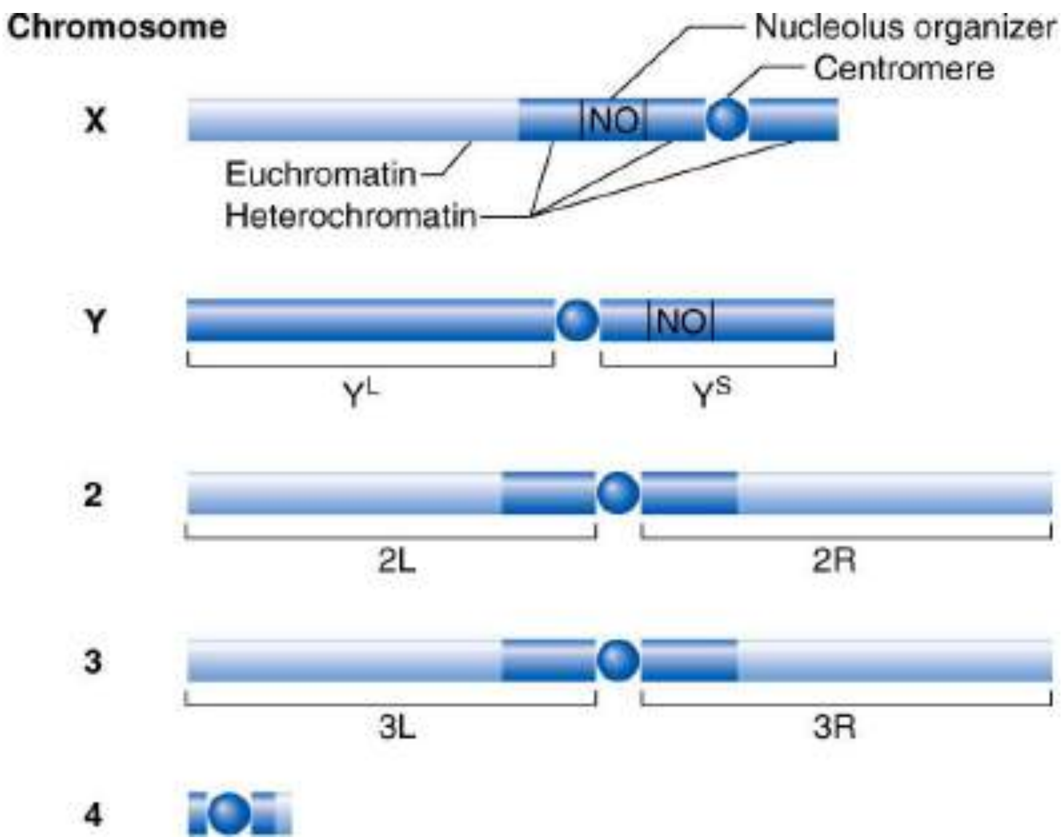
Developmental Genetics
in
Drosophila melanogaster

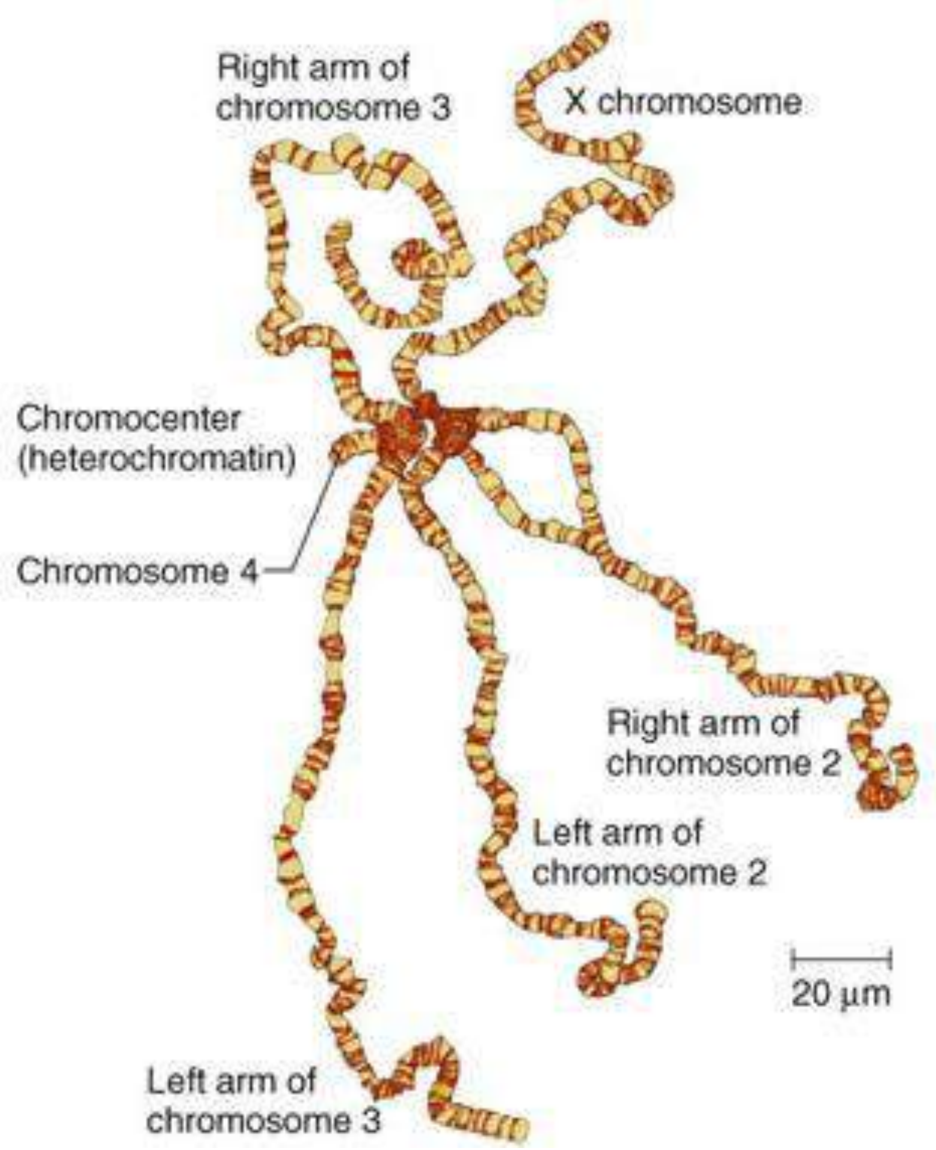
Outline

- Structure and organization of genome
- Life cycle
- Drosophila genome project
- Genetic analysis of body plan development in *Drosophila*

Structure of the *Drosophila* Genome



- Chromosomes of Drosophila
  - Four chromosomes designated 1-4
  - XY sex determination (XX females, XY males)
  - Sex determined by X:A ratio

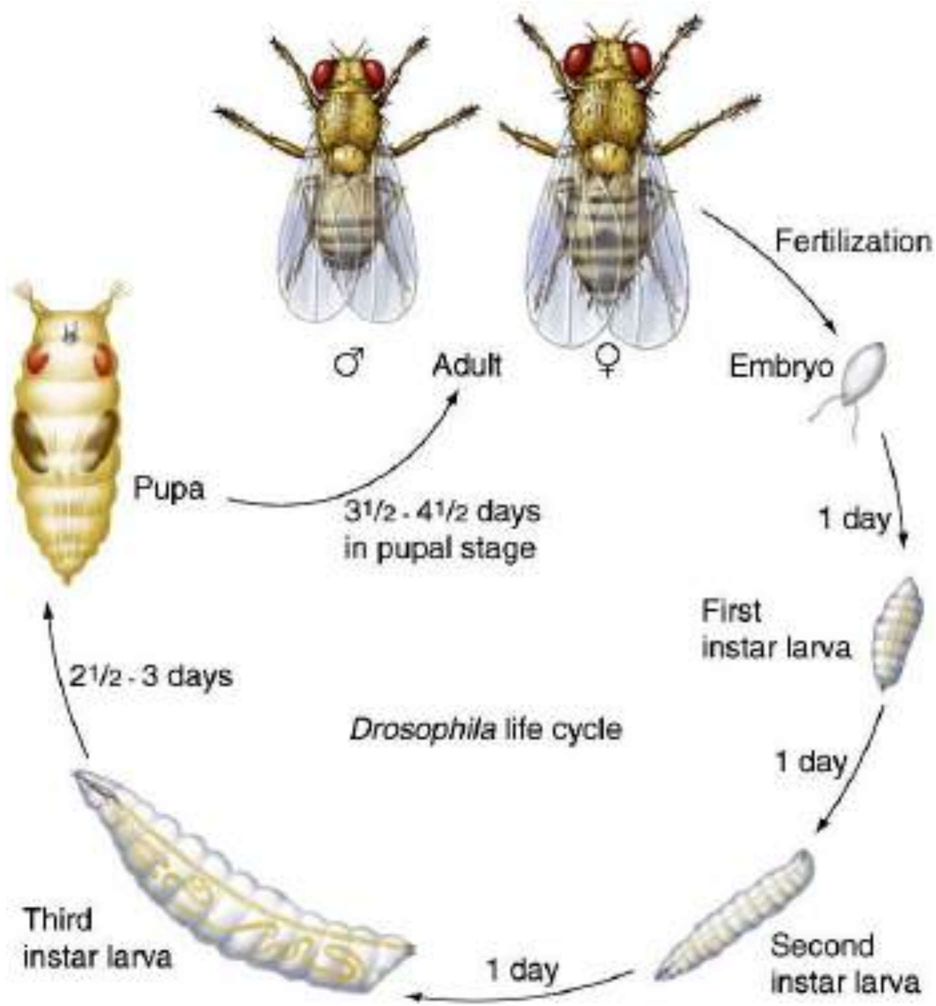


- Giant polytene chromosomes of larval salivary gland are key tools
  - Replicate 10-11 times
  - 1024-2048 sister chromatids stay associated under perfect lateral register
  - Homologous chromosome stay tightly synapsed
  - Chromocenter – common region where centromeres coalesce

