Cell Engineering Lecture 3: Multi cellular control

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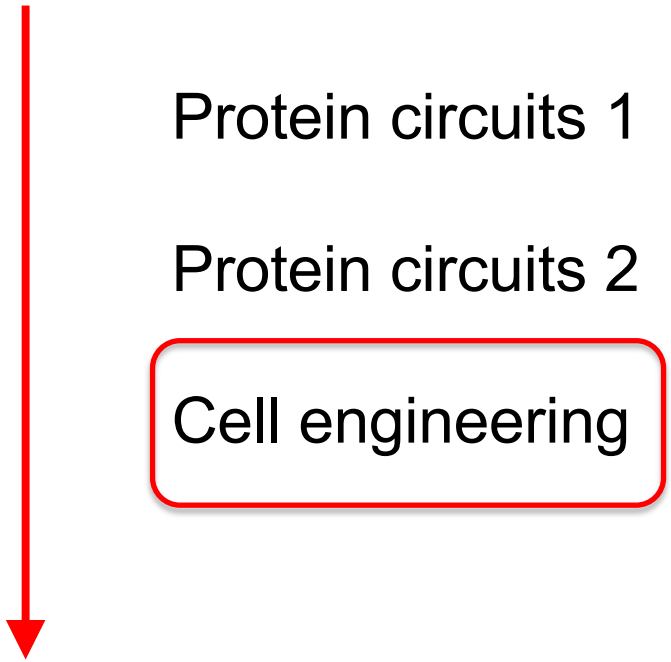
BIOENG-320

Synthetic Biology: Lecture series

From molecules to circuit to cell engineering



Cell Engineering is one of the ultimate goals in synthetic biology

Minimal self replicating living system / factory

Multicellular systems

Programming multi cellular systems through intercellular communications

Principles of intercellular communications

Intercellular communication => **coordinate the behavior** of individual cells in **multicellular** communities

Impact of such communication in prokaryotes and eukaryotes?

Prokaryotes: consortia of prokaryotes control their behaviour based on the total population density through quorum sensing for example

Eukaryotes: Multicellular organisms use signalling to organize, synchronize and differentiate their specialized tissues

What kind of signals?

Electrical, mechanical, diffusible chemicals *(secreted from the senders and recognized by the receivers through autocrine, paracrine or endocrine paths)*

Lecture structure

Programming population control

Programming multicellular organization

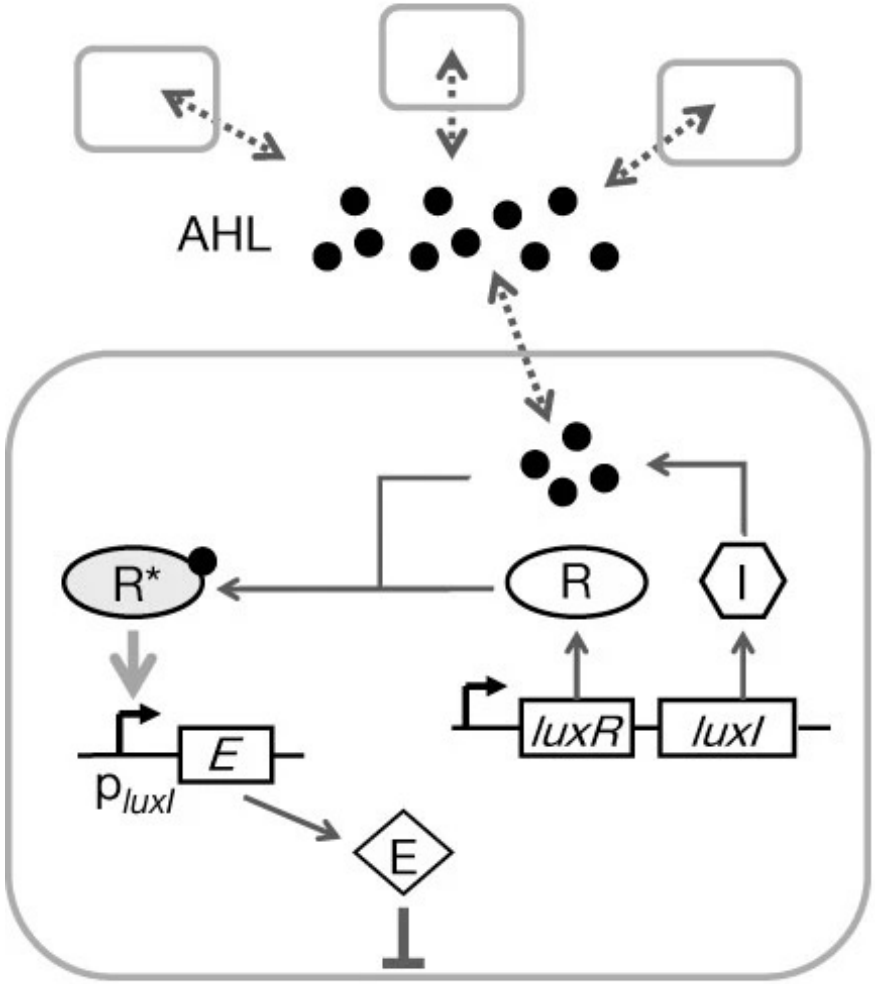
Programming multicellular patterning

Population control by the environment

Common scenario in biology: **Population control by the environment** (e.g. nutrient supply):

=> bacterial population needs to **maintain a cell density** that is **lower than the limits** imposed by the **environment**

Synthetic circuit for programmed population control by cell–cell communication and regulated killing (model system: E Coli)



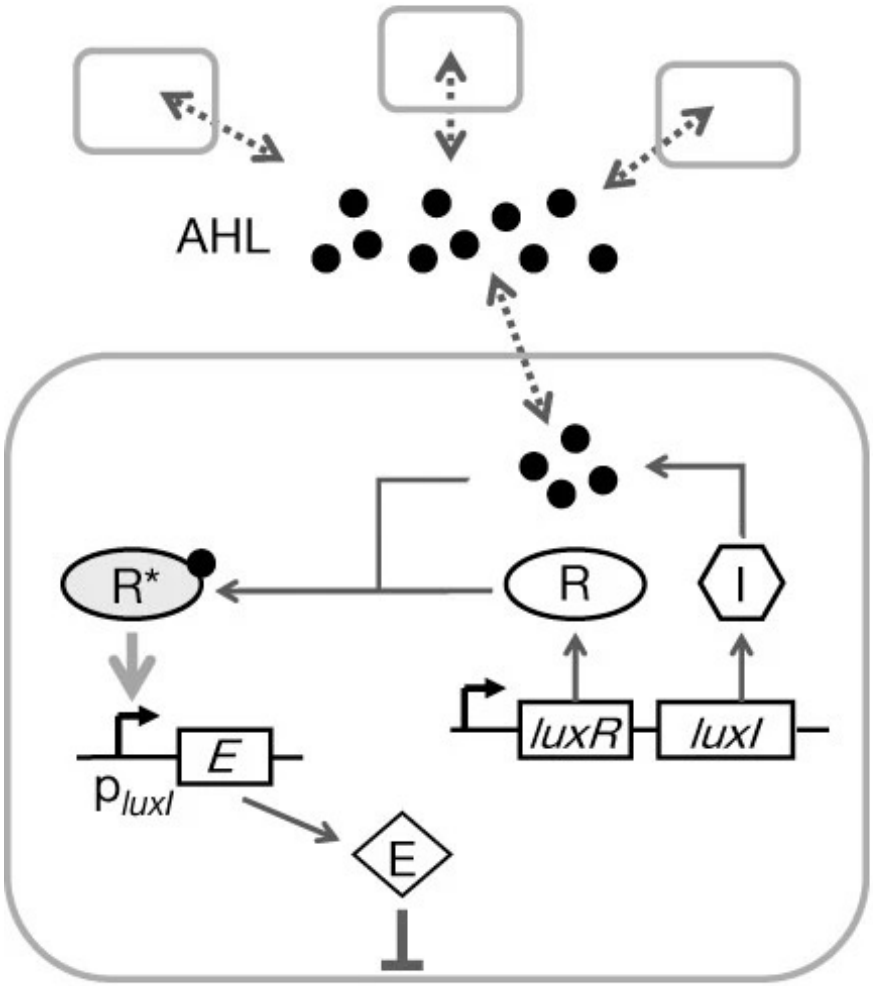
Population control circuit:

the circuit programmes a bacterial population to maintain a cell density that is lower than the limits imposed by the environment

by coupling gene expression to cell survival and death using cell–cell communication

(You Nature 2004)

Programmed population control by cell–cell communication and regulated killing (model system: E Coli)



1. LuxI (I) produces a small, **diffusible AHL signalling molecule**

2. AHL accumulates in the experimental medium and inside the cells as the **cell density increases**.

3. **high [AHL]** => LuxR (R) activation => **killer gene (E) expression** under the control of a luxI promoter (pluxI).

4. high [E] => **cell death**

Programmed population control by cell–cell communication and regulated killing: the model

Equations modeling the major kinetic events dictating the circuit function

Cell growth and death

$$\frac{dN}{dt} = kN(1 - N/N_m) - dEN \quad (1)$$

Production and degradation of the killer protein

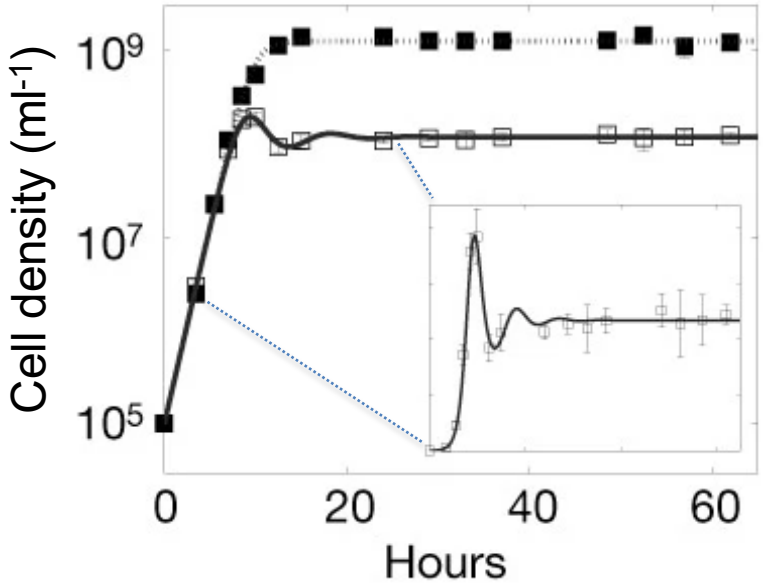
$$\frac{dE}{dt} = k_E A - d_E E \quad (2)$$

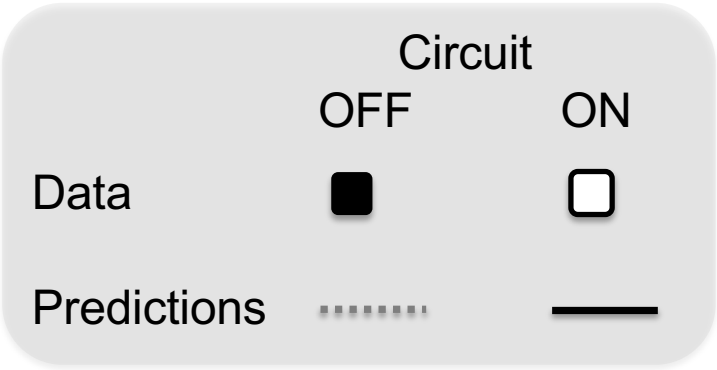
AHL signal

$$\frac{dA}{dt} = v_A N - d_A A \quad (3)$$

intracellular concentration of the killer protein (E)
AHL concentration (A); viable cell density (N)

Tight & dynamic coupling between population dynamics and killer protein expression





Why these Oscillation patterns?

