Axis Specification

in *Drosophila*

July 9, 2008
Drosophila Development - Overview

Fertilization

Cleavage

Gastrulation

Drosophila body plan

Oocyte formation

Genetic control of axis specification
  Anterior-posterior
  Dorsal-ventral

Segmentation genes

Homeotic genes
**Drosophila Fertilization**

Eggs are activated prior to fertilization.
- oocyte nucleus has resumed meiotic division
- stored mRNAs begin translation

Eggs have begun to specify axes by the point of fertilization.

Sperm enter at the micropyle.
- probably prevents polyspermy

Sperm compete with each other!

*Drosophila bifurca*
Superficial Cleavage

**Syncytial blastoderm stage**
- zygotic nuclei undergo 8 divisions
- nuclei migrate to periphery
  - karyokinesis continues

**Cellular blastoderm stage**
- following division 13, oocyte plasma membrane folds inward
- partitions off each nucleus and associated cytoplasm
- constricts at basal end
Superficial Cleavage in Drosophila cellular blastoderm
Gastrulation

(A) Internal ectoderm
   Amnioserosal covering of embryo
   Pole cells (primordial germ cells)

(B) Endoderm
   Nervous system
   Epidermis
   posterior midgut invagination

(C) Neuroectoderm
   Germ cells
   anterior midgut invagination

(D) Mesoderm

(Gastrulation)
Gastrulation - Ventral Furrow
Early Gastrulation

Ventral

Dorsal

Anterior midgut invagination

Cephalic furrow

Ventral furrow

Pole cells

Pole cells in posterior midgut invagination
Mid-Gastrulation

fullest germ band extension: just prior to segmentation

germs band cells:
- form trunk of the embryo
- thorax and abdomen
Late Gastrulation / Segmentation

- organogenesis
- segmentation
- segregation of imaginal discs
- nervous system development
Establishing the Drosophila Body Plan

Segments form along the anterior-posterior axis, then become specialized. Specification of tissues depends on their position along the primary axes. A/P and D/V axes established by interactions between the developing oocyte and its surrounding follicle cells.
Drosophila Body Plan - Egg Stage

Body axes are determined in the egg by distribution of maternal mRNAs and proteins.

Translation leads to formations of patterning protein (e.g. morphogen) gradient within the embryo.

How are asymmetric distributions of messages and proteins established in the egg?
Oocyte Formation (A-P, D-V Axes)

**Drosophila ovariole**
- oogonium divides into 16 cells
  - 1 oocyte
  - 15 nurse cells
  - all interconnected

nurse cells contribute mRNA, proteins cytoplasm

Nurse cells synthesize *gurken* message
- *gurken* mRNA transported toward oocyte nucleus (in posterior region)

Gurkin protein localized between nucleus and cell membrane
- Note – Gurken diffuses only a short distance

**Torpedo** (Gurken receptor) present on follicular cells

Gurkin binding results in “*posteriorization*” of follicles
- posteriorized follicles re-organize egg microtubules; (-) = anterior
Microtubules

Tubulin

α

β
dimer

protophilament microtubule

growth disassembly

kinesin

dynein

(+)

(-)
A-P Axis: *bicoid / Oskar / nanos*

Nurse cells manufacture *bicoid* and *nanos* mRNA - deliver cytoplasm into oocyte

*bicoid* binds to *dynein* - moves to non-growing (-) end of microtubules

*oscar* mRNA forms complex with *kinesin I* - moves toward growing (+) end of microtubules

Oskar binds *nanos* mRNA - retains *nanos* in posterior end

“posteriorized” follicles produce organized (+/-) microtubules

Anterior follicle cells

Microtubules

Posterior follicle cells

*bicoid* mRNA

Anterior border cells

Polarized microtubules

*oscar* mRNA in association with *kinesin I*
The oocyte nucleus (with associated gurken) moves anteriorly along the dorsal margin.

**Gurkin/Torpedo** interactions “*dorsalize*” follicle cells.
D-V Polarity

Gurken/Torpedo inhibits Pipe synthesis in dorsal cells.
Pipe (ventral cells) eventually triggers nuclear Toll receptor activity;
Dorsal determines ventral fates.
Distribution of Dorsal

Dorsal activates genes that create mesodermal phenotype
- transcribed only in cells with highest Dorsal concentrations
  - these genes have low affinity enhancers (lots of Dorsal necessary)
Dorsal also inhibits dorsalizing genes

Dorsal:
- large amount = mesoderm
- lesser amount = glial/ectodermal

mesodermal cells that will invaginate to form ventral furrow
Zygotic Patterning Genes

decapentaplaegic (dpp), zerknüllt (zen), tolloid are dorsal patterning genes
- repressed by Dorsal

Intermediate dorsal activates rhomboid (no Twist or snail)
- determines neural ectoderm
- rhomboid + twist = glial cells

Intermediate dorsal activates fgf8
- fgf8 repressed by snail
- promotes mesodermal ingression

High Dorsal – activates twist and snail (low affinity enhancers)
- mesoderm determinants

Dorsal (TF) – expressed ventrally; establishes diffusion gradient dorsally
Anterior-Posterior Body Plan

Drosophila use a *hierarchy* of gene expression to establish the anterior-posterior body plan.

