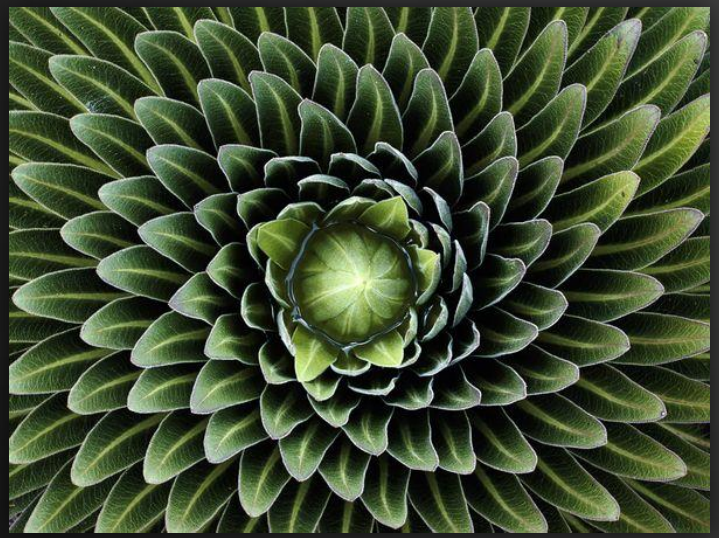
Advanced Synthetic Biology

Applications

Pattern Formation

Biological Patterns



Biological Patterns

Biological Patterns

Lecture Content

In this lecture we'll learn about:

1. What is morphogenesis
2. The reaction diffusion equation
3. A JAVA simulation of the reaction diffusion equation
4. Theoretical systems that generate patterns – Turing Patterns

5. An iGEM project to generate bacteria that swim into patterns
6. A synthetic biology pattern using communicating cells

7. Potential applications of synthetic pattern formation

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

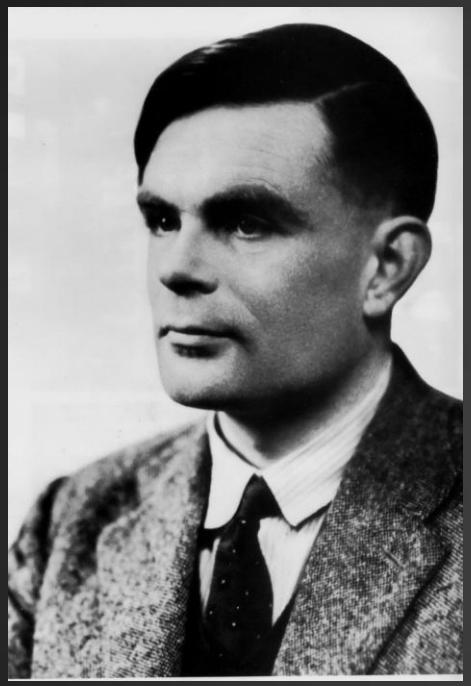
Pattern formation is mathematically important

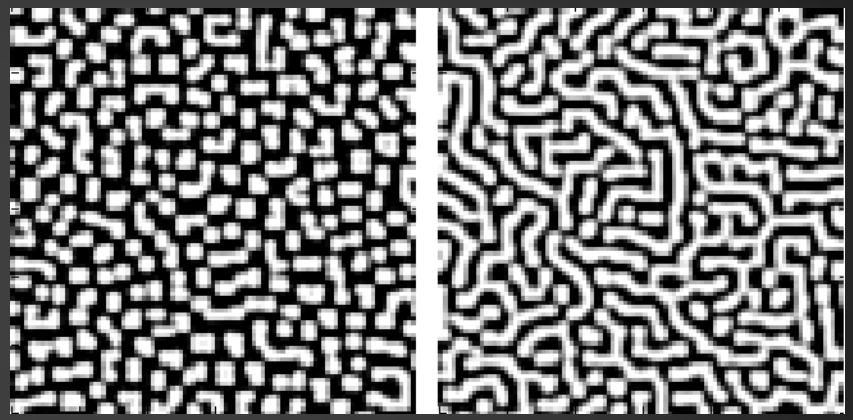
How is order formed from chaos?

- MORPHOGENESIS first explored by Alan Turing
- He initiated the nonlinear theory of biological growth

- First attempts to mathematically explain biology
- Effectively led to the topic of Chaos Theory

Pattern formation is order from chaos





Minor fluctuations give rise to sustained patterns

Pattern formation by morphogenesis

REVIEW

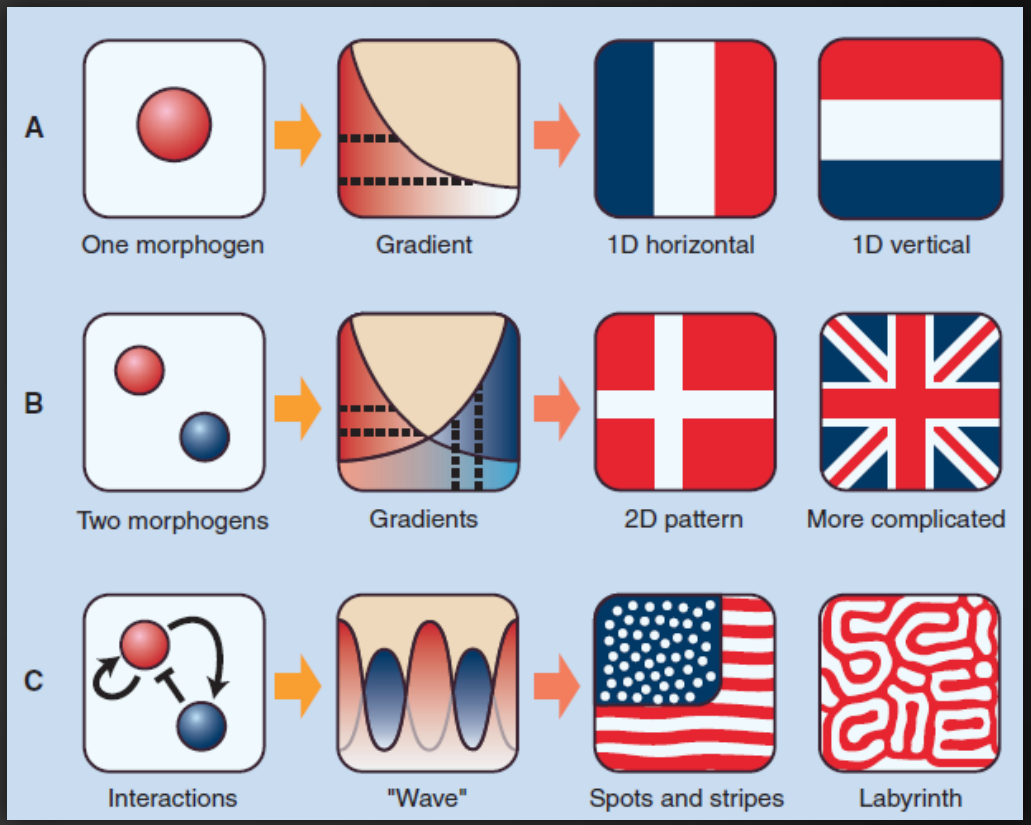
Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation

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

Essential read:
Science 2010 (v.329)

please also read the supplementary online material

And play with the JAVA program



The Reaction-Diffusion Equation

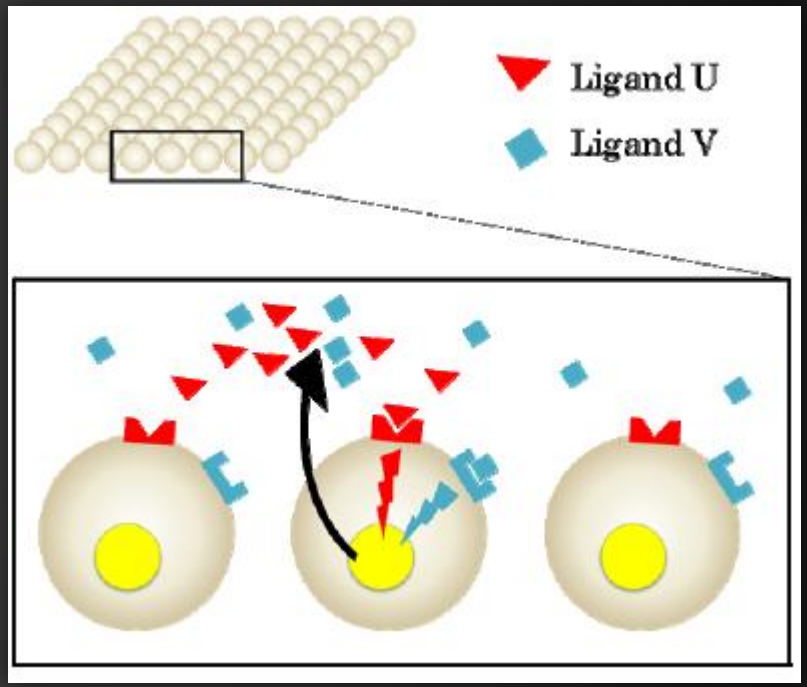
Two interacting molecules diffusing can generate stable patterns – Turing 1952

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

$$\frac{\partial v}{\partial t} = G(u,v) - d_v v + D_v \Delta v$$

Rate of concentration change | Production | Degradation | Diffusion

Reaction

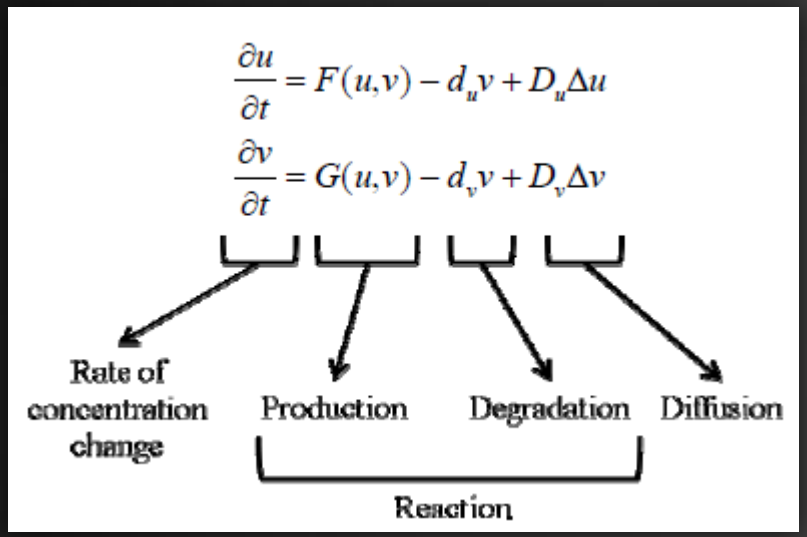


u and *v* are the local concentration of ligands U and V at each position.

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



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.

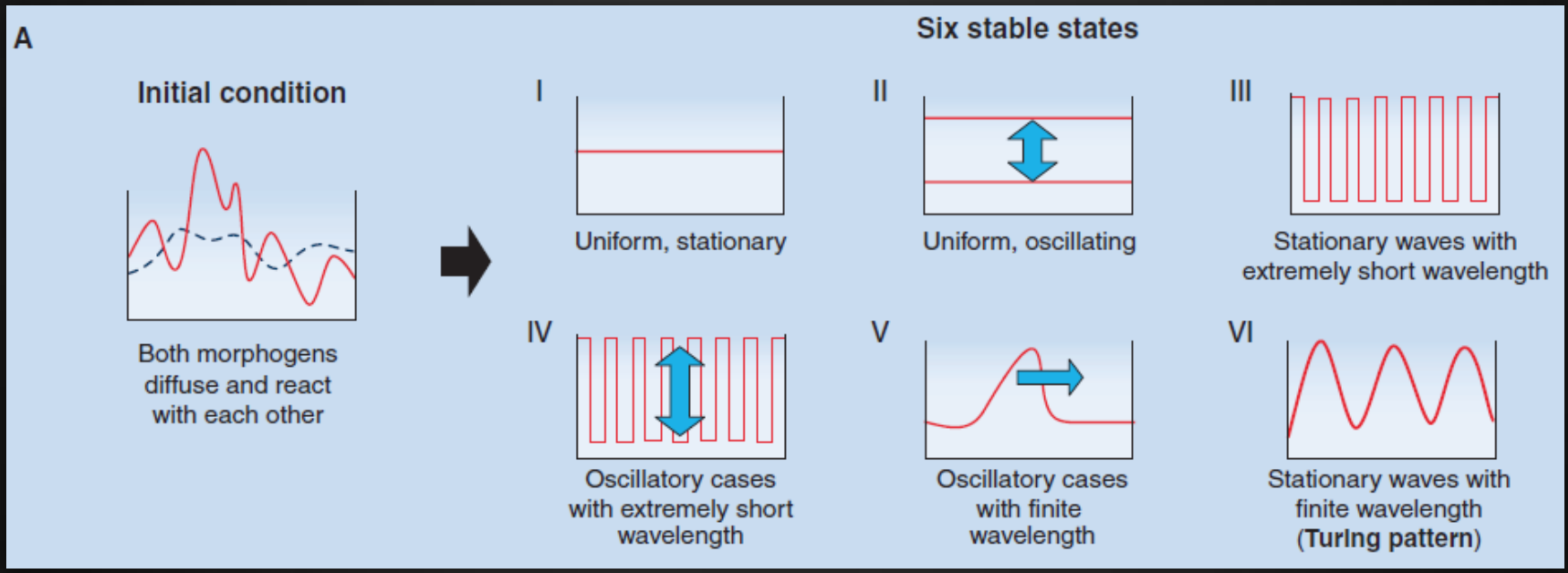
Replacing F and G by following linear function, we get the partial differential equation identical to that of Turing's original paper.

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

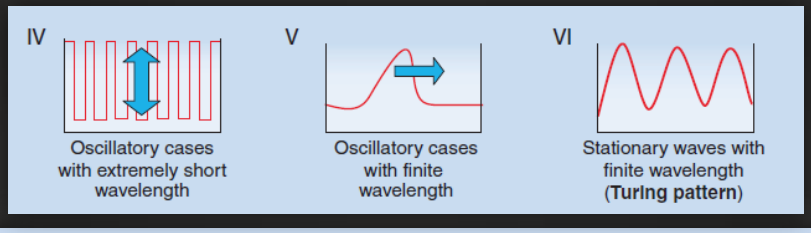
The Reaction-Diffusion Equation

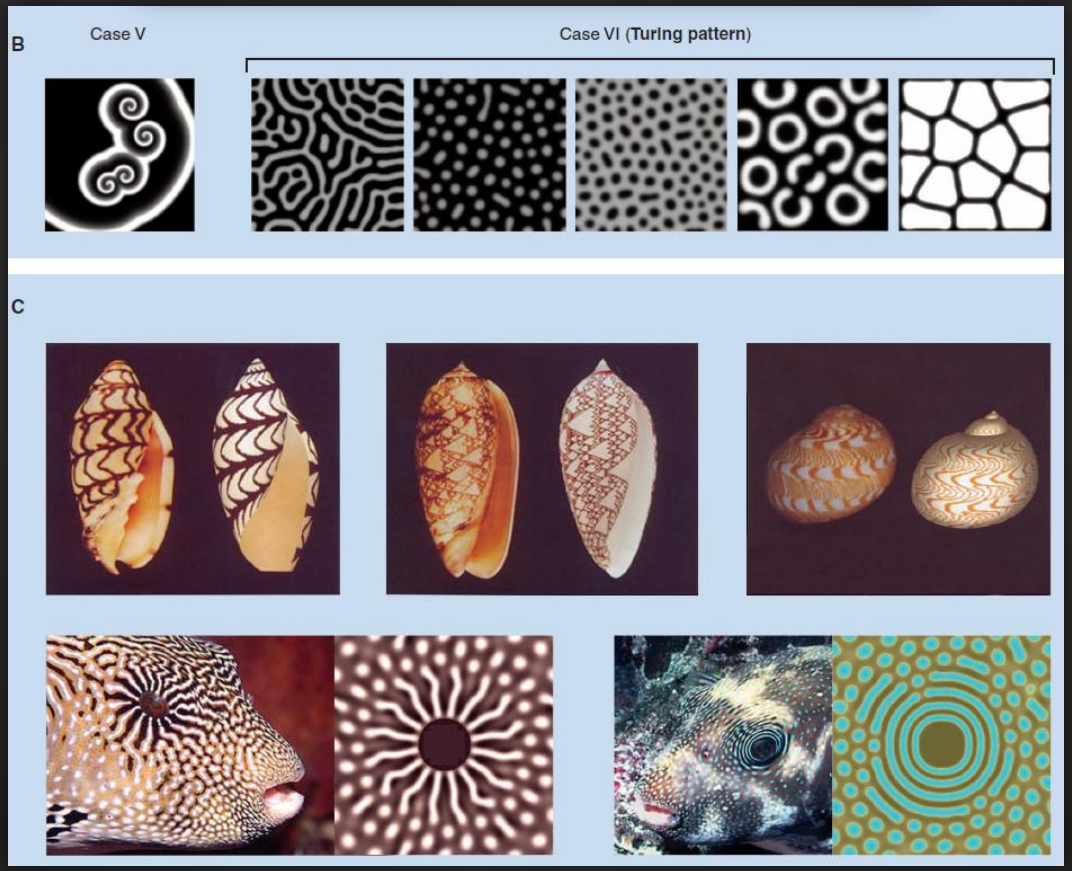
Turing found that if you solved these differential equations the system can take on six stable states depending on the parameters you use.