1. At killer protein level < steady state => population growth

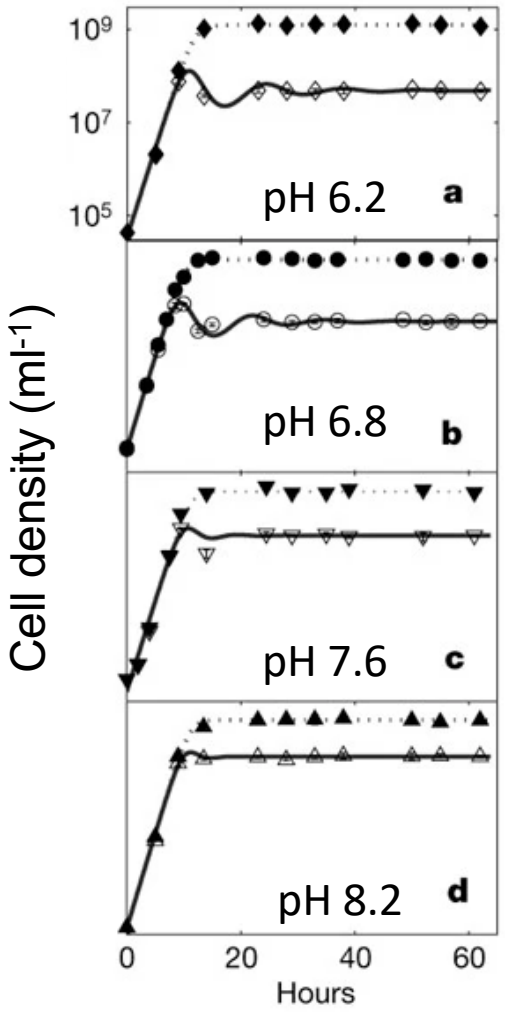
2. Then, killer protein level > steady state => decreases cell density

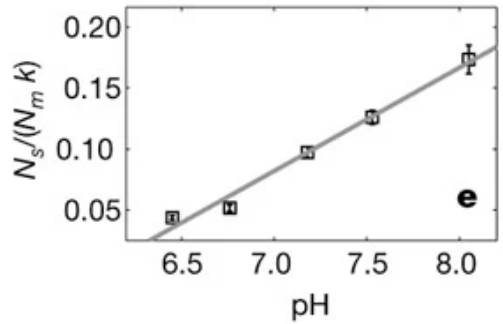
3. After some delay, cell density declines => decrease in [AHL] => reduced levels of the killer protein => cell population recovery

Prediction: steady-state cell density increases nearly proportionally with the AHL degradation rate constant

AHL serves as an external ‘dial’ to operate the circuit

Degradation of AHL is facilitated by increasing pH





Effect of pH on circuit parameters

Table 1 **Effects of pH on circuit parameters**

Medium pH	Steady-state culture pH*	k† (h^{-1})	N_m per 10^9‡ (CFU ml^{-1})	N_s per 10^7§ (CFU ml^{-1})	d_A‖ (h^{-1})
6.2	6.45	0.885	1.25 ± 0.06	4.86 ± 0.02	0.274
6.6	6.76	0.928	1.17 ± 0.05	5.59 ± 0.03	0.304
7.0	7.18	0.970	1.24 ± 0.10	11.7 ± 0.6	0.639
7.4	7.53	0.897	1.16 ± 0.10	13.1 ± 0.6	0.791
7.8	8.05	0.936	1.20 ± 0.07	19.5 ± 1.3	1.19

*Measured at about 50 h after growth initiation in ON cultures.
†Obtained by fitting the exponential phase of growth curves of OFF cultures.
‡Average of the stationary-phase cell density of OFF cultures between 28 h and 62 h.
§Average of the circuit-ON cell density between 28 h and 62 h.
‖Obtained by solving equation $N_s = \frac{N_m d_A d_E k}{N_m v_A k_E d + d_A d_E k}$ with d_A as the only unknown.
¶Average of LacZ activity of ON cultures between 28 h and 62 h.

At steady state: $E_s = k / d\ (1 - N_s / N_m) \sim k / d$

k /d : the ratio of the growth and killing rate constants (assuming that the circuit-ON cell density is far below the carrying capacity; that is, $N_s / N_m << 1$).

Now, some thinking

By coupling gene expression with population dynamics, cell–cell communication integrates the **entire population** as an essential **component** of the population-control circuit.

This coupling => the circuit functions reliably at the population level by **exploiting cell heterogeneity** in terms of their size, age, plasmid copy number, gene expression and response to the killer protein.

Why are such phenotypic variations required for the circuit to function?

The outcome of circuit function is **binary for any given cell**: it lives or dies (judged by the ability to form a colony).

If all cells had the **same phenotype**, the circuit would **fail to achieve a stable cell density** because the population would become extinct once the killer protein concentration reached a critical threshold.

Now, some thinking

Because of the coupling between gene expression and population dynamics, the circuit can function **only at the population level**

Is this statement verified experimentally?

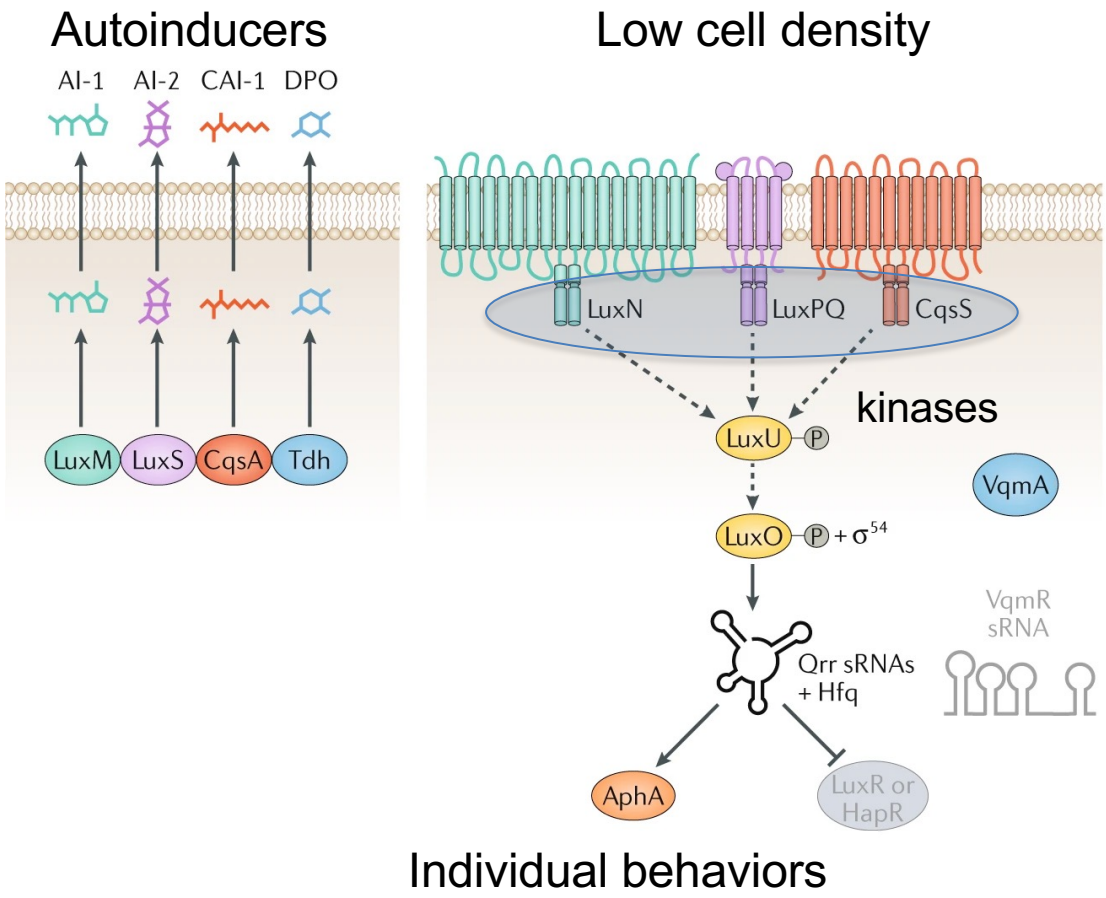
This is supported by experimental data: under each set of conditions, growth of the circuit-ON culture only deviated from that of the OFF culture when cell density was sufficiently high => **cell-cell communication is key**

What would happen if the circuit had functioned inside each cell autonomously and why?