1. **Maternal effect genes** (e.g. *bicoid*, *nanos*)
   - mRNAs differentially placed in eggs
   - transcriptional or translational regulatory proteins
     - diffuse through syncytial cytoplasm
     - activate or repress zygotic genes

2. Gap genes
3. Pair-rule genes
4. Segment polarity genes

**embryonic segmentation genes**
Anterior Specification - 1

**Stage**

- **Mid-oogenesis**
  - Nurse cells
  - *bicoid* mRNA
  - Oocyte

- **Completion of oogenesis**
  - *bicoid* mRNA

- **Syncytial blastoderm**
  - Bicoid protein
  - Caudal protein

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**Caudal** specifies posterior domain

**Bicoid** binds to *caudal* 3’UTR; prevents translation
### Anterior Specification - 2

**Hunchback – anterior patterning**

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(A) ANTERIOR: BICOID</th>
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</thead>
<tbody>
<tr>
<td><strong>Cellular blastoderm</strong></td>
<td>Hunchback protein gradient</td>
</tr>
<tr>
<td></td>
<td>Pole cells</td>
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<tr>
<td></td>
<td>Anterior gap gene mRNA</td>
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<td></td>
<td>Embryonic cells</td>
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<tr>
<td><strong>Regional specification</strong></td>
<td>Wild-type</td>
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<tr>
<td></td>
<td>bicaoid-deficient</td>
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<tr>
<td></td>
<td>Acron</td>
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<tr>
<td></td>
<td>Head Thorax Abdomen</td>
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<tr>
<td></td>
<td>Telson</td>
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<tr>
<td></td>
<td>Telson</td>
</tr>
<tr>
<td><strong>External phenotype</strong></td>
<td>Wild-type</td>
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<tr>
<td></td>
<td>bicaoid-deficient</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
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</tbody>
</table>
Bicoid Mutants

Normal development

Wild-type

bicoid mRNA

A H T Ab Te

Head Tail

Wild-type phenotype

Development of bicoid-deficient mutant

bcd−

Te Ab Te

Tail Tail

bicoid-deficient phenotype: two tails

A Acron  H Head  T Thorax  Ab Abdomen  Te
Manipulating Bicoid

Experiment: Add *bicoid* mRNA to embryos

1. Add to anterior end of mutant
   - *bcd* −
   - Normal development: Head, Tail

2. Add to middle of mutant
   - *bcd* −
   - "Head" in middle: Tail, Head, Tail

3. Add to posterior of wild-type embryo
   - Wild-type
   - Two "heads": Head, Tail, Head

Legend:
- A: Acron
- H: Head
- T: Thorax
- Ab: Abdomen
- Te: Telson
Posterior Specification - 1

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(B) POSTERIOR: NANOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-oogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>nanos mRNA</td>
</tr>
<tr>
<td></td>
<td>oskar mRNA</td>
</tr>
<tr>
<td></td>
<td>Staufen protein</td>
</tr>
<tr>
<td>Completion of oogenesis</td>
<td>Maternal hunchback mRNA</td>
</tr>
<tr>
<td></td>
<td>nanos mRNA</td>
</tr>
<tr>
<td>Syncytial blastoderm</td>
<td>Hunchback protein</td>
</tr>
<tr>
<td></td>
<td>Nanos protein</td>
</tr>
</tbody>
</table>

**nanos trap:**
Staufen allows oskar translation
Oskar binds nanos

Nanos prevents hunchback translation
Posterior Specification - 2
Model of Anterior-Posterior Patterning

**mRNA in oocytes** (maternal messages)

![Graph showing mRNA concentrations](image)

**Early cleavage embryo proteins**

* **hunchback** translation repressed by Nanos

* **caudal** translation repressed by Bicoid
Terminal Specification - 1

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(C) TERMINAL: TORSO</th>
<th>Torso – transmembrane RTK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-oogenesis</td>
<td>Torso-like protein</td>
<td>Torso uniformly distributed</td>
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<tr>
<td>Completion of oogenesis</td>
<td>Torso protein</td>
<td>Torso activated by Torso-like protein - located only at ends of egg</td>
</tr>
<tr>
<td>Syncytial blastoderm</td>
<td>Activated Torso protein</td>
<td></td>
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### Terminal Specification - 2

