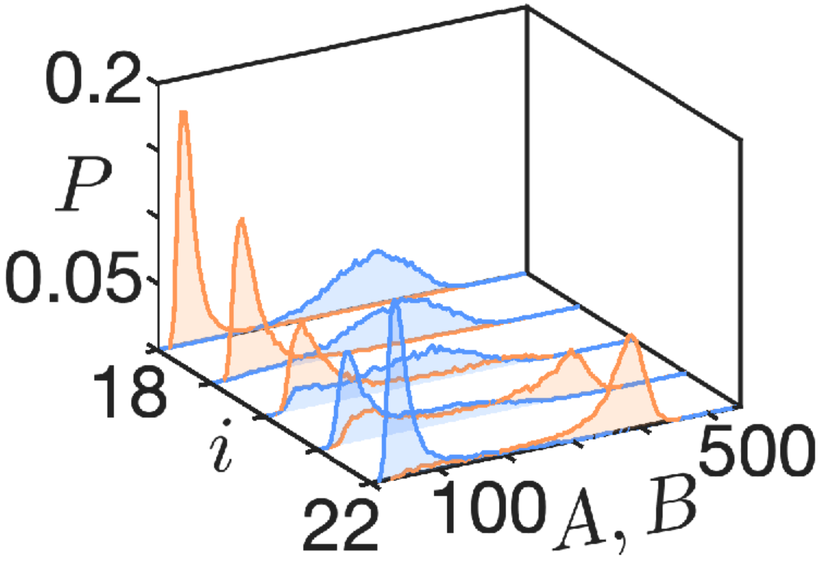
Clear separation between fate domains.



S inhibits B

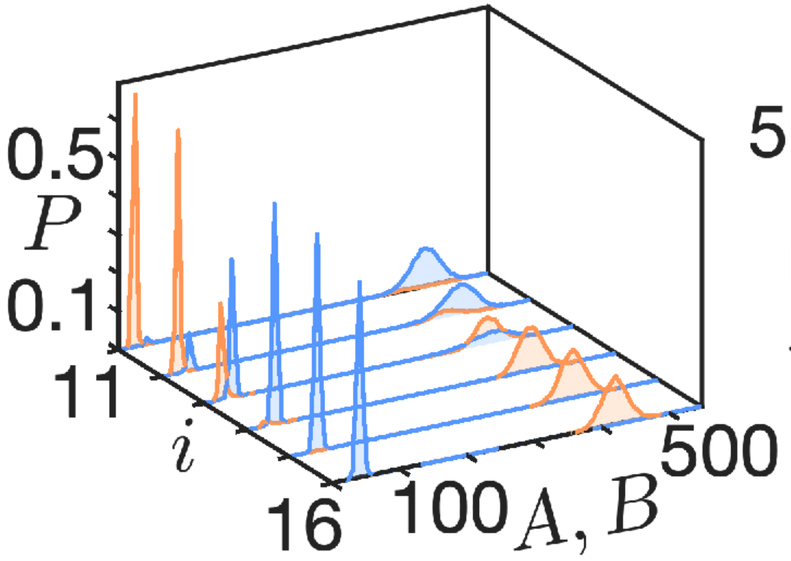
Increase in precision and robustness of the response

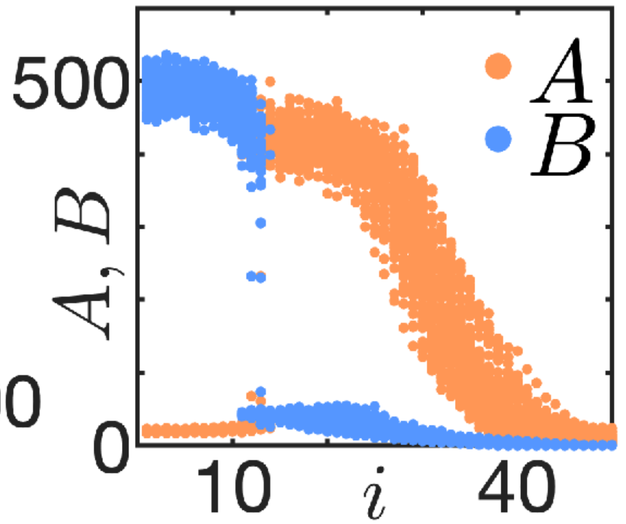
Steady-state distributions for the expression levels of A and B across realizations are extremely broad with a high degree of overlap near the boundary.



Uncoupled

Sharply defined peaks in the distributions at high and low expression levels, with a clearly identifiable dominant gene at every cell, and a robust well-defined fate boundary.





