Finding a morphogen gradient using forward genetics:

Dorsal-ventral patterning by BMPs in Drosophila embryos

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Fruit flies...

*Drosophila melanogaster*

Artist’s conception
Drosophila life cycle: two distinct developmental phases

The life cycle of *Drosophila melanogaster*

- Embryonic development
- Metamorphosis
  - Embryo
  - 1st instar larva
  - Prepupa
  - 2nd instar larva
  - 3rd instar larva
  - Pupa

FlyMove
Drosophila development: two different bodies for two different purposes.

Dispersal and reproduction

Feeding

The life cycle of *Drosophila melanogaster*
• Why Drosophila genetics?
• What is a morphogen gradient?
• Inference of a morphogen gradient from genetics
• Identification of signaling pathway from genetics
• Visualization of a gradient from signal transducers
• Shaping the gradient
• Computational predictions of molecular mechanisms
Why Drosophila genetics?

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Why I chose to study Drosophila development: the most powerful system to dissect the logic of development

Nusslein-Volhard and Wieschaus

1980 Nobel prize 1995
Ed Lewis:
Homeotic mutations

“...and when that happened a revolution occurred in the field of biology”
- Scott Gilbert

Homeobox genes establish body plans for all multicellular organisms

Why people choose Drosophila today:

- Multicellular organisms use the same tools
  - e.g. the gang of five for organogenesis:
    - TGFβ ligands: BMPs*
    - Receptor tyrosine kinase ligands: EGFs and FGFs
    - Hedgehogs*
    - Wnts*
    - Notch ligands*

- It is easier to understand a simple system
  - e.g. 7 TGFβ ligands in flies
    - 57 in mice
    - 34 in zebrafish
Practical reasons to study flies:

- They have a 10 day generation time
- They are inexpensive to rear
- Genetic tools are sophisticated and easy to use

Because flies are fast and inexpensive you can:

- Develop a developmental hypothesis
- Test it
- Define a molecular mechanism
- Test it

all in one graduate student’s thesis project
Practical reasons to study flies:
• They have a 10 day generation time
• They are inexpensive to rear
• Genetic tools are sophisticated and easy to use
• Regulators don’t care about flies
• People share reagents, even before publication

• Because flies are fast and inexpensive you can
  • Develop a developmental hypothesis
  • test it
  • Define a molecular mechanism
  • Test it
    all in one graduate student’s thesis project
• Why Drosophila genetics?

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Different cell fates can be determined by different concentrations of a morphogen, a diffusible molecule present at variable concentrations (A. Turing, 1952)
A cell decides its fate based on its position within the tissue. The pattern of cell fates is established through a coordinate system of positional information. (L. Wolpert, 1969)
Thresholds for responses
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The *Drosophila* body plan is prepatterned in the egg

By RNA and protein from the mother
The *Drosophila* body plan is visible in the exoskeleton (cuticle)
A set of maternal genes establishes each aspect of the body plan.
Maternal genes were identified by embryo phenotypes

Anterior posterior termini and dorsal-ventral

Figure 3. The Cuticular Patterns of Wild-Type and Mutant Embryos
(a) wild-type, (b) anterior (bicoid), (c) posterior (oskar), (d) terminal (torso-like), (e) dorsalized (dorsa?), (f) ventralized (cactus).
Dorsal-ventral axis is patterned by sequential morphogen gradients

Maternal genes: NF-KB gradient subdivides mesoderm/ectoderm

Zygotic genes: BMP gradient subdivides ectodermal territories

St. Johnston & Nüsslein-Volhard, 1992, Cell 68. 201
Why a gradient of Dpp in embryos?

- Uniform RNA levels, protein undetectable

  An inference from the genetic analysis of ectoderm patterning

- Dpp was known to be a BMP, and hypothesized as a morphogen for appendage development.
Why a gradient of Dpp in embryos?