Drosophila Genome

- 170,000 kb of DNA
- 21% is highly repetitive satellite DNA in heterochromatin and Y chromosome
- 3% repeated genes for rRNA, 5s RNA and histones
- 9% is 50 families of transposons 2-9 kb in length
- Telomeres do not have simple repeats
- Telomeres have transposable element sequences
- 67% of genome-unique sequences in euchromatin comprising about 13,600 genes

Life cycle


Fertilization
♂
Adult
♀
Embryo
Pupa
3½ - 4½ days
in pupal stage
1 day
First
instar larva
2½ - 3 days
Drosophila life cycle
1 day
Third
instar larva
1 day
Second
instar larva

The *Drosophila* Genome Project

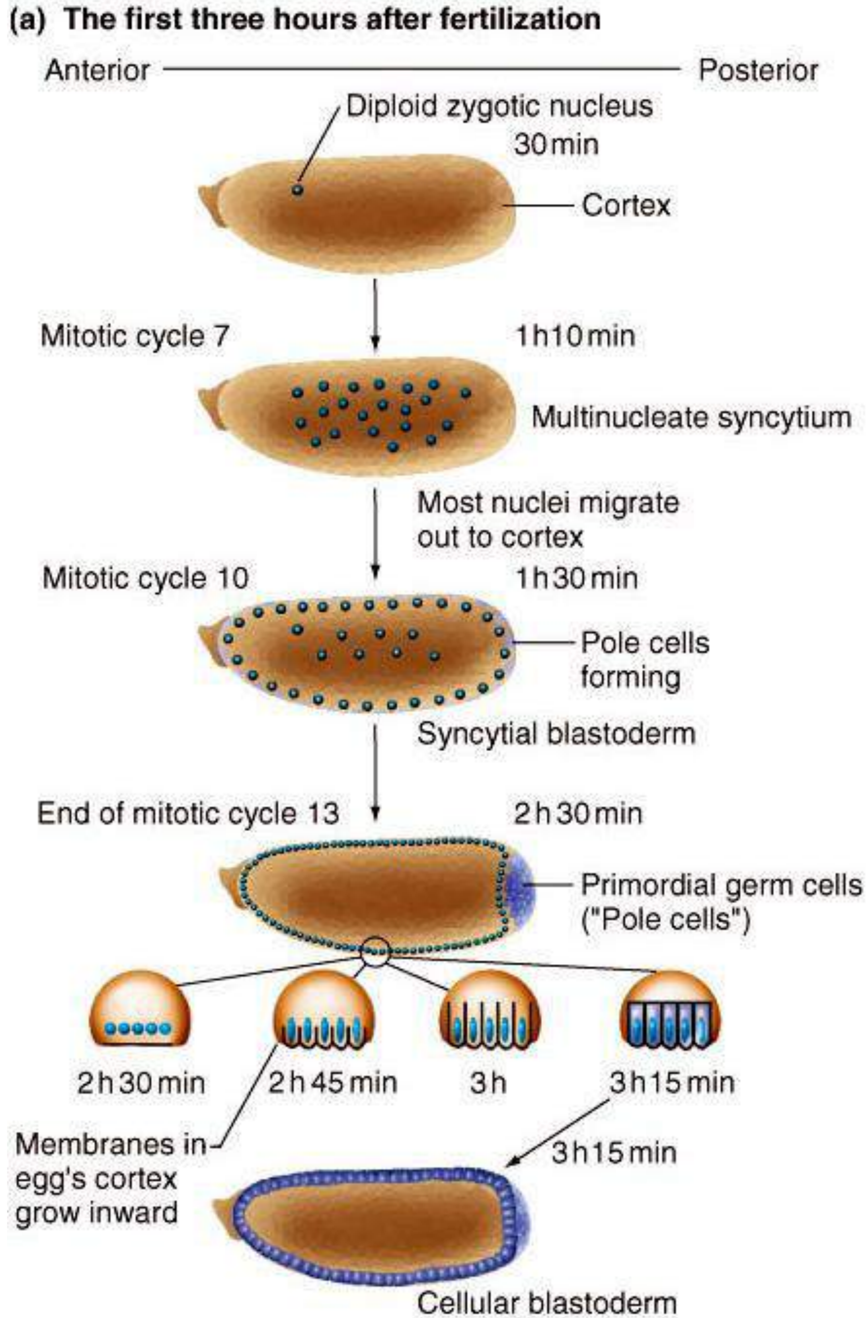
- 13,600 known or predicted genes present
- One gene every 9 kb
- Half of fly proteins homologous with mammalian proteins
- One third homologous to nematodes
- 61% of human disease genes have homologues in flies
- 30% of genes unrelated to genes in other organisms
- Only 4000 genes essential for viability

Genetic Analysis of Body Plan Development

- How body becomes specialized along both anterior-posterior (AP) and dorsal-ventral (DV) axes
- Segmentation genes subdivide body into an array of identical body segments
- Homeotic genes assign a unique identity to each body segment
- How does the developing animal establish the proper number of body segments?
- How does each body segment know what kinds of structures it should form and what role it should play in the animal's body?

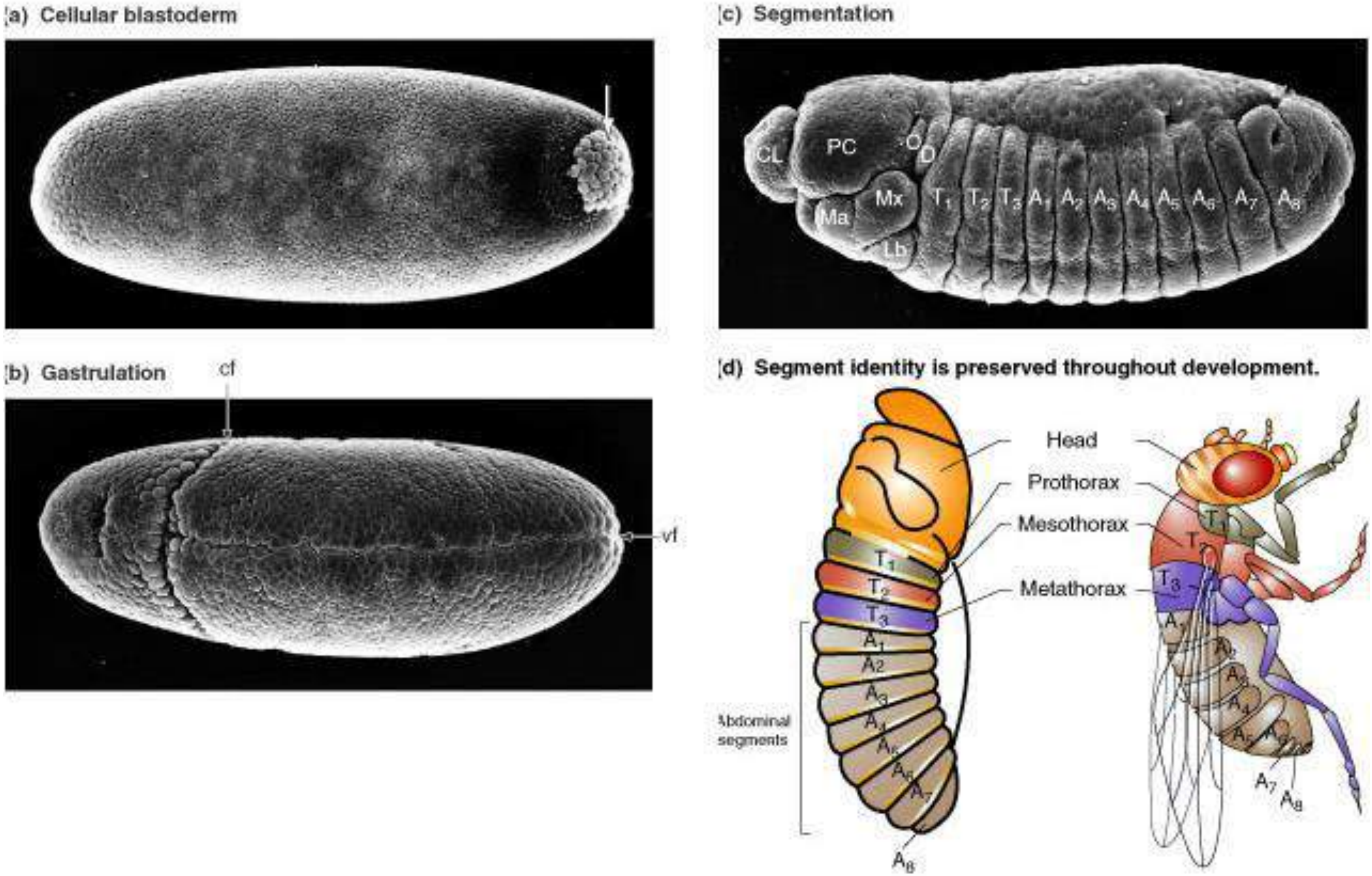
Embryonic development in *Drosophila*:

