Lecture 18: Drosophila melanogaster

Polytene chromosome

Life cycle

P elements and transformation

Embryogenesis

Read 565-578; Fig. 13.17-34

"Molecular Biology of the Cell" ed. By Bruce Albert et al. (free online through ncbi books)

Drosophila melanogaster (fruit fly)

Small genome 170 Mb (5% of human genome)

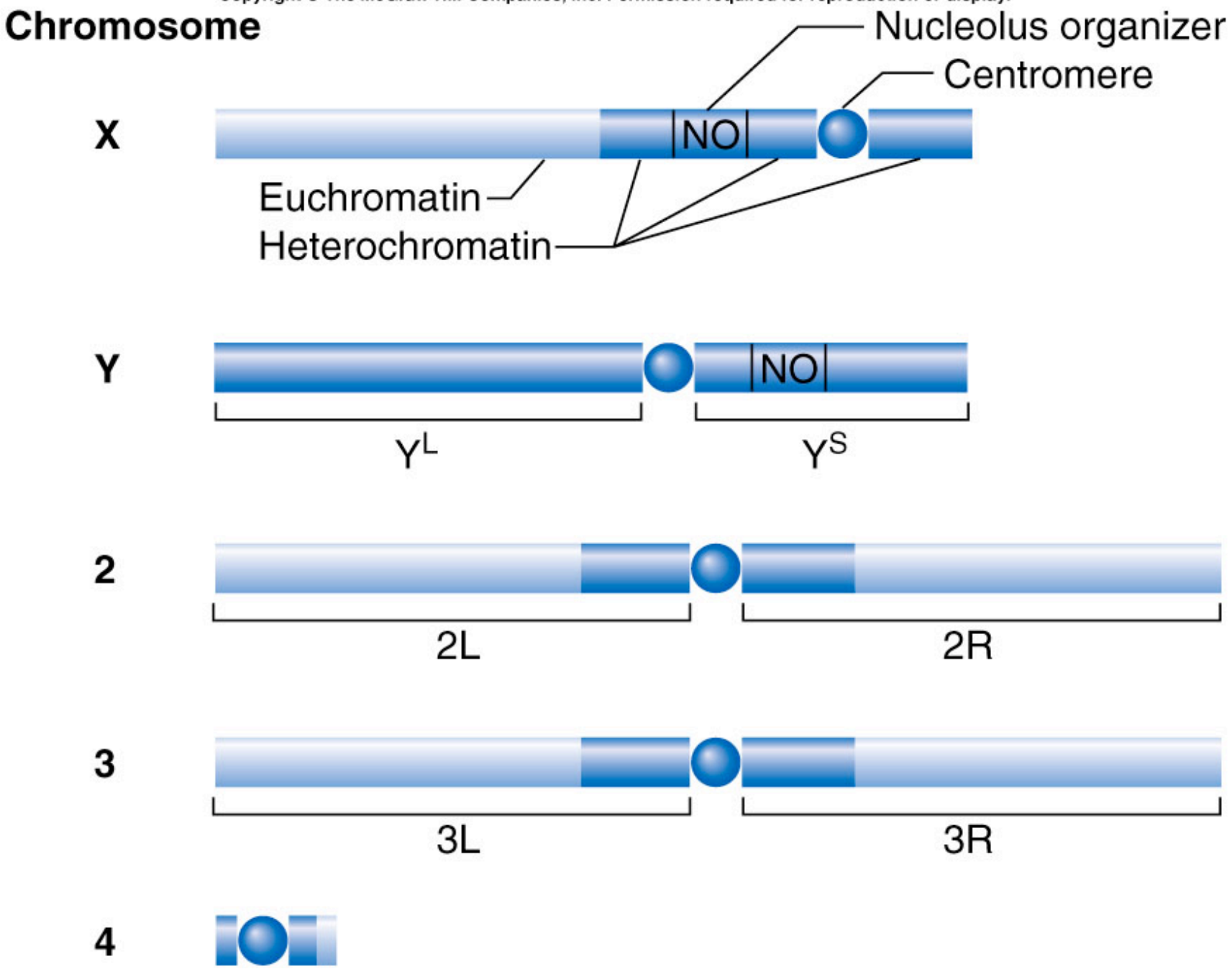
33% repetitive DNA

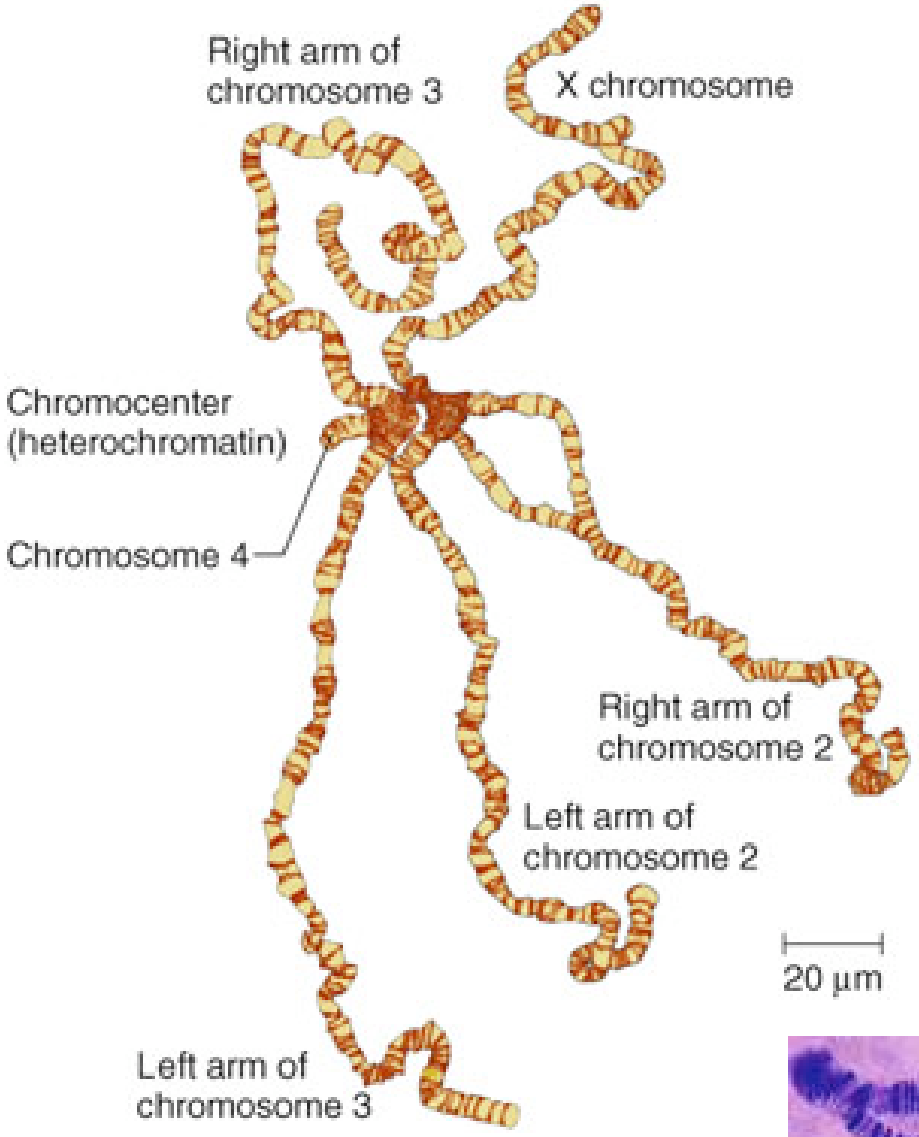
13,600 genes

Three autosome + X and Y (small chr. number)

Giant polytene chromosome

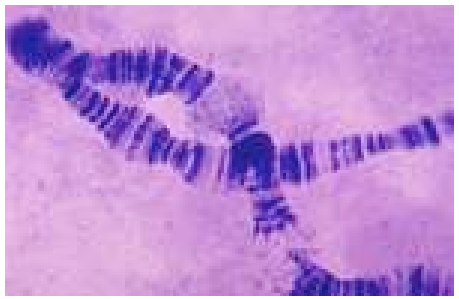
Fast life cycle (10 days)





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



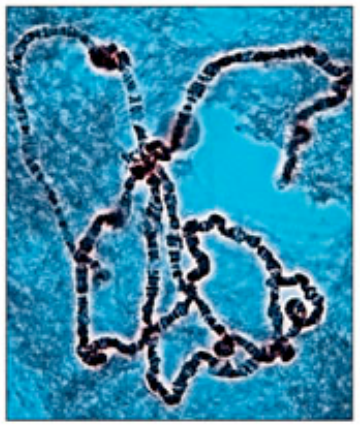
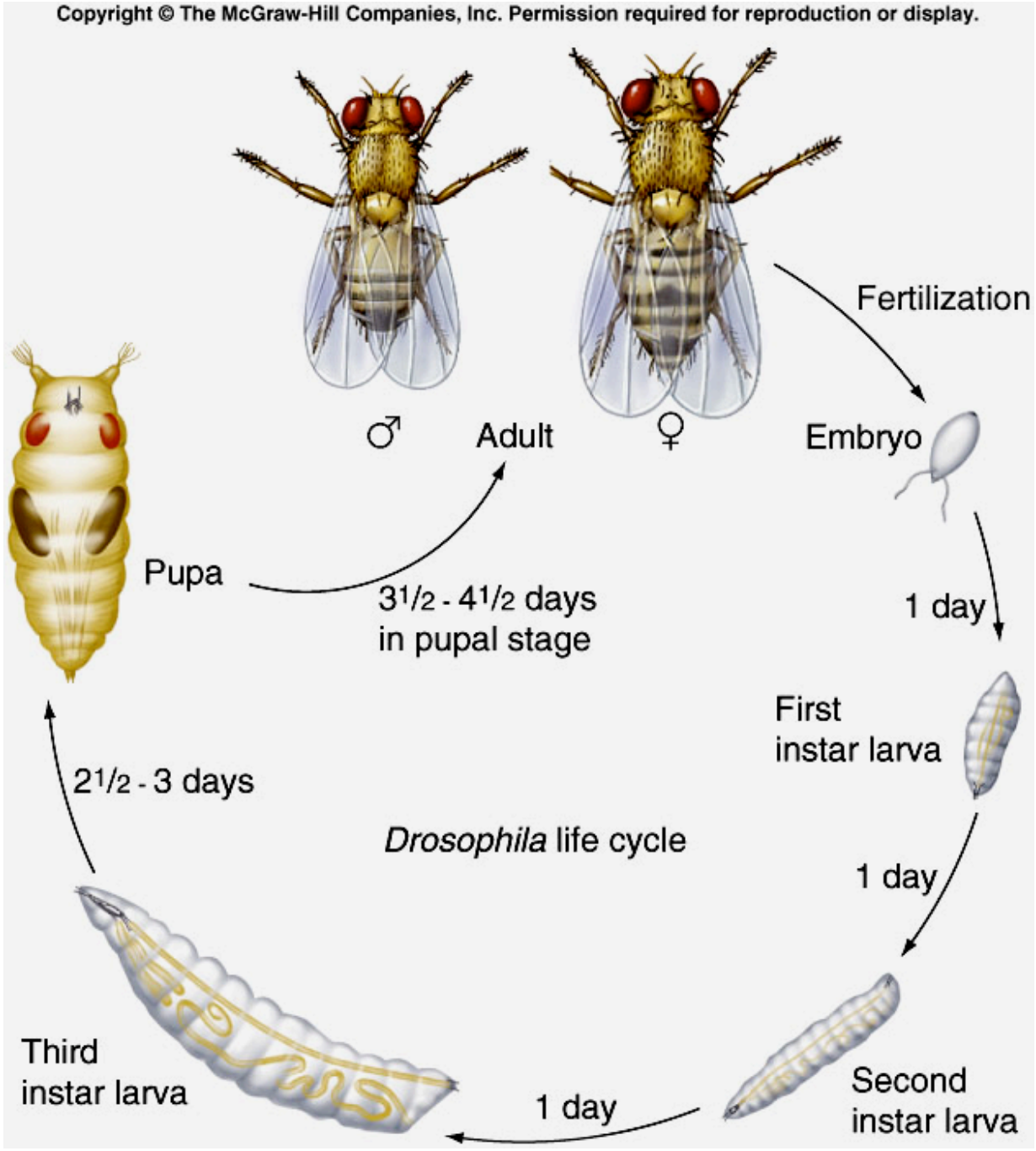


