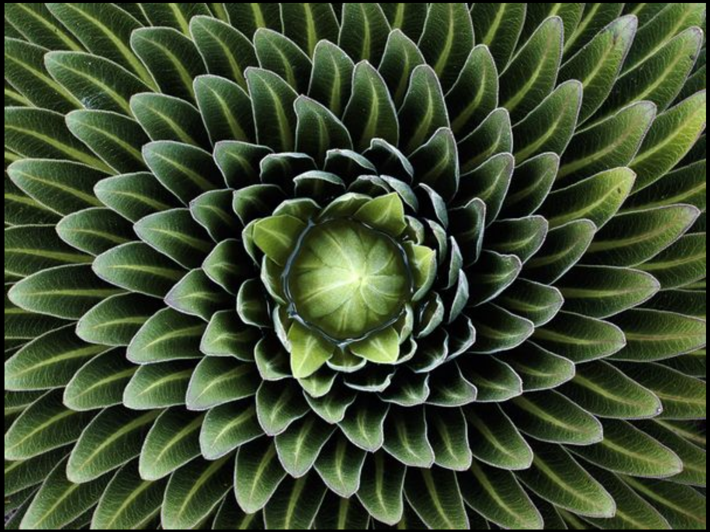
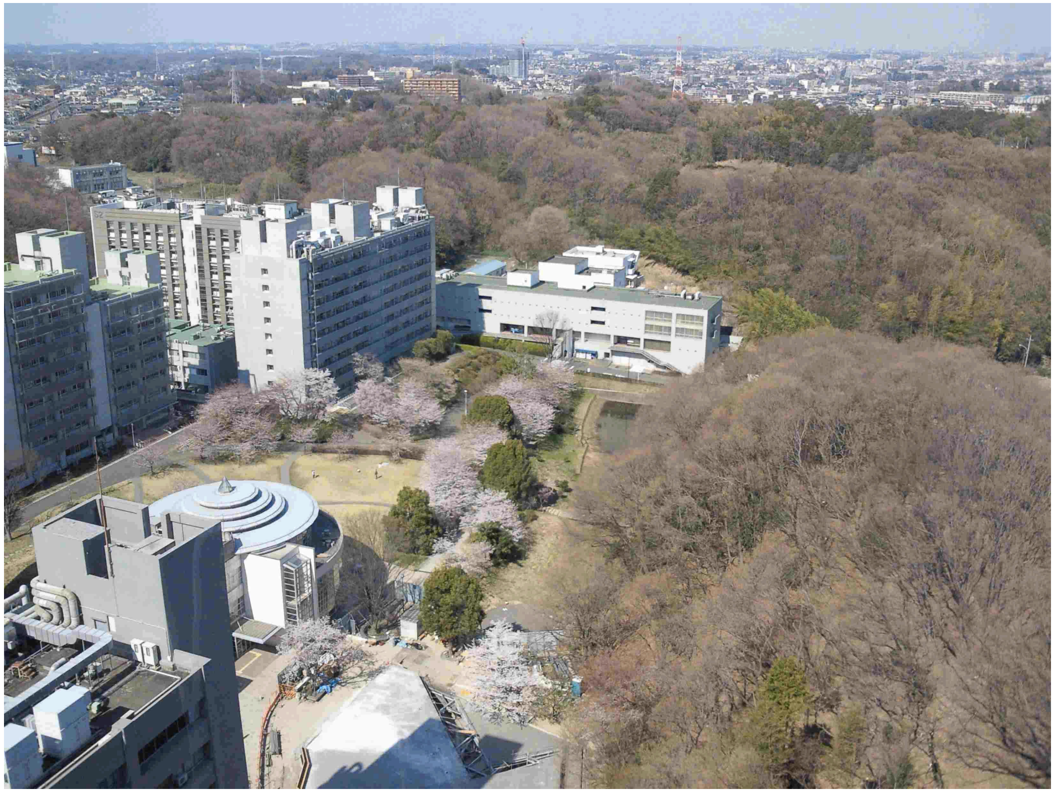
Advanced Synthetic Biology Applications: Pattern Formation



Mark Isalan – on sabbatical



Pattern Formation - Overview

This lecture will cover:

- **Theories of pattern formation**
- **French flag gradient systems versus Turing patterns**
- **An iGEM project to generate bacteria that swim into patterns**

Advanced Synthetic Biology Applications: Pattern Formation

- **Aims**

  To present the main mechanisms of biological pattern formation.

  To show how synthetic biologists are building stripe-forming systems.

- **Learning Outcomes**

  To be able to describe the main mechanisms behind biological pattern formation, and the similarities and differences between them.

  To be able to describe (briefly), with illustrations, synthetic biology implementations of different stripe forming mechanisms.

Why aren't you a big fat blob?

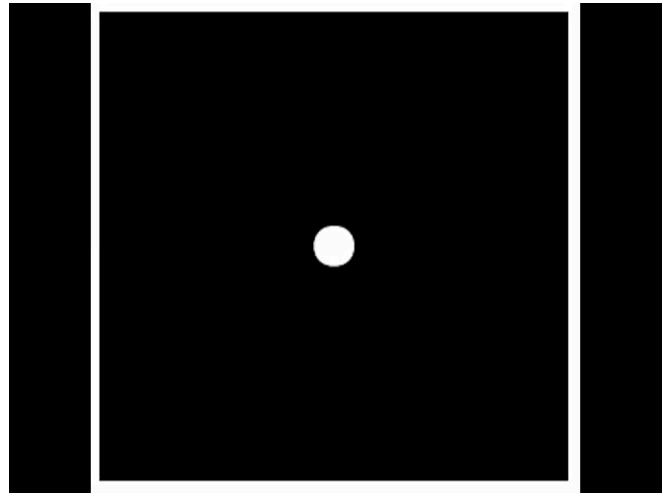
Pattern formation is biologically important

Why aren't you a big fat blob?

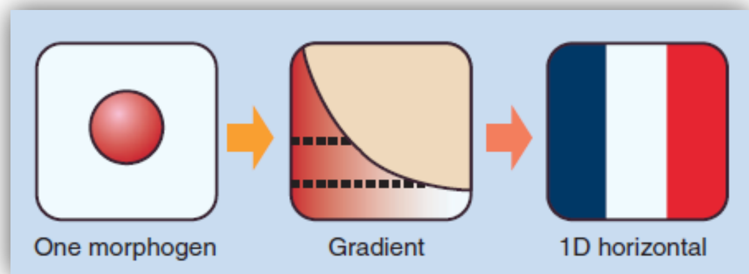
- Pattern formation is MORPHOGENESIS
- It is the mechanism behind cell differentiation
- Multicellularity (you, me, the mushrooms) requires pattern formation
- Gives rise to specialisation and division of labour:
  the body plan

Patterning theories

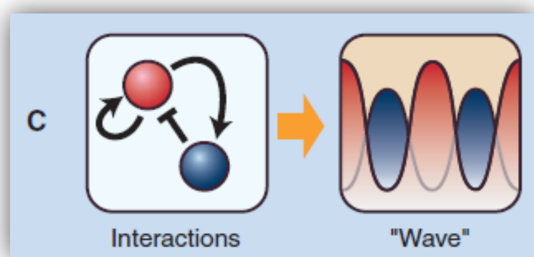
- **Growth, clock and rule-based (e.g. dendrite growth)**

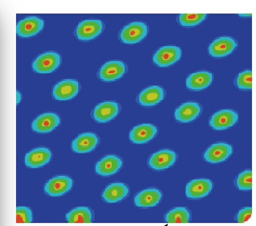


- **Gradient models: morphogen sensors (French flags)**

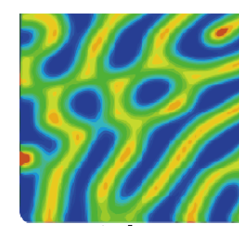


- **Reaction-diffusion systems (Turing patterns)**





spots



stripes

Pattern formation by morphogenesis

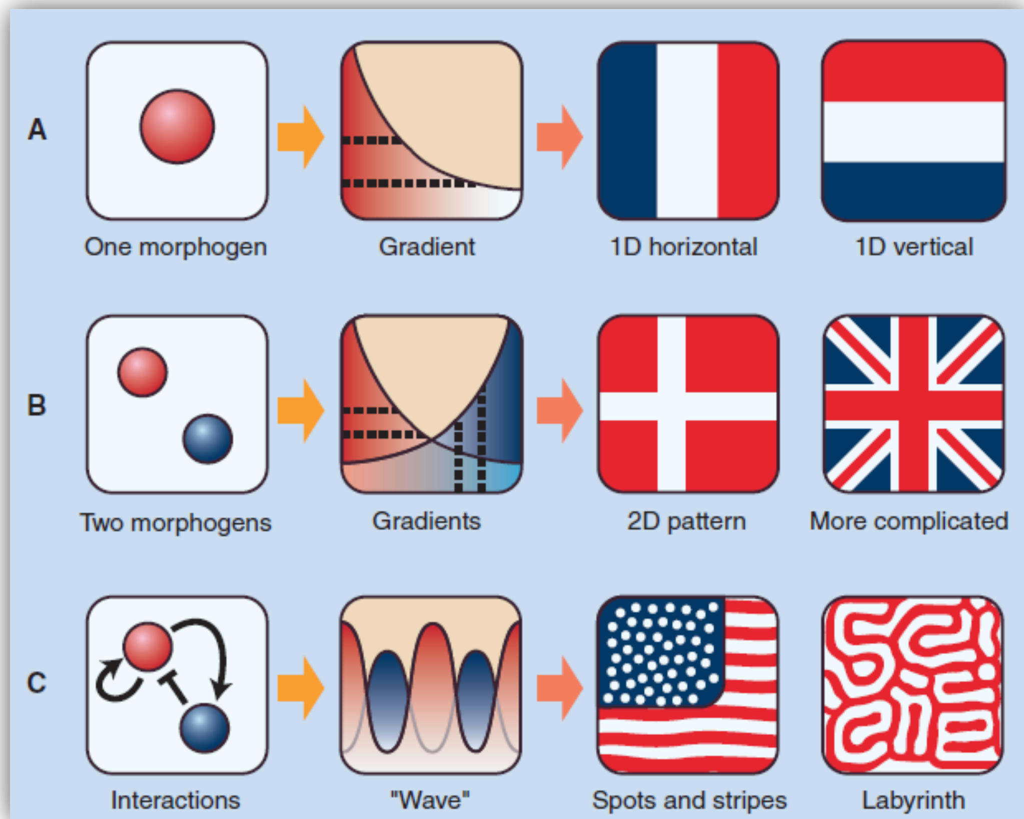
REVIEW

Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation

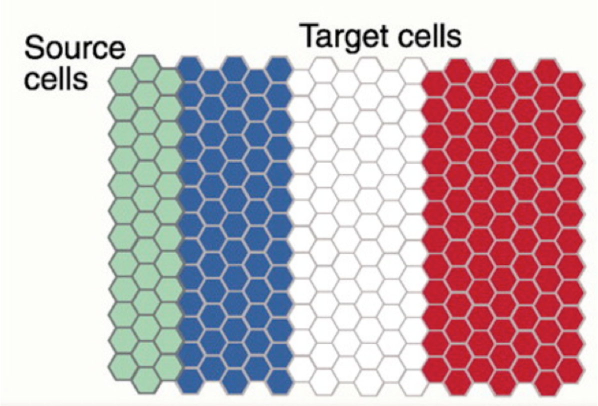
Shigeru Kondo[1]* and Takashi Miura[2]

Useful read:
Science (2010) **329**, p1616-p1620

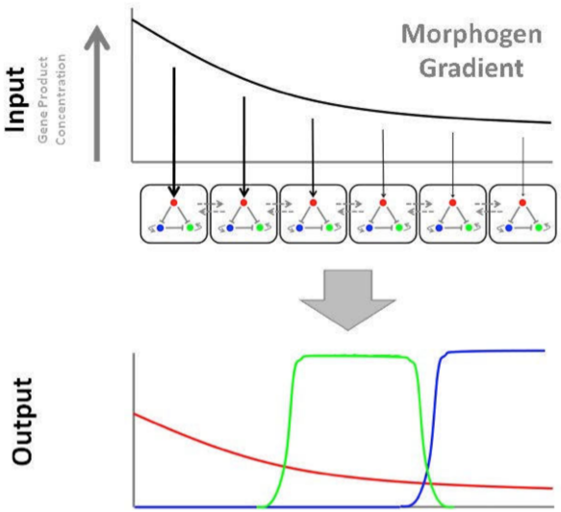
also read the supplementary online material and play with the JAVA program



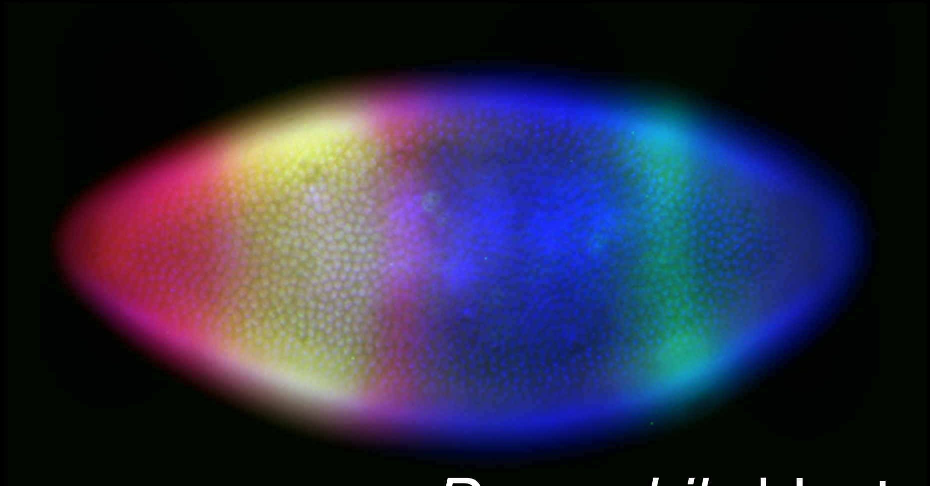
The French Flag model: how to make stripes in a morphogen gradient

