EARLY DROSOPHILA DEVELOPMENT

Fertilization
1) *Drosophila* egg activation occurs at ovulation
   - eggs are ovulated a few minutes before fertilization
   - oocyte nucleus has resumed meiotic division; cytoplasm begins translation from stored mRNAs
2) eggs begin to specify axes by the time of fertilization
3) sperm can enter only at the **micropyle** (a narrow tunnel in the chorion)
   - micropyle probably prevents polyspermy by allowing entry of only one sperm at a time
   - NOTE - no cortical granules in *Drosophila* eggs; although cortical changes seen
4) sperm competition; sperm can be many times as long as the adult fly
   - e.g. *Drosophila melanogaster* sperm tail is 1.8 mm; ~ as long as adult; 300X human sperm
   - entire sperm gets incorporated into egg

Cleavage

**Syncytial blastoderm**
Most insect eggs undergo **superficial cleavage**; large mass of centrally located yolk confines cleavage to the cytoplasmic rim of the egg
- zygote nucleus undergoes eight divisions (256 nuclei) averaging 8 min each
- nuclei then migrate to periphery of the egg
  - mitosis continues (but at a slower rate)

- during the ninth cycle, ~5 nuclei reach the posterior pole
  - these nuclei become enclosed by membranes
  - generate the **pole cells**
    - pole cells give rise to the **primordial germ cells**, then gametes

- most other nuclei arrive at the periphery of the embryo at cycle 10
  - undergo four more divisions

- cytoplasm is non-uniform throughout the cell
  - each nucleus within syncytial blastoderm is contained within its own territory of cytoskeletal proteins
  - each surrounded by microtubules and microfilaments
    - nuclei and surrounding cytoplasm = **energids**

**Cellular Blastoderm**
- following division 13, oocyte plasma membrane folds inward between the nuclei
- eventually partitions off each somatic nucleus into a single cell
- actin-membrane complex begins to constrict at what will become the basal end of the cell
- cellular blastoderm consists of ~6000 cells
- formed within 4 hours of fertilization

**Mid-blastula transition**
- nuclear transcription starts at about cycle 11
- speed of division decreases
- eventually becomes asynchronous

**Gastrulation**
- begins shortly after MBT
- first movements segregate the presumptive mesoderm, endoderm, and ectoderm
  - prospective mesoderm: ~1000 cells constitute the ventral midline of the embryo fold inward to produce the **ventral furrow**
  - ventral furrow eventually pinches off from the surface to become a ventral tube
  - prospective endoderm invaginates to form two pockets at the anterior and posterior end of ventral furrow
  - **pole cells** are internalized along with the endoderm

- embryo bends to form the **cephalic furrow**

- ectodermal cells on the surface and the mesoderm undergo convergence and extension
  - migrate toward the ventral midline to form the **germ band**
  - germ band: collection of cells along the ventral midline
    - includes all cells that will eventually form the trunk of the embryo
  - germ band extends posteriorly
    - wraps around the dorsal surface of the embryo
    - at the end of germ band formation, the cells destined to from the most posterior larval structures are located immediately behind the future head region
  - body segments begin to appear: dividing ectoderm and mesoderm
  - germ band retracts; places presumptive posterior segments at the posterior tip of the embryo

- key morphogenic events (occurring while germ band is in its extended position)
  - organogenesis
  - segmentation
  - segregation of imaginal discs
  - nervous system forms from two regions of ventral ectoderm
    - neuroblasts differentiate from neurogenic ectoderm within each segment
    - also form in non-segmented area of head ectoderm
    - **NOTE** - in insects (and other arthropods) nervous system located ventrally
- Drosophila body plan: groups of repeated segments
  - head
  - thorax
    - formed from three segments
      - first thoracic segment (T1) - legs only
      - T2 - legs and wings
      - T3 - legs and halteres (balancing organs; modified wings)
  - abdomen (tail region)