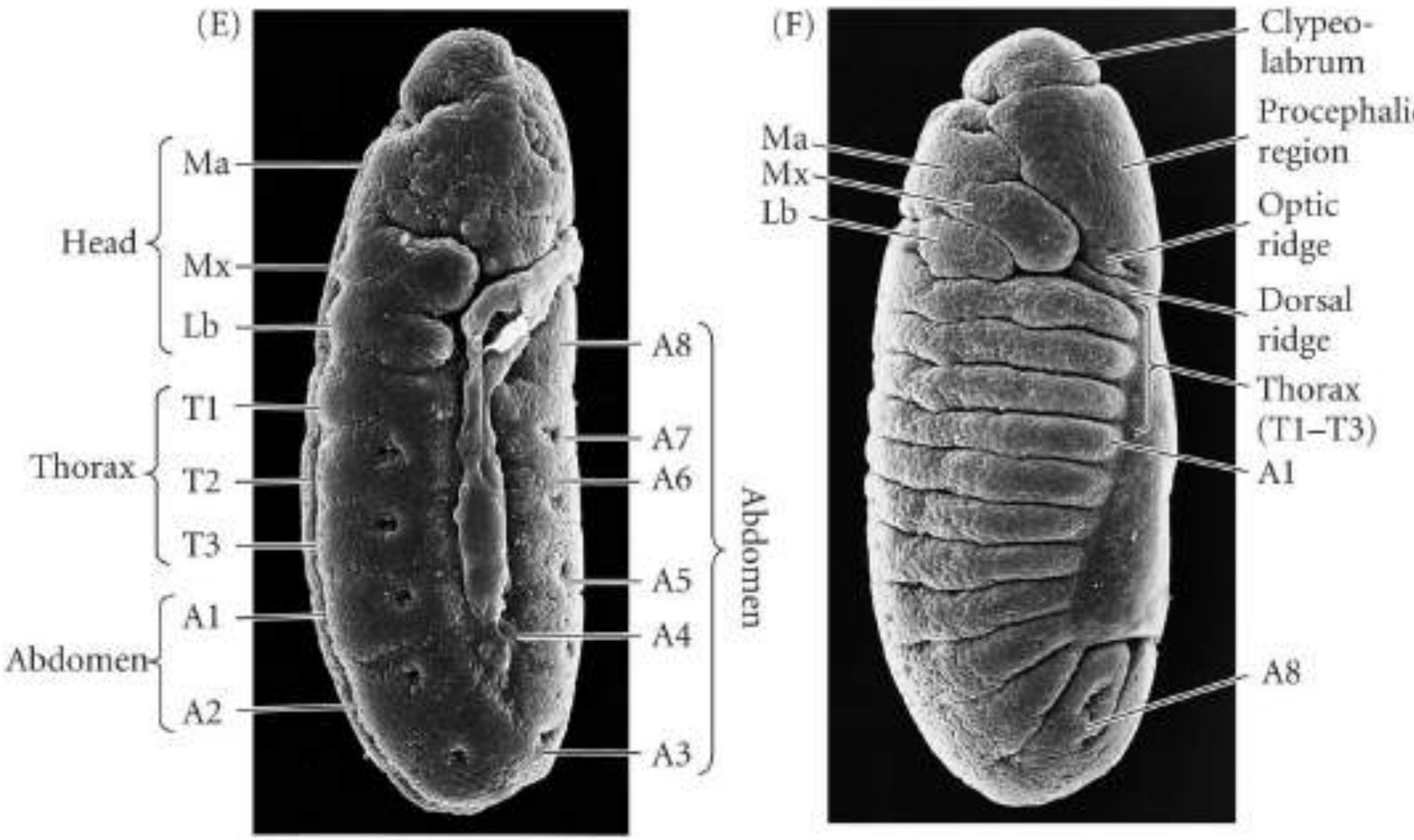
- Development begins with fertilization.
- Prior to fertilization, molecular gradients exist within the eggs. Polar cytoplasm occurs at the posterior end---example of maternal effect.
- 2 nuclei fuse after fertilization to form a zygote.
- 9 mitotic divisions occur without cell division, and after 7 divisions, some nuclei migrate to the polar cytoplasm (posterior) creating germ-line precursors.
- Other nuclei migrate to the cell surface and form blastoderm precursor.
- 4 more mitotic divisions occur and all nuclei are separated by cell membranes.


(a) The first three hours after fertilization
Anterior
Posterior
Diploid zygotic nucleus
30 min
Cortex
Mitotic cycle 7
1 h 10 min
Multinucleate syncytium
Most nuclei migrate out to cortex
Mitotic cycle 10
1 h 30 min
Pole cells forming
Syncytial blastoderm
End of mitotic cycle 13
2 h 30 min
Primordial germ cells ("Pole cells")
2 h 30 min
2 h 45 min
3 h
3 h 15 min
Membranes in egg's cortex grow inward
3 h 15 min
Cellular blastoderm

b) Nuclear divisions start without cell division in *Drosophila* (superficial cleavage)

Zygotic gene expression begins


(a) Cellular blastoderm
(b) Gastrulation
cf
vf
(c) Segmentation
CL
PC
O
D
Ma
Mx
Lb
T1 T2 T3 A1 A2 A3 A4 A5 A6 A7 A8
(d) Segment identity is preserved throughout development.
Head
Prothorax
Mesothorax
Metathorax
Abdominal segments
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 9.5 (Part 3)

Three major classes of genes control development and differentation

1. Maternal effect genes
2. Segmentation genes
3. Homeotic genes

Function in a hierarchy that progressively subdivides the embryo into successively smaller units

Maternal effect genes

Expressed by the mother during egg production; they control polarity of the egg and thus the embryo.

bicoid gene

- Regulates formation of anterior structures (mutants possess posterior structures at each end).
- Gene is transcribed during egg production, and expressed after fertilization.

nanos gene

- Regulates abdomen formation (mRNAs collect in posterior of the egg).

torso gene

- Transcription and translation occur during egg production.
- Occurs throughout the eggs, but is only active at the poles.

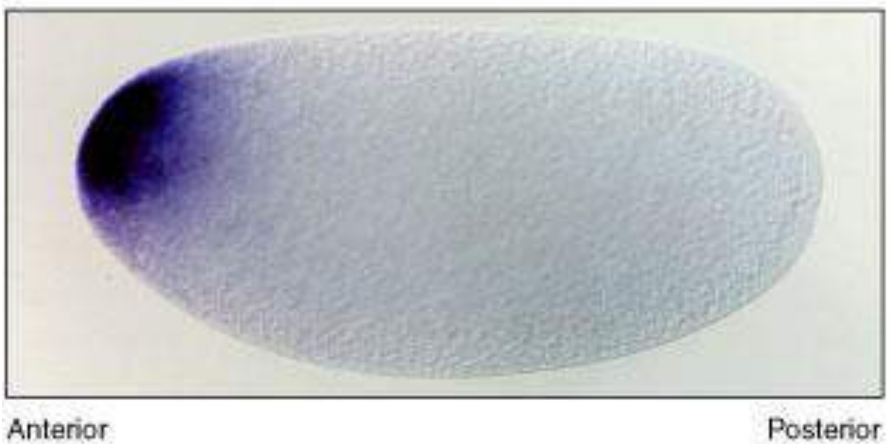
Maternal Genes Interact to Produce Morphogen Gradients

- Maternal-effect mutations
  - Recessive mutations in maternal genes that influence embryonic development
- Maternally supplied components account for formation of body plan between fertilization and end of 13 syncytial divisions
- Nusslein-Volhard and Wieschaus screened thousands of mutagen treated chromosomes by examining phenotypes of embryos from homozygous mutant mothers
- Focuses on stocks with homozygous mutant sterile females
- Identified large number of maternal genes
- Nobel Prize in medicine

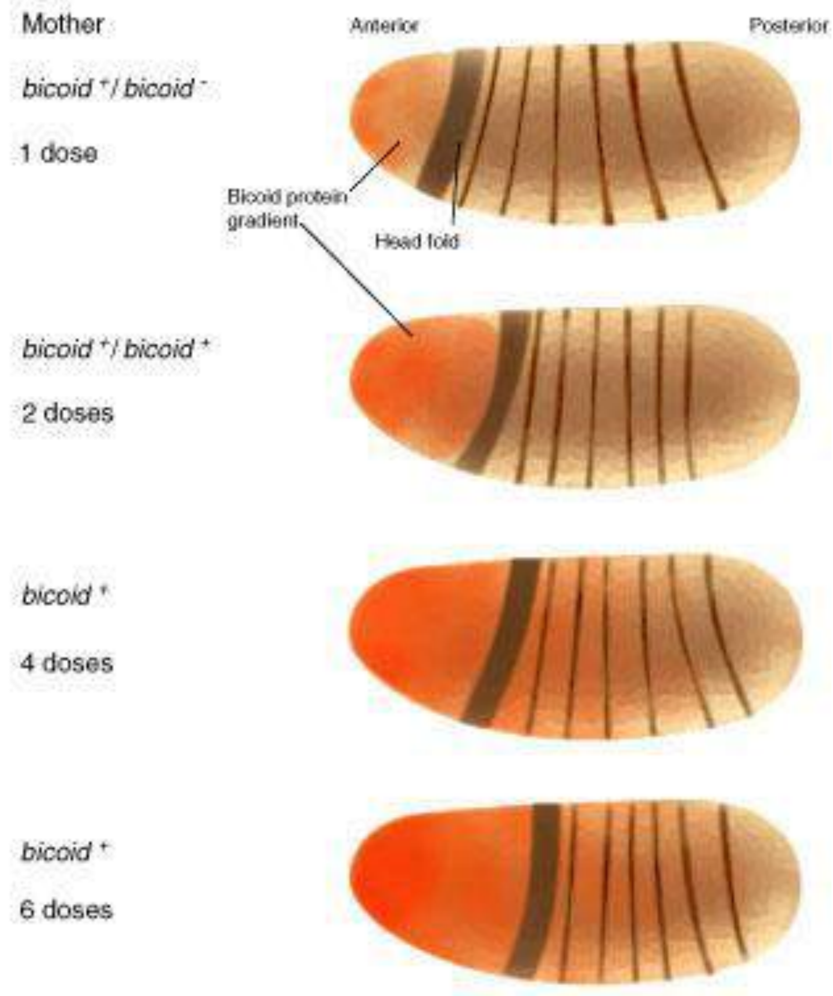
- Morphogen – substances that define different cell fates in a concentration-dependent manner
- Interaction of two signaling centers located in the anterior and posterior poles of the egg pattern insect body axis

