Segmentation and patterning in *Drosophila*

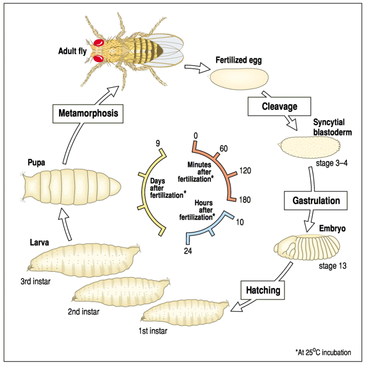
BCH 512 Developmental Genomics

Figures drawn primarily from Gilbert *"Developmental Biology"* and Wolpert *"Principles of Development"*

Why Flies?

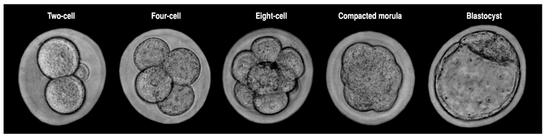
- short generation time (~ 12 days)
- many visible phenotypes
- only 4 chromosomes (3 autosomes, X&Y)
- good genetic and physical maps (polytene chromosomes)
- easy transgenesis
- 12 sequenced genomes

The Drosophila Life Cycle

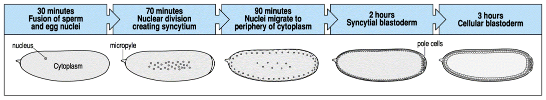


*At 25°C incubation

Cleavage vs. Syncytial Development



mouse



fly

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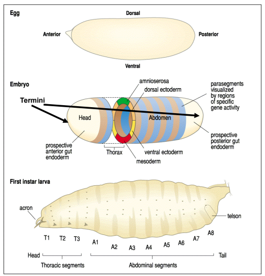
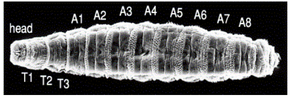
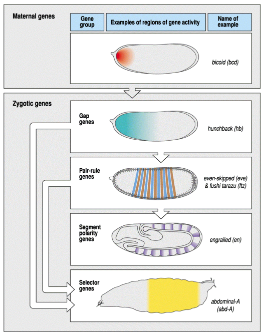
Main body axes and patterning by maternal effect genes

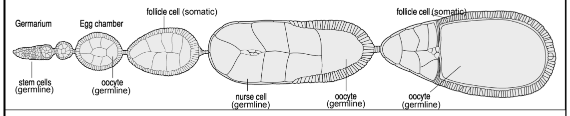




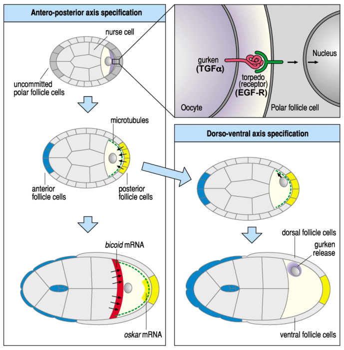
Sequential expression of different sets of genes establishes the body plan along the antero-posterior axis

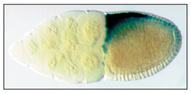


Egg development in *Drosophila*



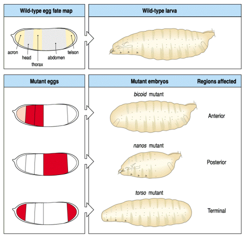
The oocyte has an intrinsic polarity, specified by the position of the nucleus

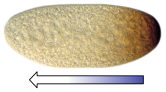




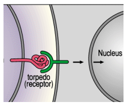
Anterior-Posterior Axis

- Separate groups of genes pattern the A-P axis (anterior, posterior, terminal)
- the syncytial nature of the embryo is critical here
- much of the process is driven by RNA localization and repression of translation (leading to protein gradients)
- zygotic genes respond to these gradients



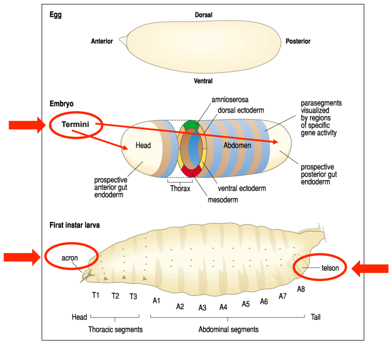


syncytium allows diffusion/transport



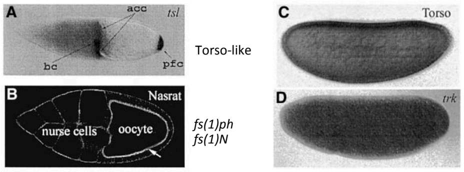
cellularization requires signalling

The main body axes and patterning by maternal effect genes



Terminal group genes: *fs(1)ph, fs(1)N, torso-like, trunk, torso, tll, hkb*

- mutations in these genes give similar terminal phenotypes
- Torso (Tor) is a receptor tyrosine kinase *(RTK→Ras→Raf→MEK→MAPK)*
- Torso and Trunk are evenly distributed around the embryo
- activating (gain-of-function) mutations in Torso or Trunk convert the entire embryo into anterior and posterior termini