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



The Reaction-Diffusion Equation

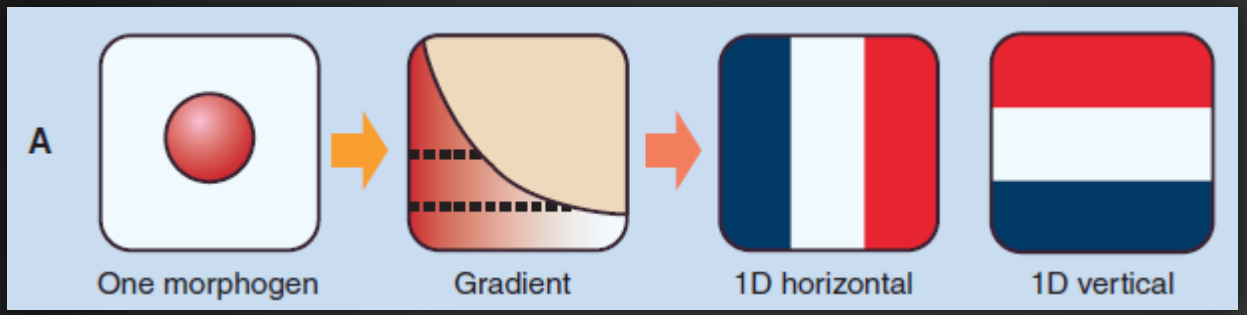


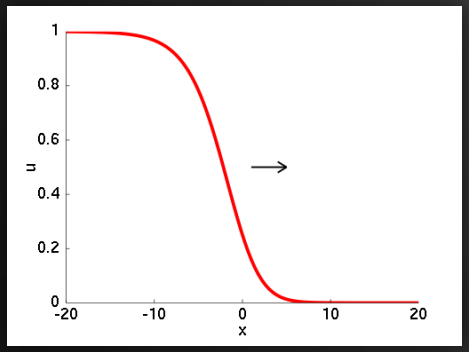


Minimal Mathematical Patterning

- One component systems are the simplest (effectively just diffusion equations)

Morphogen Gradient



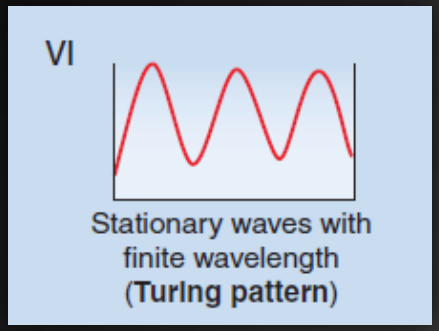


Fisher's Equation for simulation of propagation of a gene in a population

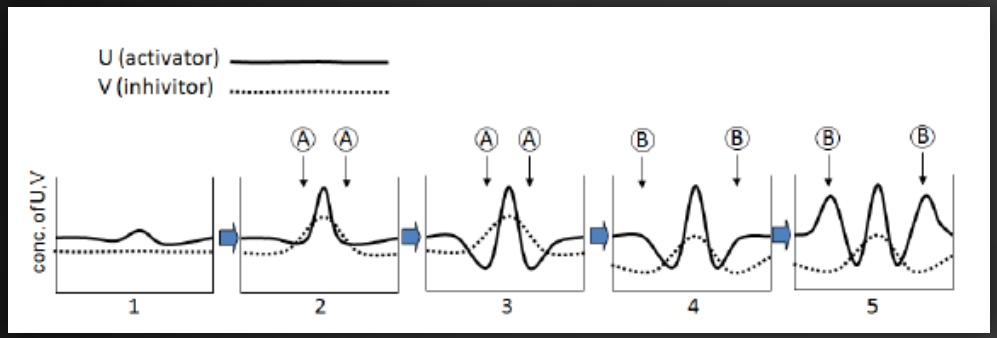
Generates a curved wave-front

$$\frac{\partial u}{\partial t} = \Delta u + f(u),$$

Minimal Mathematical Patterning

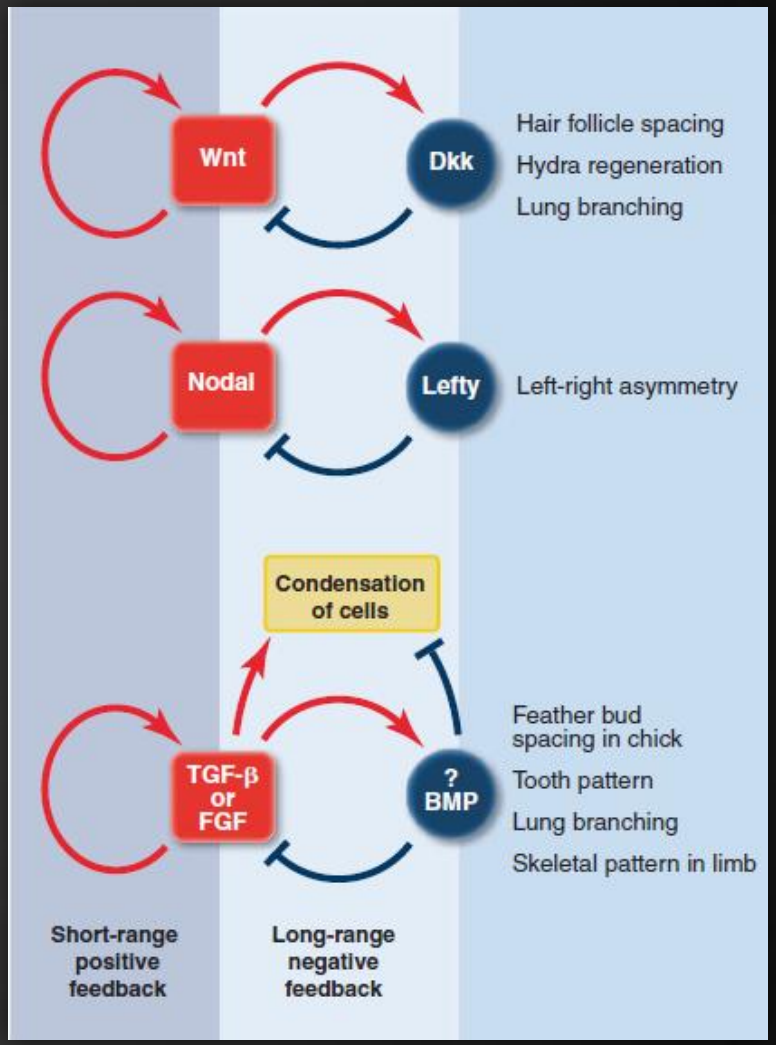


Two component system with feedback gives the most interesting case: Turing Pattern



Requires:

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



Minimal Mathematical Patterning

- Two-component systems give rise to complex patterns

Example: activator-inhibitor systems

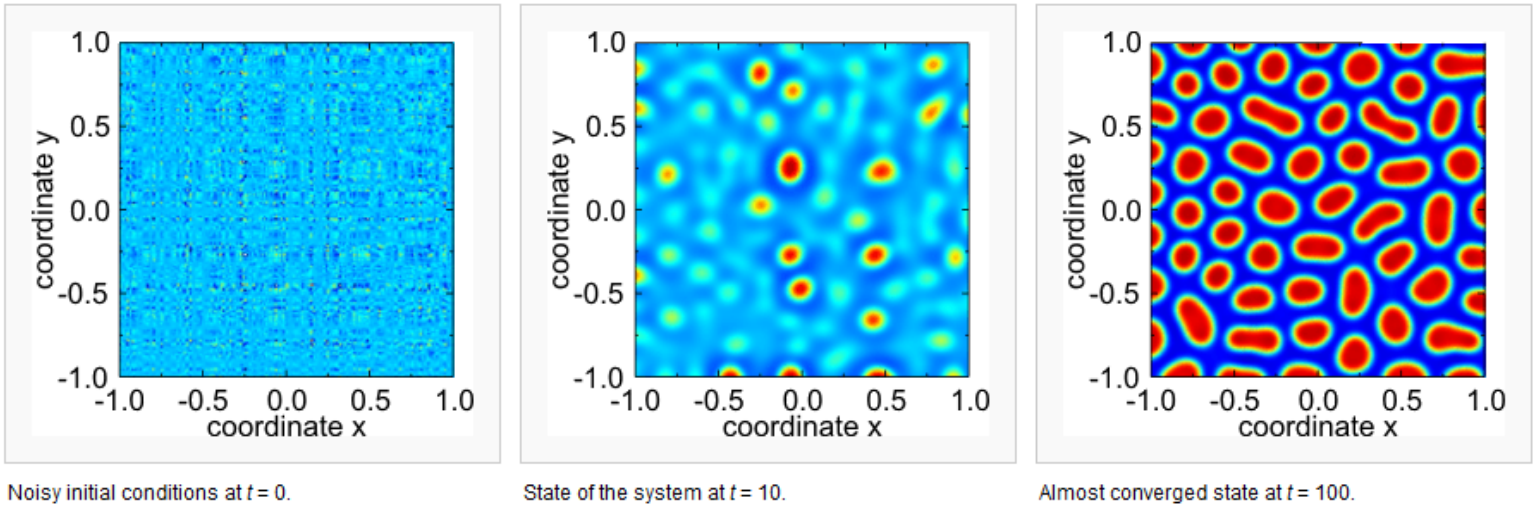
One component stimulates the production of both components while the other one inhibits their growth. (action potential travelling through nerve)

FitzHugh–Nagumo equation

$$\partial_t u = d_u^2 \nabla^2 u + f(u) - \sigma v,$$
$$\tau \partial_t v = d_v^2 \nabla^2 v + u - v$$

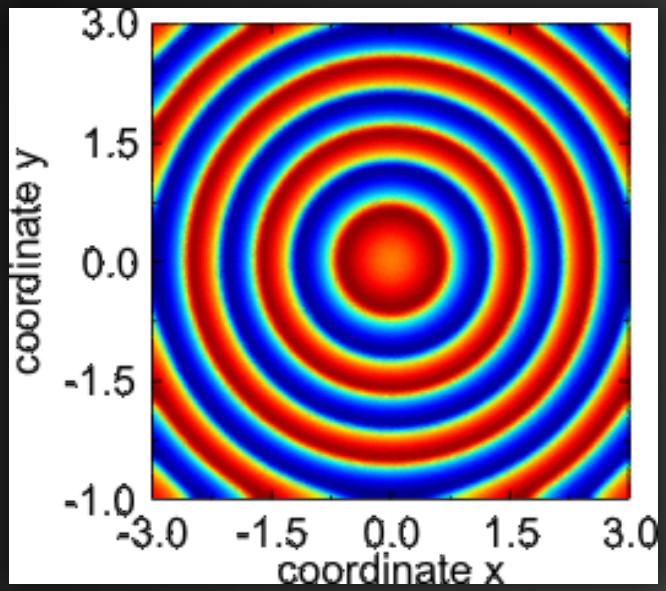
with $f(u) = \lambda u - u^3 - \kappa$

Subcritical Turing bifurcation: formation of a hexagonal pattern from noisy initial conditions in the above two-component reaction-diffusion system of Fitzhugh-Nagumo type.



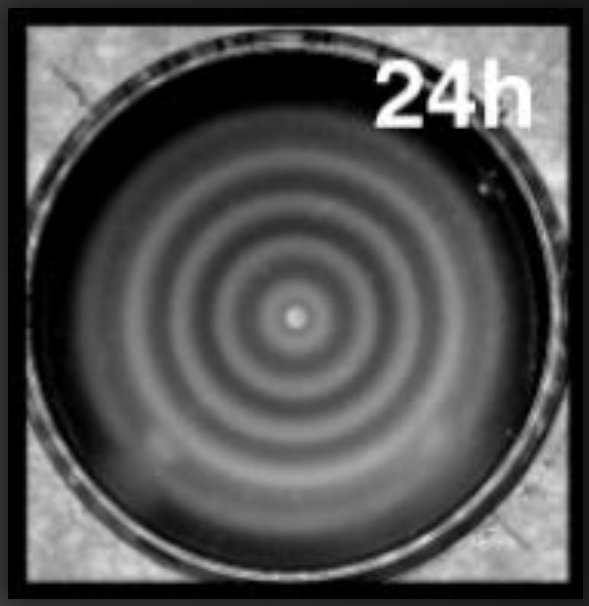
Noisy initial conditions at $t = 0$. State of the system at $t = 10$. Almost converged state at $t = 100$.

Synthetic Biology Patterning



Simulated Turing Pattern

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



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:

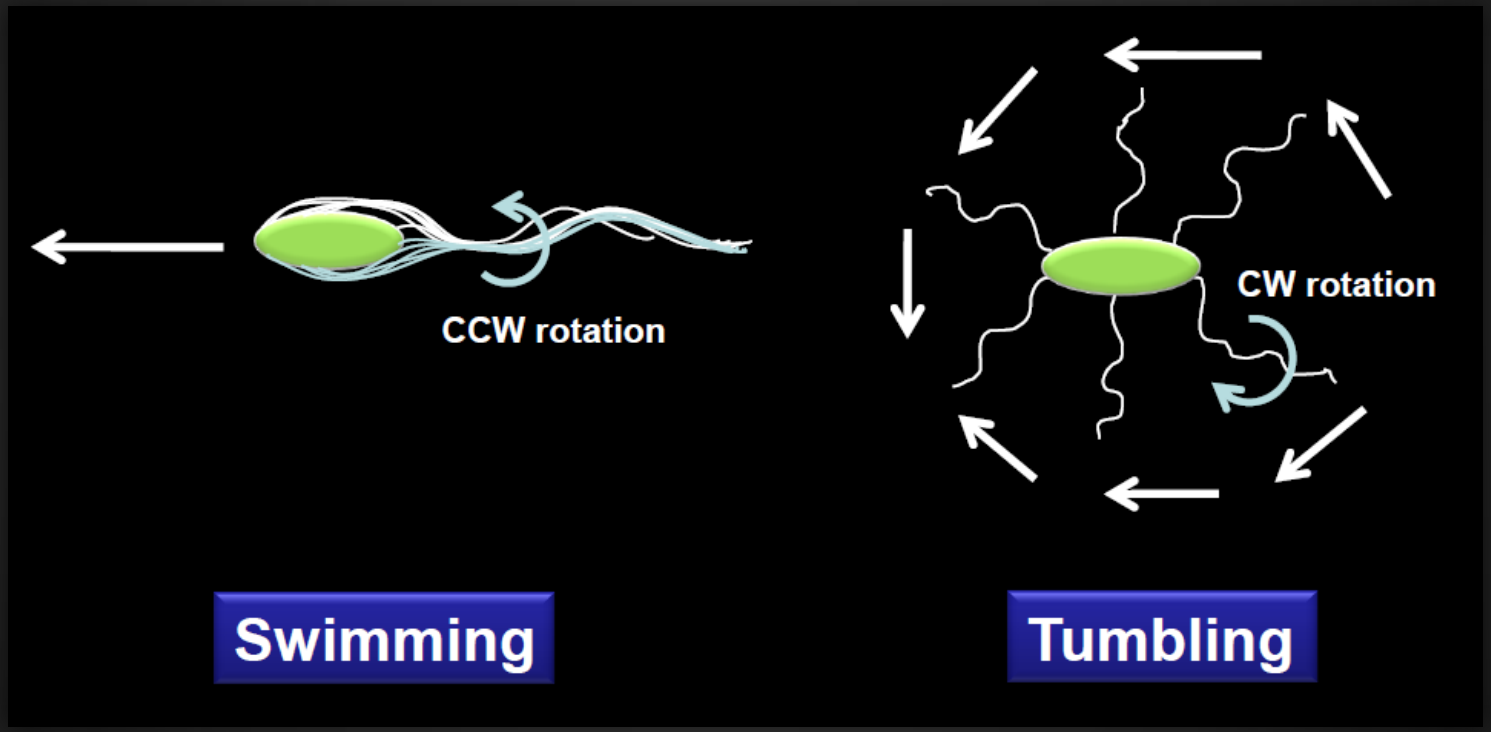
1. Control over cell motility
2. Ability to sense cell density
3. Methods for tuning key parameters