growth of the ON and OFF cultures would have deviated from the beginning

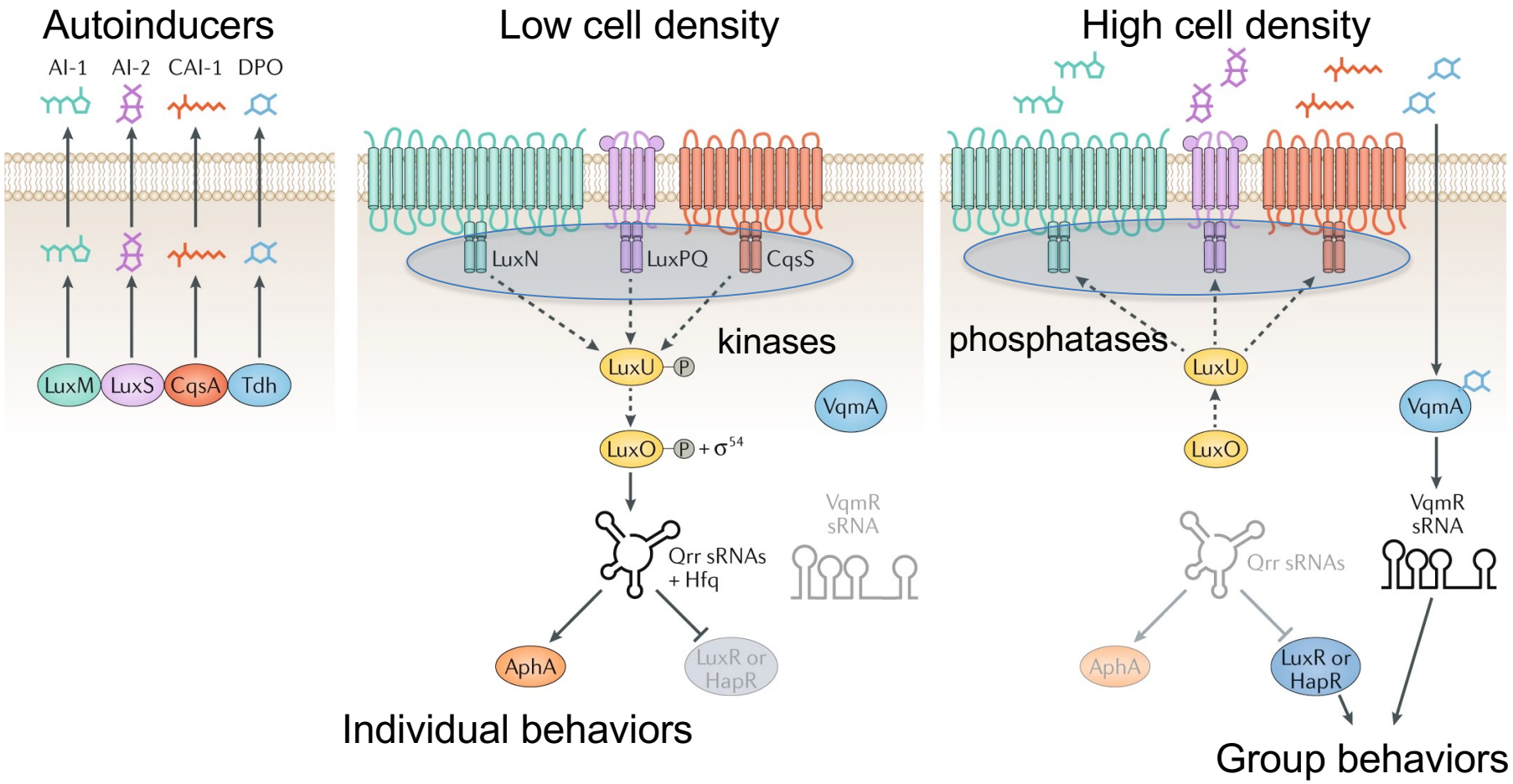
Native mechanisms for controlling cell population?

Similar mechanisms for controlling natural population through quorum sensing



Other mechanisms for controlling cell population?

Similar mechanisms for controlling natural population through quorum sensing



Large diversity of quorum-sensing modules: multichannel feedback systems where population densities are coupled to communications

Lecture structure

Programming population control

Programming multicellular organization

Programming multicellular patterning

Programming self-organizing multicellular structures with synthetic cell-cell signaling

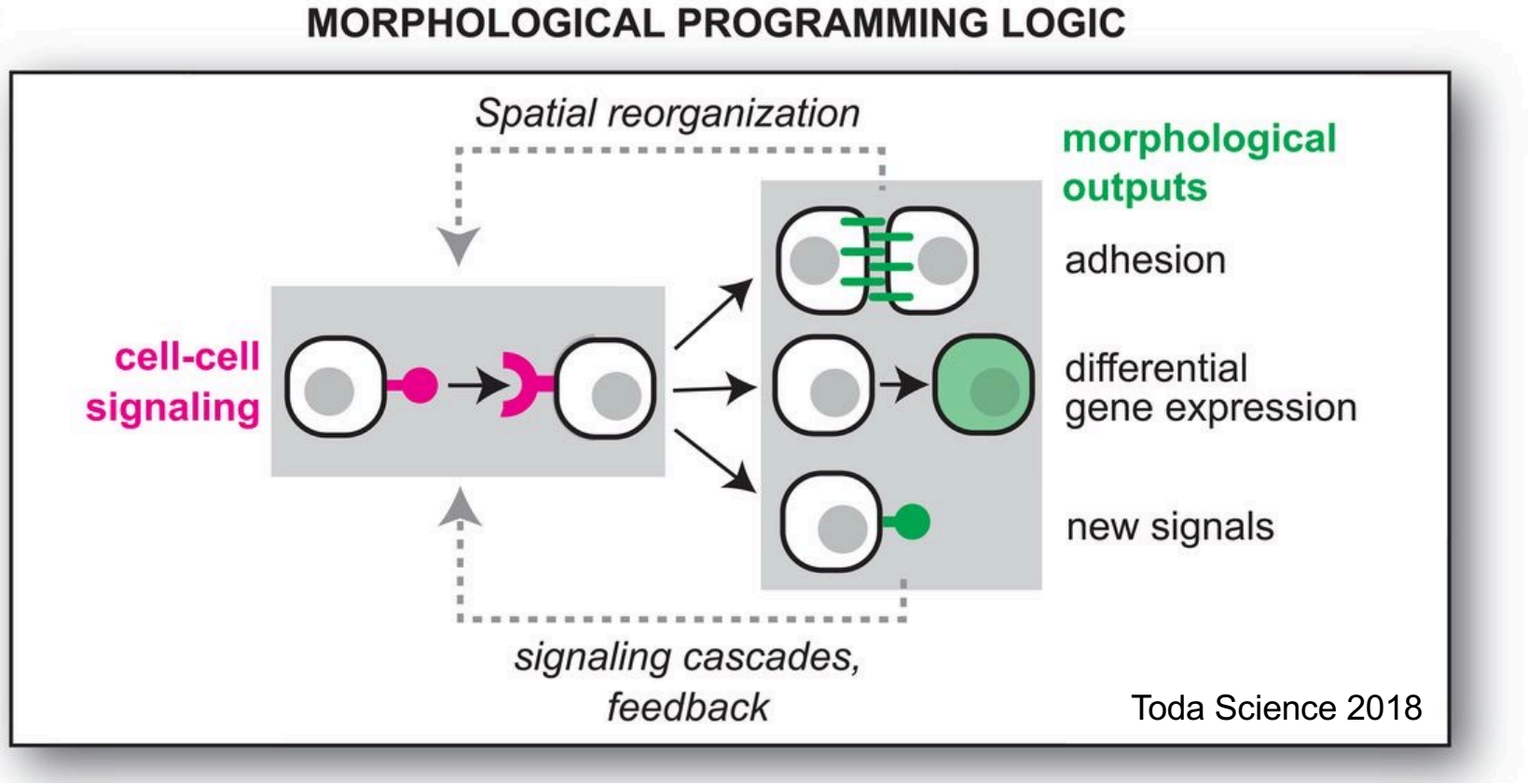
Even with **minimal external instructions**, cells proliferate, diverge into distinct cell types, and **spatially self-organize** into complex structures and patterns.

How and why are these structures different from human-made ones?

They are not assembled from **preexisting parts** that are physically linked according to a **defined Cartesian blueprint**.

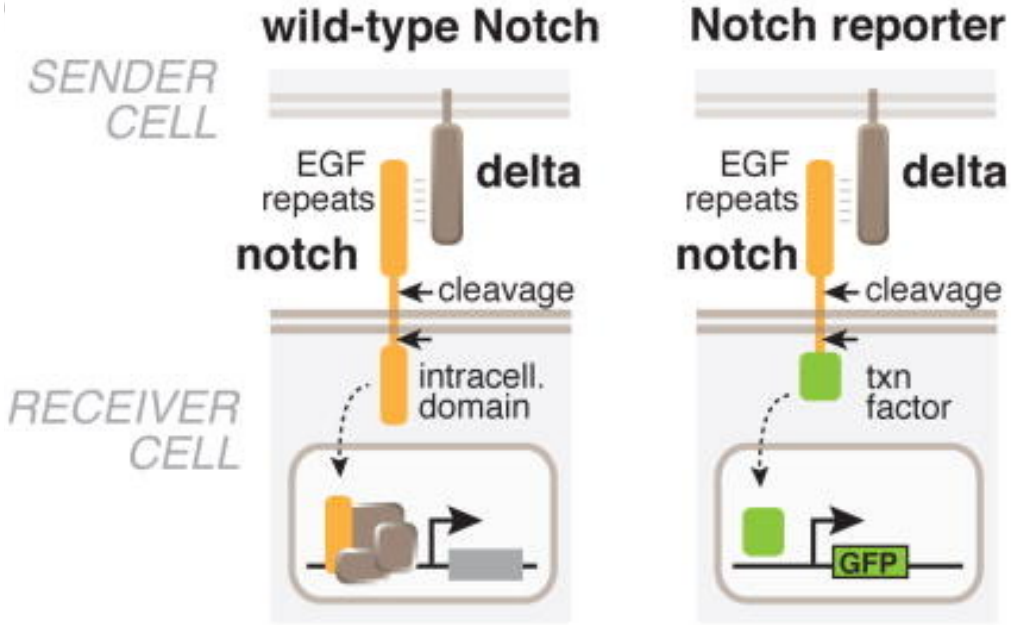
They **emerge** through a series of **genetically programmed sequential** events.

Can simple synthetic circuits in which morphological changes are driven by cell-cell signaling interactions generate self-organizing multicellular structures?



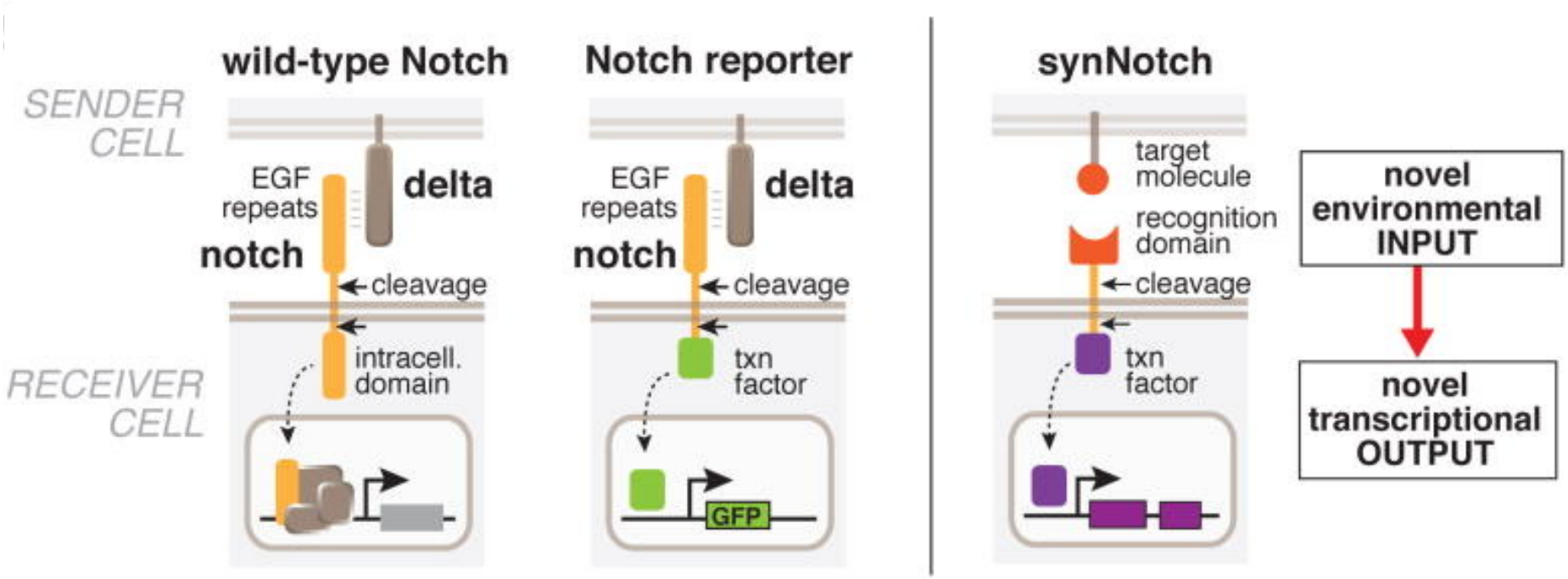
Engineered cell-cell signaling is used to drive changes in cell adhesion, differentiation, and production of new cell-cell signals. These outputs can subsequently be propagated to generate new cell-cell signaling relationships.