GENES THAT PATTERN THE DROSOPHILA BODY PLAN
Overview:
- dorsal-ventral and anterior-posterior axes established by interactions between the developing oocyte and its surrounding follicle cells
- dorsal-ventral patterning gradients formed within the embryo
  - gradients specify different tissue types
- segment formation along anterior-posterior axis
  - segments become specialized
- specification of tissues depends on position along primary axes

Primary Axis Formation during Oogenesis
Anterior-posterior polarity in the oocyte
Oocytes are derived from the oogonium
- single cell surrounded by follicle cells
  - oogonium divides (with incomplete cytokinesis: cells connected by cytoplasmic bridges) into
    - 1 oocyte precursor and 15 nurse cells
  - oocyte positioned at posterior of the follicle

- nurse cells synthesize gurken gene (homologue of vertebrate EGF)
  - gurken message transported to oocyte nucleus
  - localized between nucleus and cell membrane (at posterior end)
  - translated into Gurken protein

- Gurkin signal received by follicle cells
  - Torpedo protein (homolog of vertebrate EGF receptor)
  - NOTE - Gurken diffuses a very short distance, so only follicle cells in close proximity to nucleus will be affected

- Gurkin signal results in “posteriorization” of follicle cells
  - follicle cells differentiate into dorsal follicle cells
  - send signal (protein kinase A) back into the oocyte
    - PKA activity reorganizes the cytoskeleton (microtubules)
    - microtubules oriented specifically with their minus (cap) ends anterior and plus (growing) ends posterior
- at the same time, nurse cells transport cytoplasm to oocyte; increase oocyte size at the expense of nurse cells
- nurse cell cytoplasm contains messages
  - messages transported via microtubules to distinct oocyte locations; e.g.
    - **bicoid** - anterior
      - **bicoid** message binds to **dynein** (actually binds to Exuperantia, another protein that binds to dynein); associated with non-growing end of microtubules
      - dynein moves **bicoid** message to anterior end of egg; stabilizes there
    - **nanos** - posterior
      - **oskar** message complexes with kinesin I; moves towards the growing end of microtubules
      - kinesin I moves **oskar** to posterior end of oocyte
      **Oskar** protein binds **nanos** message; retains it in the posterior region

**Dorsal-ventral patterning in the oocyte**
- as oocyte volume increases, the nucleus moves to an anterior dorsal position
  - **gurken** message becomes localized in a crescent between the oocyte nucleus and oocyte cell membrane
  - Gurken protein forms anterior-posterior gradient along dorsal surface of the oocyte
    - Gurken protein signals cells to become dorsal follicle cells
    - establishes follicle cell dorsal-ventral polarity
- Gurken-Torpedo signal specifying **dorsalization** of follicle cells initiates a cascade of gene activities creating dorsal-ventral axis of embryo
  - activated Torpedo receptor inhibits expression of **pipe** gene
    - **Pipe** protein made only in ventral follicle cells
  - Pipe activates **Nudel** protein
    - Nudel activates **serine proteases**
      - serine proteases secreted into perivitelline space by embryo
      - proteases secreted as inactive forms; activated by peptide cleavage
  - series of protein cleavages initiate by Nudal protein results in cleavage (activation) of **Spätzle** protein
- protease cleavage (activation) must be limited to most ventral portion of the embryo
  - accomplished by the secretion of a protease inhibitor from the follicle cells
  - inhibitors found uniformly throughout perivitelline space surrounding embryo
    - therefore, protease activity limited to ventral areas where highest protease concentration occurs
- cleaved Spätzle binds **Toll** receptor in oocyte cell membrane
  - Toll protein is a maternal product; evenly distributed throughout oocyte membrane
- activated only on the ventral side of the egg
- therefore, Toll receptors on ventral side of egg are transducing a signal into the egg; Toll receptors on dorsal side are not
- localized activation establishes the dorsal-ventral polarity of the oocyte