Fig. D.4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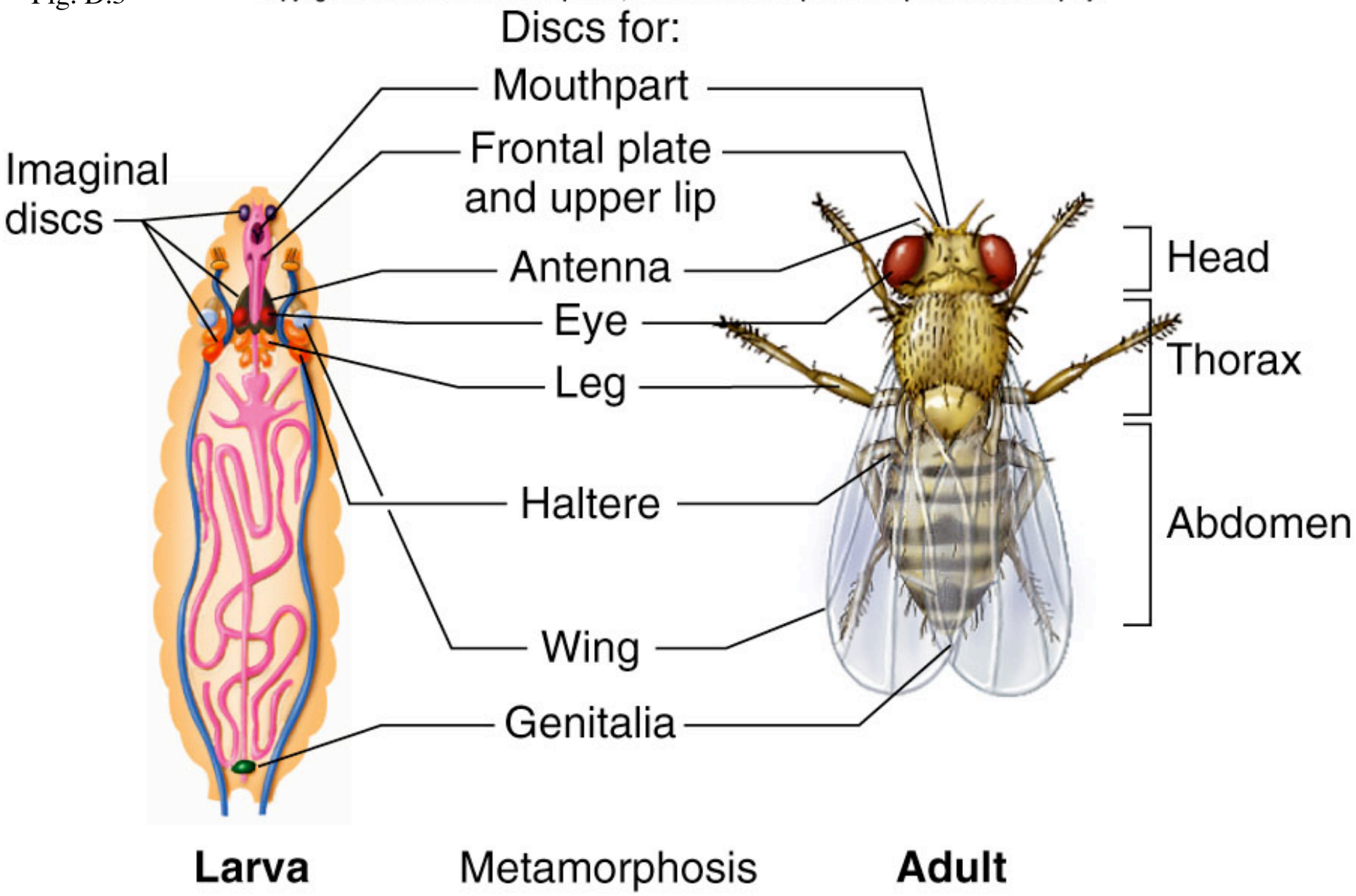
♂
Adult
♀
Fertilization
Embryo
1 day
First instar larva
1 day
Second instar larva
1 day
Third instar larva
2½ - 3 days
Pupa
3½ - 4½ days in pupal stage
Drosophila life cycle

Fig. D.5



P-element transposons are critical tools in molecular genetics

- Hybrid dysgenesis
  - Males from Drosophila strains carrying P elements crossed to females that lack P elements
  - P element becomes highly mobile in germ line of F1 hybrids
  - Chromosome breakage reduces fertility in hybrids
  - Progeny of F1 flies carry many new mutations induced by P element insertions
  - Molecular details
    - P element primary transcript encodes transposase that catalyzes transposition
    - Cross between P and M strain causes hybrid dysgenesis
    - Cross between P and P strain does not
      - Eggs produced by P female have repressor protein that prevents transposition
      - Repressor coded for by alternatively spliced P element mRNA

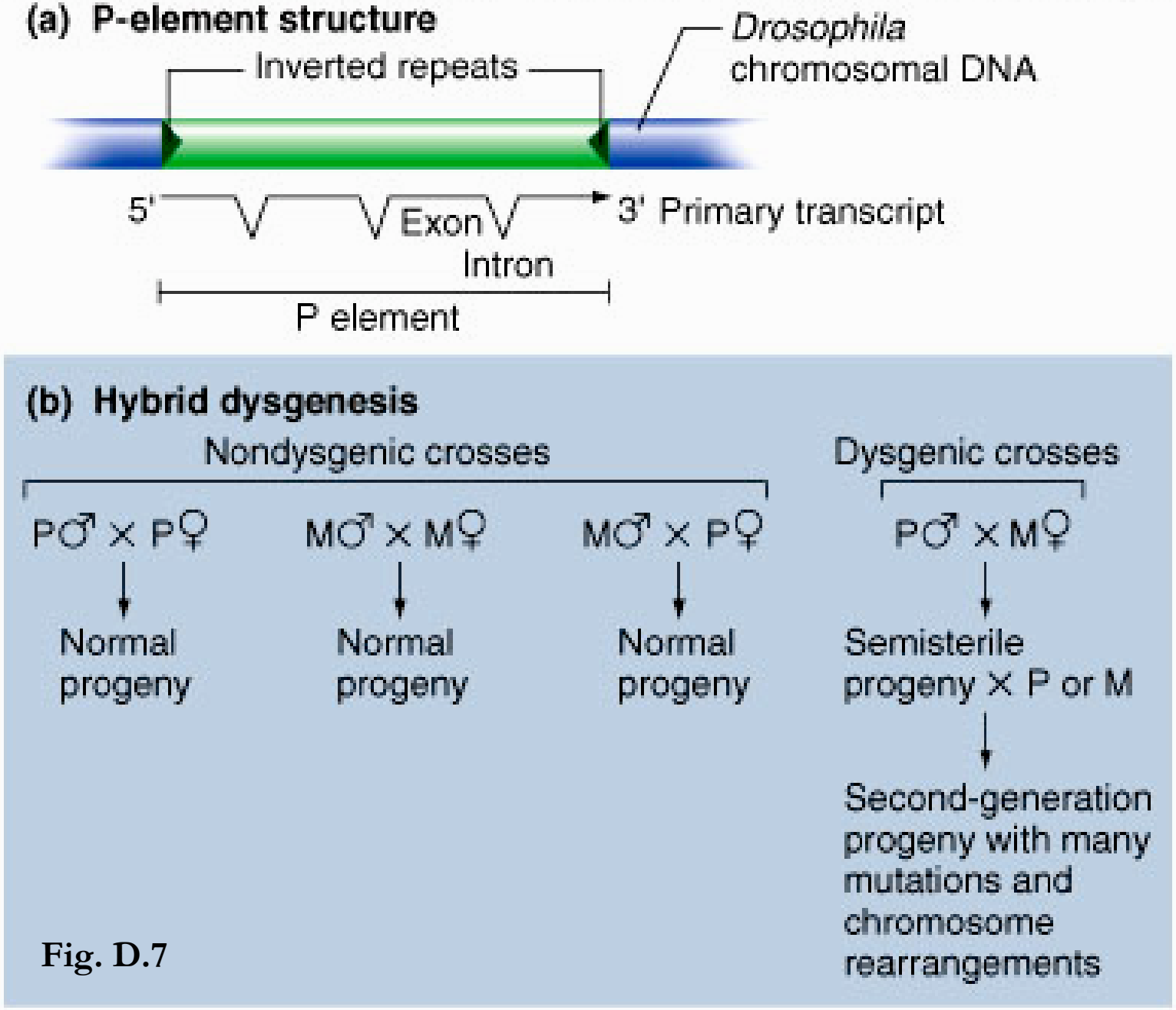
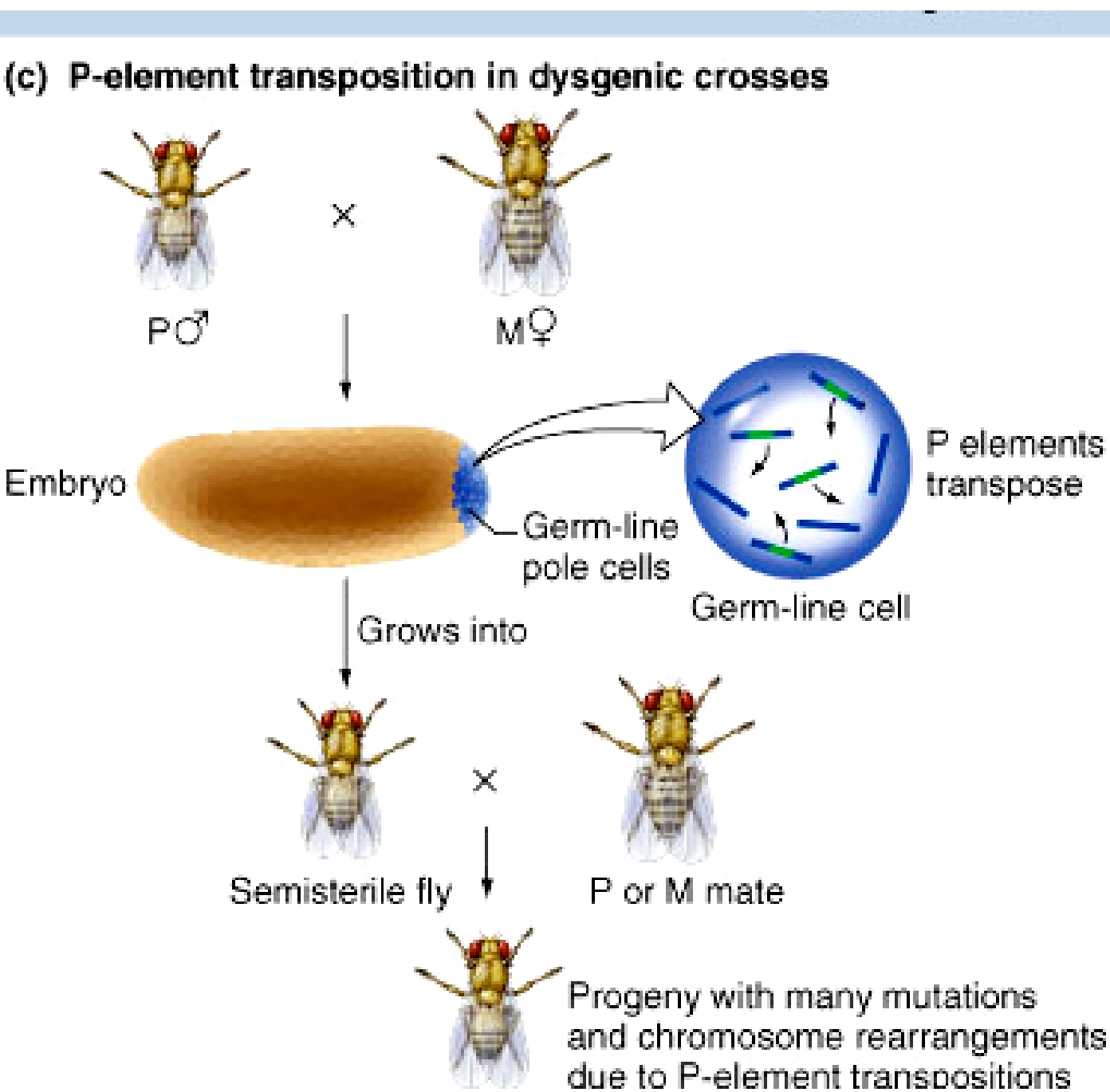
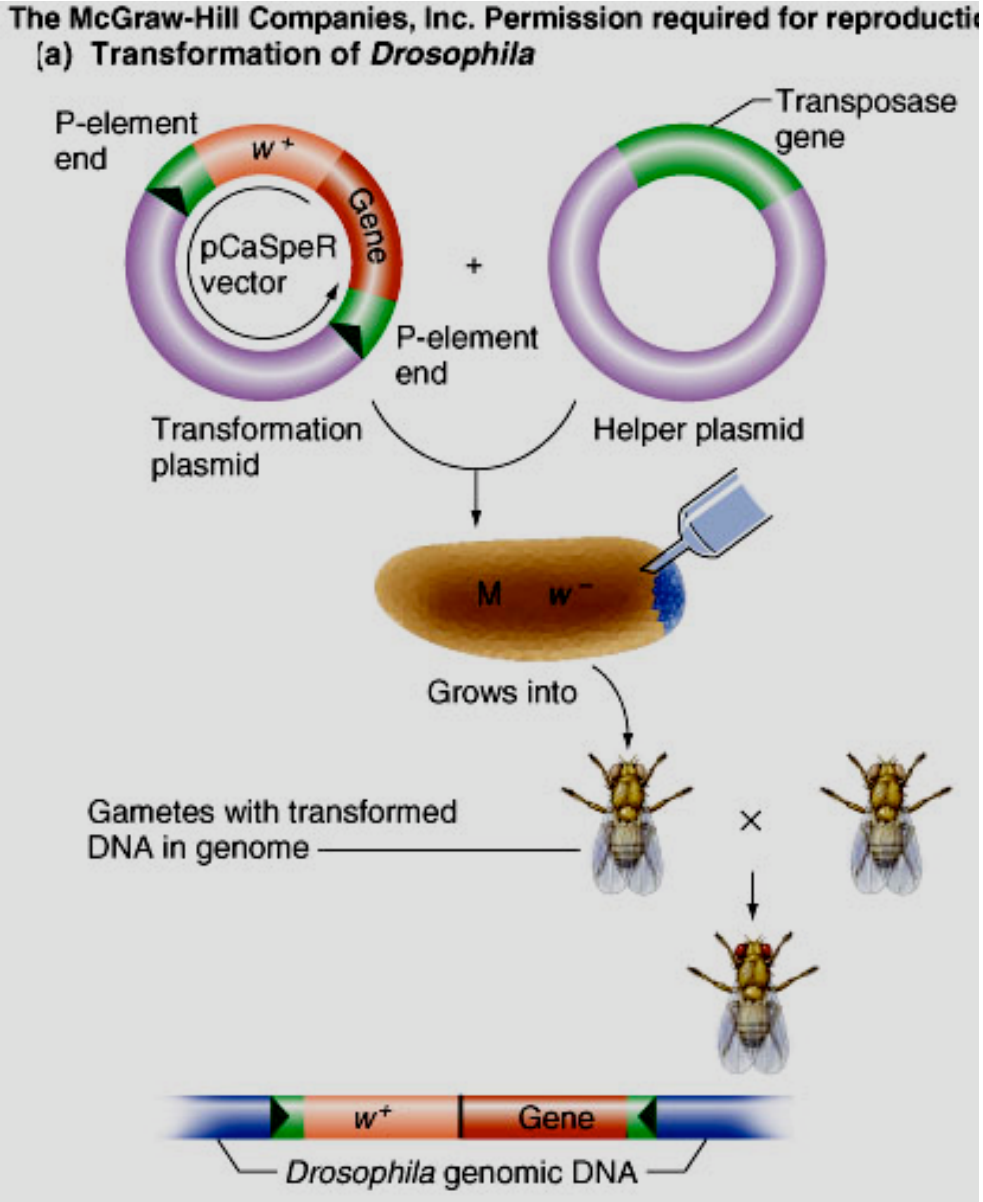