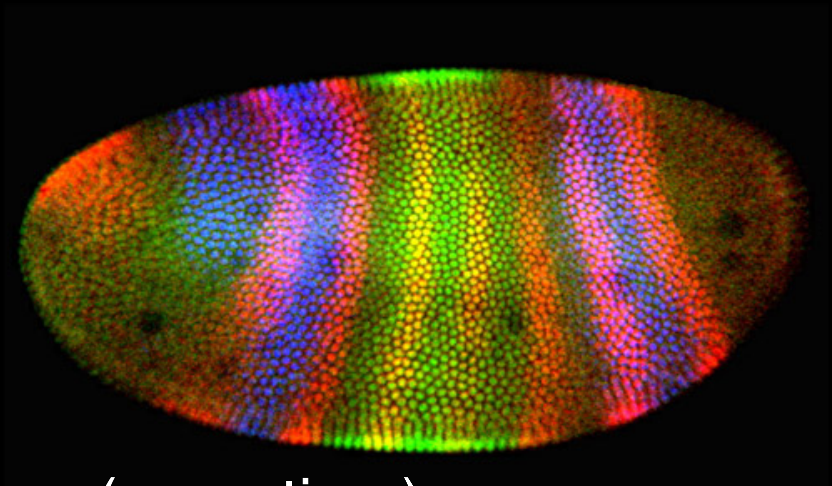


Lewis Wolpert, 1969



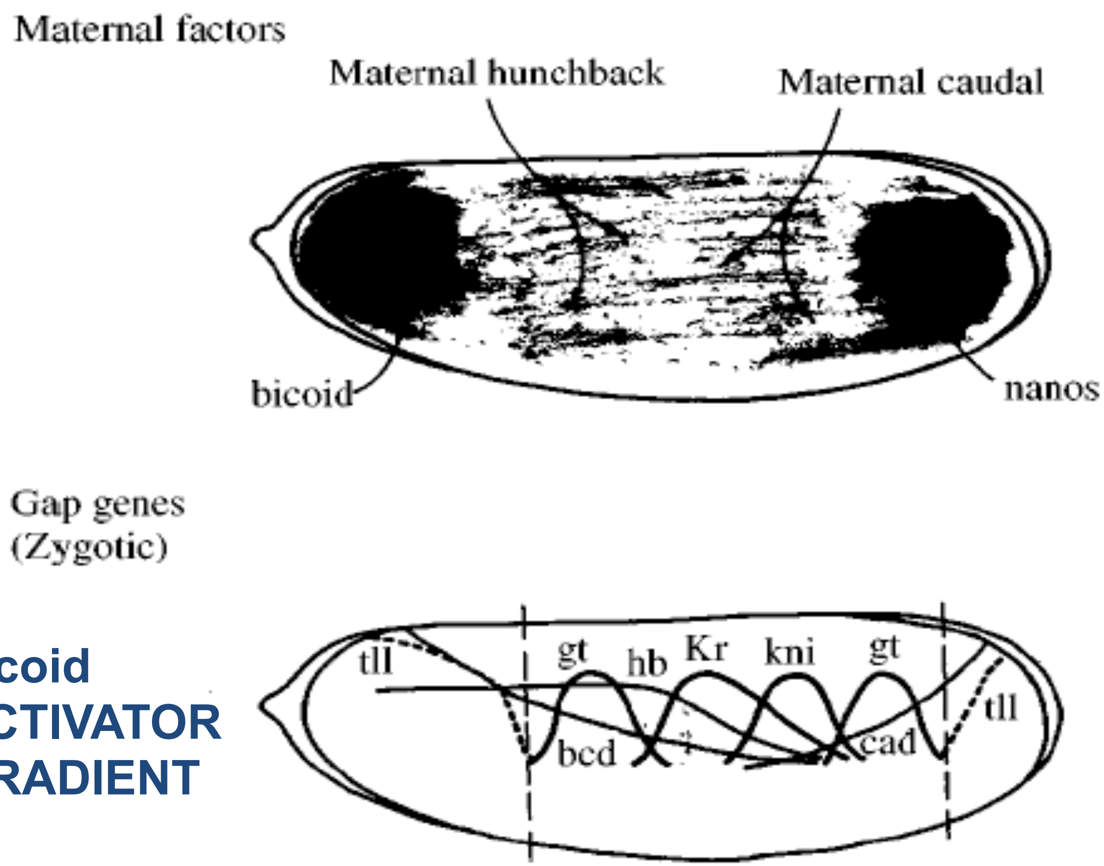
Drosophila morphogen gradients





Drosophila blastoderm (syncytium)
maternal *bicoid* and *caudal* gradients establish gap gene patterns

Drosophila - morphogen gradients and gap gene expression domains



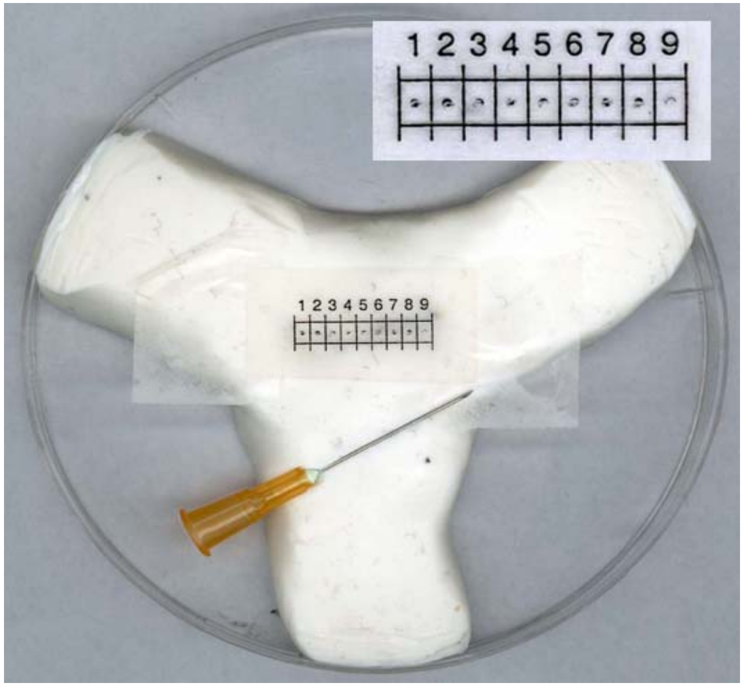
bicoid ACTIVATOR GRADIENT

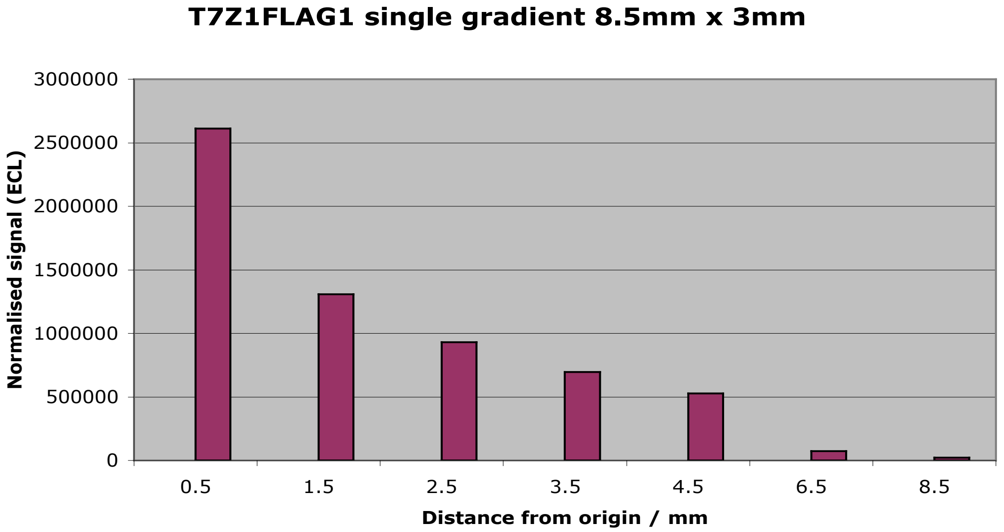
caudal ACTIVATOR GRADIENT

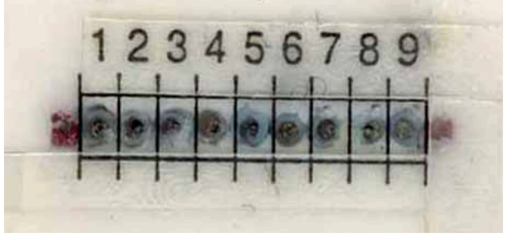
DOSE-DEPENDENT repressors

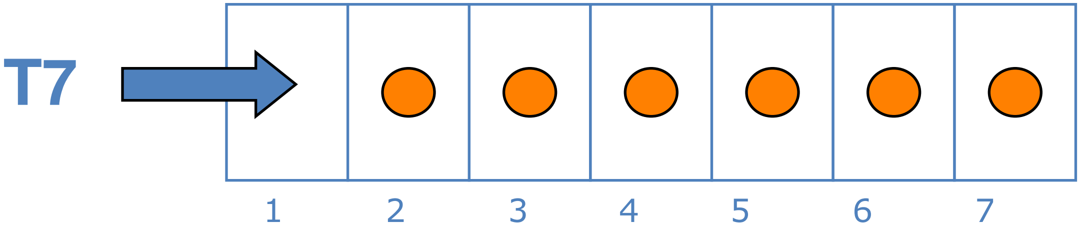
Synthetic biology: Drosophila on a chip

Engineering Gene Networks to Emulate Drosophila Embryonic Pattern Formation. Isalan et. al, PLoS Biol. (2005)



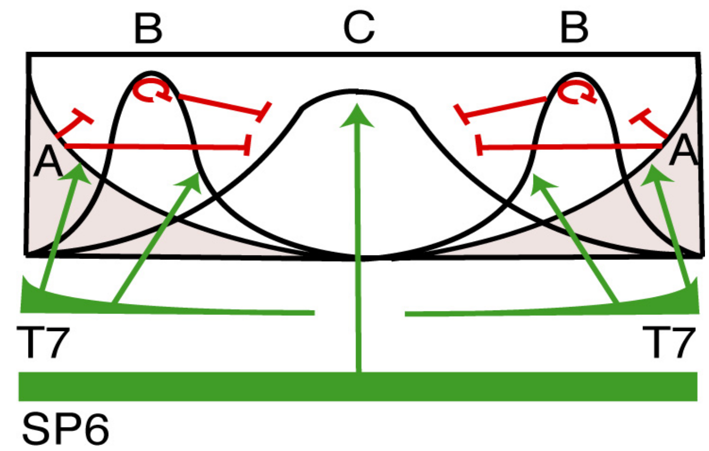


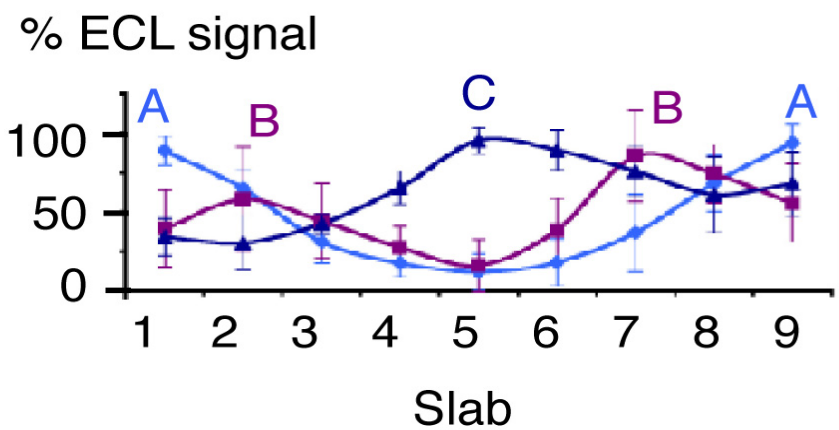


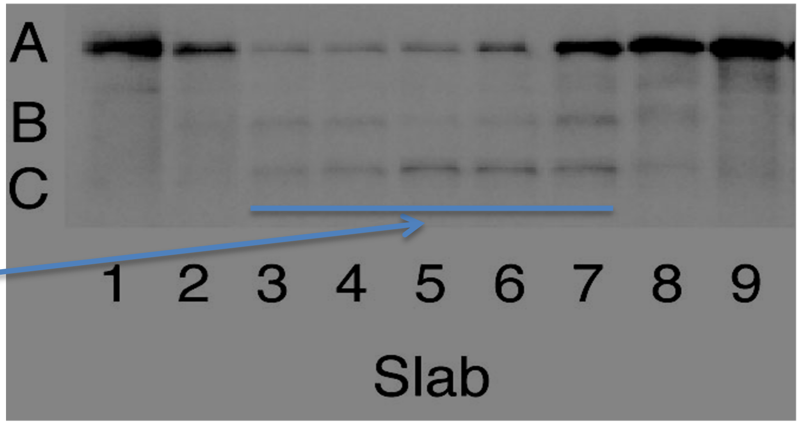


Synthetic gap gene network

- Inject polymerases to start the system
- Trancription-translation extract expresses genes in a gel
- After 1 hour, cut gel out, assay by Western blot
- Central stripe "C"







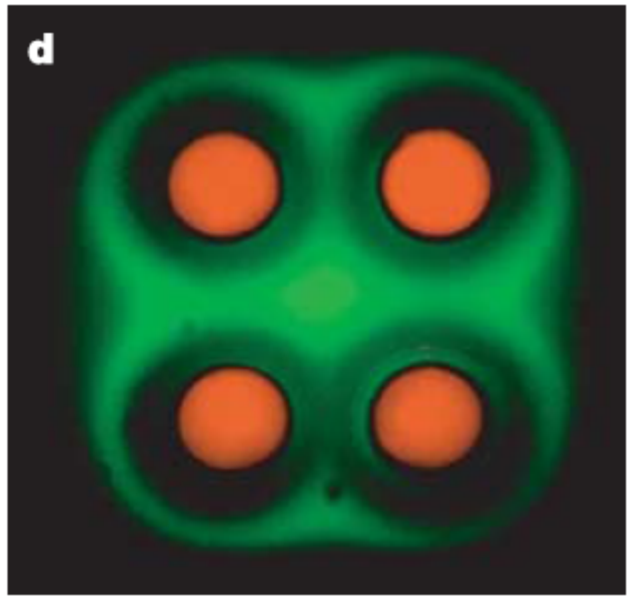
Gradients forming stripes: from *cell-free* to *cell-based* models

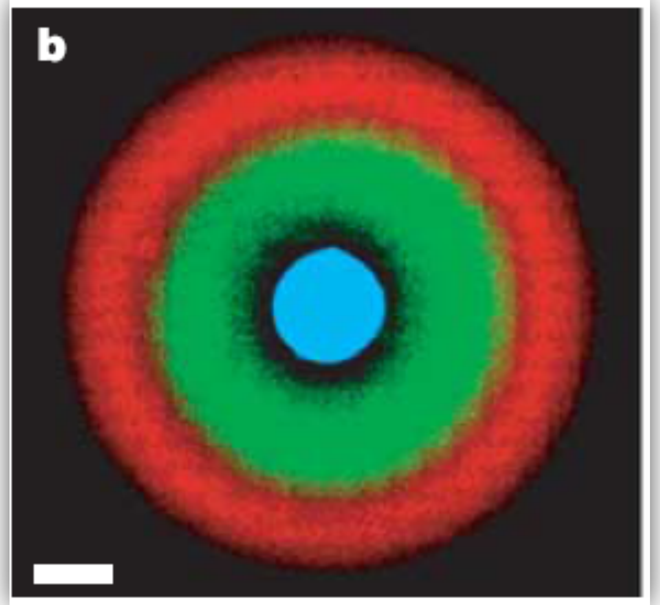
French flag systems on lawns of bacteria

Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R. A synthetic multicelluar system for programmed pattern formation Nature. 2005;434:1130-4.

