Development in multicellular organisms is controlled by intercellular signalling.

Inductive vs. Morphogen Signals

Working hypothesis: these are regulated with different logic.

Concentration independent
Threshold
Binary

Concentration dependent
Direct relationship between signal intensity and outcome

Both can produce 'complex' patterns
The initiation of signalling is regulated by ligand availability

Freeman and Gurdon 2002
Few pathways control development

- Receptor tyrosine kinases
- Notch
- Hedgehog
- Wnt
- TGFb
- G-protein coupled receptors
- PI-3 kinase/phosphoinositide

A cell’s response to a signal is determined by its developmental context, not by the nature of the signal.
Multiple cell types in the developing ommatidium are determined by EGFR

EGFR triggers each cell type

Spitz

PR  CC  PC

DN-EGFR

Dr. Matthew Freeman, Cambridge (KITP Bio Networks 2/13/03)
Signal regulation is paramount

Precise
Robust/stable
Versatile
Integrated control of EGF signalling regulates developmental patterning.

Genetics provides an unbiased method for identifying physiologically-significant components/regulators.
EGFR ligands in Drosophila

- TGF\(\alpha\)
- Neuregulin (Grk, Ker, Spitz)
- EGFR ligands

Argos blocks Egfr activation

- Argos is first Egfr antagonist
- Argos expression is dependent on Egfr activation
- Negative feedback
Remote (or local) inhibition by Argos

Short range activator, long range inhibitor

Properties:
Range of inhibition could be determined by duration of a cell’s susceptibility to Argos
Can potentially stabilise a gradient of activity, or convert graded signal to binary output

NB

Our models of signalling logic are based on experiment and intuition
They are not mathematically tested and may be wrong
Sprouty: an intracellular inhibitor of Ras signalling

- Potent inhibitor of Ras activation
- MAP kinase target
- Binds Gap1 and Drk
- Tightly bound to inner face of plasma membrane
- Acts in negative feedback control of signalling
- Mammalian homologues

Novel feedback inhibitors of EGFR

Argos
- Extracellular
- Diffusible
- Egfr-specific

Kekkon-1
- Ghiglione et al. 1999
- Membrane-spanning
- Egfr-specific
- Binds Egfr

Sprouty
- Intracellular
- Blocks all RTKs
- Binds membrane
- Binds Drk (Grb2)
- Binds Gap1
Signalling Review

Negative feedback in 3rd century BC

Waterclock by Ktesibios of Alexandria

Negative feedback

Stabilises

Limits

Patterns
Rhomboid and Star are principal activators of Egfr signalling

Rhomboid is a key activator of EGFR signalling

Necessary
Sufficient
Expression prefigures activation
Grk signals to Egfr to specify dorsal/anterior follicle cells

Role of Rhomboid?
How are appendages positioned?

Spitz is required in the follicle cells
spitz is expressed in follicle cells

-ve control

spitz probe
detail

spitz increases intensity of signal
Also increases range of signal

What are patterning implications?

Autocrine amplification of initial paracrine signal

Nucleus

EGFR

Rho

Spi

Grk

Oocyte

Positive feedback loop

\[ \text{Spitz increases intensity of signal} \]
\[ \text{Also increases range of signal} \]
\[ \text{What are patterning implications?} \]
argos is expressed in dorsal anterior of oocyte

Stage 12 oocyte

Pattern generation by EGFR
- Initiation
- Amplification & Broadening
- Inhibition

Net signalling
- argos threshold
- Gurken threshold
Loss of argos causes a wide central appendage

MAPK activation evolves from a single peak into two

Stage 10  Stage 12
Interlocking negative and positive feedback loops pattern the egg

An automatic pattern-generating circuit
Possible Phenotypes:

effect of input parameters

Positive feedback

Produces binary response

Amplifies

Can lead to dangerous instability

(don’t forget transcriptional autoregulation)
Signalling Review

Tacoma Narrows Bridge November 1940

ErbB receptors in disease

Cancer
- brain
- head/neck
- oesophagus
- mouth
- breast
- lung
- stomach
- pancreas
- kidney
- colon
- prostate
- cervix
- ovary

Other
- psoriasis
- atherosclerosis

Therapeutic opportunities
- wound healing
- nerve injuries

Significant role for positive feedback

Dr. Matthew Freeman, Cambridge (KITP Bio Networks 2/13/03)
Lewis, you mentioned earlier that the types of forces that cells generate are very few, and that small changes in any of them can have profound influences on the end result. One would think, then, that there should be lots of feedback mechanisms, to ensure reproducibility...