Fig. D.7


(c) P-element transposition in dysgenic crosses
P♂
×
M♀
Embryo
Germ-line
pole cells
P elements
transpose
Germ-line cell
Grows into
Semisterile fly
×
P or M mate
Progeny with many mutations
and chromosome rearrangements
due to P-element transpositions

Transformation: the introduction of cloned DNA into flies



- P-elements used as vectors
- Insert fly DNA into intact P element and then into plasmid
- Inject into syncytial embryos from M strain mothers
- Cross to P males
- Mimicking hybrid dysgenesis

Fig. D.8a

Drosophila embryogenesis

Four classes of genes responsible for formation of segments

- Maternal genes
- Gap genes
- Pair-rule genes
- Segmentation polarity genes
- Function in a hierarchy that progressively subdivides the embryo into successively smaller units

Drosophila oocyte

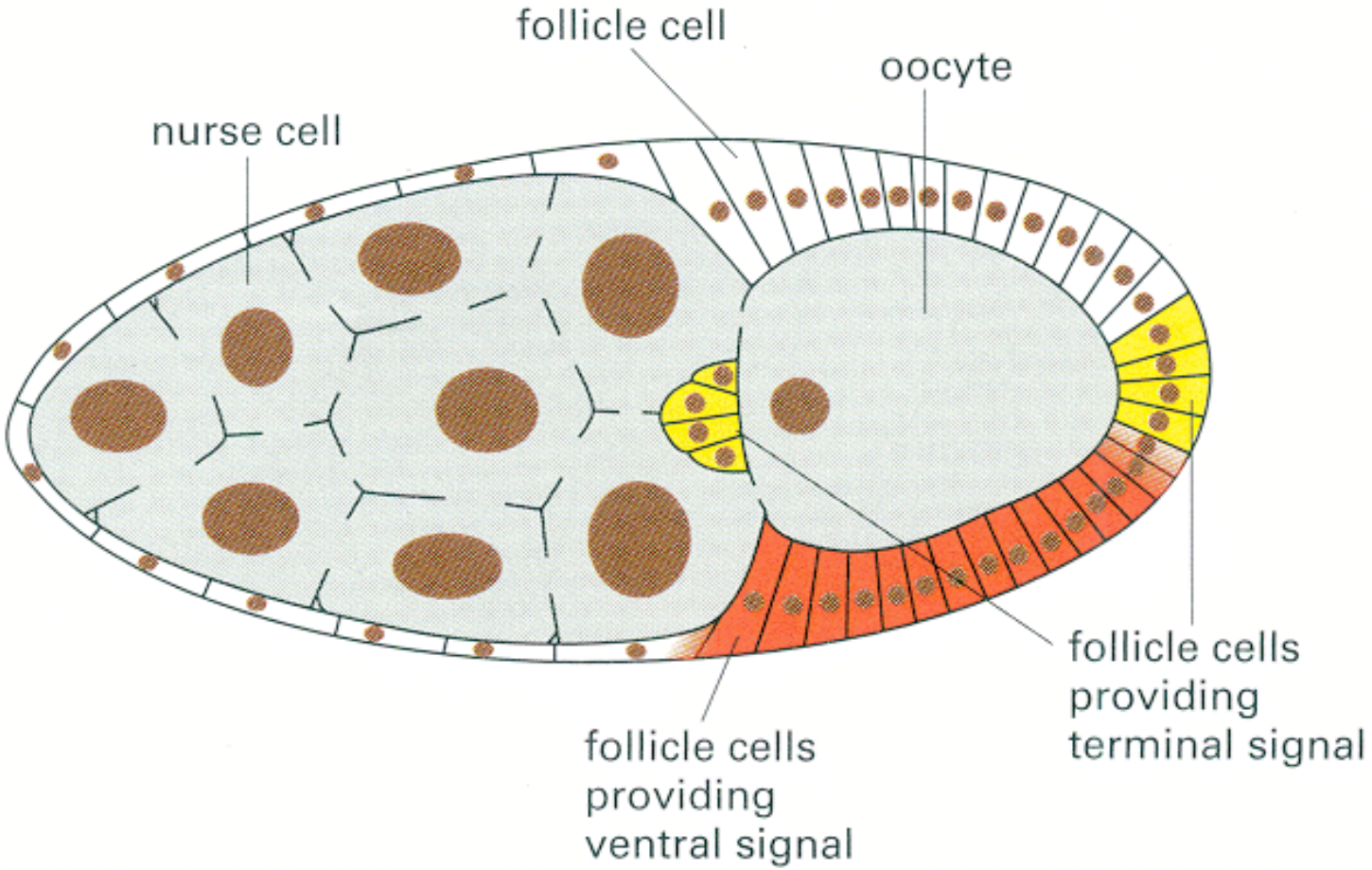
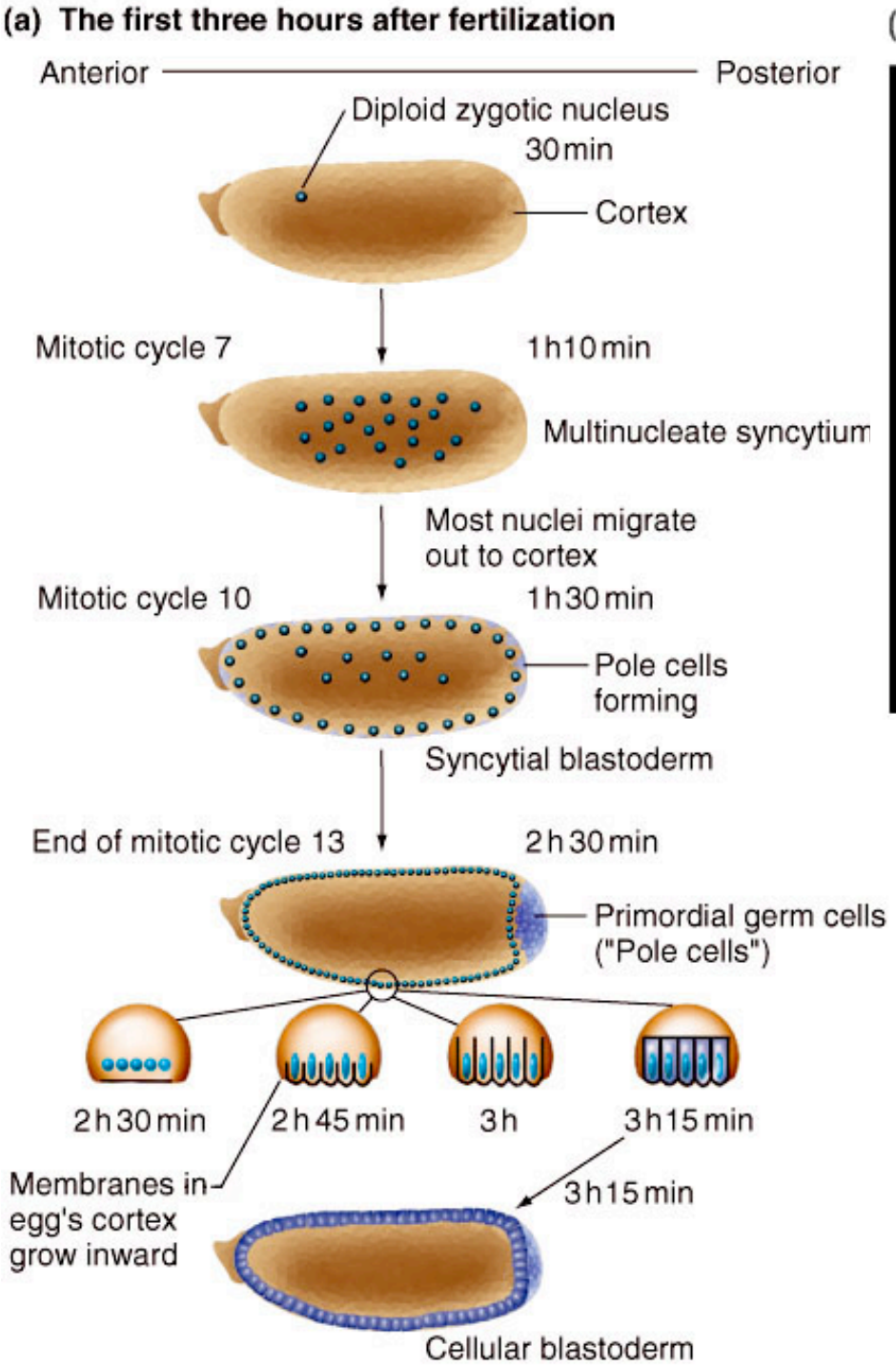
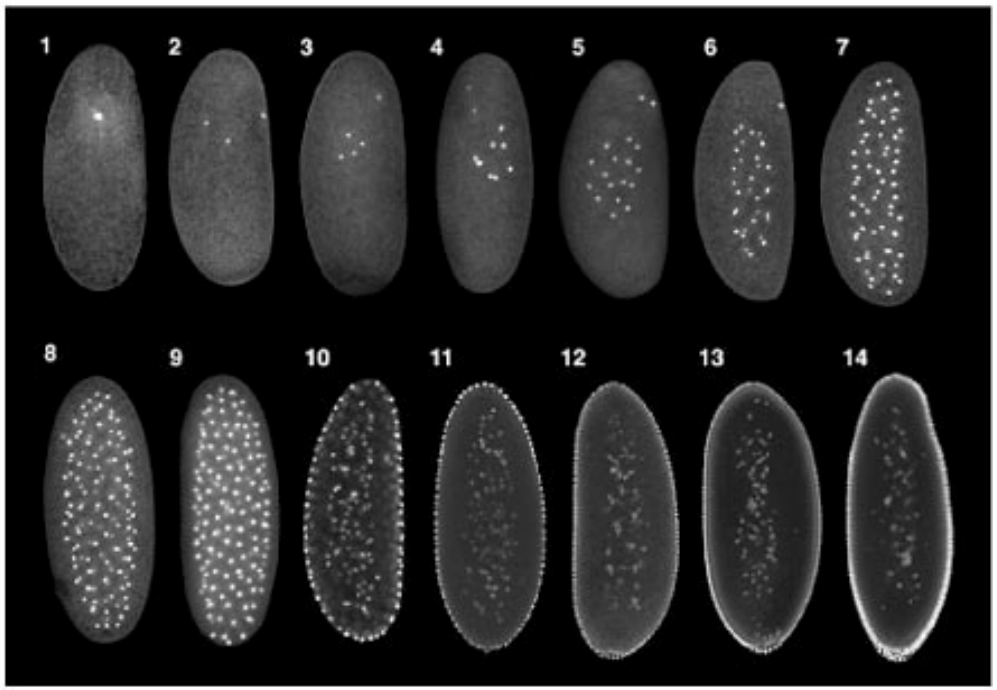


Figure 21-30. A Drosophila oocyte in its follicle. The oocyte is derived from a germ cell that divides four times to give a family of 16 cells that remain in communication with one another via cytoplasmic bridges (gray). One member of the family group becomes the oocyte, while the others become nurse cells, which make many of the components required by the oocyte and pass them into it via the cytoplasmic bridges. The follicle cells that partially surround the oocyte have a separate ancestry. As indicated, they are the sources of terminal and ventral egg-polarizing signals. (**From Bruce Albert Book)**