bicoid (*bcd*) Encodes the Anterior Morphogen

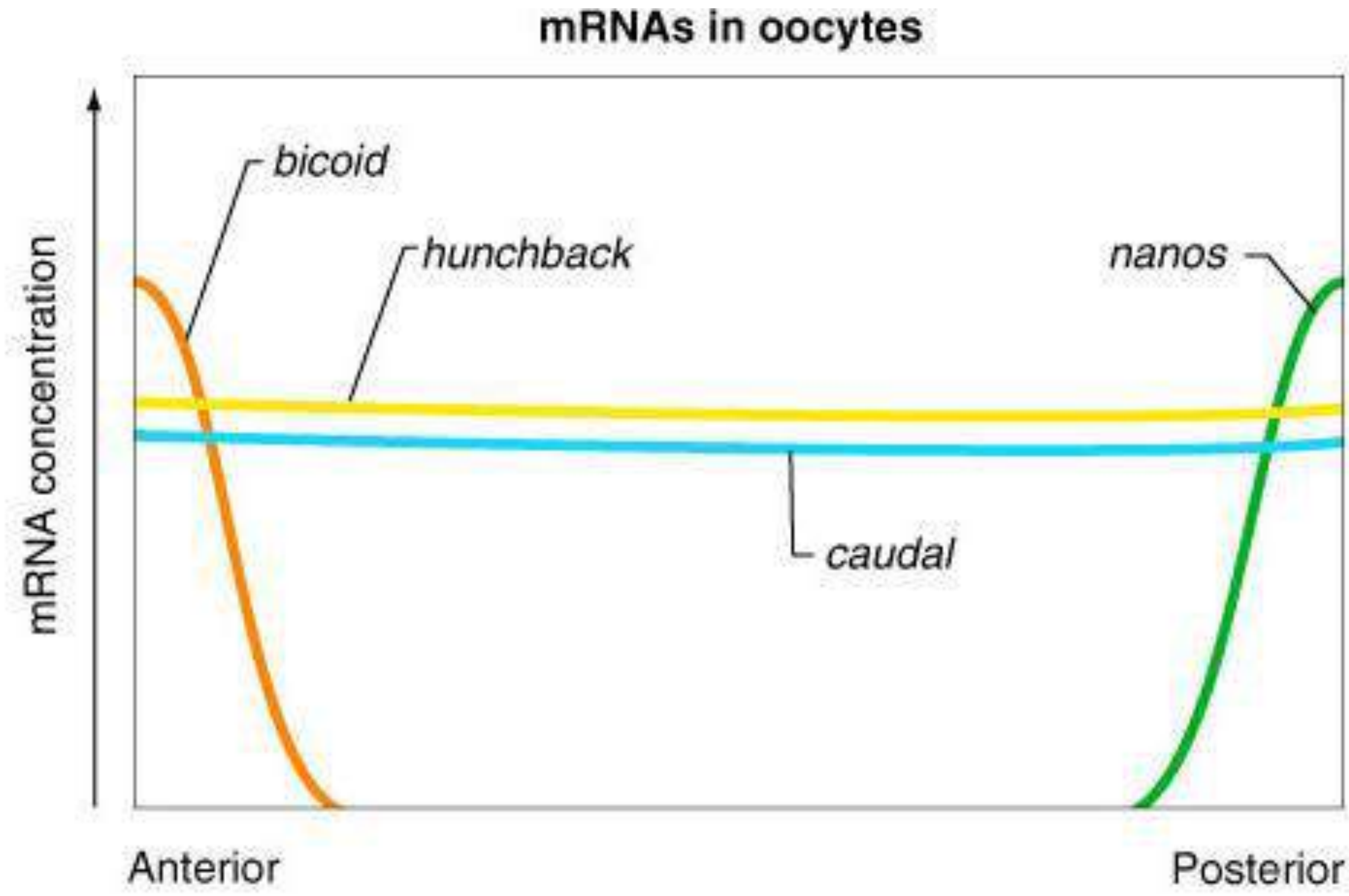
(a) Localization of *bicoid* mRNA

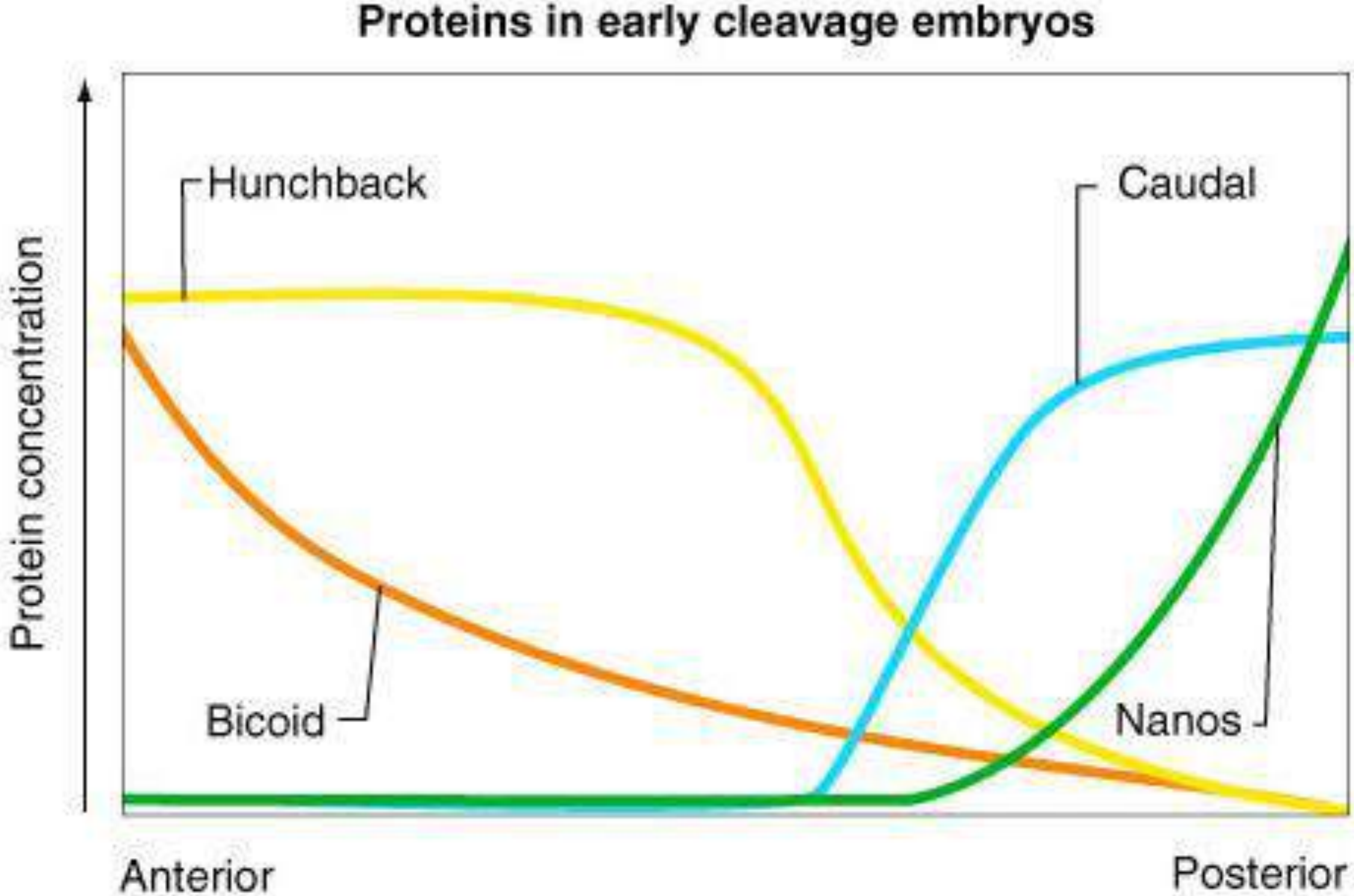


(c) Bicoid protein is a morphogen.