Coupling cell-cell communication with genetic programs



Morsut Cell 2016

Coupling cell-cell communication with genetic programs



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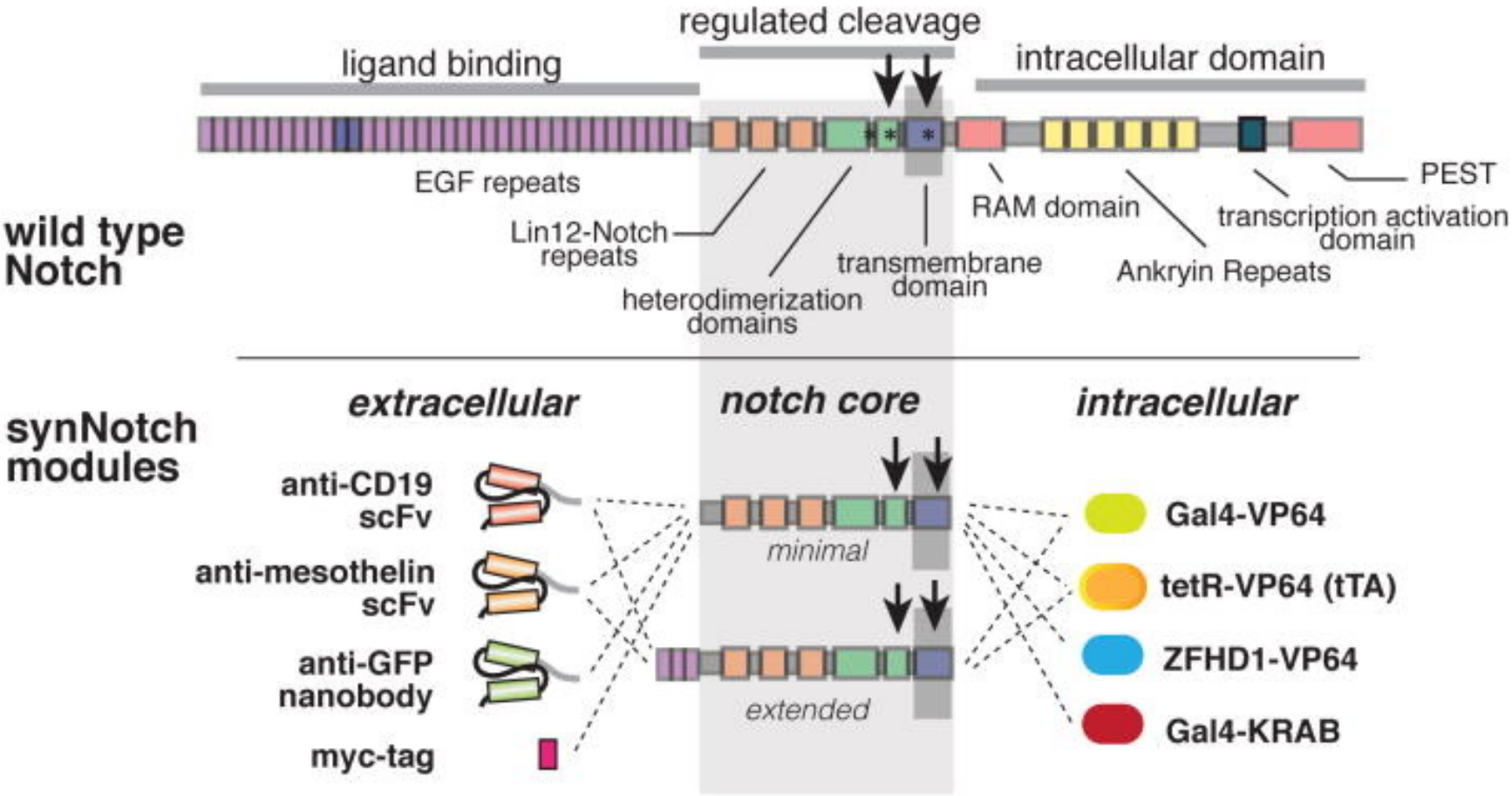

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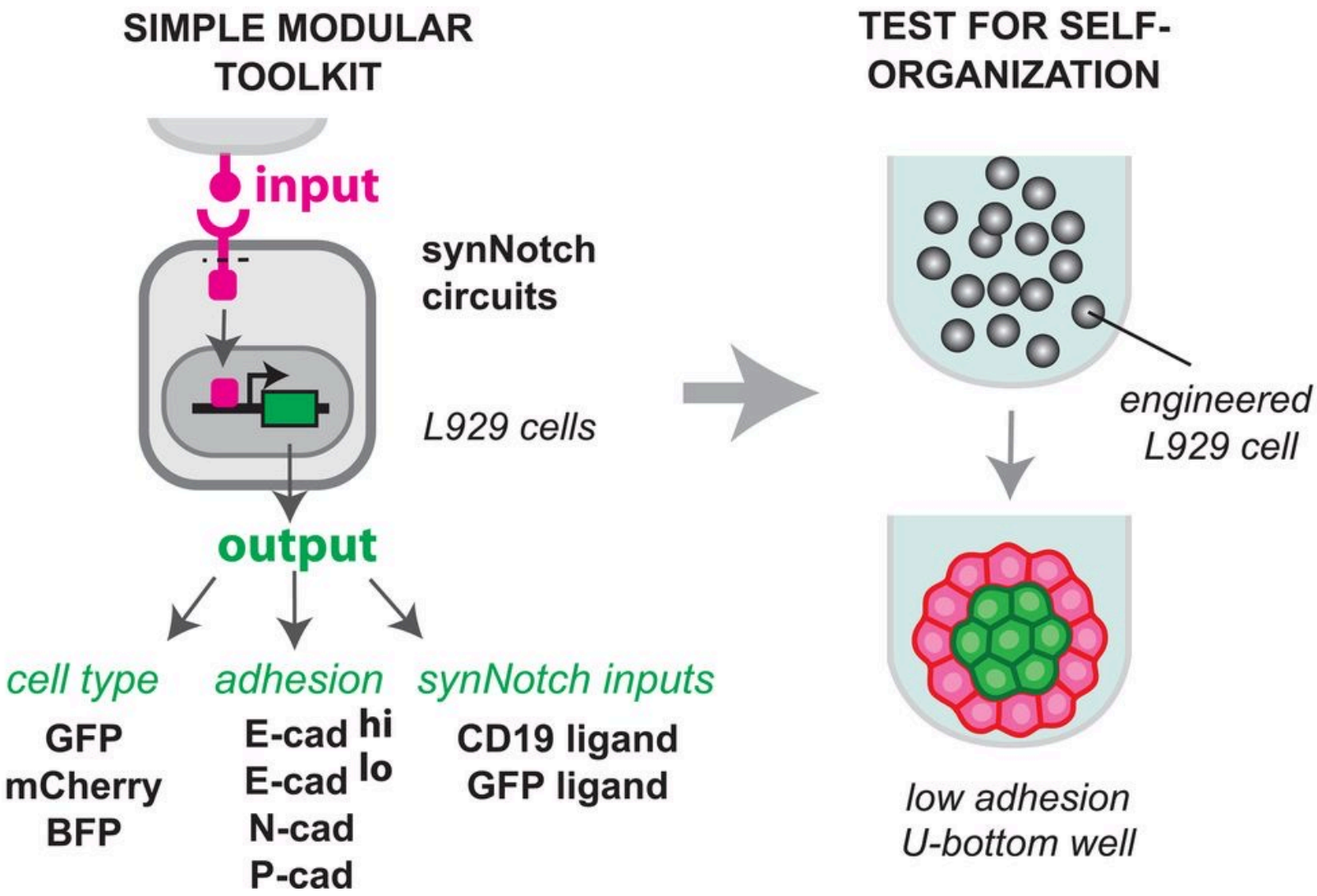
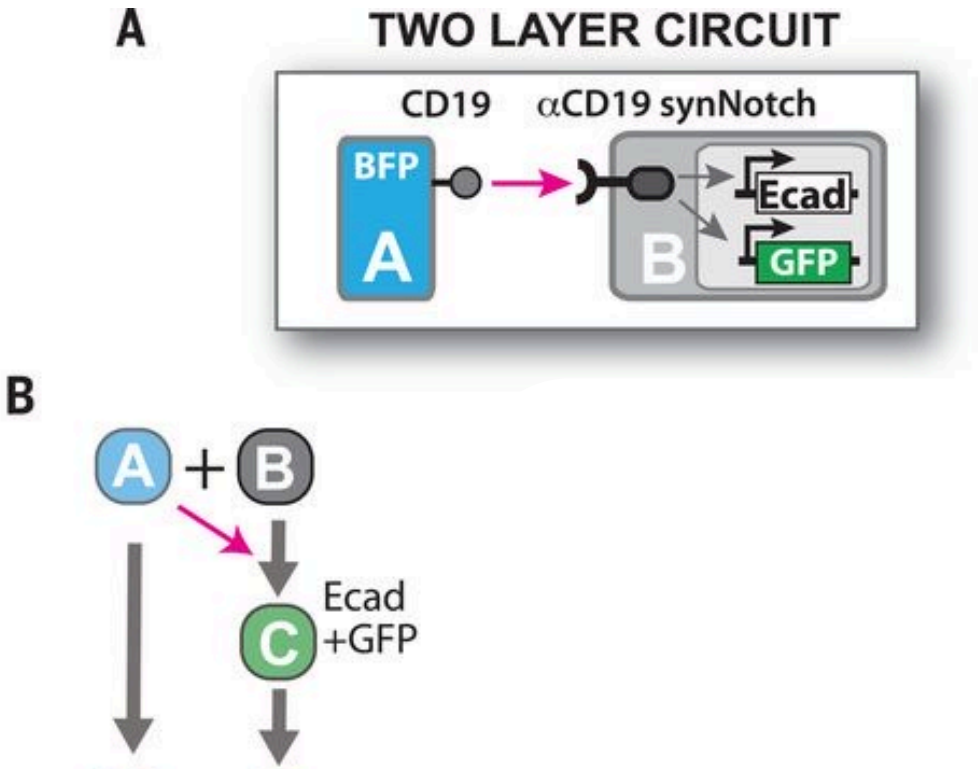
Simple morphological circuits



Engineering self-organizing multilayered spheroids



An A-type sender cell expressing CD19 ligand induces a B-type receiver cell to express E-cadherin and GFP

SynNotch cell-cell signals drive receiver cells to express E-cadherin (Ecad), which leads to their segregation into a central layer. **The system starts with two disordered cell genotypes but organizes to form a structure with two distinct spatial compartments**.