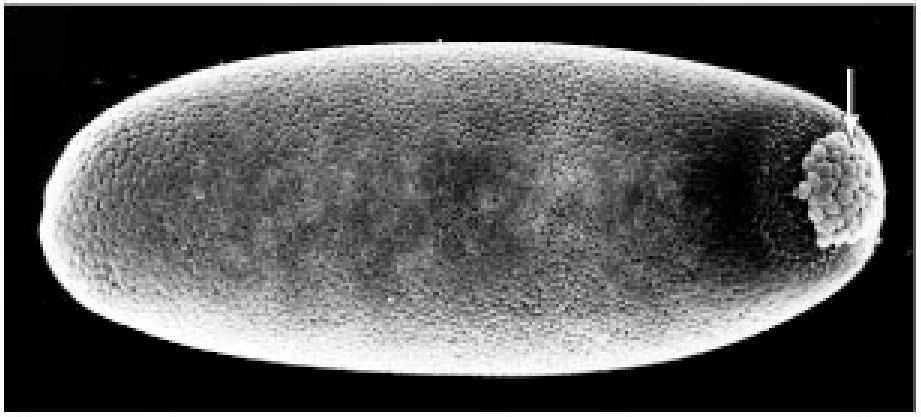




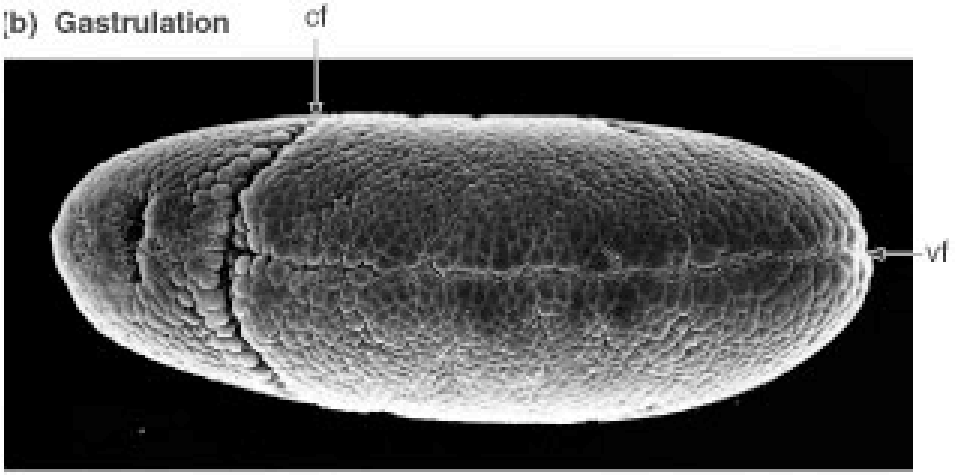
Drosophila Embryogenesis

Fig. D.18

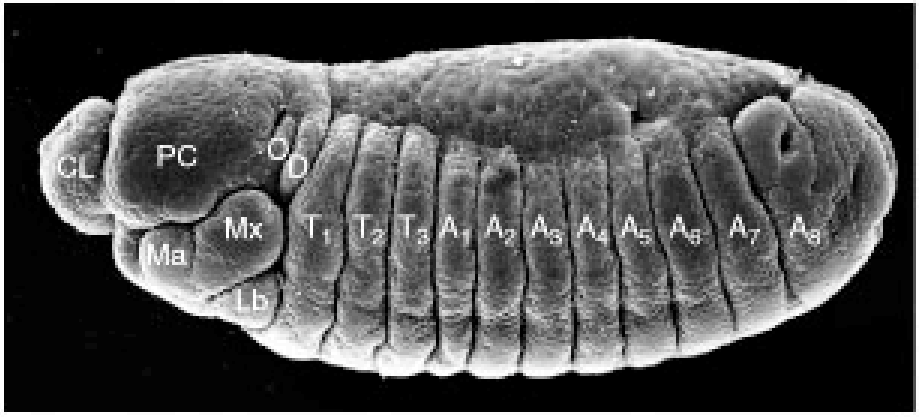
(a) Cellular blastoderm



(b) Gastrulation



(c) Segmentation



(d) Segment identity is preserved throughout development.

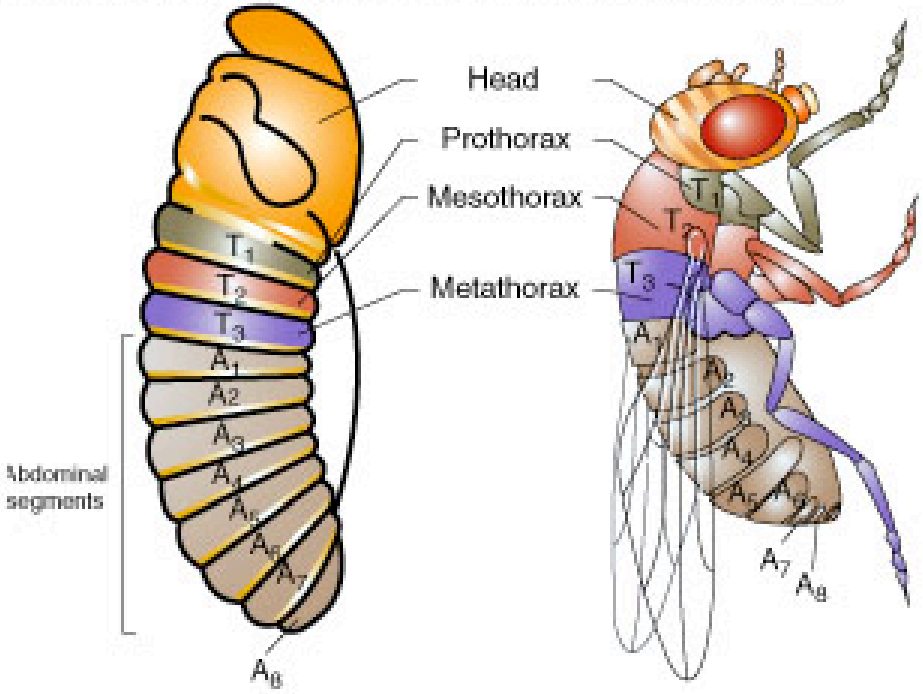
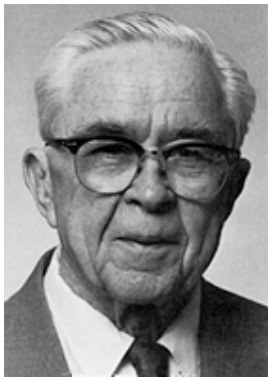


Fig. D.19

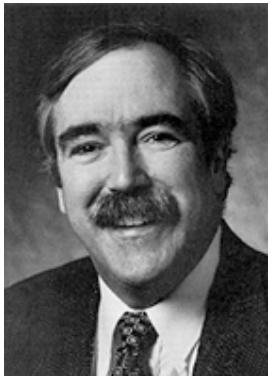
A parade of 1995 Nobels:

for their discoveries concerning the genetic control of early embryonic development