How Bcd Protein Works




Proteins in early cleavage embryos
Protein concentration
Hunchback
Caudal
Bicoid
Nanos
Anterior
Posterior

Specification of Segment Number through Activation of Zygotic Genes in Successively More Sharply Defined Regions of the Embryo

- Zygotic genes
  - Transcribed and translated from DNA in nuclei of embryonic cells
  - Expression begins in syncytial blastoderm stage (roughly cycle 10)
- Nusslein-Volhard and Wieschaus performed zygotic mutant screen
- Three classes of genes
  - 9 gap genes
  - 8 pair-rule genes
  - 17 segment-polarity genes
- Hierarchy of gene expression

Segmentation genes:

Determine the segments of the embryo and adult, and thus divide the embryo into regions that correspond to the adult segmentation patterns.

1. Gap genes
   - Subdivide the embryo along the anterior-posterior axis.
   - Mutation results in the deletion of several adjacent segments.

2. Pair rule genes
   - Divide the the embryo into regions, each containing parasegments.
   - Mutations cause deletions of the same part of a pattern in every other segment.

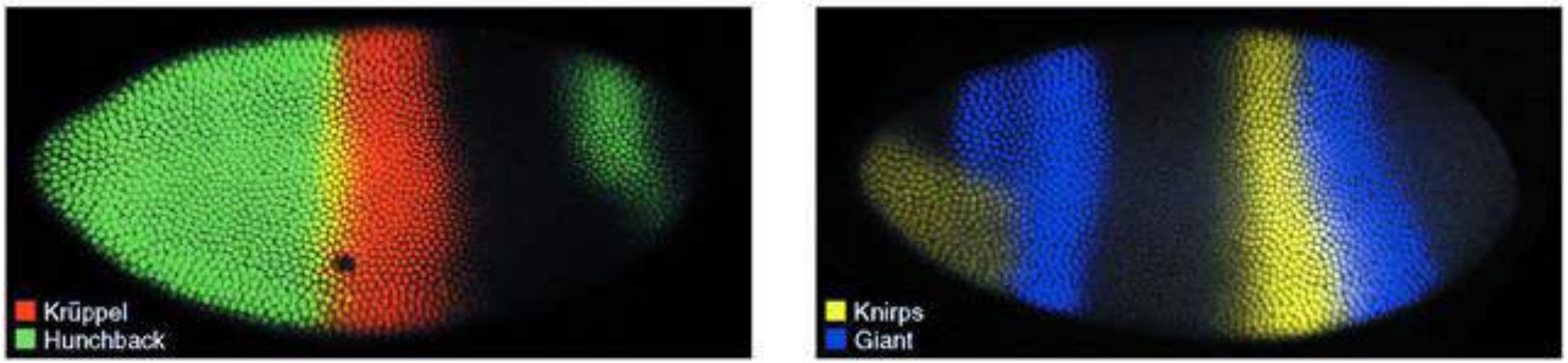
3. Segment polarity genes
   - Determine regions that become segments of larvae and adults
   - Mutants possess parts of segments replaced by mirror images of adjacent half segments.

1. Gap genes

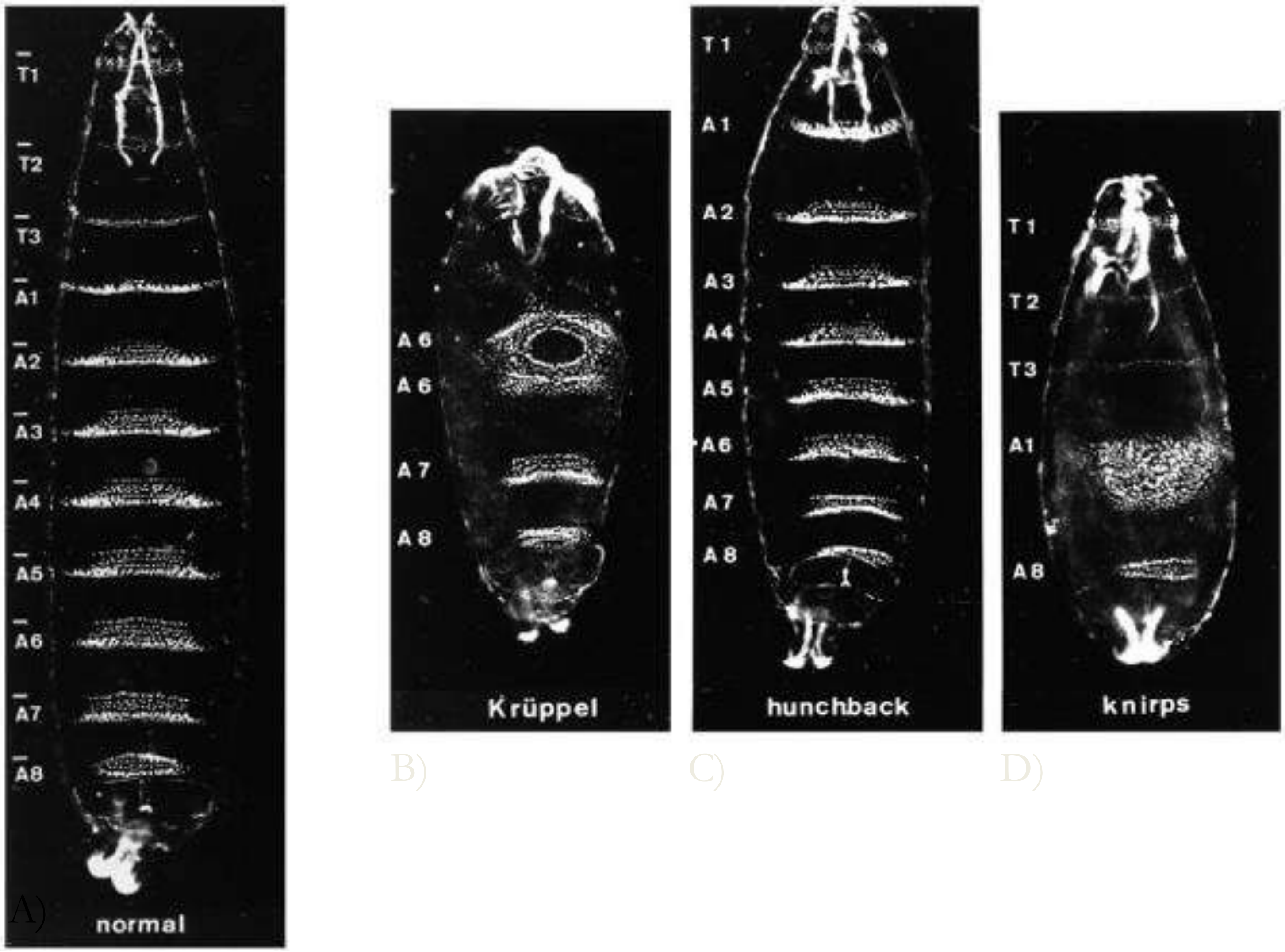
- Expressed first
- Gap mutants show a gap in segmentation pattern at positions where particular gene is absent
- Binding sites in promoter have different affinities for maternal transcription factors
- Gap genes encode transcription factors that influence expression of other gap genes

Zones of Expression of Four Gap Genes: *Hunchback*, *Kruppel*, *Knirps*, and *Giant* in Late Syncytial Blastoderm Embryos