S inhibits B

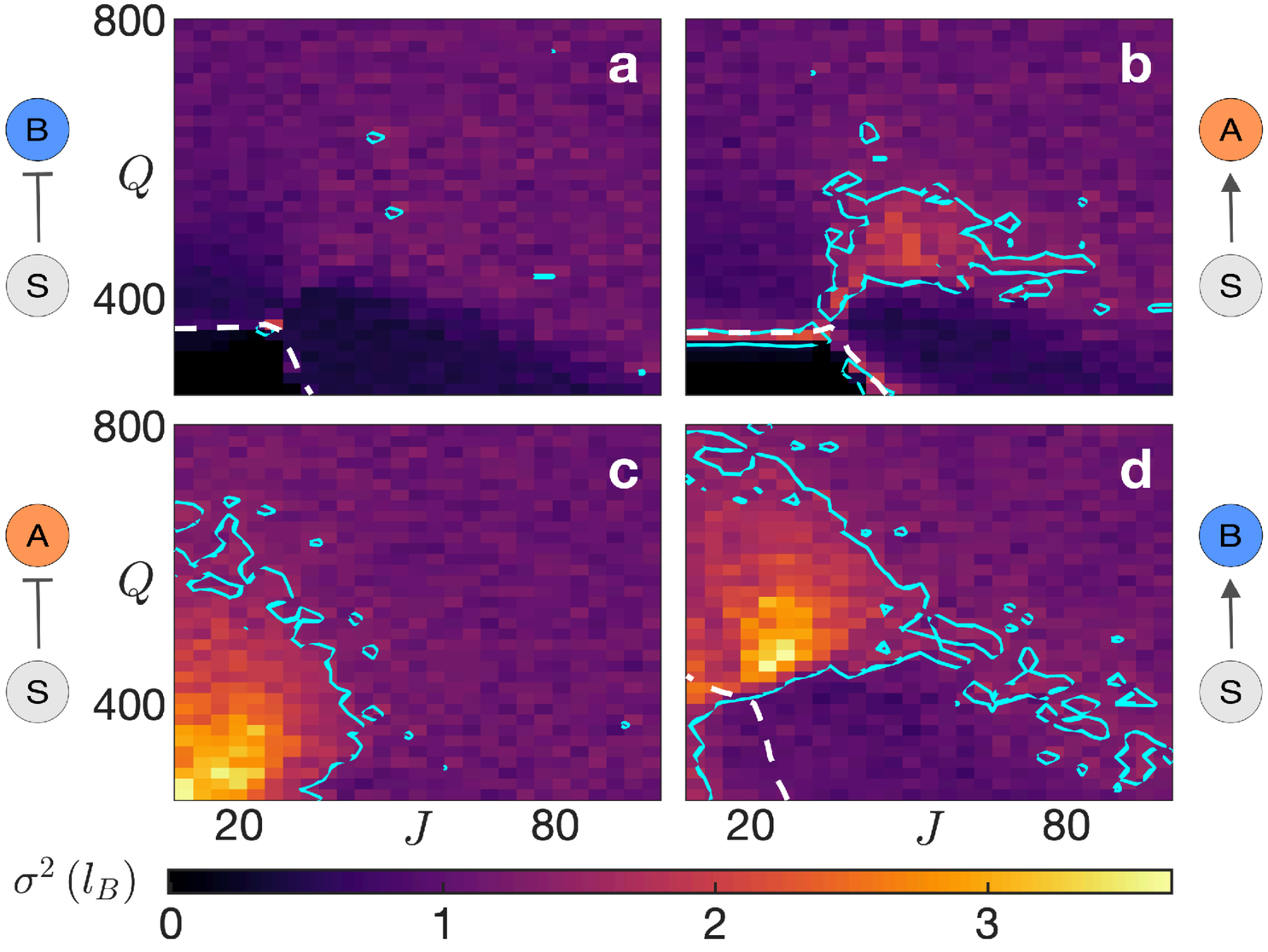
Role of downstream effector on variability in boundary location

We consider the location of the fate boundary l_B for each of the four scenarios.

The variance of this quantity across realizations, $\sigma^2(l_B)$, is substantially reduced when $S \to A$ or $S \dashv B$.

Contours: Variance in the absence of coupling, $\sigma^2(l_B) \approx 1.38$.