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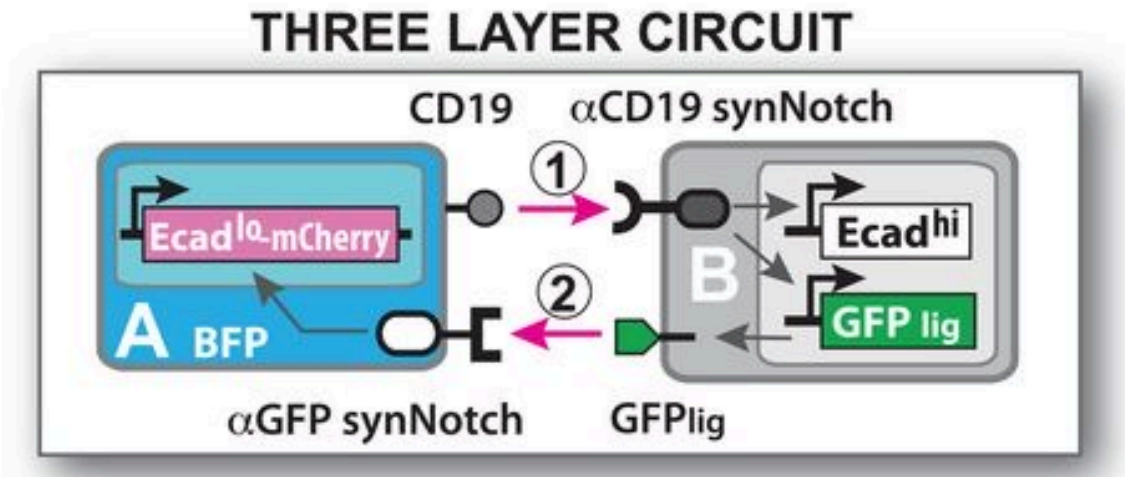
Engineering self-organizing multilayered spheroids

2 genotypes

3 phenotypes



(1) An A-type cell can send signals to a B-type cell using CD19 ligand, which induces **expression of E-cadherin (high expression) and GFP_{lig} (surface-expressed GFP)**.

(2) The induced B-type cell can then send reciprocal signals to the A-type cell; GFP_{lig} serves as ligand to stimulate anti-GFP synNotch receptor expressed in the A-type cell. This reciprocal interaction is programmed to drive a **low level of E-cadherin and mCherry**.

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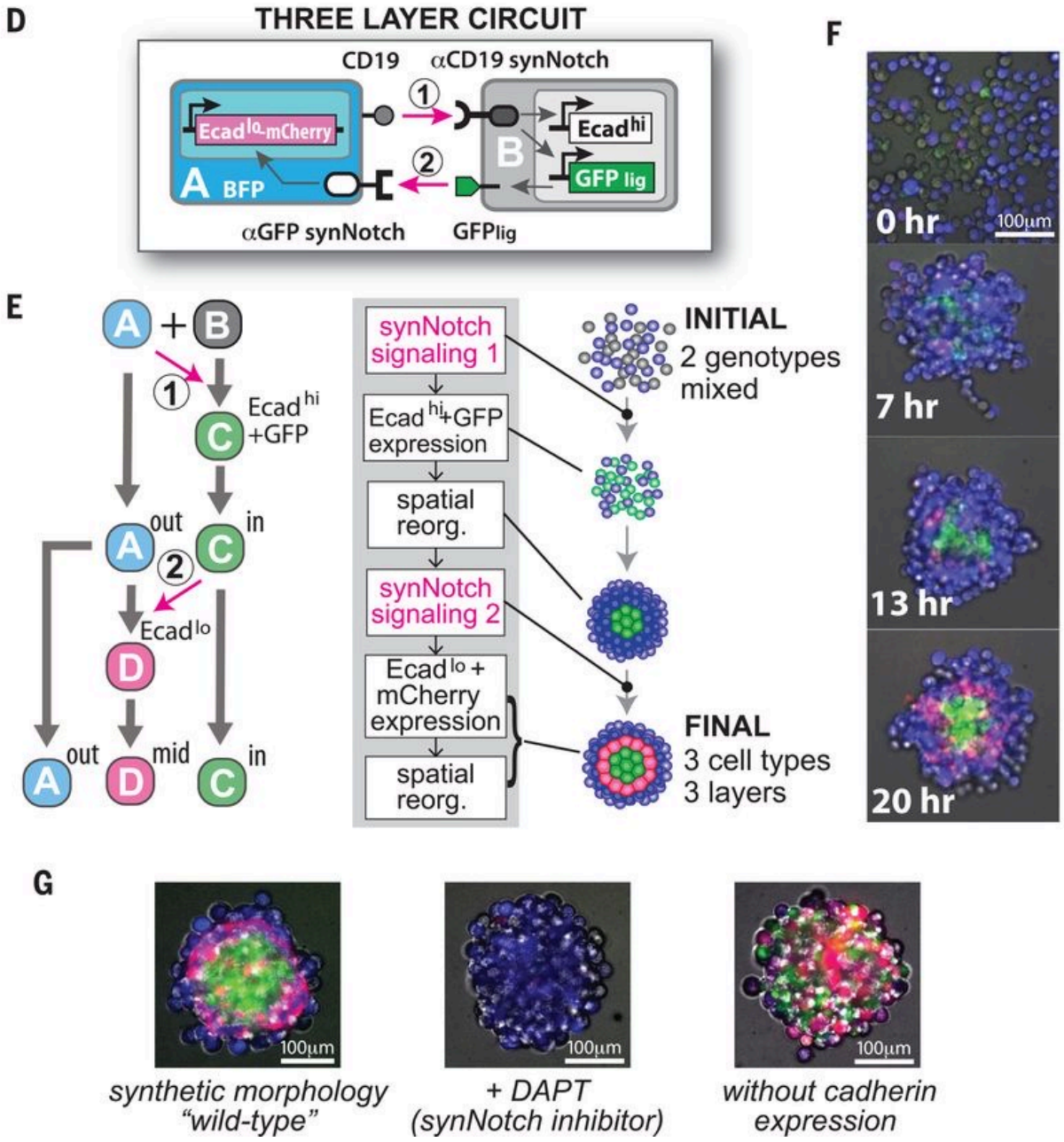
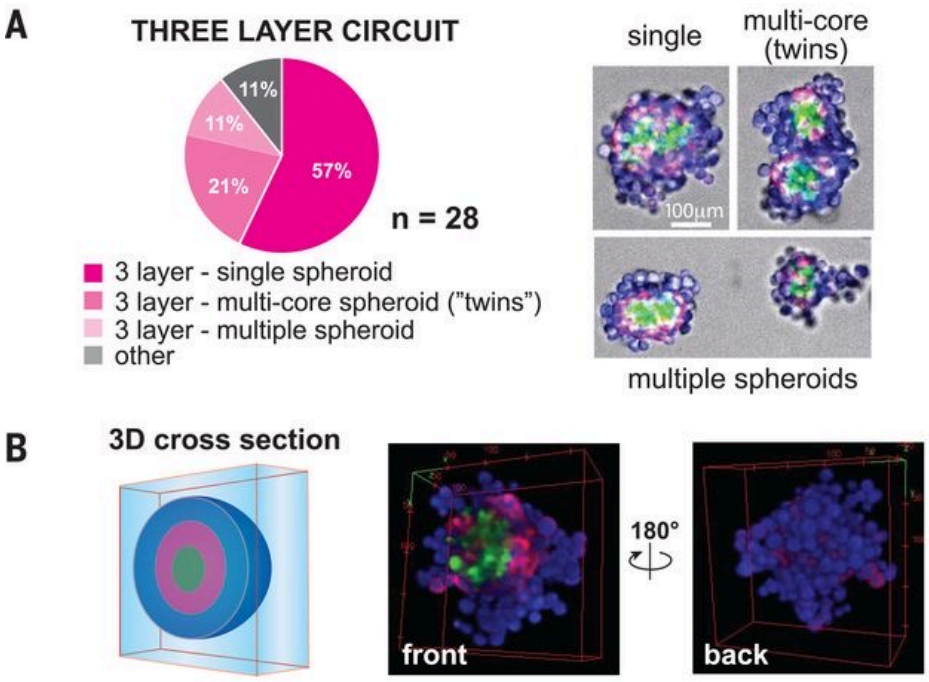
Engineering self-organizing multilayered spheroids

