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Programming Biology

Neil Dalchau

Pattern formation in multicellular systems









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Pattern formation in single cells



Even in a single cell, polarization and asymmetrical division can initiate distinct developmental programs

munrolab.bsd.uchicago.edu

Morphogen gradient vs. Turing model



Kondo & Miura, Science 2000

THE CHEMICAL BASIS OF MORPHOGENESIS

By A. M. TURING, F.R.S. University of Manchester

Vol. 237. B. 641. (Price 8s.)

5

[Published 14 August 1952



- Alan Turing's seminal work on morphogenesis introduced the idea of diffusible molecules being capable of generating stable spatial patterns
 - Two morphogens
 - Activation-inhibition





Figure S7: The network of the Activator-Inhibitor model

Turing pattern behind digit formation





Sheth et al., Science 2012



Raspopovic et al., Science 2014

Turing pattern behind digit formation



Complexity of evolved systems



How can we understand biological patterning independent of the complications of evolution and development *in vivo*?

Programming biological systems



Computer Aided Design software

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Programming pattern formation in cells

- To what extent can we program static/dynamic/spatial behaviours in cell colonies?
- Can we *implement* **Turing patterns**?
 - Independent cell-cell signalling channels
 - Activation-inhibition network
 - Differential diffusion



University of Cambridge



James Brown



Jim Haseloff



Using established biological components



Chen et al., Genome Biology 2012

Multiple signalling channels

Predator-prey interaction based on two AHL communication channels



Cycles of high prey abundance then high predator abundance



Balagaddé et al., Mol. Syst Biol. 2008

Two signalling channels in the same cell?

C6-HSL C12-HSL LuxR LasR **C6** C12 LuxR LasR C6 C12 LuxR LasR **C6** C12 LasR LuxR LasR LuxR Plux Plas

"A central goal of synthetic biology is to achieve multi-signal integration and signal processing in living cells for diagnostic, therapeutic and biotechnology applications."

Daniel et al., Nature 2012

Measuring response of P_{Lux} to HSL signals





Identifying cross-talk



Chemical reaction model

$$\frac{a_{0} + a_{Rk}K_{GRk}r^{2}\left(\frac{K_{Rk}C_{k}}{1 + K_{Rk}C_{k}}\right)^{n} + a_{Sk}K_{GSk}s^{2}\left(\frac{K_{Sk}C_{k}}{1 + K_{Sk}C_{6}}\right)^{n}}{1 + K_{GRk}r^{2}\left(\frac{K_{Rk}C_{k}}{1 + K_{Rk}C_{k}}\right)^{n} + K_{GSk}s^{2}\left(\frac{K_{Sk}C_{k}}{1 + K_{Sk}C_{k}}\right)^{n}}$$



Controlling LuxR and LasR



Mutants for exclusive luxR/lasR responses



Candidate mutants





Response to increased LuxR levels

PCat PBad BBa_114033 BBa 10500 **Iux**R K_{GR} LuxR B0034 B0034 20 | i i 30-C6-HSL ii POLux POLux 15 **Relative promoter activity** 10 B0034 K_{R12} K_{GR} (Promoter 76) ii 30-C12-HSL -POLux 5 0 20 | **iii** iv iii 30-C6-HSL K_{R6} LuxR POLas 15 POLas 10 B0034 iv 30-C12-HSL LasR - K_{GS} (Promoter 81) 5 **POLas** 0 10² 10² 10⁰ 10⁰ 10⁴ 10⁴ [30C6-HSL] (nM) [30C12-HSL] (nM)

Arabinose-inducible LuxR

Response to increased LasR levels



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Double receiver cells



Minimize impact of chemical crosstalk: Selection of optimal luxR/lasR levels



Minimize impact of chemical crosstalk: Selection of optimal luxR/lasR levels



 $\max_{R,S} \left\{ \frac{f_6^{P76}(R,S)}{f_{12}^{P76}(R,S)} \cdot \frac{f_{12}^{P81}(R,S)}{f_6^{P81}(R,S)} \right\}$

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Independent detection of signal propagation

Experiment design



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A spatial model

Parameterising the spatial model

- Used RFP measurement to parameterise cell growth
- Fit HSL diffusion and HSL sender cells to the spatial data

Patterning with intercellular signalling

Model simulation

Model analysis

- Inferred parameters did not show the correct qualitative behaviour
 We had to increase the HSL synthesis rates
- System is near a bifurcation
 - i.e. inducible signalling proceeds upon reaching a critical threshold

Summary

- By altering promoter sequence, specificity for regulators can be altered
 - We created two mutant promoters: one LuxR-specific and one LasRspecific
 - Mathematical modelling helped to describe and quantify the sources of crosstalk
- By controlling the expression of receiver protein, we can optimise for independent signalling
 - Predicted levels that maximise *signal-to-noise* using the model
- Demonstrated independent signalling by constructing **double** reporter cells, which simultaneously recognise and report two different signals
 - Spatiotemporal dynamics well predicted by the model
- The philosophy behind synthetic biology offers a new way to understand cell biology and developmental patterning
- Next up... Turing patterns?