**Generating Dorsal-Ventral Pattern in the Embryo**

**Dorsal, the ventral morphogen**
- **Dorsal** protein distinguishes **dorsum** (back) from **ventrum** (belly)
- but, dorsal message is evenly distributed throughout the embryo
  - dorsal mRNA placed in the oocyte by the nurse cells
  - protein not synthesized until ~90 min post fertilization
- Dorsal acts as a transcription factor
  - activates or represses transcription
- however, Dorsal is translocated into nuclei only in ventral part of the embryo
- in the dorsal region, gene transcription specifying dorsal region remains active; ventral genes are not transcribed
- Dorsal protein is complexed with **Cactus** protein
  - as long as Dorsal is bound to Cactus, it cannot enter nucleus
- **Toll receptor** activation results in a series of transcriptions that result in Cactus degradation and Dorsal release
  - Toll (receptor) is activated by a gradient of **Spätzle** protein (ligand) that is highest in the ventral region; therefore, highest concentrations of Dorsal occur in the most ventral cell nuclei

**Effects of the dorsal protein gradient**
A fate map can be generated based on the Dorsal gradient; starting from the highest concentration in the most ventral areas and moving to the lowest concentration in the most dorsal areas
- large amounts of Dorsal instruct the cells to become mesoderm
- lesser amounts instruct the cells to become glial or ectodermal tissue
- first morphogenetic event of *Drosophila* gastrulation is the invagination of the 16 ventral-most cells of the embryo
  - all body muscles, fat bodies, gonads derive from these mesodermal cells
- Dorsal specifies these cells to become **mesoderm** in two ways
  1. Dorsal activates specific genes that create the mesodermal phenotype; e.g.
     - **twist**, **snail**, Fgf8, FGF8 receptor, **rhomboid**
     - transcribed only in nuclei receiving highest Dorsal concentration
     - enhancers bind Dorsal at low affinity
     - some of these gene products bind to and inhibit the others
- therefore, interactions between gene products differentially initiated by Dorsal are also involved with specification

- intermediate levels of nuclear Dorsal activate transcription of other genes; lead to formation of neural differentiation into **neural ectoderm**

2. Indirect determination of mesoderm - Dorsal inhibits dorsalizing genes; e.g. - **zerknüllt (zen)** and **decapentaplegic (dpp)**

- thus, in the same cells Dorsal can act as an activator of some genes and a repressor of others
- depends on structure of the genes enhancers
- **zen** enhancer has a silencer region that contains a Dorsal binding site, as well as binding sites for two other TFs
- the two other proteins allow Dorsal to bind transcriptional repressor protein **Groucho** and bring it to the DNA

**Segmentation and the Anterior-Posterior Body Plan**

*Drosophila* use a hierarchy of genes to establish anterior-posterior polarity

- **maternal effect genes** - mRNAs differentially placed in eggs
  - encode transcriptional and translational regulatory proteins that diffuse through the syncytial blastoderm; activate or repress expression of zygotic genes

- first zygotic genes expressed: **gap genes**
  - expressed in broad, partially overlapping domains; about 3 segments wide

- differing combinations and concentrations of the gap gene proteins regulate transcription of **pair-rule genes**
  - divide embryo into periodic units
  - results in striped pattern of seven transverse bands perpendicular to the anterior-posterior axis

- pair-rule proteins activate transcription of **segment polarity genes**
  - mRNA and protein products divide the embryo into 14-segment-wide units, establishing periodicity of embryo

- gap, pair-rule, segment polarity proteins interact to regulate **homeotic selector genes**
  - determines developmental fate of each segment

**Maternal gradients: Polarity regulation by oocyte cytoplasm**

- two (at least) **organizing centers** in the inset egg: anterior and posterior
- ligation of the egg early in development results in one anterior half and one posterior half; no middle
- ligature later in development resulted in more of middle segments represented

- destruction of mRNA in anterior or posterior resulted in embryos lacking head/thorax or abdomen/telsons, respectively, but developing mirror-image anterior-anterior or posterior-posterior morphologies
  - evidence suggested the existence of two mRNA/protein gradients, originating in anterior and posterior