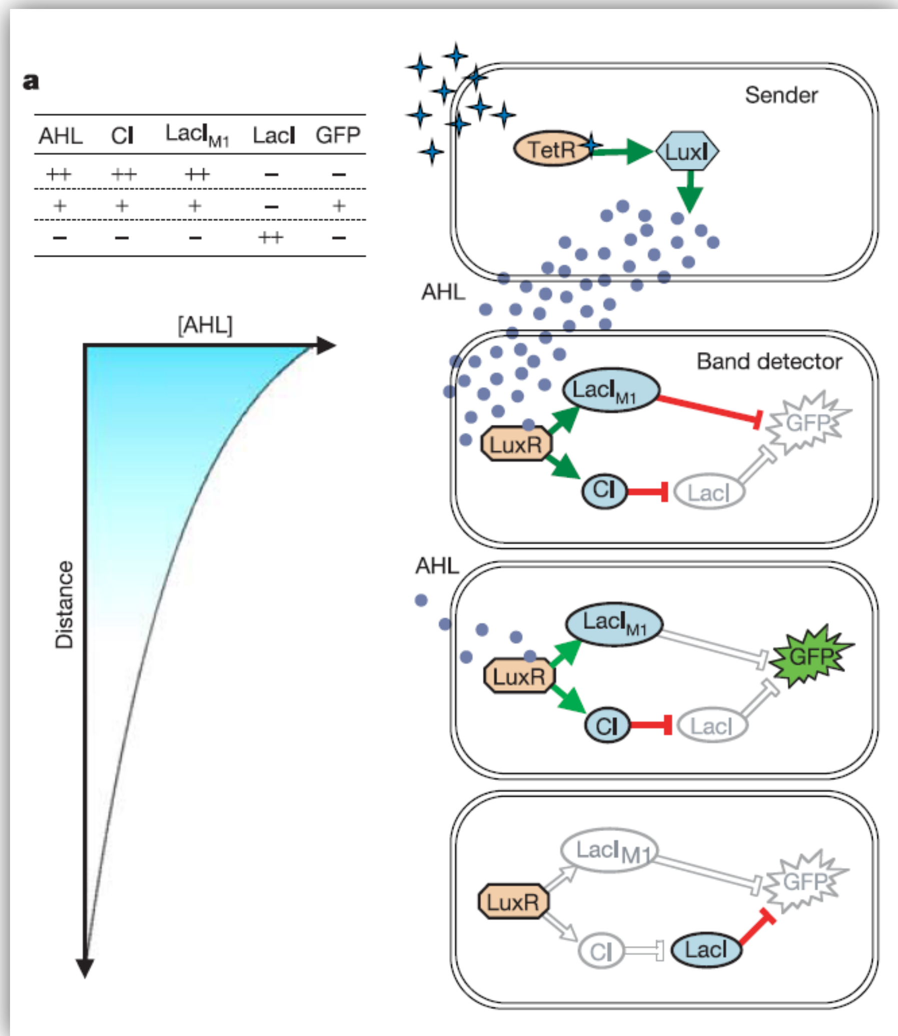
A synthetic multicellular system for programmed pattern formation

Subhayu Basu[1], Yoram Gerchman[1], Cynthia H. Collins[3], Frances H. Arnold[3] & Ron Weiss[1,2]





Basu *et al.* Nature 2005

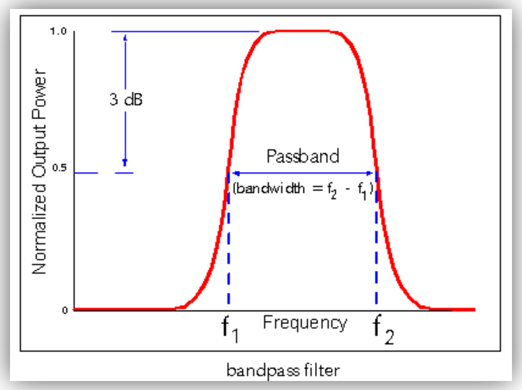


Band-detect multicellular system

Sender cell produces AHL

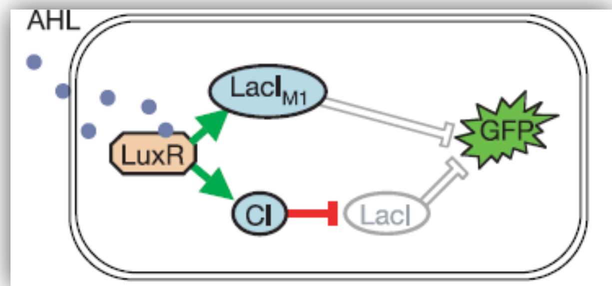
Receiver cell contains a feed-forward loop responsive to AHL (band-pass filter)

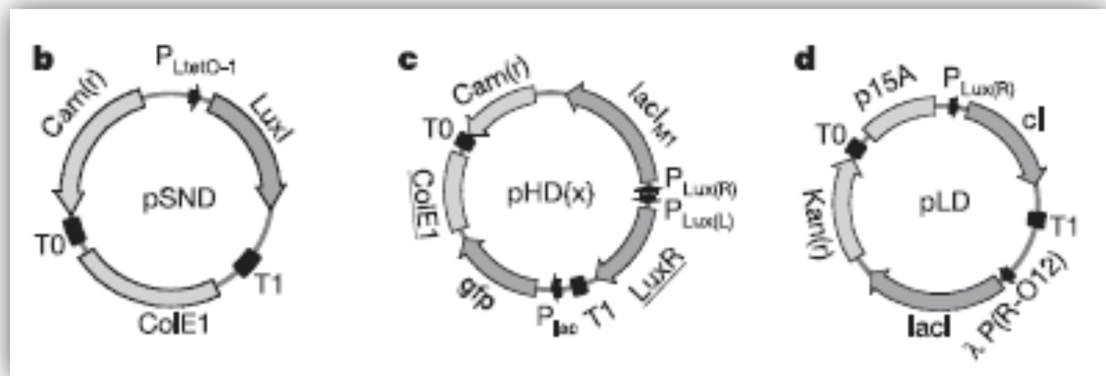
Receiver cell produces GFP only at medium AHL levels



Basu *et al.* Nature 2005

Modular System allows for different versions to be made and mixed



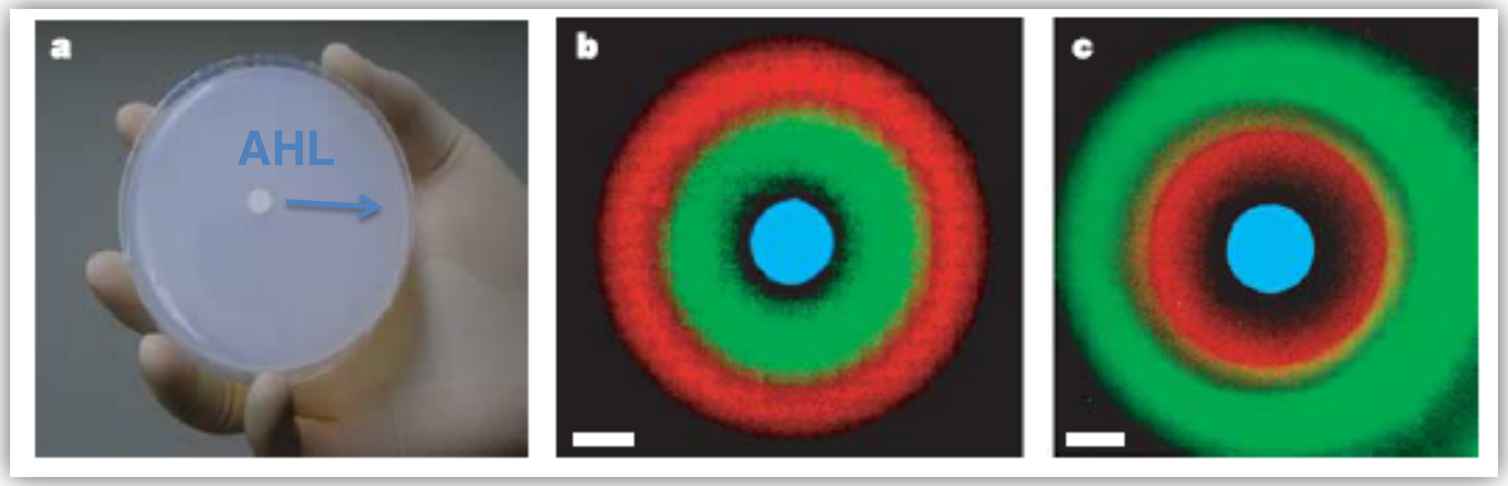


Sender High-detect Low-detect

- HD1 – mutation in LuxR that makes it hyper-sensitive
- HD2 – normal plasmid
- HD3 – mutation in ColE1 means cells have lower-copy number

- GFP can be swapped with RFP to change the colour of the cells

Basu *et al.* Nature 2005



Immobilized plug of sender cells placed in centre of petri dish

Rest of agar inoculated with a mix of receiver cells:

b. BD2-RFP mixed with BD3-GFP

c. BD1-GFP mixed with BD2-RFP

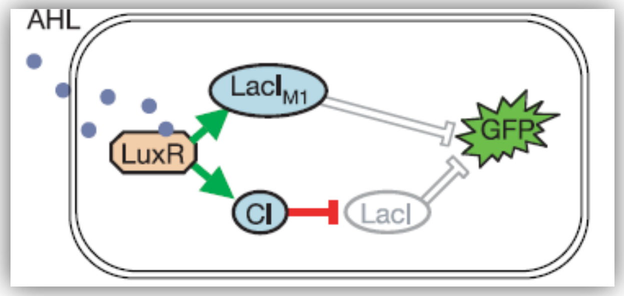
Sender disk impregnated with AHL signal chemical (cyan colour)

Basu *et al.* Nature 2005

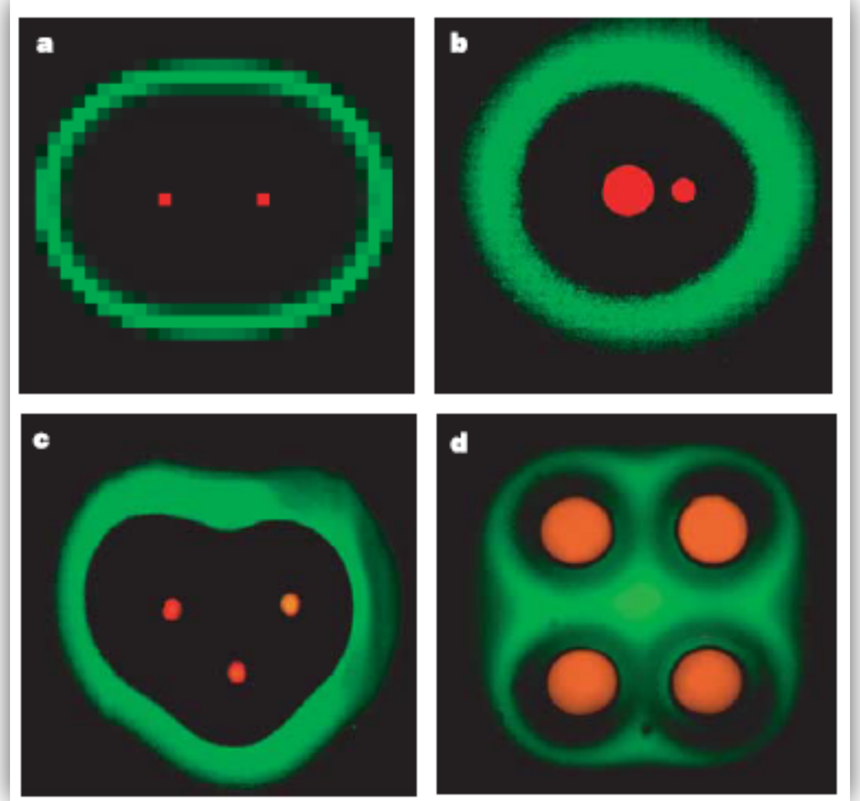
Modelling can predict complex patterns from two or more spots

Parameter analysis of the model also determines the critical parts of the network:

e.g. LacI stability (half-life)



I2 feedforward loop network

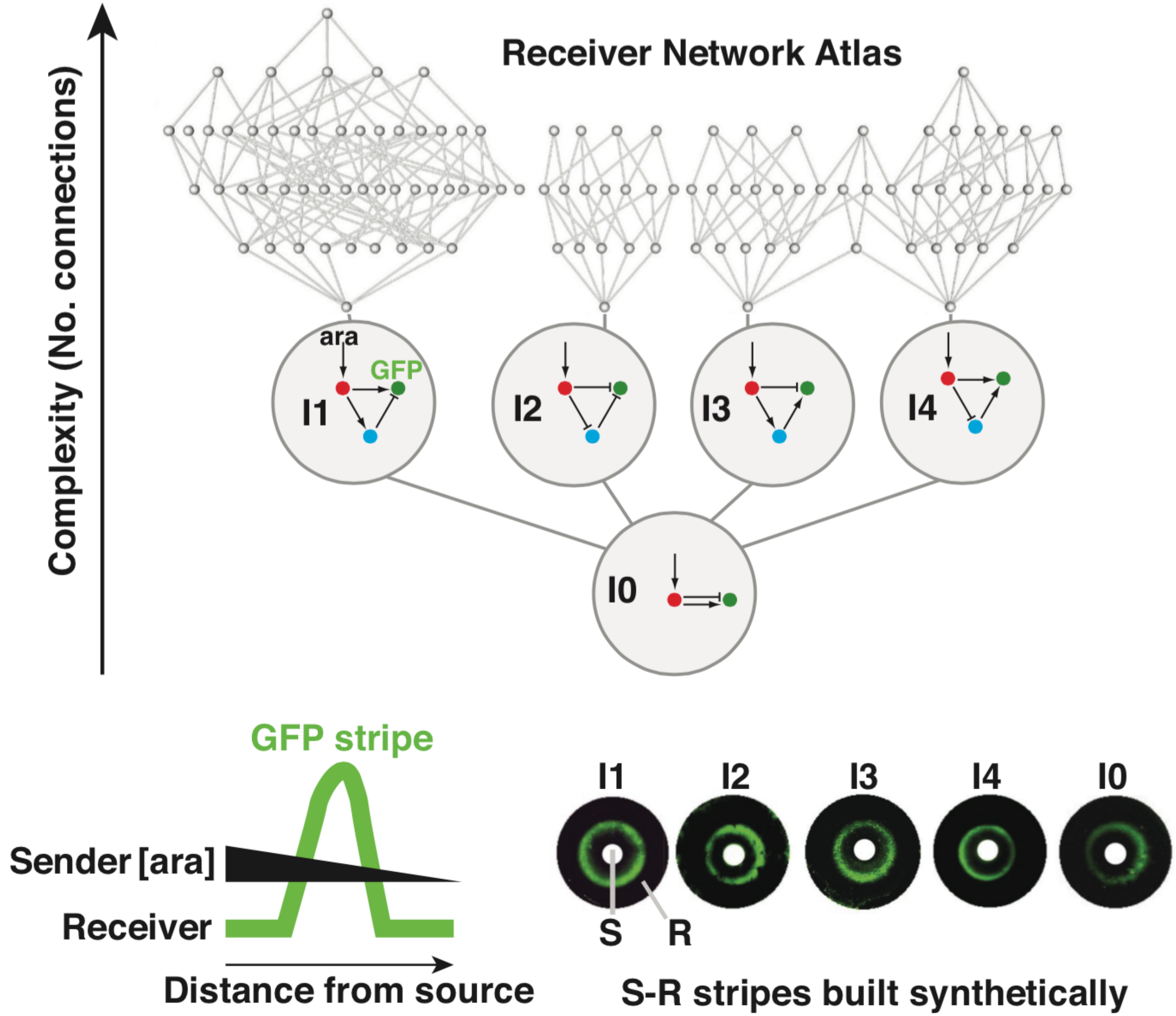


a = Simulation

How many ways can you make a stripe? A network atlas approach

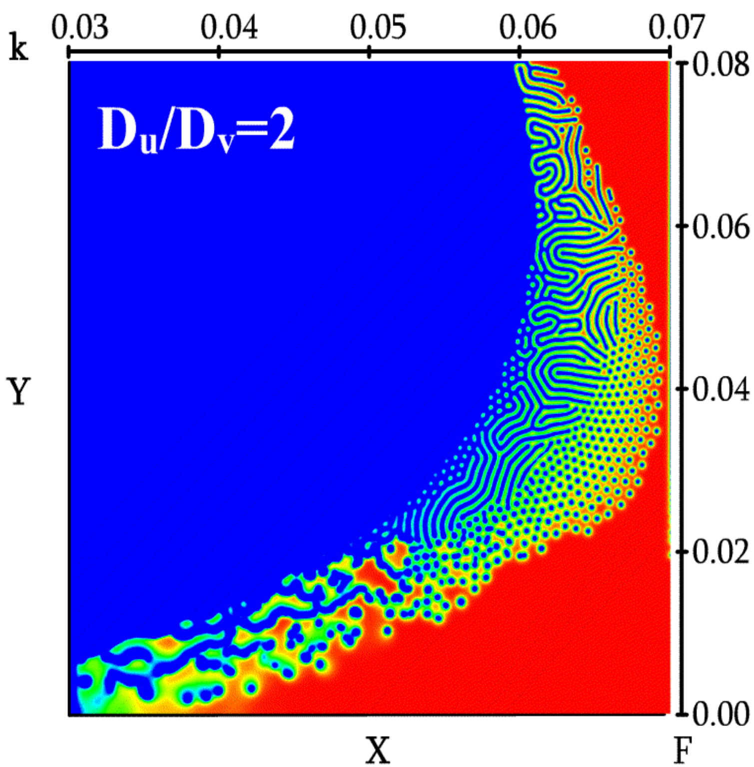
Schaerli, Y. et al. A unified design space of synthetic stripe-forming networks. *Nat. Commun.* **5**:4905 doi: 10.1038/ncomms5905 (2014).