- Uniform RNA levels, protein undetectable
- An inference from the genetic analysis of ectoderm patterning
- Different Dpp mutations cause loss of more or fewer dorsal ectoderm structures
  - Wharton et al, 1993, Development

- Dpp was known to be a BMP, and hypothesized as a morphogen for appendage development.
Different mutations in dpp eliminate different amounts of dorsal fates

Dpp is necessary to make dorsal ectoderm and amnioserosa

Irish & Gelbart, 1987, Genes Dev. 1, 868
Wharton et al. 1993, Development 117, 807
The less DPP, the fewer dorsal structures

Wharton et al. 1993, Development 117, 807
Why a *gradient* of Dpp in embryos?

- Uniform RNA levels, protein undetectable

- Different Dpp mutations cause loss of more or fewer dorsal ectoderm structures
  - Wharton et al, 1993, Development

- Injections of Dpp RNA induced dorsal fates
  - Ferguson and Anderson, 1992, Cell
Dpp RNA is *sufficient* to induce dorsal fates

*zen* is expressed in amnioserosa primordium

*zen* expression is induced in ventral cells by Dpp RNA

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Mutational screens identified classes of genes with shared D-V patterning defects

tld and scw are almost as severe as dpp

Arora et al, 1992, Development 114, 1003
Dpp activity is modulated by \textit{tld} and \textit{sog}

- \textit{tld} regulates Dpp which in turn regulates \textit{zen}
- \textit{sog}

Ferguson & Anderson, 1992, Development 114, 583
Dpp and Screw are BMPs
(bone morphogenetic proteins)

TGFβ proteins organized according to sequence similarity:
A phylogenetic tree

Mouse 57
Fly 7
Worm 4
Experimental tests say that the most similar BMPs are from different species:

Fly DPP can induce bone formation in rats.

Mouse BMP2 can replace fly DPP in dorsal-ventral patterning.

**DPP, BMP2, BMP4 are orthologs**
BMPs regulate nested patterns of gene expression

reviewed by Raftery & Sutherland, 2003 Trends Genet. 19, 701
Model: Sog removes BMPs at low end of gradient

Screw is everywhere

Jazwinska et al, 1999, Development 126, 3323

reviewed by Bier, 1999, Nature 398, 375
Model: a BMP activity gradient at blastoderm induces dorsal ectoderm fates

2 BMPs: Dpp+Screw

Amnioserosa  Dorsal Ectoderm  Ventral Neurectoderm
How do cells respond to the BMP gradient?
Mad and Medea identified in screens for genes that interact with Dpp

Raftery et al, 1995, Genetics 139, 241
Smad proteins transduce TGFβ family signals to the nucleus

Reviewed by Raftery & Sutherland, 1999, Dev. Biol. 210, 251
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How can we visualize a BMP activity gradient?

- Dpp RNA expression is low
- Dpp protein hasn’t been visualized
- Graded distribution of diffusible antagonist, Sog

Srinavasan et al, 2002, Dev. Cell 2, 91

Look at distribution of activated signal transducers
Medea nuclear localization and phospho-Mad predicted to indicate level of BMP activity *in vivo*
A dorsal midline stripe of strong nuclear Medea

End blastoderm
Side view
2.75hr

Gastrulation
Dorsal view
3.25hr

Sutherland et al, 2003, Development 130, 5705
BUT...?

Stripe of intense response is only wide enough to account for amnioserosa induction...

Can we detect a broader signal?
A low level signal in some blastoderm embryos

Medea DNA

uniform cellular blastoderm
cephalic furrow initiated

32 cells wide 3-9 cells wide

Sutherland et al, 2003
co-Smad response domain narrows as peak forms at the dorsal midline

Ross et al, 2001 and Rushlow et al, 2001 have similar observations for PMad
Both co-Smad and phospho-Mad responses elevate during gastrulation.

Sutherland et al, 2003
During gastrulation, peak co-Smad response is further elevated and more cells are involved

- **peak nuc. Med + P-Mad**
- **declining nuc. Med + PMad**
- **uniform Med**
- **no nuclear signal**

The graph shows the number of cells involved:
- 3-4 cells
- About 18 cells
uniform Medea+ low P-Mad
wanng nuc. Med +P-Mad
No detectable signal

CFI
1st gradient transition
ADG
BEH
Phase IPhase IIPhase III
2nd gradient

peak nuc. Med +P-Mad
wanng nuc. Med +P-Mad
uniform Medea+ low P-Mad
No detectable signal

Raftery and Sutherland, 2003
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How is the BMP gradient shaped?