Practical requirements:

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

iHKU iGEM 2008

Motility of *E. coli*



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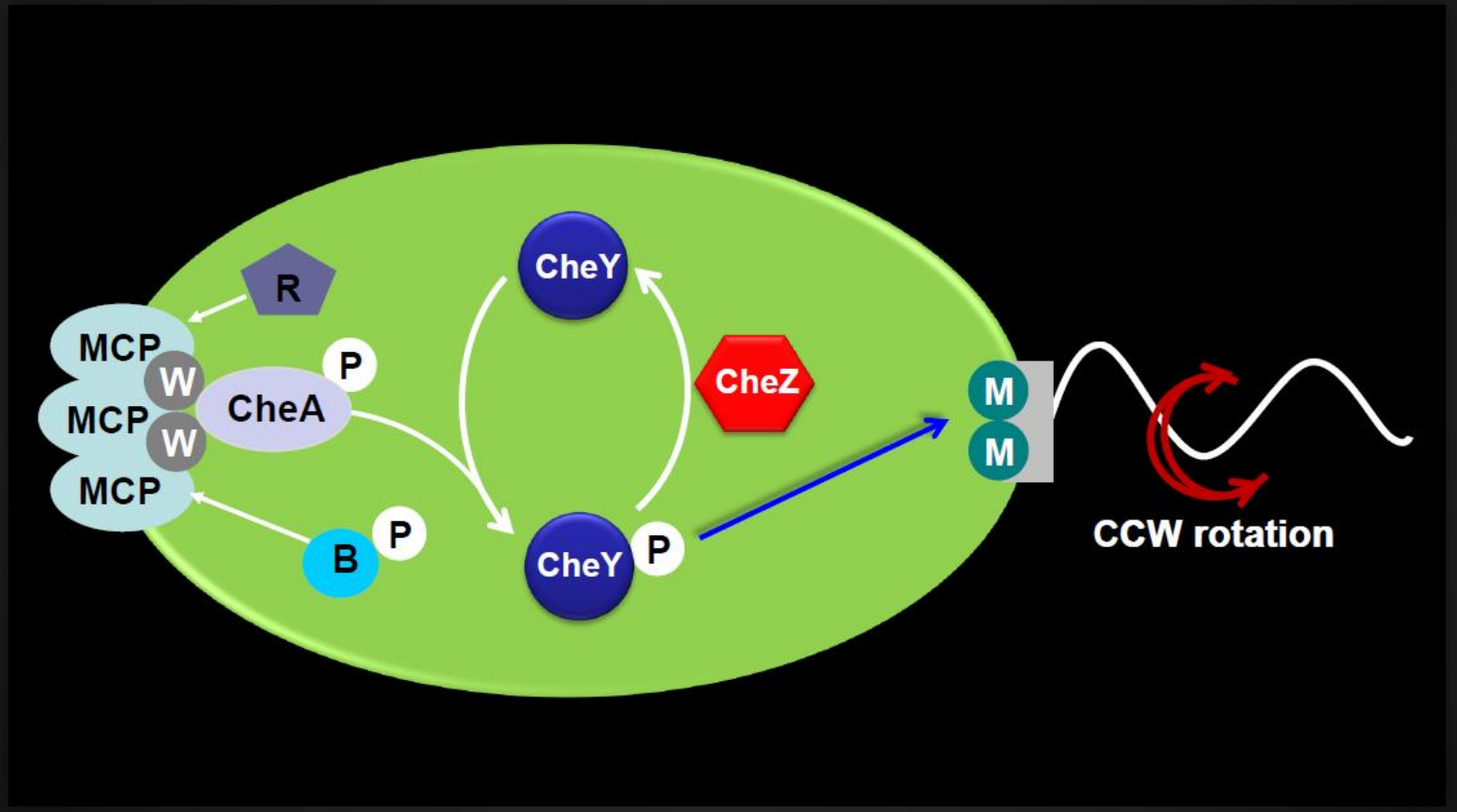
Mechanism of motility in *E. coli*



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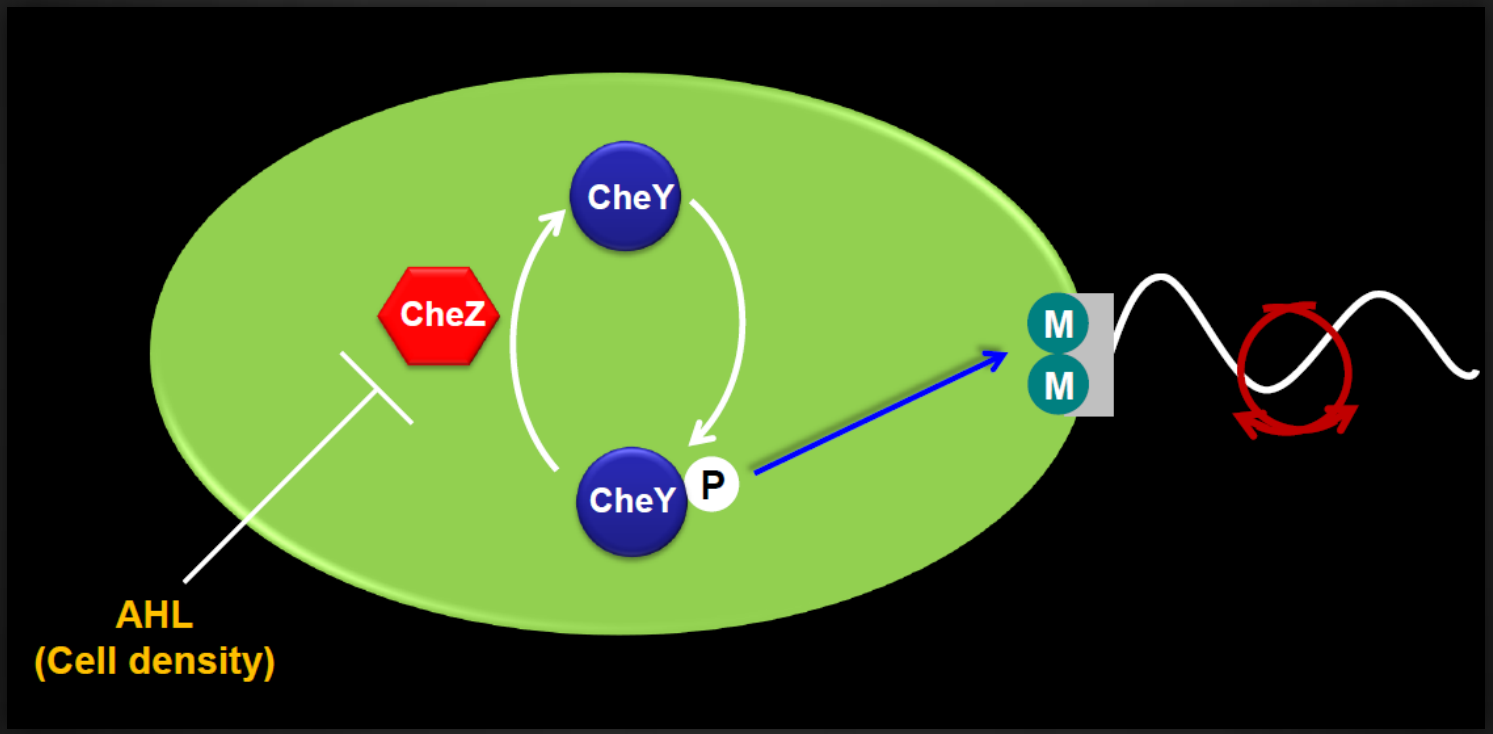
Synthetic control of CheZ = control of motility in *E. coli*



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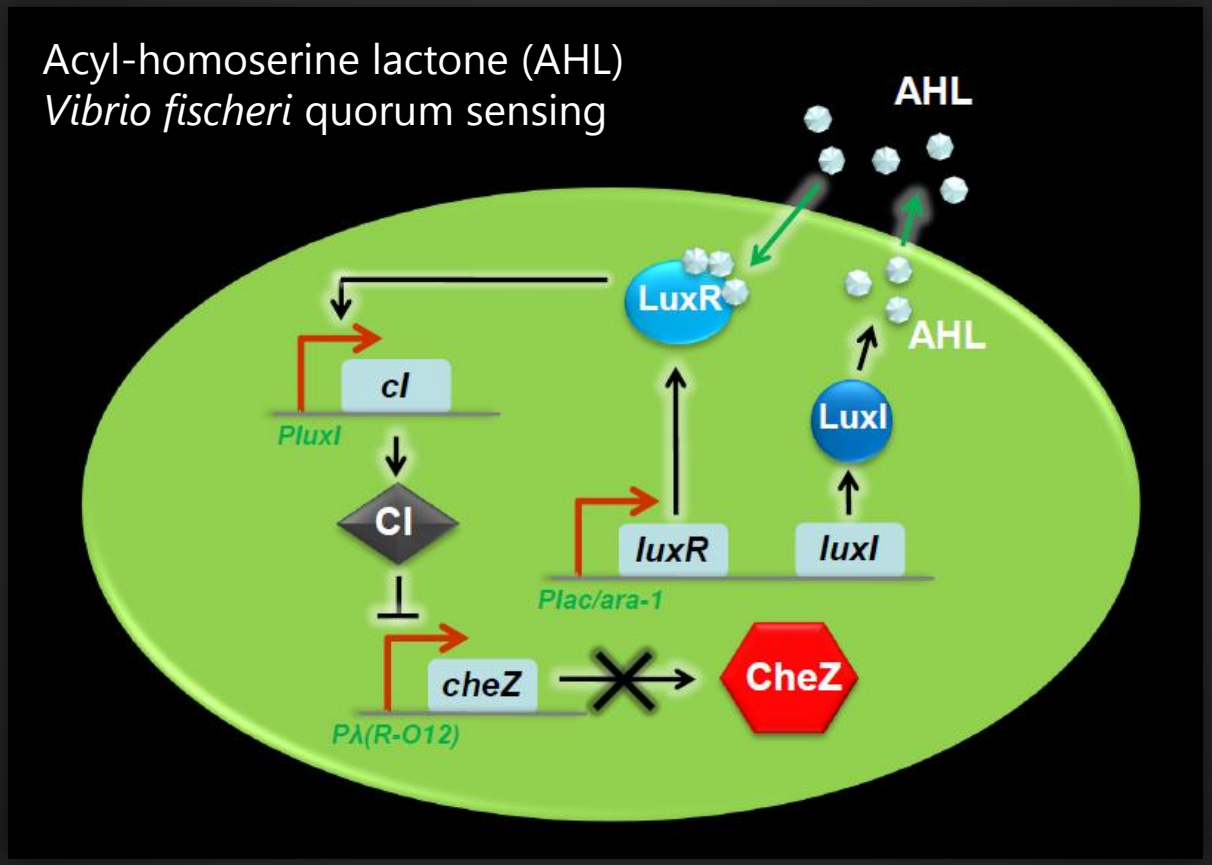
Density-dependent control of CheZ



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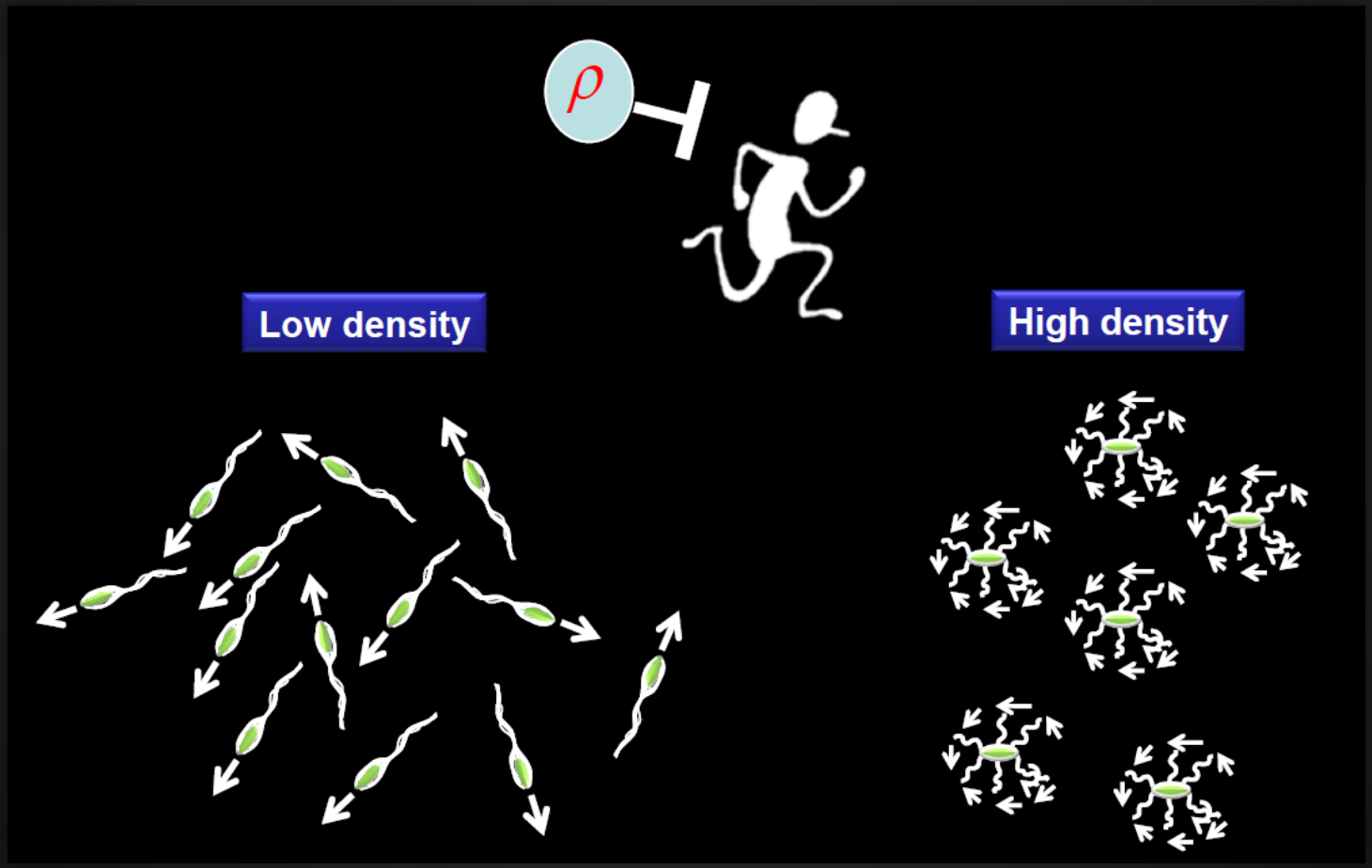
Creation of a low-density mover