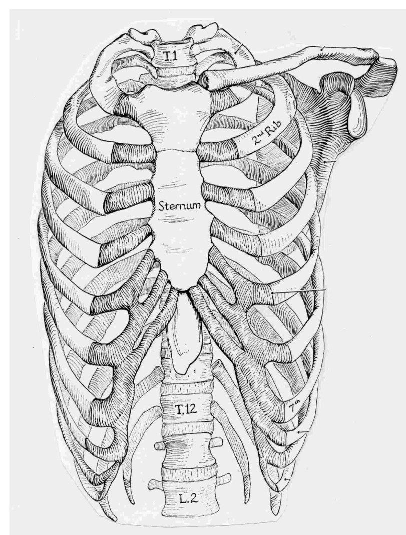
2897 topologies
100,000 parameter sets
109 solutions
4 "stalactites"



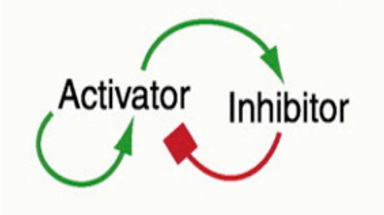
What about more complex patterns?

Reaction-diffusion: Turing patterns





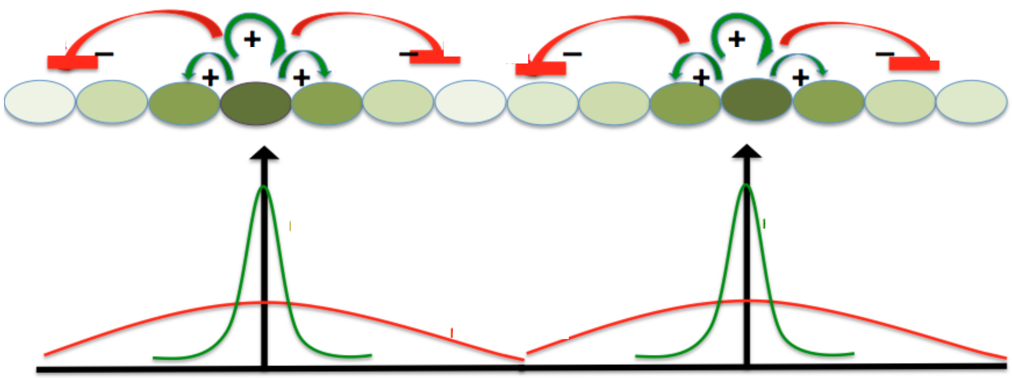
Turing and Gierer-Meinhardt patterns

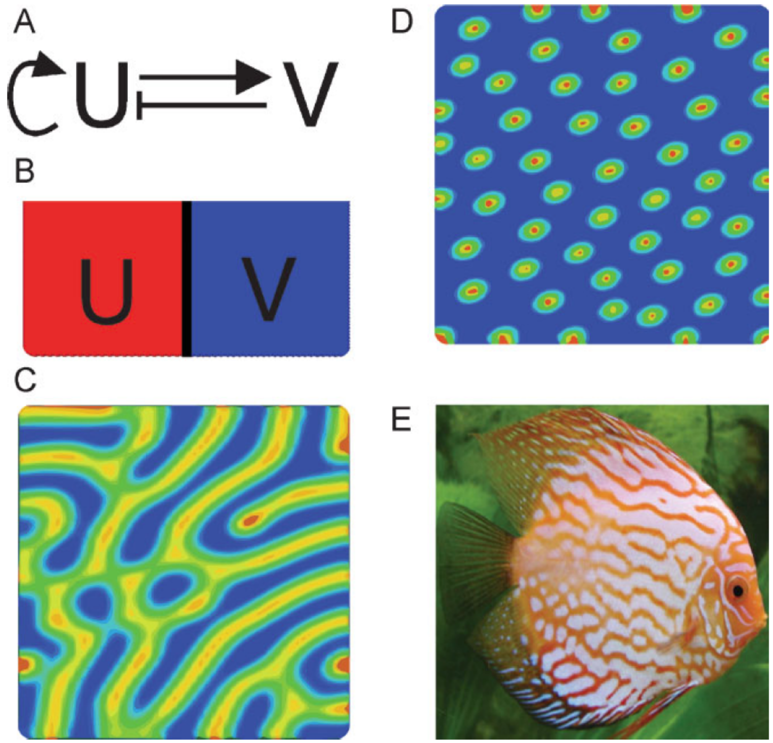


Turing, 1952-3
Gierer & Meinhardt, 1972

slow diffusing non-linear auto-activator

fast diffusing inhibitor ('lateral inhibition')





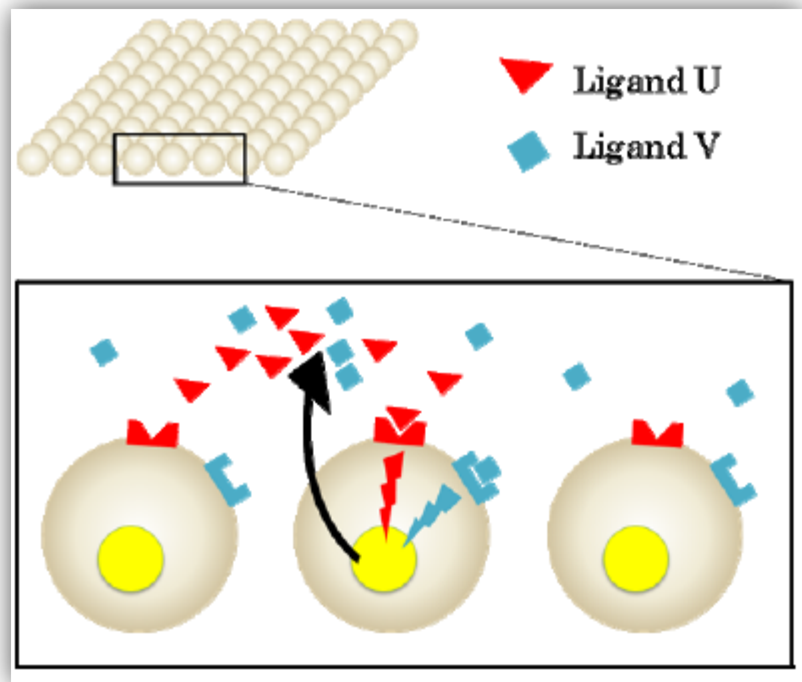
initiated by noise
self-organising and self-repairing

The Reaction-Diffusion Equation

Two interacting molecules diffusing can generate stable patterns – Turing 1952

$$\frac{\partial u}{\partial t} = F(u,v) \; -d_u u \; + D_u \Delta u$$

$$\underbrace{\frac{\partial v}{\partial t}}_{\text{Rate of concentration change}} = \underbrace{\underbrace{G(u,v)}_{\text{Production}} \underbrace{- d_v v}_{\text{Degradation}}}_{\text{Reaction}} + \underbrace{D_v \Delta v}_{\text{Diffusion}}$$



u and *v* are the local concentration of ligands U and V at each position.

F and *G* are the functions governing the production rates. *du* and *dv* are the degradation rates.

The Reaction-Diffusion Equation

Two interacting molecules diffusing can generate stable patterns – Turing 1952

$$\frac{\partial u}{\partial t} = F(u,v) - d_u u + D_u \Delta u$$

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u and *v* are the local concentration of ligands U and V at each position.

F and *G* are the functions governing the production rates. *du* and *dv* are the degradation rates.

Replacing F and G by the following linear function, we get the partial differential equation identical to that of Turing's original paper.

$$F(u,v) - d_u u = a_u u + b_u v + c_u$$

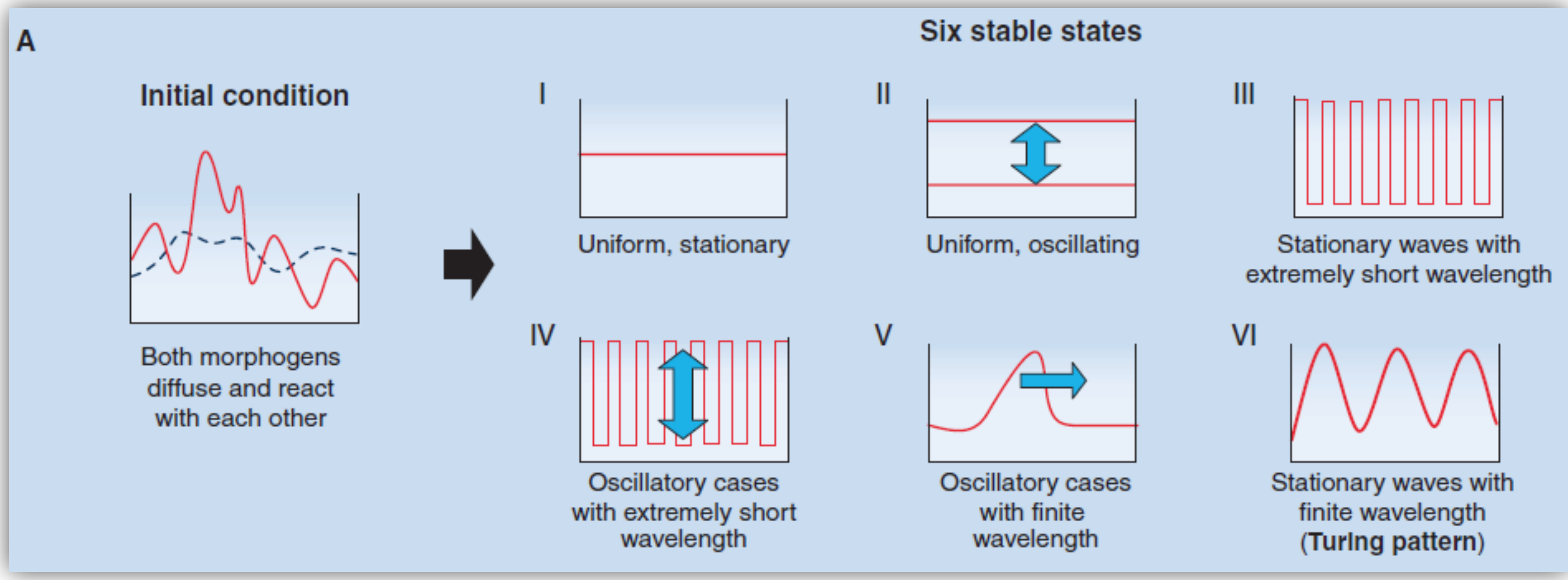
$$G(u,v) - d_v v = a_v u + b_v v + c_v$$

Useful read:
Science (2010) ***329****, p1616-p1620*
also read the supplementary online material and play with the JAVA program

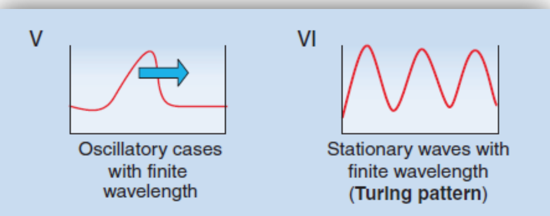
The Reaction-Diffusion Equation

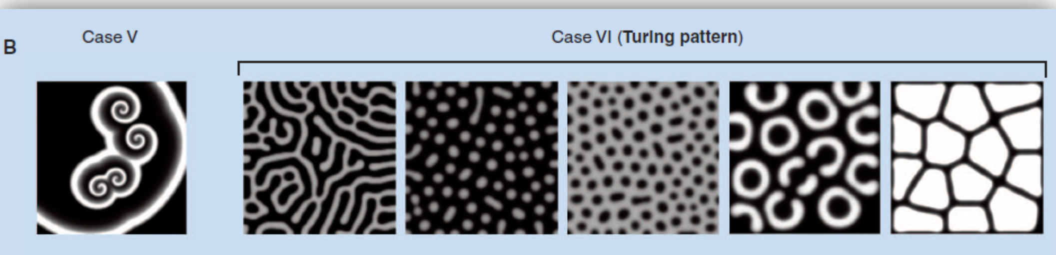
Turing found that if you solved these differential equations the system can take on six stable states depending on the parameters you use.

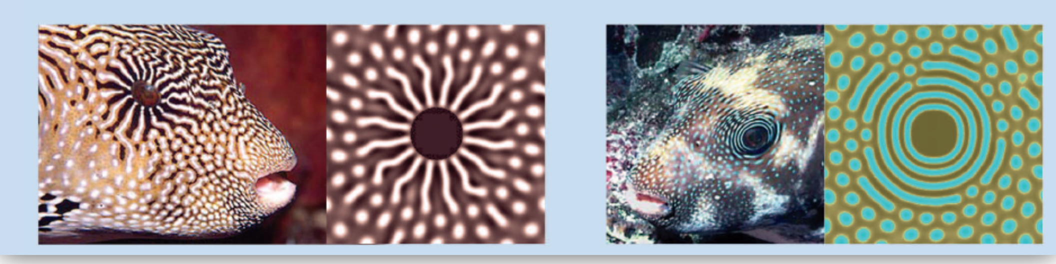
$$F(u,v) - d_u u = a_u u + b_u v + c_u$$
$$G(u,v) - d_v v = a_v u + b_v v + c_v$$



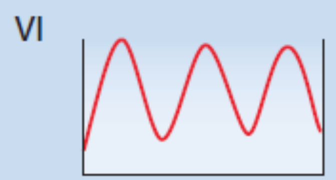
The Reaction-Diffusion Equation



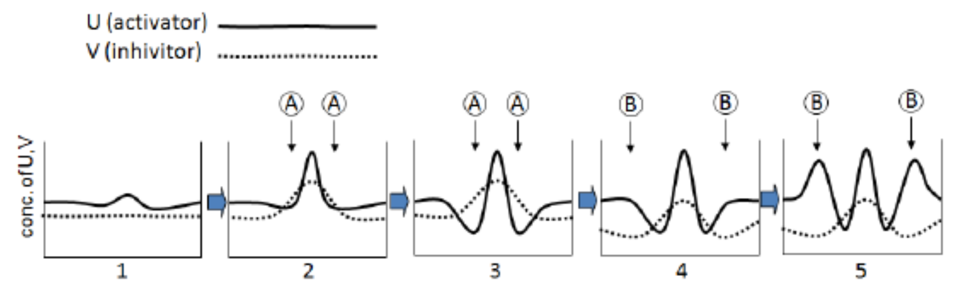




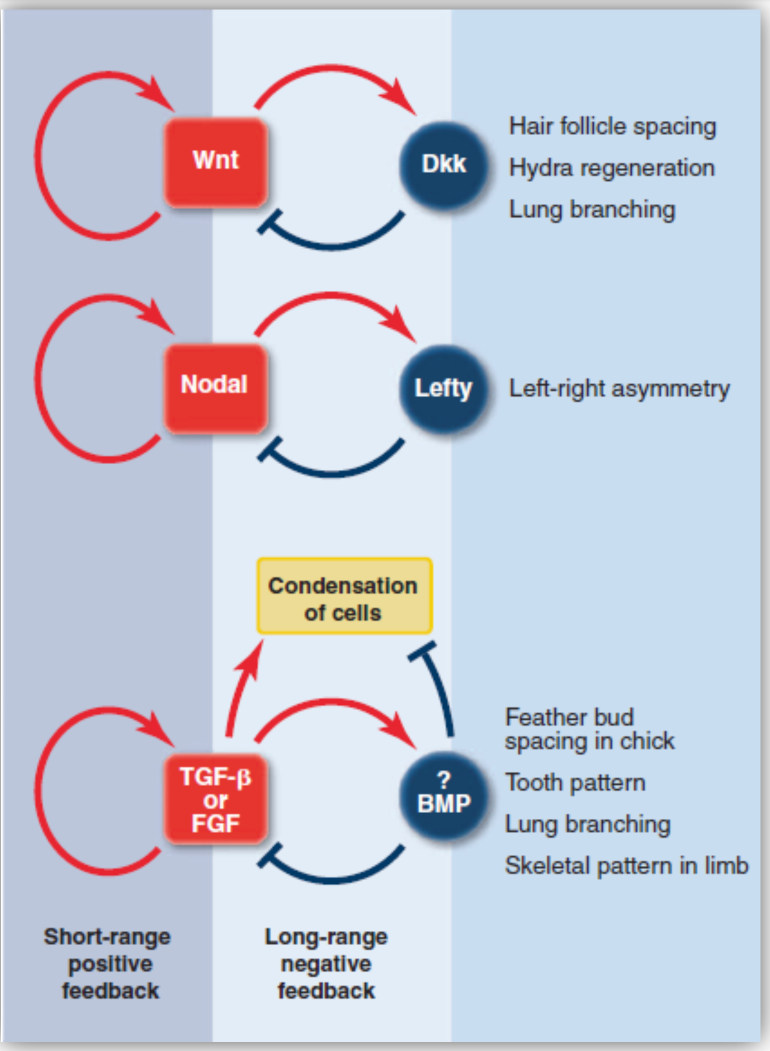
Turing patterns in biology



Stationary waves with
finite wavelength
(Turing pattern)