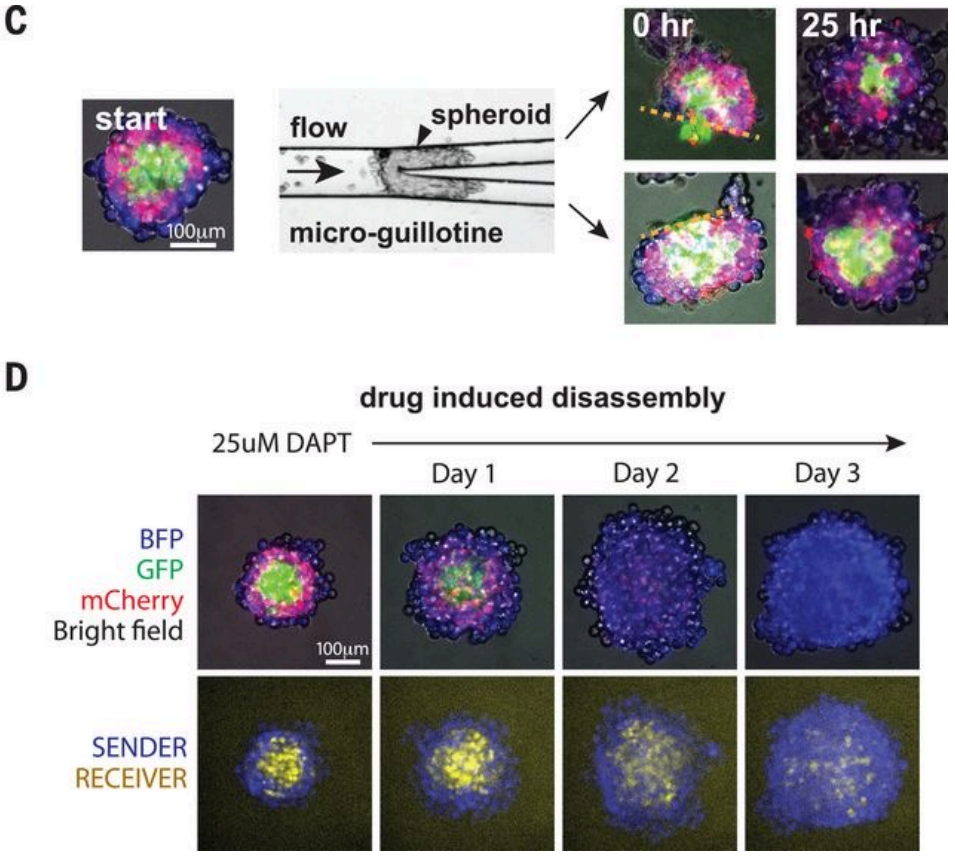
2 genotypes

3 phenotypes

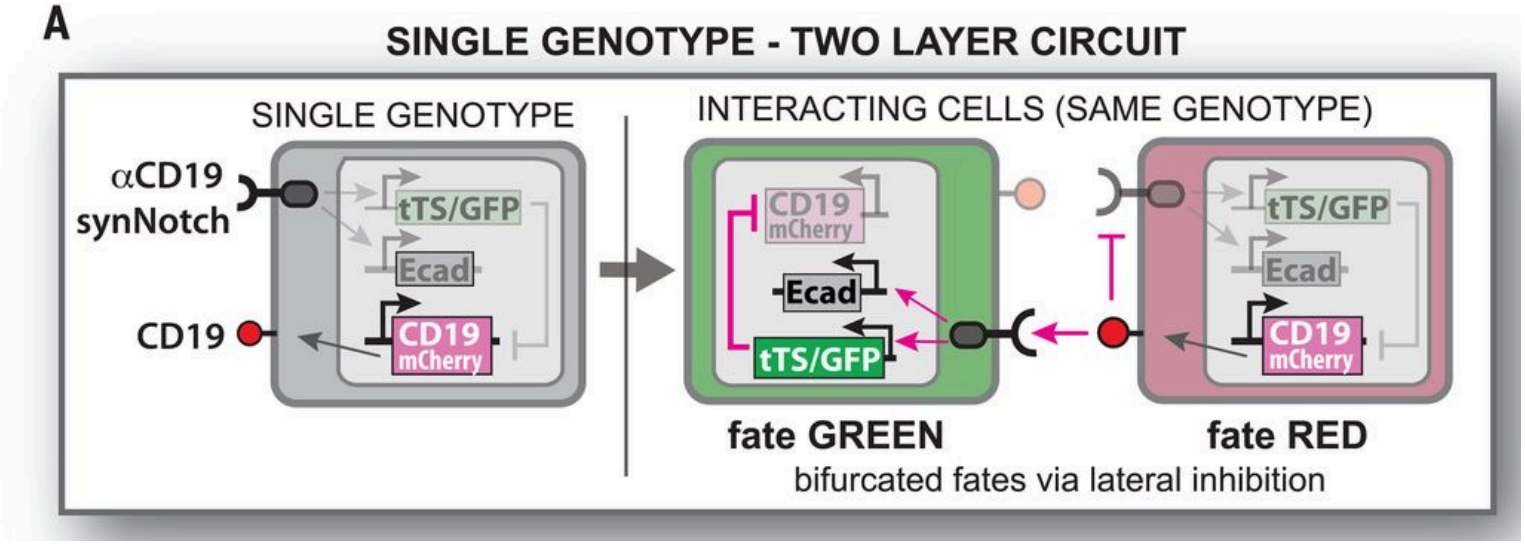


Three-layer self-organized structure is robust, reversible, and self-repairing





Single-genotype circuit that induces fate bifurcation and spatial ordering into a two-layer structure



Design of **single-genotype** circuit with lateral inhibition between sender ($CD19^+$) and receiver (antiCD19-synNotch–activated) states.

The cell encodes both CD19 and anti-CD19 synNotch, but activated synNotch receptor drives expression of tet repressor (tTS), which inhibits CD19 expression.

Thus, neighboring cells will drive each other into opposite states indicated by red and green fluorescent markers (fate RED and GREEN).

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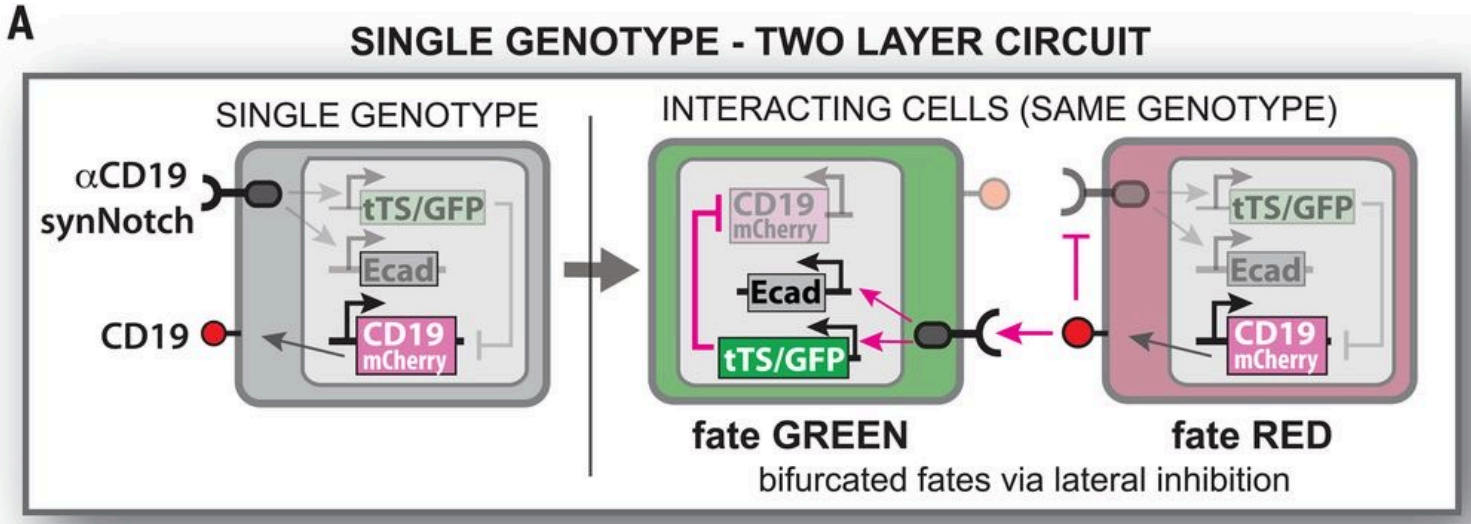
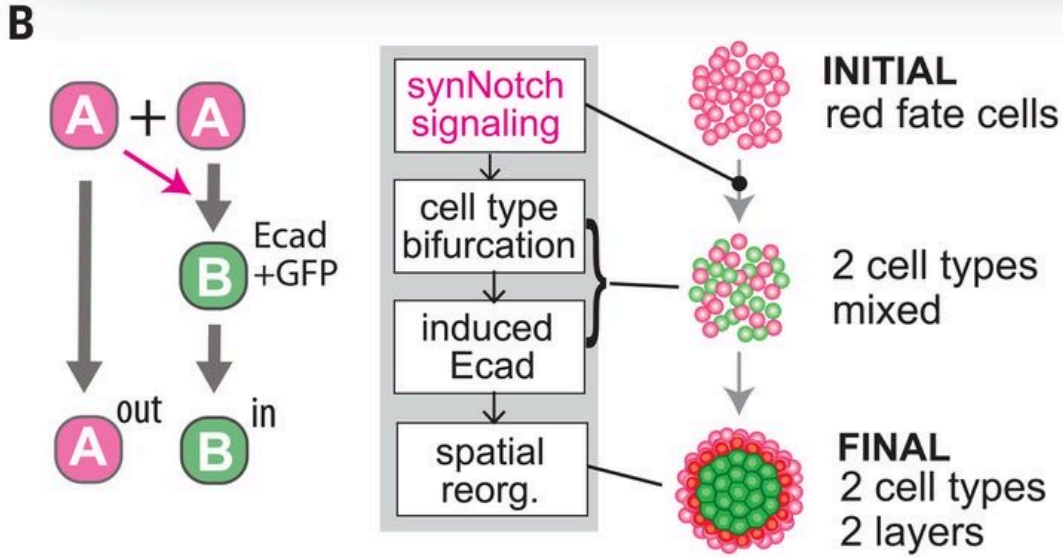
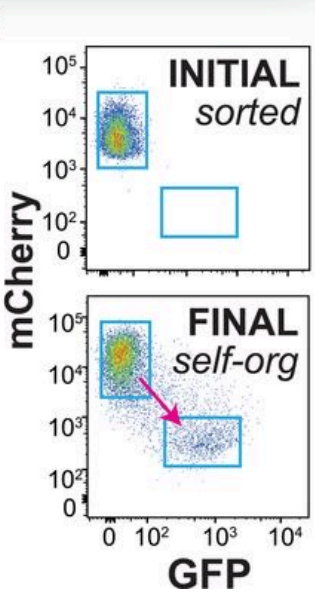
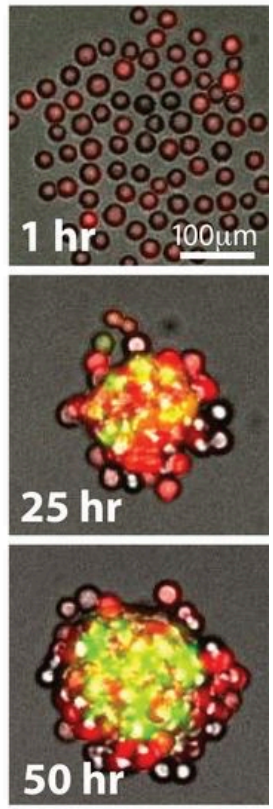
Single-genotype circuit that induces fate bifurcation and spatial ordering into a two-layer structure









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Lecture structure

Programming population control

Programming multicellular organization

Programming multicellular patterning

Engineering synthetic morphogen systems that can program multicellular patterning

Morphogens provide **positional information** during tissue development. For this behavior to occur, morphogens must spread out and form a **concentration gradient**

Can synthetic morphogens be used to program **de novo multidomain tissue patterns** independently from endogenous morphogen pathways?

