Synthetic Biological Systems

1. Pattern Formation
Biological Patterns
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
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

Essential read:
Science 2010 (v.329)

please also read the supplementary online material
Two interacting molecules diffusing can generate stable patterns – Turing 1952

\[
\begin{align*}
\frac{\partial u}{\partial t} &= F(u,v) - d_u v + D_u \Delta u \\
\frac{\partial v}{\partial t} &= G(u,v) - d_v v + D_v \Delta v
\end{align*}
\]

- \( u \) 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

\[
\frac{\partial u}{\partial t} = F(u,v) - d_u v + D_u \Delta u
\]
\[
\frac{\partial v}{\partial t} = G(u,v) - d_v v + D_v \Delta v
\]

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

\[
\begin{align*}
F(u,v) - d_u u &= a_u u + b_u v + c_u \\
G(u,v) - d_v v &= a_v u + b_v v + c_v
\end{align*}
\]
The Reaction-Diffusion Equation

IV
Oscillatory cases with extremely short wavelength

V
Oscillatory cases with finite wavelength

VI
Stationary waves with finite wavelength (Turing pattern)

B
Case V
Case VI (Turing pattern)

C

20/01/2012
Dr Tom Ellis
Minimal Mathematical Patterning

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

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 \( f(u) = \lambda u - u^3 - \kappa \)
Simulated Turing Pattern

\[
\begin{align*}
\partial_t u &= d_u^2 \Delta u + \lambda u - u^3 - v + \kappa, \\
\tau \partial_t v &= d_v^2 \Delta v + u - v
\end{align*}
\]

Engineered *E. coli* growth pattern

iHKU iGEM team 2008
Chenli Liu *et al.* Science 2011
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
Motility of *E. coli*
Mechanism of motility in *E. coli*
Synthetic control of CheZ = control of motility in *E. coli*
Density-dependent control of CheZ

Acyl-homoserine lactone (AHL)
*Vibrio fischeri* quorum sensing
Creation of a low-density mover
Traffic is a low-density mover
iHKU iGEM 2008

Design, Model and Some Results – but only a Bronze
ChenLi Liu et al. Science 2011

3 years later.....
ChenLi Liu et al. Science 2011
Model of the system

3 partial differential equations

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)

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

2. Synthesis, diffusion and turnover of AHL $h(x, t)$

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

3. Consumption and diffusion of nutrient $n(x, t)$

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

$\alpha = \text{AHL synthesis rate}$  $\beta = \text{AHL degradation rate}$  $\gamma = \text{cell growth rate}$  $\mu(h) = \text{AHL-dependent motility}$
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

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

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

\[
\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \tag{3}
\]
Model of the system

Getting the parameters

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

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

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

\[
\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \tag{3}
\]
Model of the system

3 partial differential equations

\[
\frac{\partial \rho}{\partial t} = \nabla^2 [\mu(h) \rho] + \frac{\gamma n^2 \rho}{n^2 + K_n^2} \quad [1]
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\frac{\partial n}{\partial t} = D_n \nabla^2 n - \frac{k_n \gamma n^2 \rho}{n^2 + K_n^2} \quad [3]
\]
ChenLi Liu et al. Science 2011

Simulation

Experiment
Only the complete system works

Patterns are robust
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!
As with Turing Patterns, the patterns emerge when there is more noise in the system.

Low cell numbers means higher stochasticity.
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?
Example 1
Pancreatic Beta Cells Send out Insulin
A synthetic multicellular system for programmed pattern formation

Subhayu Basu¹, Yoram Gerchman¹, Cynthia H. Collins³, Frances H. Arnold³ & Ron Weiss¹,²
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
Feed-forward loops

Band-pass filter is generated by the difference between the CI and LacI<sub>M1</sub> 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
Modular System allows for different versions to be made and mixed

- 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

5 species model

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

\[
\frac{dG}{dt} = \frac{\alpha_G}{1 + (L/\beta_L)^3} - \gamma_G G
\]

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

\[
\frac{dC}{dt} = \frac{\alpha_C \cdot R^3}{(\theta_R)^3 + R^3} - \gamma_C C
\]

\[
\frac{dR}{dt} = \rho_R [\text{LuxR}]^2 A^2 - \gamma_R R
\]

\[
\frac{dA_{xy,z}}{dt} = \xi (A_{x-1,y,z} + A_{x+1,y,z} + A_{x,y-1,z} + A_{x,y+1,z} + A_{x,y,z-1} + A_{x,y,z+1} - 6A_{x,y,z}) - \gamma_A
\]

\[G = \text{GFP} \quad L = \text{LacI} \quad C = \text{CI} \quad R = \text{LuxR/AHL} \quad A = \text{AHL}\]

LuxR alone is at a fixed concentration

Equation 5 captures the diffusion and degradation of AHL
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
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)
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

Antennapedia complex

Bithorax complex

Deformed (dfd)
Sex combed reduced (Scr)
Antennapedia (Antp)

Abdominal A ( AbdA)
Abdominal B ( AbdB)
Ultrabithorax (Ubx)

T1, T2, T3, A6, A7, A8
1, 2, 3, 4, 5, 6, 7, 8
9, 10, 11, 12, 13, 14

(a) (b) (c)
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
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

### Synthetic Drosophila Patterning - DNA

<table>
<thead>
<tr>
<th>Unrepressed control constructs</th>
<th>Simple repressed constructs</th>
<th>Mutually repressed constructs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T7 promoter</strong></td>
<td><strong>T7 promoter</strong></td>
<td><strong>T7 promoter</strong></td>
</tr>
<tr>
<td>ATG G</td>
<td>ATG G</td>
<td>ATG G</td>
</tr>
<tr>
<td>Gene A or B</td>
<td>Gene A</td>
<td>Gene A</td>
</tr>
<tr>
<td><strong>ATTTAGTGA</strong></td>
<td><strong>ATTTAGTGA</strong></td>
<td><strong>ATTTAGTGA</strong></td>
</tr>
<tr>
<td>Gene C</td>
<td>Gene C</td>
<td>Gene C</td>
</tr>
</tbody>
</table>

- **Different regulation – different patterns**
For each species $E$, the dynamics of its concentration at time $t$ and position $s$ are governed by the following classical difference equation:

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

* 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.
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, diffusion, and some kind of functional network connection → Minimal system
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
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. A synthetic mimic of Drosophila pattern formation.
7. 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


Sequential Establishment of Stripe Patterns in an Expanding Cell Population – Science

A synthetic multicellular system for programmed pattern formation - Nature

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