Torso-like

fs(1)ph
fs(1)N

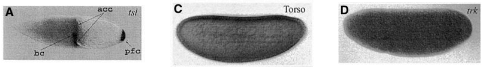
Torso

Trunk

Furriols and Casanova EMBO J. 22:1947 (2003)

Terminal group genes: *fs(1)ph, fs(1)N, torso-like, trunk, torso, tll, hkb*

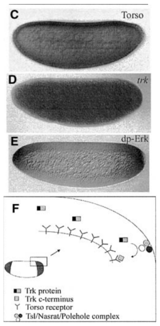
- loss-of-function mutations in *torso-like (tsl), torso (tor)* and *trunk* (*trk)* have similar phenotypes
- ectopic expression of Tsl leads to ectopic activation of Tor



- Trk looks like a ligand
- Trk is uniformly distributed in the oocyte
- Trk has a putative cleavage site which creates a null mutation when mutated

- Trk C-terminal can activate Torso even in absence of *tsl*, *fs(1)ph* and *fs(1)N*
- on the other hand, ectopic *tsl* expression still requires *trk, fs(1)ph* and *fs(1)N* to induce gain-of-function phenotypes

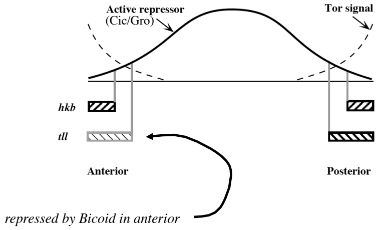
The termini are determined by localized activation of the Torso receptor tyrosine kinase



Furriols and Casanova EMBO J. 22:1947 (2003)

soma
fs(1)ph
fs(1)N
vitelline membrane x-linking
tsl accumulation/stablility
↓
Torso-like (tsl)
↓
germline
Trunk (ligand)
↓
Torso (RTK)
↓
Ras/Raf/MAP Kinase/etc.
⊥
capicua (cic) + gro
⊥
hkb
tll

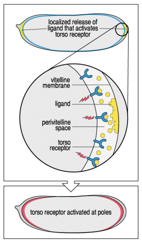
The termini are determined by localized activation of the Torso receptor tyrosine kinase



repressed by Bicoid in anterior

Key concepts in terminal patterning

- *Local activation of a global receptor*
- The end result is a *gradient of signaling* leading away from the poles—target genes respond to higher or lower levels of MAPK signaling via *relief of repression* from a global repressor



Anterior-Posterior Axis

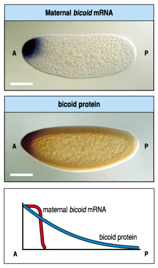
- Separate groups of genes pattern the A-P axis (anterior, posterior, terminal)
- the syncytial nature of the embryo is critical here
- much of the process is driven by RNA localization and repression of translation (leading to protein gradients)
- zygotic genes respond to these gradients

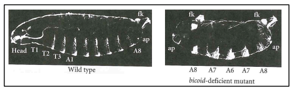
Anterior group genes:
bicoid (bcd)
exuperantian (exu)
swallow (swa)

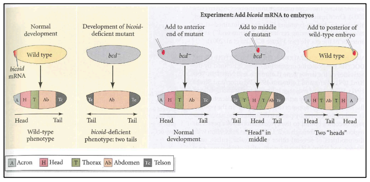
Posterior group genes:
nanos (nos)
tudor (tud)
oskar (osk)
vasa (vas)
valois (val)
pumilio (pum)
caudal (cad)

Anterior-Posterior Axis: The Anterior Morphogen

- the syncytial nature of the embryo is critical here
- much of the process is driven by RNA localization and repression of translation (leading to protein gradients)

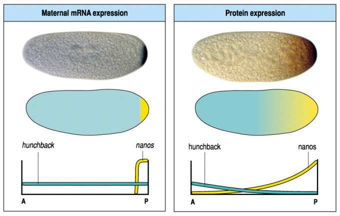


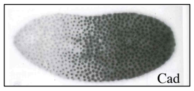




Anterior-Posterior Axis: The Posterior Morphogens

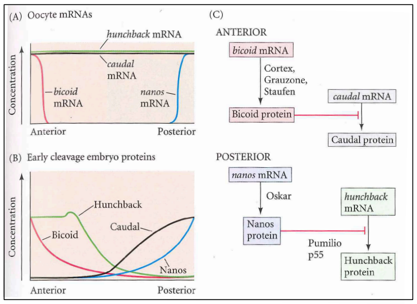
- the syncytial nature of the embryo is critical here
- much of the process is driven by RNA localization and repression of translation (leading to protein gradients)