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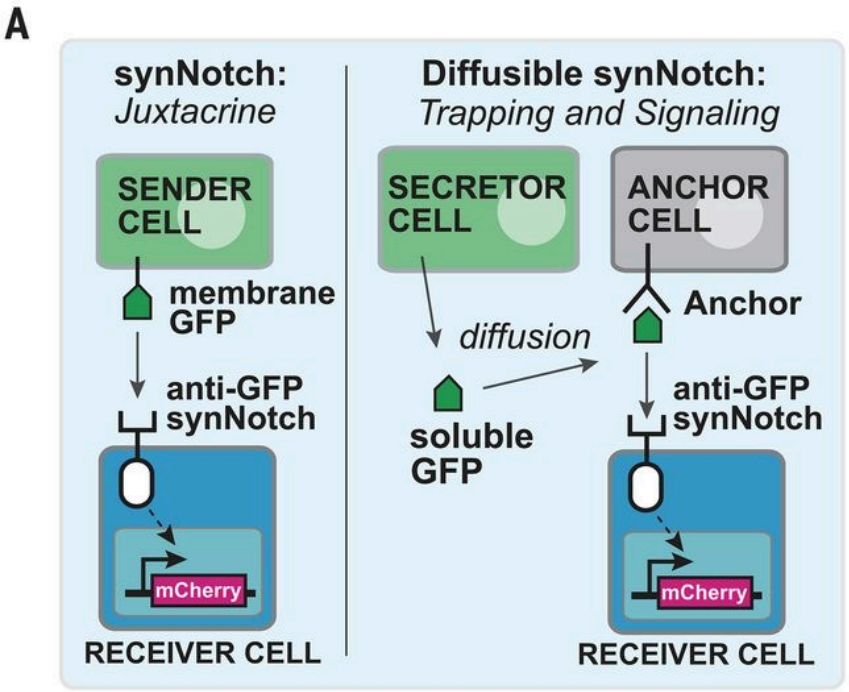
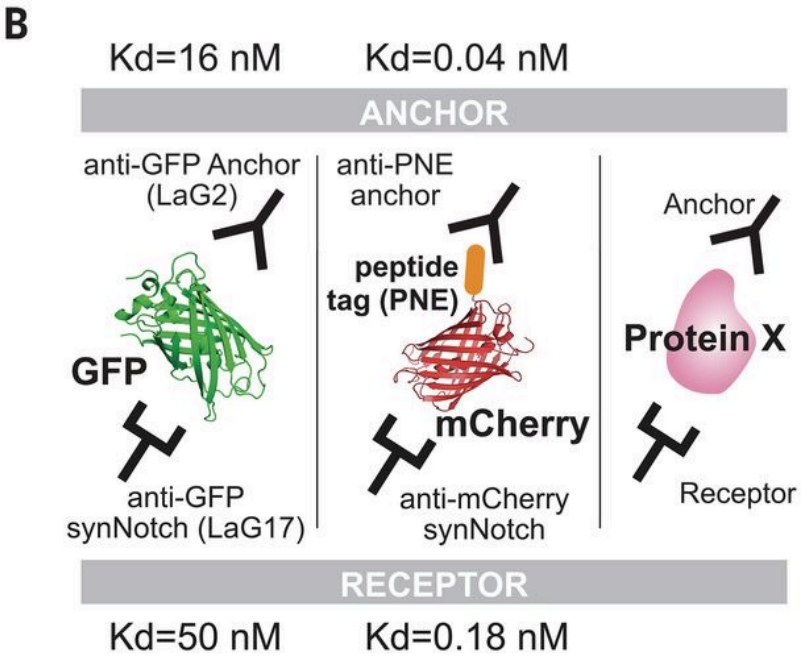
Turning arbitrary proteins into synthetic morphogens





Multiple arbitrary proteins with two recognition sites could be converted into synthetic morphogens

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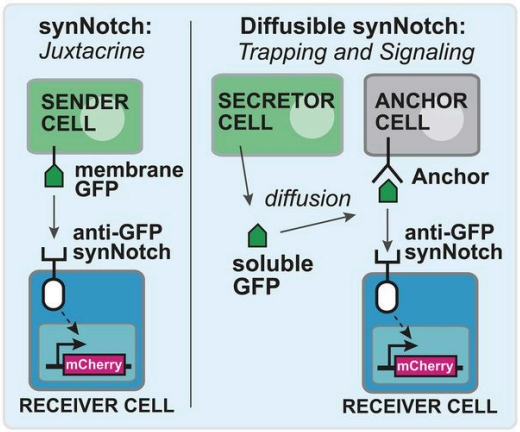
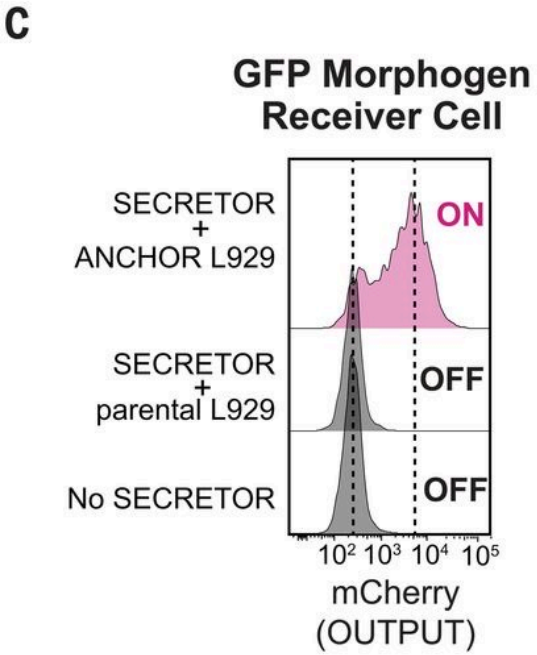
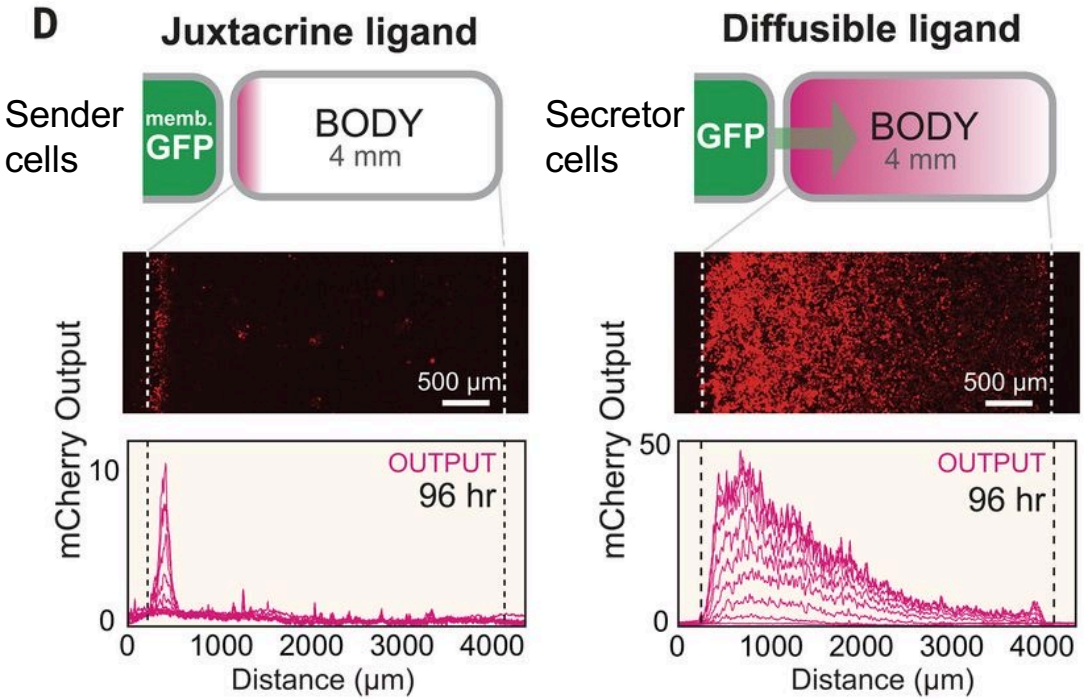
Engineering synthetic morphogen systems that can program multicellular patterning