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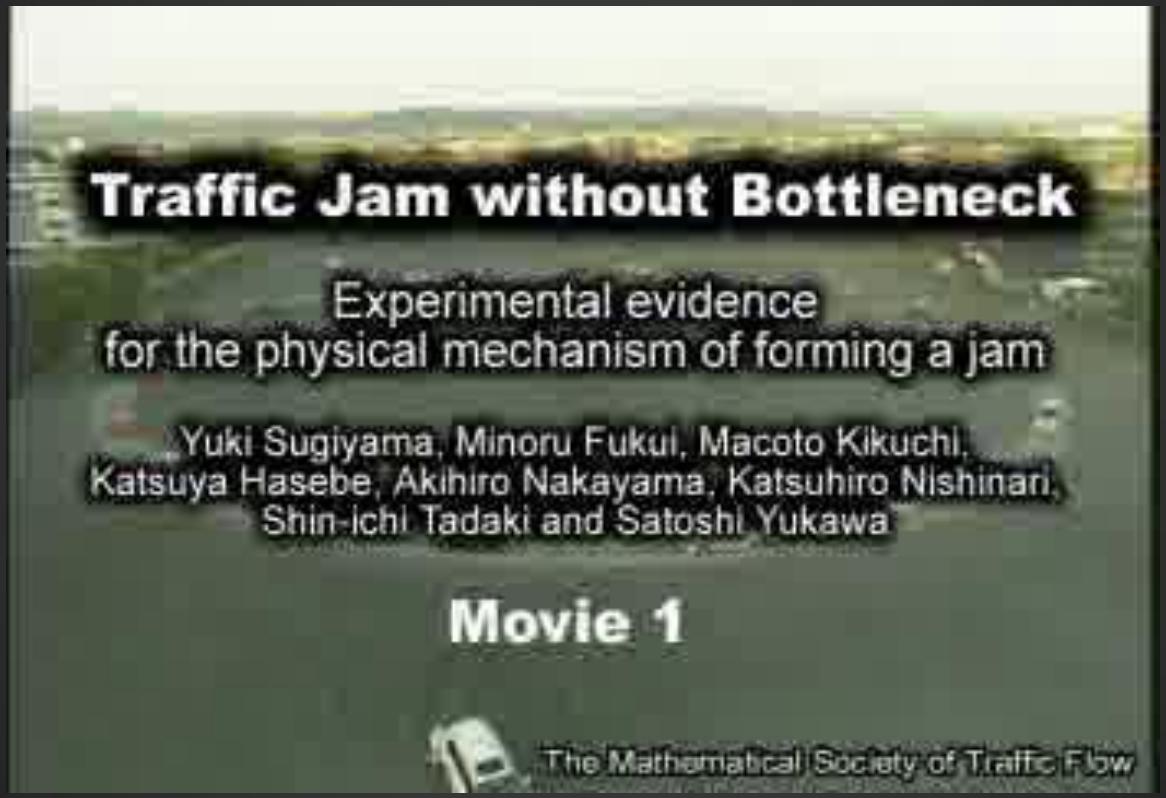
Traffic is a low-density mover



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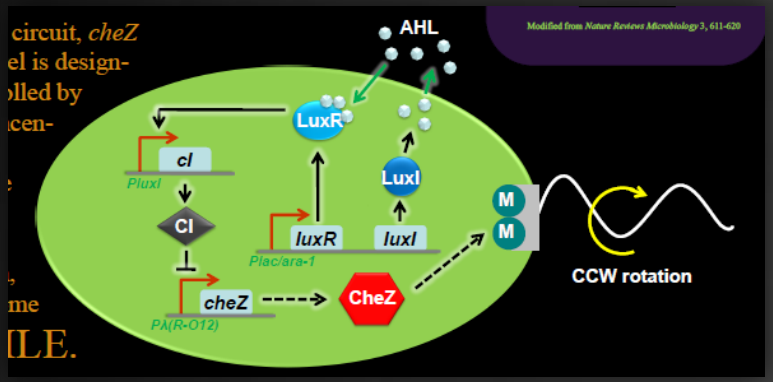
Design, Model and Some Results – but only a Bronze

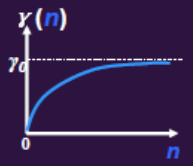


$$\frac{\partial \rho}{\partial t} = \nabla^2 (D_\rho(h)\rho) + \gamma(n)\rho$$

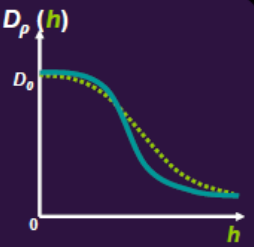
$$\frac{\partial h}{\partial t} = D_h \nabla^2 h + \lambda\rho - \beta h$$

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \gamma(n)\rho$$

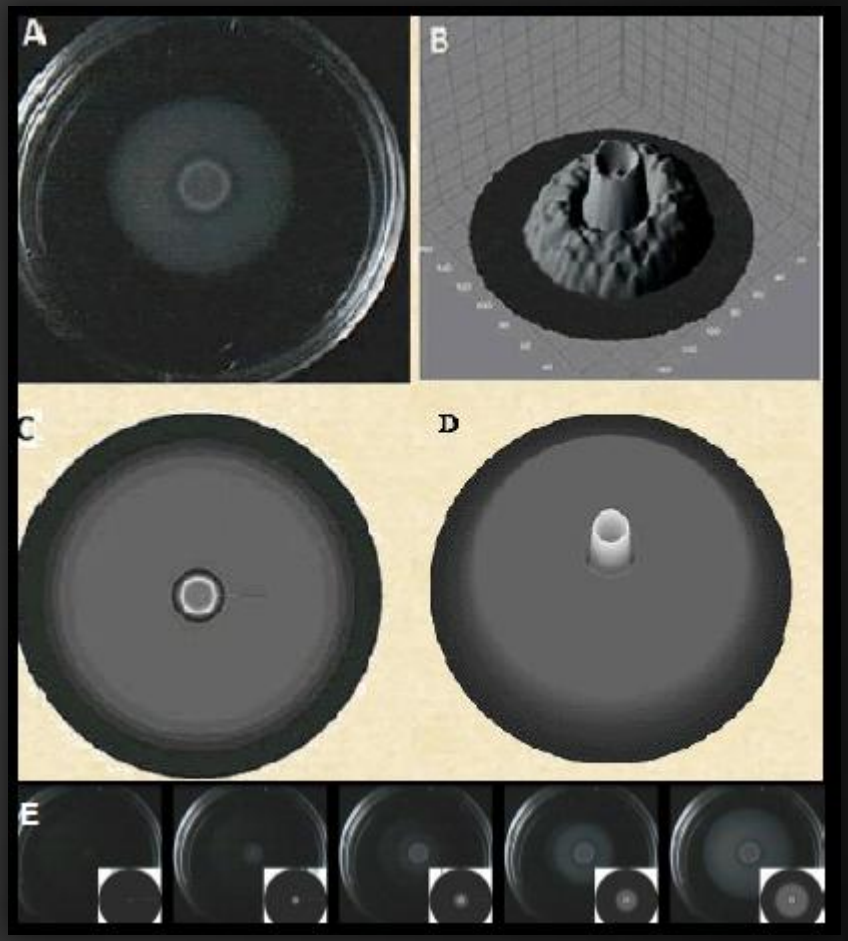
ρ: Cell Density
h: Density of AHL
n: Density of Nutrient
β:AHL Degradation Rate
λ:AHL Synthesis Rate
γ:Cell Growth Rate



The cell growth ratey is a function of nutrient concentration (n).



Unlike the wild type *E. coli*, whose D_ρ is a constant, the cell motility is determined by the AHL concentration via our designed genetic circuit. Therefore, we tried tow decrease Hill functions $D_\rho(h)$ to simulate the ring patterns (Results). The one decrease more sharply near the threshold gave a multiple-ring pattern.



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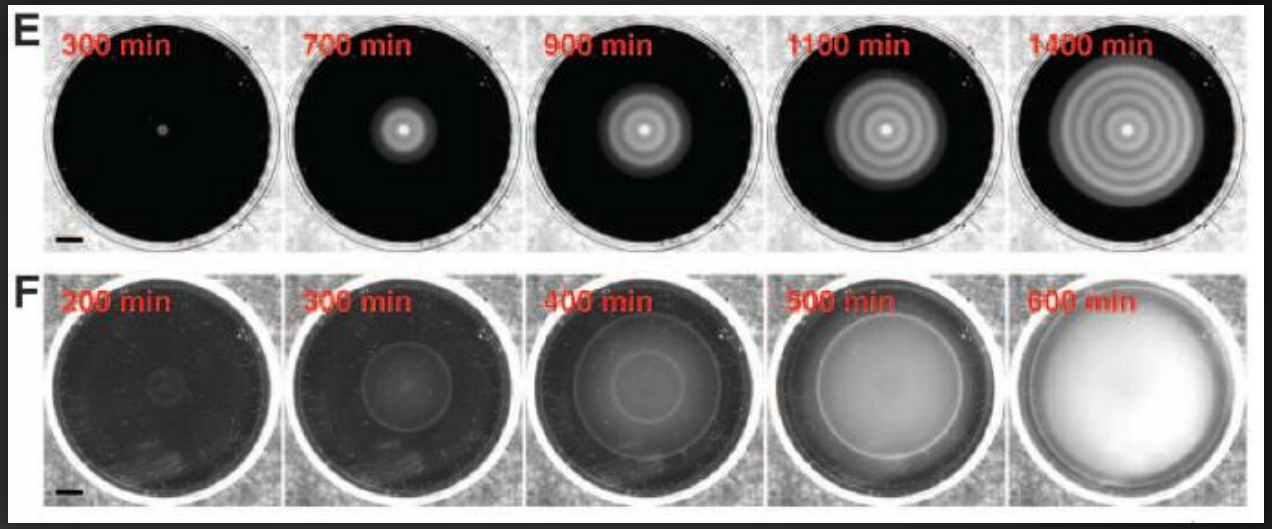
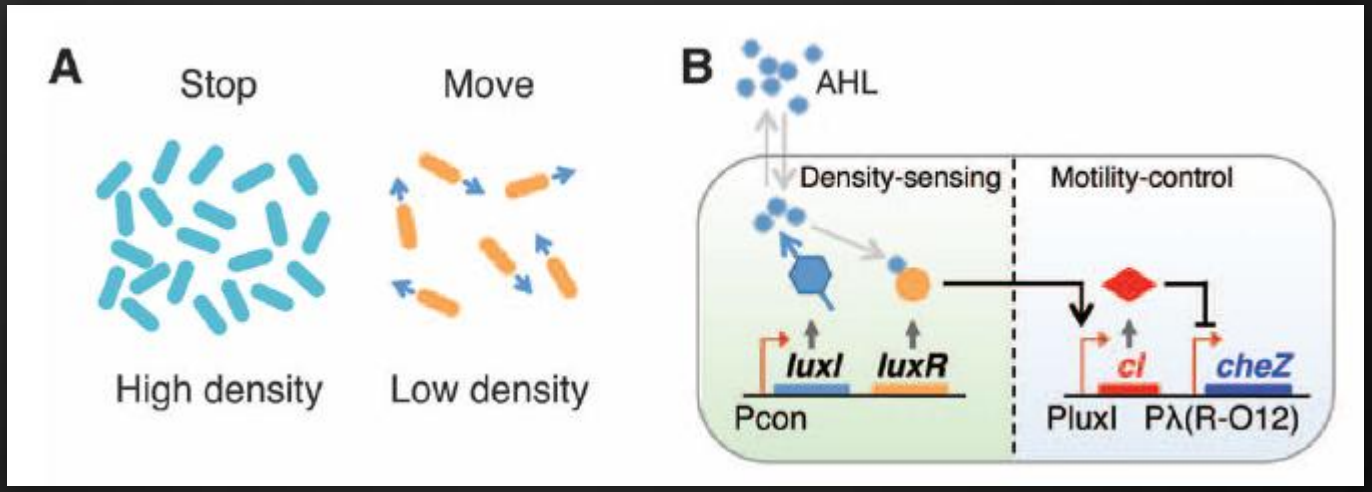
3 years later.....





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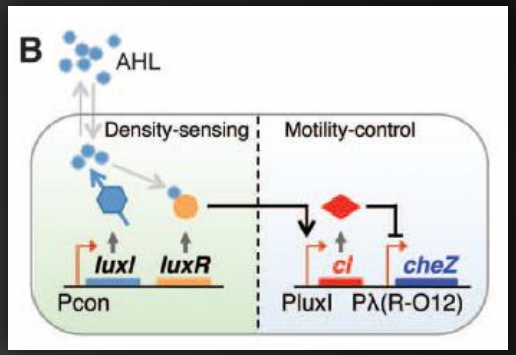

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Model of the system

3 partial differential equations

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

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

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

(shown here is the updated 2011 model)

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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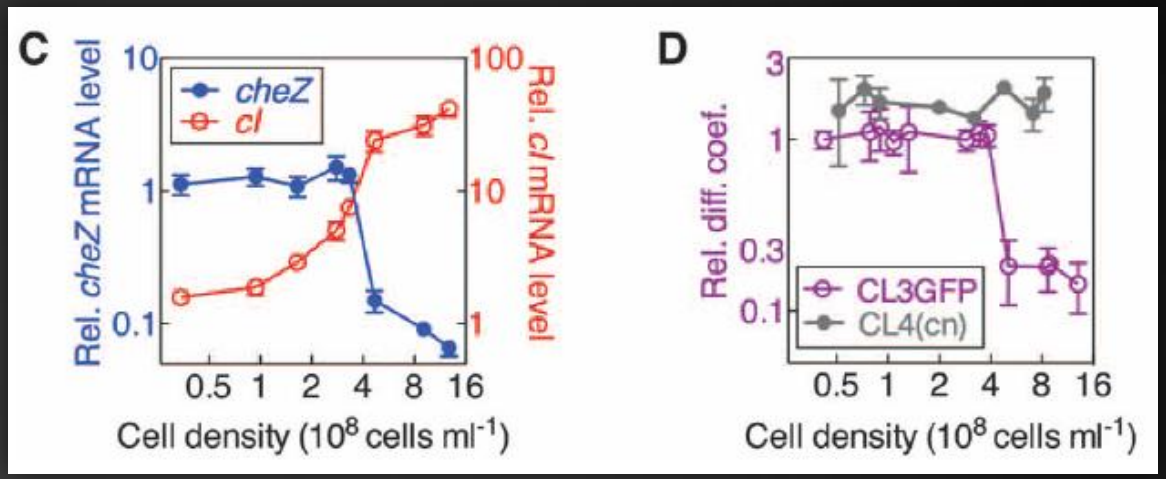
Model of the system

Getting the parameters

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



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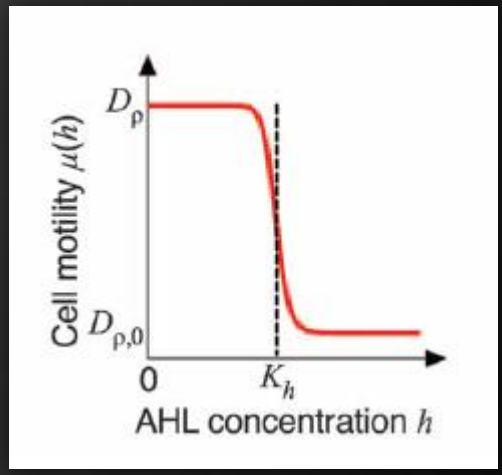

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Model of the system

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 $\mu m^2\ s^{-1}$	The normal value (200-1,000 $\mu m^2\ s^{-1}$) (*49-51*)
$D_{\rho,0}$	10 $\mu m^2\ s^{-1}$	Fig. 1D shows it is almost zero
D_h	400 $\mu m^2\ s^{-1}$	As small molecular diffusion (100-1,000 $\mu m^2\ s^{-1}$) (*56, 57*)
D_n	800 $\mu m^2\ s^{-1}$	As small molecular diffusion (100-1,000 $\mu m^2\ s^{-1}$) (*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 of the system

3 partial differential equations

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

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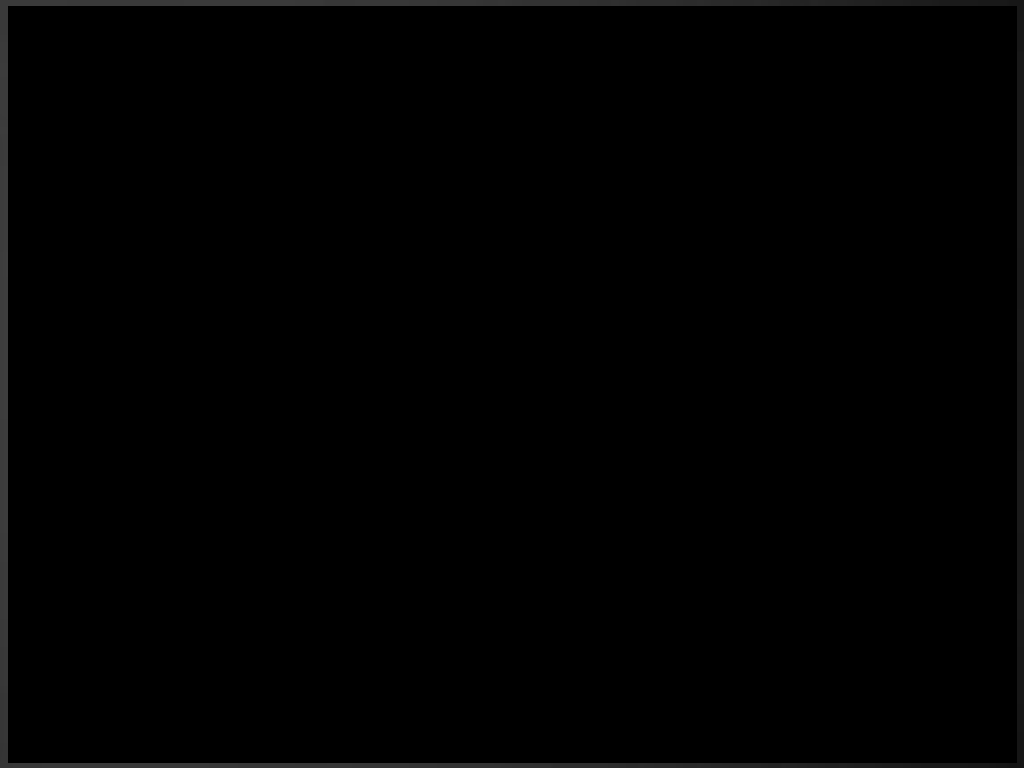
$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \quad [3]$$