Acknowledgements

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University of Cambridge

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Tools home

Computational Science

new tools for new science

Tools

Unify biological hypotheses with models and experiments

Easy visualization of scientific data: drag, drop, filter, slide, view, zoom, share

http://research.microsoft.com/science/tools

Easily work with multidimensional datasets: NetCDF, text, memory or remote, all from within your code

DSD: DNA Strand Displacement language

A language for designing and simulating computational devices made of DNA

Visualize your data over the web and add complex dynamic graphs and maps to your web applications

Retrieve climatic and environmental information with the click of a button or a few lines of code

Fit complex models to hetereogenous data: Bayesian and likelihood analysis made easy

GEC: Genetic Engineering of Cells language

A language for designing and simulating genetic devices to reprogram cell behaviour

Changing our understanding of animal behaviour: novel hardware, analysis and software tools

Visualizing and Modelling Food Webs and other Complex Network

A language for modelling and simulating complex biological processes in a modular way

Domain-specific programming languages

GEC: Genetic Engineering of Cells language

Modelling Engine

 $\mu\left(1-x^2\right)y-x$

OSLO: Open Solving Library for ODEs

Simulation

- ODEs
- PDEs
- Stochastic
- Chemical Master Equation

Table 1: Datasets used for wild-type promoter. Throughout, the tag "123" indicates the wildtype promoter.

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Codename	Description	Files	Platereader
P _{cat} -LL123	luxR and lasR transcribed	char130508_Pcat_LL123_CI_Edited.csv	Ajioka
	constitutively via P _{cat}	char130530_Pcat_LL123.csv	Ajioka
	-	char140624_Pcat_LL123.csv	Haseloff
P _{cat} -R123	luxR transcribed constitutively	char130607_Pcat_R123.csv	Haseloff
	via P _{cat}	char130718_Pcat_R123.csv	Ajioka
		char130822_Pcat_R123.csv	Ajioka
		char140625_Pcat_R123.csv	Haseloff
		char140702_Pcat_R123.csv	Haseloff
P _{cat} -S123	lasR transcribed constitutively	char130614_Pcat_S123.csv	Ajioka
	via P _{cat}	char130624_Pcat_S123.csv	Haseloff
		char130718_Pcat_S123.csv	Ajioka
		char140702_Pcat_S123.csv	Haseloff
P _{bad} -LuxR-S123	luxR transcribed inducibly via	char140419_S123.csv	Haseloff
	P _{bad} and lasR transcribed	char140430_S123.csv	Haseloff
	constitutively via P _{cat}	char140507_S123.csv	Haseloff
P _{bad} -LasR–R123	lasR transcribed inducibly via	char140527_R123.csv	Haseloff
	P _{bad} and luxR transcribed	char140601_R123.csv	Haseloff
	constitutively via P _{cat}	char140612_R123.csv	Ajioka

Table 2: Datasets used for mutant promoter 76.

Codename	Files	Platereader
P _{cat} -LL76	char130530_Pcat_LL76_Edited.csv	Ajioka
	char130719_Pcat_LL76.csv	Ajioka
	char130822_Pcat_LL76.csv	Ajioka
	char130923_Pcat_LL76_CI_Edited.csv	Haseloff
	char140702_Pcat_LL76.csv	Haseloff
P _{cat} -R76	char130607_Pcat_R76.csv	Haseloff
	char130719_Pcat_R76.csv	Haseloff
	char140624_Pcat_R76.csv	Haseloff
P _{cat} -S76	char130614_Pcat_S76_CI.csv	Ajioka
	char130624_Pcat_S76.csv	Haseloff
	char140624_Pcat_S76.csv	Haseloff
P _{bad} -LuxR-S76	char140422_Pbad_LuxR_S76.csv	Haseloff
	char140428_Pbad_LuxR_S76.csv	Ajioka
	char140506_Pbad_LuxR_S76.csv	Ajioka
P _{bad} -LasR-R76	char140607_Pbad_LasR_R76.csv	Haseloff
	char140610_Pbad_LasR_R76.csv	Ajioka
	char140611_Pbad_LasR_R76.csv	Haseloff

Table 3: Datasets used for mutant promoter 81.

Codename	Files	Platereader
P _{cat} -LL81	char130530_Pcat_LL81_Edited.csv	Ajioka
	char130822_Pcat_LL81.csv	Ajioka
	char130923_Pcat_LL81_CI_Edited.csv	Haseloff
	char140702_Pcat_LL81.csv	Haseloff
P _{cat} -R81	char130607_Pcat_R81.csv	Haseloff
	char130822_Pcat_R81.csv	Ajioka
	char140625_Pcat_R81.csv	Haseloff
	char140703_Pcat_R81.csv	Haseloff
P _{cat} -S81	char130626_Pcat_S81.csv	Haseloff
	char140625_Pcat_S81.csv	Haseloff
Pbad-LuxR-S81	char140424_Pbad_LuxR_S81.csv	Haseloff
	char140429_Pbad_LuxR_S81.csv	Haseloff
	char140502_Pbad_LuxR_S81.csv	Haseloff
P _{bad} -LasR–R81	char140611_Pbad_LasR_R81.csv	Haseloff
	char140612_Pbad_LasR_R81.csv	Haseloff
	char140616_Pbad_LasR_R81.csv	Haseloff

Posterior parameter distributions

Fitting wild-type promoter data alone

Posterior parameter distributions

Fitting wild-type and mutant promoters data together

Relay signal spread