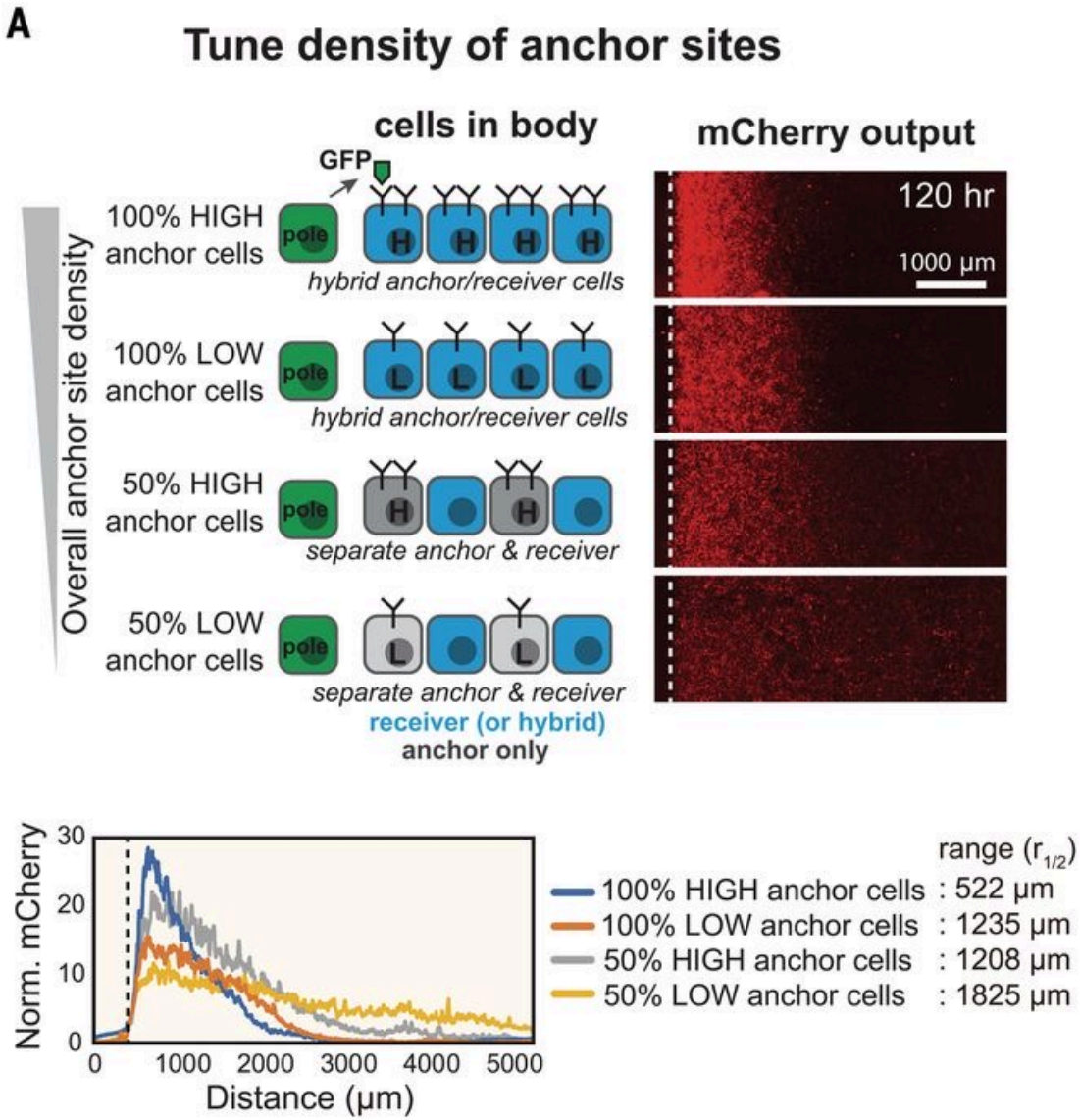


Body contains:
Anchor + Receiver cells





Systematic control over distance range of synthetic morphogen gradient



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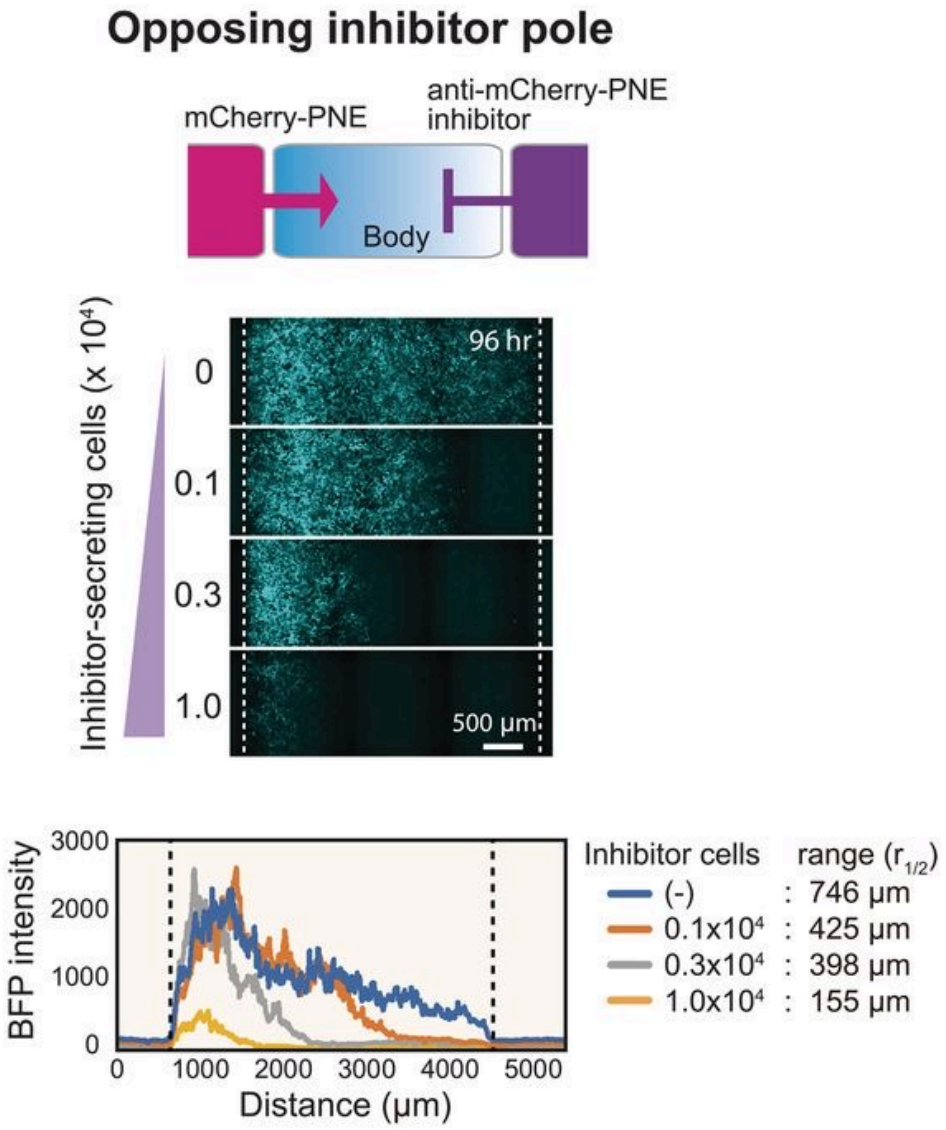
Controlling signaling range of mCherry-PNE morphogen with inhibitor

Morphogen pole:
mCherry-PNE–secreting cells

Body:
anti-PNE anchor cells and
anti-mCherry synNotch receiver cells

Inhibitor pole :
cells expressing
anti–mCherry-PNE inhibitor



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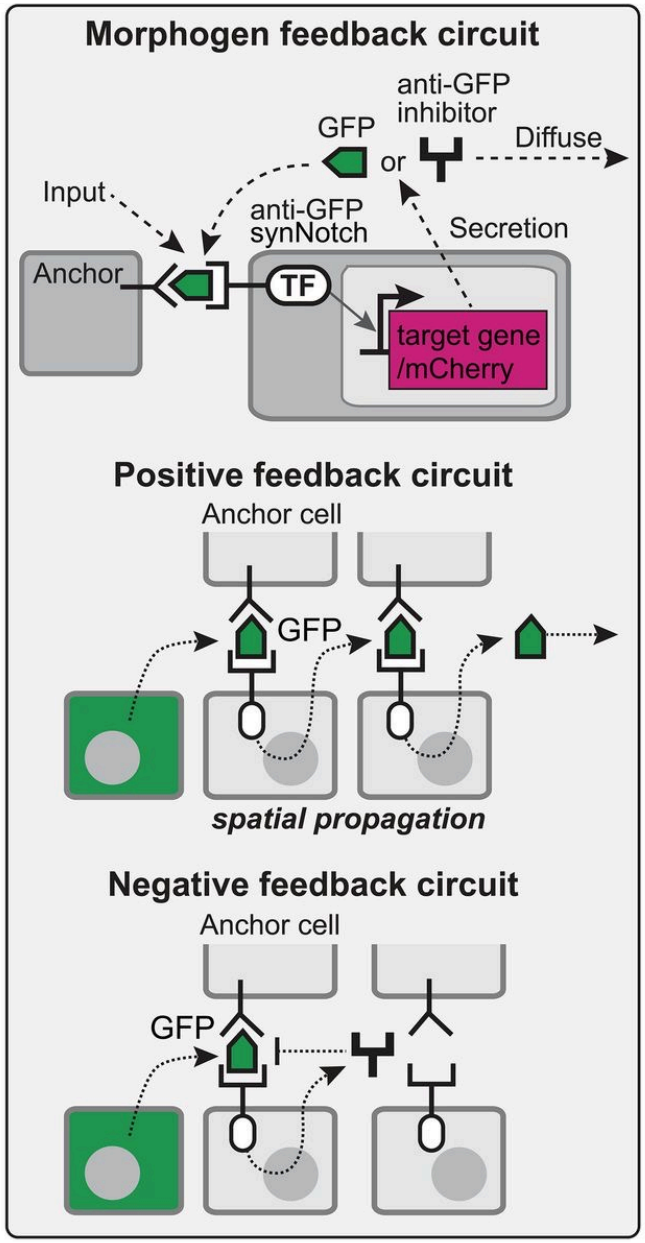
Reshaping morphogen interpretation with positive or negative feedback

In a **positive feedback** circuit, GFP morphogen activates receiver cells to induce the secretion of more GFP.

In a **negative feedback** circuit, GFP morphogen induces the expression of antimorphogen inhibitor by receiver cells.

TF, transcription factor



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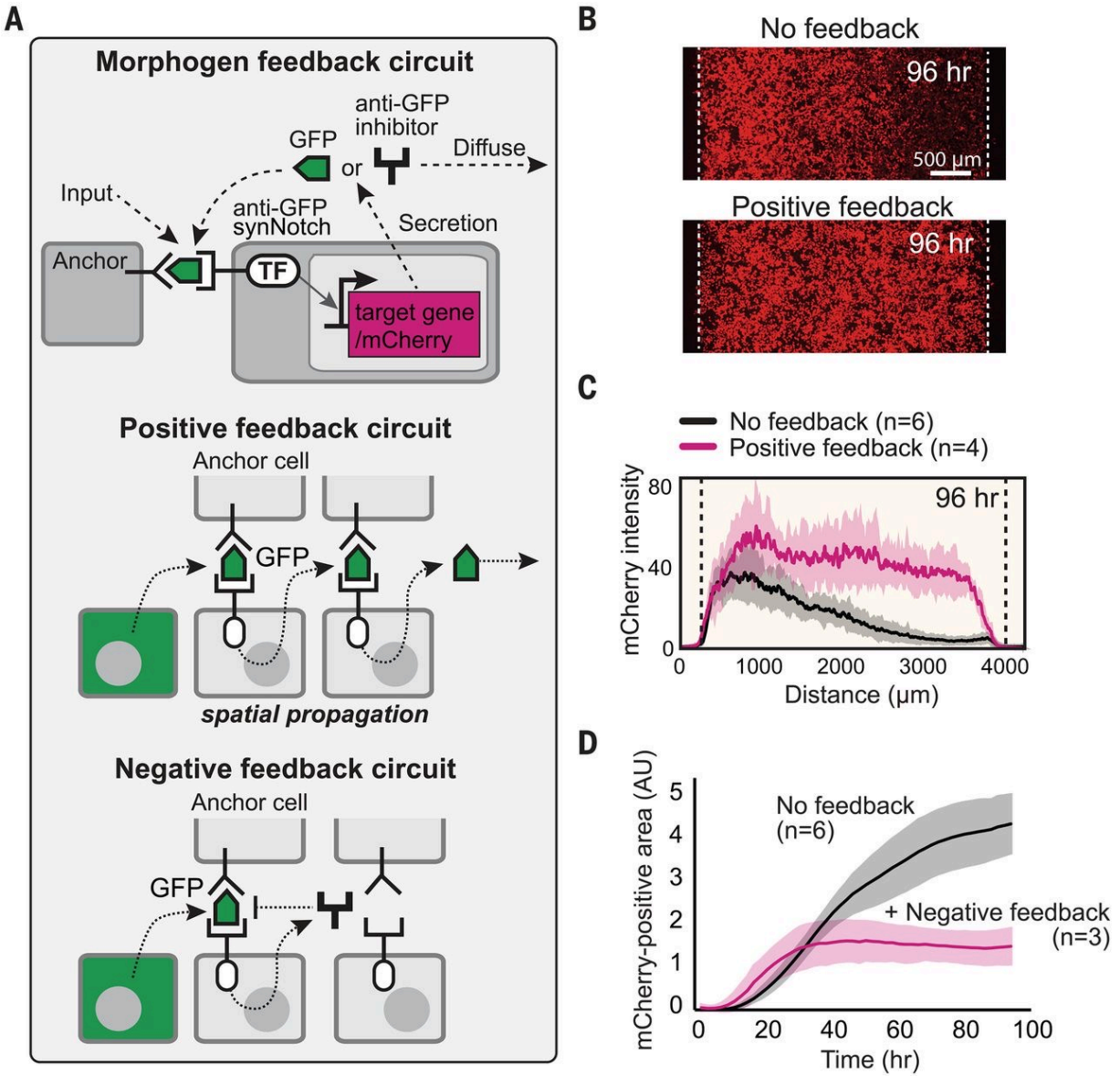
Reshaping morphogen interpretation with positive or negative feedback



Take home message

Cell-cell communication through cell surface attached or diffusible ligands can control:

- Cell population
- Multicellular Morphology
- Multicellular Patterning

Take home message

Open book exam with a large fraction inspired from exercises and mini protein design project.

Mock exam to follow at the end of the lectures
(more info on Moodle soon)

- No need to remember the precise details of circuits
- Learn and understand the principles, concepts and goals underlying the different types of circuits