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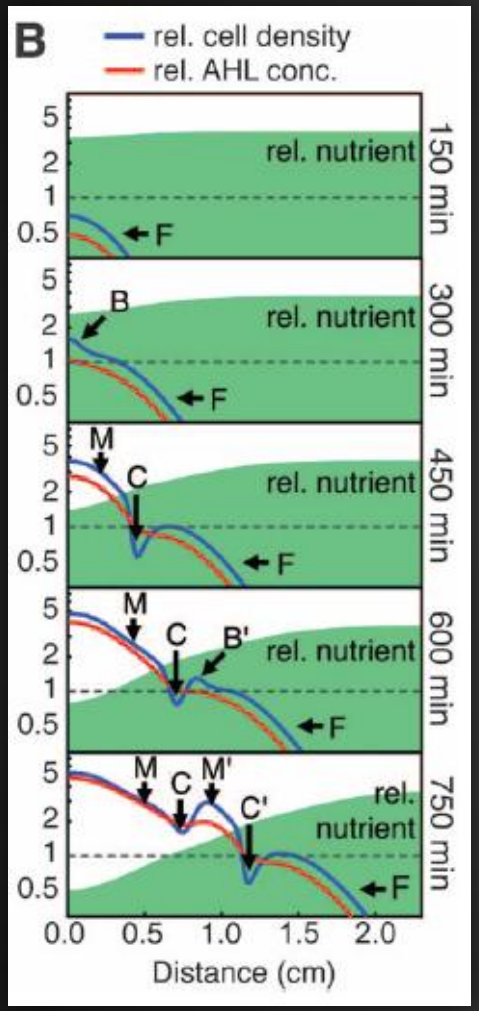
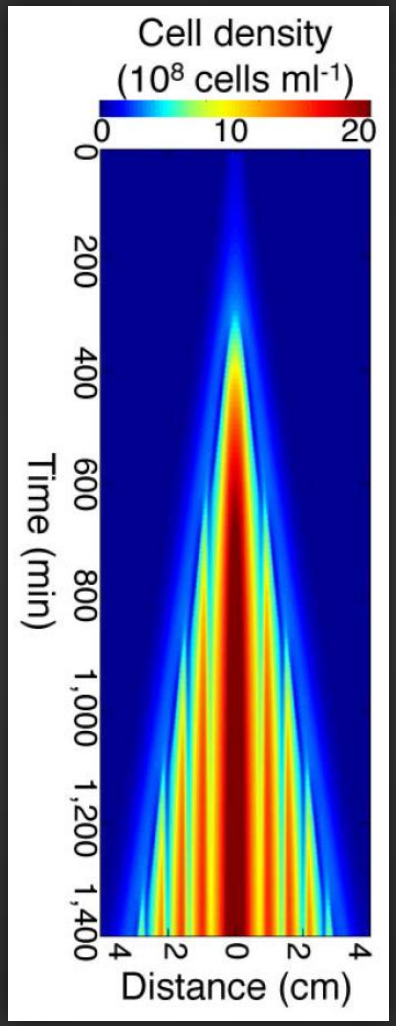
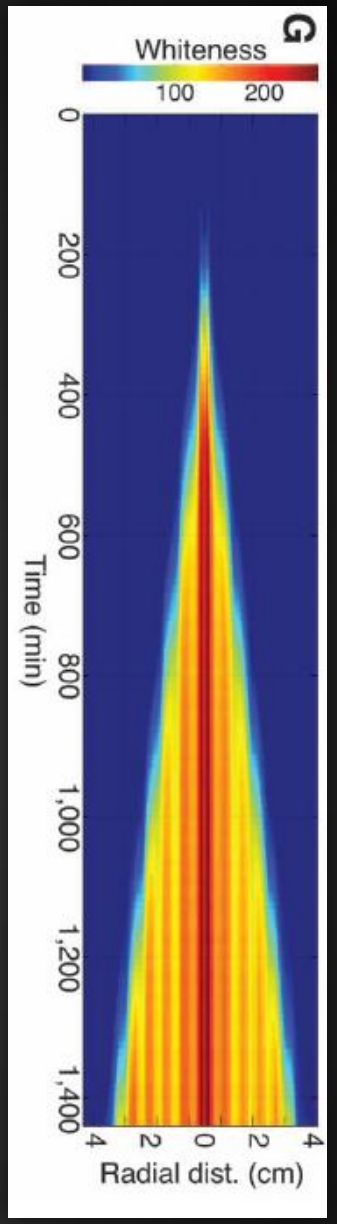




Simulation



Experiment

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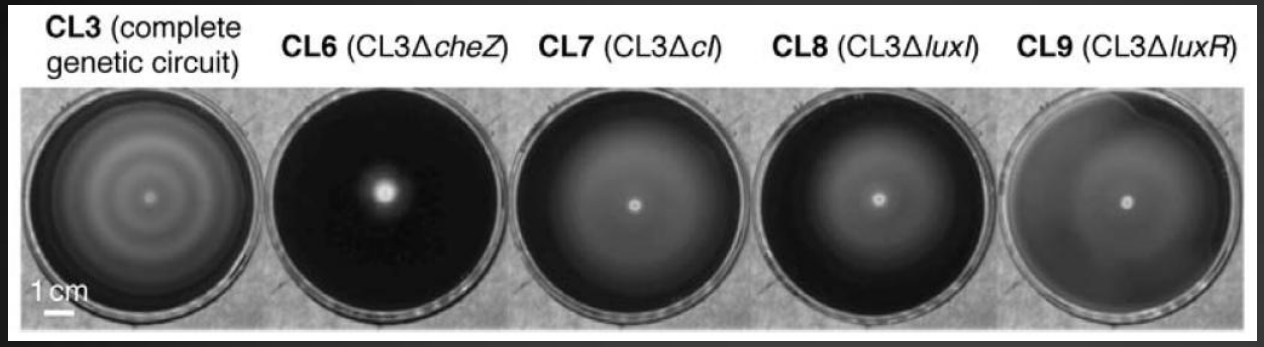
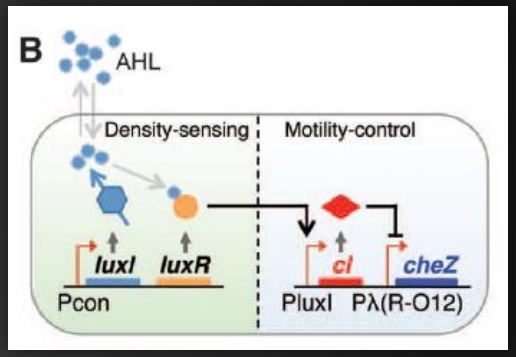
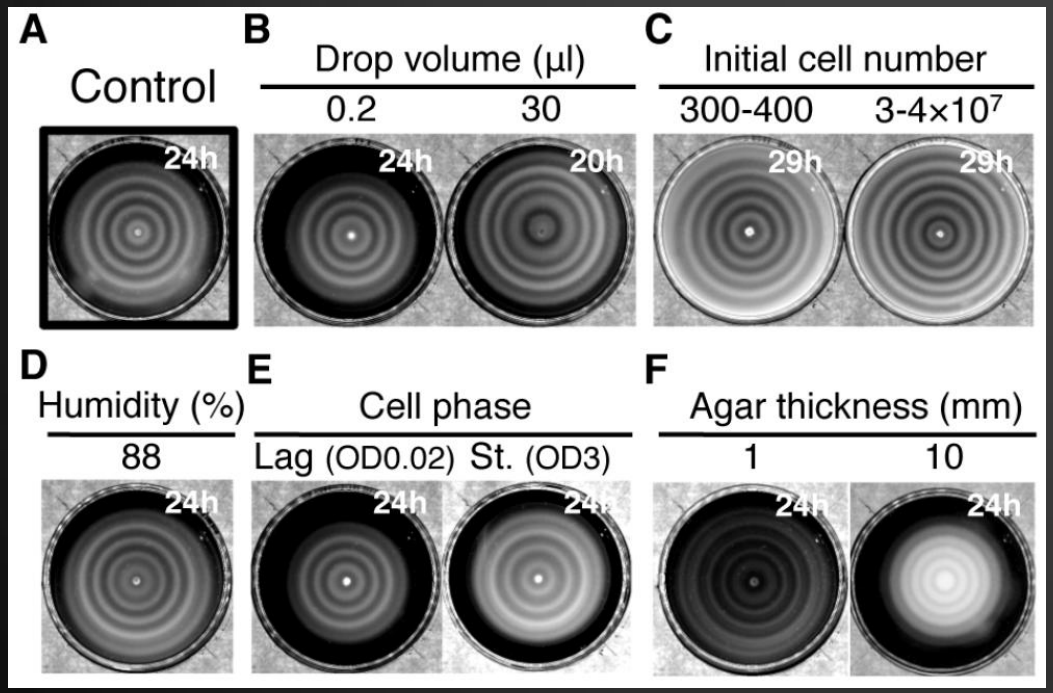
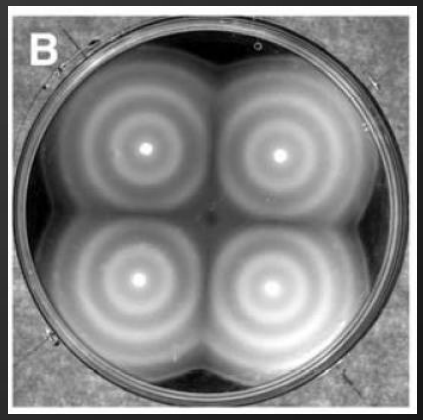


Only the complete system works





Patterns are robust



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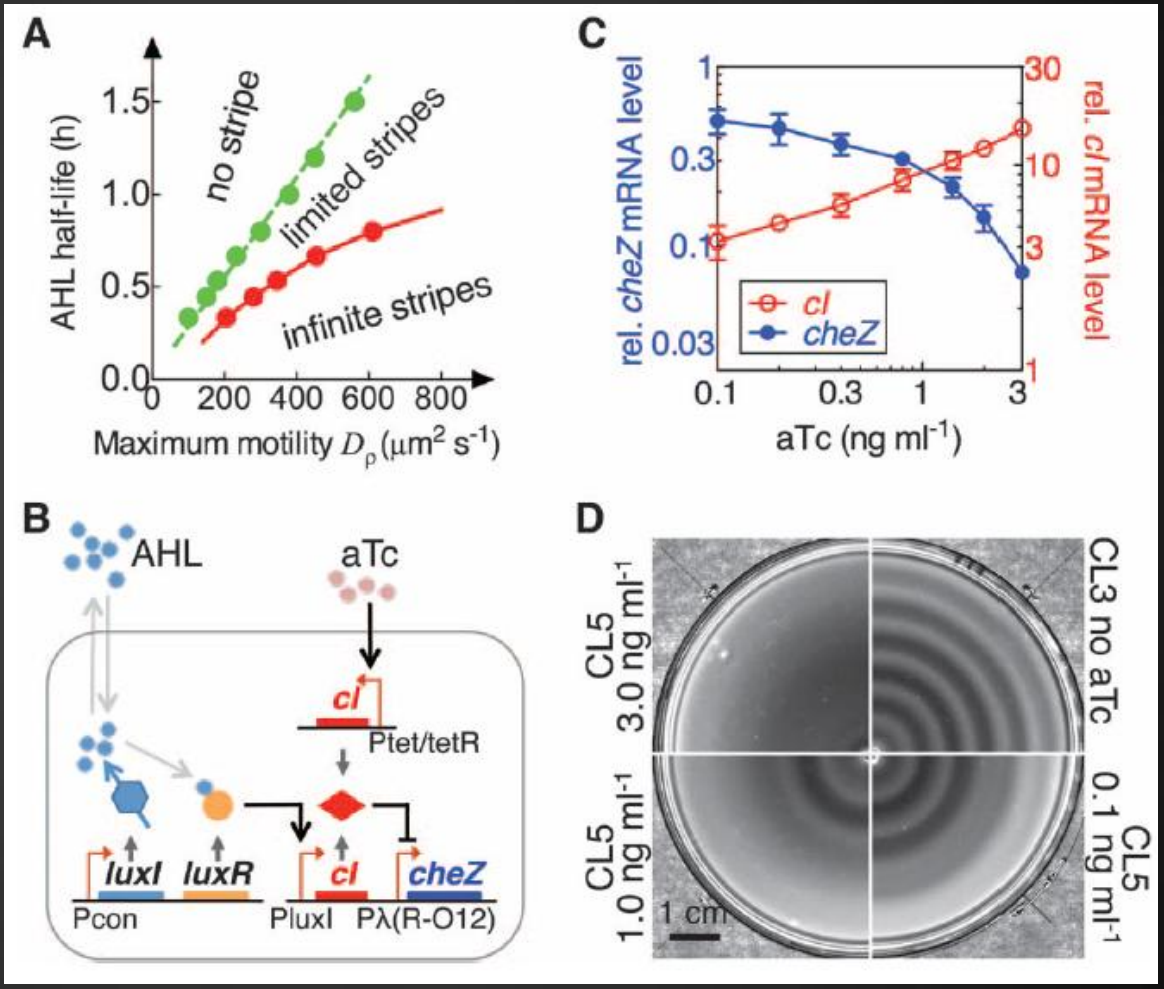
Model predicts that occurrence of stripes is dependent on two factors:

1. AHL half-life
2. Maximum motility

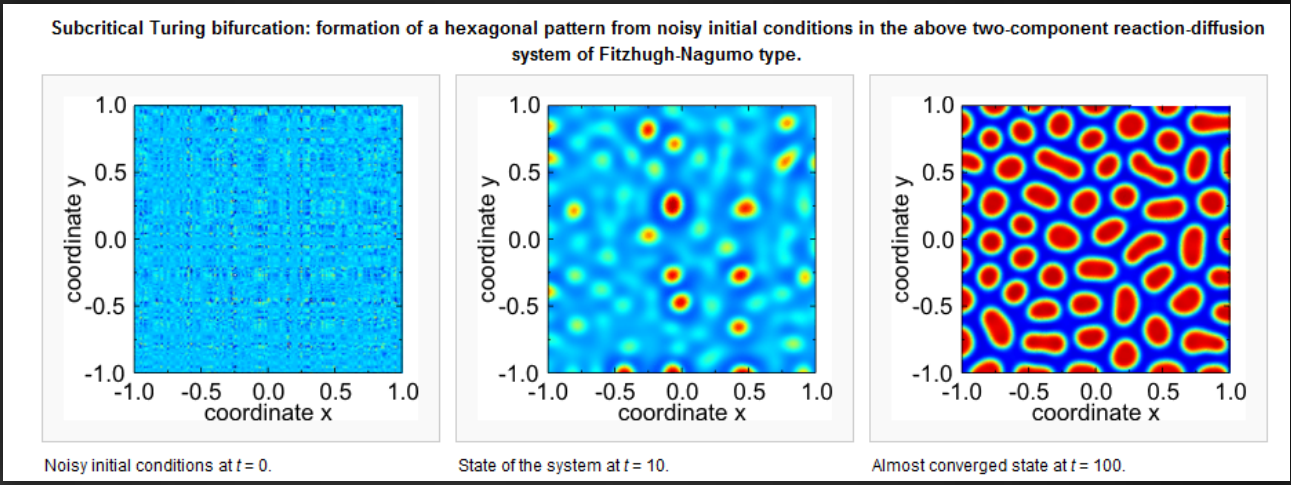
Synthetic biology allows these to be tested!

