# Lecture 20 continued: Drosophila embryogenesis

Embrygenesis

Four classes of genes:

- Maternal genes
- Gap genes
- Pair-rule genes
- Segment polarity genes

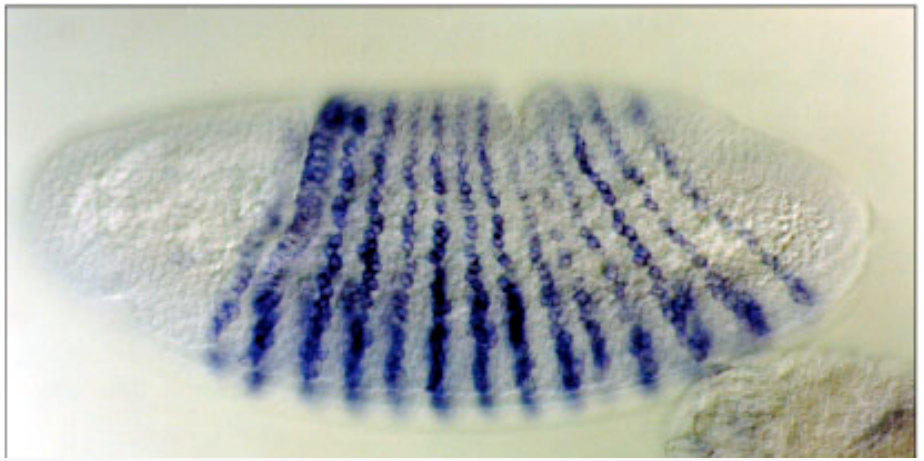
Homeotic genes

Read 826-837Fig. D18-D27; 19.2; 19.16

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

# Segment polarity genes

(a) Distribution of Engrailed protein



(b) Segment polarity genes establish compartment borders.