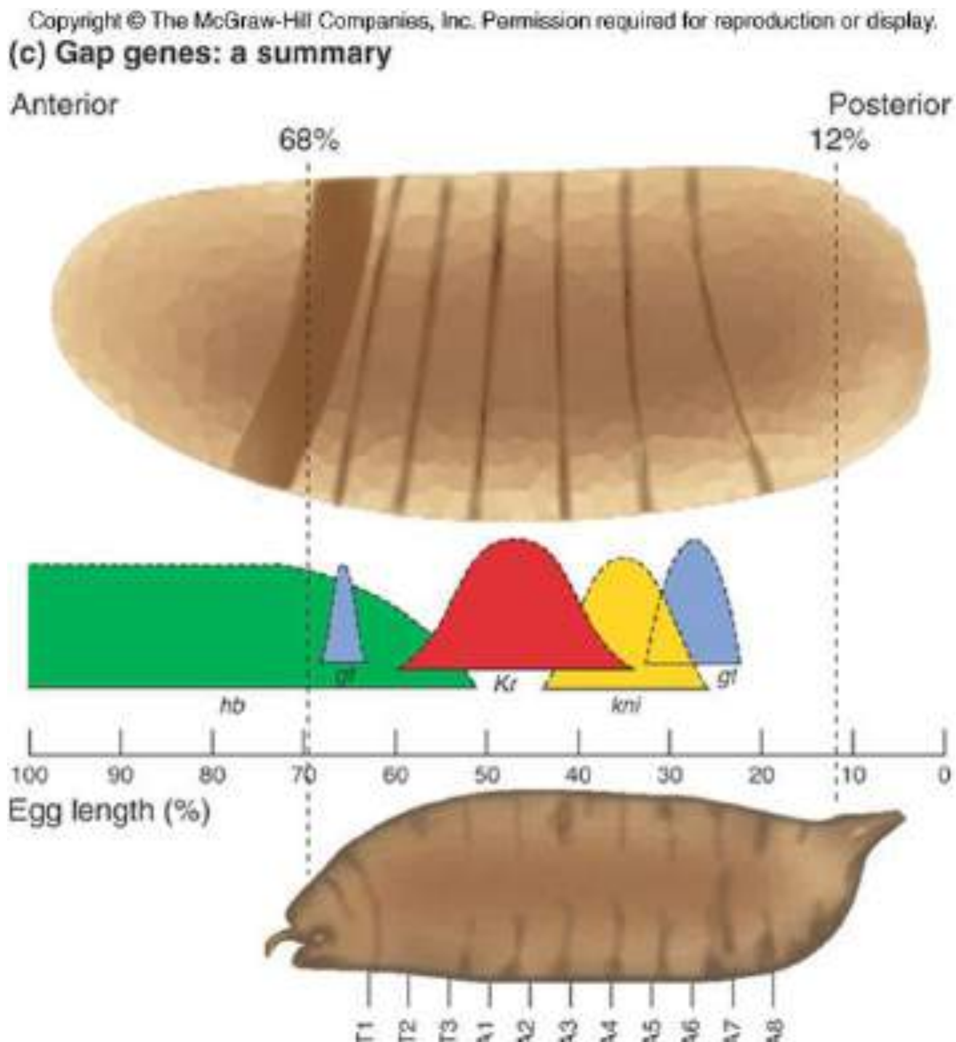
(a) Zones of gap gene expression



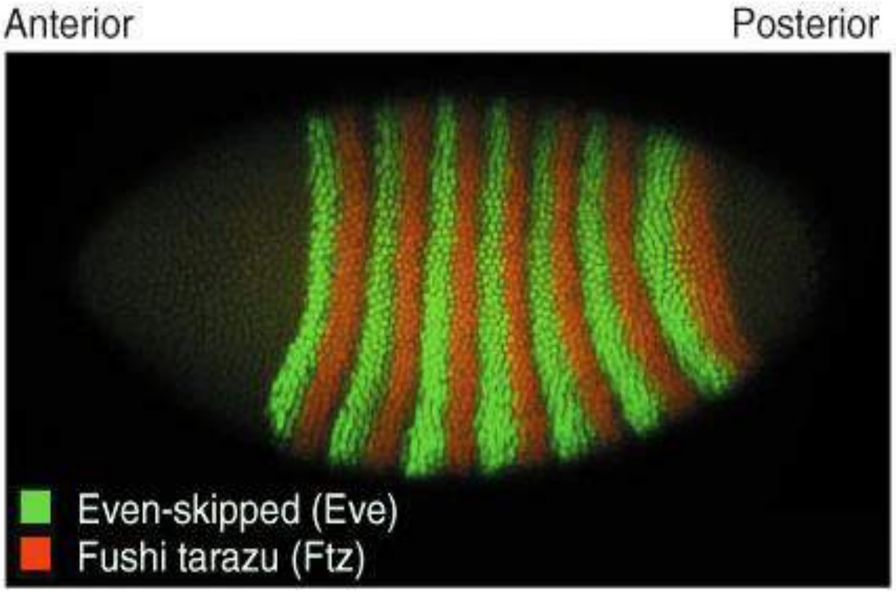
Defects in Segmentation from Mutations in Gap Genes



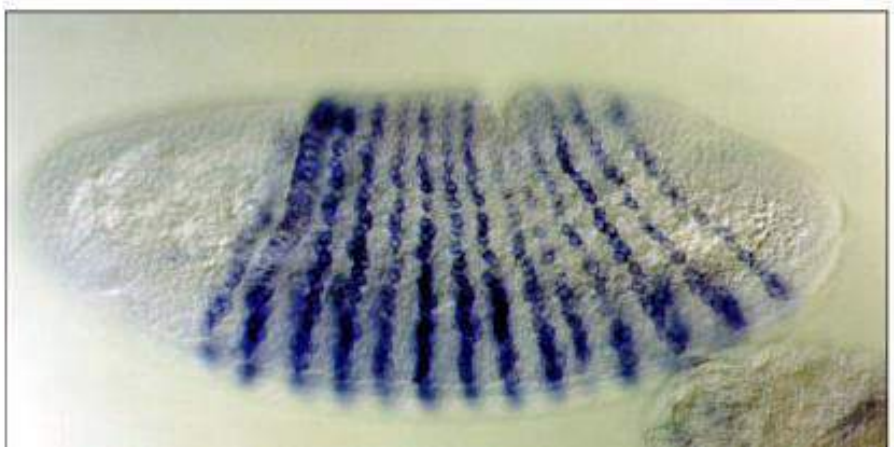
Mutations in Gap Gene Result in Loss of Segments Corresponding to Zone of Expression



2. Pair-rule Genes



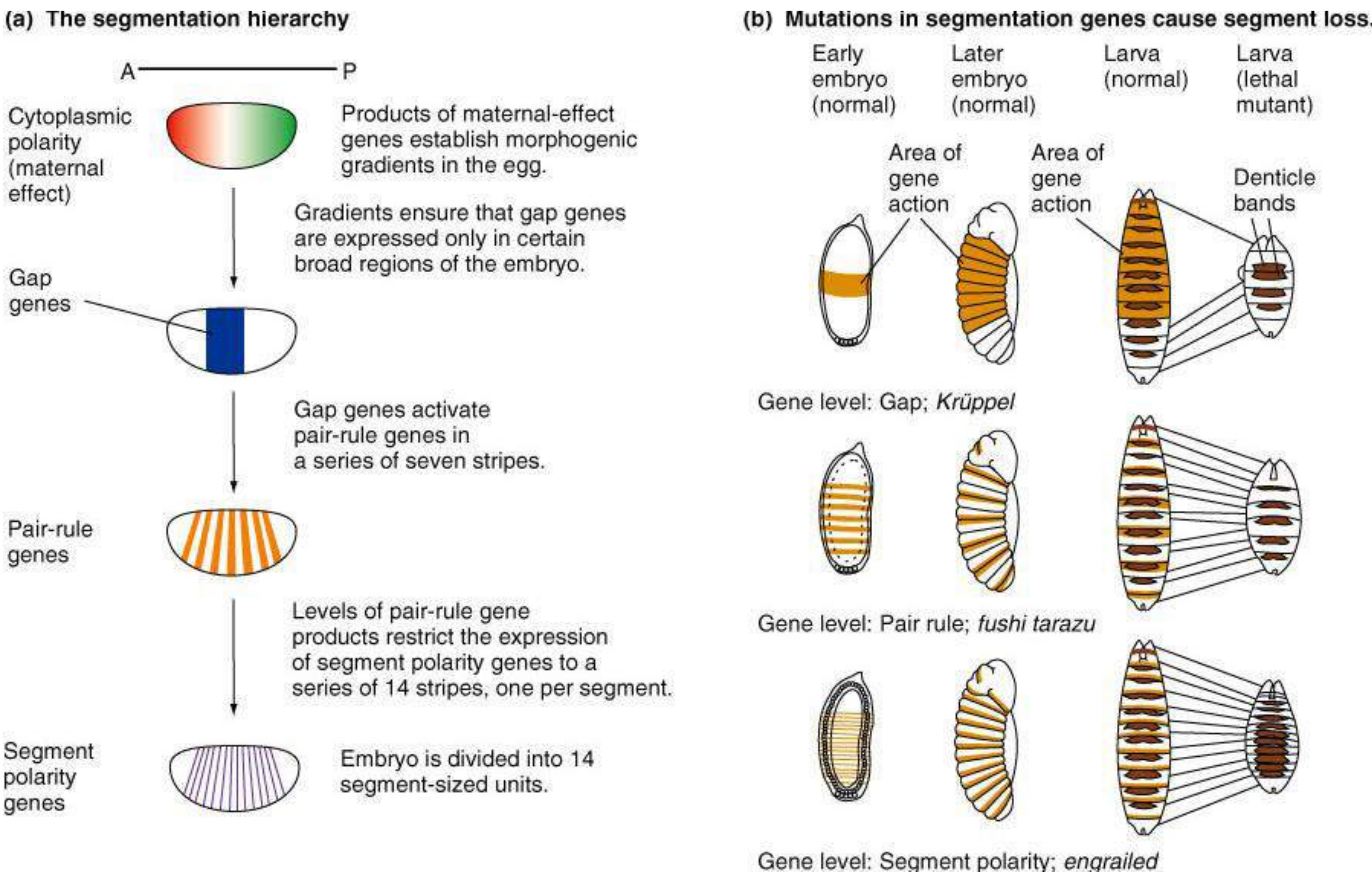
(a) Distribution of Engrailed protein



- (a) Zones of expression at beginning of blastoderm stage
  - Each gene expressed in seven stripes
- (b) formation of Eve stripe 2 requires activation by Bcd and Hb proteins and repression by Gt and Kr proteins
- 700 bp upstream regulatory region of *eve* gene that directs the Eve second stripe contains multiple binding sites