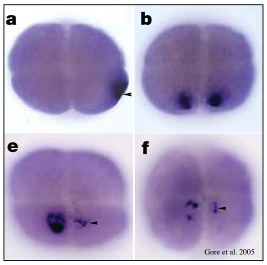


Key concepts in anterior/posterior patterning:

- •localized RNA *(bcd, nos)*
- •translation inhibition *(cad, hb* by *bcd, nos)*
- •morphogen gradients and diffusion/transport

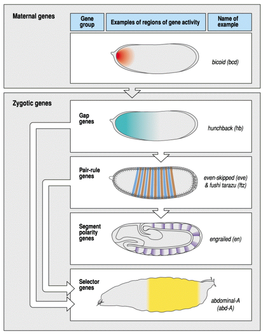


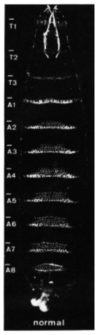
Maternal localization of *sqt* RNA in zebrafish presages the dorsal axis



Gore et al. 2005

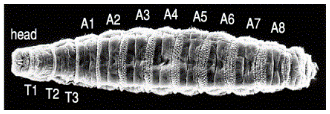
Sequential expression of different sets of genes establishes the body plan along the antero-posterior axis

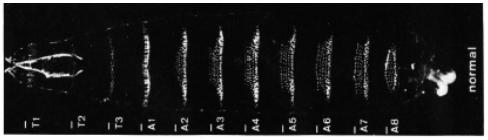




Segmentation I : The power of genetics

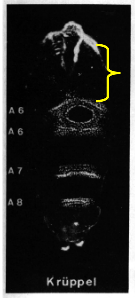
- saturation mutagenesis of *Drosophila* by Nüsslein-Volhard, Wieschaus, et alia
- scored pattern of larval denticle belts in lethal mutations

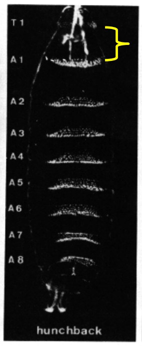


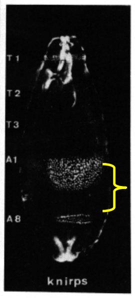


anterior posterior

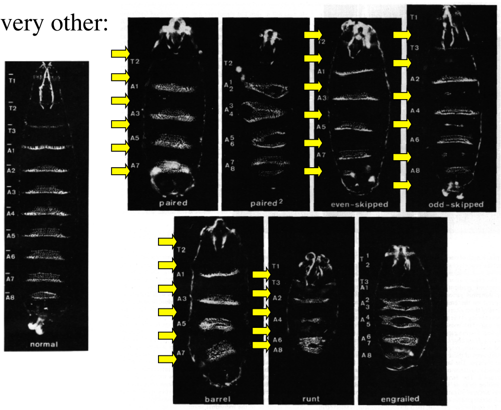
Gaps:



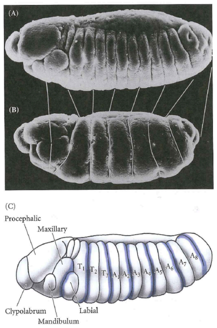




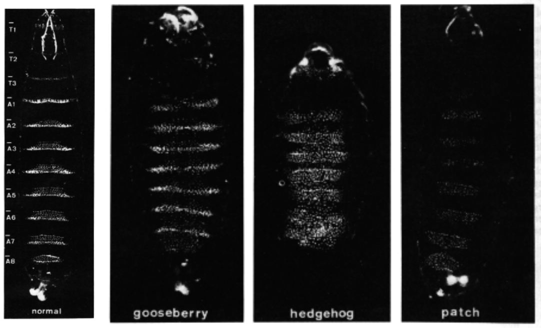
Every other:

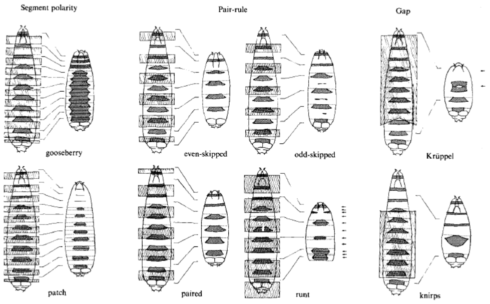


Every other: *ftz*



Mirroring:





Summary of gene classes in segmentation

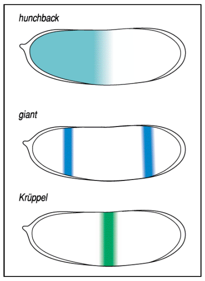
- **Gap genes** set up broad regions along the A-P axis
- **Pair-rule genes** establish the parasegments
- **Segment polarity genes** divide the parasegments into anterior and posterior compartments