The molecular model: Protein gradients in the early embryo
- bicaud mRNAs located near the anterior tip of the unfertilized egg
- nanos mRNA located at posterior tip
  - both transported by microtubule network in developing oocyte
- after ovulation and fertilization, bicaud and nanos messages translated into proteins that diffuse in the syncytial blastoderm
  - form gradients

bicaud localization
- bicaud 3'UTR binds Exuperantia and Swallow proteins while in the nurse cells
- bicaud -Exuperantia complex transported out of nurse cells via microtubules
  - complex rides on a kinesin ATPase
  - once in oocyte, complex attaches to dynein proteins that are maintained at the microtubule organizing center (the minus end) at the anterior of the oocyte

nanos localization
- nanos mRNA gets “trapped” in the posterior end of oocyte by passive diffusion
- nanos message bound to cytoskeleton in the posterior region of the egg through its 3' UTR and association with other proteins e.g. Oskar, Valois, Vasa, Staufen, Tudor proteins
- nanos-specific RNA trap at posterior via the Oskar protein
  - oskar message and Staufen protein transported to posterior end of oocyte by kinesin I
    - bound to actin microfilaments of the cortex
    - Staufen allows translation of the oskar message
      - Oskar protein binds nanos message

- NOTE - most nanos is not trapped
  - but is bound in cytoplasm by translation inhibitors Smaug and CUP

- Nanos and Bicoid proteins diffuses toward center of embryo
  - create two opposing gradients

- hunchback (hb) and caudal (cad); maternal mRNAs which are critical in patterning anterior and posterior regions, respectively
- synthesized by nurse cells
- distributed uniformly throughout syncytial blastoderm
- however, *hb* and *cad* translation is repressed by Bicoid and Nanos proteins

- in anterior regions, Bicoid binds to caudal 3'UTR; prevents translation
  - if *caudal* is expressed in anterior, head and thorax do not form properly
  - Caudal is critical in specifying the posterior domains of the embryo
    - activates genes responsible for invagination of the hindgut

- posterior: Nanos prevents hunchback translation
  - normal *hb* translation involves interaction of 3'UTR with Pumilio protein
  - Nanos protein binds to Pumilio; deadenylates *hb* mRNA; prevents translation

Summary: four maternal protein gradients
- anterior-to-posterior gradient of Bicoid protein
- anterior-to-posterior gradient of Hunchback protein
- posterior-to-anterior gradient of Nanos protein
- posterior-to-anterior gradient of Caudal protein

- Bicoid, Hunchback, Caudal proteins are transcription factors
  - relative concentrations activate or repress zygotic genes

The anterior organizing center: The Bicoid and Hunchback gradients
- Bicoid mutants (homozygous, *bcd*−) show telson-abdomen-abdomen-telson morphology
  - *exuperantia* and *swallow* genes responsible for keeping bicoid at anterior pole
  - absence of *exuperantia* and *swallow* allows the Bicoid protein to diffuse farther into posterior of the egg
    - Bicoid gradient less steep in *exuperantia* and *swallow* mutants
    - phenotype similar to *bicoid*-deficient embryos, but less severe

- injection of purified bicoid mRNA could rescue anterior structures of *bicoid*-deficient embryos
  - any location in an embryo where bicoid message was injected became the head
    - including middle (thorax-head-thorax)
    - also in posterior region (head-thorax-abdomen-thorax-head)

- Bicoid acts to inhibit translation of *caudal*
  - *caudal* expression is critical for specifying posterior
- Bicoid acts primarily as a TF to activate expression of genes in the anterior
  - Bicoid binds to an activates *hunchback*
    - *hunchback* expression seen only in anterior
  - transcription reinforces gradient of maternal Hunchback protein produced by Nanos-independent translational repression
- mutants deficient in both maternal and zygotic \textit{hb} genes lack mouth parts and thorax structures

\textbf{The terminal gene group}

The unsegmented extremities of the A-P axis are the \textbf{acron} (anterior) and \textbf{telson} (posterior)

- \textit{torso} activity involved with both acron and telson formation
  - Torso protein is a transmembrane \textbf{receptor tyrosine kinase}
  - \textit{torso} mRNA synthesized by ovarian cells, deposited in the oocyte and translated after fertilization
  - however, since Torso protein is evenly distributed throughout the plasma membrane, and
    - \textit{torso} mutants lack both acron and telson, and
    - \textit{torso} gain-of-function mutants (impart constitutive activity) convert entire anterior half of the embryo into an acron; entire posterior half into a telson
  - Torso must be activated only at the ends of the eggs by another activity

