Gastrulation III - establishment of body axes

- Anterior-Posterior Axis Formation in Drosophila.
  - Bicoid, the molecular interpretation of a gradient
- Anterior-Posterior Axis Formation in Mouse.
  - Formation of the primitive streak
  - Anterior Visceral Endoderm

Drosophila melanogaster

Fly Anatomy

The Axis is Established Through a Graded Reduction in Pattern

Drosophila melanogaster

The Axis is Established Under Maternal Control

Mutations Affecting the Anterior-Posterior Axis

Bicoid (Bcd) Controls Drosophila A-P Patterning
Evidence the \( Bcd \) is the Key Regulatory Gene

1. Strong \( Bcd \) alleles lead to a complete loss of head structures.
2. \( Bcd \) mutants can be completely rescued by injection of wild-type anterior cytoplasm.
3. Size of head directly related to \( Bcd \) gene dosage.

\[ \text{Hypothesis: } \textcolor{red}{Bcd} \text{ establishes the anterior-posterior axis through the establishment of a morphogen gradient.} \]

\[ \text{Morphogen} \quad \text{An inducing factor that can evoke more than one cell state from the responding tissue.} \]
Gradient
The asymmetric distribution of a protein or protein activity in a tissue.

Hypothesis: Bcd establishes the anterior-posterior axis through the establishment of a morphogen gradient.

Prediction: Bcd must be a cytoplasmic determinant localized to the anterior pole.

Establish A-P axis through localization of Bcd RNA and protein in anterior pole

Establish A-P axis through localization of Bcd RNA and protein in anterior pole

Posterior pole must be cleared of hunchback by nanos.

Posterior: Posterior pole must be cleared of hunchback

Main Function of nanos is to Block Hunchback

How does the embryo interpret the Bcd gradient?

The Conversion of Maternal Information to Zygotic readout.

**Drosophila melanogaster**

Quantitative Relationship Between the Number of *Bcd* and the Pattern of the Embryo

*Bcd* can Activate and Repress Target Genes at Defined Thresholds

The Axis is Established Through a Graded Reduction in Pattern

**Gastrulation III - establishment of body axes**
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  - Gradients and Morphogenesis
  - *Bicoid*
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**Specification #Fate**

Hypothesis: Must exist region a third signal that modifies mesodermal cell types.

**The 3-Signal Model (Smith and Slack)**

**Mus Musculus domesticus**

3-Signal Model Circa 2008
Drosophila vs. Xenopus

Xenopus vs. Mouse

Mouse Node

Node verses Organizer

Node verses Organizer

Conclusion: Must be other source of organizing signals responsible for anterior structures in the mouse.

The AVE is Required for Proper Anterior Patterning

AVE Patterns the Anterior Portion of the Embryo

Formation of the AVE
Does gene expression in the AVE correlate with anterior organizing function?

Evidence:
1. Transplant mouse node get a duplicated axis but secondary axis lacks a head.
2. Genes found to be expressed exclusively within the AVE prior to primitive streak formation.
3. Remove AVE headless embryos results in a duplicated axis but secondary axis lacks a head.
4. Mutations in genes expressed in AVE (e.g., Hoxd1, Lim1, Otx2) associated with anterior truncation.
5. Chicken eggs treated with TGF-ß lack an organizer but retain some anterior patterning.
6. Removal of nodal only from the VE results in anterior truncations.

Removal of nodal only from the VE results in anterior truncations

Basis of experiments:
1. Decedents of tetraploid blastocysts only give rise to extraembryonic tissue.
2. ES cells injected into blastocysts give little to no contribution to the visceral endoderm and trophectoderm.

Restriction of Gene expression Patterns in AVE Precedes Streak Formation

Chimeric Studies to Test the Requirement for nodal in the AVE

AVE Patterns the Anterior Portion of the Embryo

ES Cell Descendants

Pathological Tissue

Visceral Endoderm

Wildtype ES Cell Descendants

Nodal -/- Blastocyst Descendants

Nodal and Anterior Patterning

Removal of the TGF-ß family member nodal only from the VE results in anterior truncations
Chimeric Studies to Test the Requirement for Otx2 in the AVE

AVE Patterns the Anterior Portion of the Embryo

Wildtype ES Cell Descendants

Embryonic Ectoderm

Visceral Endoderm

AVE

Otx2 -/- Blastocyst Descendants

AVE

Wildtype Blastocyst Descendants

Otx2 and Anterior Patterning

Otx2 is Required Both in the AVE and in the Underlying Ectoderm

AVE Patterns the Anterior Portion of the Embryo

Wildtype ES Cell Descendants

Embryonic Ectoderm

Visceral Endoderm

AVE

Otx2 -/- Blastocyst Descendants

AVE

Wildtype Blastocyst Descendants

Current Model for Anterior Patterning

Segmentation

Drosophila (graded reduction)
- Gap Genes
- Pair-Rule Genes
- Segmentation Genes
- Homeobox Genes

Mouse (one segment at a time)
- Somites
- Homeobox Genes
- Notch Pathway
- Brussel et al., 2004
The Drosophila Axis is Established Through a Graded Reduction in Pattern

Gap proteins except hunchback are extremely short-lived.

All gap proteins except Giant are members of the Zn finger transcription family.

Giant Leucine Zipper family

Three Aspects of Body Patterning That are Dependent on Gap Genes
1. Polarity of the body: i.e. sequence of body parts. The sequence can be reversed in the absence of a gap gene.
2. Properly spaces the parasegment primordia. Segments can be fused or entirely absent in gap gene mutants.
3. Proper differentiation into body parts.

From: The Making of a Fly by Lawrence

Pair-Rule Genes

Pair-Rule Genes activated during cell cycle 13.

Mutate loose every other segment.

Expression divides area into the precursors of the segmented body plan.

If pair-rule genes, 3 of which are directly regulated by the gap genes: hairy, even-skipped, and run.

Aspects of Body Patterning That are Dependent on Pair-Rule Genes
• Major role of pair-rule genes is to allocate cells to the 14 parasegments (also have minor role in head development).
• Mutations in pair rule affect every other alternate segment and the affect is to eliminate a specific part of the cuticle pattern.

Pair-Rule Pattern is Established Through Gradual Refinement of Genes to Parasegments
Complex interaction among Pair-Rule genes

Segment Polarity Genes

First set of genes to be expressed after cellularization; i.e. genes involved in signal transduction.

Segment Polarity genes encode genes that are members of either the Wnt or Hedgehog pathway.

Delete Segment Polarity Gene, segment is transformed into mirror image of neighbor.

Aspects of Body Patterning That are Dependent on Segment Polarity Genes

- Establish boundaries and compartment between parasegments.
- Required for ALL segments.

Fly Segmentation Parasegments vs Segments

Homeotic Verses Homeobox

Homeotic: transformation of one tissue type to another.

Homeobox: Proteins containing a Helix-Loop-Helix domain.

Homeobox Code
Co-linearity of the HOX Code

Hox Genes Establish Somite Identity

Co-linearity of the HOX Code