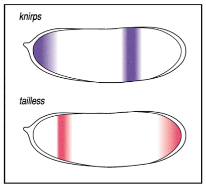
Segmentation II: molecular bio

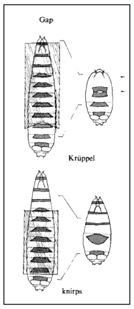
- Most have been cloned
- These discoveries have had an enormous impact and drive large parts of today's research in vertebrate development and in medicine
- The molecular data are quite striking…

Gap Genes:

- are expressed in discrete domains
- are transcription factors

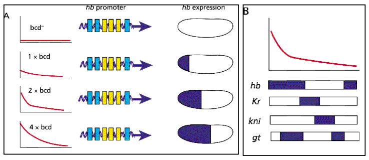


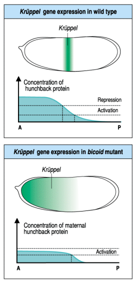




Gap Genes continued:

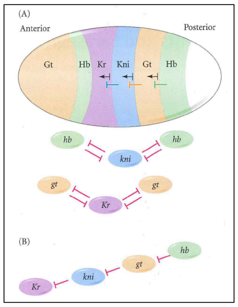
- read the maternal gradients of *bcd*, *hb*, and *cad* (dependent on syncytial nature of embryo)

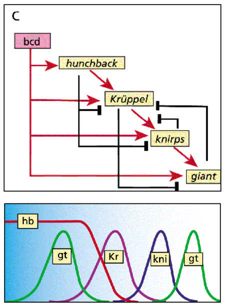


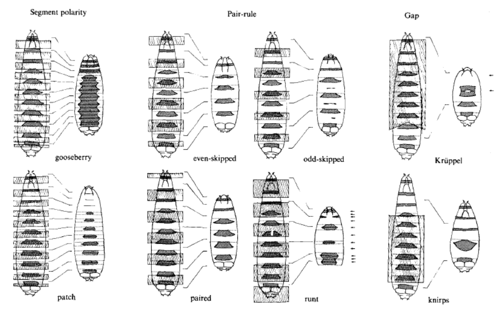


Gap Genes continued:

- exhibit mutual repression that refines their patterns of expression

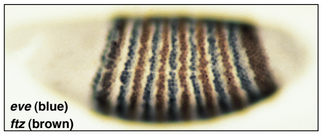


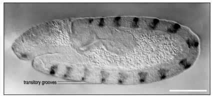


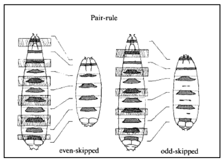


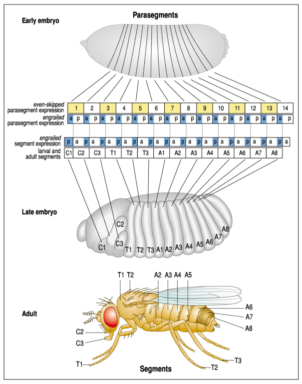
Pair-rule Genes

- also transcription factors
- define and are expressed in alternating parasegments
  - the 14 parasegments are visible as transient grooves following gastrulation
  - initially all same but will ultimately have unique identities
  - offset from segments by ~1/2


eve (blue)
ftz (brown)

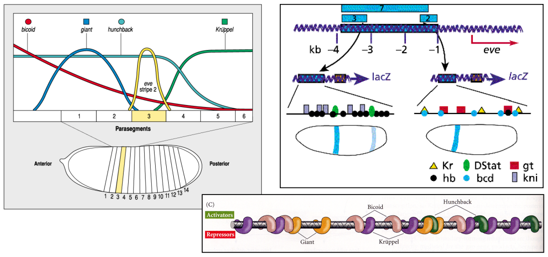




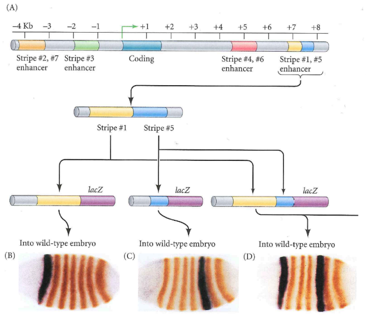


More pair-rule genes

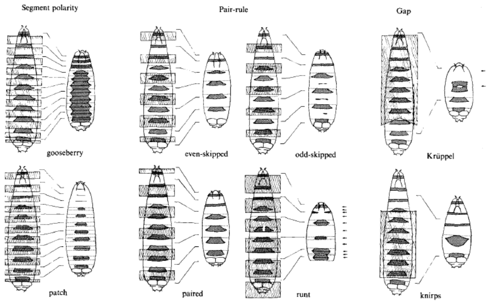
- pair-rule gene expression is regulated by the gap genes, and the maternal genes (still in syncytium)
- competition between activators and repressors is important
- stripes are independently regulated



More pair-rule genes

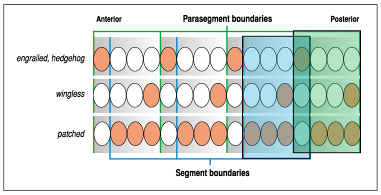


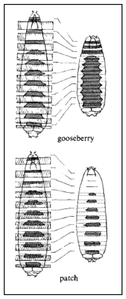
what about in a *gt* mutant?