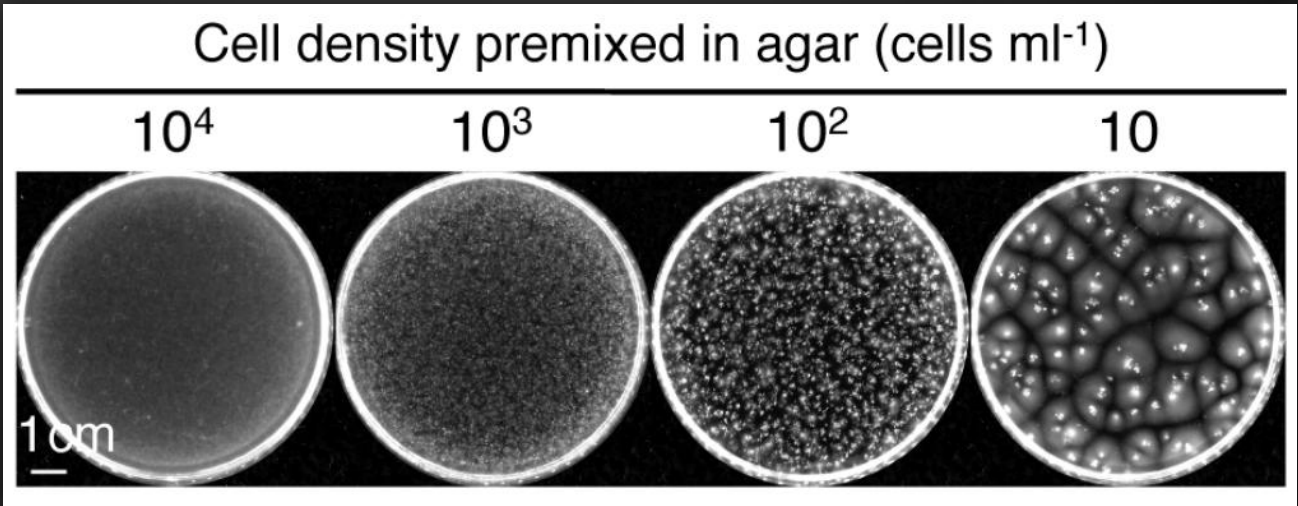


ChenLi Liu *et al.* Science 2011

As with Turing Patterns, the patterns emerge when there is more noise in the system

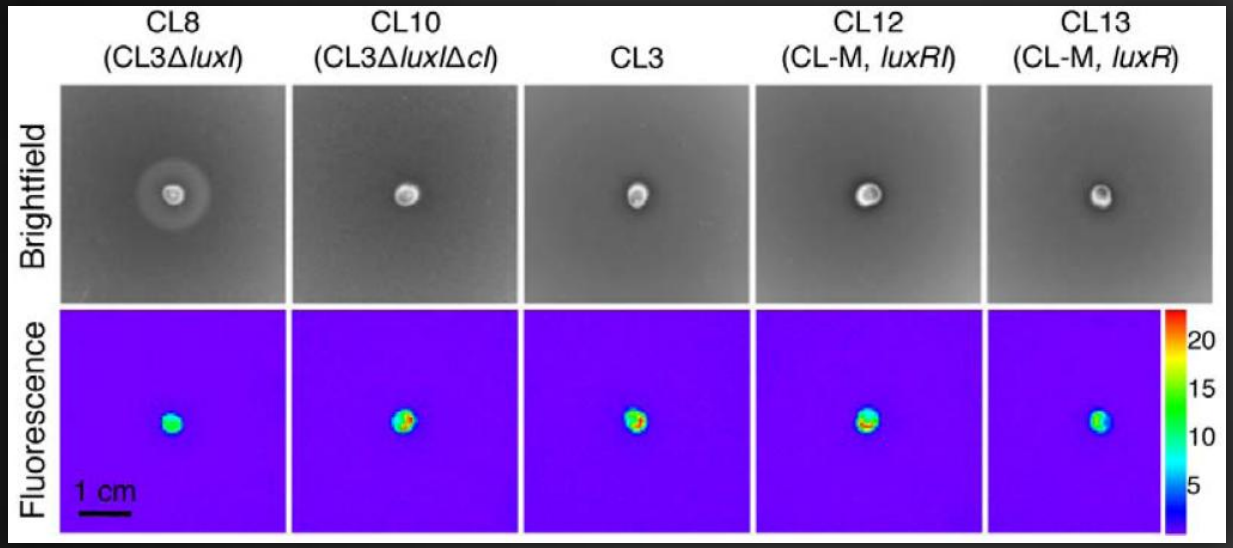
Low cell numbers means higher stochasticity





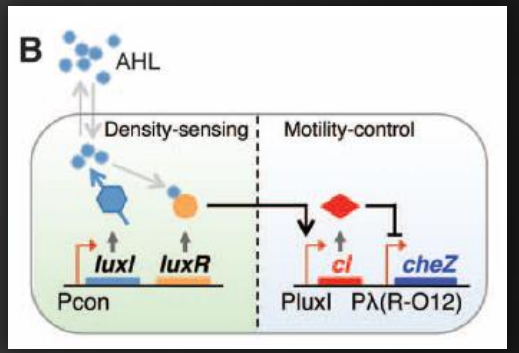
ChenLi Liu *et al.* Science 2011

One final experiment... pattern forms due to 'effective aggregation'



Prove this using split system of:

a) Sender cells
b) Receiver cells



Cleft zone results from an aggregation phenomenon driven by density-dependent motility. Cells can diffuse freely in semisolid agar when the cell density is low. As cells proliferate and the local AHL level exceeds the threshold, cell motility slows down as programmed. These cells cannot move away, but neighbouring cells may continue to move into this high-density region and become non-motile leading to a net cell flow toward the high-density region.

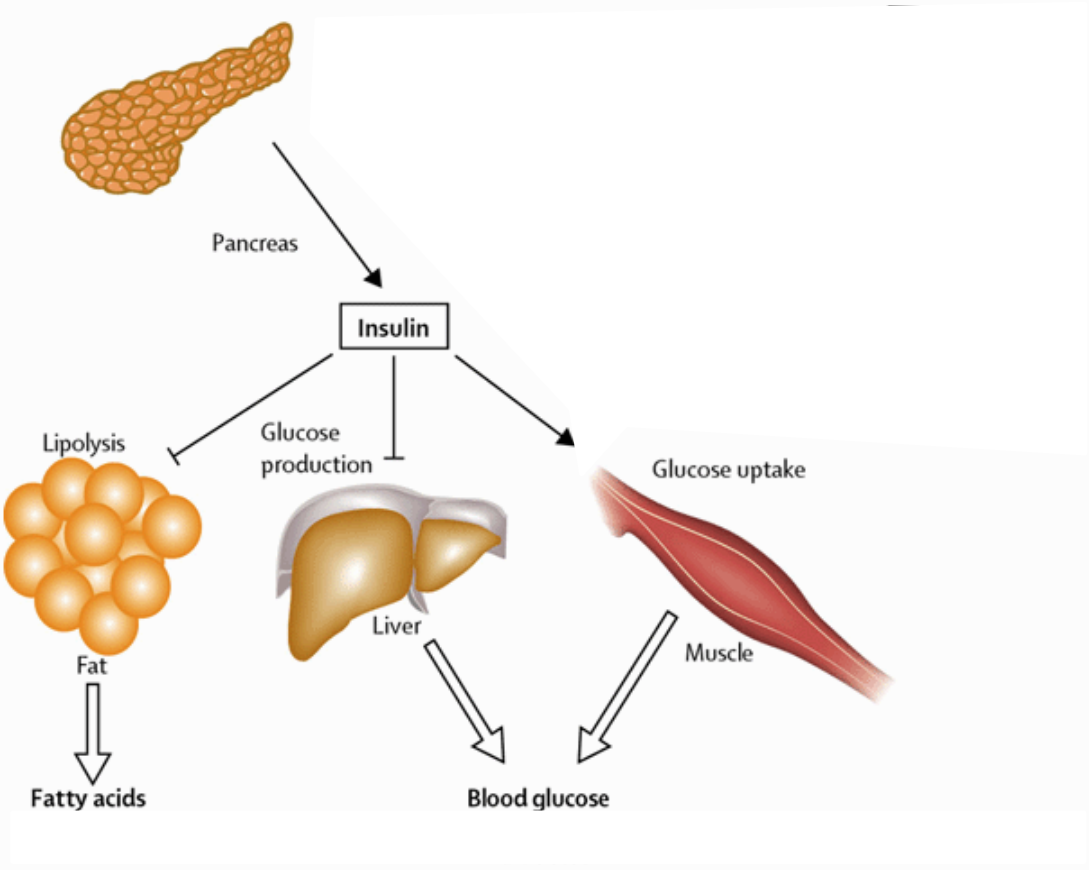
Sender and Receiver Cells

- Simple route to complex multi-cellularity
- Division of labour between cells
- Diffusion of a message
- Reaction of acting upon message

What examples can you think of from nature?

Sender and Receiver Cells

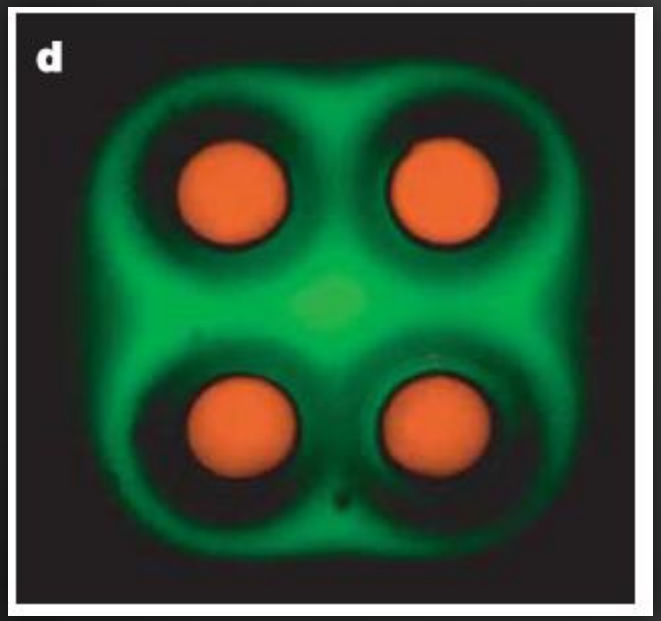
Example 1 Pancreatic Beta Cells Send out Insulin

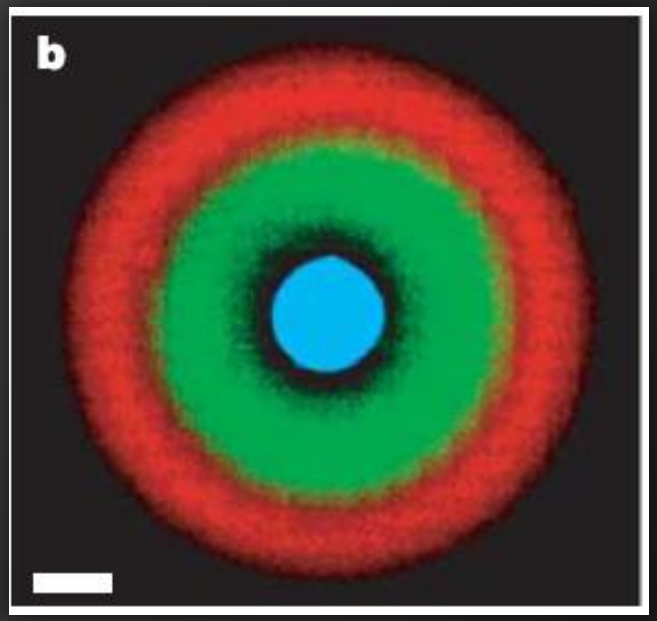


Basu *et al.* Nature 2005

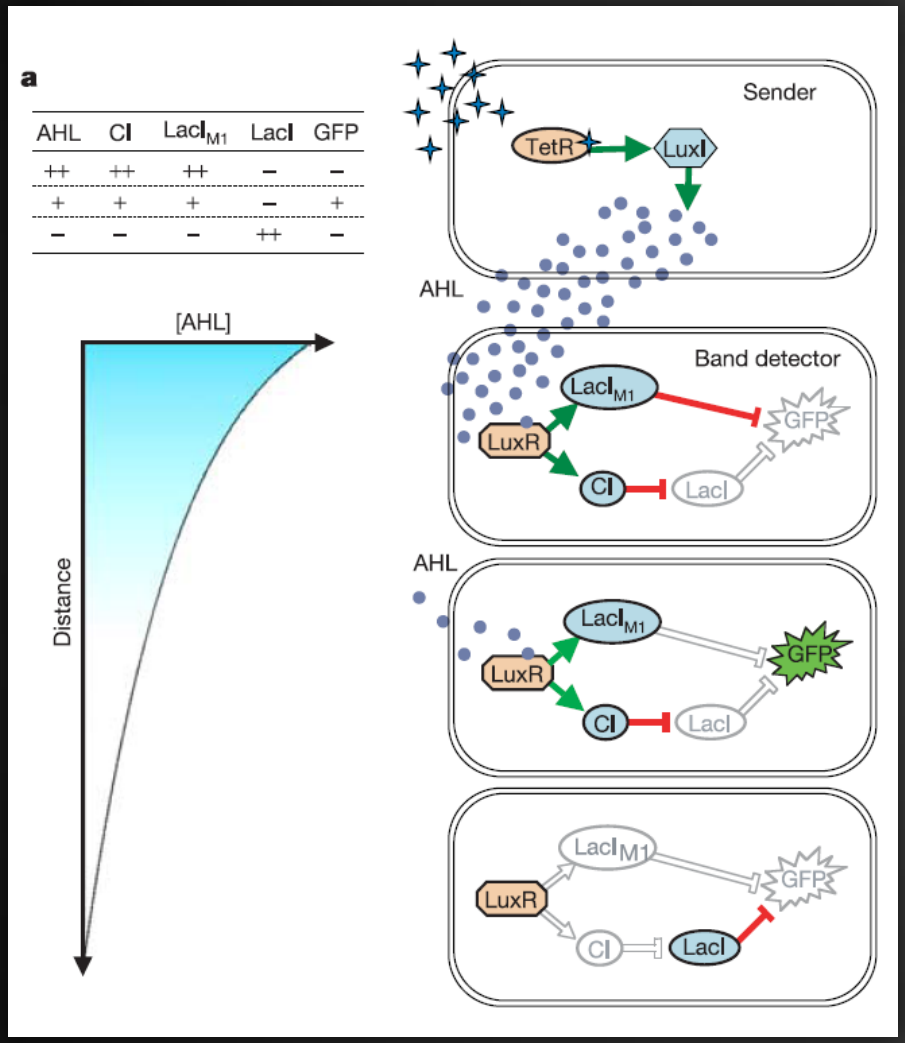
A synthetic multicellular system for programmed pattern formation

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





Basu *et al.* Nature 2005

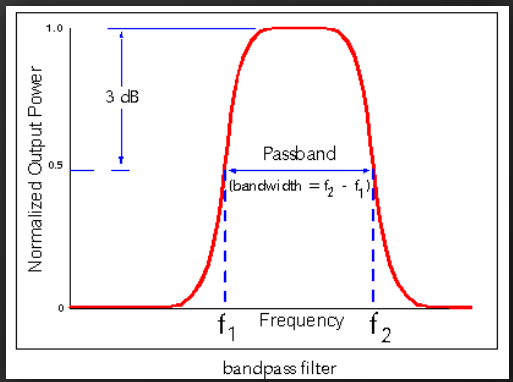


Band-detect multicellular system

Sender cell produces AHL

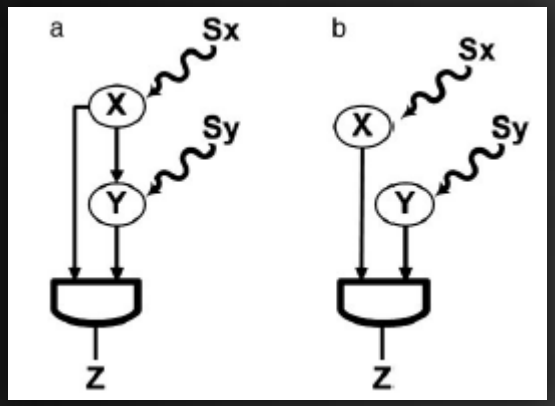
Receiver cell contains a feed-forward loop responsive to AHL (band-pass filter)