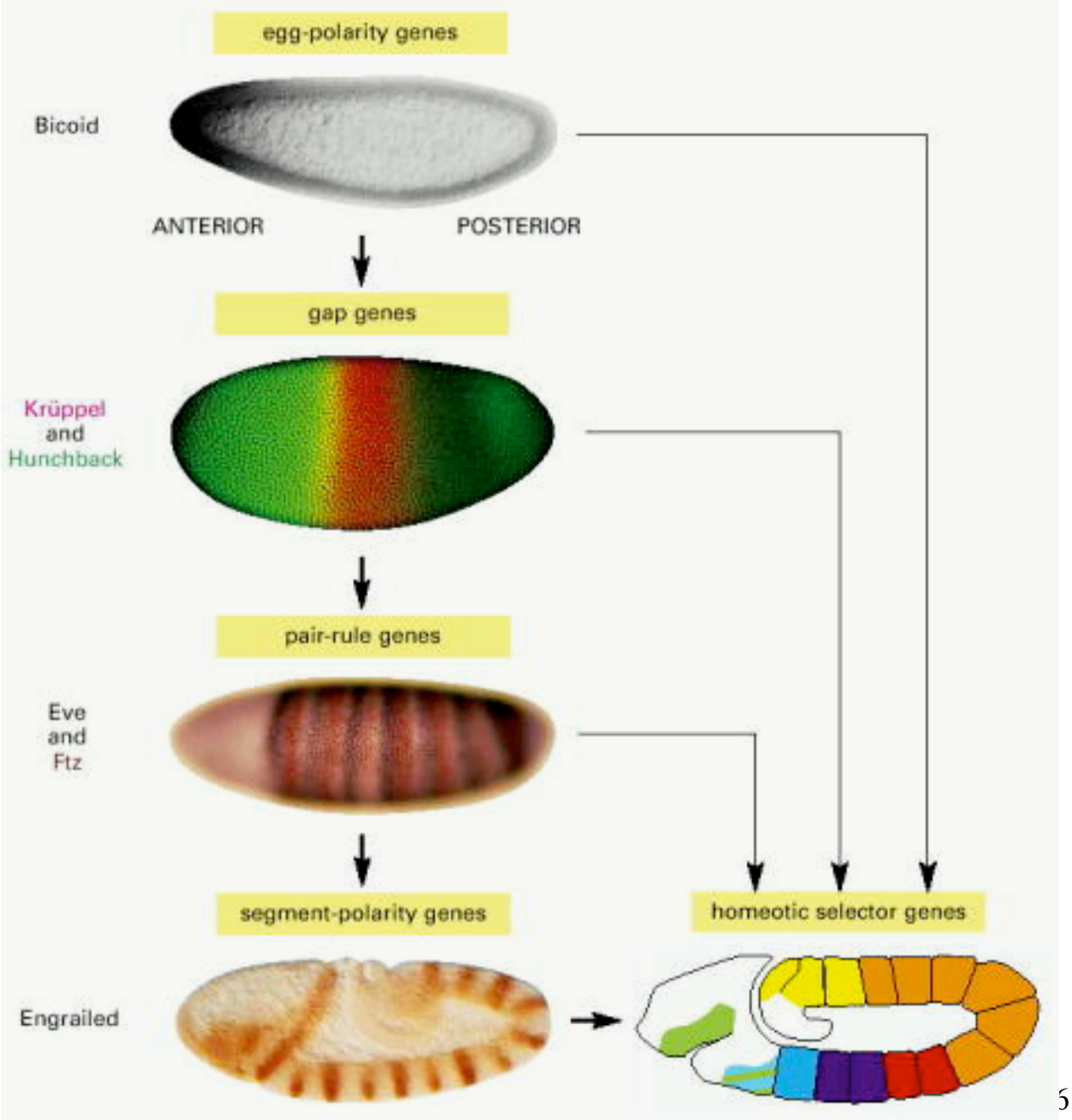
Edward B. Lewis

Christiane Nüsslein-Volhard

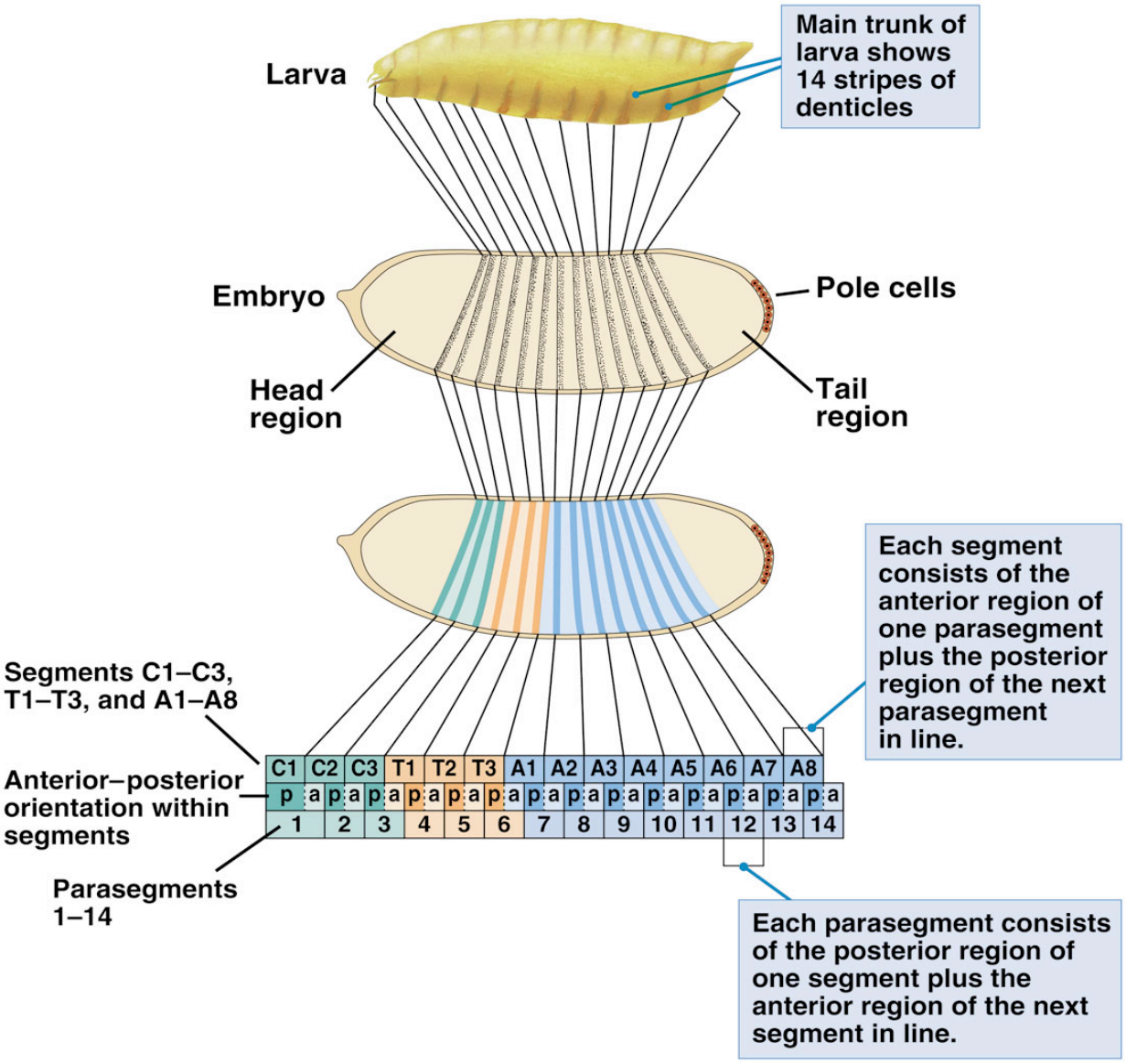


Eric F. Wieschaus

From Bruce Albert Book



The segmentation pattern of *Drosophila* larva



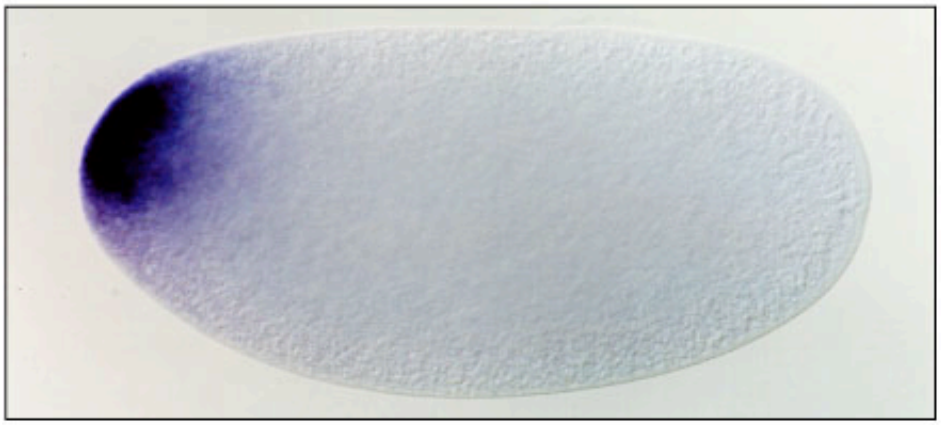
Morphogens: Substances that define different cell fate in a concentration-dependent manner

Klaus Sander proposed:

- Each pole of the egg produces a different substance
- These substance form the opposing gradients by diffusion
- Concentrations of these substances determine the type of structure produced at each position long the body axis

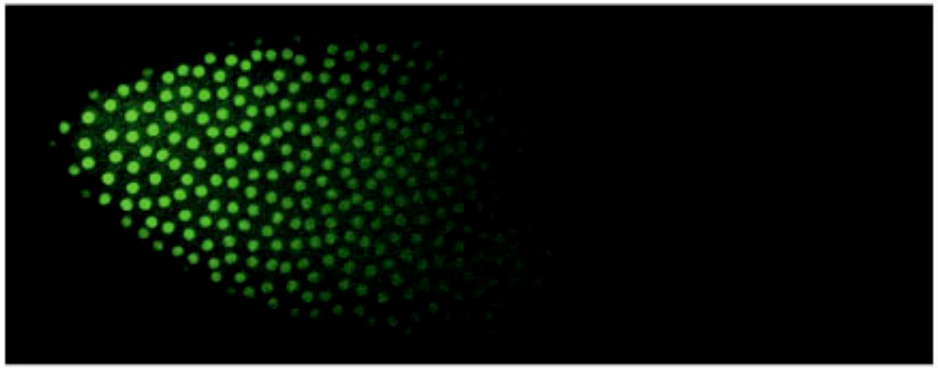
Bicoid (Bcd) is a morphogen

(a) Localization of *bicoid* mRNA



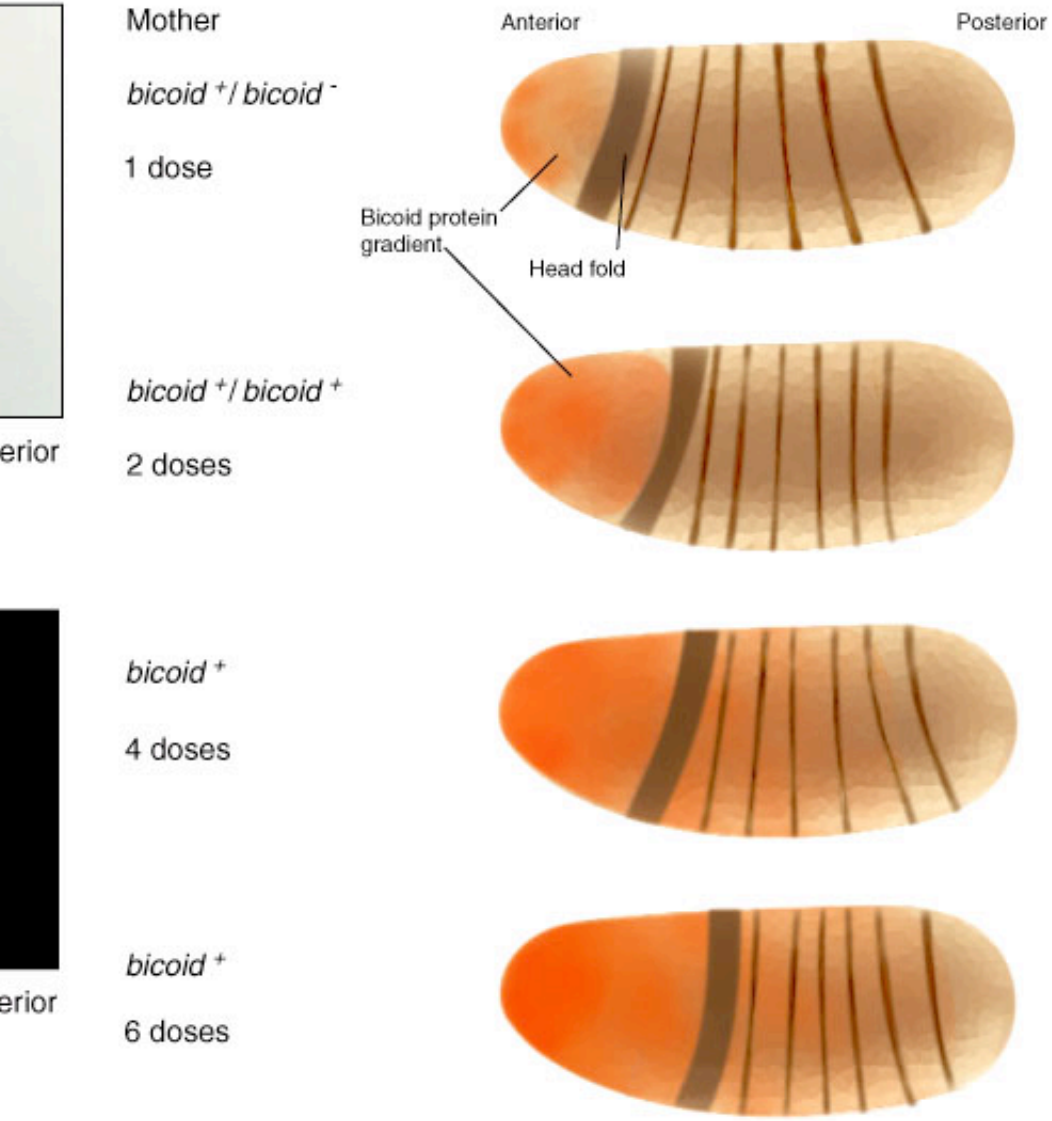
Anterior Posterior

(b) A gradient of Bicoid protein

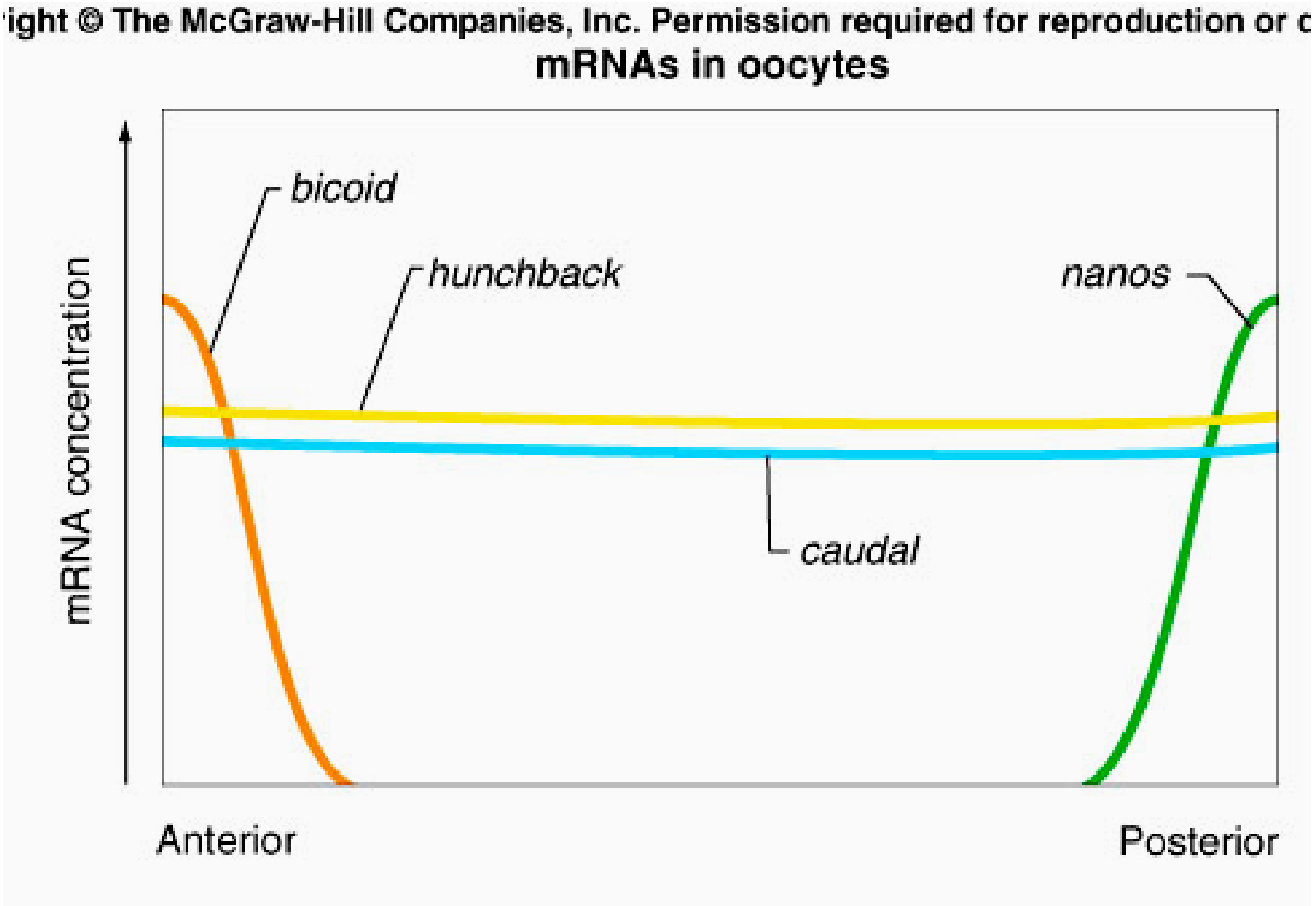


Anterior Posterior

(c) Bicoid protein is a morphogen.