- \textbf{Torso-like protein} activates \textit{torso}
  - \textit{Torso-like} gene usually expressed only in the anterior and posterior follicle cells
  - secreted Torso-like protein can cross the perivitelline space to active Torso in egg membranes
  - phosphorylated end products of Torso-activated RTK-kinase cascade diffuse into the cytoplasm at both ends of the embryo
  - kinases inactivate the \textbf{Groucho} protein
    - Groucho is a transcriptional inhibitor of the \textit{tailless} and \textit{huckebein} gap genes
    - \textit{tailless} and \textit{huckebein} specify the termini of the embryo
    - distinction between anterior and posterior is due to the presence of Bicoid
  - if \textit{tailless} and \textit{huckebein} act alone, the terminal region differentiates into a telson
    - if Bicoid is present, terminal region forms an acron

\textbf{Segmentation Genes}

Cell fate commitment has two phases: \textbf{specification} and \textbf{determination}

- early in development cell fate depends on interactions with protein gradients
- specification is flexible; can alter in response to signals from other cells
- eventually cells undergo a transition from loose commitment to irreversible determination
  - fate becomes cell-intrinsic (i.e. cells are committed to fate, regardless of their environment)
- the transition from specification to determination in \textit{Drosophila} is mediated by the \textbf{segmentation genes}
- Segmentation genes divide the early embryo into a repeating series of segmental primordia along the anterior-posterior axis

- Three groups of segmentation genes (names are based on mutant phenotypes)
  1. **Gap genes** - mutants lack large regions of the body (several contiguous segments)
  2. **Pair-rule genes** - mutants lack portions of every other segment
  3. **Segment polarity genes** - mutants show defects (deletions, duplications, polarity reversals) in every segment

**Segments and Parasegments**

Gene expression patterns do not always follow segment boundaries
- Some expression patterns occur in the posterior half of one segment and the anterior half of the next segment
- These “ transegmental” units are termed **parasegments**
- Expression patterns in early embryos are delineated by parasegmental boundaries; not segmental boundaries
  - Parasegments appear to the fundamental unit of embryonic gene expression; e.g.
  - Segment boundaries are seen in adult epidermis and musculature, but
  - Parasegmentation is seen in adult nerve cord (*Drosophila*)

- Segments and parasegments are two different ways of organizing the **compartments** (anterior and posterior) along the A-P axis
  - Cells of one compartment do not mix with cells from their neighboring compartments
  - Segments and parasegments are out of phase by one compartment

- Organization may be necessary for efficient movement; e.g.
  - Nerves are organized by parasegments; cuticle grooves and musculature are segmental
  - If segmental border is a movable hinge; shift in frame by one compartment allows muscle on both sides of any particular epidermal segment to be coordinate by the same ganglion
  - Allows rapid and coordinated muscular movement for locomotion

**NOTE** - Similar phenomenon in vertebrate somites: posterior portion of anterior somite combines with anterior portion of the next somite

**The gap genes**

**Gap genes** are activated or repressed by **maternal effect genes**
- Gap genes e.g. *hunchback, Krüppel, knirps, giant, tailless, huckebein*
- Expressed in one of two broad domains along the anterior-posterior axis
- Expression patterns correlate with regions of embryo that are missing in gap mutations
- expression patterns of gap genes are highly dynamic, i.e.
  - genes usually show low, fairly uniform expression pattern in the early embryo
  - and expression is consolidated later in development as cleavage continues
    - e.g. Hunchback gradient important in establishing initial gap gene pattern
    - by the end of nuclear division 12 Hunchback protein is found at high levels across
      the anterior embryo
      - Hunchback forms a steep gradient through ~15 nuclei (near the middle of
        the embryo); last third of embryo has undetectable Hunchback
        protein levels
  - transcription patterns of anterior gap genes are initiated by the different concentrations
    of Hunchback and Bicoid proteins
    - high Bicoid and Hunchback induce expression of giant
    - Krüppel appears over the region where Hunchback begins to decline
    - high Hunchback also represses posterior gap genes (e.g. knirps, giant) in anterior
    - Caudal protein (highest in posterior) is responsible for activating abdominal gap
      gene knirps and giant in the posterior embryo
  - gap gene expression patterns are stabilized by mutual repression of non-adjacent gap
    proteins
    - i.e. proteins from non-adjacent expression domains repress each others
      expression
      - this repression can be very strong; results in precise expression patterns
      - results in precise placement of gap protein domains, but permits overlaps
        between adjacent gap genes