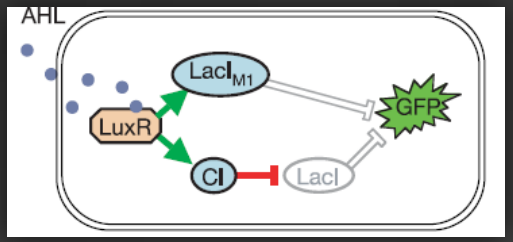
Receiver cell produces GFP only at medium AHL levels



Basu *et al.* Nature 2005

Feed-forward loops





Band-pass filter is generated by the difference between the CI and LacI$_{M1}$ repression efficiencies and an incoherent feed-forward loop (type II)

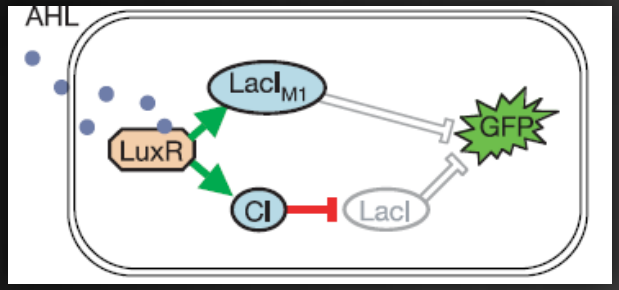
Species	Incoherent type 1 Structure	Incoherent type 1 Abundance	Incoherent type 2 Structure	Incoherent type 2 Abundance	Incoherent type 3 Structure	Incoherent type 3 Abundance	Incoherent type 4 Structure	Incoherent type 4 Abundance
E. coli	X, Y, Z	5	X, Y, Z	0	X, Y, Z	1	X, Y, Z	1
S. cerevisiae	X, Y, Z	21	X, Y, Z	3	X, Y, Z	1	X, Y, Z	0
Z logic →	AND		AND		AND		AND	
Steady-state Z(Sx,Sy)	$S_x \wedge \bar{S}_y$		$\bar{S}_x \wedge \bar{S}_y$		0		0	
Pulse								
Sx on step	Weak		—		—		Strong	
Sx off step	—		Weak		Strong		—	
Sy effect	Destroy		Destroy		Enable		Enable	
Response acceleration								
Sx on step	Accelerate		—		—		Accelerate	
Sx off step	—		Accelerate		Accelerate		—	

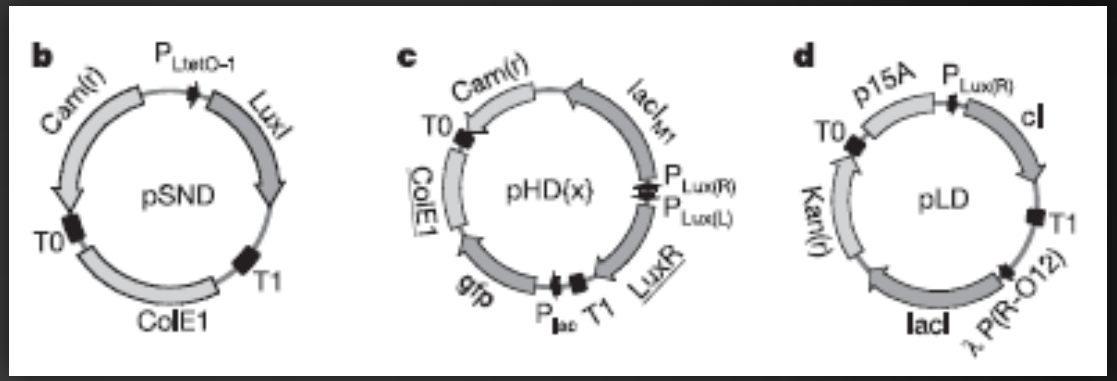
Structure and function of the feed-forward loop network motif

S. Mangan and U. Alon PNAS 2003

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

$$\frac{dG}{dt} = \frac{\alpha_G}{1 + (L/\beta_L)^{\gamma 1}} - \gamma_G G \quad (1)$$

$$\frac{dL}{dt} = \frac{\alpha_{L1}}{1 + (C/\beta_C)^{\gamma 2}} + \frac{\alpha_{L2} \cdot R^{\gamma 3}}{(\theta_R)^{\gamma 3} + R^{\gamma 3}} - \gamma_L L \quad (2)$$

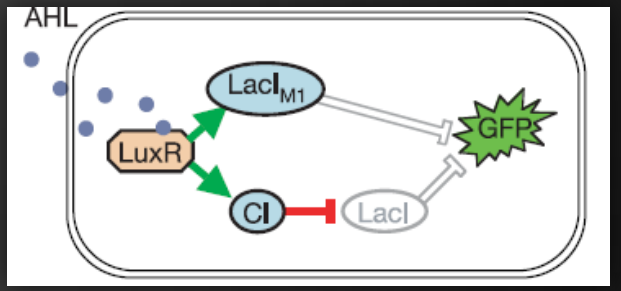
$$\frac{dC}{dt} = \frac{\alpha_C R^{\gamma 3}}{(\theta_R)^{\gamma 3} + R^{\gamma 3}} - \gamma_C C \quad (3)$$

$$\frac{dR}{dt} = \rho_R [\text{LuxR}]^2 A^2 - \gamma_R R \quad (4)$$

$$\frac{dA_{x,y,z}}{dt} = \xi (A_{x-1,y,z} + A_{x+1,y,z} + A_{x,y-1,z} + A_{x,y+1,z} + A_{x,y,z-1} + A_{x,y,z+1} - 6A_{x,y,z}) - \gamma_A \quad (5)$$

5 species model

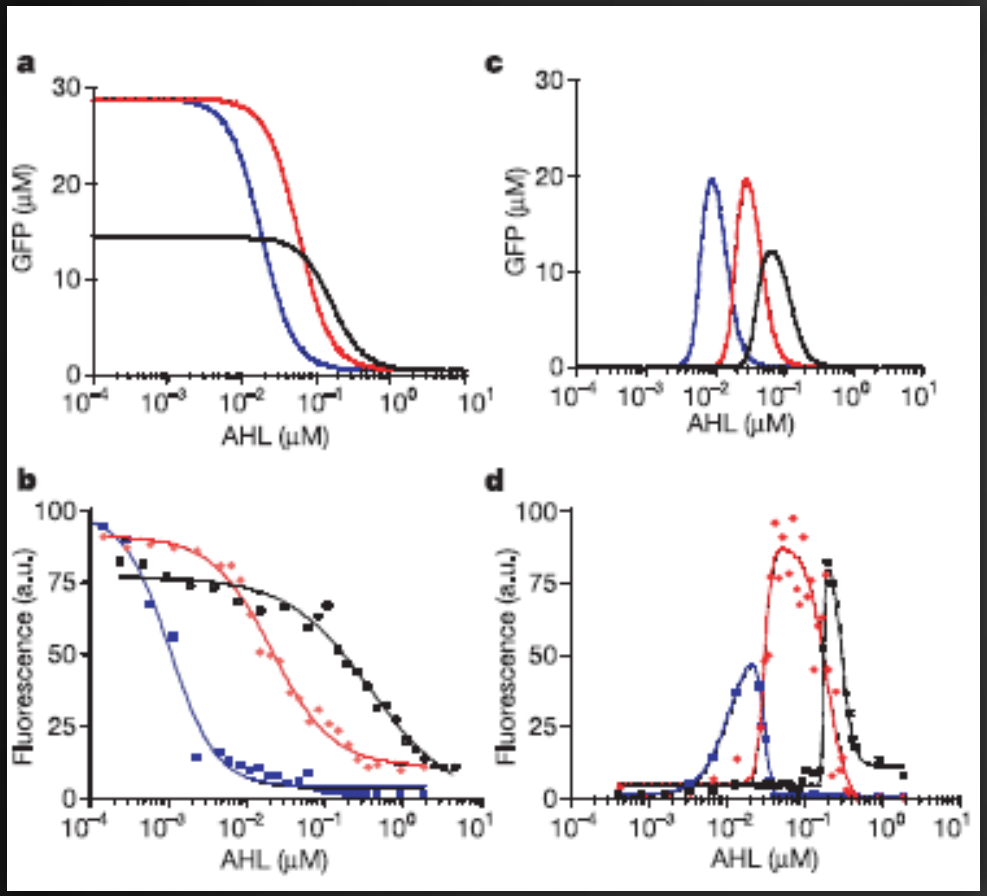
Ordinary Differential Equations with Hill functions that capture the activation and repression of protein synthesis



G = GFP L = LacI C = CI R = LuxR/AHL A = AHL
LuxR alone is at a fixed concentration

Equation 5 captures the diffusion and degradation of AHL

Basu *et al.* Nature 2005

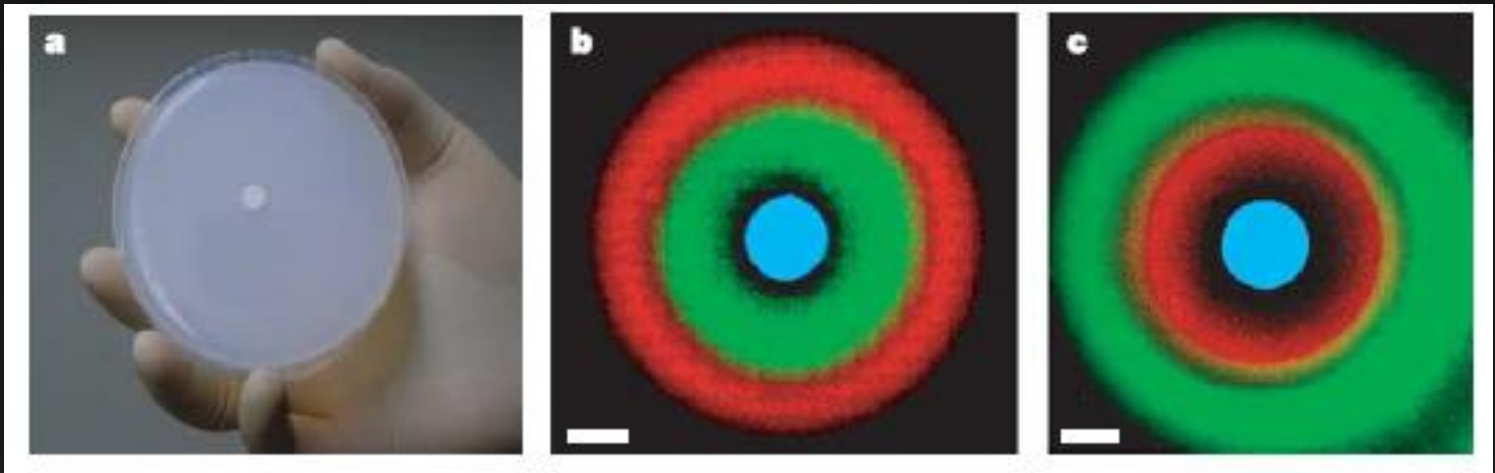


5 species model

Ordinary Differential Equations with Hill functions that capture the activation and repression of protein synthesis

Parameters chosen to fit the data and based on previous research

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

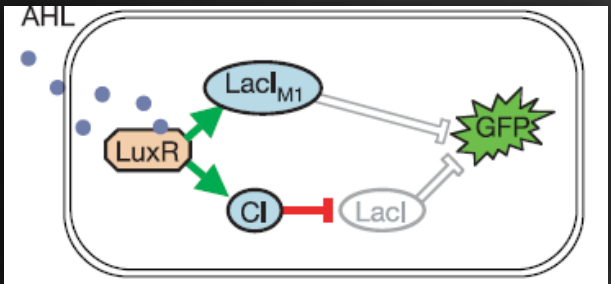
Sender cells express CFP (cyan colour)

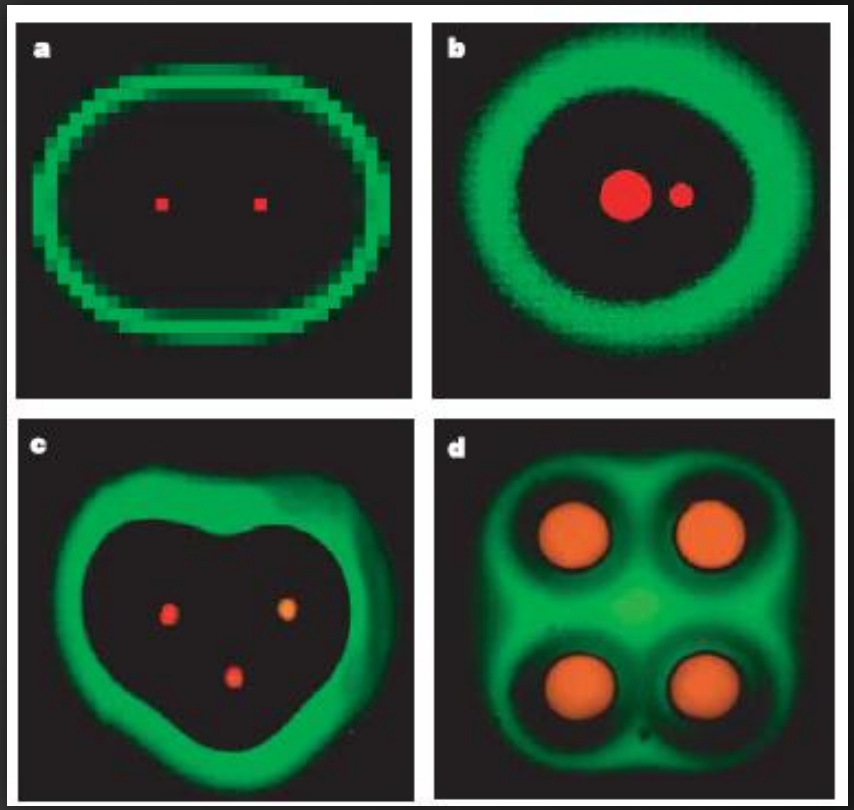
Basu *et al.* Nature 2005

The model can predict complex patterns from two or more spots

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

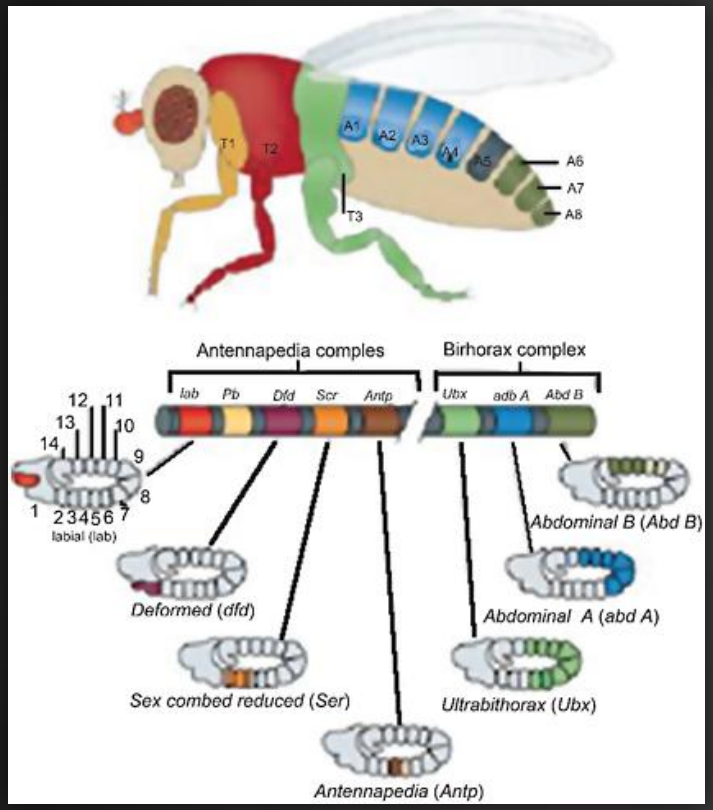
- LacI stability (half-life)