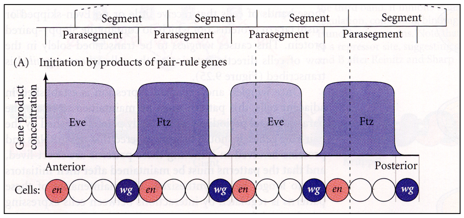
Segment polarity genes

- not all tx factors—most are involved in cell-cell signaling *(this makes sense, because no longer have syncytial embryo)*
- expressed in a portion of *each* parasegment
- pattern the segments and clarify boundaries

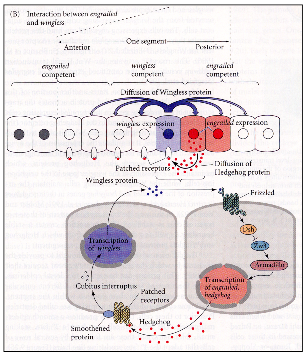




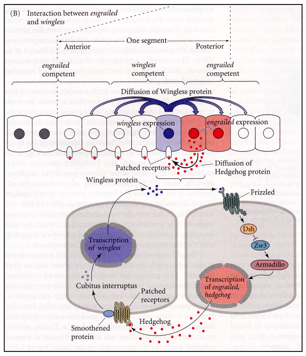
Segment Polarity Genes: Initial expression is regulated by the pair-rule genes

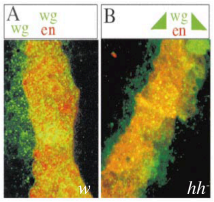


Segment Polarity Genes: subsequent expression depends on reciprocal signaling between *wg* and *en/hh* expressing cells



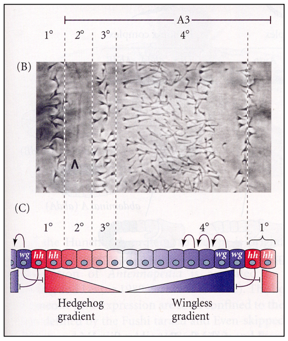
Segment Polarity Genes: asymmetry of the Wg and Hh morphogen gradients leads to differential cell fates



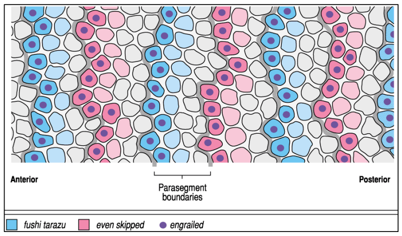


Sanson et al. (1999). *Cell* **98**:207–216

Segment Polarity Genes: asymmetry of the Wg and Hh morphogen gradients leads to differential cell fates

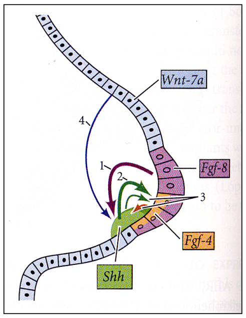


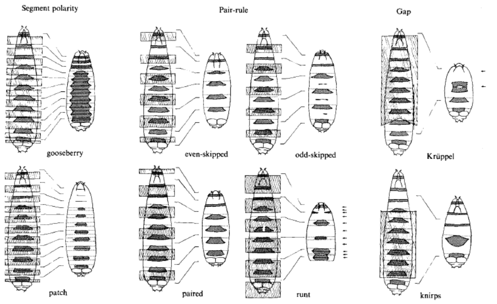
Segment Polarity Genes: *engrailed (en)* establishes a true compartment boundary (i.e., no mixing of cells between parasegments)



The developing vertebrate limb

- Interactions between Wg (Wnt) and HH (Shh), like we saw in the fly embryo

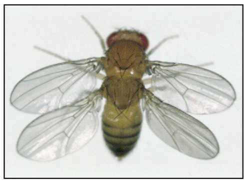




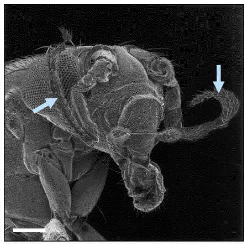
Segmental Identity

Homeotic mutation (Bateson, 1894):
A mutation in which one normal
body part is replaced with another