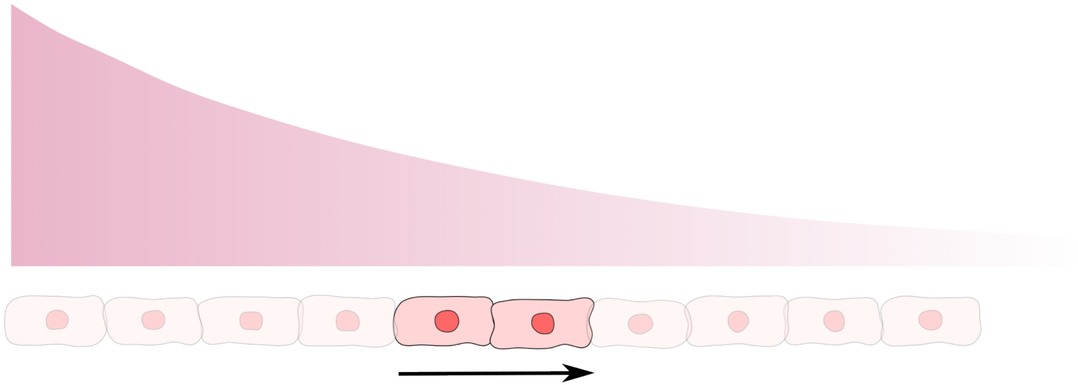
Regions below dashed lines: Here $\langle l_B \rangle$ lies outside the range $10 \leq \langle l_B \rangle \leq 30$, where $\langle l_B \rangle = 20$ is seen in the uncoupled case.



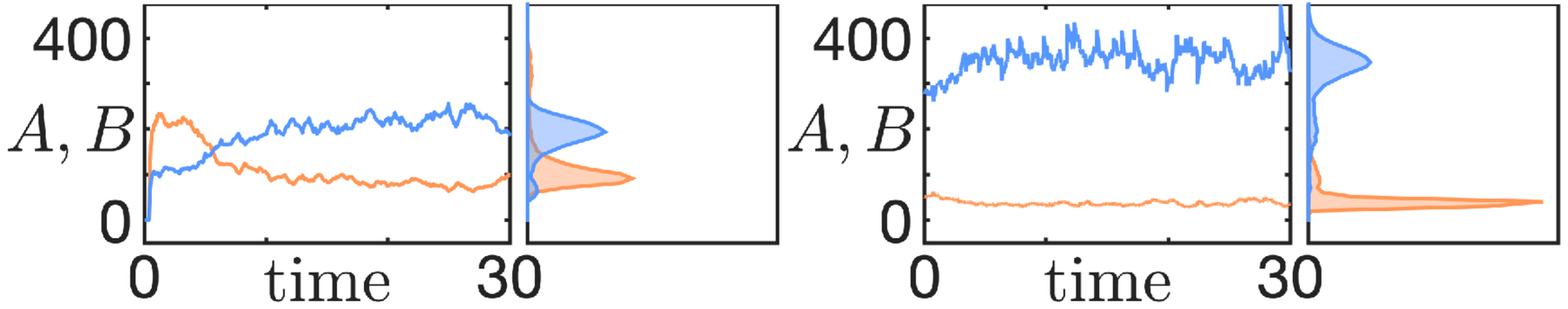
Focusing on the boundary between cell-fates

We consider the dynamics of a pair of coupled cells, located at adjacent positions along the morphogen gradient.

The main observations of the full system can be reproduced in this simplified setting, allowing us to obtain analytical insights from this system.



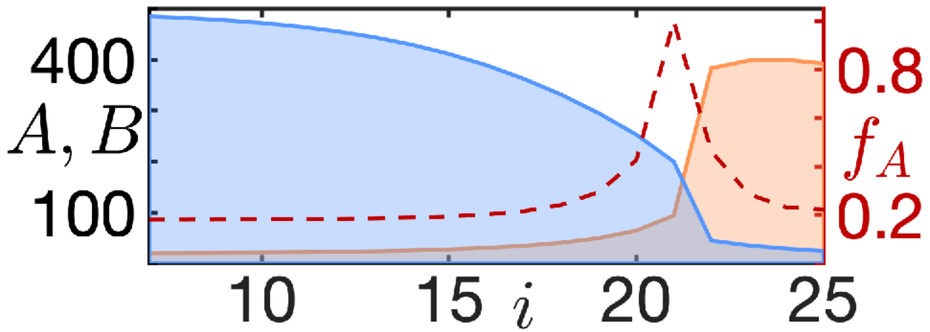
Coupling leads to an increased separation between the levels of A & B.