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(C) TERMINAL: TORSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular blastoderm</td>
<td>Torso kinases inactivate an inhibitor of <em>tailless</em> and <em>huckebein</em></td>
</tr>
<tr>
<td>Regional specification</td>
<td>Tailless and Huckebein specify termini</td>
</tr>
<tr>
<td>External phenotype</td>
<td>Distinction between anterior and posterior = <strong>Bicoid</strong></td>
</tr>
</tbody>
</table>

**Bicoid** = **acron** formation
Segmentation Genes

Cell fate commitment:
- Phase 1 – **specification**
- Phase 2 – **determination**

- early in development cell fate depends on interactions among protein gradients
- specification is flexible; it can alter in response to signals from other cells
- eventually cells undergo transition from loose commitment to irreversible determination

The transition from specification to determination in *Drosophila* is mediated by the **segmentation genes**.
- these divide the early embryo into a repeating series of segmental primordia along the anterior-posterior axis
Drosophila use a *hierarchy* of gene expression to establish the anterior-posterior body plan.

1. **Maternal effect genes** (e.g. *bicoid, nanos*)
   - mRNAs differentially placed in eggs
   - transcriptional or translational regulatory proteins
     - diffuse through syncytial cytoplasm
     - activate or repress zygotic genes

2. **Gap genes**: first zygotic genes expressed
   - expressed in broad, partially overlapping domains
     - about 3 segments wide
   - activated or repressed by maternal effect genes

Hunchback

Krüppel

Overlap
Anterior-Posterior Body Plan

Drosophila use a hierarchy of gene expression to establish the anterior-posterior body plan.

3. **Pair-rule genes**; differing combinations of gap genes regulate transcription
   - divide the embryo into periodic units
   - results in a pattern of seven transverse bands

4. **Segment polarity genes**; activated by pair-rule genes
   - divide embryo into 14 segment-wide units

5. **Homeotic selector genes**; stimulated by interactions of gap, pair-rule, and segment polarity proteins
   - determines **developmental fate** of each segment
Maternal effect genes

- bicoid
- nanos

Gap genes

- huckebein
- hunchback
- giant

Pair-rule genes

- even-skipped
- fushi tarazu

Segment polarity genes

- engrailed
- hedgehog
- wingless
- patched

(A) Gap: Krüppel

(B) Pair rule: fushi tarazu

(C) Segment polarity: engrailed
Segments and Parasegments

Expression patterns in early embryos are not delineated by segmental boundaries, but by

- **parasegments**: fundamental units of embryonic gene expression

Segments and parasegments organized from A/P **compartments** out of phase

Cells of adjacent compartments **do not mix**

<table>
<thead>
<tr>
<th>Segments</th>
<th>Ma</th>
<th>Mx</th>
<th>Lb</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>A1</th>
<th>A2</th>
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<tr>
<td>Compartments</td>
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<tr>
<td>Parasegments</td>
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<td>5</td>
<td>6</td>
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<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

*fitz*^+^  

*fushi tarazu* – pair-rule gene
Segments and Parasegments - Adult

Segments

Segments 6 5 4 3 2 1

Segmental hinges

Ganglia

Ganglia 5 4 3 2

Segments

Segments

Compartments

Parasegments

Parasegments

muscles/hinge

nerves
Pair-Rule Gene Regulation

e.g. even-skipped (eve)
- each stripe regulated by a different set of enhancers
- expression patterns are stabilized by interactions among other gene products
  e.g. even-skipped expression limited by Giant
Homeotic Selector Genes

Pair-rule and gap genes interact to regulate the homeotic selector genes
- homeotic selector genes determine the identity of each segment

Homeotic genes specify:
- head segments
- labial palps
- antennae
- thoracic segments
  - wings
  - halteres
  - legs
- abdominal segments
Homeotic Gene Expression

Antennapedia complex

- lab
- Dfd
- Scr
- Antp

bithorax complex

- Ubx
- abdA
- AbdB

distal-less – jaws, limbs

Antennapedia – thoracic

Ultrabithorax – abdomen
Ultrabithorax Mutant

$Ubx^{-/-}$ transforms 3$^{rd}$ thoracic segment (halteres)...

...into duplicate 2$^{nd}$ thoracic segment (wings).
Antennapedia Mutant

(A) 

(B)