Some of the famous ones…



Ultrabithorax (Ubx)

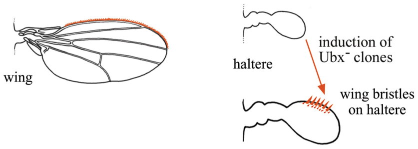


Antennapedia (Antp)

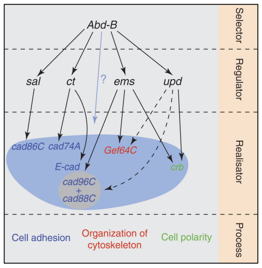
The *Hox* genes specify the identity of a field (segment), not the formation of the field.

Contrast this to the segmentation genes, which establish the segments themselves.

The *Hox* genes act cell autonomously, working within cells to select their developmental fate.



Thus they are sometimes called "selector" genes—they select the future developmental path of the segment, controlling whole later developmental programs



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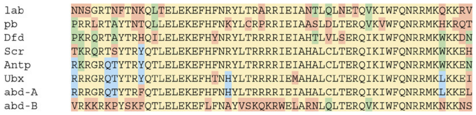
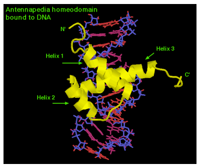
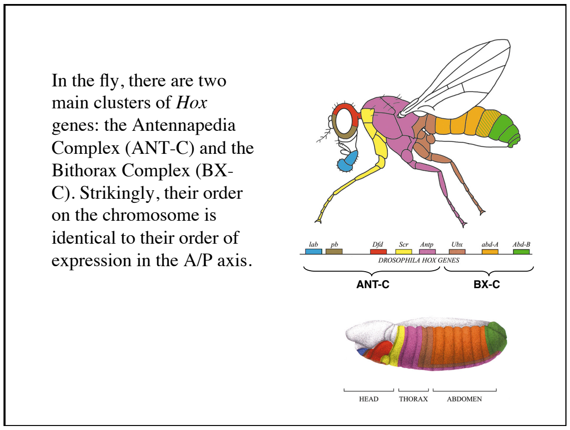
The *Hox* genes are all *transcription factors* and are related in their DNA binding domains, the *homeobox*

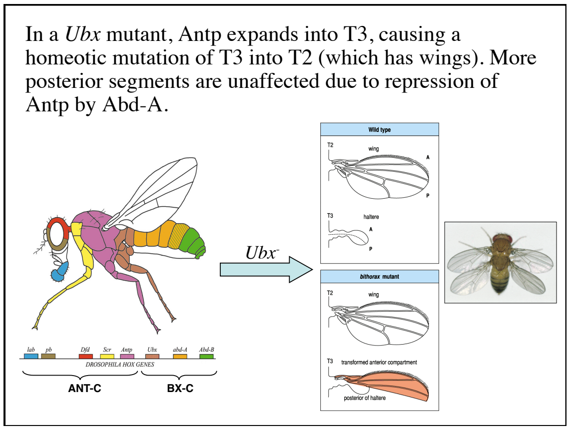


```
lab     NNSGRTNFTNKQLTELEKEFHFNRYLTRARRIEIANTLQLNETQVKIWFQNRRMKQKKRV
pb      PRRLRTAYTNTQLLELEKEFHFNKYLCRPRRIEIAASLDLTERQVKVWFQNRRMKHKRQT
Dfd     PKRQRTAYTRHQILELEKEFHYNRYLTRRRRIEIAHTLVLSERQIKIWFQNRRMKWKKDN
Scr     TKRQRTSYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEH
Antp    RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN
Ubx     RRRGRQTYTRYQTLELEKEFHTNHYLTRRRRIEMAHALCLTERQIKIWFQNRRMKLKKEI
abd-A   RRRGRQTYTRFQTLELEKEFHFNHYLTRRRRIEIAHALCLTERQIKIWFQNRRMKLKKEL
abd-B   VRKKRKPYSKFQTLELEKEFLFNAYVSKQKRWELARNLQLTERQVKIWFQNRRMKNKKNS
```

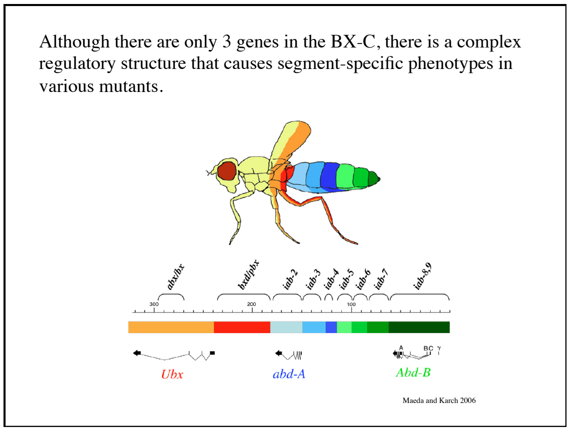




In the fly, there are two main clusters of *Hox* genes: the Antennapedia Complex (ANT-C) and the Bithorax Complex (BX-C). Strikingly, their order on the chromosome is identical to their order of expression in the A/P axis.