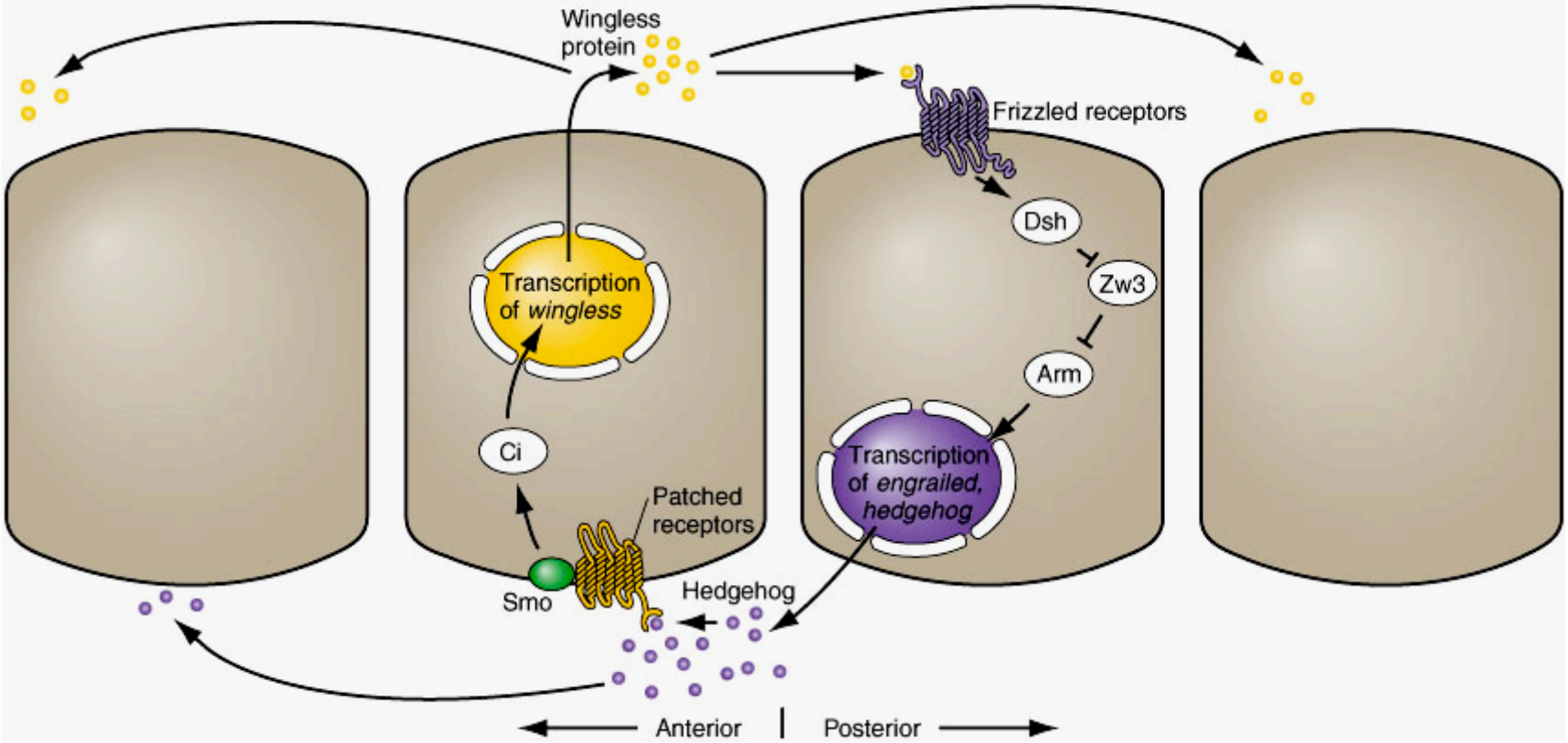



Fig. D.24

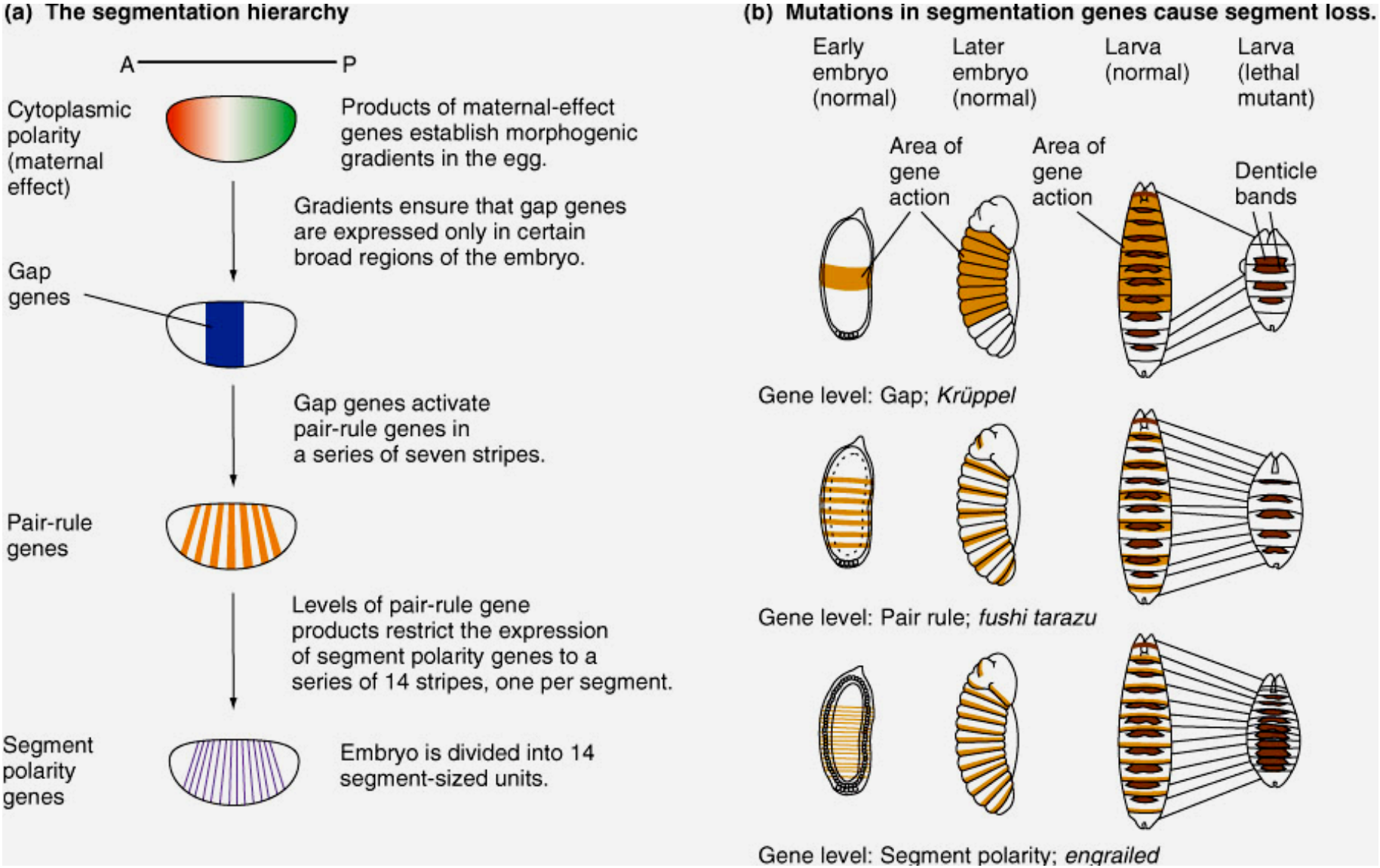

(a) The segmentation hierarchy
A
P
Cytoplasmic polarity (maternal effect)
Products of maternal-effect genes establish morphogenic gradients in the egg.
Gradients ensure that gap genes are expressed only in certain broad regions of the embryo.
Gap genes