*Mid-blastoderm*

- Wide band
  - Weak signal

*Gastrulation*

- Narrow stripe
  - Strong signal
Sog is an extracellular BMP binding protein that can block signaling

but, Sog is also required to get a normal number of amnioserosa cells

Jazwinska et al, 1999, Development 126, 3323
How does Sog affect the nuclear co-Smad signal?

2 BMPs: Dpp+Screw

[An image showing a graph with labels for Amnioserosa, Dorsal Ectoderm, and Ventral Neurectoderm]

Dpp

Sog
The BMP antagonist Sog limits the domain of signaling...

but Sog is also necessary for the stripe of peak signal.

Sutherland et al, 2003
Sog shapes the BMP activity gradient over time

WT

sog-

quenches

promotes
Modulation of BMP levels: 1992 to 2002

tld → Dpp → zen

sog

TID = Metalloprotease

Sog = BMP binding protein

Zen = transcription factor, Partner to Smads

But how does this work?
A BMP transport model:

A. Input
   dpp (in situ)
   Lateral view

B. Output
   pMad (dorsal view)
   refinement

C. Early blastoderm
   Dpp
   Scw
   Dpp/Scw
   Sog
   Tsg
   Tld

D. Late cellular blastoderm

O’Connor, M. B. et al. Development 2006;133:183-193
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Changing BMP gradient can be described mathematically

Free ligand concentration:

\[
\frac{\partial[L]}{\partial t} = D_L \frac{\partial^2[L]}{\partial x^2} - k_{on}[L](R_0 - [LR]) + k_{off}[LR]
- j_{on}[L][ST] + (j_{off} + \tau)[LST] + V_L(x)
\]

Ligand bound to receptor:

\[
\frac{\partial[LR]}{\partial t} = k_{on}[L](R_0 - [LR]) - (k_{off} + k_{deg})[LR]
\]

Sog concentration over time:

\[
\frac{\partial[S]}{\partial t} = D_S \frac{\partial^2[S]}{\partial x^2} - n_{on}[S][T] + n_{off}[ST] + V_S(x)
\]

Sog bound to Tld:

\[
\frac{\partial[ST]}{\partial t} = D_{ST} \frac{\partial^2[ST]}{\partial x^2} + n_{on}[S][T] - n_{off}[ST]
- j_{on}[L][ST] + j_{off}[LST]
\]

Tld concentration over time:

\[
\frac{\partial[T]}{\partial t} = D_T \frac{\partial^2[T]}{\partial x^2} - n_{on}[S][T] + n_{off}[ST] + \tau[LST] + V_T(x)
\]

Ligand bound to Sog and Tld:

\[
\frac{\partial[LST]}{\partial t} = D_{LST} \frac{\partial^2[LST]}{\partial x^2} + j_{on}[L][ST] - (j_{off} + \tau)[LST]
\]

Mizutani et al, 2005, Dev Cell 8:915-924
Computational modeling of the changing pattern of responses…

Can be used to predict important parameters

Mizutani et al, 2005, Dev Cell 8:915-924
Computational modeling to test the importance of molecular mechanisms...

- Degradation of ligand-receptor complexes
  Mizutani et al, 2005, Dev Cell 8:915-924

- Positive feedback to stabilize narrow, high level gradient
  O'Connor et al., 2006, Development 133:183-193

- Contribution of Screw ligand: heterodimers versus two homodimers
  Shimmi et al. 2005, Cell 120: 873-886
• Pathway genetics
• Prediction of a morphogen gradient
• Identification of signaling pathway from genetics
• Visualization of a gradient from signal transducers
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We still have questions…
How is a threshold response organized to make a tissue boundary?

amnioserosa/
dorsal ectoderm boundary