The pair-rule genes
First indication of segmentation: pair-rule gene expression during cell division cycle 13
  - pair-rule expression divides the embryo into regions that are precursors of the segmental
    body plan
  - eight pair-rule genes
    - primary (hairy, even-skipped, runt) are expressed in seven stripes
      - NOTE: regulatory regions of hairy, even-skipped, and runt expression are each
        unique! Expression in each stripe is determined by distinct enhancers and
        regulatory mechanisms.
      - enhancers are modular: contain binding sites for gap proteins
      - therefore, different concentrations of gap proteins determine whether a
        pair-rule gene is expressed or not
  - 15 divisions along the A-P axis are established by pair-rule gene expression
  - the even-skipped (eve) enhancer is composed of modular units arranged such that each unit
    regulates a separate stripe or pair of stripes
- e.g. *eve* stripe 2 is controlled by a 500 bp region that is activated by low Bicoid and Hunchback concentrations
  - repressed by Giant and Krüppel proteins
  - minimal enhancer region for *eve* stripe 2 contains 5 binding sites for Bicoid, 1 for Hunchback, 3 for Krüppel, 3 for Giant
    - the enhancer region acts a switch that can directly sense the concentration of these proteins; make on/off transcriptional decisions

- once initiated by the gap gene proteins, transcription pattern of the primary pair-rule genes becomes stabilized by interactions among their products
  - primary pair-rule genes also form the context that allows or inhibits expression of later-acting secondary pair-rule genes
    - e.g. *fushi tarazu* (*ftz*) (Japanese: “too few segments”)
      - during early development *ftz* and Fushi tarazu protein are present throughout the segmented portion of the embryo
      - as proteins from primary pair-rule genes begin to interact with the *ftz* enhancer, *ftz* gene is repressed into certain bands of nuclei to create interstripe regions
      - Ftz protein interacts with its own promoter to simulate more transcription of *ftz* where it is already present

- eight known pari-rule genes are all expressed in striped patterns, but,
  - the patterns are not coincident with each other; i.e.
    - each row of nuclei within a parasegment has its own array of pair-rule products that distinguishes it from any other row
    - these products activate the segment polarity genes

The segment polarity genes
- post syncytial embryo stage: interaction between cells are mediated by **segment polarity genes**
  - functions:
    1. reinforce parasegmental periodicity established by the earlier transcription factors
    2. cell fates established within each parasegment through cell-to-cell signaling

- segment polarity genes encode proteins that are constituents of the **Wingless** and **Hedgehog** signal transduction pathways
  - mutations = defects in segmentation and in gene expression pattern across each parasegment

- development of a normal pattern relies on only one row of cells in each parasegment expressing the Hedgehog protein and only one row expressing the Wingless protein
  - the key to this pattern is the activation of the **engrailed** gene in those cells that are going
to express Hedgehog
- *engrailed* gene is activated in cells that have high levels of Even-skipped, Fushi tarazu, or Paired transcription factors
- *engrailed* is repressed in those cells with high levels of Odd-skipped, Runt, or Sloppy-paired proteins
- as a result, the Engrailed protein is found in 14 stripes across the anterior-posterior axis of the embryo

- stripes of *engrailed* transcription mark the anterior compartment of each parasegment (and the posterior compartment of each segment)
- *wingless* gene is activated in those bands of cells that receive little or no Even-skipped or Fushi tarazu protein, but which do contain Sloppy-paired
  - this pattern causes *wingless* to be transcribed solely in the row of cells directly anterior to the cells where *engrailed* is transcribed