How Bcd protein works



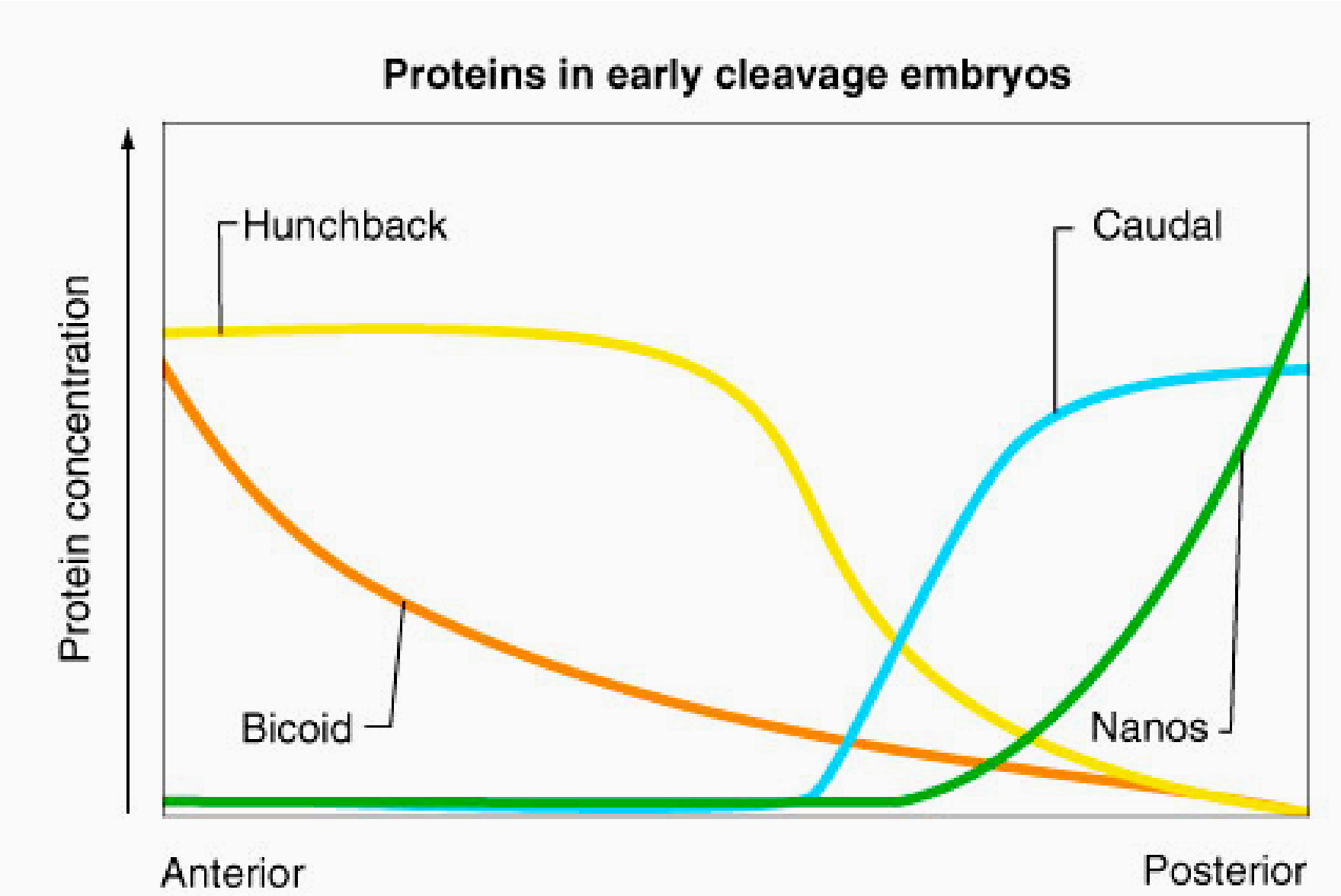
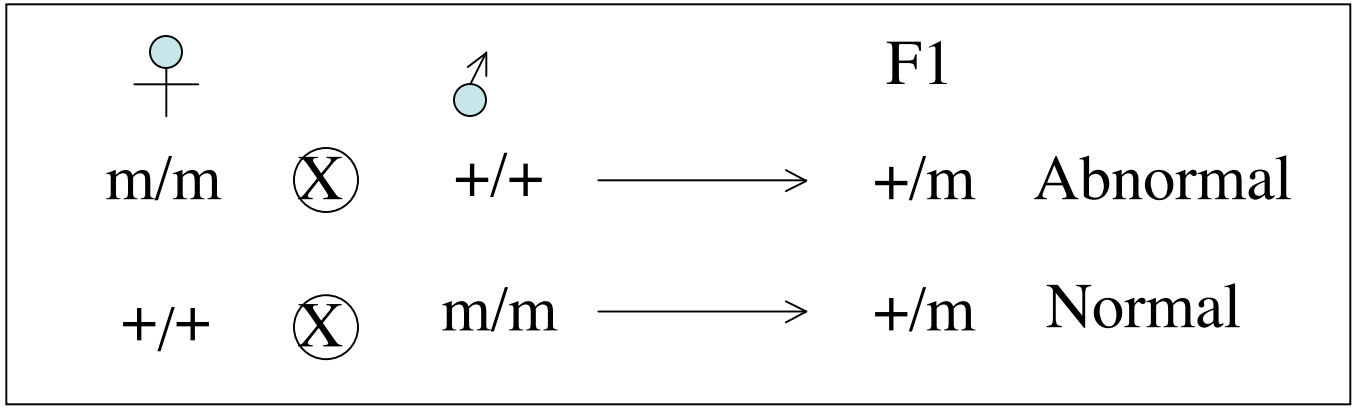


Fig. D.21 bottom

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**

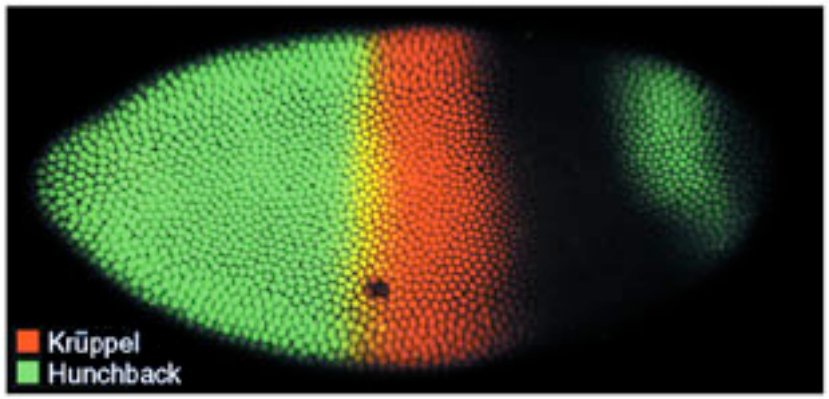


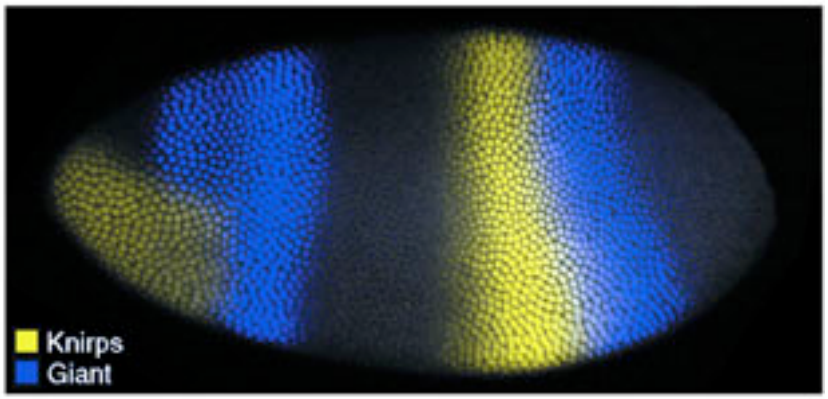
Gap genes

Zones of expression of four gap genes: *hunchback*, *Kruppel*, *knirps*, and *giant* in late syncytial blastoderm embryos

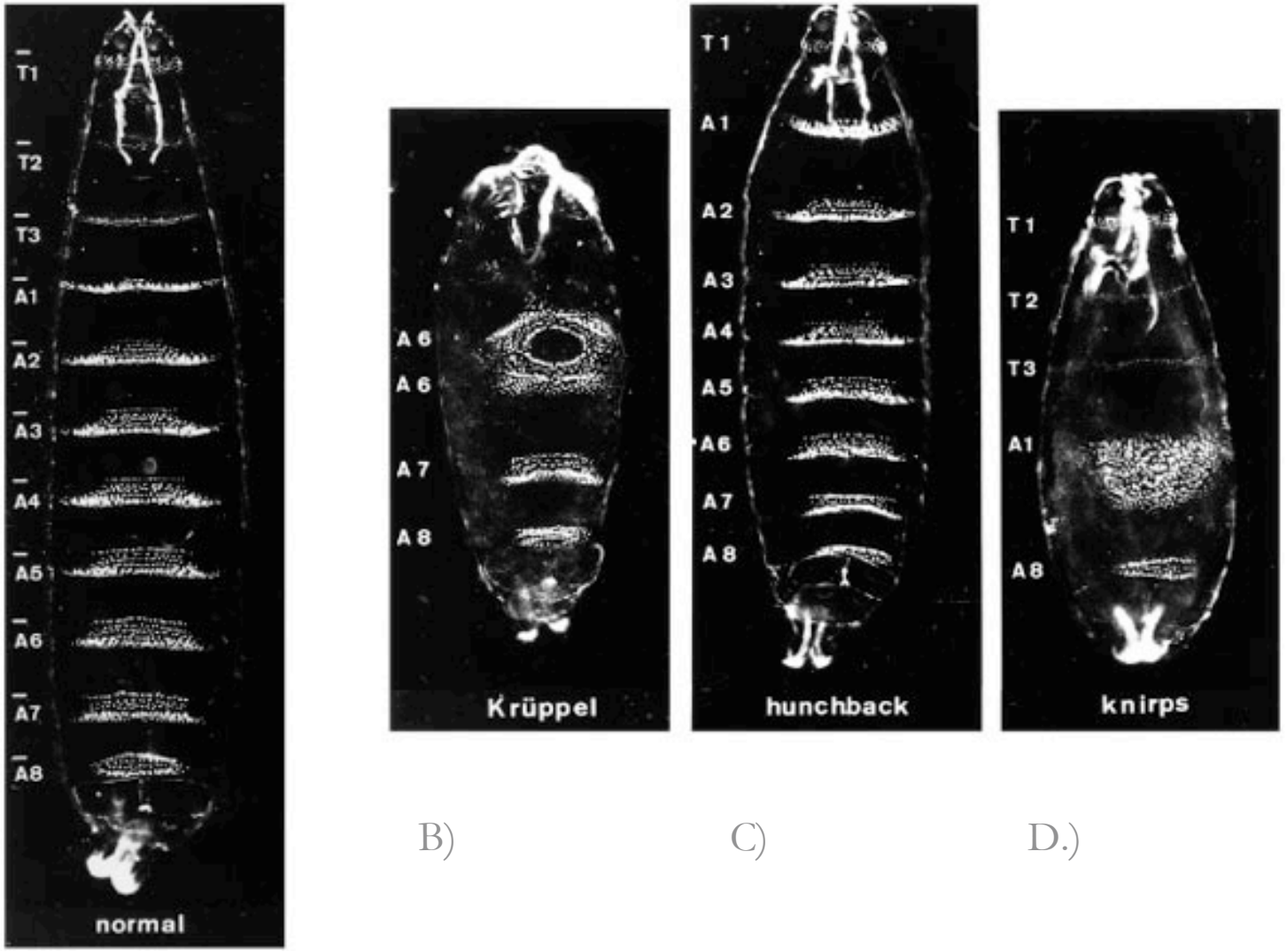
These are zygotic genes

(a) Zones of gap gene expression





Defects in segmentation from mutations in gap genes



A) B) C) D.)