Gap genes activate pair-rule genes in a series of 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

Fig. D.25

# 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

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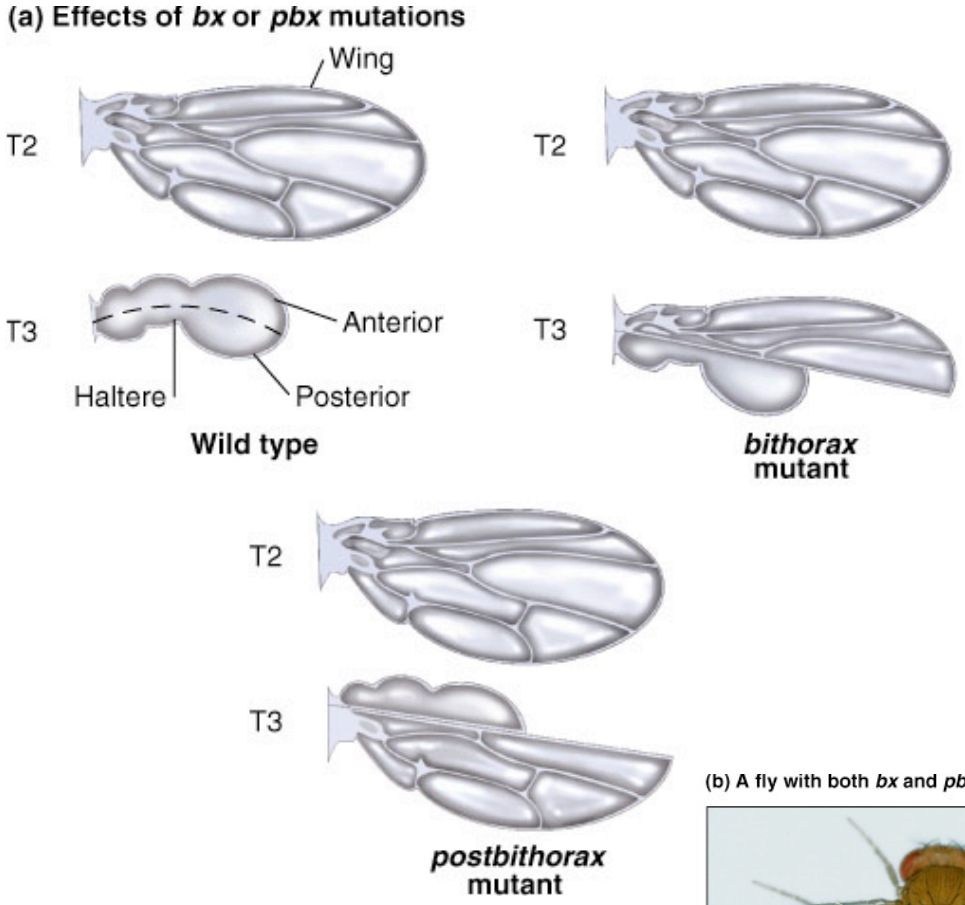
# Each segment establishes own identity through activation of homeotic genes