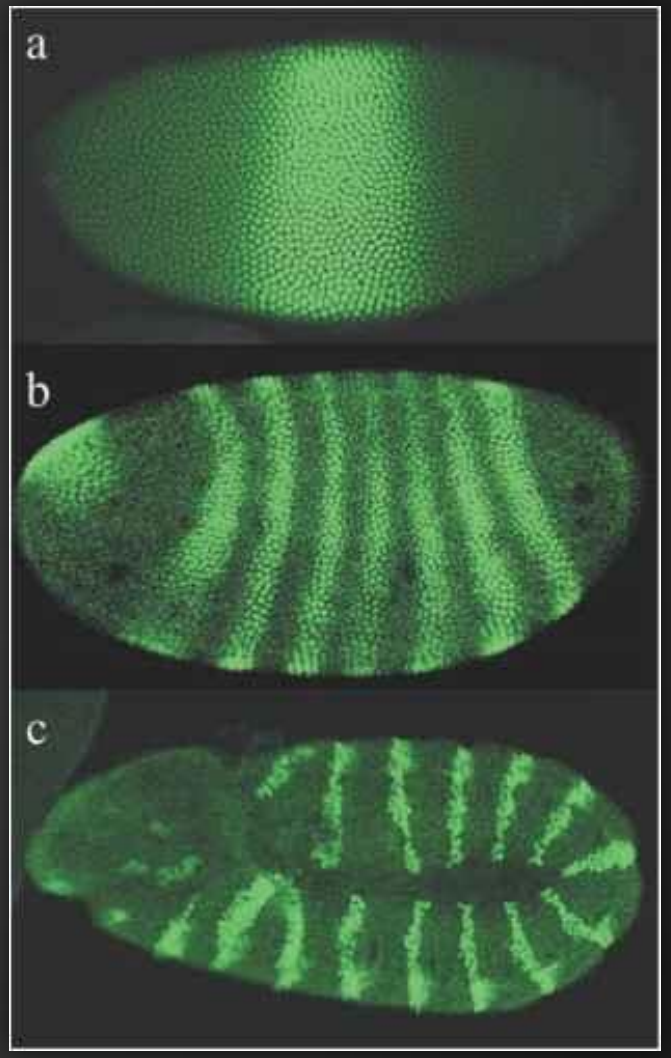




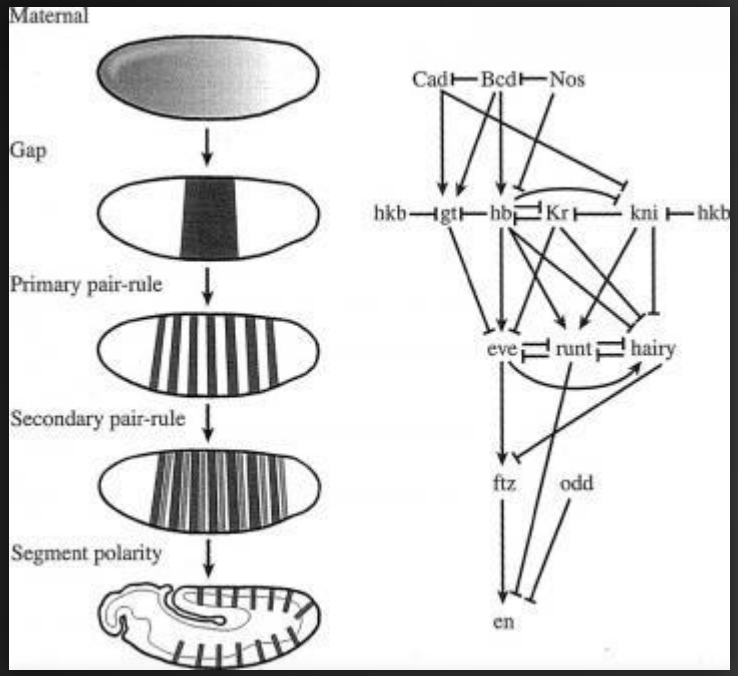
a = Simulation

Natural Example - Drosophila Patterning

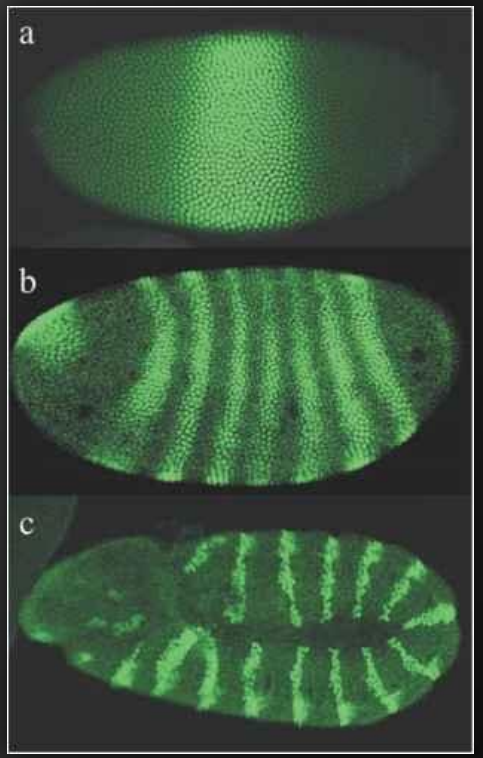


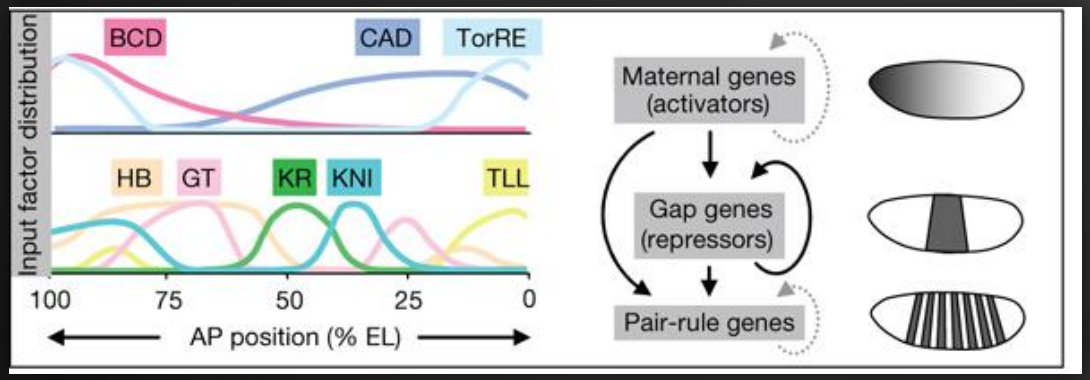


Natural Example - Drosophila Patterning



Initially a Morphogen Gradient
(simple diffusion model)

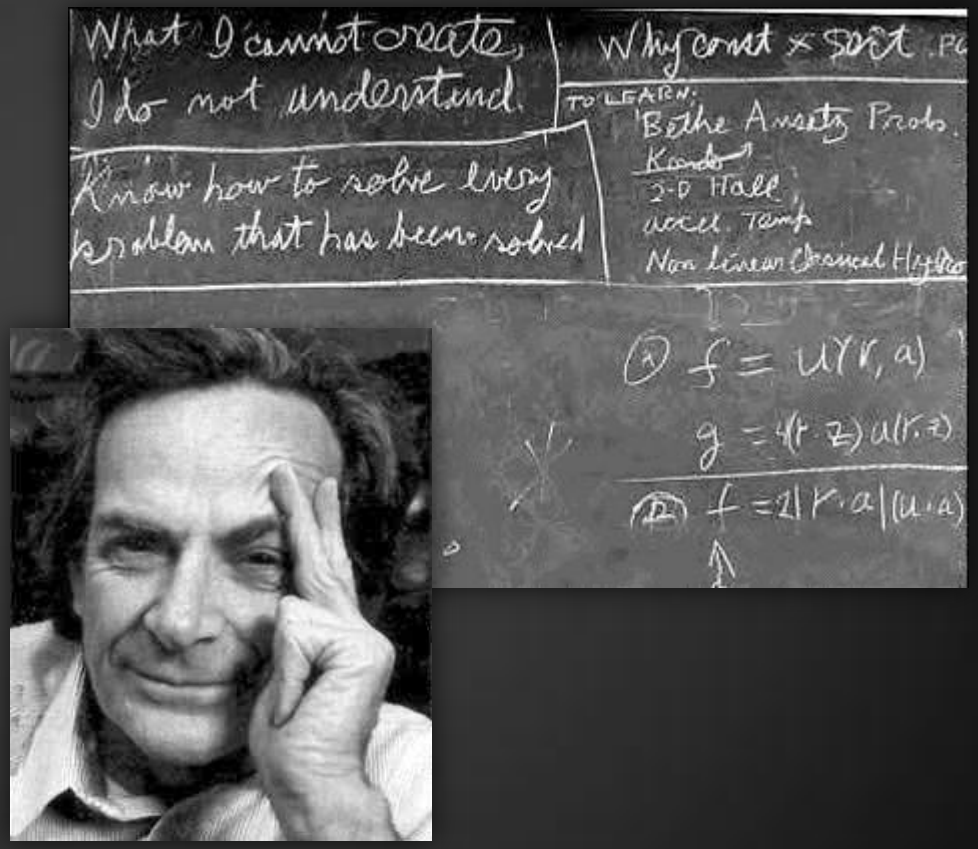




Applications of pattern formation

1. Investigation of natural phenomena

- Drosophila Patterning
- Embryonic Differentiation
- Plant growth
- Species habitation
  (e.g. predator/prey models)



- Turing patterns
- Traffic jams

Applications of pattern formation

2. Artificial versions of natural systems

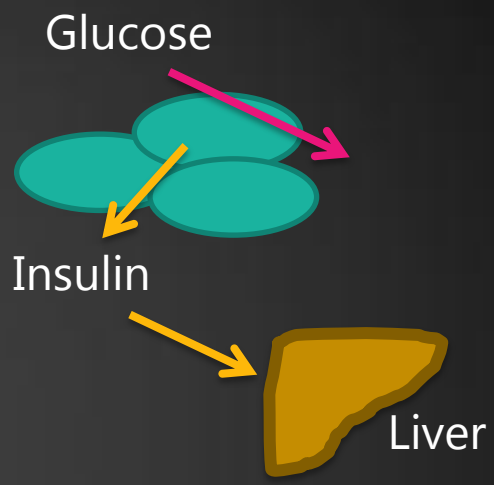
- **Synthetic Insulin Production**

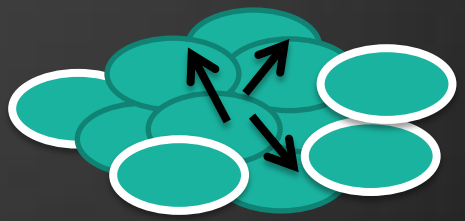
Program implanted stem cells to sense blood glucose and release insulin to replace pancreatic cells

How to stop stem cells over-proliferating?

- Need to sense their own size

- Quorum sensing – e.g. AHL





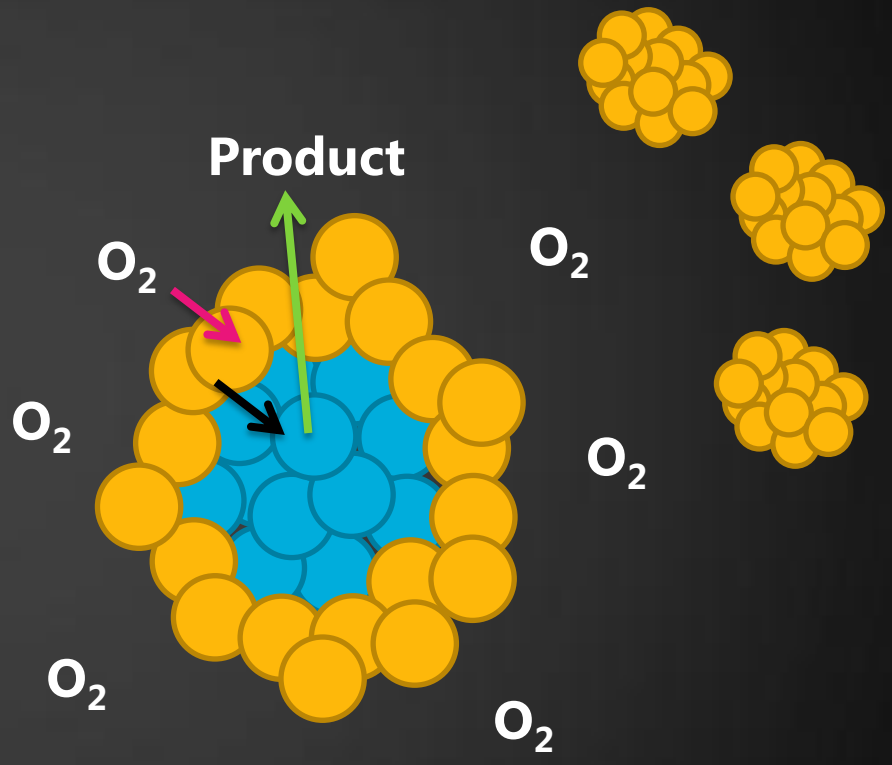
Applications of pattern formation

3. Generation of specialist environments

DIVISION OF LABOUR

Cell engineered to have two versions:
- Surface lovers
- Centre lovers

Activation of different processes can be tied to the version of cell
- Surface lovers use oxygen to make a metabolite
- Centre lovers perform anaerobic reaction on this metabolite to make product



Applications of pattern formation

4. Other applications we've not thought of...

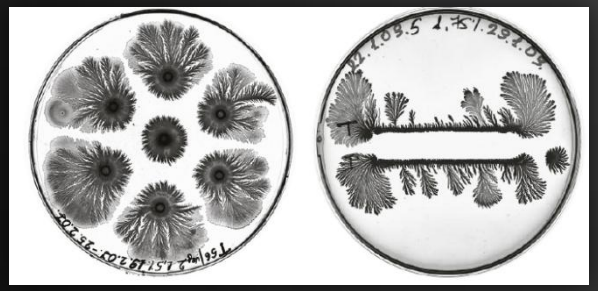
Art / Fashion / Architecture / Technology

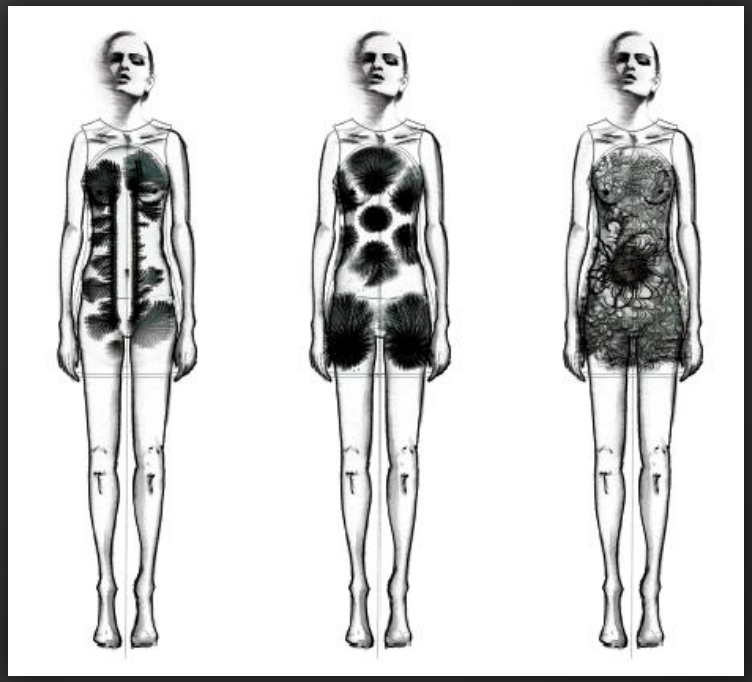
ANY IDEAS?

Applications of pattern formation

4. Other applications we've not thought of...

Art / Fashion / Architecture / Technology





Nicola Morgan
RCA 2011

What you should now know and read up on!

You could get exam questions on...

1. What is morphogenesis and how does nature do it?
2. The reaction-diffusion equation for minimal pattern formation
3. Turing Patterns
4. Bacterial density-dependent patterns generated by HKU iGEM 2008 and ChenLi Liu *et al.* 2011
5. Sender and receiver cell systems as used by Basu *et al.* 2005
6. Examples and Applications of synthetic pattern formation

How would you use multicellularity?

Reading – useful sources and papers

Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation - Science
Kondo S and Miura T (2010) – Also read the supplementary online material

iHKU 2008 iGEM Team Wiki: http://2008.igem.org/Team:iHKU

iHKU 2008 iGEM Team Presentation: http://2008.igem.org/files/presentation/iHKU.pdf

Sequential Establishment of Stripe Patterns in an Expanding Cell Population – Science
Chenli Liu, Fu X, Liu L, Ren X, Chau CKL, Li S, Xiang L, Zeng H, Chen G, Tang L-H, Lenz P, Cui X, Huang W, Hwa T, Jian-Dong Huang (2011)

A synthetic multicellular system for programmed pattern formation - Nature
Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R (2005)

Engineering Gene Networks to Emulate Drosophila Embryonic Pattern Formation – PLoS Biology
Isalan M, Lemerle C, Serrano L (2005)

Essential Cell Biology (Alberts *et al.*) Chapter 8 page 282