- *wingless* and *engrailed* expression patterns maintained by reciprocal interaction between neighboring cells
  - cells secreting Hedgehog activate the expression of *wingless* in its neighbor
  - Wingless protein maintains *hedgehog* expression in neighboring cells

**The homeotic selector genes**
- after the parasegmental boundaries are set up, pair-rule and gap genes interact to regulate the
  - **homeotic selector genes**
    - homeotic selector genes determine the identity of each segment

- in *Drosophila*, two regions of chromosome 3 contain most of the homeotic genes
  - the **Antennapedia** complex contains *labial, Antennapedia, sex combs reduced, Deformed, proboscopedia*
    - *labial* and *Deformed* genes specify head segments
    - *sex combs reduced* and *Antennapedia* specify thoracic segments
    - *proboscopedia* acts only in adults: in its absence, labial palps of the mouth are transformed into legs

  - the **bithorax** complex contains:
    - *Ultrabithorax* - specifies the third thoracic segment
    - *abdominal A (abdA), Abdominal B (AbdB)*- specify the segmental identity of abdominal segments

  - homeotic mutations; e.g.
    - *Ultrabithorax* mutant:
      - Ultrabithorax specifies 3rd thoracic segment (characterized by halteres)
      - in the Ultrabithorax mutant, the 3rd thoracic segment is transformed into another
2nd thoracic segment; results in wings instead of halteres

- *Antennapedia* mutant:
  - Antennapedia specifies 2nd thoracic segment
  - when expressed in head, results in legs rather than antennae

**Initiating the patterns of homeotic gene expression**
- the initial domains of homeotic gene expression are influenced by the gap genes and pair-rule genes; e.g.
  - expression of *abdA* and *AbdB* genes are repressed by the gap gene proteins Hunchback and Krüppel
  - Hunchback and Krüppel prevent abdomen-specifying genes from being expressed in the head and thorax

- conversely, the *Antennapedia* gene is activated by particularly high levels of Hunchback (amount needed requires both maternal and zygotically transcribed messages)
  - *Antennapedia* is originally transcribed in parasegment 4, specifying mesothoracic (T2) segment

**Maintaining the patterns of homeotic gene expression**
- expression of homeotic genes is a dynamic process: e.g.
  - *Antennapedia* gene initially expressed in presumptive parasegment 4 also appears in parasegment 5;
  - as the germ band expands *Antp* is expressed in the presumptive neural tube as far as parasegment 12
  - *Antp* expression then contracts
    - transcripts localized strongly to parasegments 4 & 5

- *Antp* expression is strongly negatively regulated by all of the homeotic gene products expressed posterior to it
  - e.g. each of the bithorax complex genes represses the expression of *Antp*
    - thus, if *Ultrabithorax* gene is deleted, *Antp* activity extends through the region that would normally have expressed *Ubx* and stops where *Abd* region begins

- identities of segments (i.e. specified by transient expression of gap and pair-rule genes) are stabilized so that differentiation can occur
  - once transcription patterns of homeotic genes have been stabilized, they are “locked” into place by alteration of the chromatic conformation in these genes
  - repression of gene appears to be maintained by the *Polycomb* family of proteins
  - active chromatin conformation appears to be maintained by the *Trithorax* proteins
Realisator genes
- **realisator genes** are homeotic gene targets
  - function to form specified tissue or organ primordia
  - e.g. Antennapedia protein binds to an represses the enhancers of at least two genes:
    - *homothorax* and *eyeless*
      - *homothorax* and *eyeless* code for transcription factors that are critical for antenna and eye formation, respectively
      - Antennapedia suppresses genes that would trigger antenna and eye development
- Ultrabithorax protein represses expression of wingless gene in haltere cells
- *distal-less* gene (also a homeobox-containing gene) is a target of homeotic proteins
  - necessary for limb development
  - active only in thorax
    - *distal-less* is repressed in the abdomen by a combination of UBx and AbdA proteins and pair-rule genes;
      - these proteins bind to the *distal-less* enhancer; block transcription