3. Segment Polarity Genes are Lowest Level of Segmentation Hierarchy

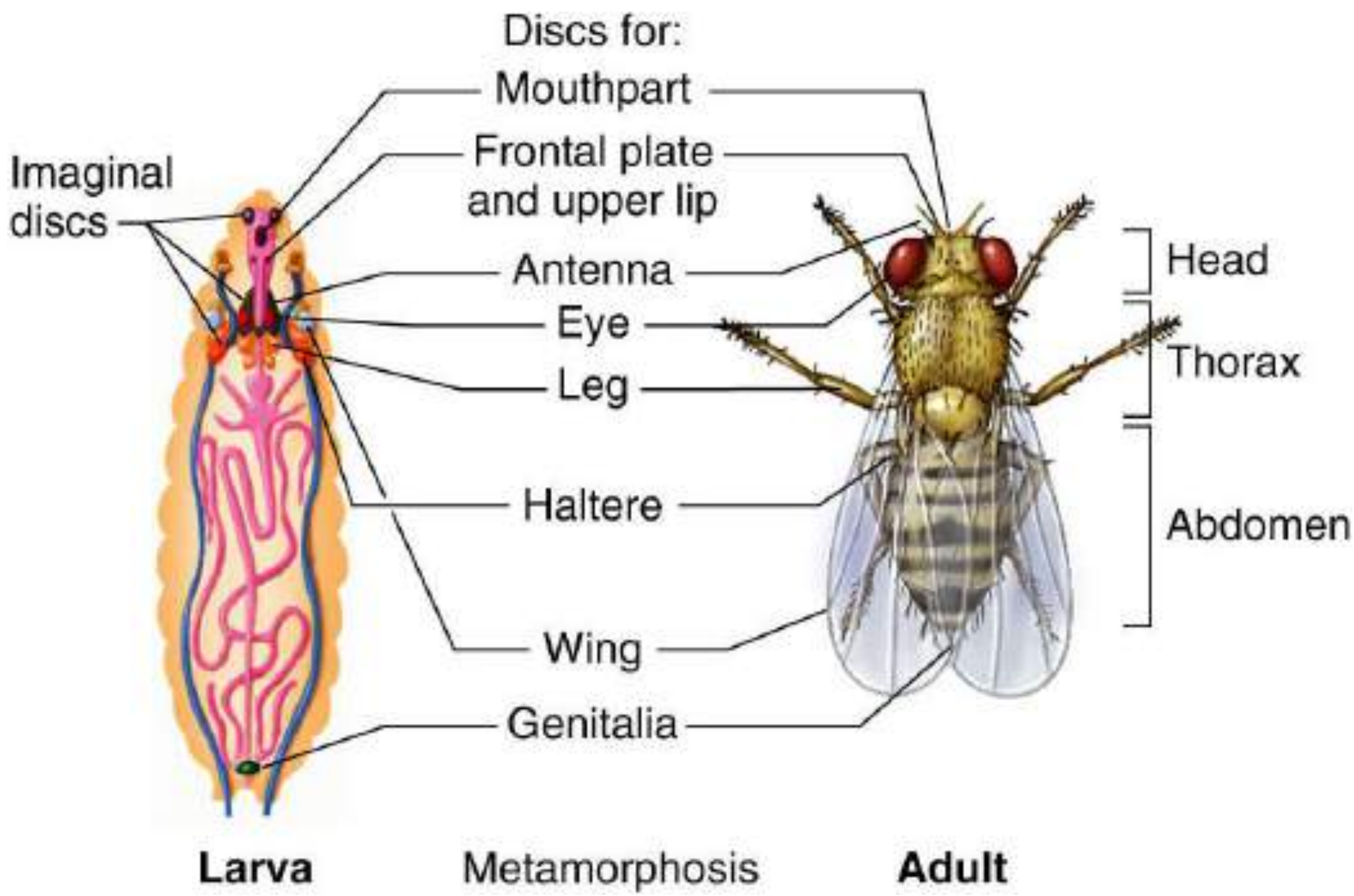
- Mutations in segment polarity genes cause deletion of part of each segment and its replacement by mirror image of different part of next segment
- Regulatory system complex
  - Transcription factors encoded by pair-rule genes initiate pattern by regulating segment polarity genes
  - Interactions between cell polarity genes maintain periodicity later in development
  - Activation occurs after cellularization of embryo is complete
  - Diffusion of transcription factors within syncytium ceases to play a role


(a) The segmentation hierarchy
A
P
Cytoplasmic polarity (maternal effect)
Products of maternal-effect genes establish morphogenic gradients in the egg.
Gradients ensure that gap genes are expressed only in certain broad regions of the embryo.
Gap genes
Gap genes activate pair-rule genes in a series of seven stripes.
Pair-rule genes
Levels of pair-rule gene products restrict the expression of segment polarity genes to a series of 14 stripes, one per segment.
Segment polarity genes
Embryo is divided into 14 segment-sized units.
(b) Mutations in segmentation genes cause segment loss.
Early embryo (normal)
Later embryo (normal)
Larva (normal)
Larva (lethal mutant)
Area of gene action
Area of gene action
Denticle bands
Gene level: Gap; Krüppel
Gene level: Pair rule; fushi tarazu
Gene level: Segment polarity; engrailed

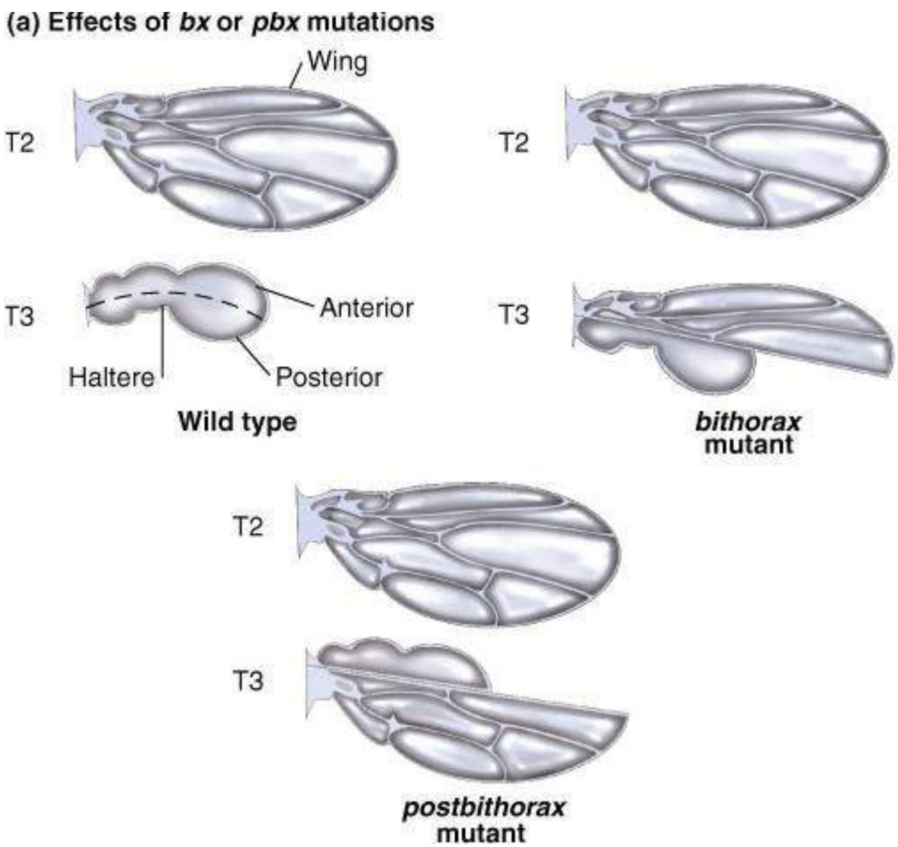
Homeotic genes:

- Homeotic genes specify the body part to develop at each segment.
- Adult body parts develop from undifferentiated larval tissues called imaginal discs.
- Homeotic mutants develop a different body part at a particular segment (imaginal disc) than the usual body part.
- Different homeotic gene groups share similar sequences of ~180 bp called homeoboxes that code proteins.
- Homeoboxes regulate development and produce proteins that bind upstream of the gene units.
- Homeotic gene complexes are abbreviated *Hox*.
- *Hox* genes also specify body plans in vertebrates and plants.