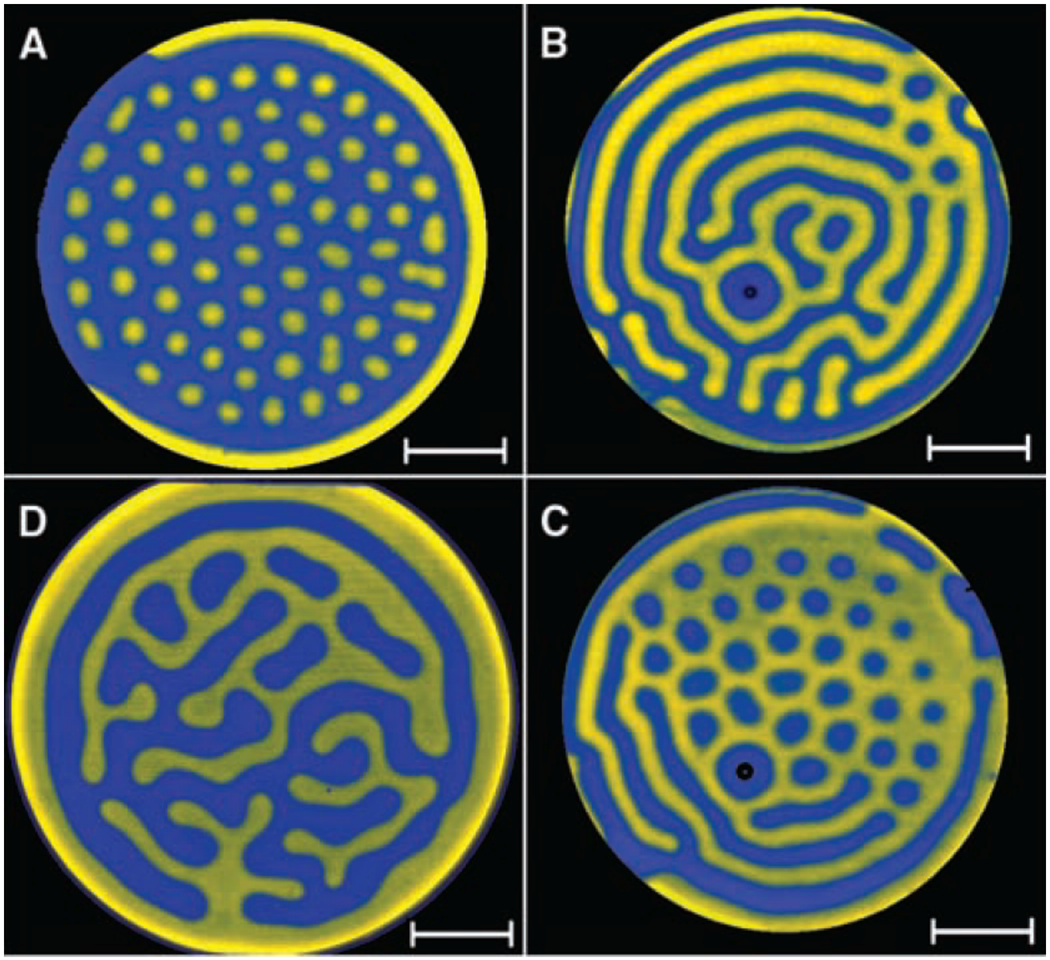


Requires:
Initial stochasticity
Short-range positive feedback
Long-range negative feedback



How close are we to engineering Turing patterns?

Chemical Turing patterns have been synthesised

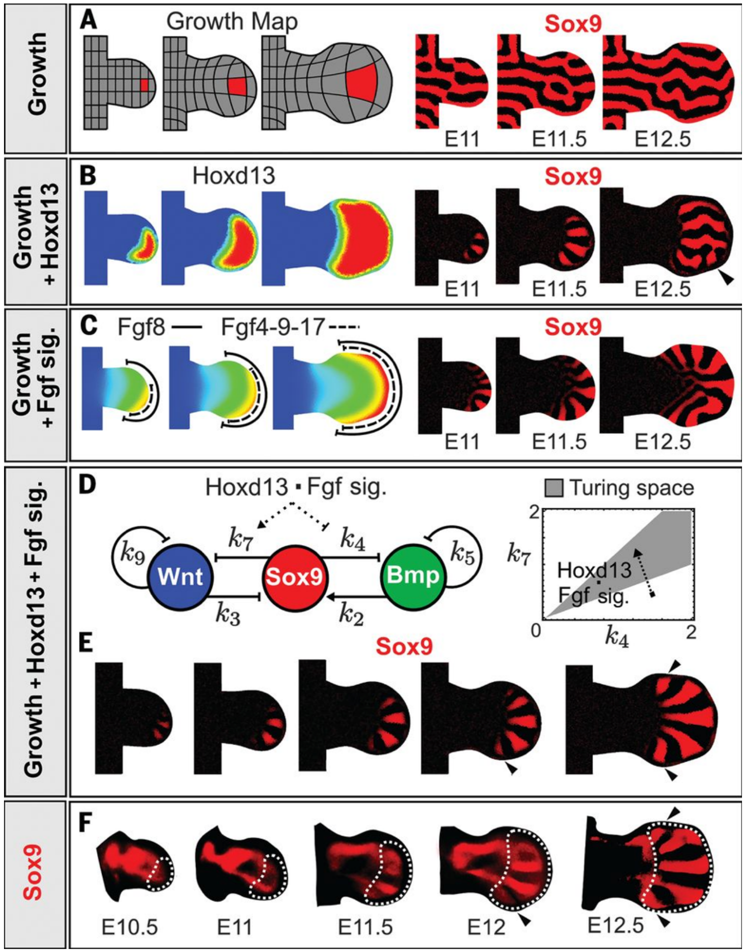


$$IO_3^- + 3HSO_3^- \rightarrow 3SO_4^{2-} + I^- + 3H^+ \quad (1)$$

$$IO_3^- + 6H^+ + 6Tu \rightarrow I^- + 3Tu_2^{2+} + 3H_2O \quad (2)$$

Science **324**, 772-775, 2009

Despite underlying many biological processes, no-one has yet built biological Turing patterns *ab initio*

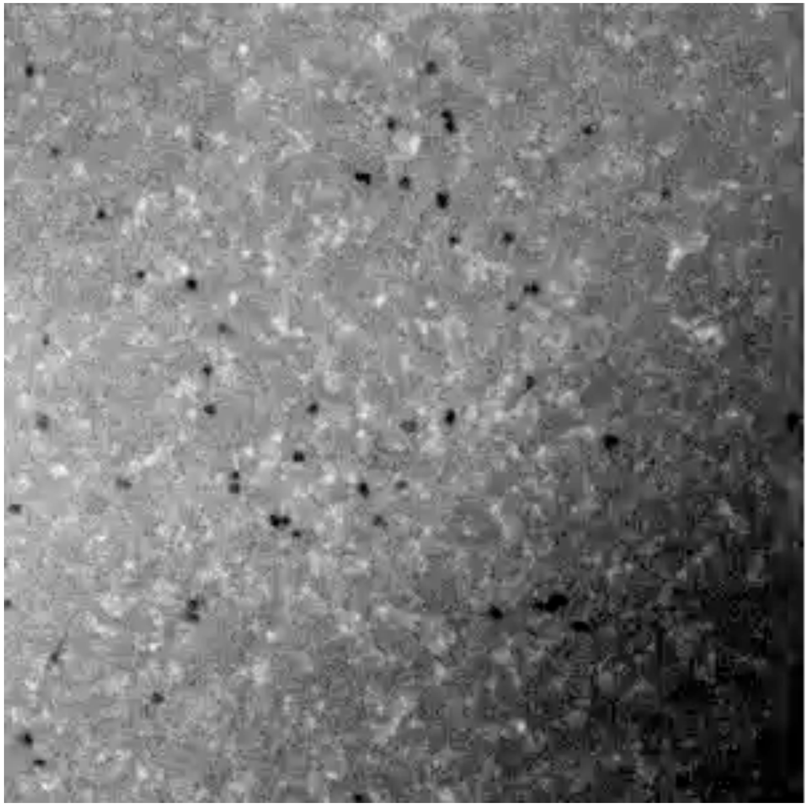


SELF-CORRECTION

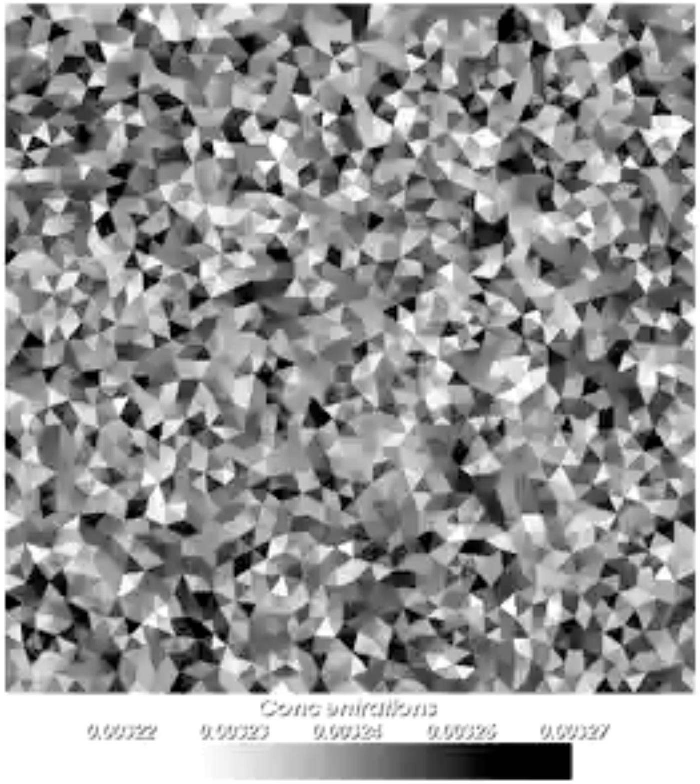
bigger field size: more repeats

Science **345**, 566-570, 2014

Sox9-BMP look like a Turing system

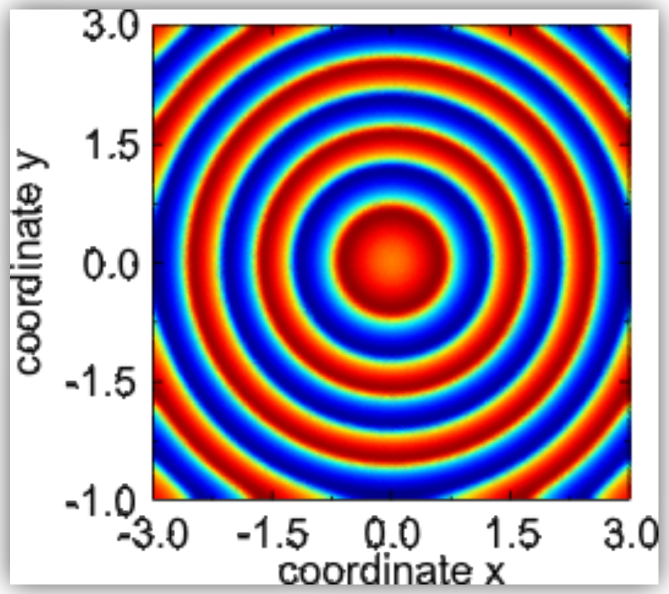


sox9-GFP micromass culture
(cells from mouse limb bud)



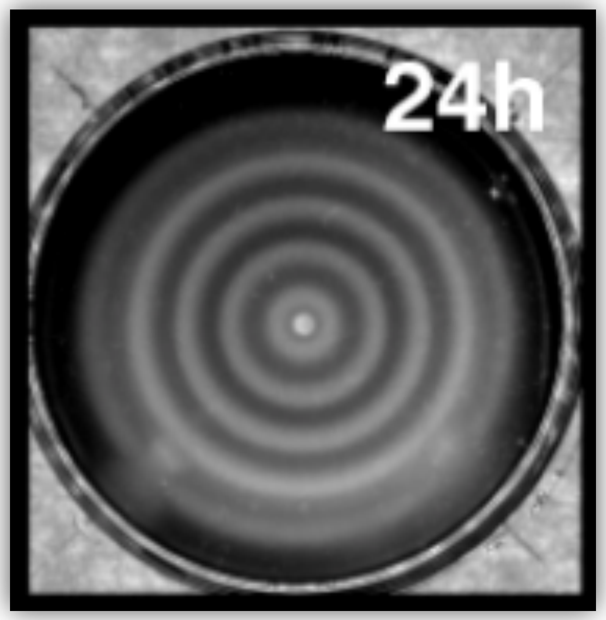
Science **345**, 566-570, 2014

Synthetic Biology Patterning



Simulated Turing Pattern
(FitzHugh–Nagumo type)

$$
\begin{aligned}
\partial_t u &= d_u^2 \Delta u + \lambda u - u^3 - v + \kappa, \\
\tau \partial_t v &= d_v^2 \Delta v + u - v
\end{aligned}
$$



Engineered *E. coli* growth pattern

iHKU iGEM team 2008
Chenli Liu *et al.* Science 2011

iHKU iGEM 2008

“Formation of New Patterns by Programmed Cell Motility”

‘Diffusion’ = Cell motility (swimming bacteria)

‘Reaction’ = Stop swimming when in high-density

Engineering requirements:

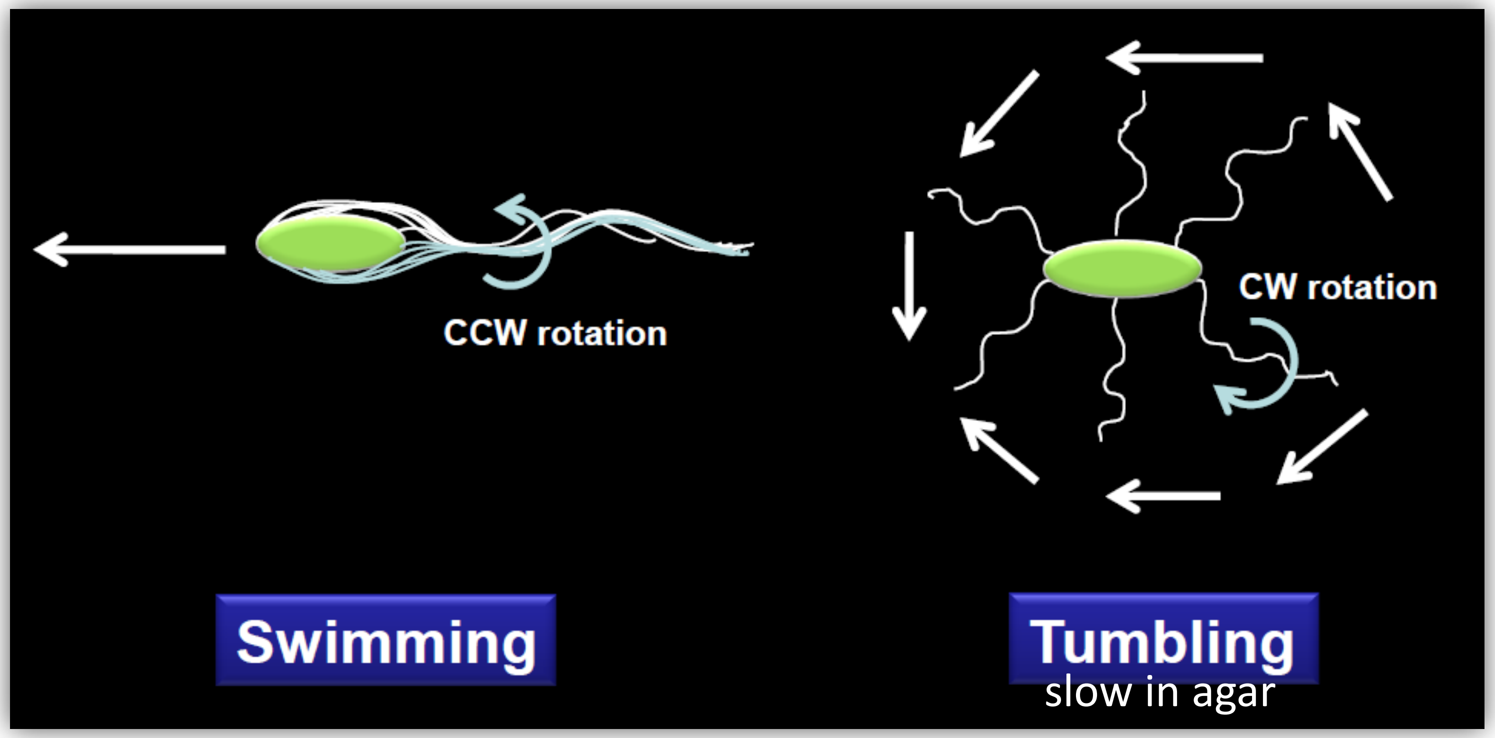
1. Control over cell motility
2. Ability to sense cell density
3. Methods for tuning key parameters

Practical requirements:

1. Low-density agar plates for swimming
2. Time-lapse imaging

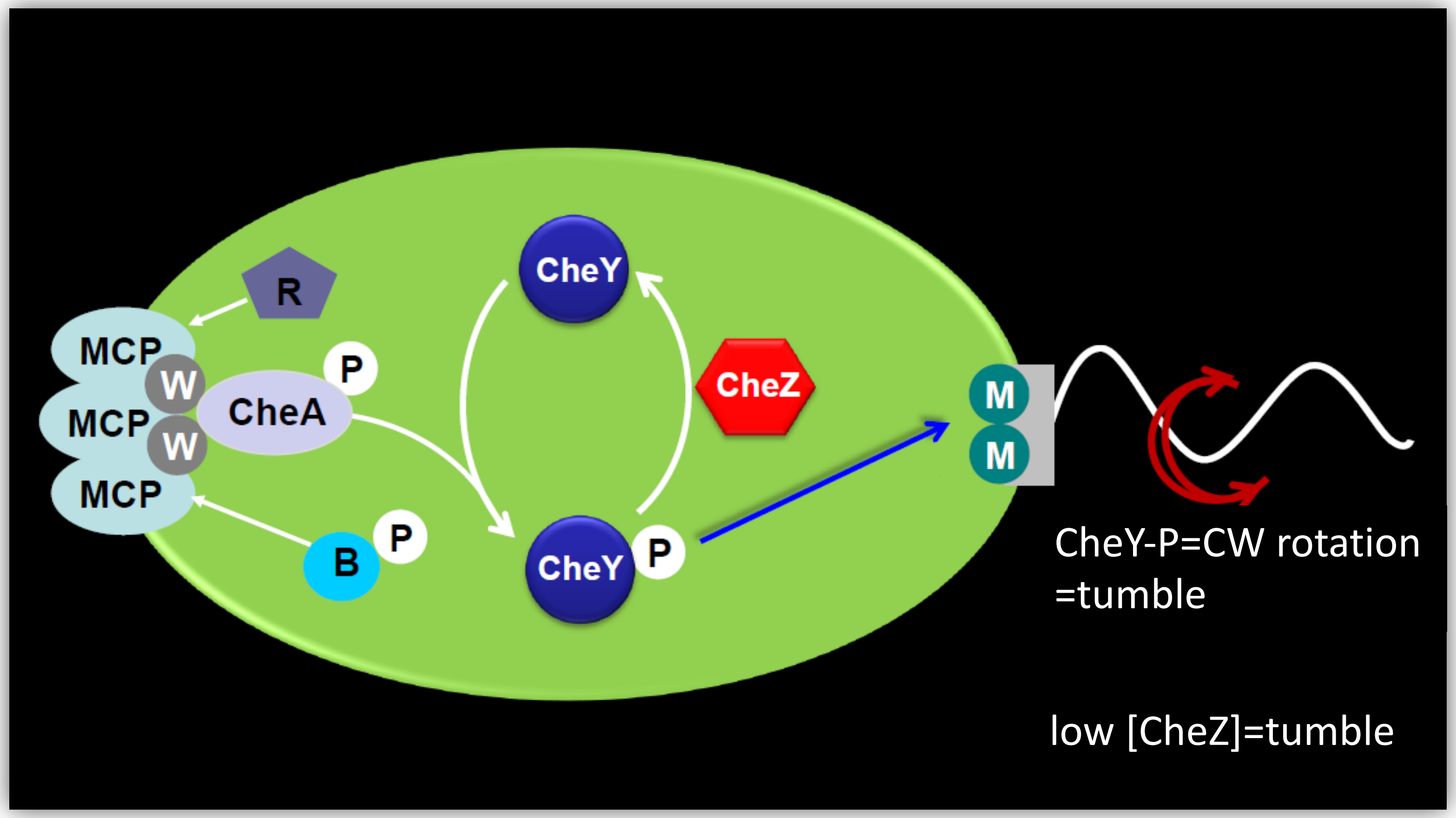
iHKU iGEM 2008

Motility of *E. coli*



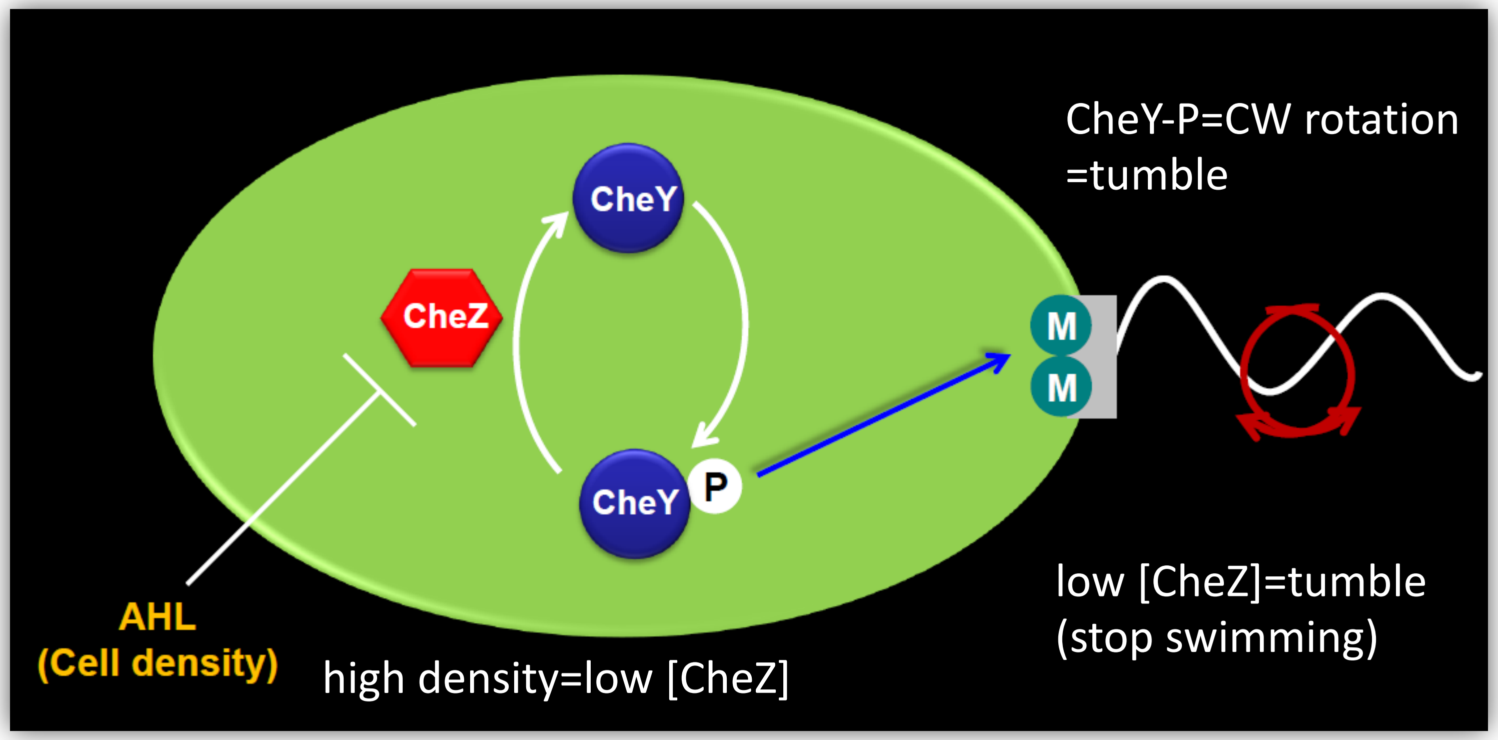
iHKU iGEM 2008

Mechanism of motility in *E. coli*



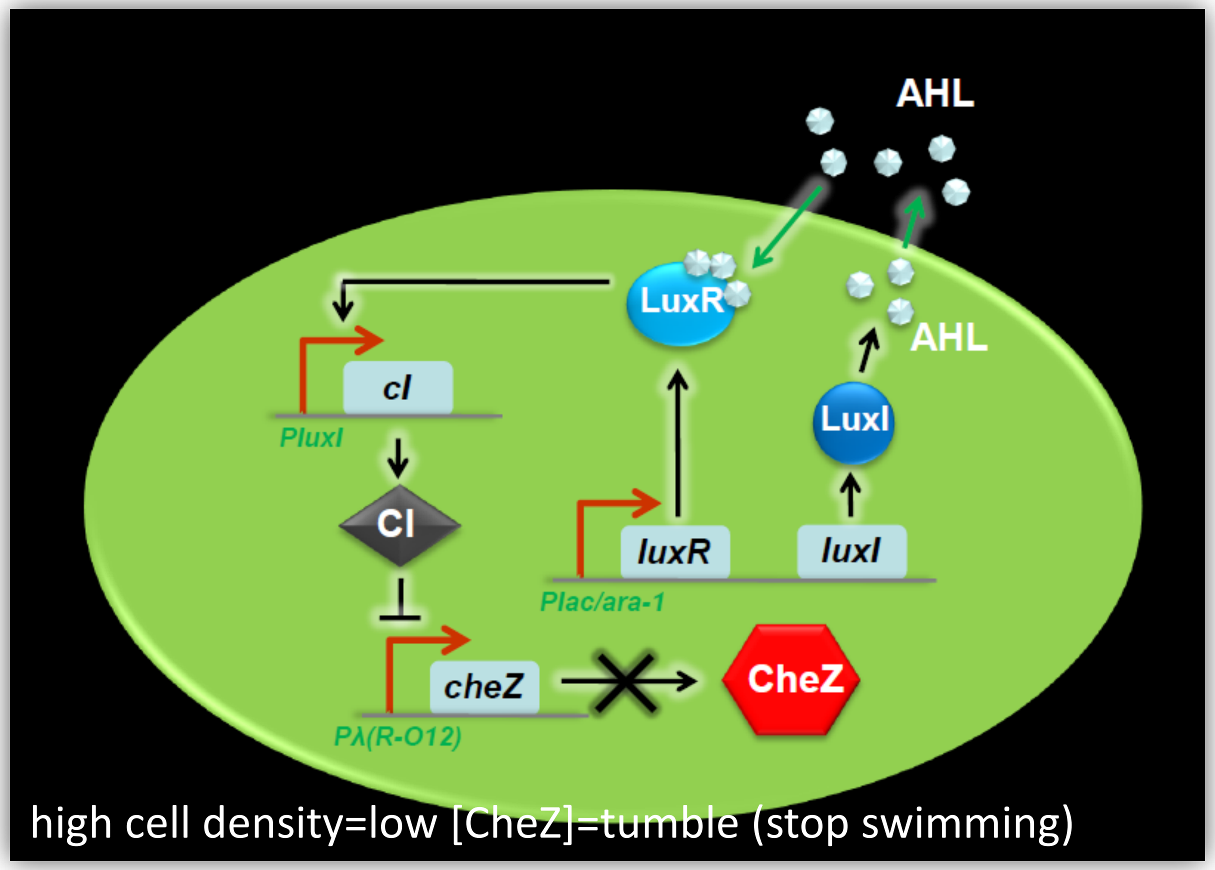
iHKU iGEM 2008

Synthetic control of CheZ = control of motility in *E. coli*



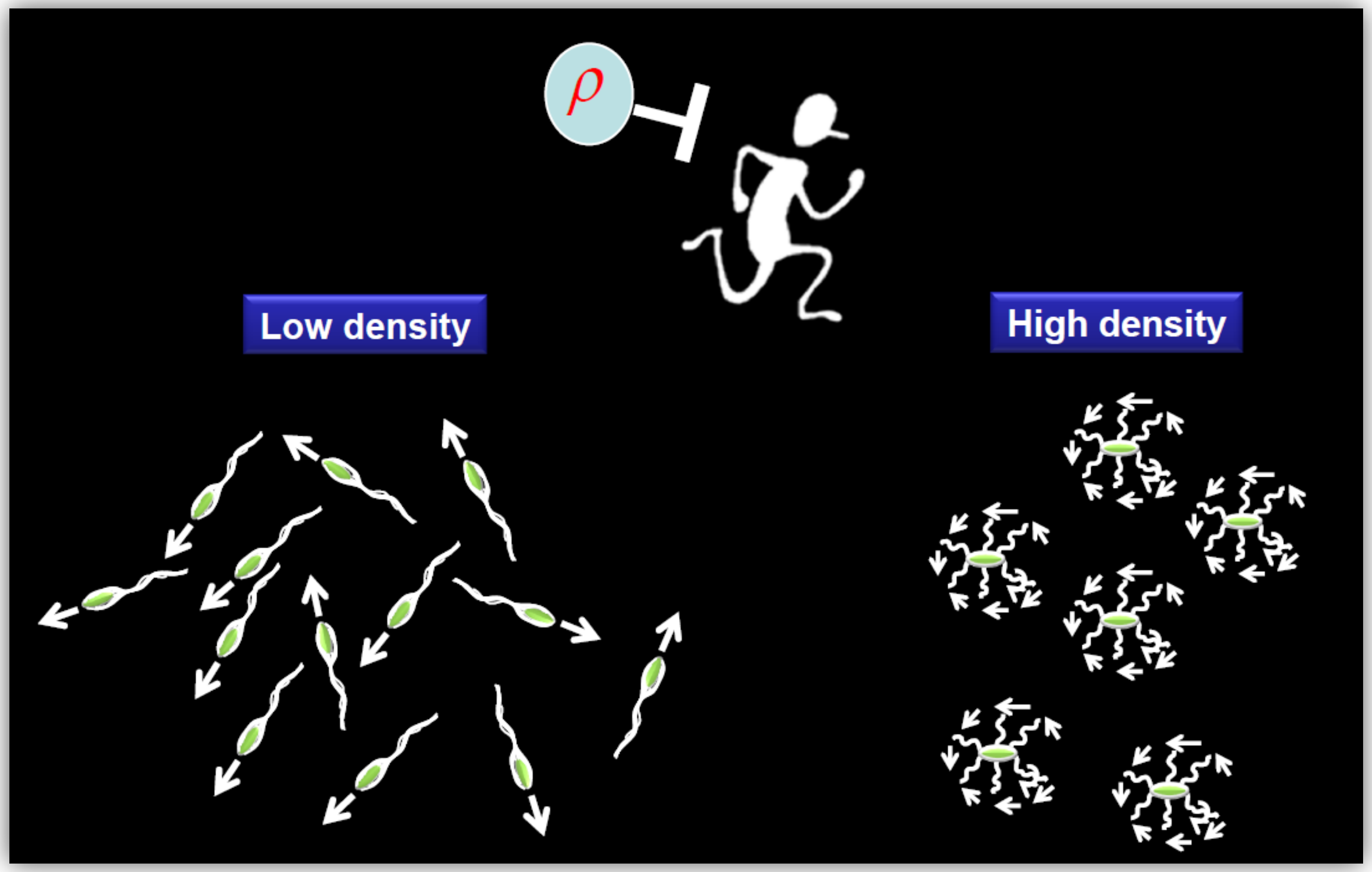
iHKU iGEM 2008

Density-dependent control of CheZ



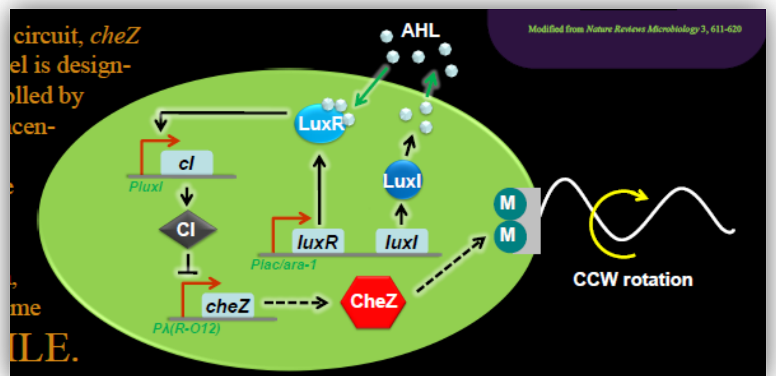
iHKU iGEM 2008

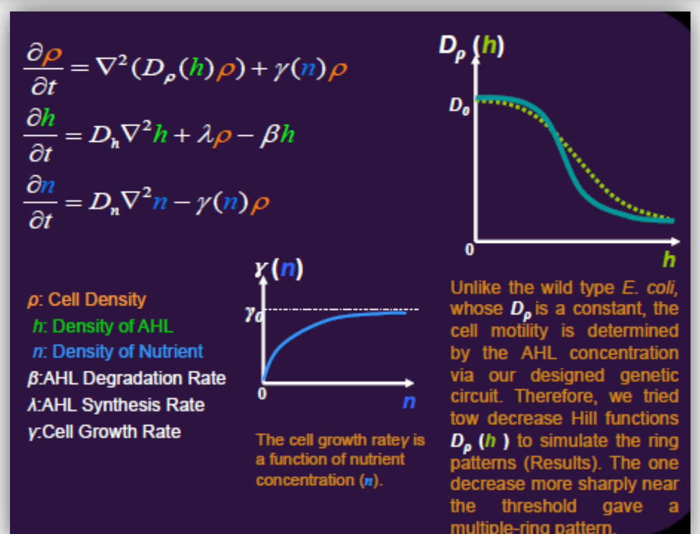
Creation of a low-density mover

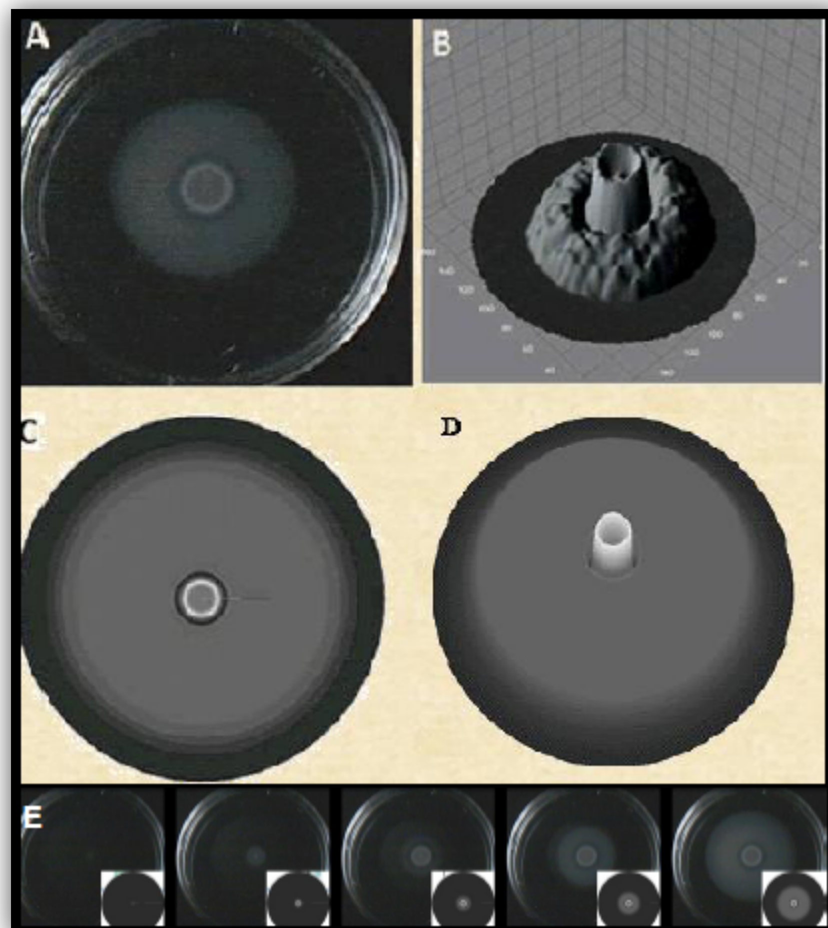


iHKU iGEM 2008

Design, Model and Some Results – but only a Bronze

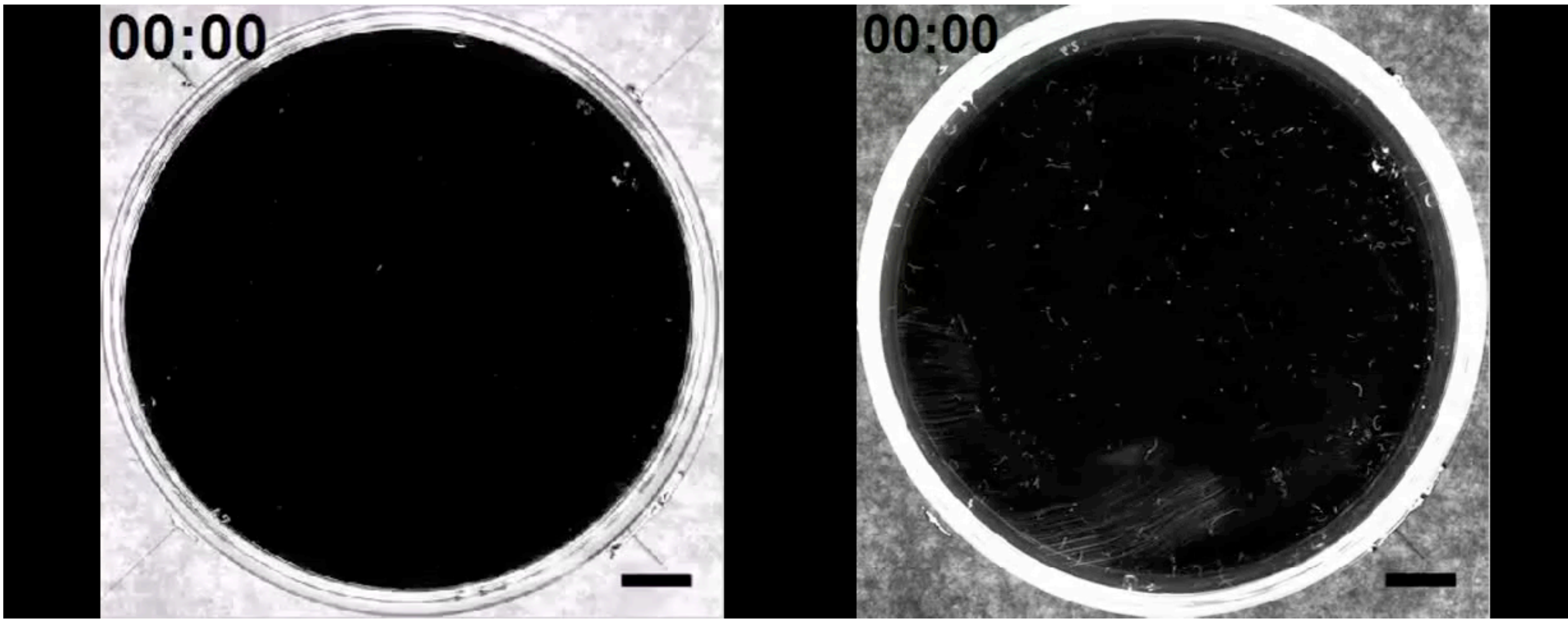






3 years later.....

Video S1,S2 from Chenli Liu et al. Science 2011



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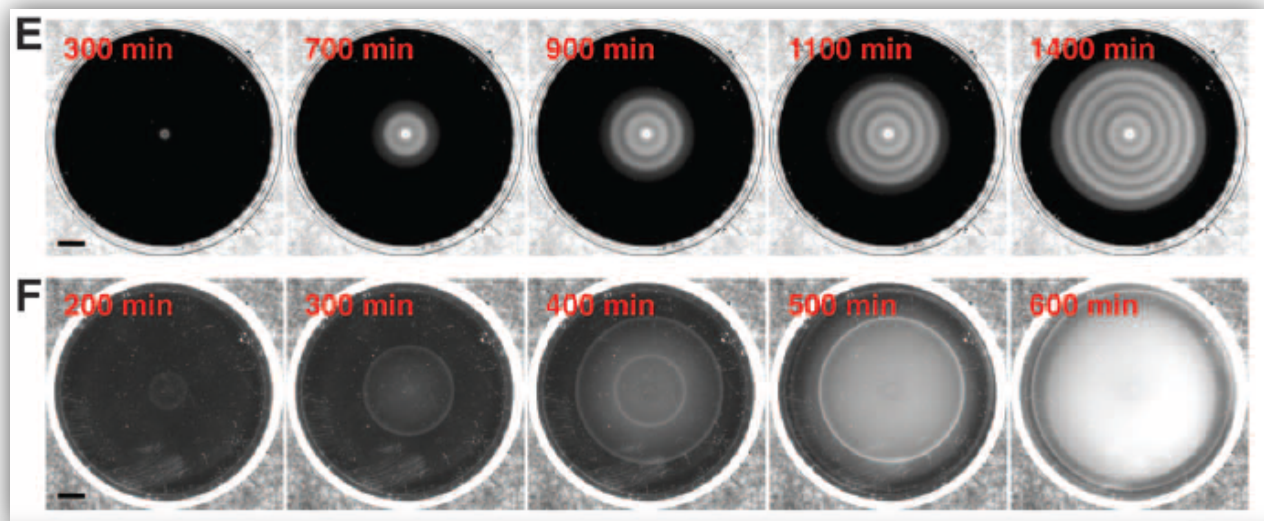
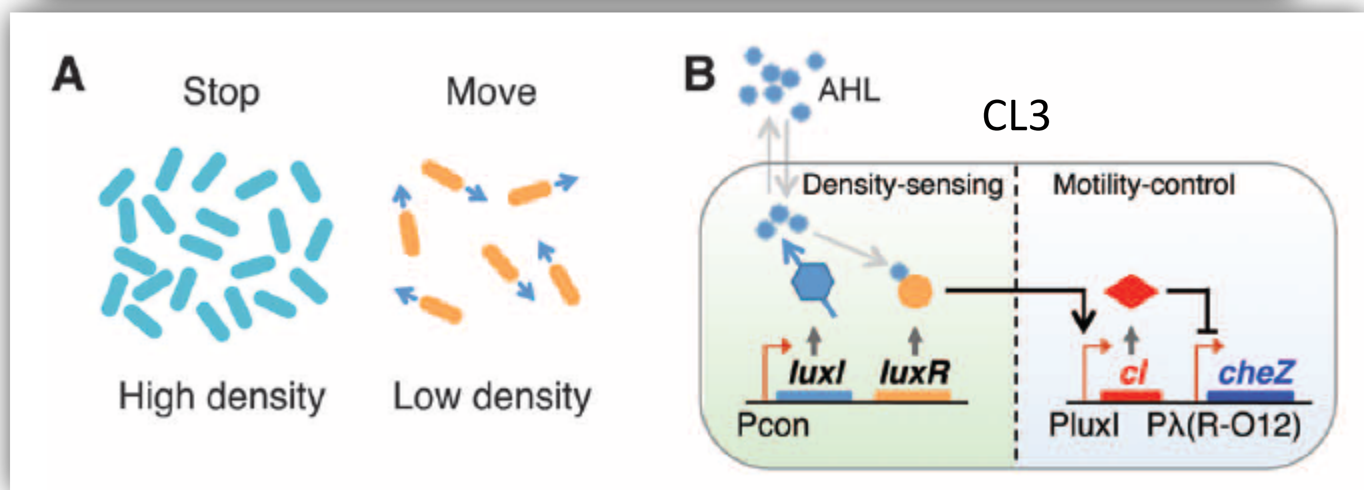


CL3
(engineered strain)

CL4
(control strain: wt+density sensing module)



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Model of the system

(shown here is the updated 2011 model)

3 partial differential equations

$$\frac{\partial \rho}{\partial t} = \nabla^2[\mu(h)\rho] + \frac{\gamma n^2 \rho}{n^2 + K_n^2} \qquad [1]$$

$$\frac{\partial h}{\partial t} = D_h \nabla^2 h + \alpha\rho - \beta h \qquad [2]$$

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \qquad [3]$$

1. Stochastic swim-and-tumble motion of cells described as a diffusion equation at population level for the cell density ρ(*x*, *t*) (version of Fisher's equation)
2. Synthesis, diffusion and turnover of AHL h(*x*, *t*)
3. Consumption and diffusion of nutrient n(*x*, *t*)

α = AHL synthesis rate β = AHL degradation rate
γ = cell growth rate

μ(h) = AHL-dependent motility

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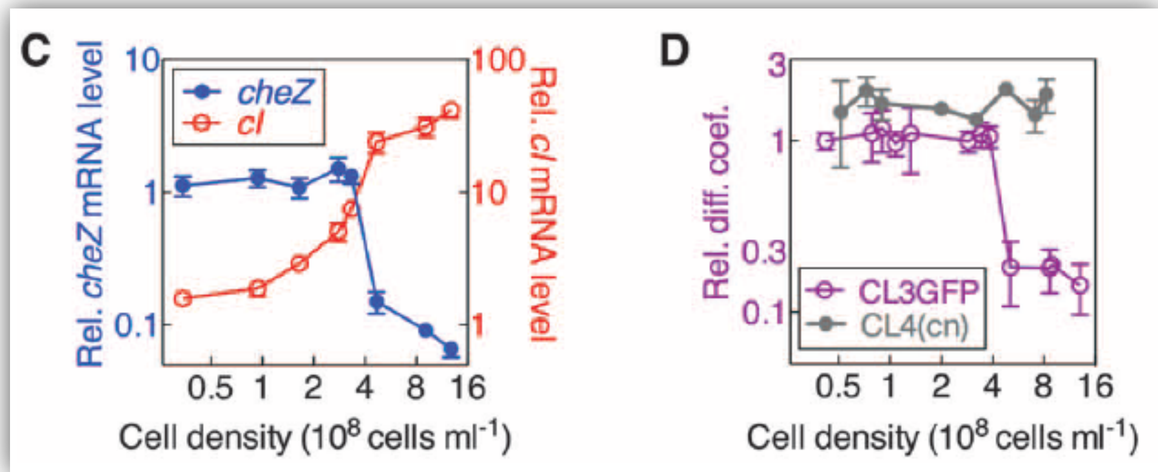
Model of the system