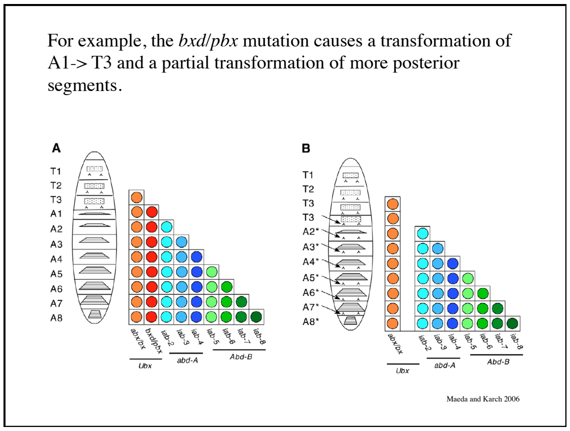


In a *Ubx* mutant, Antp expands into T3, causing a homeotic mutation of T3 into T2 (which has wings). More posterior segments are unaffected due to repression of Antp by Abd-A.



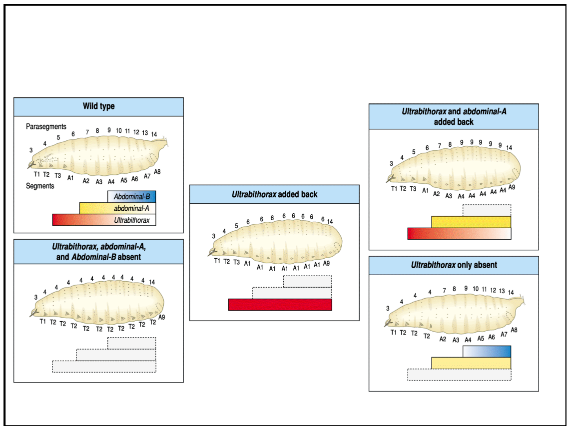
Although there are only 3 genes in the BX-C, there is a complex regulatory structure that causes segment-specific phenotypes in various mutants.

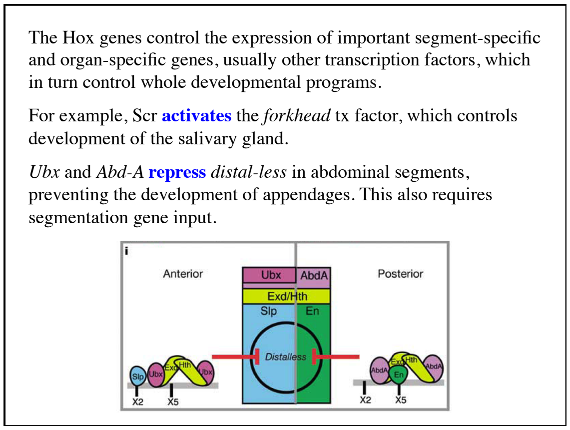
Maeda and Karch 2006



For example, the *bxd/pbx* mutation causes a transformation of A1-> T3 and a partial transformation of more posterior segments.

Maeda and Karch 2006

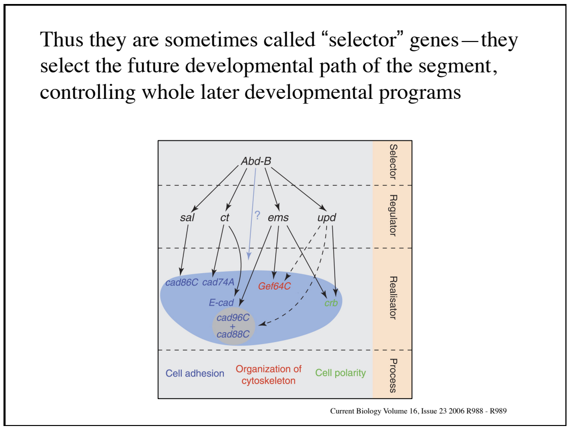




The Hox genes control the expression of important segment-specific and organ-specific genes, usually other transcription factors, which in turn control whole developmental programs.

For example, Scr **activates** the *forkhead* tx factor, which controls development of the salivary gland.

Ubx and *Abd-A* **repress** *distal-less* in abdominal segments, preventing the development of appendages. This also requires segmentation gene input.



