Axis Specification in *Drosophila*

November 6, 2007
Axis Specification in Drosophila

Fertilization
Superficial 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!
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
Gastrulation

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

(B) Endoderm
Neuroectoderm
Mesoderm
ventral furrow
germ band movement

(C) Germ cells

(D) posterior midgut invagination
Germ cells

(E) anterior midgut invagination
Nervous system
Epidermis
Early Gastrulation

Ventral:
- Anterior midgut invagination
- Cephalic furrow
- Ventral furrow

Dorsal:
- Pole cells in posterior midgut invagination

Pole cells
Mid-Gastrulation

fullest germ band extension:
just prior to segmentation

germ band cells:
- form trunk of the embryo
- thorax and abdomen
Segmentation

- organogenesis
- segmentation
- segregation of imaginal discs
- nervous system

germ band movement
Drosophila Body Plan

Thoracic segments

T1 – legs
T2 – legs & wings
T3 – legs & halteres

Head
Prothorax
Mesothorax
Mesorax
Metathorax

A-P axis
D-V axis
Segmentation

Abdominal segments
Genes that Pattern the Body Plan

**Anterior-posterior** and **dorsal-ventral axes** established by interactions between the developing oocyte and its surrounding follicle cells.

- dorsal-ventral **patterning gradients** are formed within the embryo.

**Segments** form along the anterior-posterior axis, then become specialized.

**Specification** of tissues depends on their position along the primary axes.
Oocyte Formation (A-P, D-V Axes)

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

- nurse cells synthesize **gurken** gene (an **FGF** homologue)
- transported to oocyte nucleus
- localized between nucleus and cell membrane
- **Torpedo** - Gurken receptor (FGF receptor homologue)
- Gurkin signal results in “*posteriorization*” of follicle
- follicles send signal to re-organize microtubule system
Microtubules

Tubulin

β

dimer

protofilament

microtubule

kinesin

dynein

growth

disassembly
bicoid / Oskar / nanos

Nurse cells manufacture **bicoid** 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 *polarized* (+/-) microtubules
Gurken Dorsalizes Follicle Cells

Oocyte nucleus moves anteriorly along the dorsal margin

Gurkin/Torpedo interactions “dorsalize” follicle cells
Dorsal-Ventral 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
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
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
**Anterior - 1**

### Stage: Mid-oogenesis
- **Bicoid mRNA**
- Nurse cells
- Oocyte

### Stage: Completion of oogenesis
- **Bicoid mRNA**

### Stage: Syncytial blastoderm
- **Bicoid protein**
- **Caudal protein**

**Caudal** specifies posterior domain

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

**Hunchback** – anterior patterning

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(A) ANTERIOR: BICOID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular blastoderm</strong></td>
<td>Hunchback protein gradient</td>
</tr>
<tr>
<td></td>
<td>Anterior gap gene mRNA</td>
</tr>
<tr>
<td><strong>Regional specification</strong></td>
<td>Wild-type</td>
</tr>
<tr>
<td></td>
<td>Acron</td>
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<tr>
<td></td>
<td>Head</td>
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<tr>
<td></td>
<td>Thorax</td>
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<tr>
<td><strong>External phenotype</strong></td>
<td>Wild-type</td>
</tr>
<tr>
<td></td>
<td>Telson</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
Messin’ with Bicoid

Experiment: Add *bicoid* mRNA to embryos

- Add to anterior end of mutant: 
  - bcd− → Normal development
- Add to middle of mutant: 
  - bcd− → “Head” in middle
- Add to posterior of wild-type embryo: 
  - Wild-type → Two “heads”
### Posterior - 1

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(B) POSTERIOR: NANOS</th>
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<tbody>
<tr>
<td>Mid-oogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>nanos mRNA</td>
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<tr>
<td></td>
<td>oskar mRNA</td>
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<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>
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**nanos trap:**
Staufen allows oskar translation
Oskar binds nanos

Nanos prevents hunchback translation
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<tbody>
<tr>
<td>Cellular blastoderm</td>
<td>knirps RNA</td>
</tr>
<tr>
<td></td>
<td>giant mRNA</td>
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<tr>
<td>Regional specification</td>
<td>nanos-deficient</td>
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<td>Acron</td>
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<td>Head</td>
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<td>External phenotype</td>
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Model of Anterior-Posterior Patterning

**mRNA in oocytes** (maternal messages)

**Early cleavage embryo proteins**

- *hunchback* translation repressed by Nanos
- *caudal* translation repressed by Bicoid
### Terminal - 1

<table>
<thead>
<tr>
<th>STAGE</th>
<th>(C) TERMINAL: TORSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-oogenesis</td>
<td><img src="image" alt="Diagram showing Torso-like protein uniformly distributed across egg" /></td>
</tr>
<tr>
<td>Completion of oogenesis</td>
<td><img src="image" alt="Diagram showing Torso protein activated by Torso-like protein at egg ends" /></td>
</tr>
<tr>
<td>Syncytial blastoderm</td>
<td><img src="image" alt="Diagram showing activated Torso protein" /></td>
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**Torso** – transmembrane RTK

Torso uniformly distributed

Torso activated by **Torso-like protein** - located only at ends of egg
Terminal - 2

**Terminal: Torso**

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<tr>
<th>STAGE</th>
<th>(C) TERMINAL: TORSO</th>
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<tbody>
<tr>
<td>Cellular blastoderm</td>
<td>Torso kinases inactivate an inhibitor of <em>tailless</em> and <em>huckebein</em></td>
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<tr>
<td>Regional specification</td>
<td><strong>Tailless</strong> and <strong>Huckebein</strong> 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
Maternal effect genes

- bicoid
- nanos

Gap genes

- huckebein
- hunchback
- giant

Pair-rule genes

- even-skipped
- fushi tarazu

Segment polarity genes

- engrailed
- hedgehog
- wingless
- patched
Segments and Parasegments

Expression patterns in early embryos are not delineated by segmental boundaries;
- **parasegments** appear to be the fundamental units of embryonic gene expression
- segments and parasegments organize **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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<td>Parasegments</td>
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<td>4</td>
<td>5</td>
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<td>7</td>
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<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

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

Segments: 6, 5, 4, 3, 2, 1

Segmental hinges

Ganglia: 5, 4, 3, 2
Pair-Rule Gene Regulation

- *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
  - distal-less – jaws, limbs

![Diagram of insect anatomy with labels for Antennapedia complex and bithorax complex]
Ultrabithorax Mutant

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

into duplicate 2\textsuperscript{nd} thoracic segment (wings).
Antennapedia Mutant