- 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

(b) A fly with both *bx* and *pbx* mutations

Fig. D.26

# Antennapedia Complex and Bithorax Complex

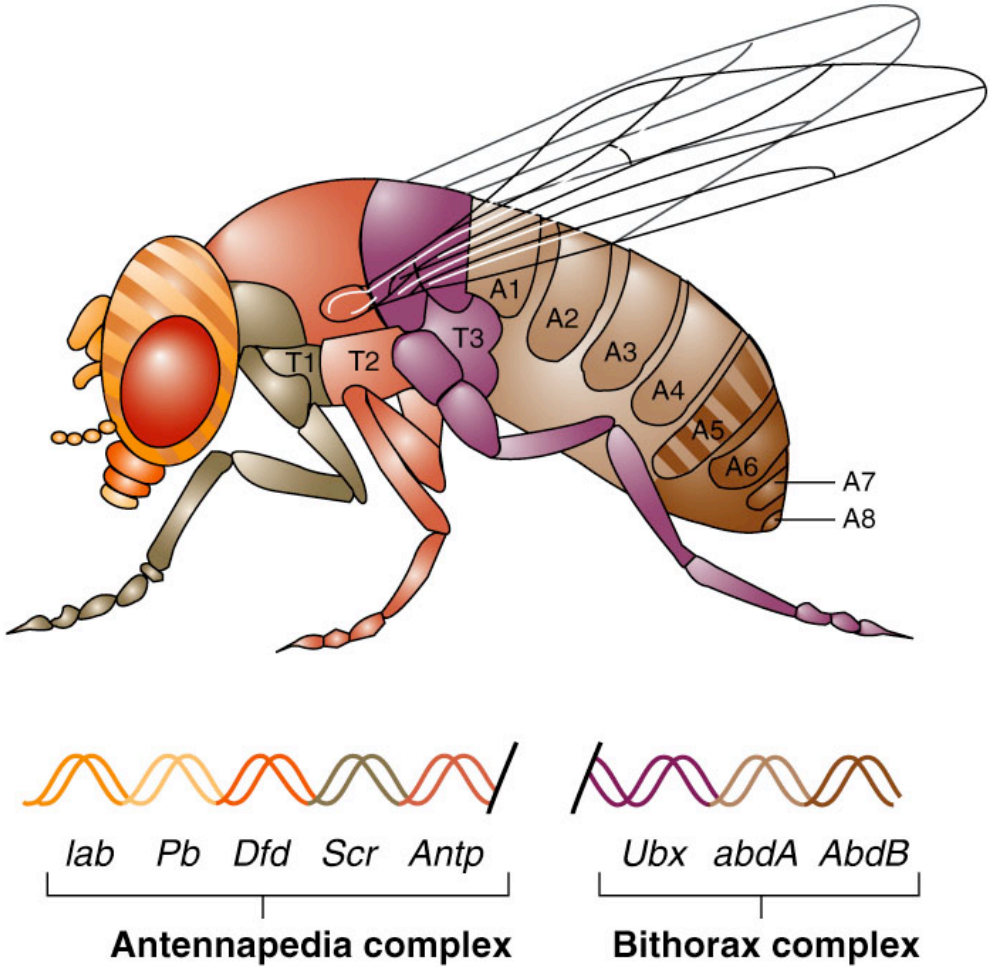



Fig. D.27

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

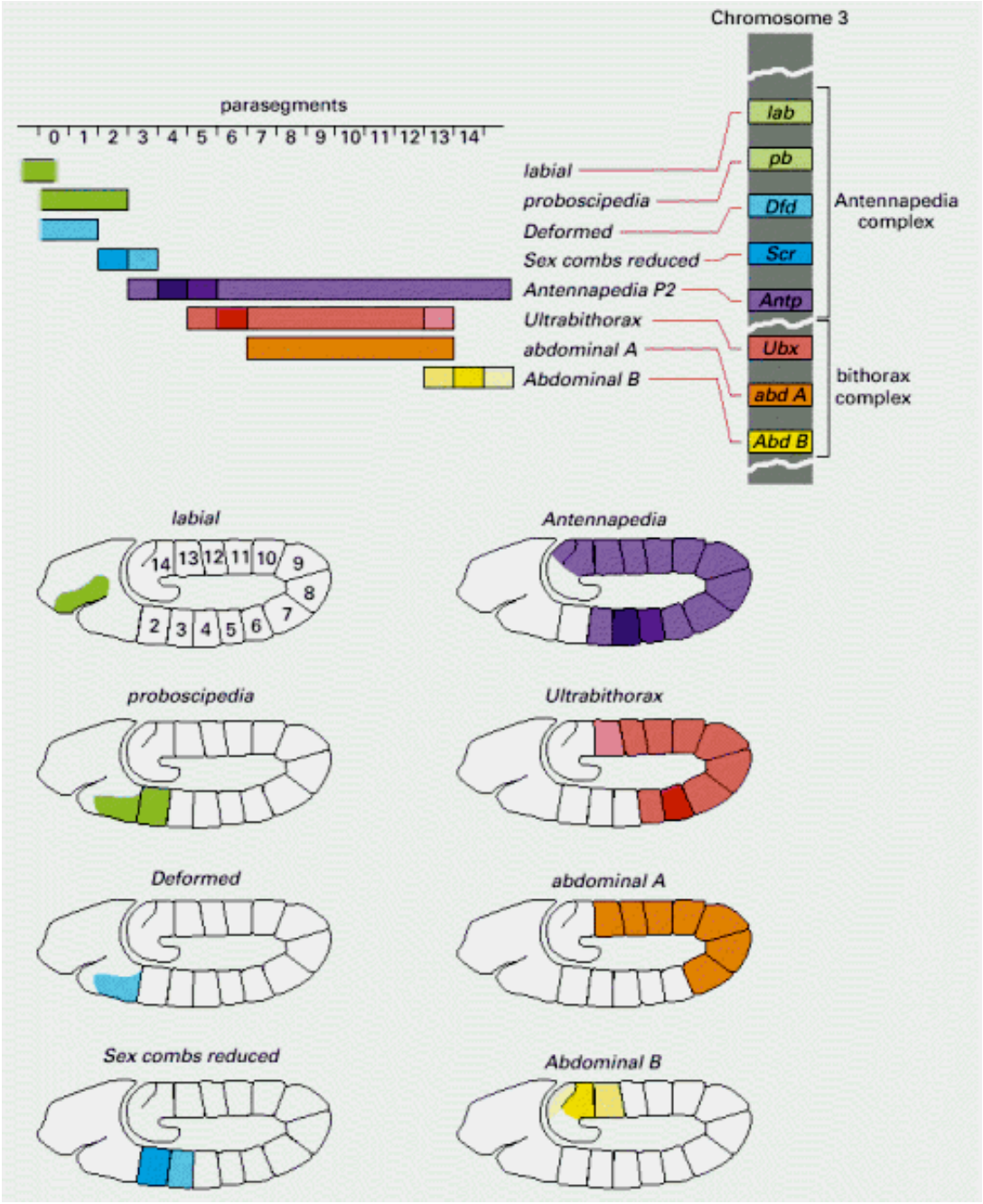
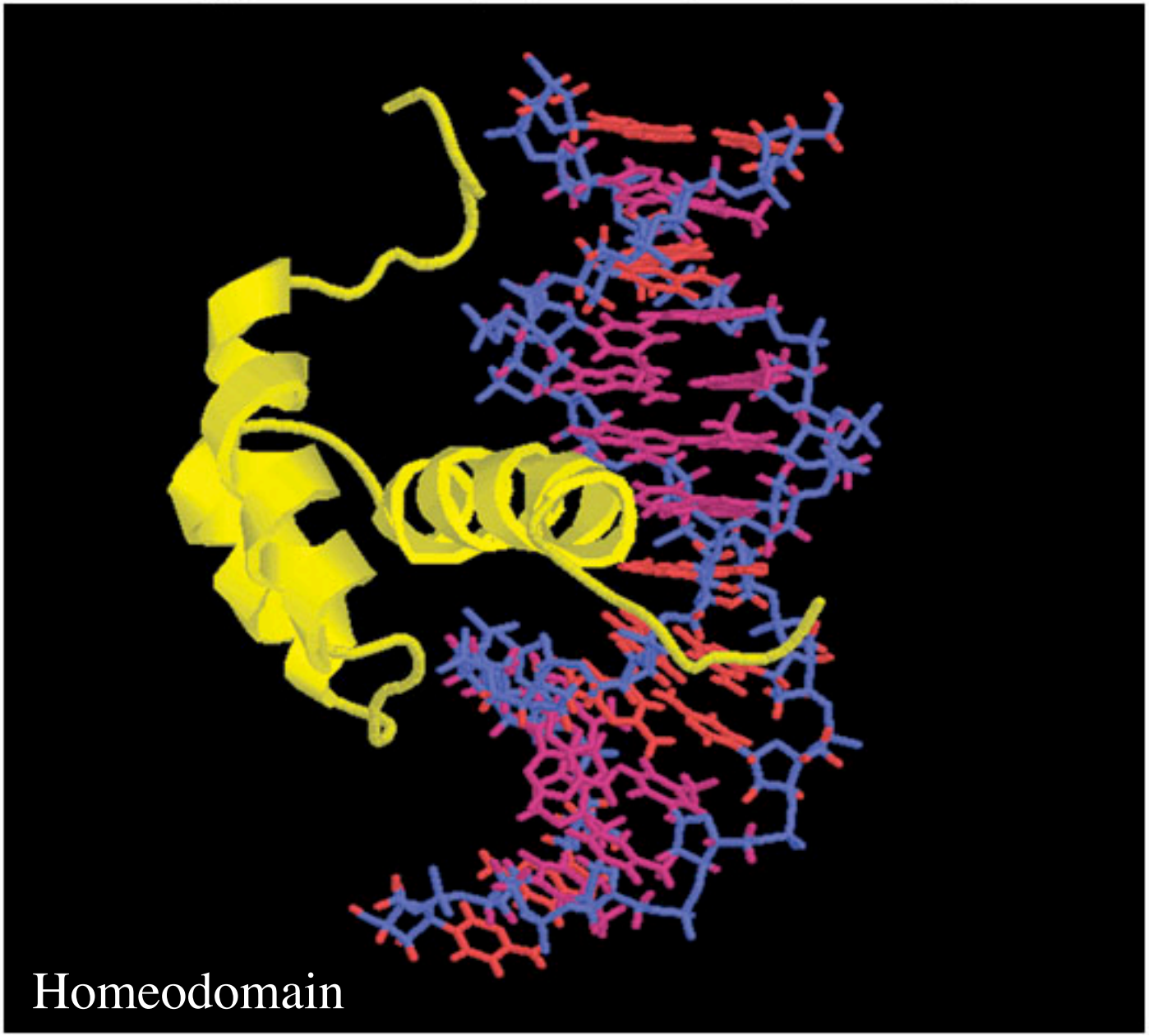



Figure 21-43. The patterns of expression compared to the chromosomal locations of the genes of the Hox complex. The sequence of genes in each of the two subdivisions of the chromosomal complex corresponds to the spatial sequence in which the genes are expressed. Note that most of the genes are expressed 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)**

Fig. 19.16





# Lecture 21 Mouse (Mus musculus)
# A Model for studying human diseases

Read 845-862

Fig. E3, 5, 6, 8, 9, 10, 11, 14, 15, 16, 17

Table E.1

**TABLE E.1 Comparison of Mice and Humans**

| Trait | Mice | Humans |
|---|---|---|
| Average weight | 30 g | 77,000 g (170 lb) |
| Average length | 10 cm (without tail) | 175 cm |
| Genome size | ~3,000,000,000 bp | ~3,000,000,000 bp |
| Haploid gene number | ~50,000 | ~50,000 |
| Number of chromosomes | 19 autosomes + X and Y | 22 autosomes + X and Y |
| Gestation period | 3 weeks | Average, 38 weeks (8.9 months) |
| Age at puberty | 5–6 weeks | Average, 624–728 weeks (12–14 years) |
| Estrus cycle | 4 days | Average, 28 days |
| Life span | 2 years | Average, 78 years |