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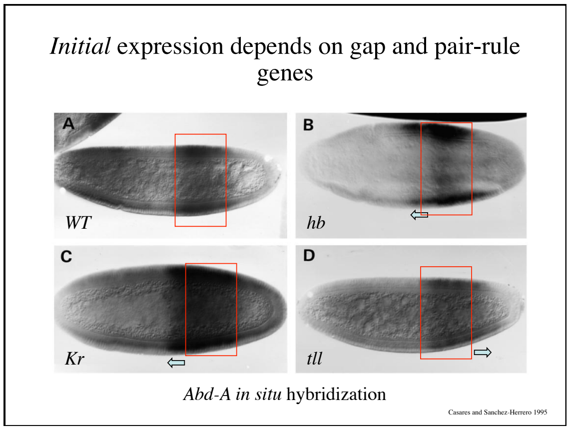


Initial expression depends on gap and pair-rule genes

Abd-A in situ hybridization

Casares and Sanchez-Herrero 1995

Maintenance of expression is via epigenetic mechanisms.

Polycomb group (Pc-G) genes keep homeotic genes repressed in segments where they were not intially activated.

Trithorax group (trx-G) genes maintain the expression of the homeotic genes in their appropriate segments.

Both groups act by modifying chromatin conformation. PcG complexes promote H3K27 tri-methylation. TrxG complexes can promote H3K4 methylation. Many details remain to be established.

In this way, patterning information conveyed by the early activity of the segmentation genes is preserved throughout the life cycle.

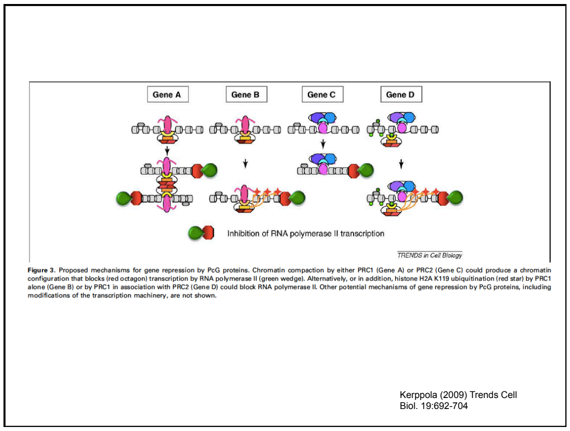
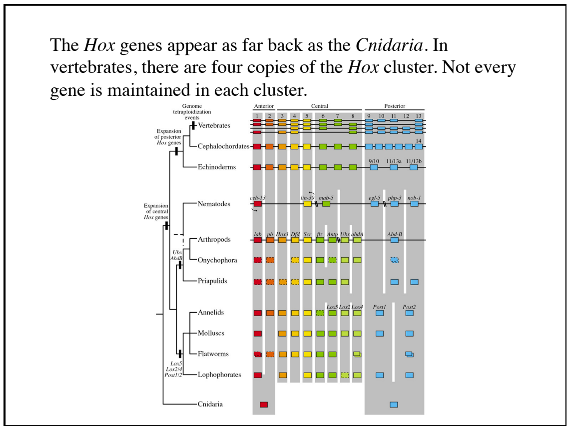
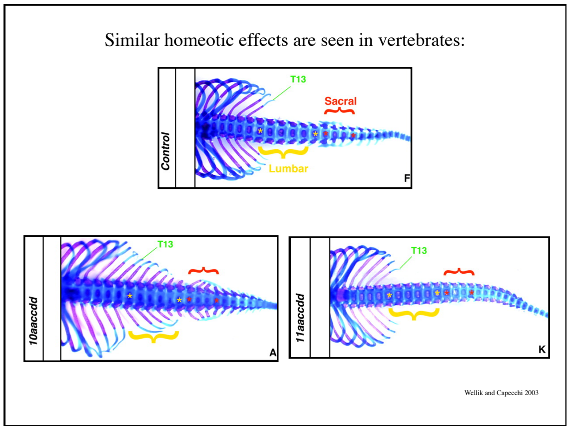


Figure 3. Proposed mechanisms for gene repression by PcG proteins. Chromatin compaction by either PRC1 (Gene A) or PRC2 (Gene C) could produce a chromatin configuration that blocks (red octagon) transcription by RNA polymerase II (green wedge). Alternatively, or in addition, histone H2A K119 ubiquitination (red star) by PRC1 alone (Gene B) or by PRC1 in association with PRC2 (Gene D) could block RNA polymerase II. Other potential mechanisms of gene repression by PcG proteins, including modifications of the transcription machinery, are not shown.

Kerppola (2009) Trends Cell Biol. 19:692-704



The *Hox* genes appear as far back as the *Cnidaria*. In vertebrates, there are four copies of the *Hox* cluster. Not every gene is maintained in each cluster.



Similar homeotic effects are seen in vertebrates:

Wellik and Capecchi 2003

Function is conserved between fly and mouse: