Synthetic Biological Systems

1. Pattern Formation

Biological Patterns

CSYNBI
Centre for Synthetic Biology and Innovation

Imperial College
London

Biological Patterns

Dr Tom Ellis

CSynBI
Centre for **Sy**nthetic **Bi**ology and Innovation

Imperial College
London

Biological Patterns

Lecture Content

In this lecture we'll learn about:

1. What is morphogenesis
2. The reaction diffusion equation
3. Theoretical systems that generate patterns – Turing Patterns

4. An iGEM project to generate bacteria that swim into patterns
5. A synthetic biology pattern using communicating cells
6. A synthetic version of the Drosophila patterning system

7. Potential applications of synthetic pattern formation

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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 <u>division of labour</u>

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
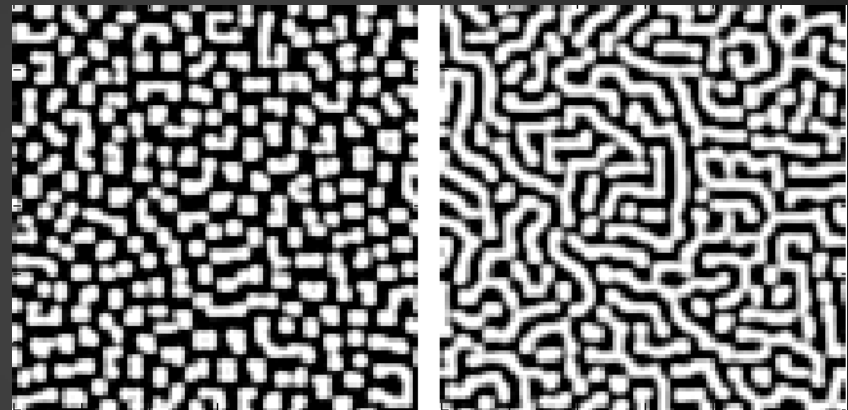
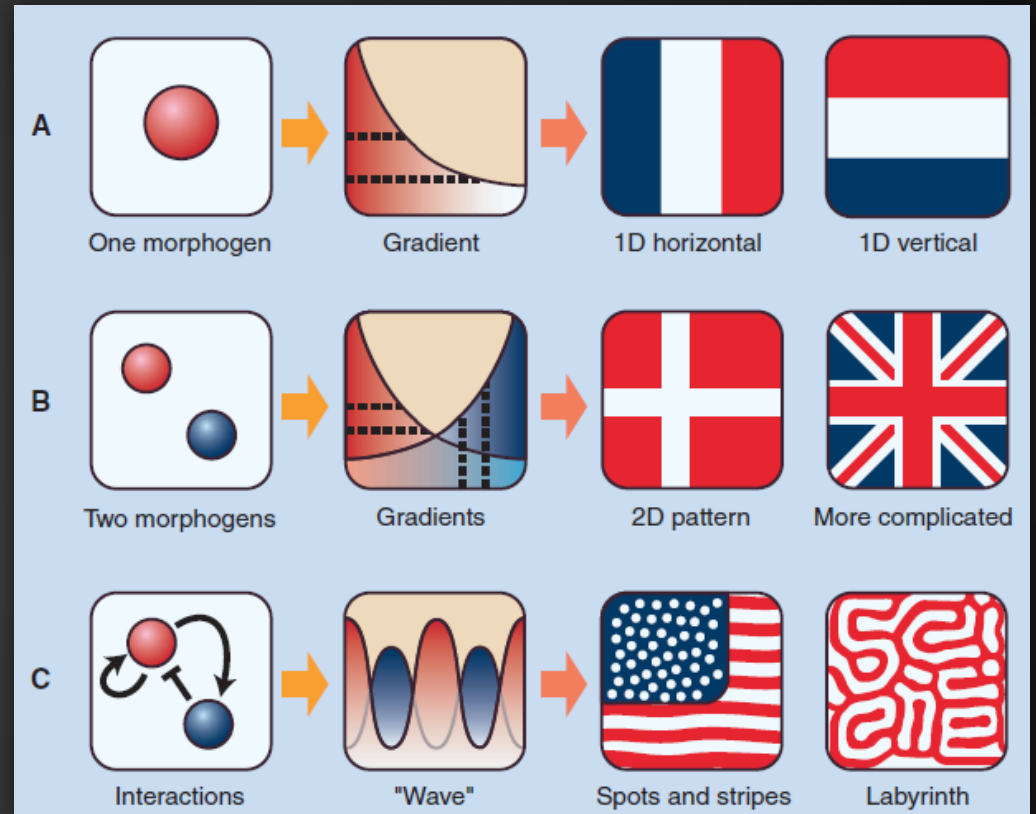Minor fluctuations give rise to sustained patterns

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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

A
One morphogen → Gradient → 1D horizontal — 1D vertical

B
Two morphogens → Gradients → 2D pattern — More complicated

C
Interactions → "Wave" → Spots and stripes — Labyrinth

The Reaction-Diffusion Equation

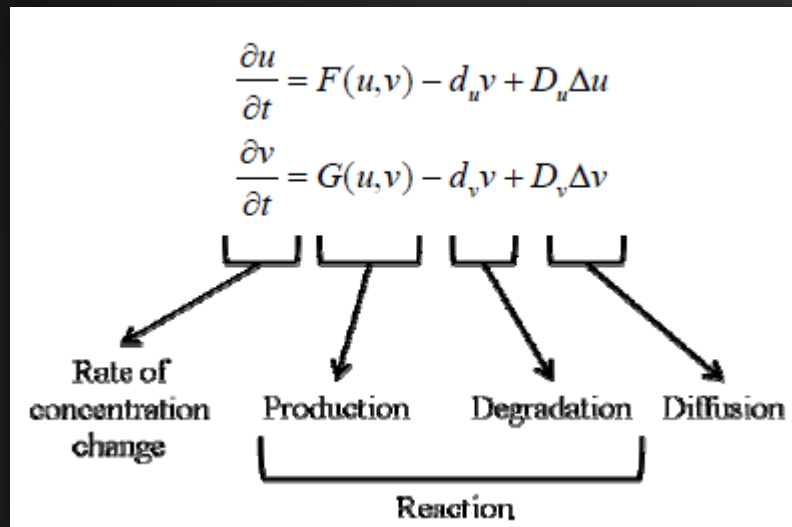Two interacting molecules diffusing can generate stable patterns – Turing 1952

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.

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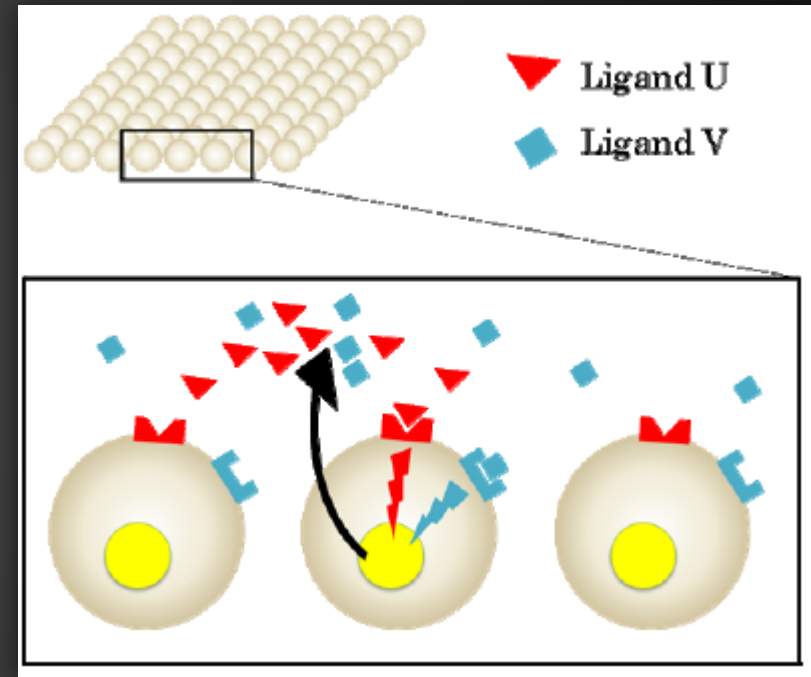
Replacing F and G by following linear function, we get the partial differential equation identical to that of Turing's original paper.

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

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

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.
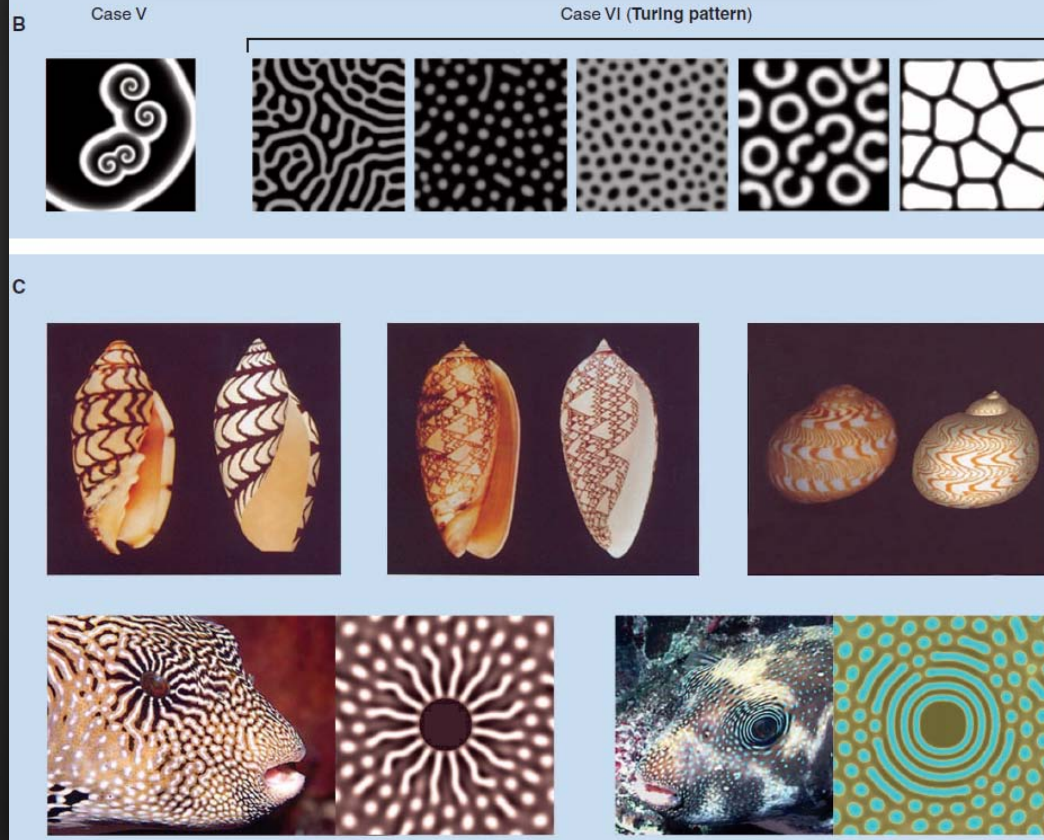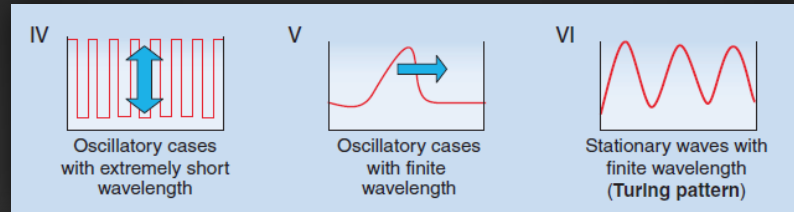
The Reaction-Diffusion Equation

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

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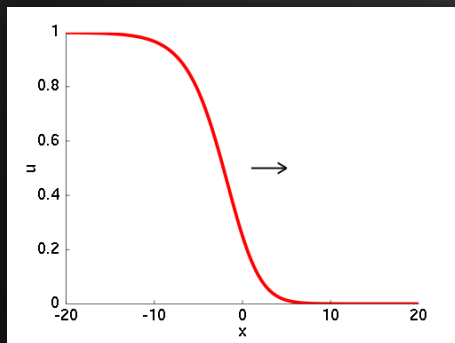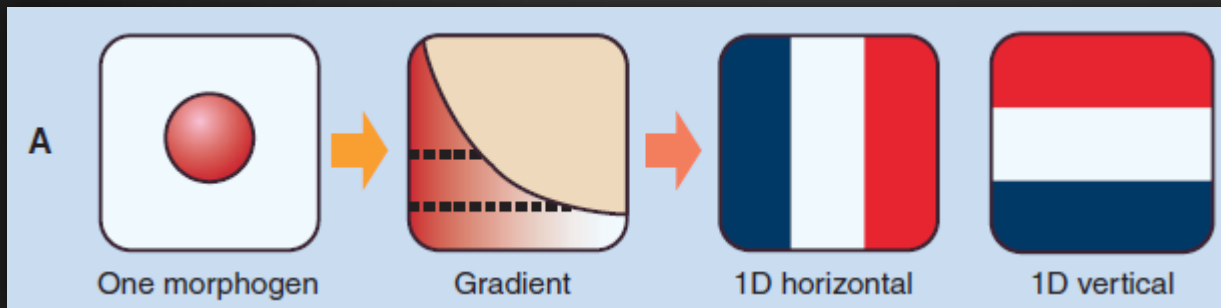$$G(u,v) - d_v v = a_v u + b_v v + c_v$$
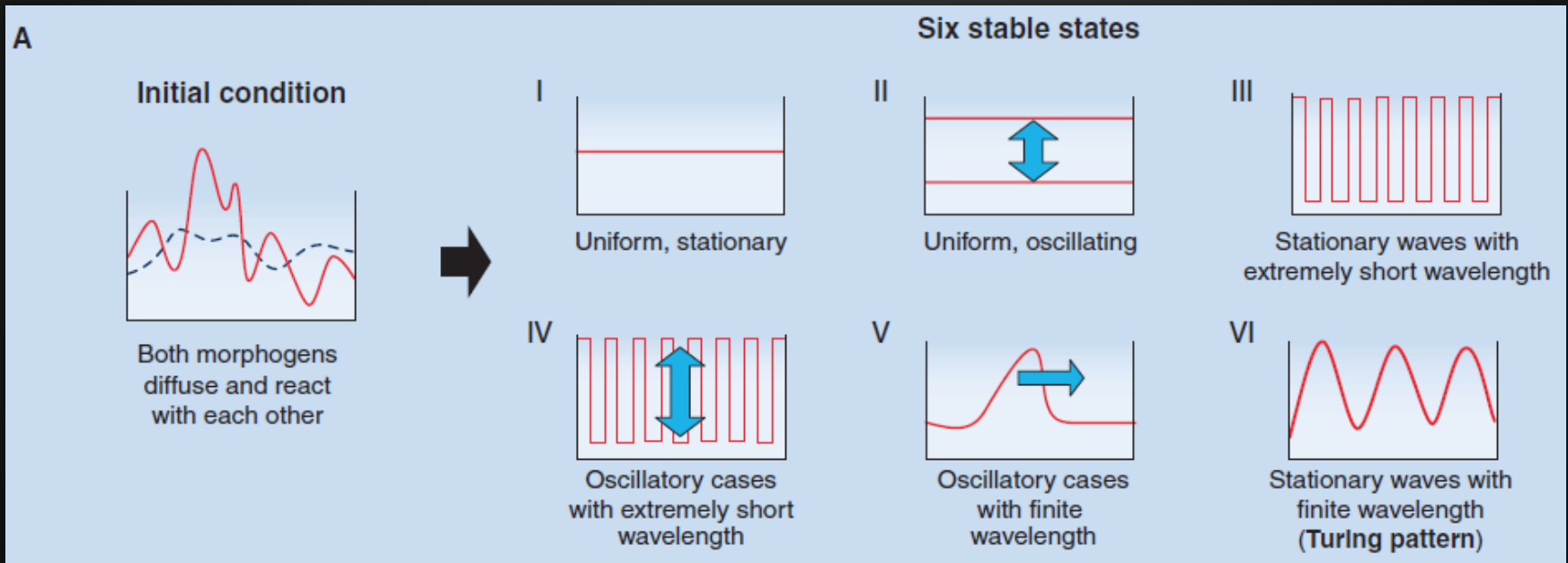
The Reaction-Diffusion Equation

Minimal Mathematical Patterning

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

Morphogen Gradient

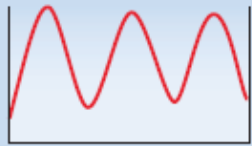Fisher's Equation for simulation of propagation of a gene in a population

Generates a curved wave-front
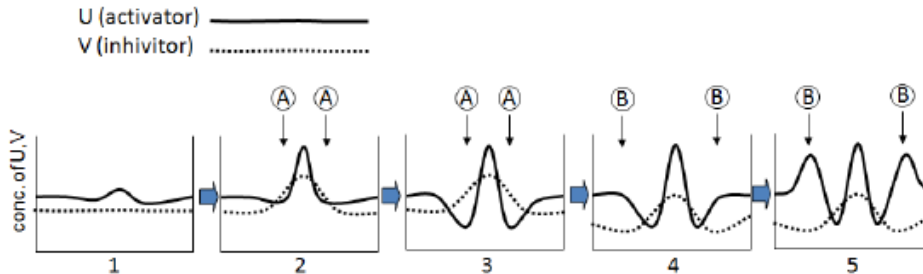
$$\frac{\partial u}{\partial t} = \Delta u + f\left(u\right),$$

Minimal Mathematical Patterning

VI

Stationary waves with finite wavelength (**Turing pattern**)

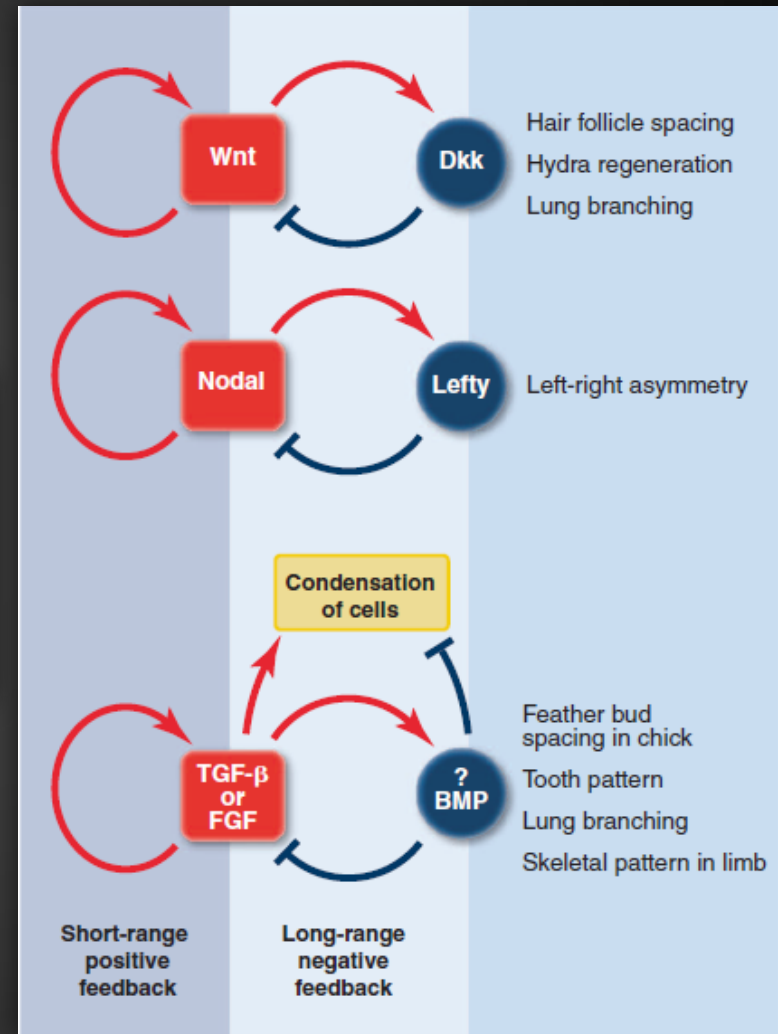Two component system with feedback gives the most interesting case: Turing Pattern

U (activator) ⎯⎯⎯
V (inhivitor) ·········

Requires:

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

Wnt — Dkk — Hair follicle spacing / Hydra regeneration / Lung branching

Nodal — Lefty — Left-right asymmetry

Condensation of cells

TGF-β or FGF — ? BMP — Feather bud spacing in chick / Tooth pattern / Lung branching / Skeletal pattern in limb

Short-range positive feedback — Long-range negative feedback

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London

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)
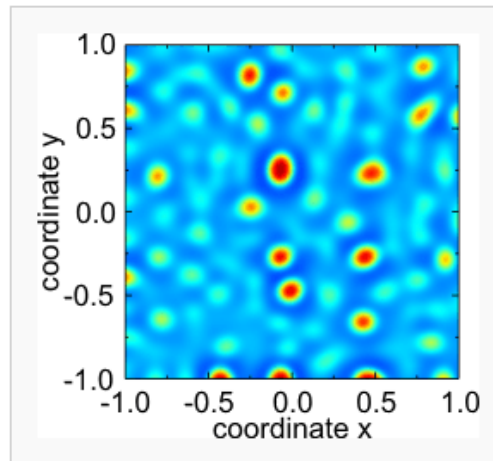
FitzHugh–Nagumo equation

$$\partial_t u = d_u^2 \nabla^2 u + f(u) - \sigma v,$$
$$\tau \partial_t v = d_v^2 \nabla^2 v + u - v$$
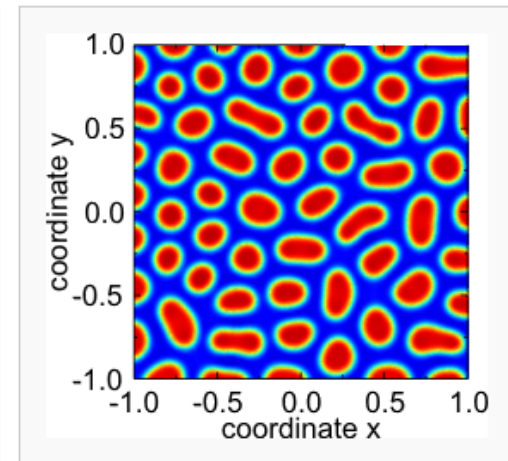
with $f(u) = \lambda u - u^3 - \kappa$

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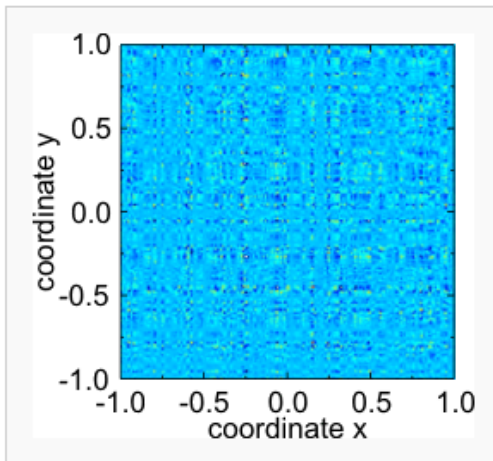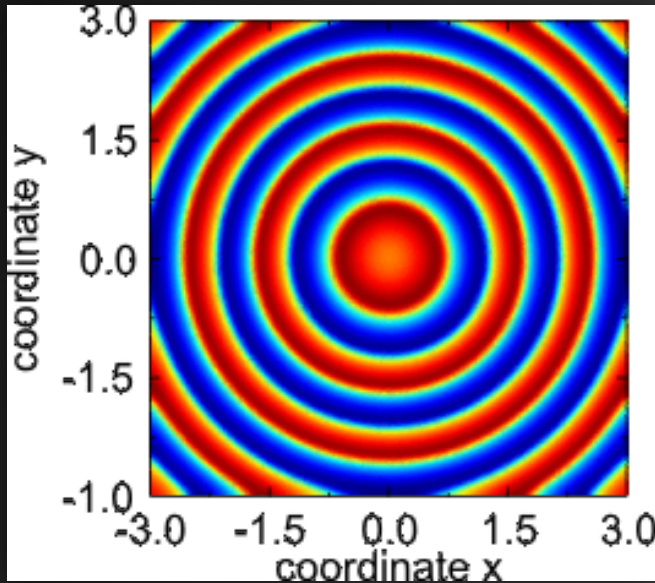

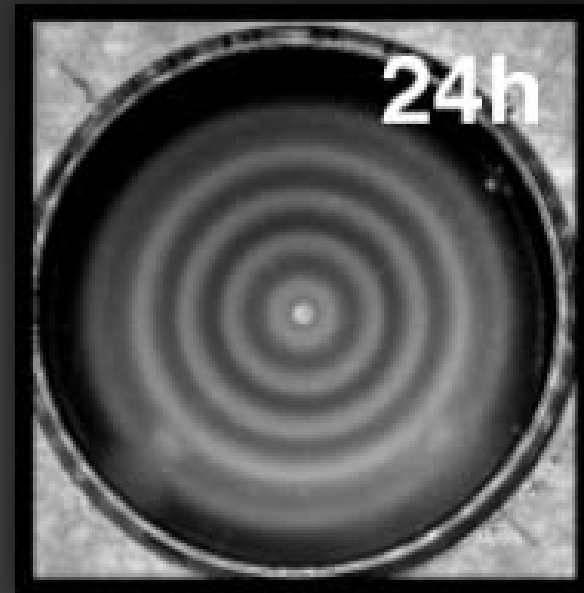Noisy initial conditions at $t = 0$.   State of the system at $t = 10$.   Almost converged state at $t = 100$.

Synthetic Biology Patterning

Simulated Turing Pattern

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

24h

Engineered *E. coli* growth pattern

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

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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
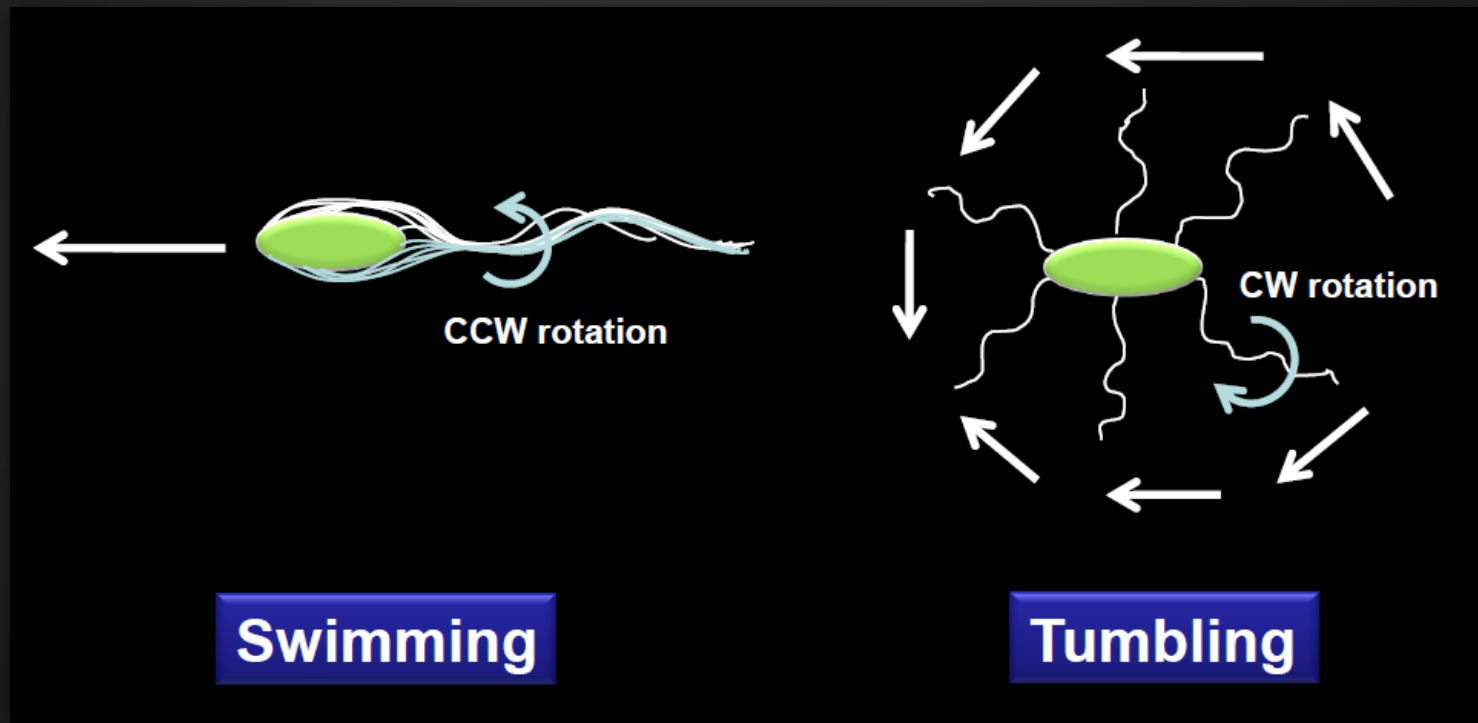3. Methods for tuning key parameters

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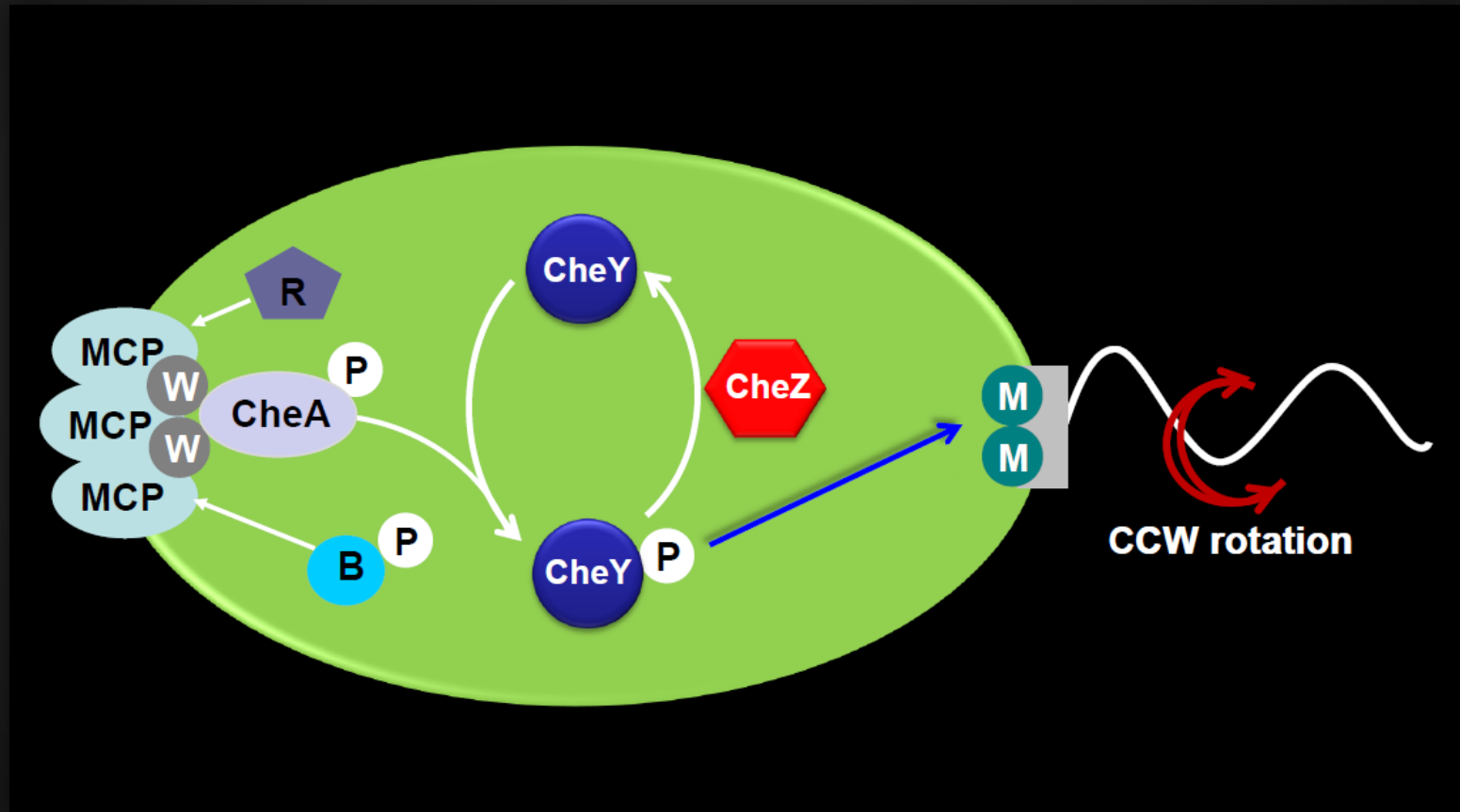2. Time-lapse imaging