Getting the parameters

- Q-RTPCR to measure mRNA expression
- Track diffusion of cells using microscopy
- Dilute cells to different cell densities

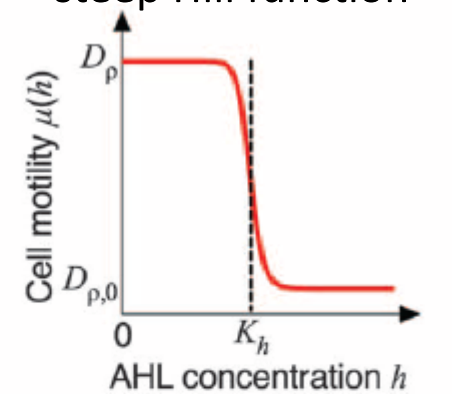


$$\frac{\partial \rho}{\partial t} = \nabla^2[\mu(h)\rho] + \frac{\gamma n^2 \rho}{n^2 + K_n^2} \quad [1]$$

$$\frac{\partial h}{\partial t} = D_h \nabla^2 h + \alpha\rho - \beta h \quad [2]$$

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \quad [3]$$

steep Hill function



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Model of the system

Getting the parameters

$$\frac{\partial \rho}{\partial t} = \nabla^2[\mu(h)\rho] + \frac{\gamma n^2 \rho}{n^2 + K_n^2} \quad [1]$$

$$\frac{\partial h}{\partial t} = D_h \nabla^2 h + \alpha\rho - \beta h \quad [2]$$

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \quad [3]$$

Table S3 The parameters most used in the simulation

Parameters	Value	Comments
D_ρ	450 μm^2 s^{-1}	The normal value (200-1,000 μm^2 s^{-1}) (*49-51*)
$D_{\rho,0}$	10 μm^2 s^{-1}	Fig. 1D shows it is almost zero
D_h	400 μm^2 s^{-1}	As small molecular diffusion (100-1,000 μm^2 s^{-1}) (*56, 57*)
D_n	800 μm^2 s^{-1}	As small molecular diffusion (100-1,000 μm^2 s^{-1}) (*56, 57*)
γ	0.7 h^{-1}	Measured shown in Fig. S16
β	1.04 h^{-1}	AHL half-life ranging 10~1,000 min (*39, 59*)
m	20	Fig. 1D shows an abrupt fall of the cell motility
n (t=0)	15×10^8 cells ml^{-1}	The saturated density cell as shown in Fig. S16
k_n	1	Rescaled with n to the unit of ρ
K_n	10^9 cells ml^{-1}	Estimated from Fig. S16
K_h	4×10^8 cells ml^{-1}	Estimated from Fig. 1D

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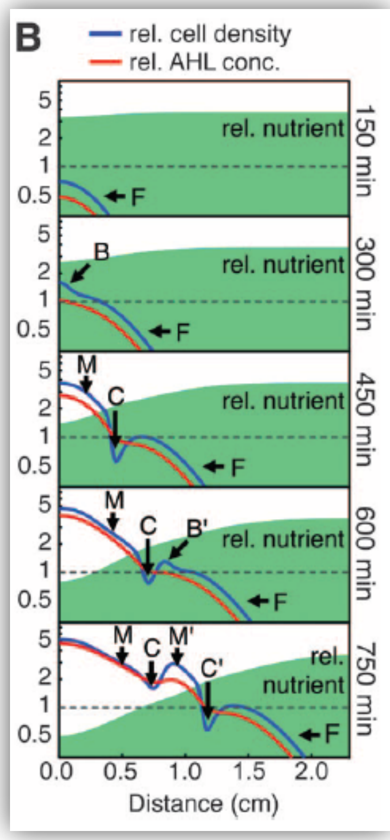
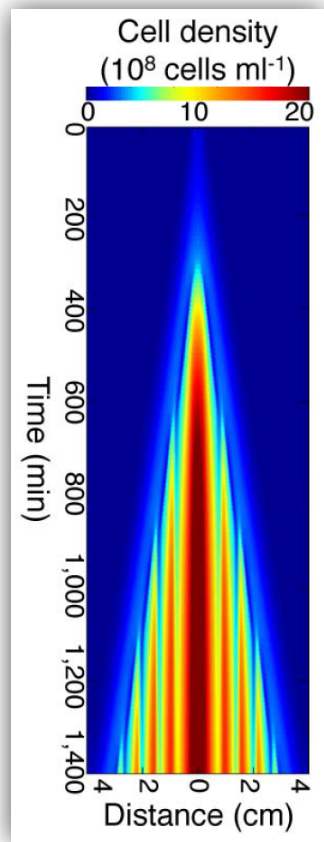
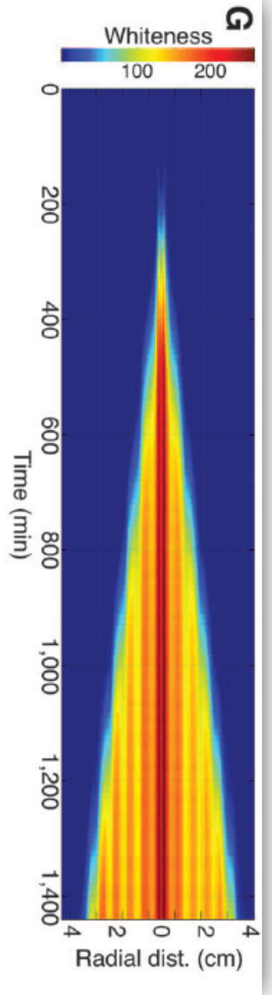


Model



Simulation



Experiment

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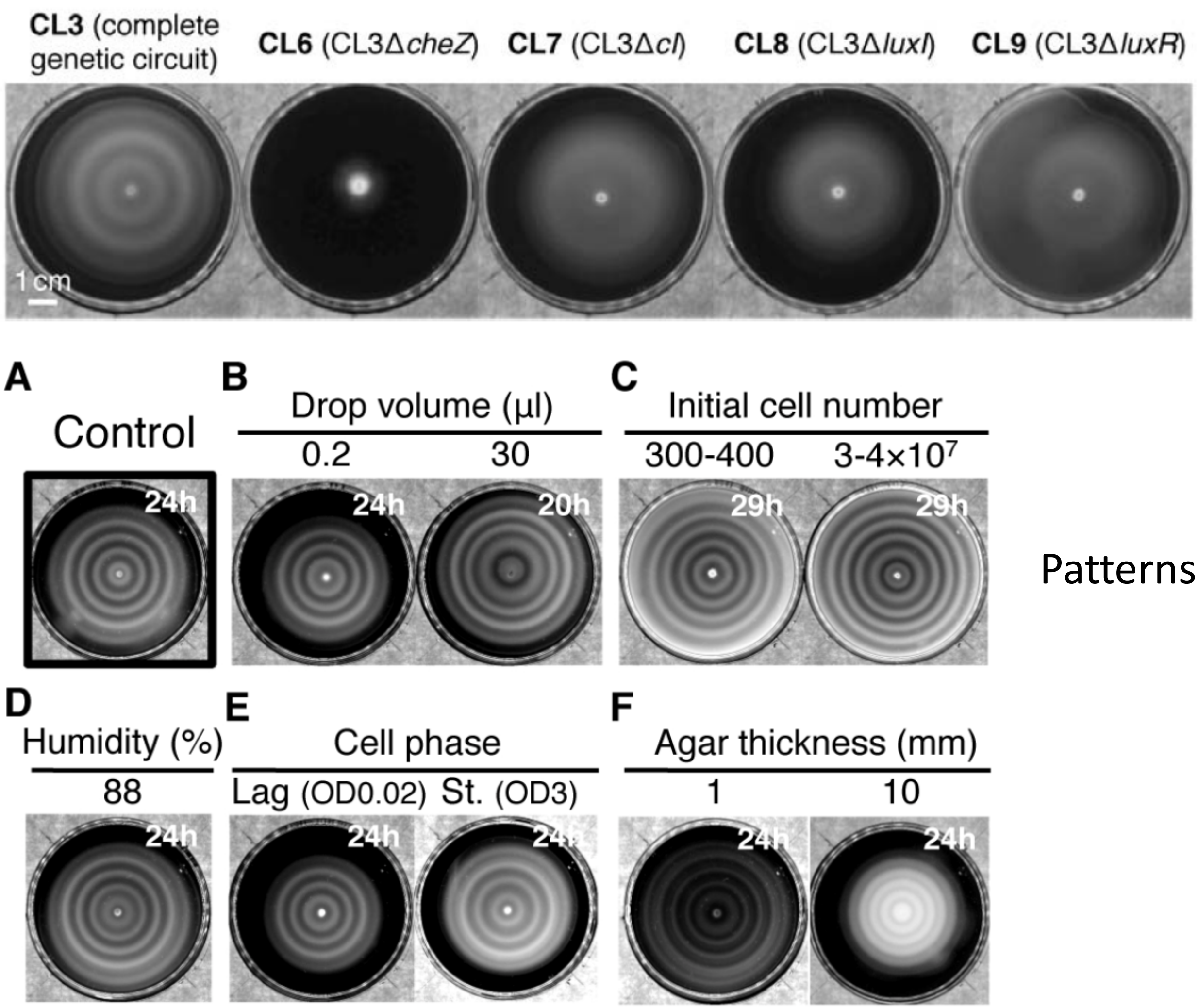
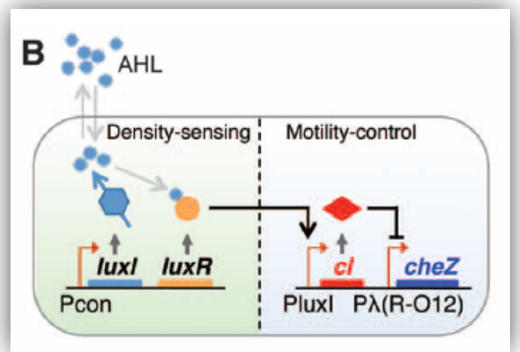
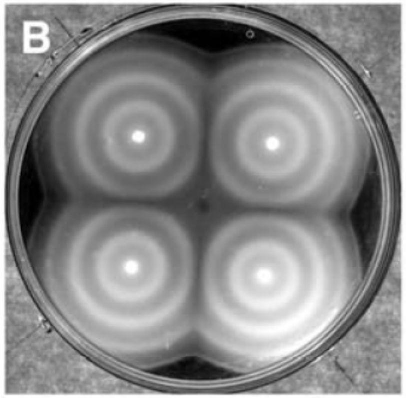


Only the complete system works



Patterns are robust



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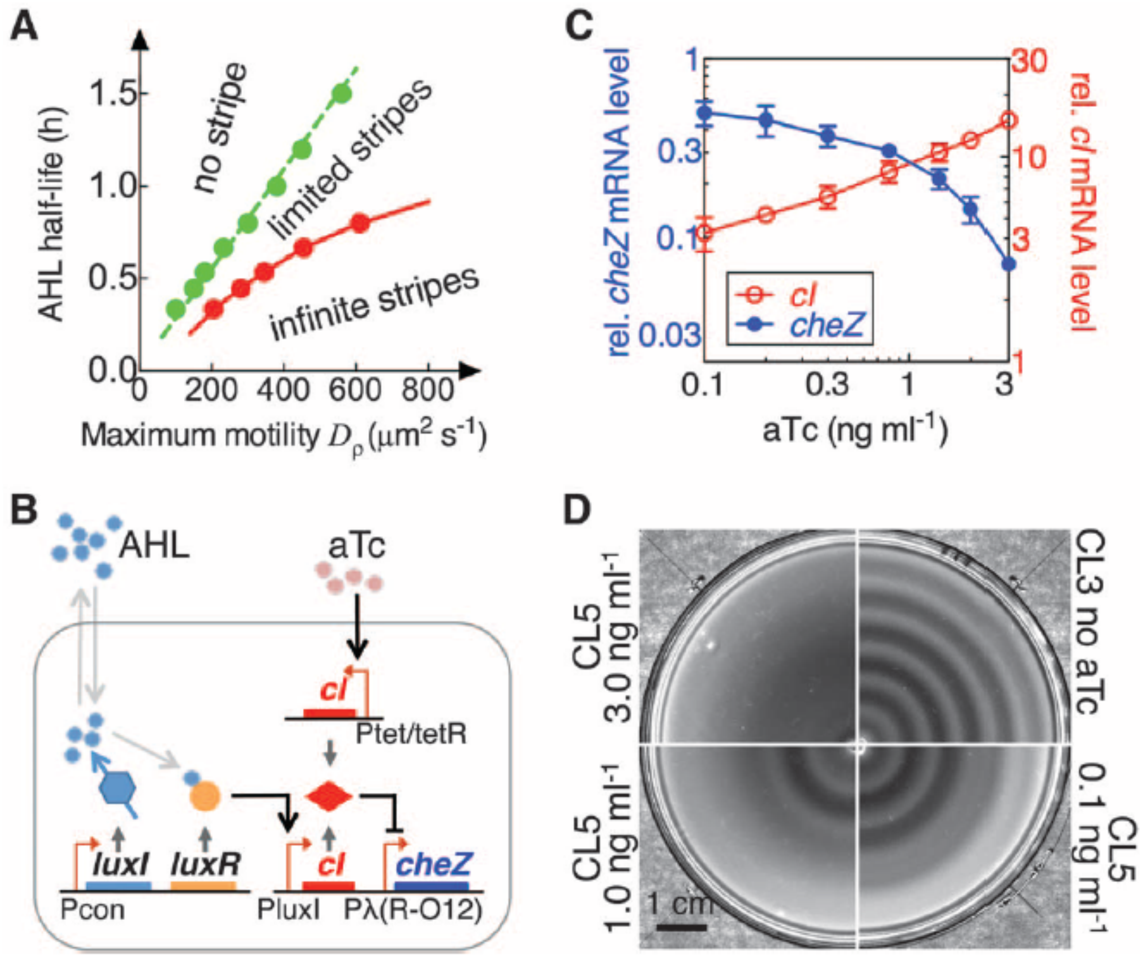
Model predicts that occurrence of stripes is dependent on two factors:

1. AHL half-life
2. Maximum motility

Synthetic biology allows these to be tested!

IMPORTANT:

- Not a Turing pattern: *not generated by noise*
- But also not a clock



tunable with aTc

Advanced Synthetic Biology Applications: Pattern Formation

- **The Gradient detection and Reaction Diffusion models are thought to underly much of biological pattern formation.**
- **Synthetic biologists have built several French flag-like systems, to test potential mechanisms.**
- **No one has built a Turing system from first principles (yet).**

Suggested reading

Review:

Kondo et al. Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation
Science (2010) **329**, p1616-p1620

Morphogen gradients and synthetic stripes

Isalan M. et al. (2005) Engineering Gene Networks to Emulate *Drosophila* Embryonic Pattern Formation. *PLoS Biol* **3(3)**: e64. doi:10.1371/journal.pbio.0030064

Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R. A synthetic multicelluar system for programmed pattern formation Nature. 2005;434:1130-4.

Schaerli, Y. et al. A unified design space of synthetic stripe-forming networks. *Nat. Commun*. **5**:4905 doi: 10.1038/ncomms5905 (2014).

Chenli Liu et al. Sequential Establishment of Stripe Patterns in an Expanding Cell Population *Science*. 2011 Oct 14;**334**(6053):238-41