Fig. D.22b

- Gap genes
  - 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

Pair-rule genes

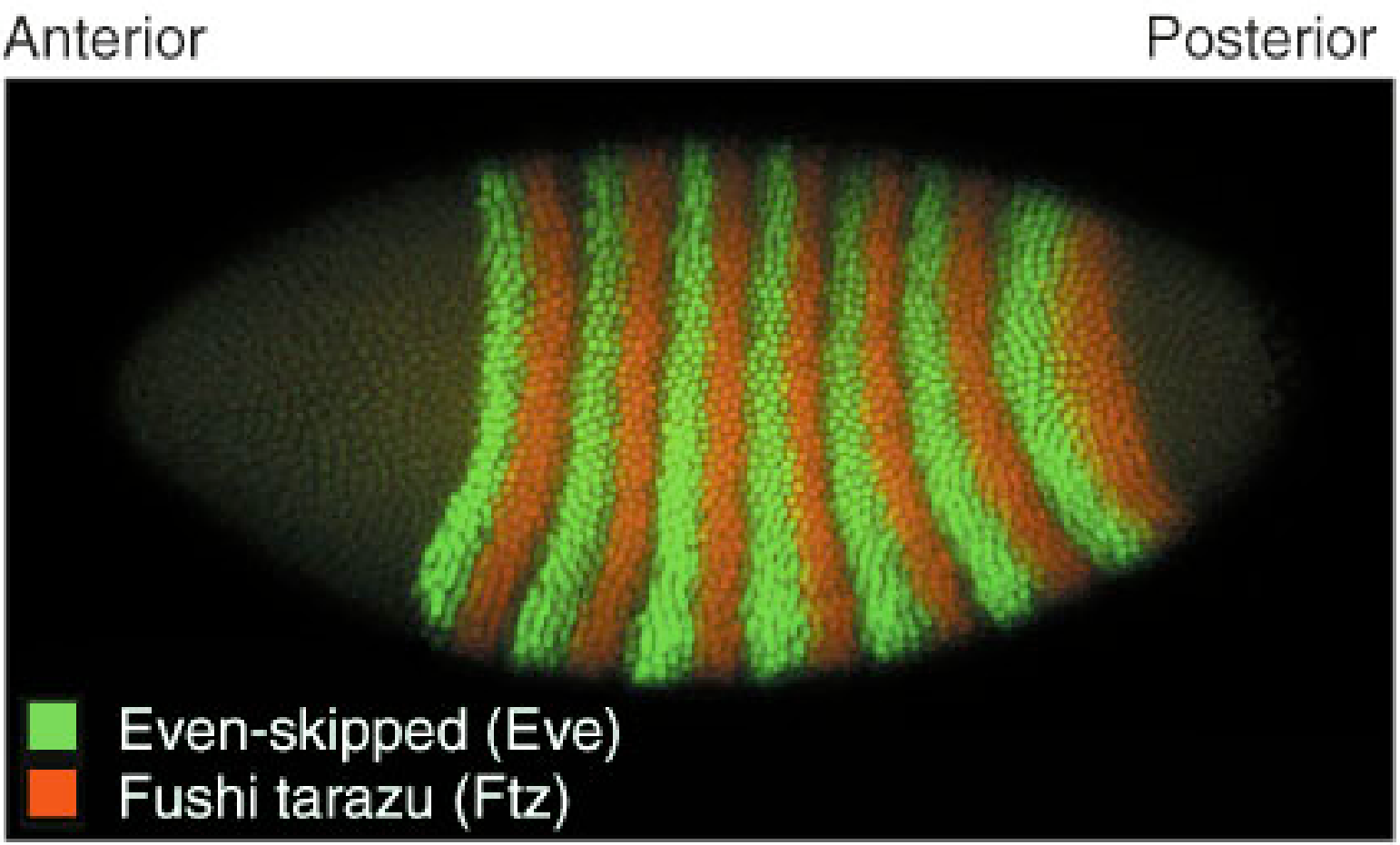
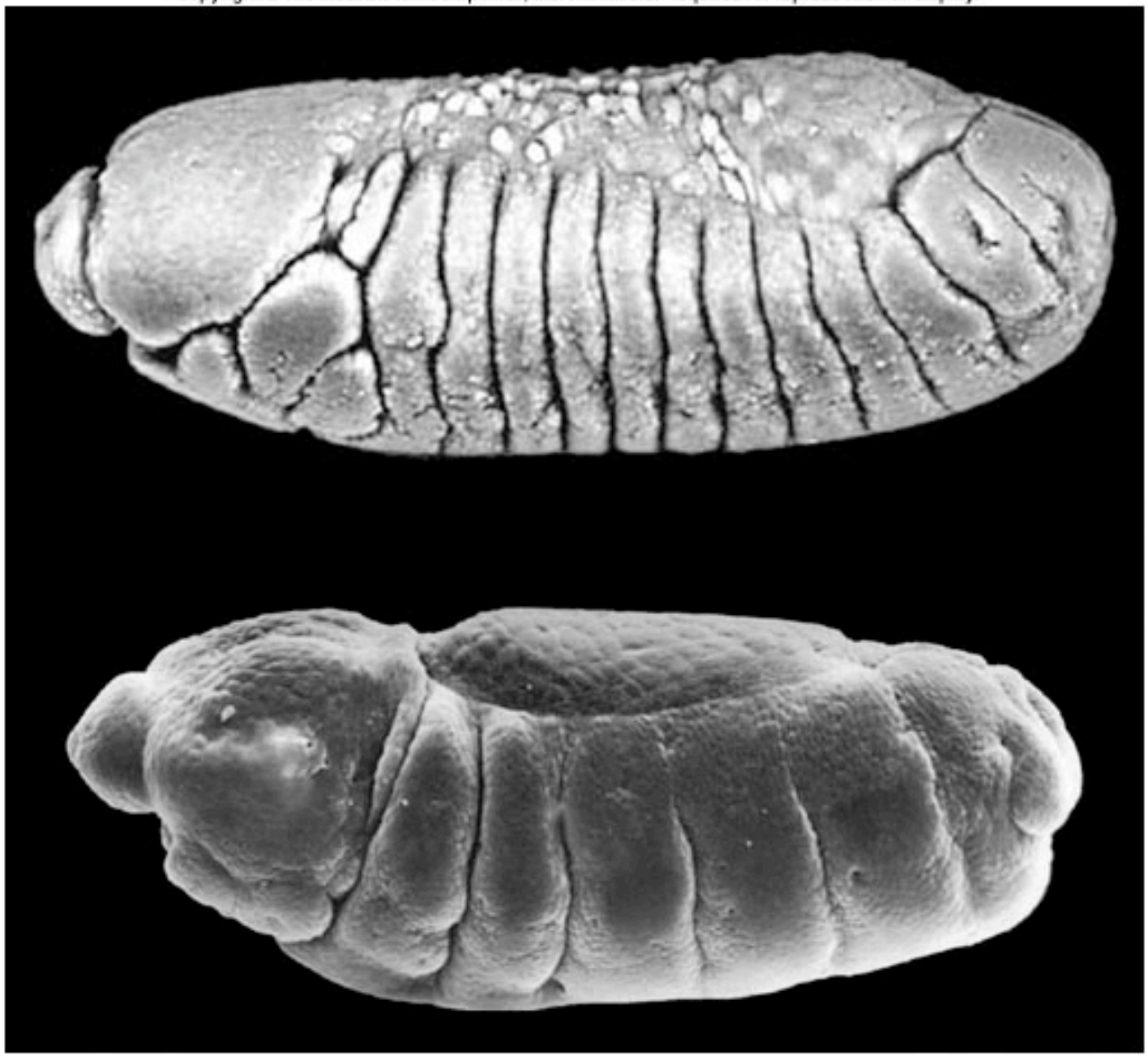


Fig. D.23

Fig. 19.2



Ftz
mutant

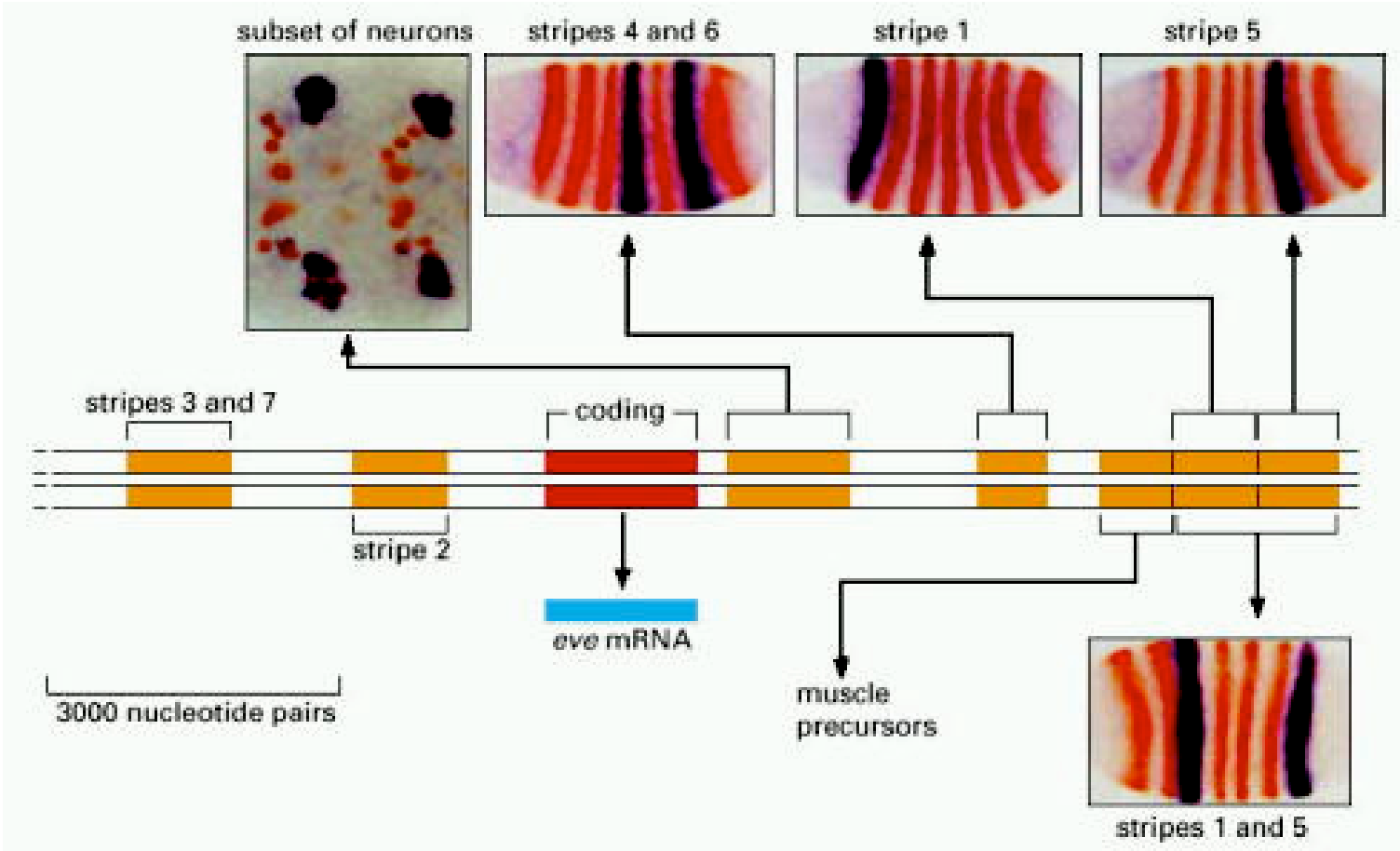


Figure 21-38. Modular organization of the regulatory DNA of the eve gene. In the experiment shown, cloned fragments of the regulatory DNA were linked to a LacZ reporter (a bacterial gene). Transgenic embryos containing these constructs were then stained by in situ hybridization to reveal the pattern of expression of LacZ (blue/black), and counterstained with an anti-Eve antibody (orange) to show the positions of the normal eve expression stripes. Different segments of the eve regulatory DNA (ochre) are thus found to drive gene expression in regions corresponding to different parts of the normal eve expression pattern. Two segments in tandem drive expression in a pattern that is the sum of the patterns generated by each of them individually. Separate regulatory modules are responsible for different times of gene expression, as well as different locations: the leftmost panel shows the action of a module that comes into play later than the others illustrated and drives expression in a subset of neurons. **(From Bruce Albert Book)**

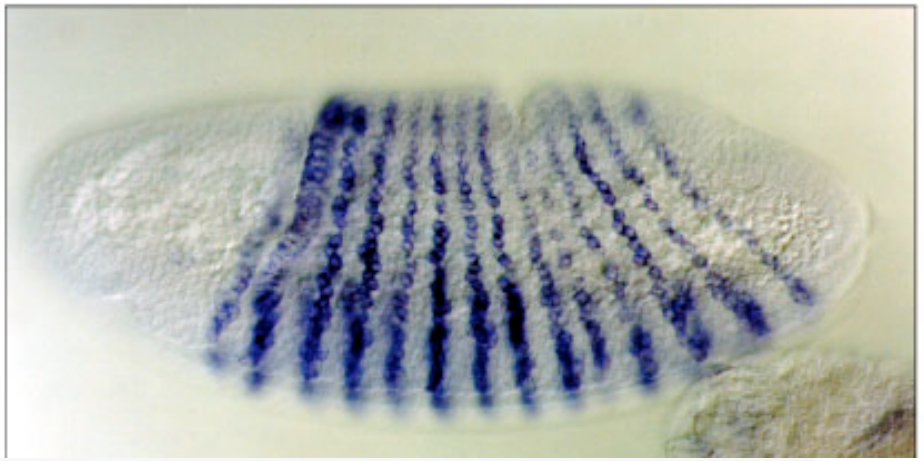
Pair-rule genes

- (a) zones of expression at beginning of blastoderm stage
  - Each gene expressed in seven stripes

- (b) Activation of Eve stripes relies on different cis-regulatory elements

Segment polarity genes

(a) Distribution of Engrailed protein



(b) Segment polarity genes establish compartment borders.