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

iHKU iGEM 2008

Motility of *E. coli*

iHKU iGEM 2008

Mechanism of motility in *E. coli*

CSYNBI
Centre for Synthetic Biology and Innovation
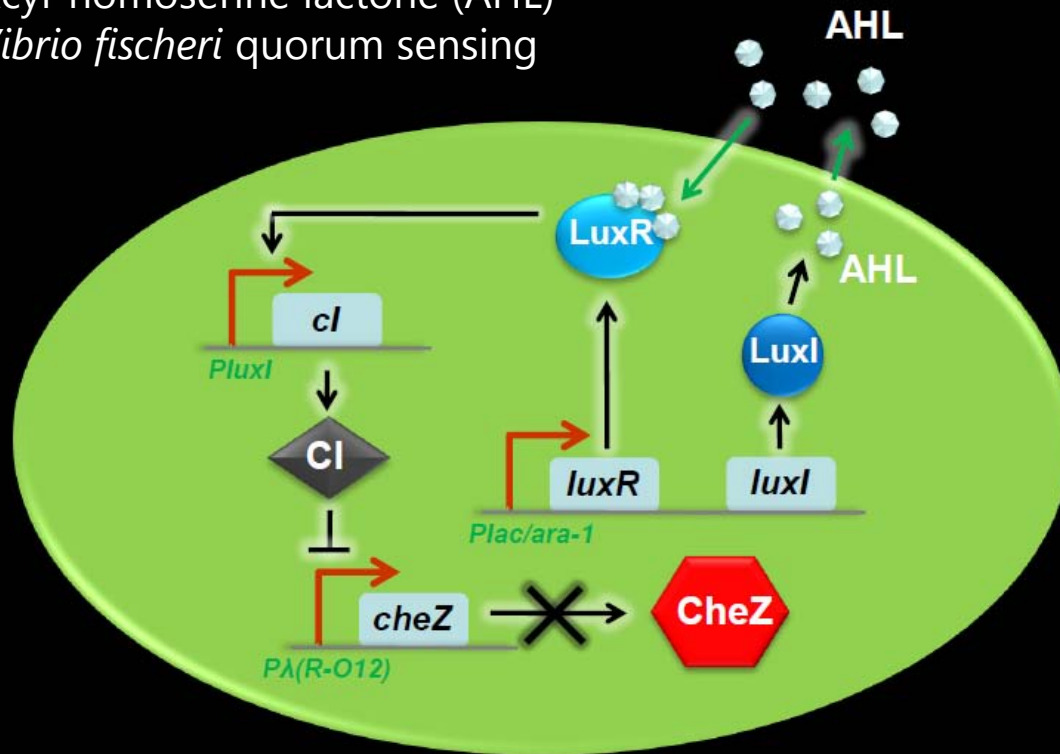
Imperial College
London

Synthetic control of CheZ = control of motility in *E. coli*
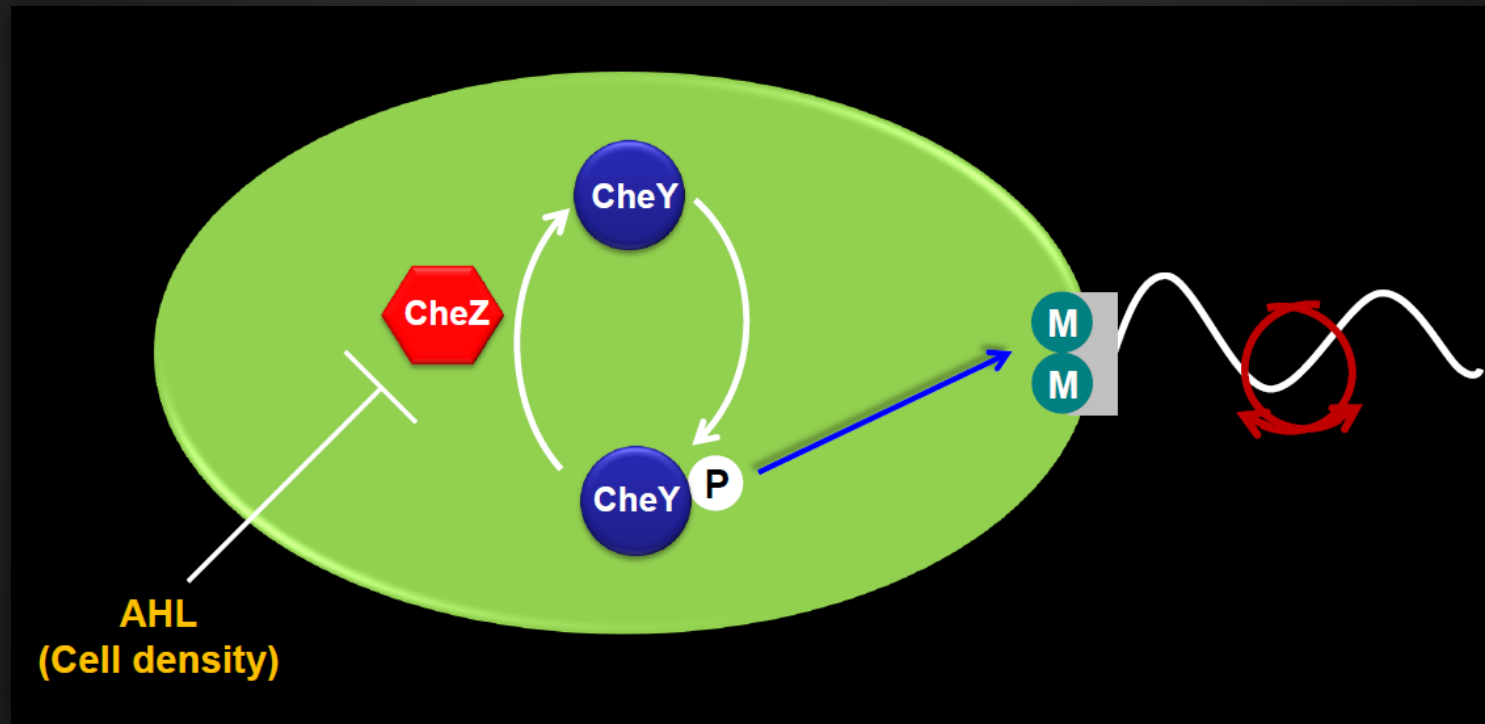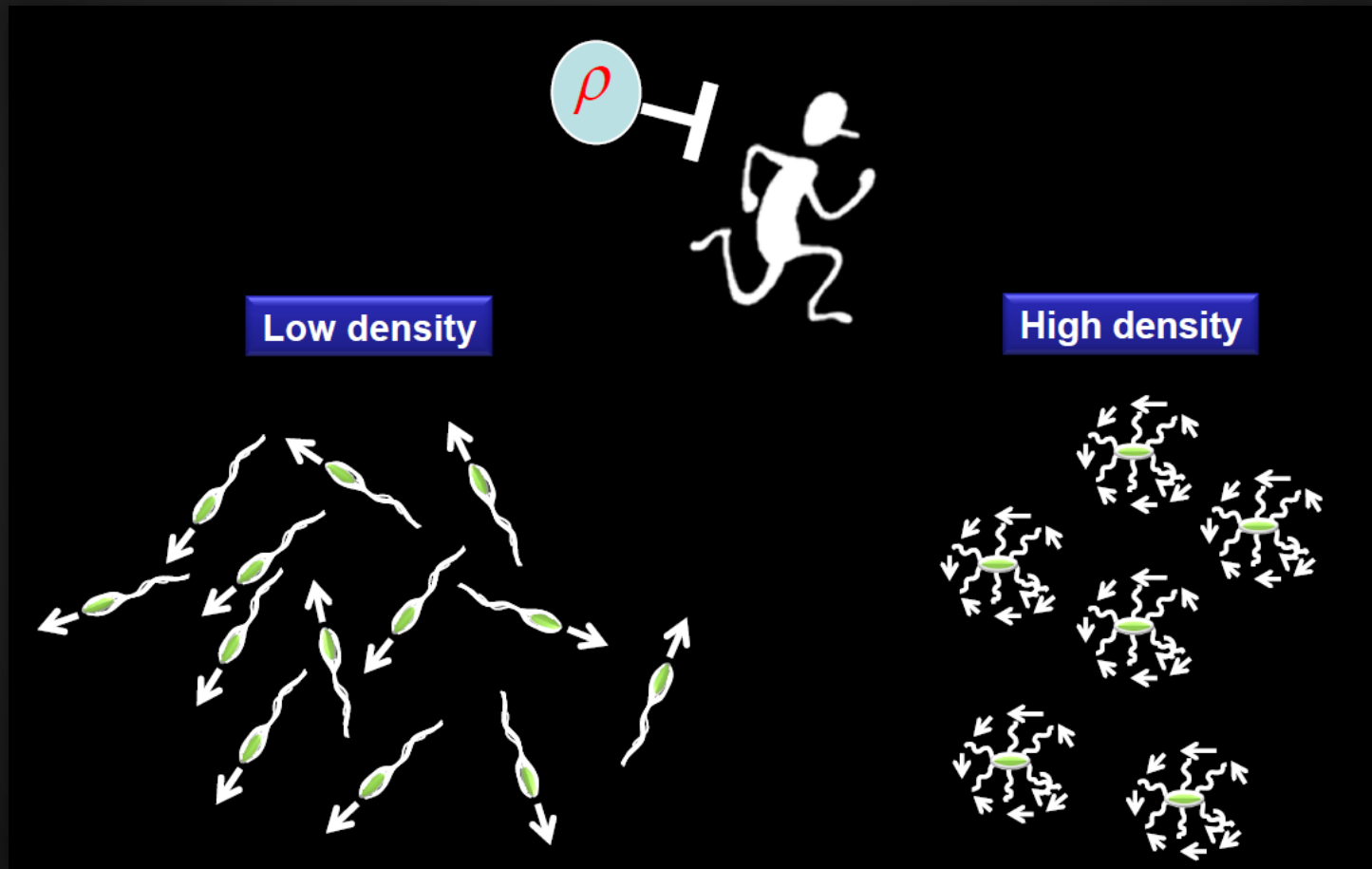
iHKU iGEM 2008

Density-dependent control of CheZ

Acyl-homoserine lactone (AHL)
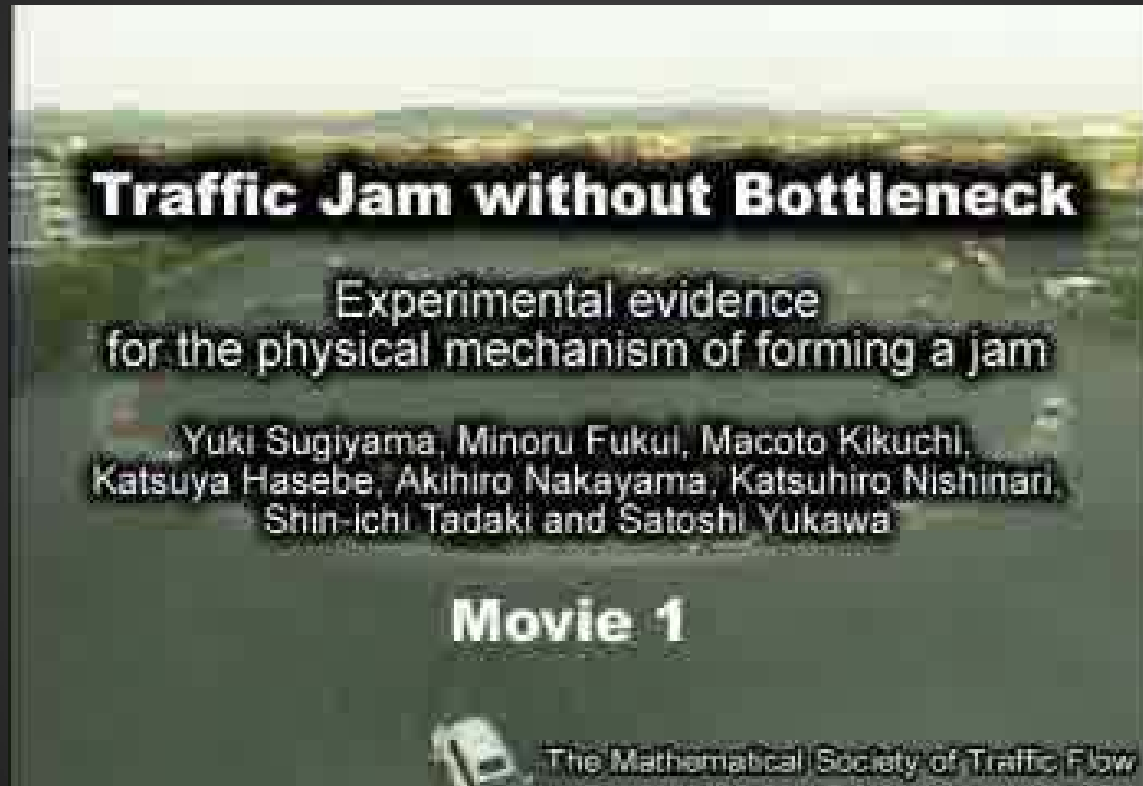*Vibrio fischeri* quorum sensing

iHKU iGEM 2008

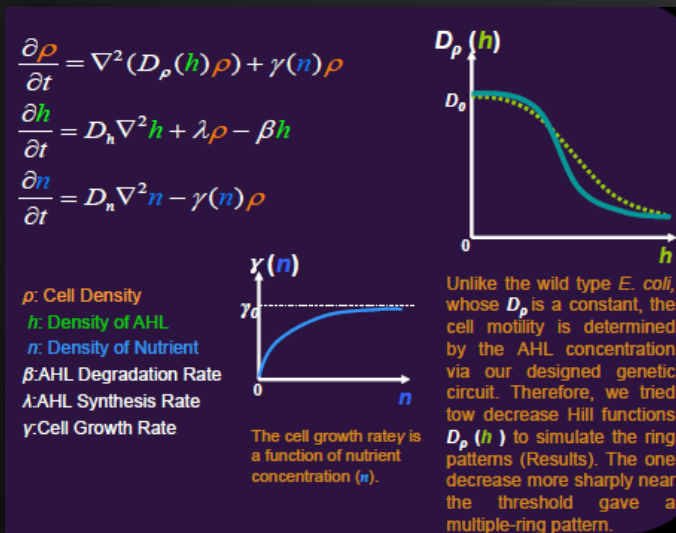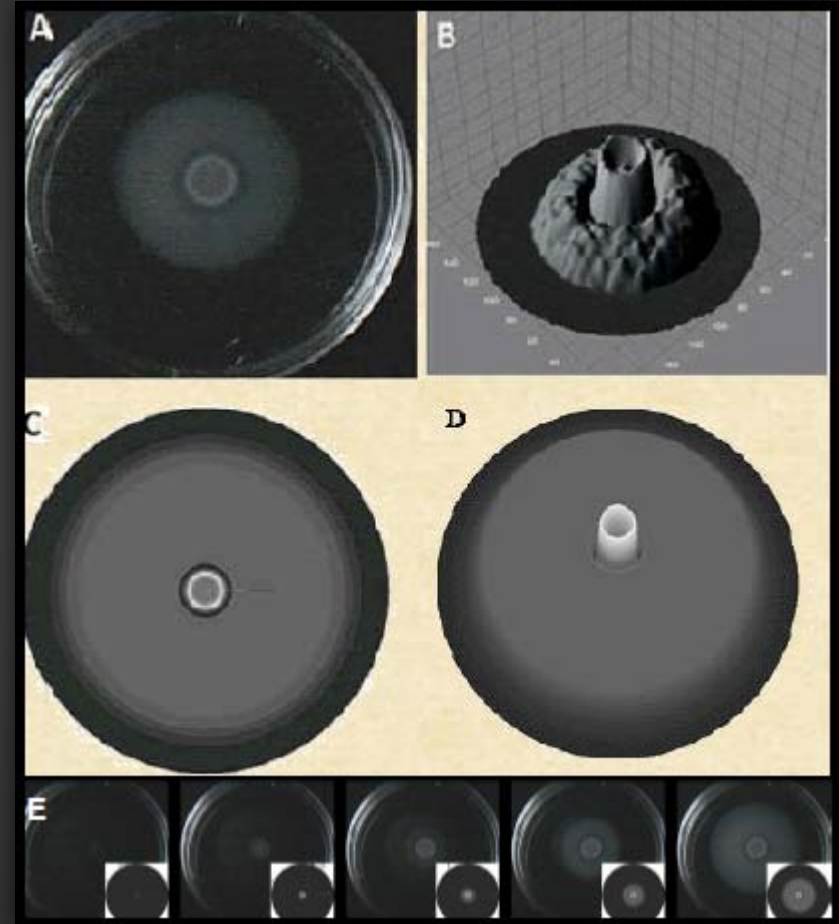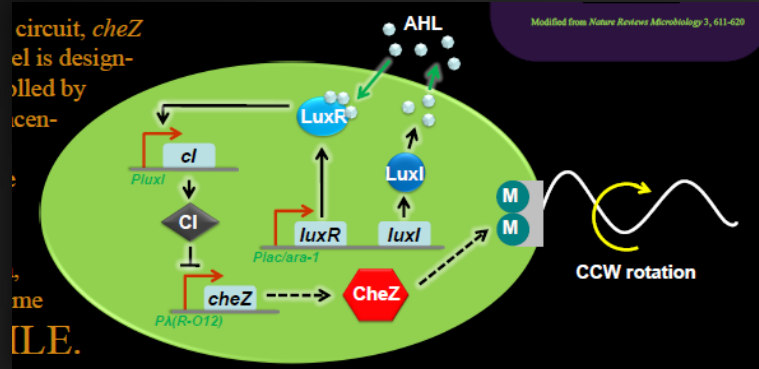## Creation of a low-density mover

iHKU iGEM 2008

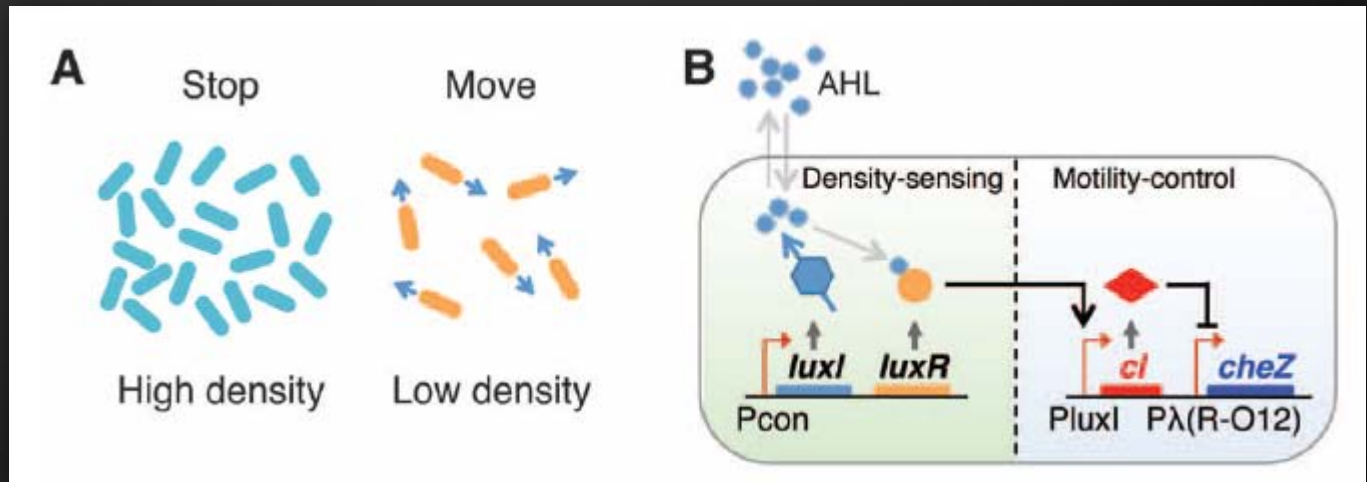## Traffic is a low-density mover

iHKU iGEM 2008

Design, Model and Some Results – but only a Bronze

CSYnBI
Centre for Synthetic Biology and Innovation
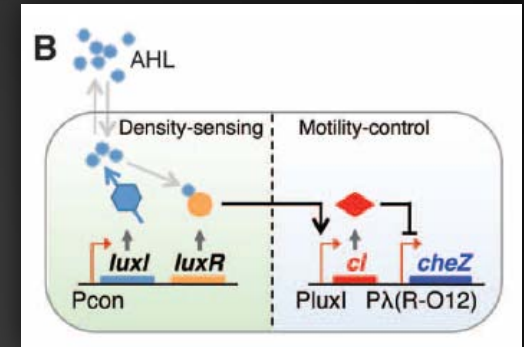
Imperial College
London

ChenLi Liu *et al.* Science 2011

3 years later.....
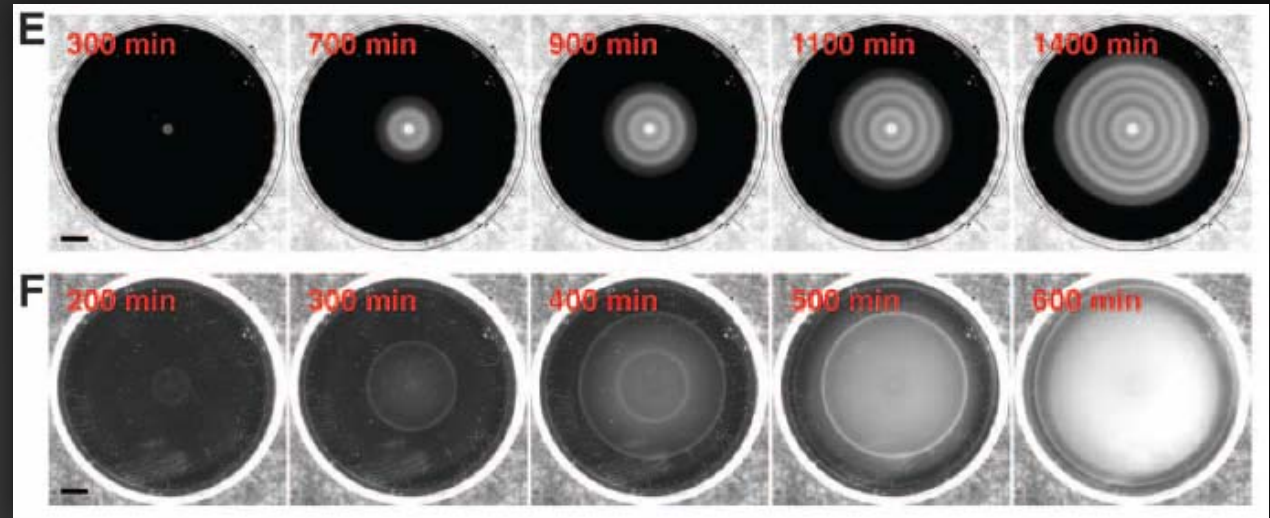
ChenLi Liu *et al.* Science 2011

ChenLi Liu *et al.* Science 2011

Model of the system   (shown here is the updated 2011 model)

3 partial differential equations

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

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

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

1. Stochastic swim-and-tumble motion of cells described as a diffusion equation at population level for the cell density $\rho(x, t)$ (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
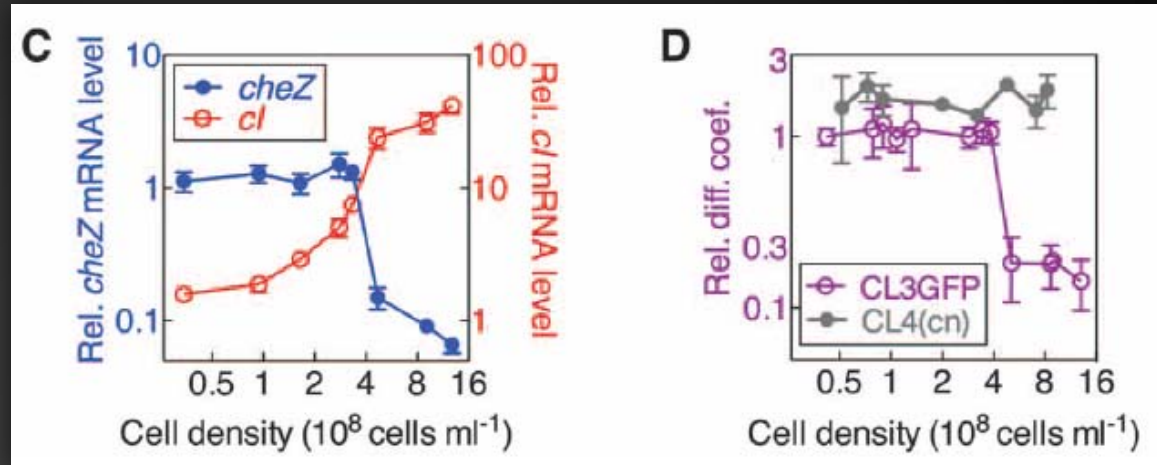 γ = cell growth rate

$\mu(h)$ = AHL-dependent motility

ChenLi Liu *et al.* Science 2011
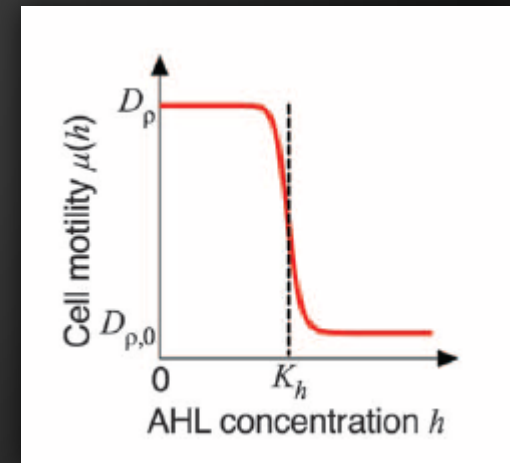
Model of the system

Getting the parameters

• Q-RTPCR to measure mRNA expression

• Track diffusion of cells using microscopy

• Dilute cells to different cell densities

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

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

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

ChenLi Liu *et al.* Science 2011

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

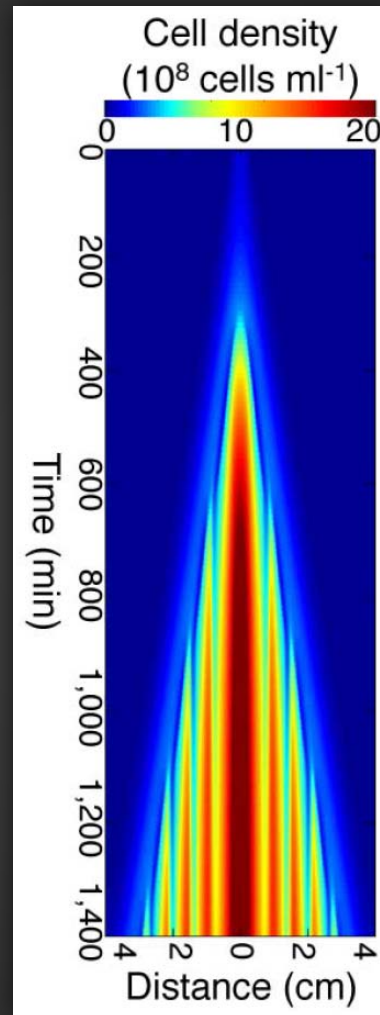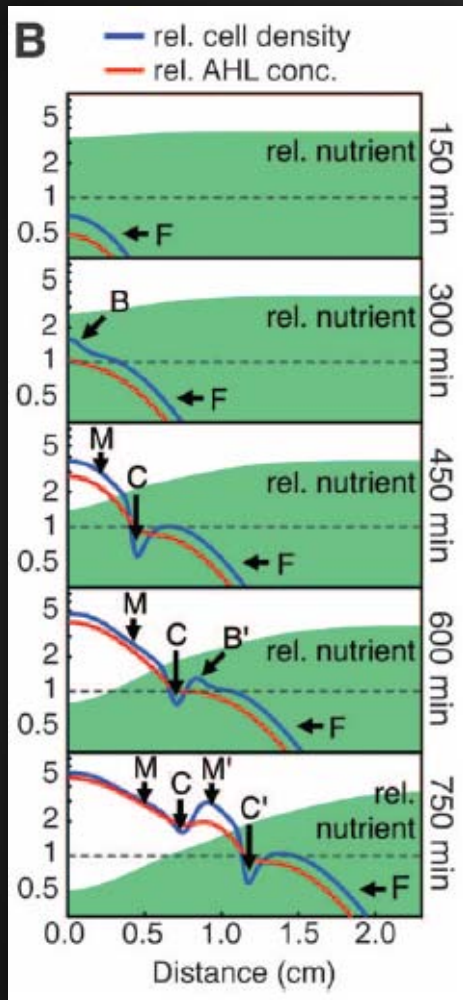# ChenLi Liu *et al.* Science 2011

Model of the system

3 partial differential equations

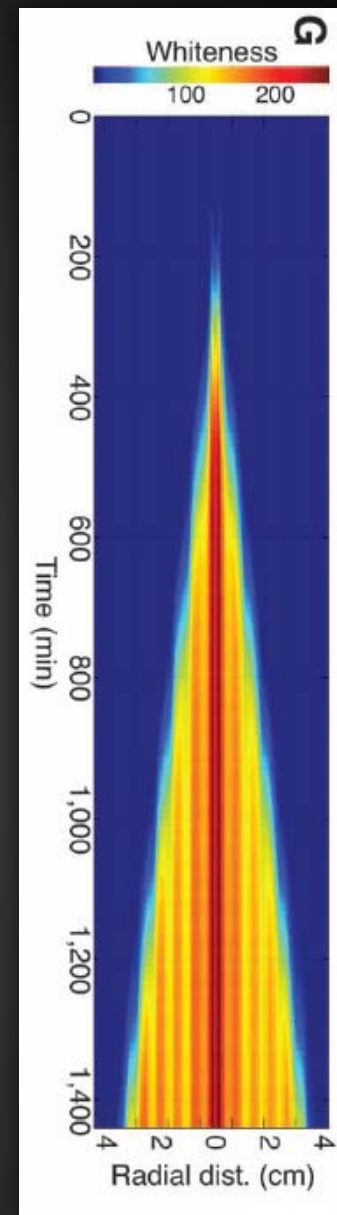$$\frac{\partial \rho}{\partial t} = \nabla^2[\mu(h)\rho] + \frac{\gamma n^2 \rho}{n^2 + K_n^2} \quad [1]$$

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

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

CSYNBI
Centre for Synthetic Biology and Innovation

Imperial College
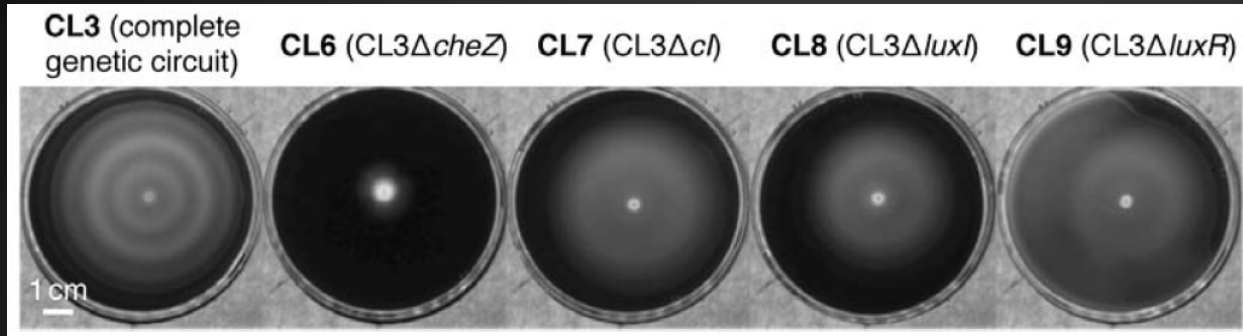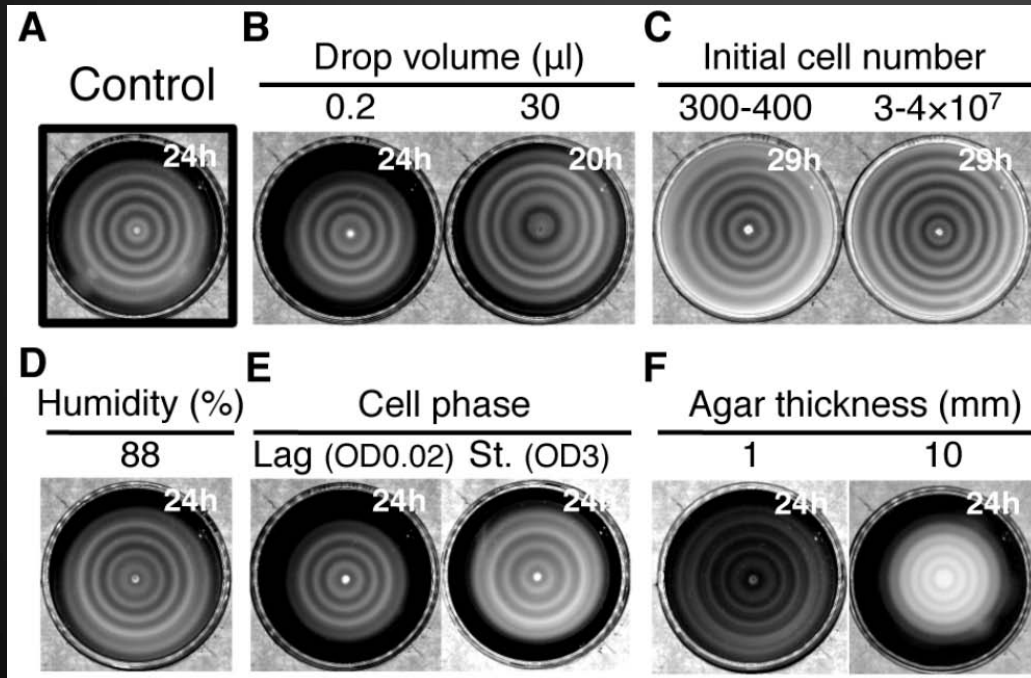London

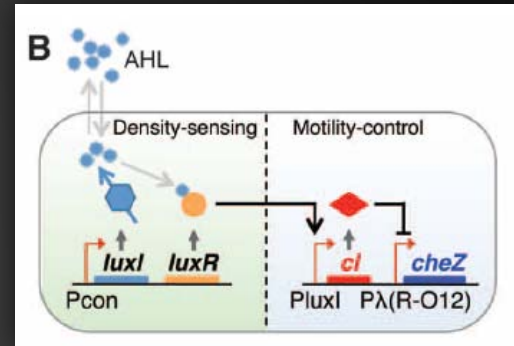# ChenLi Liu *et al.* Science 2011

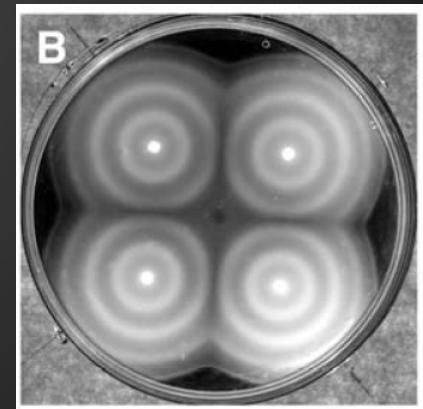

Simulation

Experiment

ChenLi Liu *et al.* Science 2011

Only the complete system works
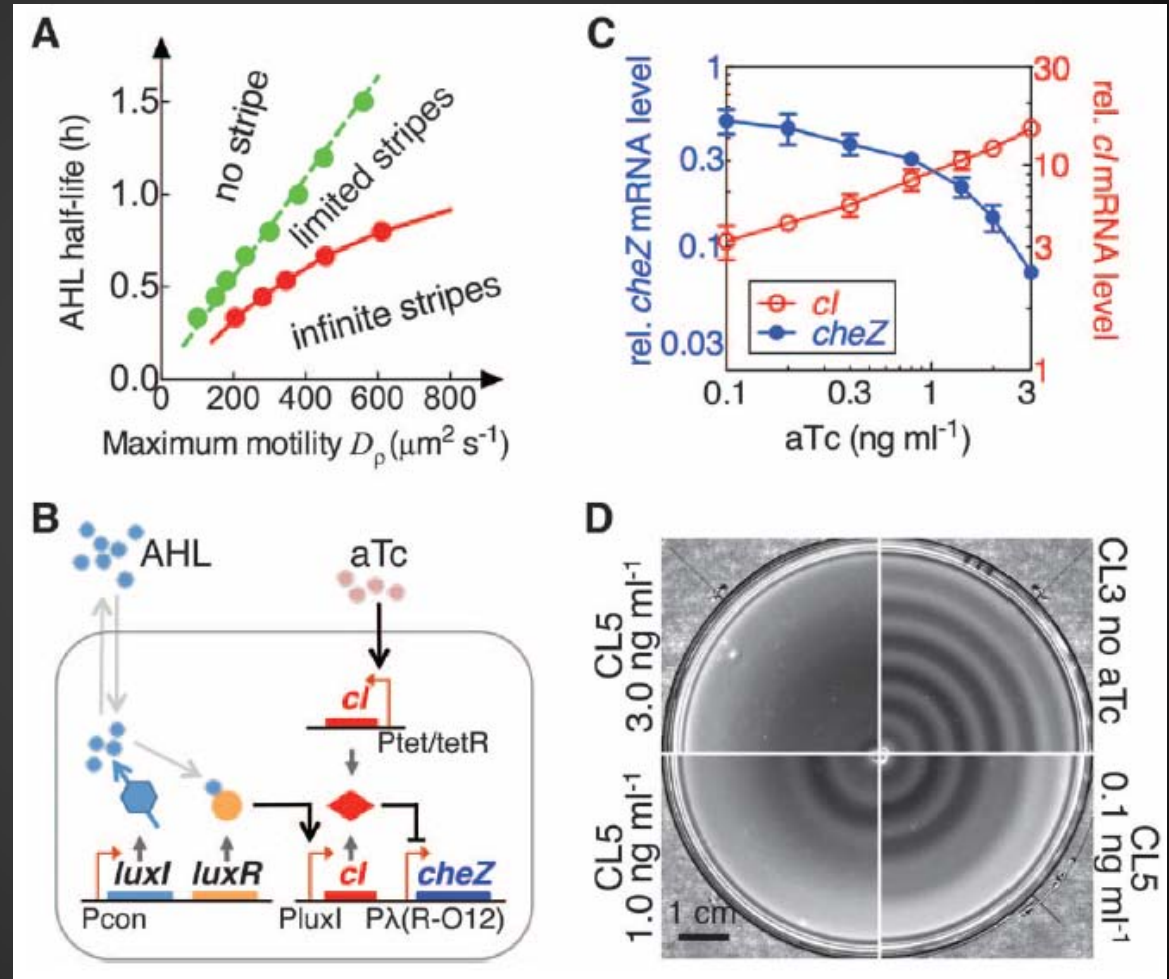
Patterns are robust

ChenLi Liu *et al.* Science 2011

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

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 $t = 100$.

Cell density premixed in agar (cells ml^{-1})

10^4 10^3 10^2 10

1 cm

CSynBI
Centre for Synthetic Biology and Innovation
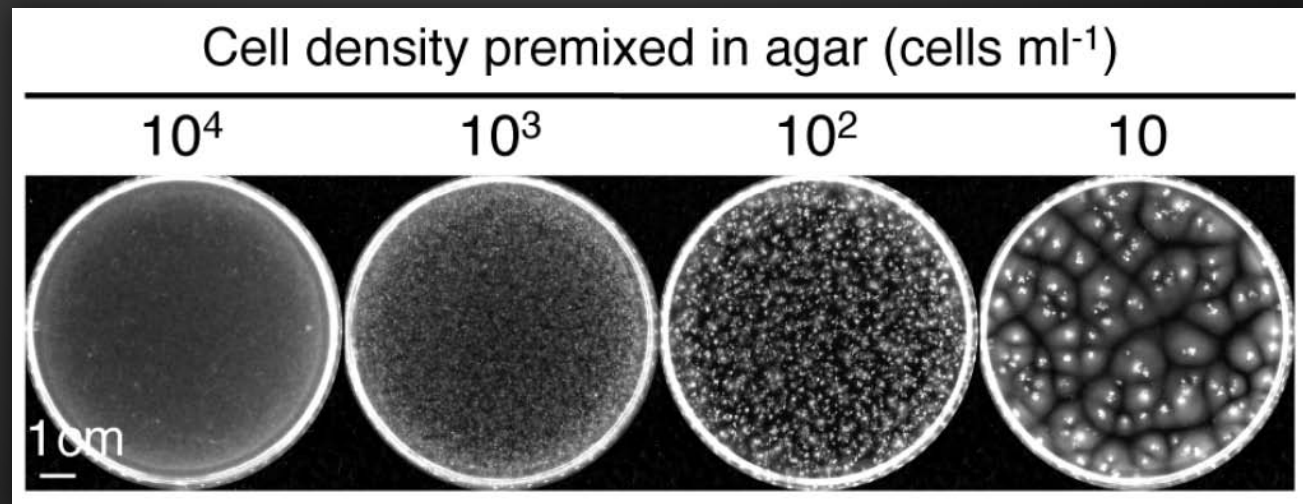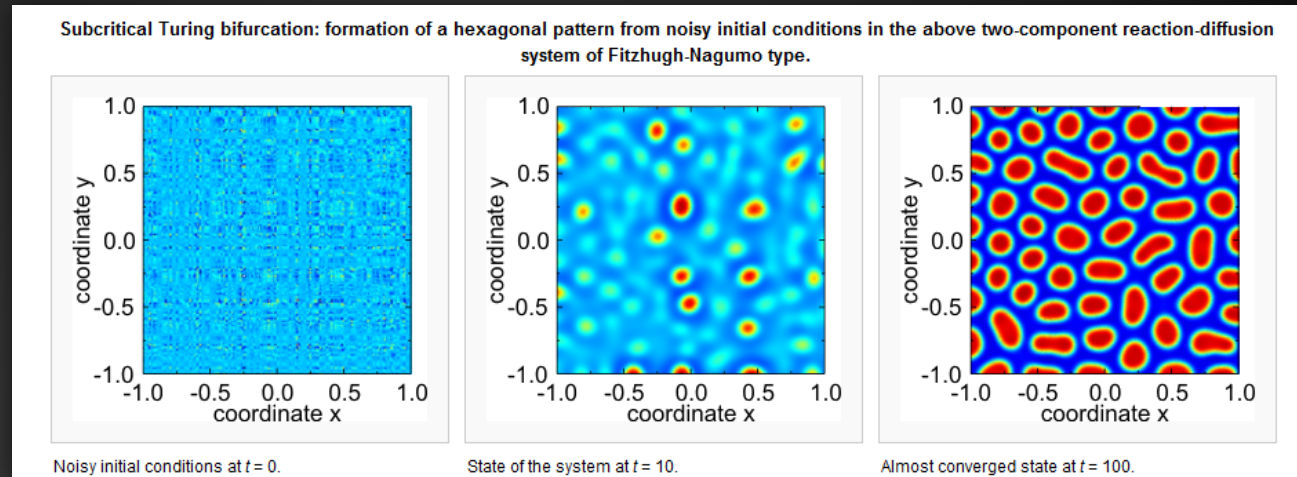
ChenLi Liu *et al.* Science 2011

One final experiment…   pattern forms due to 'effective aggregation'
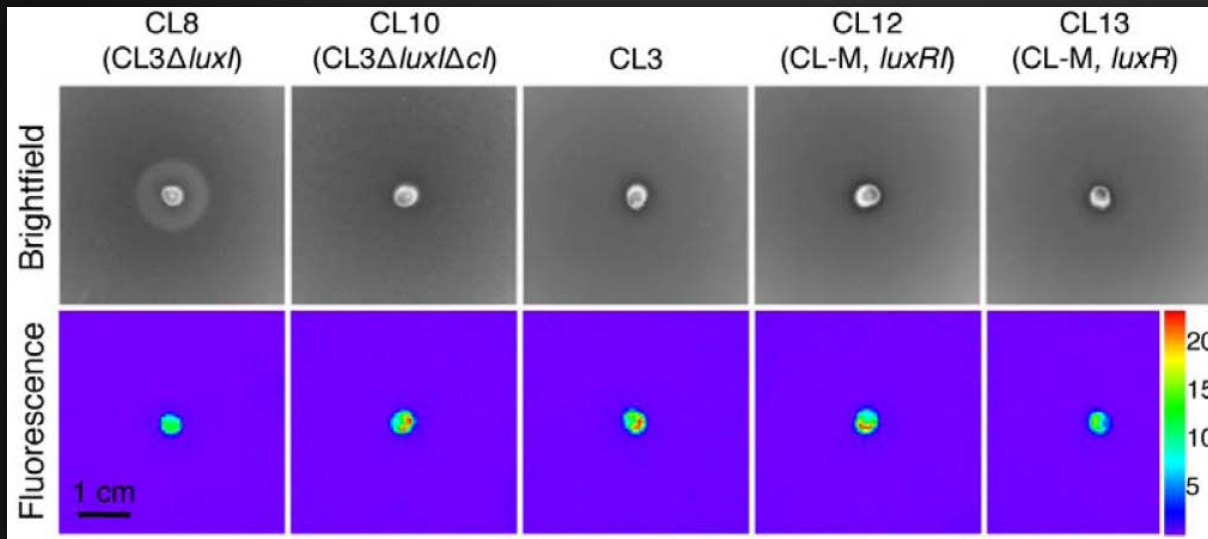
Prove this using split system of:
a) Sender cells
b) Receiver cells

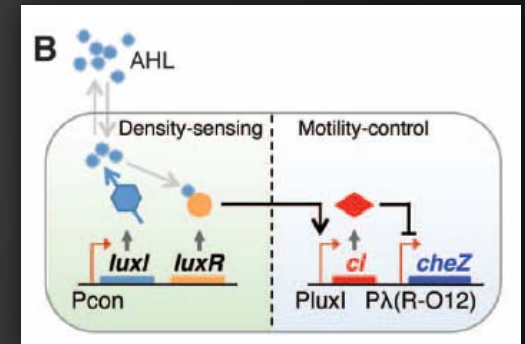
Cleft zone results from an aggregation phenomenon driven by density-dependent motility. Cells can diffuse freely in semisolid agar when the cell density is low. As cells proliferate and the local AHL level exceeds the threshold, cell motility slows down as programmed. These cells cannot move away, but 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.
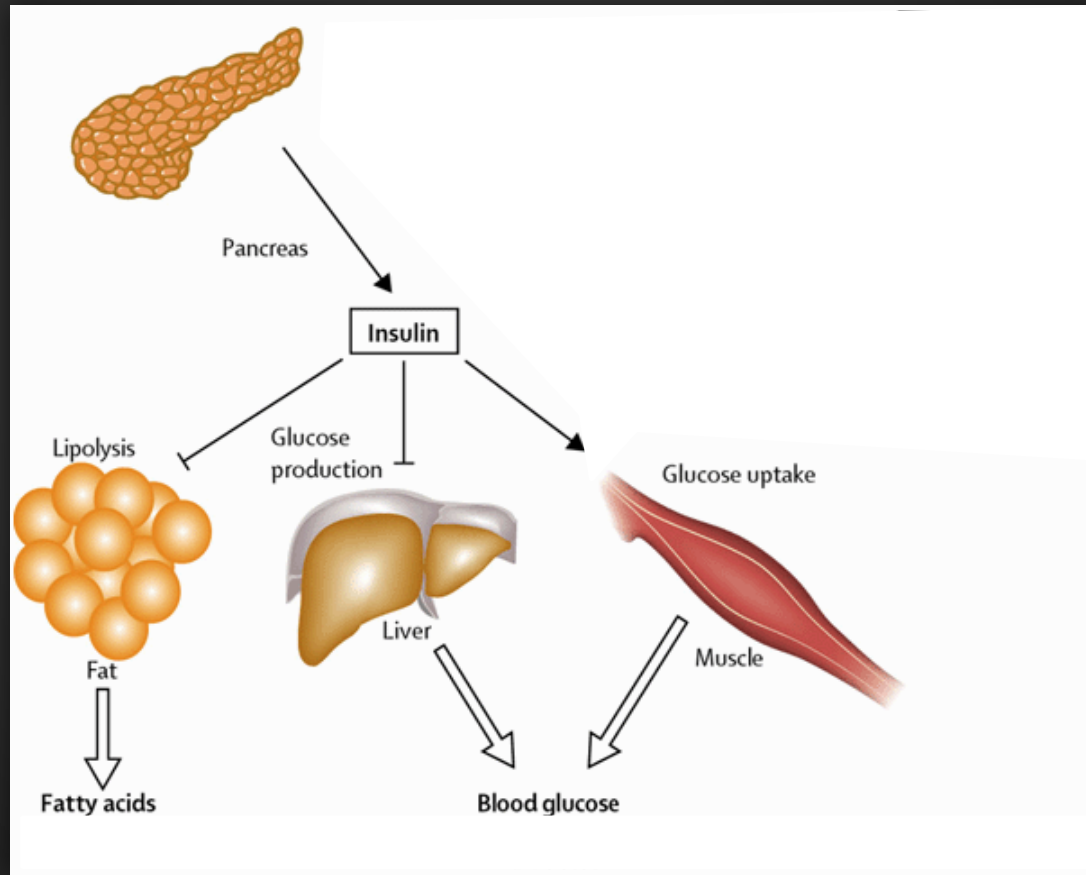
Sender and Receiver Cells

- 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

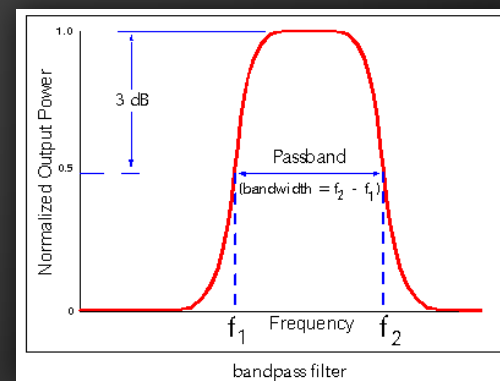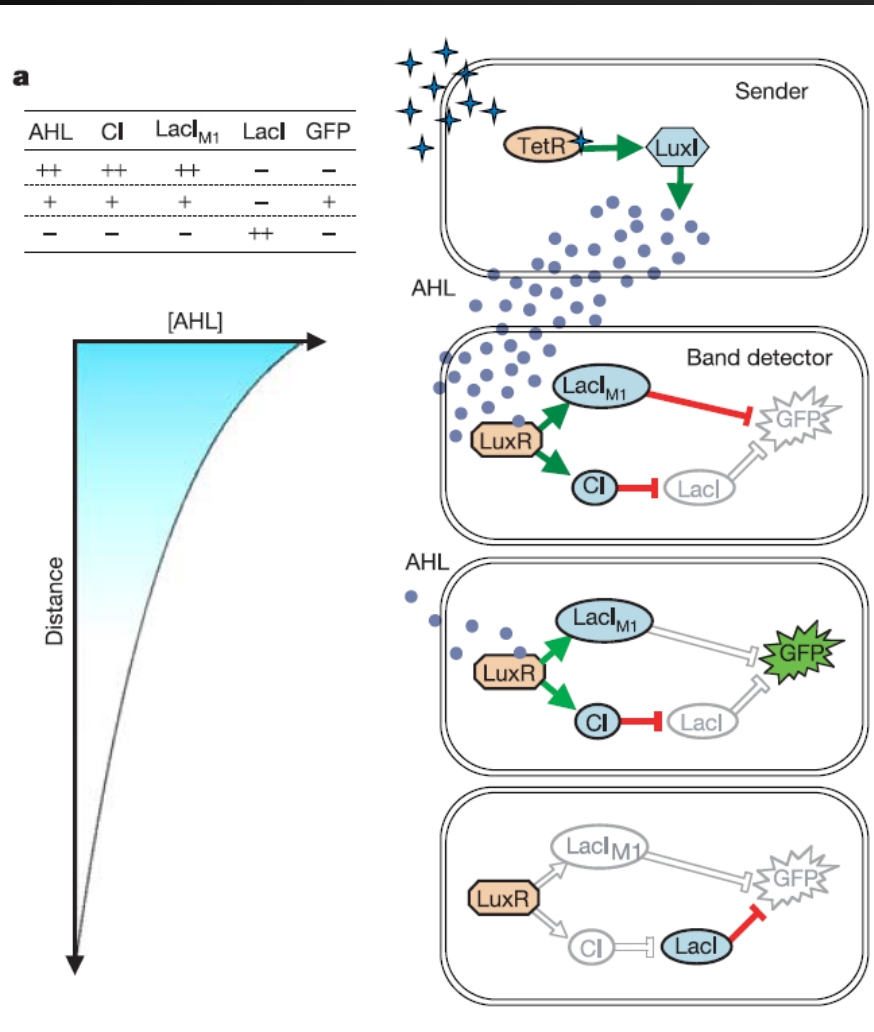# Basu *et al.* Nature 2005
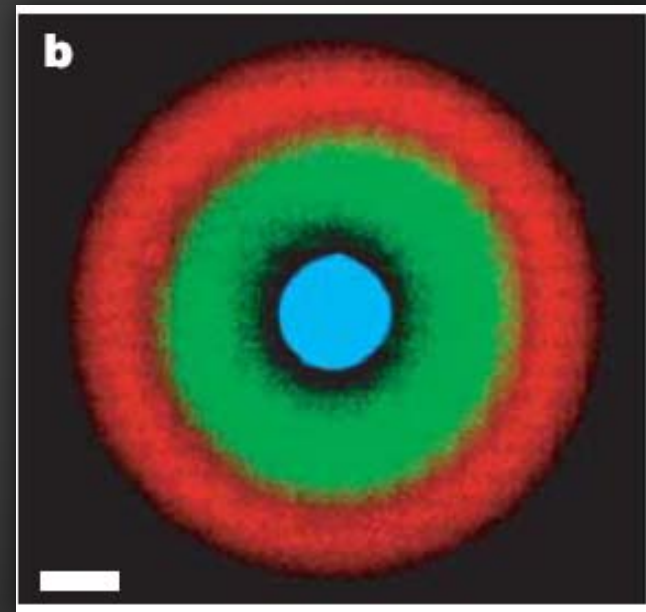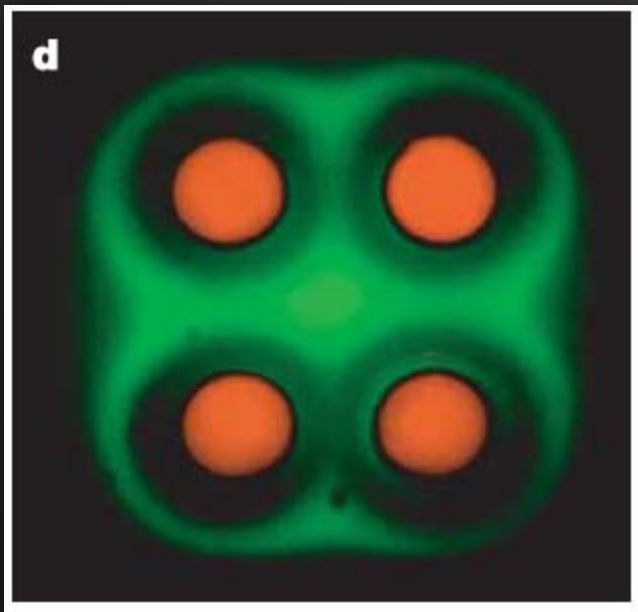
Basu *et al.* Nature 2005

Band-detect multicellular system

Sender cell produces AHL

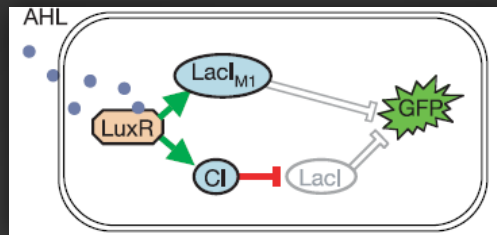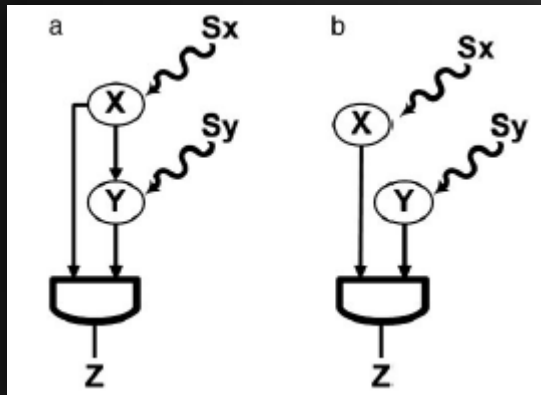Receiver cell contains a feed-forward loop responsive to AHL (band-pass filter)

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)

Structure and function of the feed-forward loop network motif

S. Mangan and U. Alon
PNAS 2003

Species	Incoherent type 1		Incoherent type 2		Incoherent type 3		Incoherent type 4	
	Structure	Abundance	Structure	Abundance	Structure	Abundance	Structure	Abundance
E. coli	X↓Y⊥Z	5	X⊥Y⊥Z	0	X↓Y⊥Z	1	X⊥Y↓Z	1
S. cerevisiae		21		3		1		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		—	

CSYNBI
Centre for Synthetic Biology and Innovation

Imperial College
London

Basu *et al.* Nature 2005

Modular System allows for different versions to be made and mixed
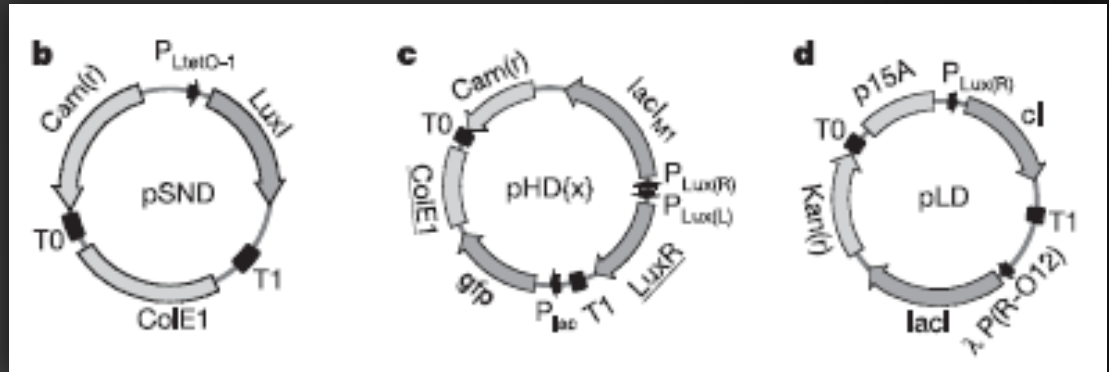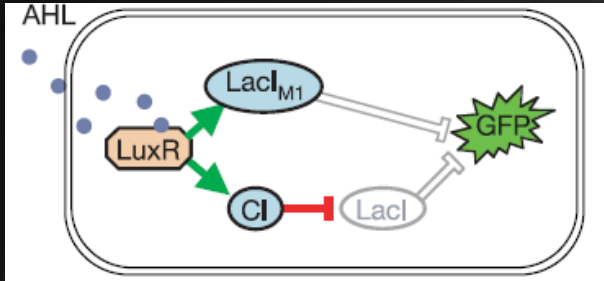
Sender   High-detect   Low-detect

- 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

$$\frac{dG}{dt} = \frac{\alpha_G}{1 + (L/\beta_L)^{\eta_1}} - \gamma_G G \qquad (1)$$

$$\frac{dL}{dt} = \frac{\alpha_{L1}}{1 + (C/\beta_C)^{\eta_2}} + \frac{\alpha_{L2} \cdot R^{\eta_3}}{(\theta_R)^{\eta_3} + R^{\eta_3}} - \gamma_L L \qquad (2)$$

$$\frac{dC}{dt} = \frac{\alpha_C R^{\eta_3}}{(\theta_R)^{\eta_3} + R^{\eta_3}} - \gamma_C C \qquad (3)$$

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

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

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
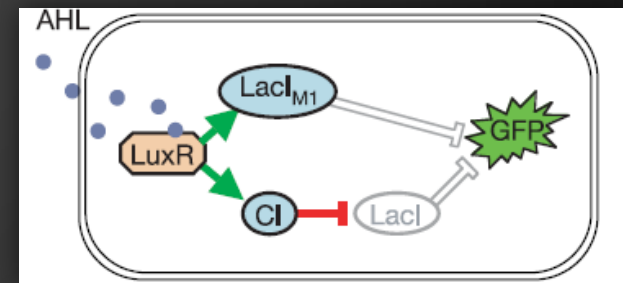
Basu *et al.* Nature 2005
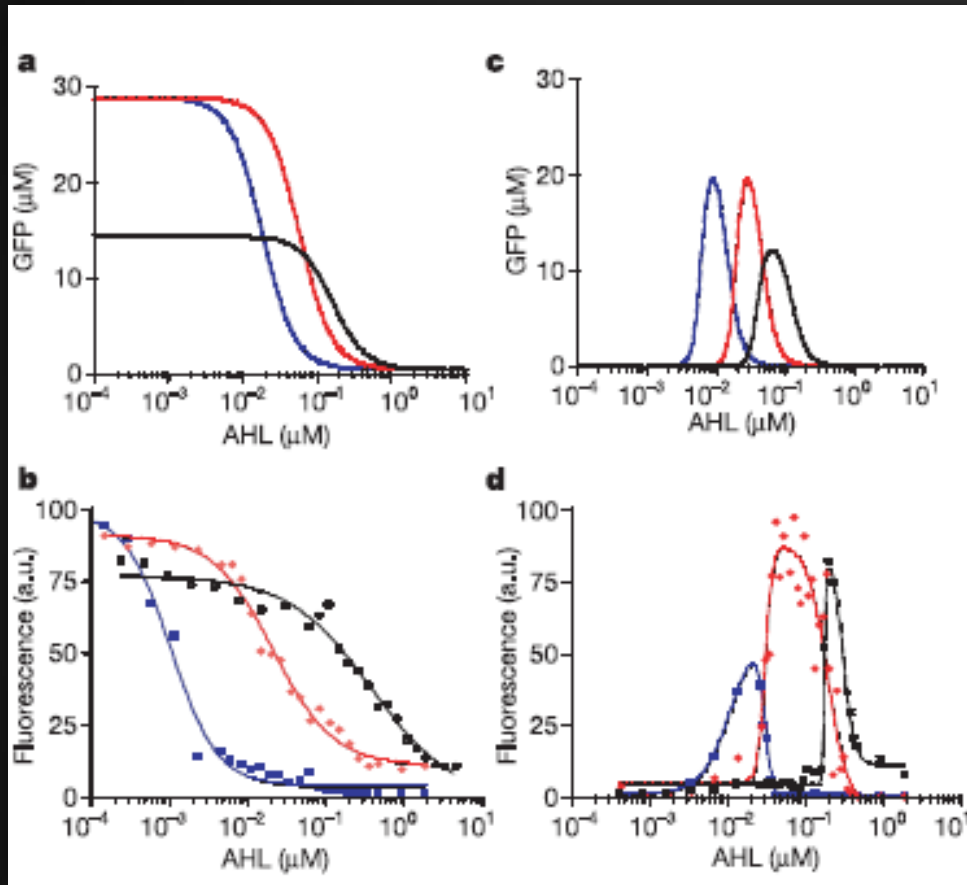
5 species model

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
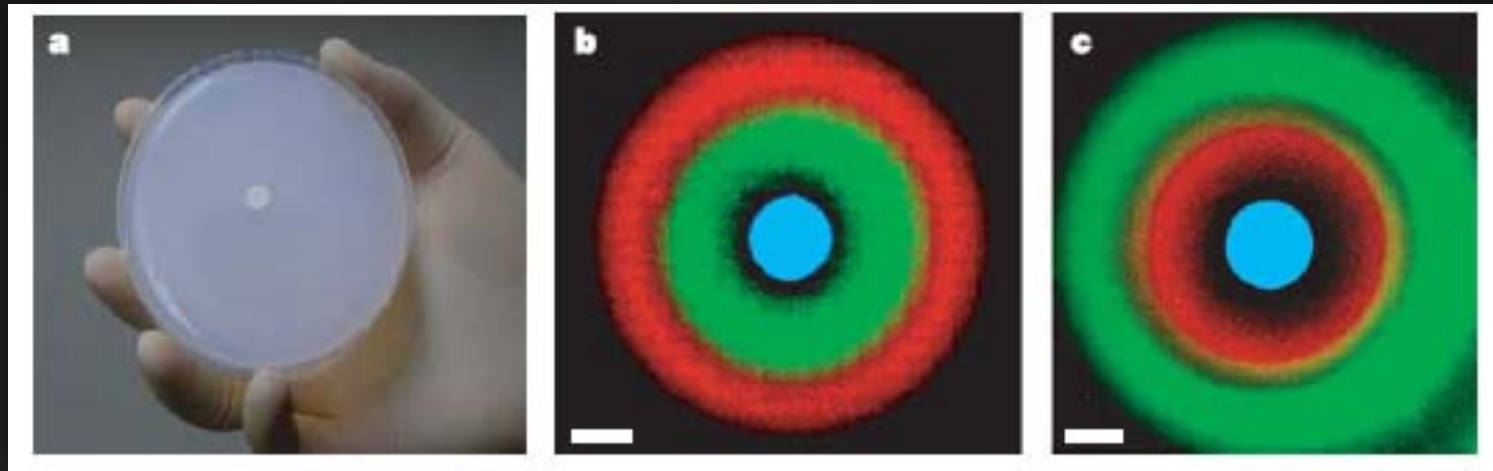
Immobilized plug of sender cells placed in centre of petri dish

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)

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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)
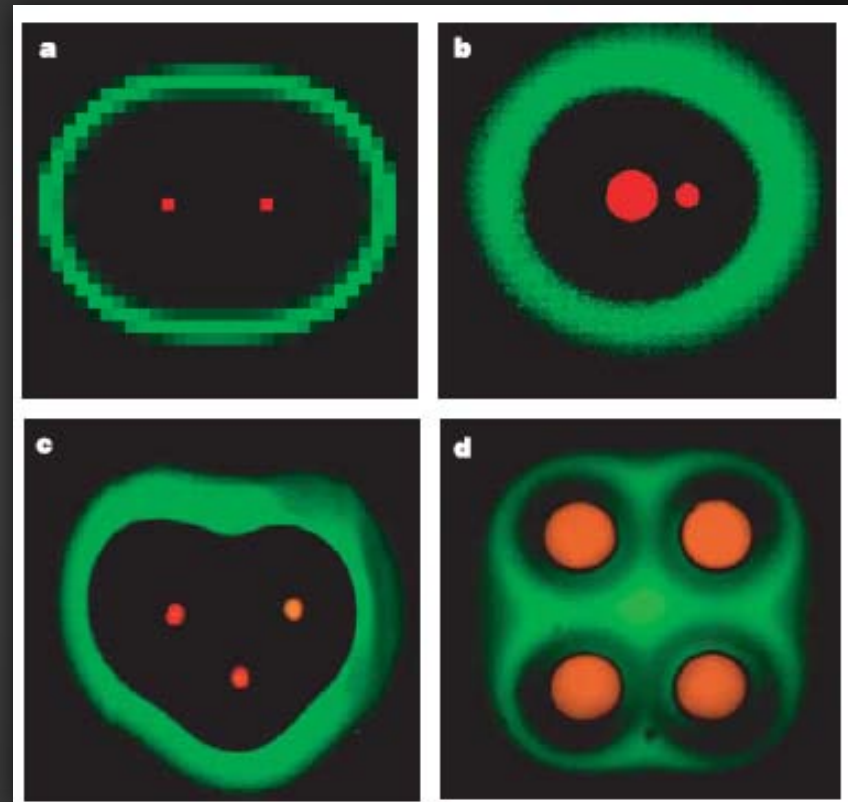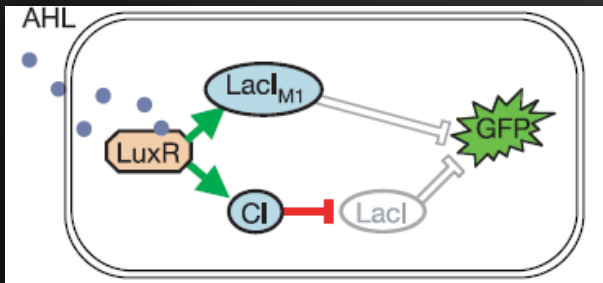
a = Simulation

Natural Example - Drosophila Patterning

Dr Tom Ellis

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London
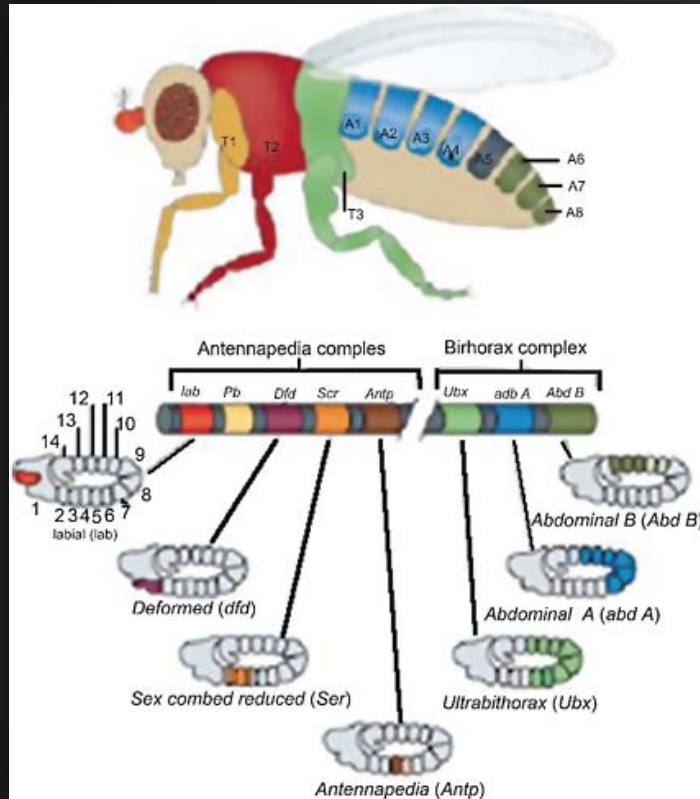
Natural Example - Drosophila Patterning
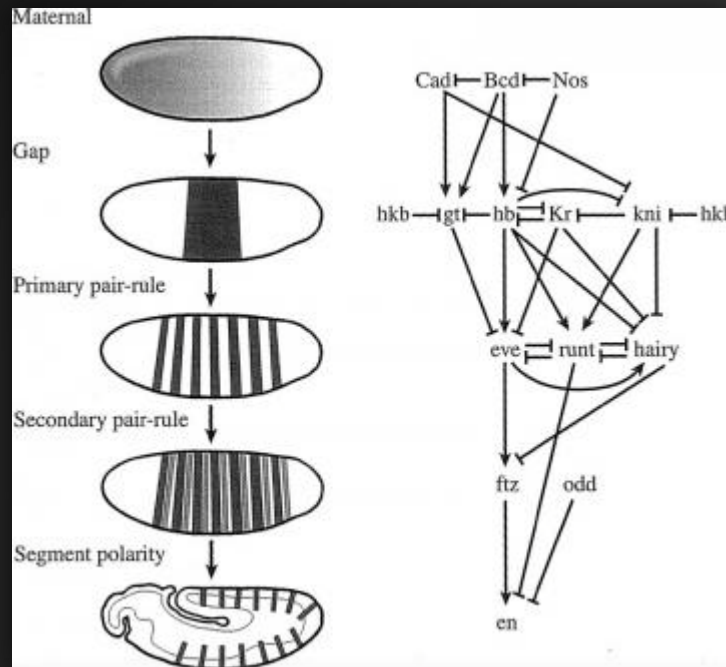
Initially a Morphogen Gradient (simple diffusion model)
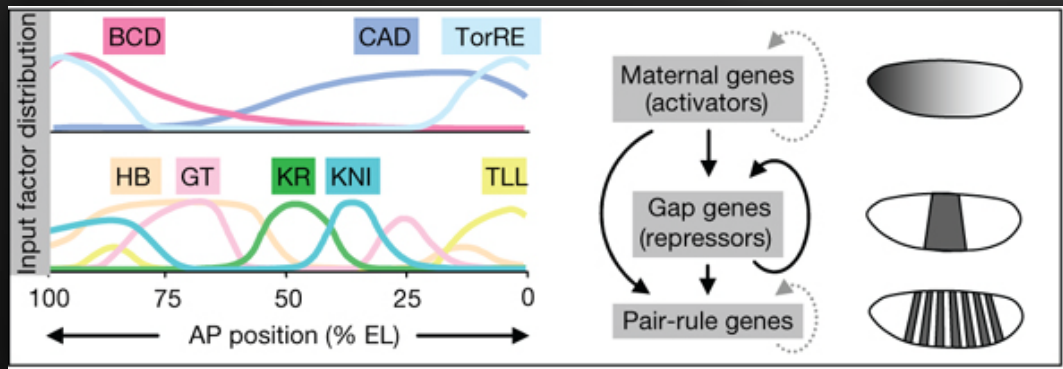
Understanding Drosophila Patterning

- Drosophila patterning is one of the most studied differentiation systems

- Gap genes, pair-rule genes have been investigated and mathematically modelled

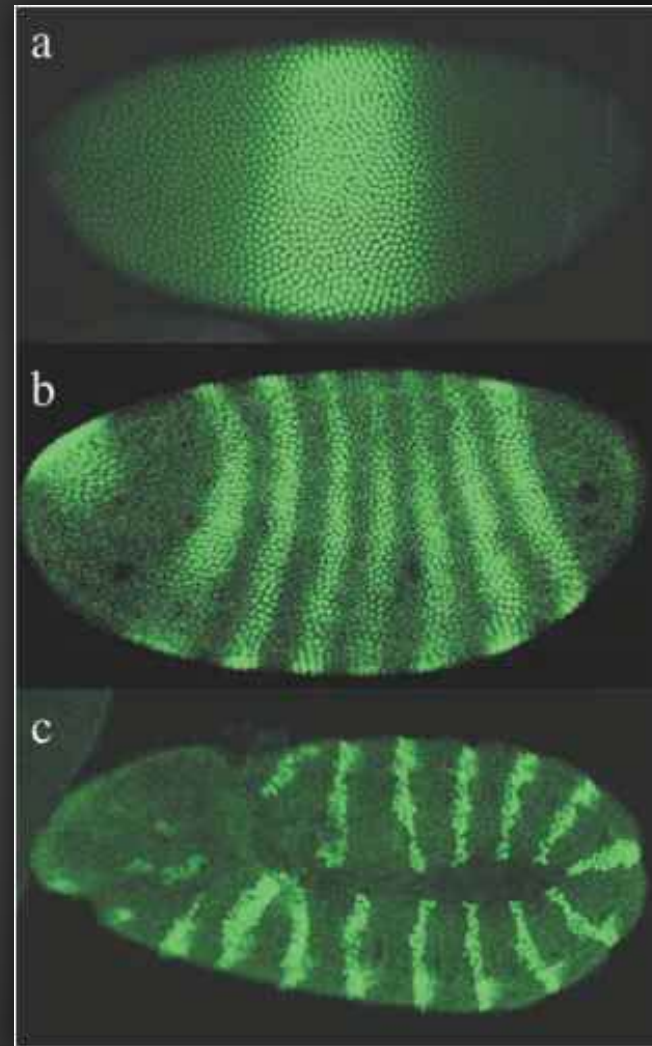- System works using diffusion of transcription factors through a giant multi-nuclei cell　　　–　　　MORPHOGEN GRADIENT

- Genes responding to these transcription factors have modular promoters

- See:

Essential Cell Biology

Chapter 8 p282

stripe 2 module: 480 nucleotide pairs

Krüppel and its binding site

Bicoid and its binding site

Giant and its binding site

Hunchback and its binding site
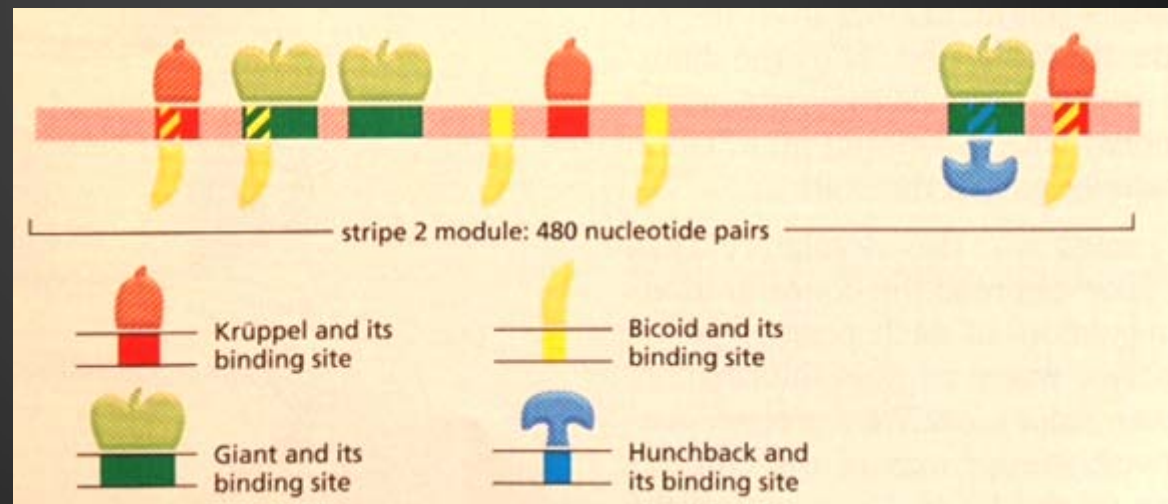
Synthetic Drosophila Patterning

- Synthetic biology - can we rebuild it and what can we learn from rebuilding?

- Engineered morphogen gradient system with phage polymerases and zinc finger repressors

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

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London
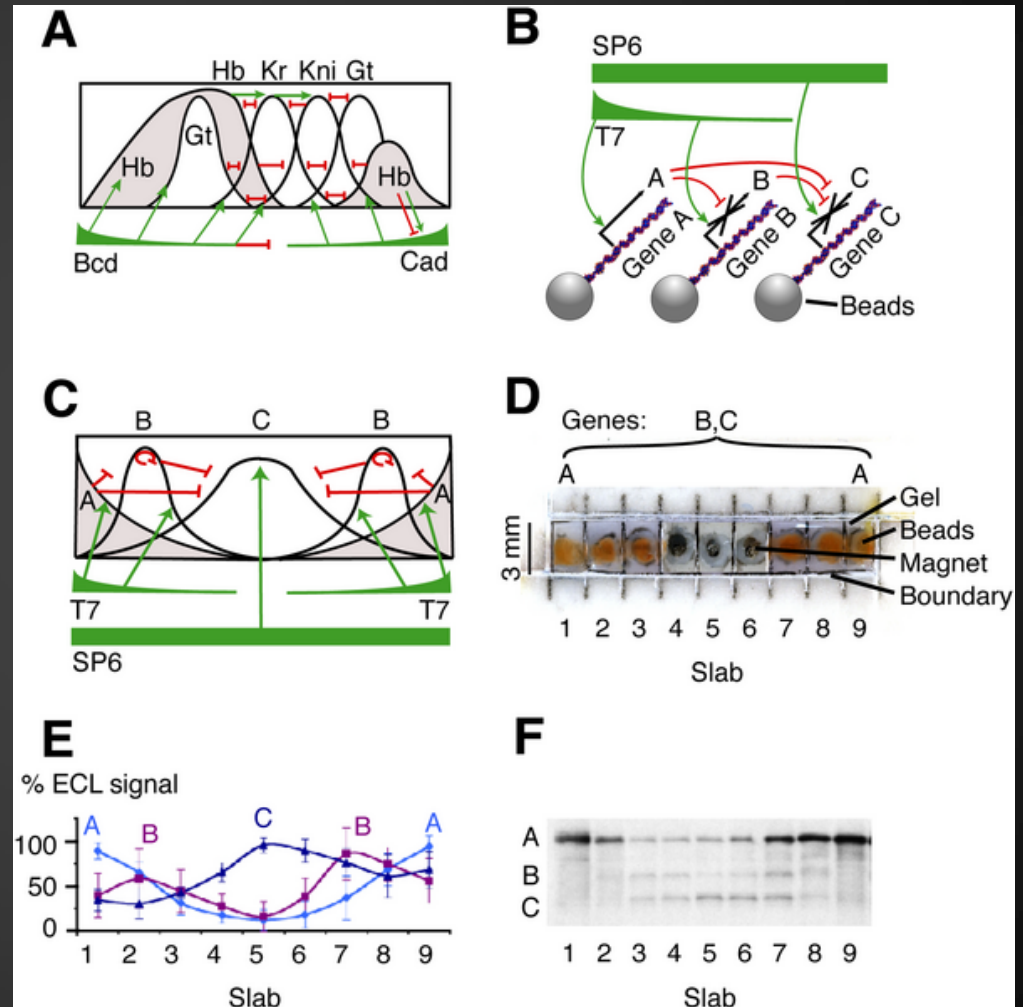
Synthetic Drosophila Patterning - DNA
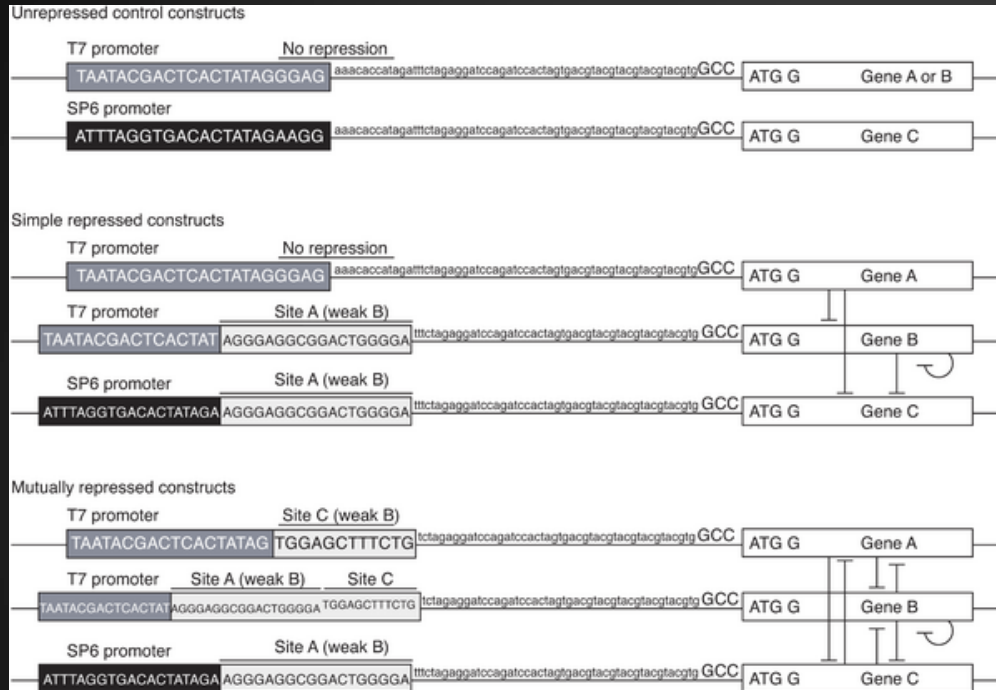
Different regulation – different patterns
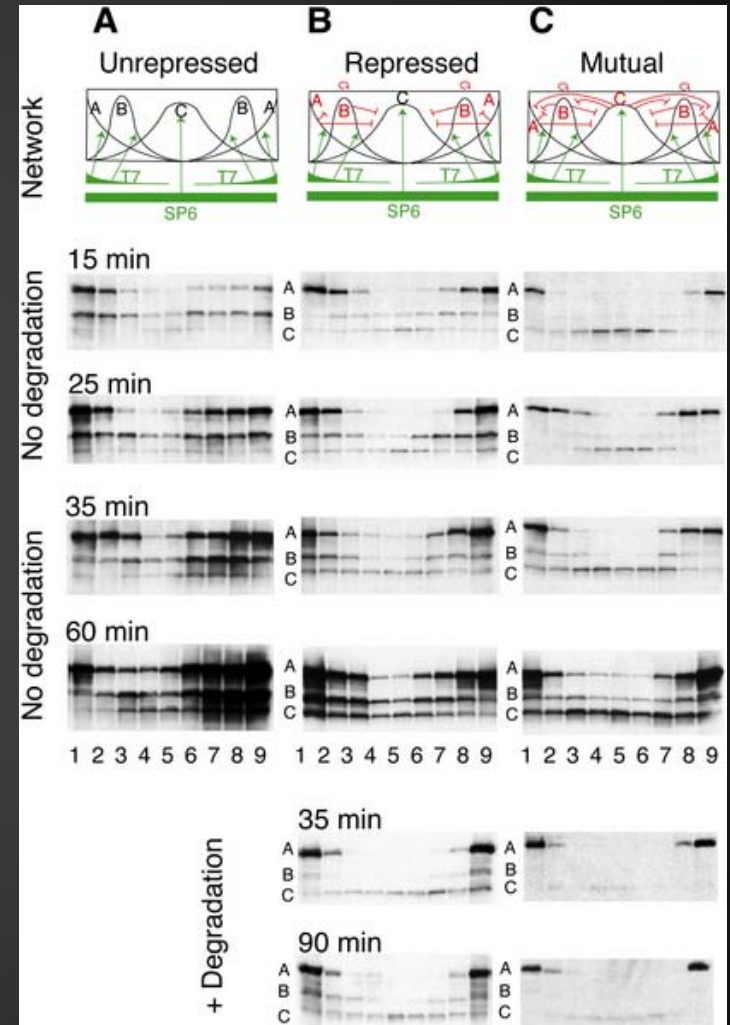
Synthetic Drosophila Patterning - Model
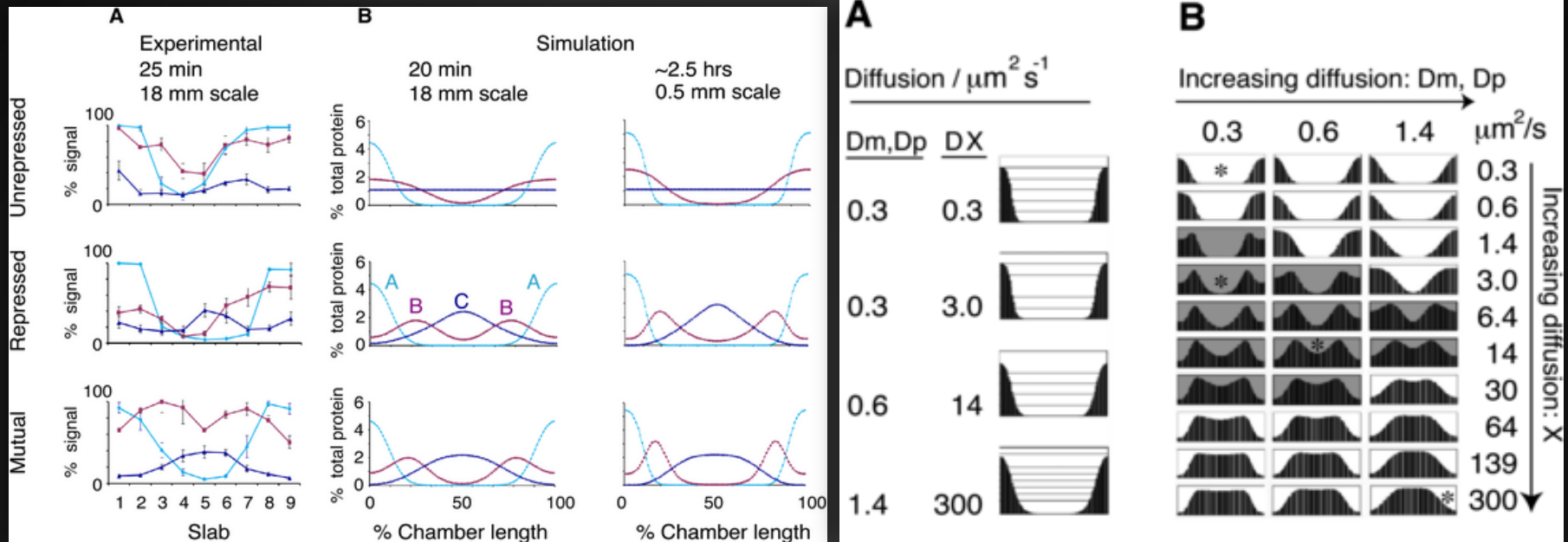
For each species E, the dynamics of its concentration at time t and position s are governed by the following classical difference equation:

$$\Delta E = E(s,t+1) - E(s,t) = \Delta t * (\text{diffusion} + \text{production} - \text{degdration})$$

* Modified equation is needed to describe T7 and to describe diffusion at the ends

Simulations were run using a Perl script. Parameters were measured or looked-up.

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London

Synthetic Drosophila Patterning - Findings

- Adding more transcription repression interactions increases the "sharpness" of the pattern while reducing overall expression levels.

- A computer model of the system allows searching for parameter sets compatible with patterning.

- Model suggests that simple diffusion may be too rapid for Drosophila-scale patterning, i.e. sublocalisation, or "trapping," is required.

- Model shows that in this system, the activator molecules must propagate faster than the inhibitors for patterning to occur.

- Model shows that adding controlled protease degradation to the system stabilizes pattern formation over time.

- Patterning may be achieved with only localisation, <u>diffusion</u>, and some kind of functional network connection   →   Minimal system

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London

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
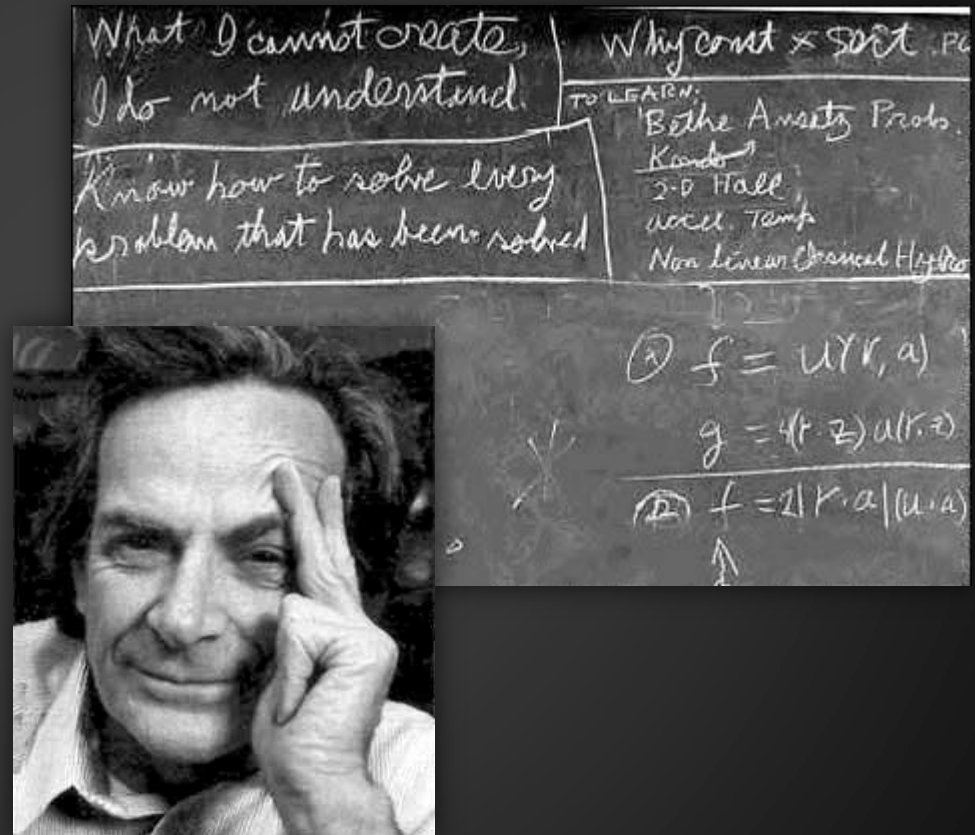
Applications of pattern formation
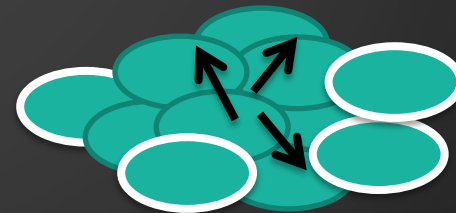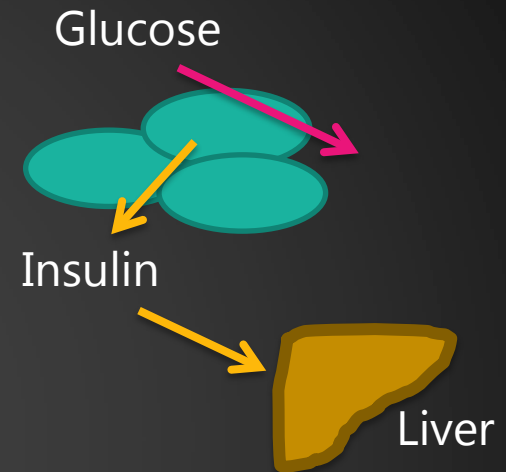
2. Artificial versions of natural systems

- **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

Glucose

Insulin

Liver

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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
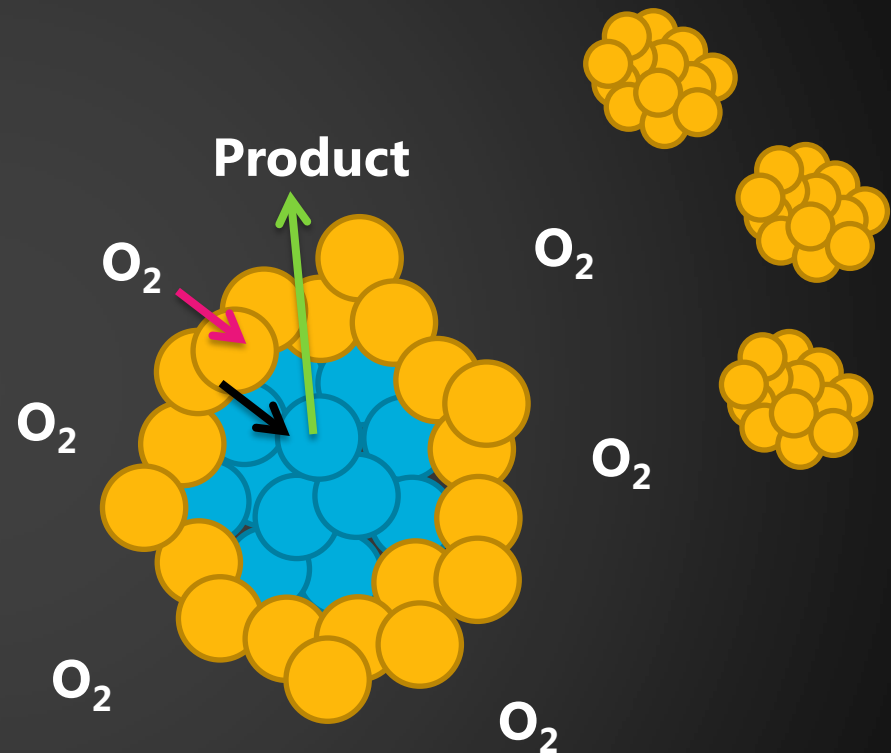
Product

O_2 O_2 O_2 O_2 O_2 O_2 O_2

CSYNBI
Centre for Synthetic Biology and Innovation

Imperial College
London

Applications of pattern formation

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

Art / Fashion / Architecture / Technology

ANY IDEAS?

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

Applications of pattern formation

4. Other applications we've not thought of…

Art / Fashion / Architecture / Technology

Nicola Morgan
RCA 2011

CSYNBI
Centre for Synthetic Biology and Innovation

Imperial College
London
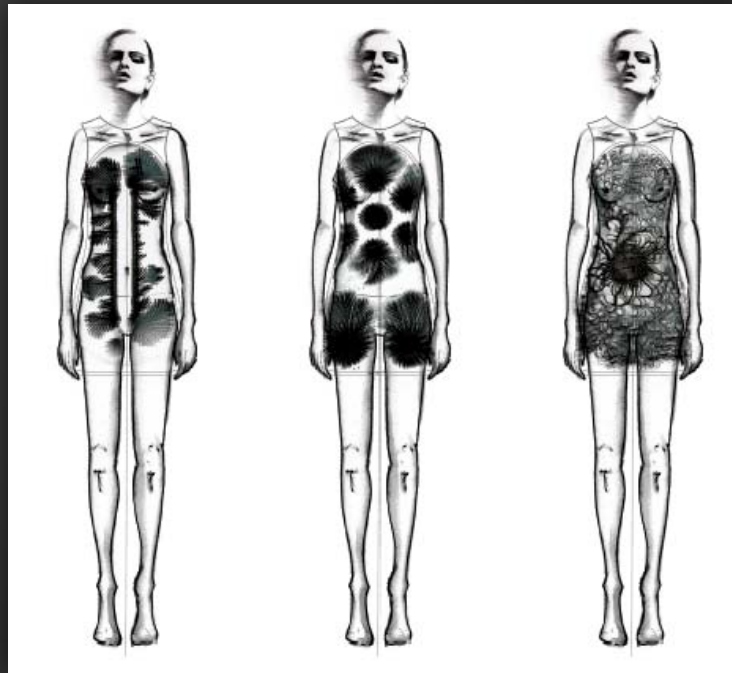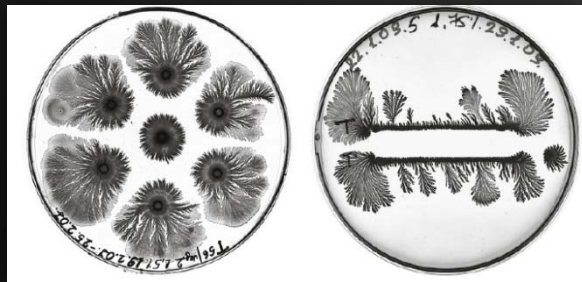
What you should now know and <u>read up</u> on!

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. A synthetic mimic of Drosophila pattern formation.
7. Applications of synthetic pattern formation

How would you use multicellularity?

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College
London

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

CSynBI
Centre for Synthetic Biology and Innovation

Imperial College London