Many Adult Structures Develop from Imaginal Discs in Larvae and Pupae



Each Segment Establishes own Identity Through Activation of Homeotic Genes

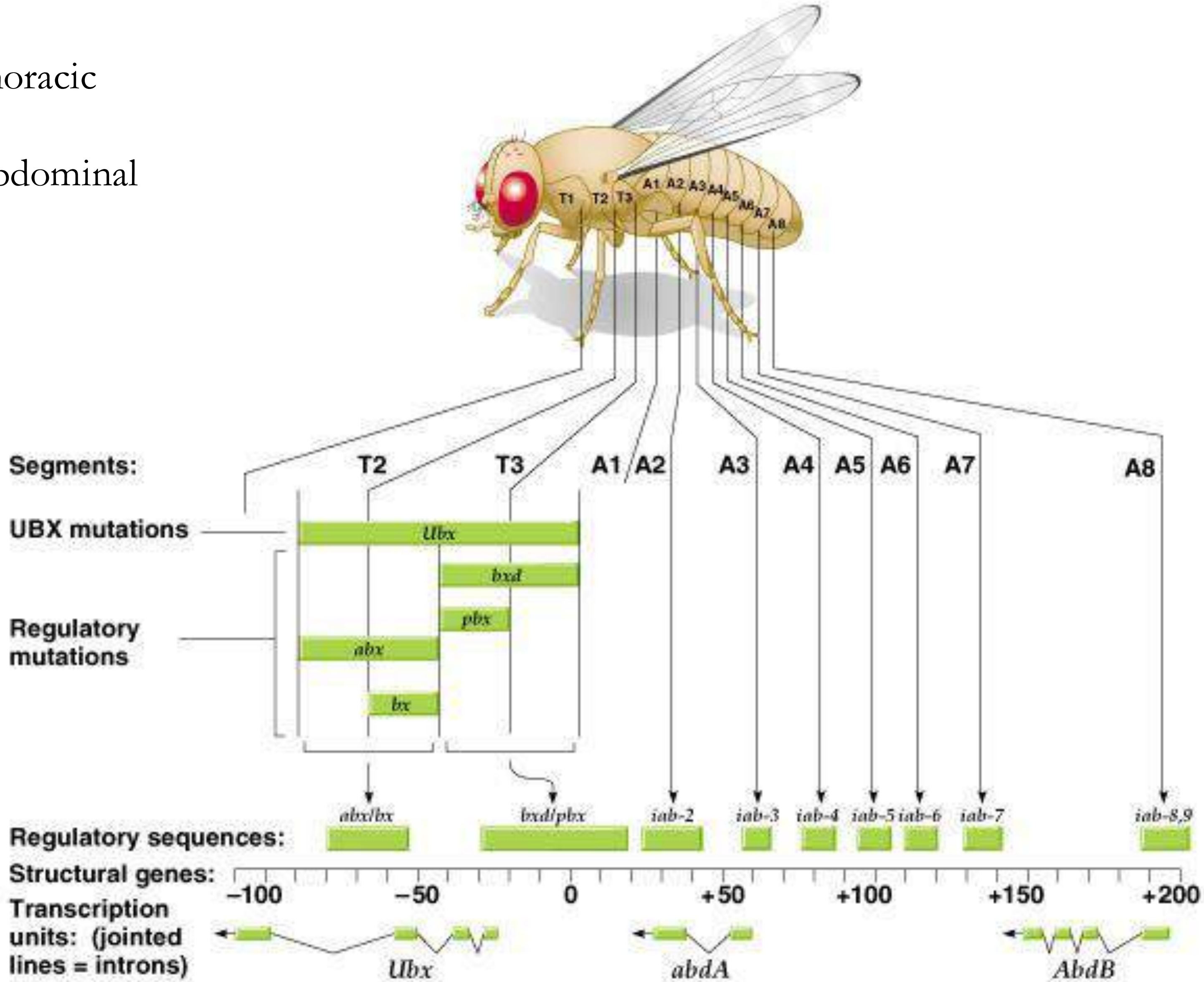


- Homeotic mutations cause different segments to develop as if located elsewhere
- *bithorax* (*bx*)
  - Anterior third thoracic segment (T3) develops like second anterior thoracic segment (T2)
  - *postbithorax* (*pbx*) posterior T3 transforms into posterior T2

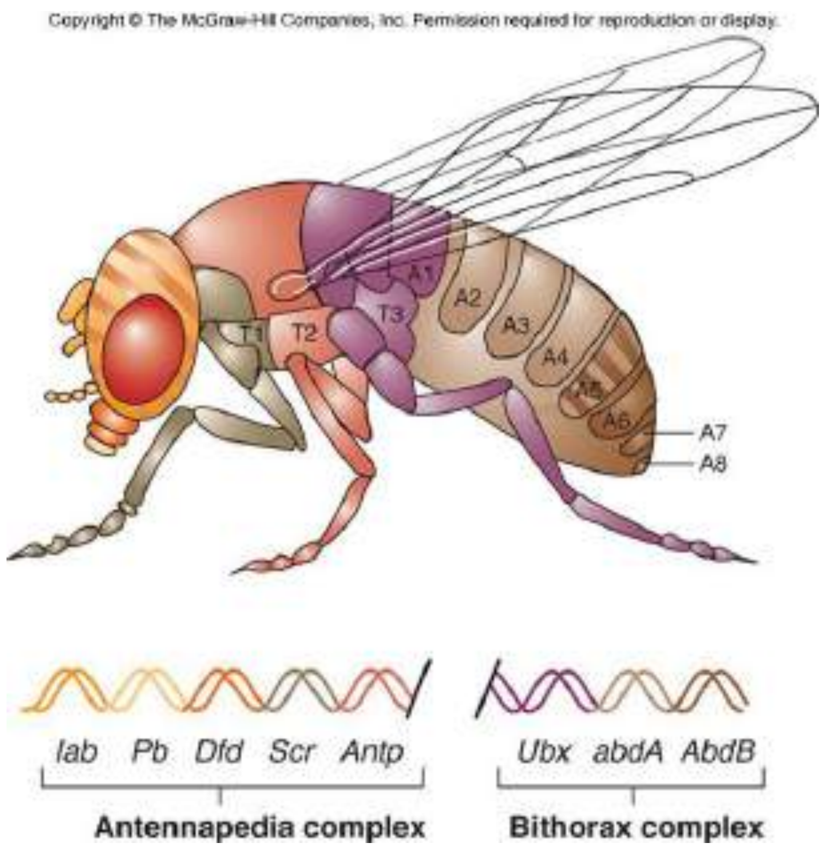
Organization of *bithorax* homeotic genes in a 300kb region of the *Drosophila* genome.

T = thoracic

A = abdominal



Antennapedia Complex and the Homeobox



- Homeotic selector genes
  - Two clusters of genes on third chromosome – antennapedia complex and bithorax complex
  - Responsible for determining segment identity

Antennapedia Complex and the Homeobox

- Specifies the identities of segments in head and anterior thorax
  - Five genes
    - *labial* (*lab*) – expressed in intercalary region
    - *proboscipedia* (*pb*) expressed in maxillary and labial segments
    - *Deformed* (*Dfd*) – expressed in mandibular and maxillary segments
    - *Sex combs reduced* (*Scr*) – expressed in labial and T1 segments
    - *Antennapedia* (*Antp*) – expressed mainly in T2, but also active in lower levels in all three thoracic segments and abdomen
  - Other genes (not homeotic)
    - *zerknullt* (*zen*) – specifies dorsal embryonic structures
    - *fushi tarazu* (*ftz*) – segmentation gene of pair-rule class
    - *bicoid* (*bcd*) – encodes maternally supplied anterior determinant

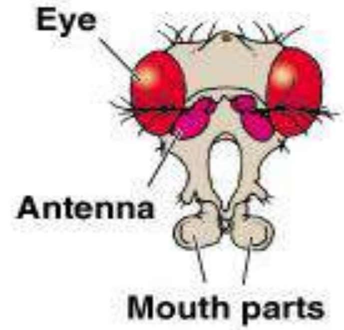
Examples of homeotic *Drosophila* mutant with the *bithorax* mutation

What is wrong with one of these flies?

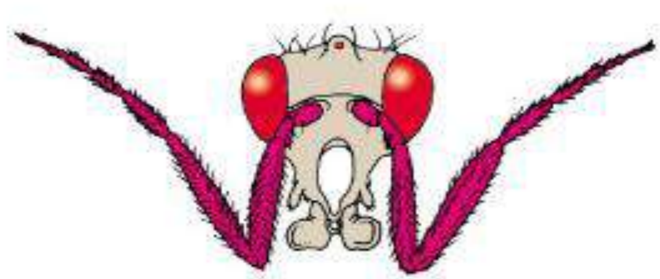
b)

c)

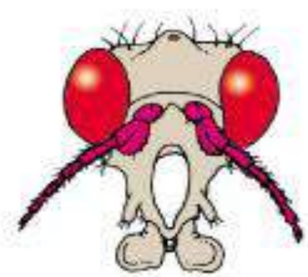
Antennapedia and *aristapedia mutants*



a) Normal



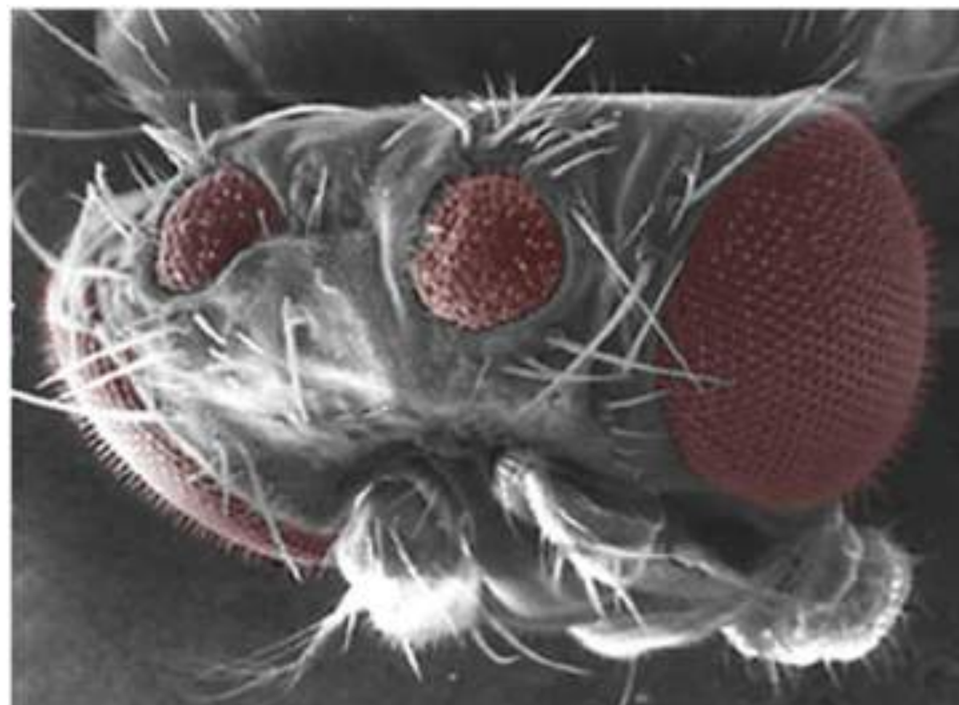
b) Antennapedia



c) Aristapedia

b) *Antennapedia*

Second set of eyes in place of antennae



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