L.W.: Very little feedback at all - one of the most remarkable features about development is the virtual total absence of feedback.

A.G.B.: but there is feedback! Development has lots of regulation, and that is a form of feedback. Most of developmental operation involve counteracting forces, they are done by antagonisms. The way the HLH products work is by titrating each other!

L.W.: Titration is not feedback: a threshold is not a thermostat. Negative feedback has a well-defined classical meaning: you actually have to measure something, and then if you have too much you make less, and if you have too little you make more. There is no feedback in development, nor even in the regulation of developmental genes: if you put extra copies of bicoid, you make more bicoid proteins.

**Inductive vs. Morphogen Signals**

**Part 2**

- **Concentration independent**
  - Threshold
  - Binary

- **Concentration dependent**
  - Direct relationship between signal intensity and outcome

- **Feedback common**
  - (adds robustness and makes response digital)

- **Much less feedback(?)**
  - (uncouples input and output signal strength)

- **Enzyme signal cascades**
  - (amplification of input signal)

- **Signal cascades more dependent on protein-protein interactions**
NB
Both morphogens and inductive signals can be used to elaborate complex patterns
Patterning the fly eye by EGFR

Multiple roles

How is the 'crystalline' array formed?
An EGFR-dependent spacing mechanism

co-starring Scabrous and Notch/Delta

once initiated, self-organising
A new molecular mechanism for intercellular signalling

Rhomboid and Star are principal activators of Egfr signalling

Necessary
Sufficient
Expression prefigures activation
A conserved family of Rhomboids

- *E. coli* GLPG
- *Arabidopsis* O81073
- *Sugar cane*
- *B. subtilis* GLPG
- *B. subtilis* YDCA
- *Mycobacterium tuberculosis* 2
- *P. aeruginosa*
- *C. elegans* E1344687
- *C. elegans* Q19821
- *Drosophila* 2
- *Drosophila* 1
- *Drosophila* 3
- *Human*
- *Rat*
- *S. cerevisiae* 246c
- *S. cerevisiae* 2
- *H. influenzae* GLPG
- *E. coli* GLPG
- *A. tumefaciens* 1
- *O. vulgaris* 246c
- *S. pombe* 1
- *M. tuberculosis* 1
- *M. tuberculosis* 2
- *M. leprae*
Rhomboid and Star-dependent Spitz cleavage in Cos cells

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Spitz is localised in ER

Dr. Matthew Freeman, Cambridge (KITP Bio Networks 2/13/03)
Rhomboid is localised in Golgi apparatus

Spitz is relocalised by Star
Regulated Intramembrane Proteolysis...

Site 2 protease
Presenilin
Polytopic membrane proteins
Metallo- and aspartyl proteases
Release cytoplasmic domains of SREBP, Notch, APP etc

Rhomboid
Polytopic membrane protein
Serine protease
Releases luminal domains eg growth factors

...a widespread signalling mechanism

Bacterial rhomboids

Providencia stuartii

AarA

Rather et al.
cma genes

AarA = Rhomboid
Gallio & Kylsten

Spitz + Star

Providencia Rhomboid cleaves Drosophila substrate

Dr. Matthew Freeman, Cambridge (KITP Bio Networks 2/13/03)
Can we search for substrates?

Spitz TMD

critical region

mutagenesis and replacement

define sequence/structural constraints (necessary and sufficient)

search genomes

Mouse genome

20-30 candidates

Testable number!
Rhomboid
intramembrane serine protease
high specificity
recognition and cleavage in TMD
rhomboids regulate mitochondrial membrane dynamics
prokaryotic rhomboids are also proteases and share specificity
mammalian rhomboids?

Drosophila EGF receptor

Spi

Egfr

Ras

MAPK

Nucleus
Regulators of Egfr signalling in flies

Regulated intercellular signalling controls development

Feedback (positive and negative) is a key regulatory principle of developmental signalling

Inductive signalling is not a poor relation of morphogen signalling: both can elaborate complex patterns