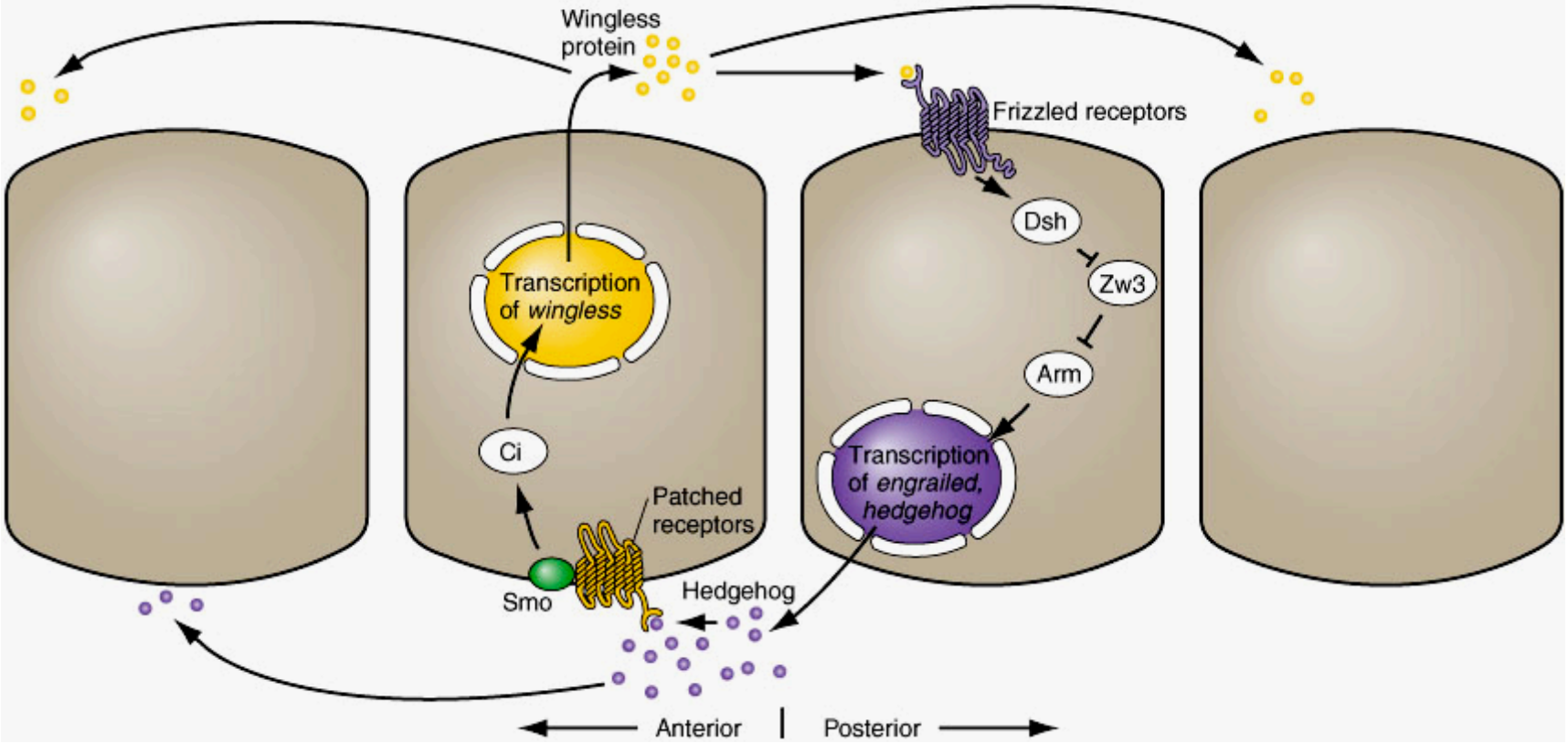


Fig. D.24

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

Segment polarity genes are lowest level of segmentation hierarchy

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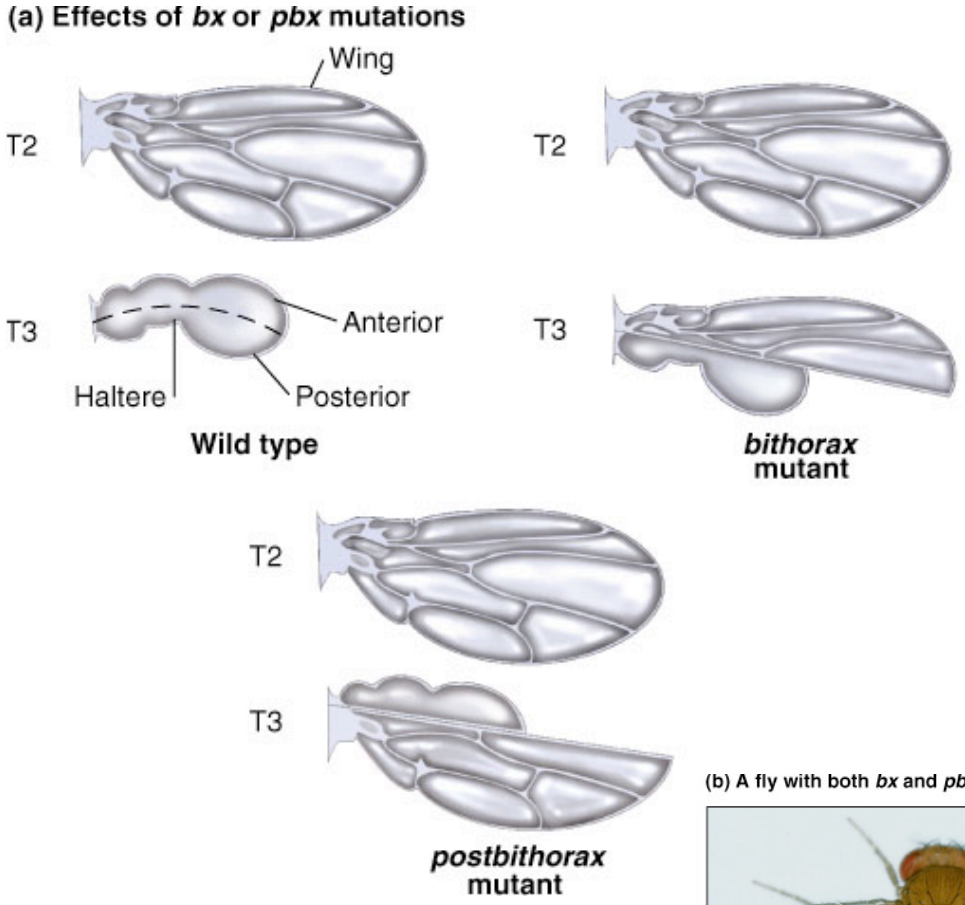
- Regulatory system complex
  - Transcription factors encoded by pair-rule genes initiate pattern by regulating segment polarity genes
  - Interactions between cell polarity genes maintain periodicity later in development

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

Fig. D.26

Antennapedia Complex and Bithorax Complex

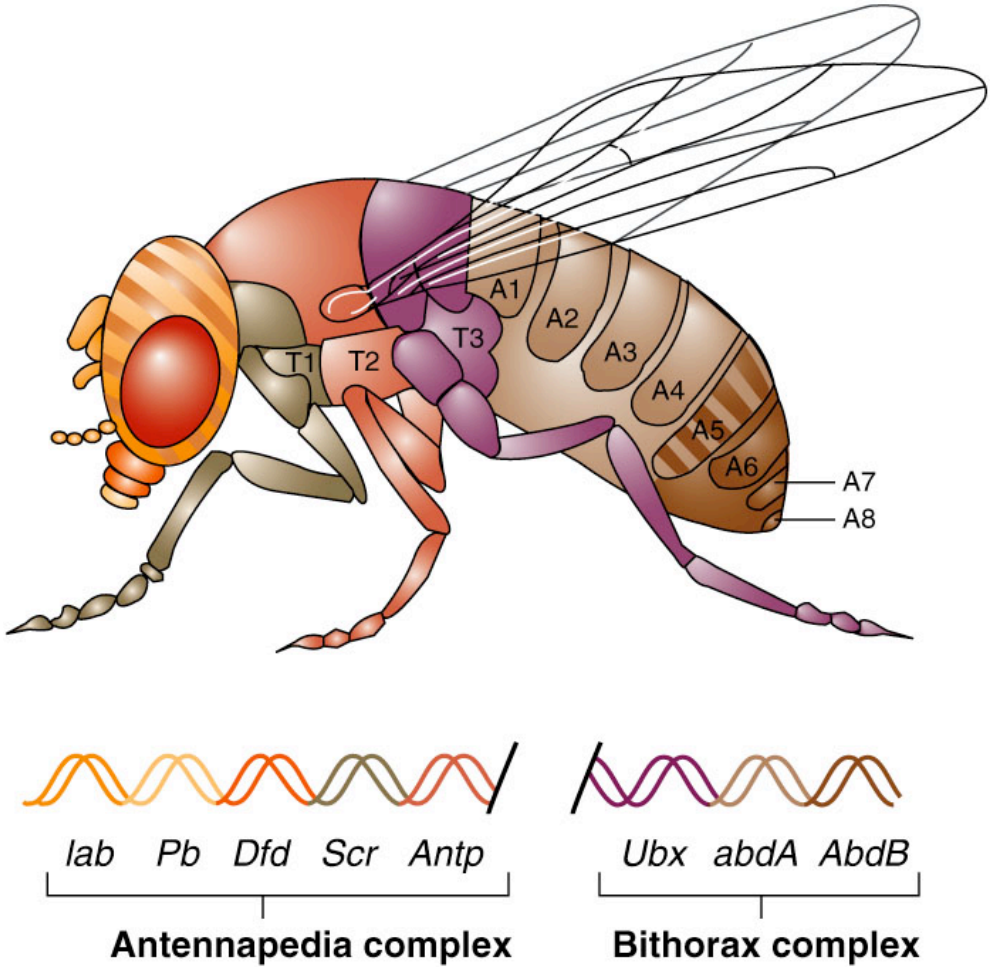


Fig. D.27

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

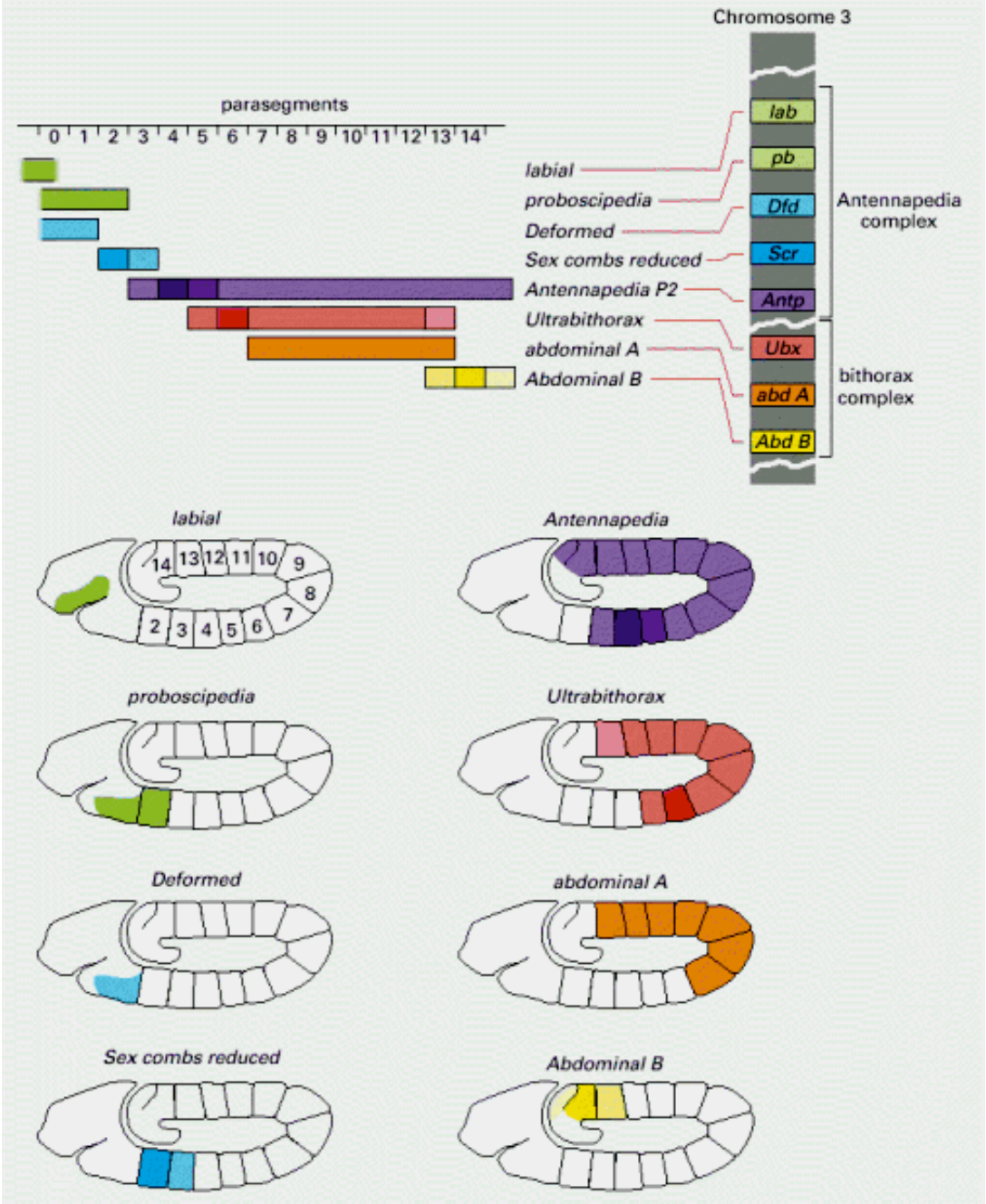


Figure 21-43. The patterns of expression compared to the chromosomal locations of the genes of the Hox complex. The sequence of genes in each of the two subdivisions of the chromosomal complex corresponds to the spatial sequence in which the genes are expressed. Note that most of the genes are expressed at a high level throughout one parasegment (dark color) and at a lower level in some adjacent parasegments (medium color where the presence of the transcripts is necessary for a normal phenotype, light color where it is not). In regions where the expression domains overlap, it is usually the most "posterior" of the locally active genes that determines the local phenotype.

(From Bruce Albert Book)