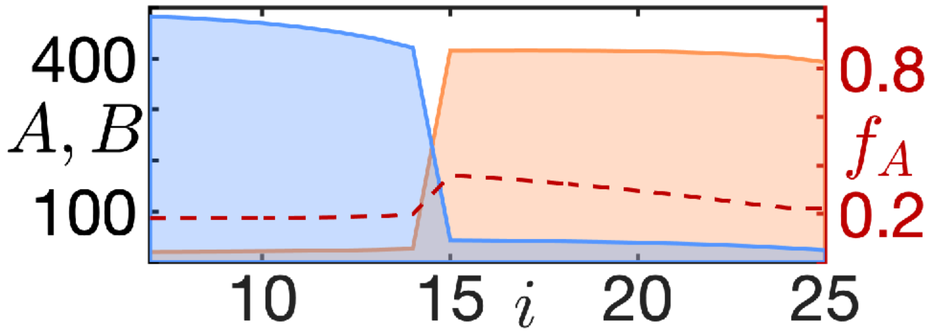
Uncoupled **S inhibits B**

Analytically capturing the noise reduction at the boundary

- We analytically capture the dynamics at the cell-fate boundary using the *linear noise approximation* (LNA).
- We formulate a set of effective single-step reactions describing the interactions between the species $\mathbf{X}$ of the two cells. We obtain the stoichiometric matrix $\mathbf{T}$ and propensities $\pi_a(\mathbf{X})$ of each reaction a.
- The LNA yields a matrix equation: $\mathbf{J}\Sigma + \Sigma\mathbf{J}^T = -\mathbf{B}\mathbf{B}^T$, where $B_{ia} = T_{ia}\sqrt{\pi_a(\mathbf{X})}$ and $\mathbf{J}$ is the Jacobian matrix, calculated at the fixed point of the deterministic system.
- We solve this to obtain the covariance matrix Σ, which contains the variance in expression levels of each of the species along the diagonal. The resulting Fano factor $\left(\langle X^2\rangle - \langle X\rangle^2\right)/\langle X\rangle$ is seen to capture the coupling-induced reduction in cell-fate variability at the boundary.

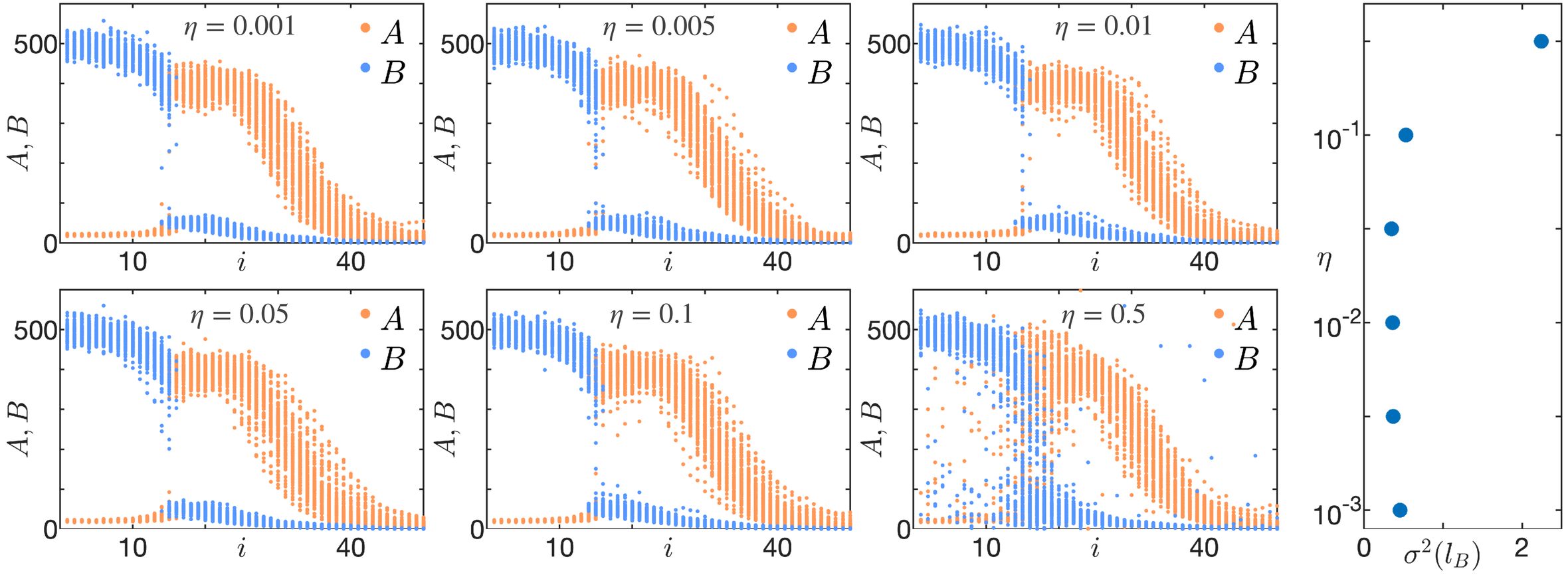


Uncoupled



S inhibits B

Robustness of fate-boundary precision to noise strength



Intercellular interactions via notch signaling can enhance boundary precision even when the noise strength η is increased over two orders of magnitude.

Conclusion (Part II)

- Our model suggests that Notch signalling can enhance the **precision** and the **robustness** of cell-fate boundaries by utilizing the *inherent asymmetry* in the response of patterning genes to an external morphogen gradient.
- An experimentally verifiable implication of our investigation is that notch signalling is more effective at reducing noise when it **upregulates** the patterning gene expressed at low morphogen concentration (or downregulates the gene that inhibits it).

Publication:

C. Kuyyamudi, S. N. Menon, and S. Sinha, *Phys. Rev. E* **107**, 024407 (2023).

Associated code:

https://github.com/boyonpointe/Notch-enhanced-cell-fate-determination

Acknowledgements

Dr Chandrashekar Kuyyamudi

The Institute of Mathematical Sciences, Chennai, India

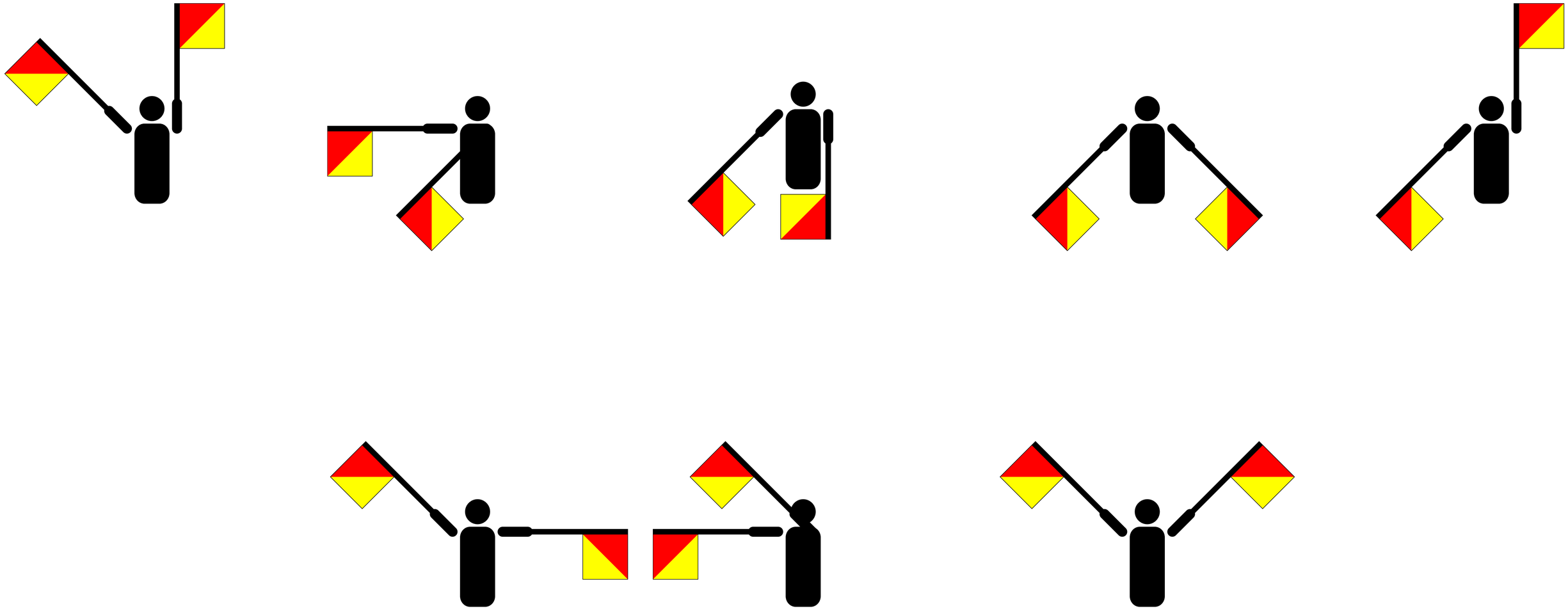
currently: Harvard University

Prof. Sitabhra Sinha

The Institute of Mathematical Sciences, Chennai, India

Funding/Support:

- This research was supported in part by the IMSc Complex Systems Project (12th Plan) and the Center of Excellence in Complex Systems and Data Science, both funded by the Department of Atomic Energy, Government of India
- The simulations required for this work were supported by the Institute of Mathematical Science's High Performance Computing (hpc.imsc.res.in) [Nandadevi]



(“Thank You” in semaphore!)