# Synteny Between mouse and human genome



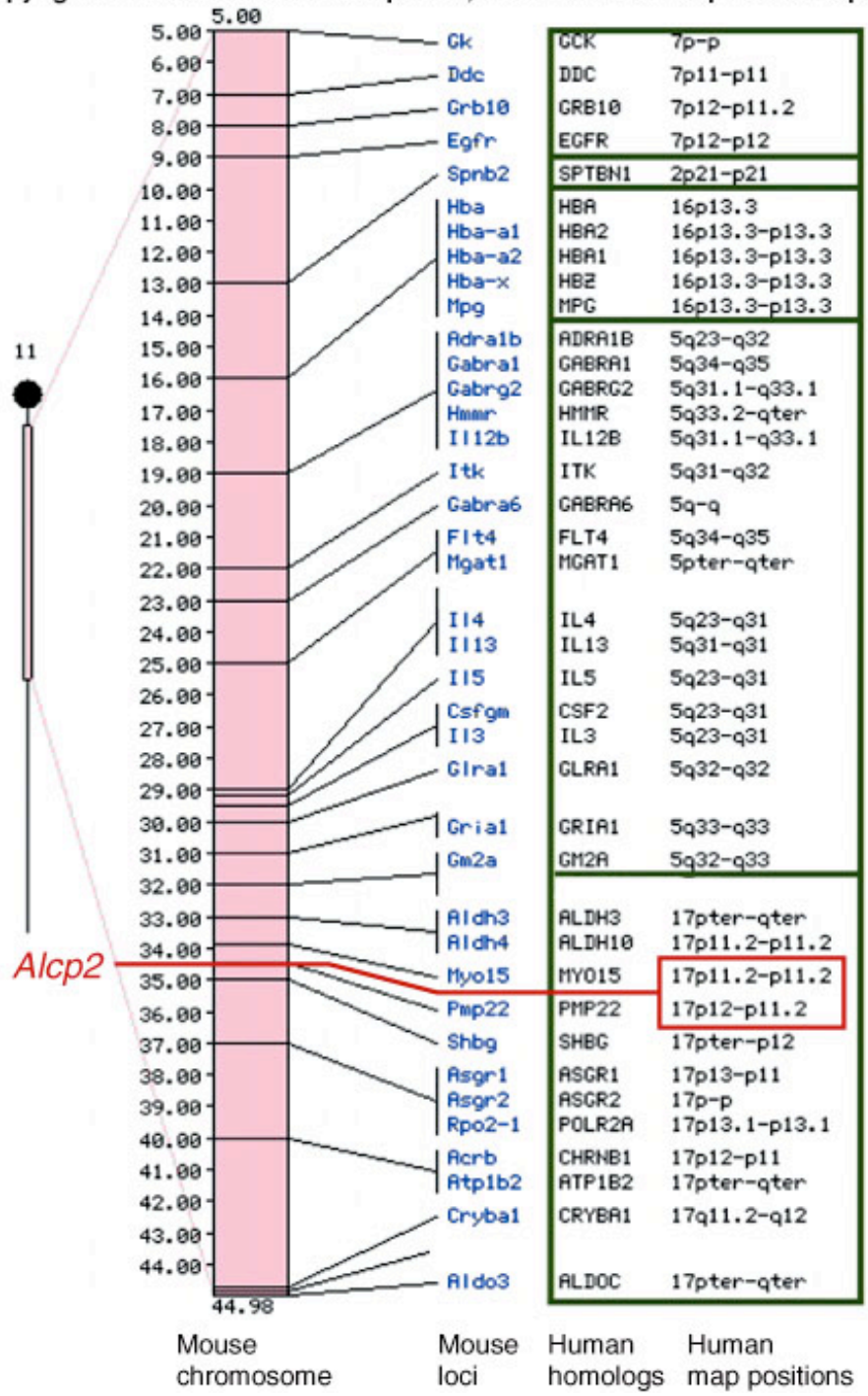
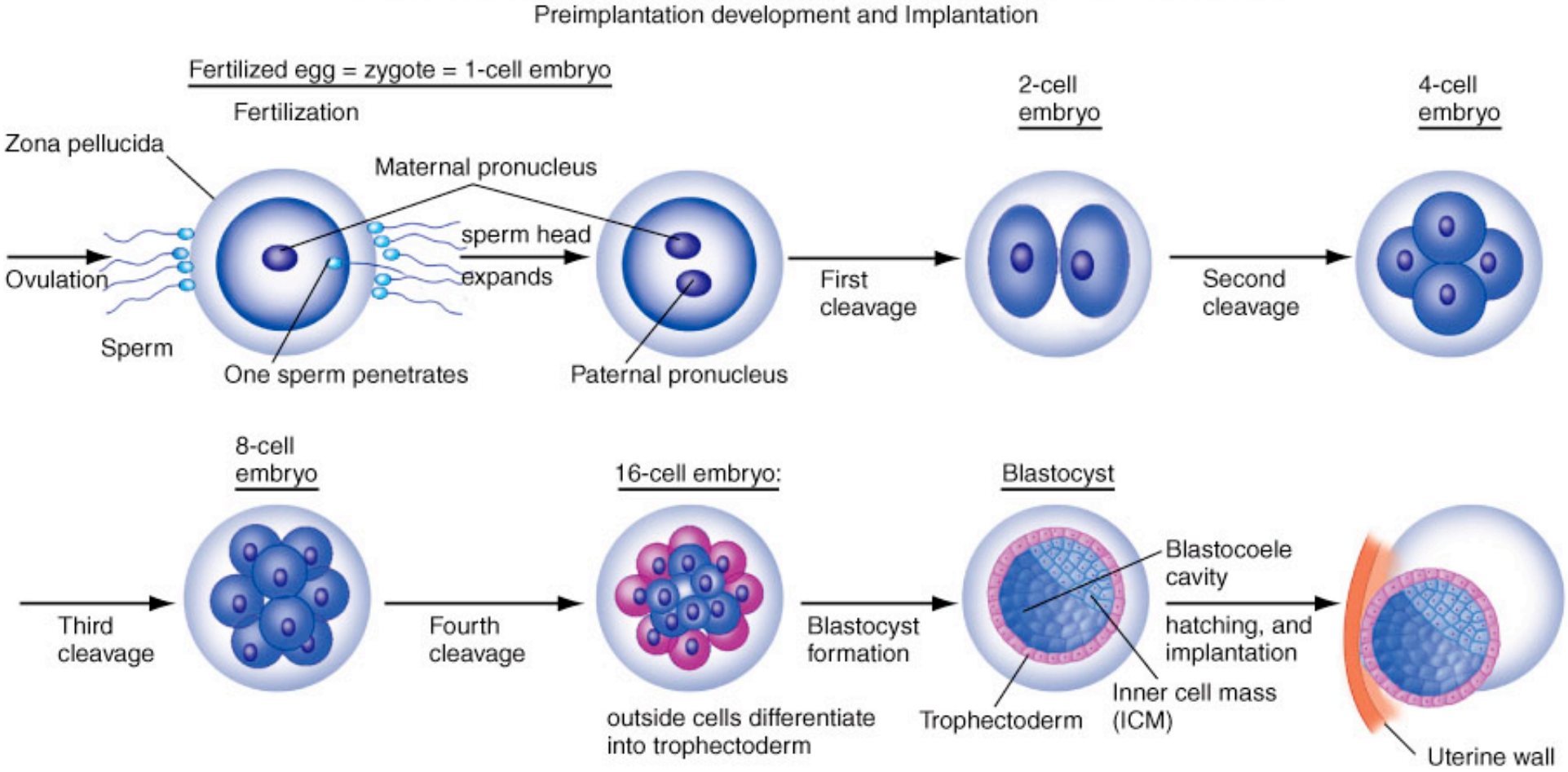



Fig. E.3

Fig. E.5

# Mouse embryogenesis





# Cleavage stage cells are totipotent

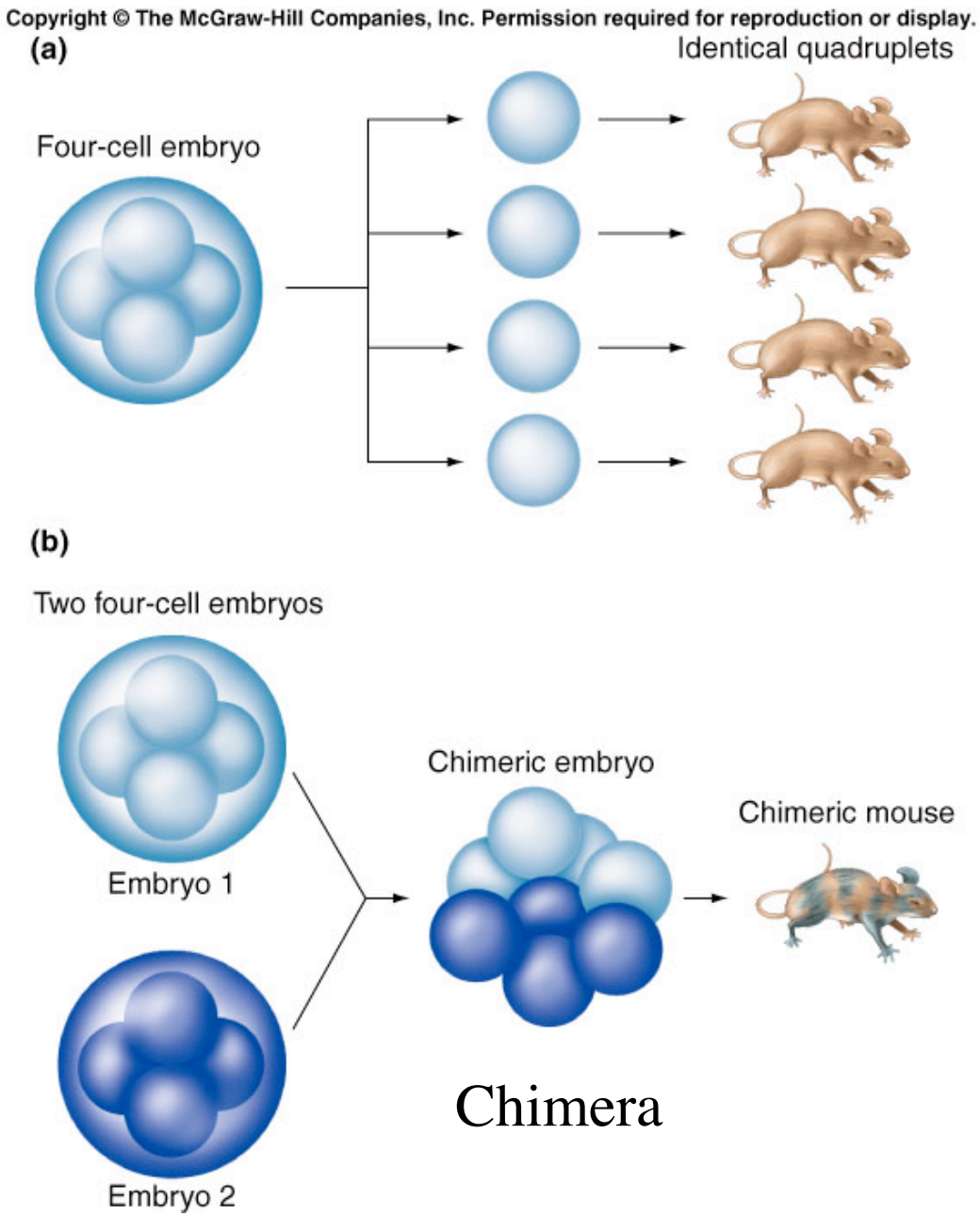
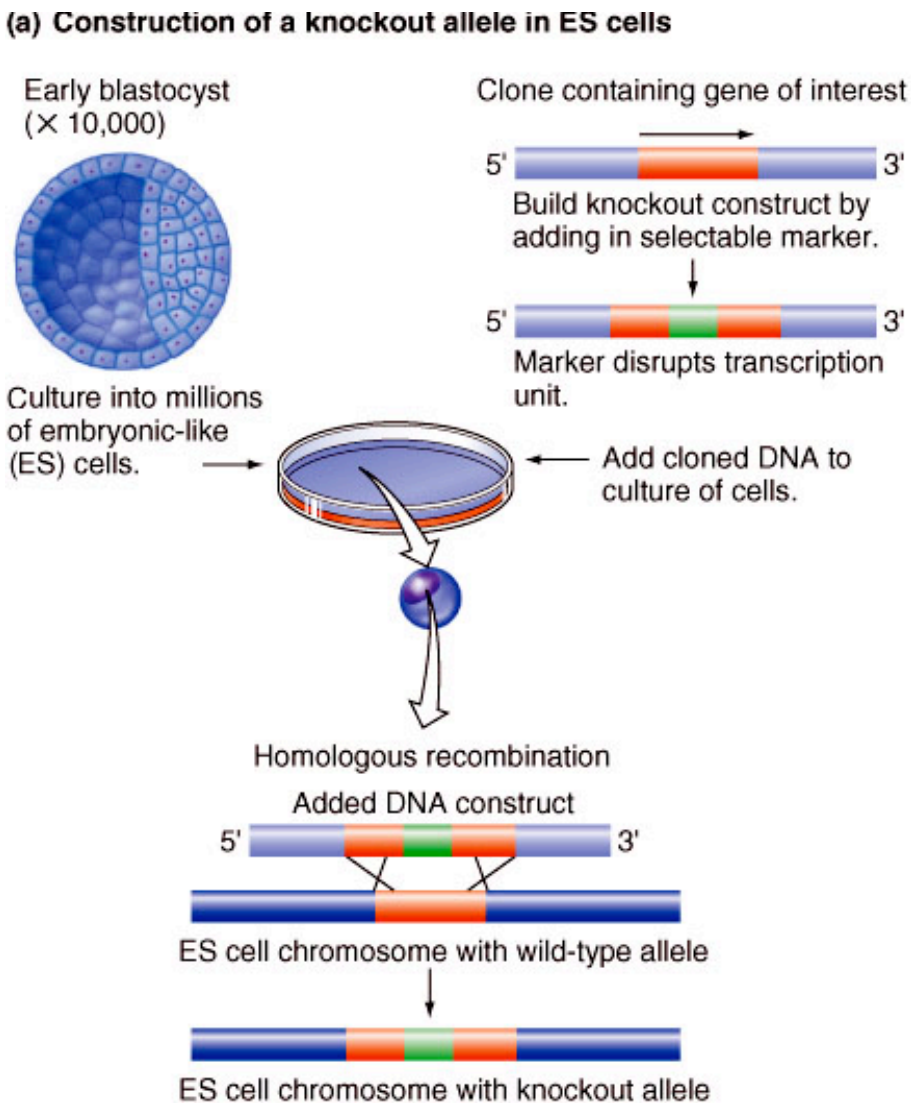
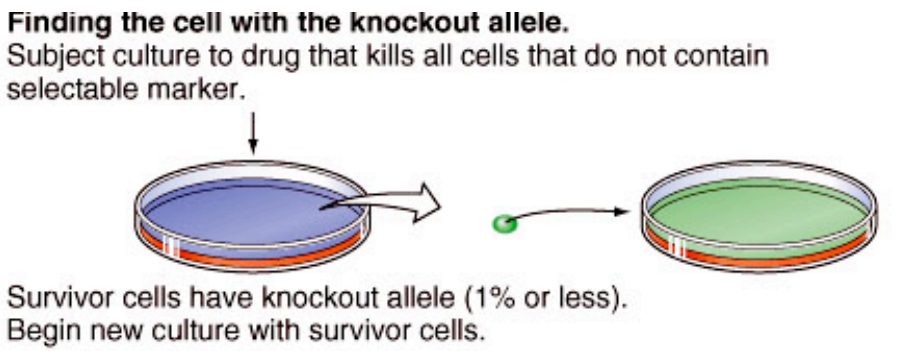



Fig. E.6

# Knocking out a gene in ES cells




(b)

# Using transgenic tools

## (1) verify gene cloning

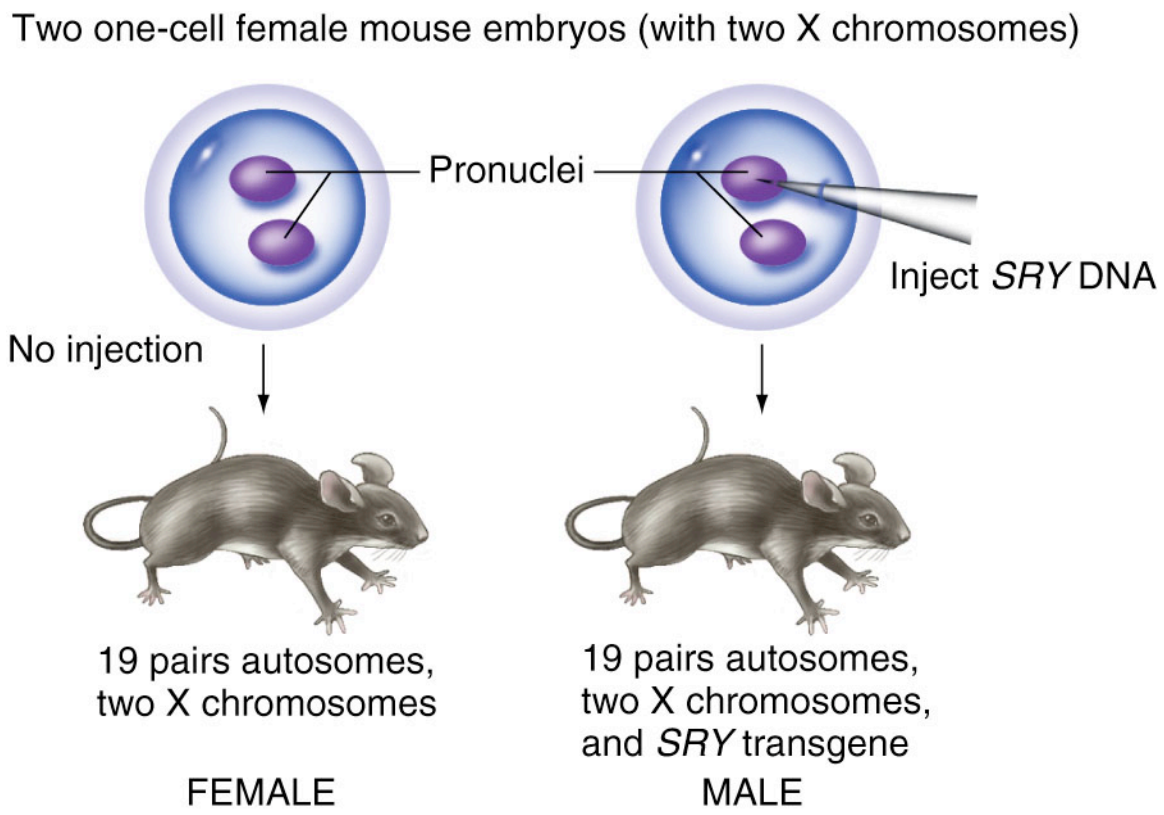
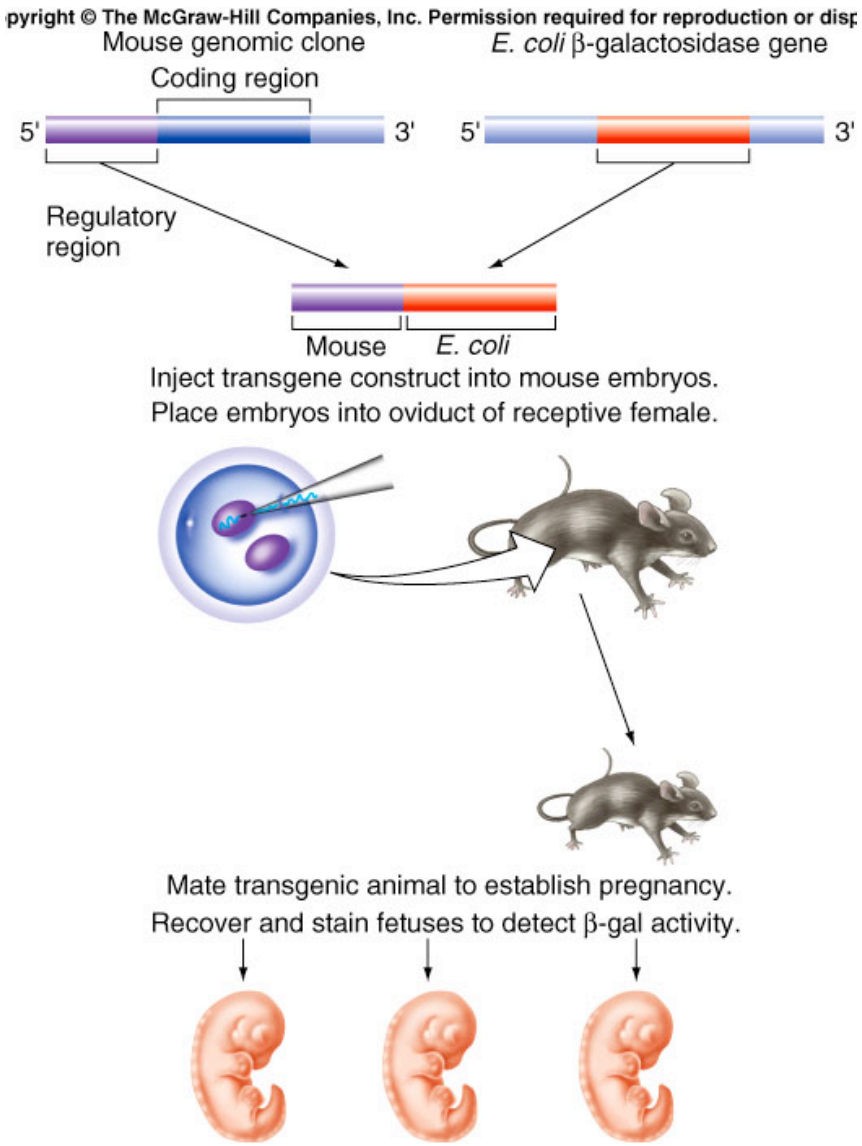



Fig. E.9

# Using transgenic technology
## (2) characterize regulatory regions

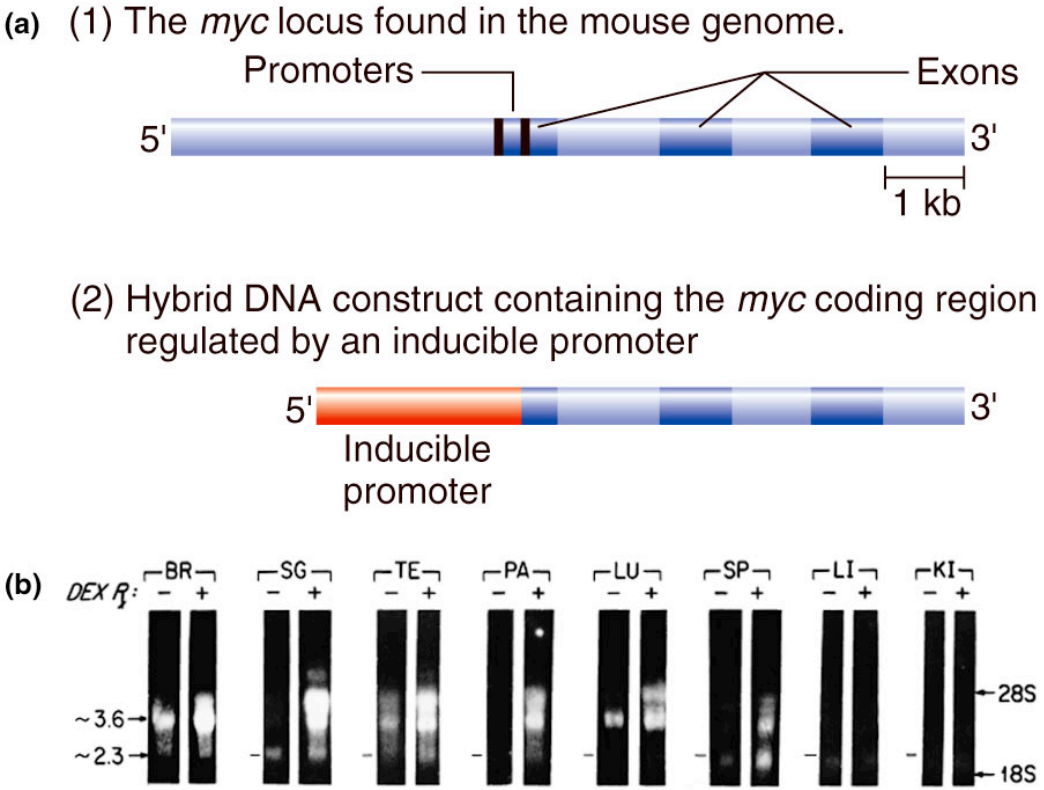



- DNA construct containing mouse regulatory region of interest is attached to *E. coli* reporter gene.
- Function ascertained by β-gal expression in transgene fetus

Fig. E..11

# Using transgenic technology

## (3) mis-express genes




- Transgenic expression of myc gene provides information on gene's role in tumor formation

Fig. E.10

Fig. E.14a-c

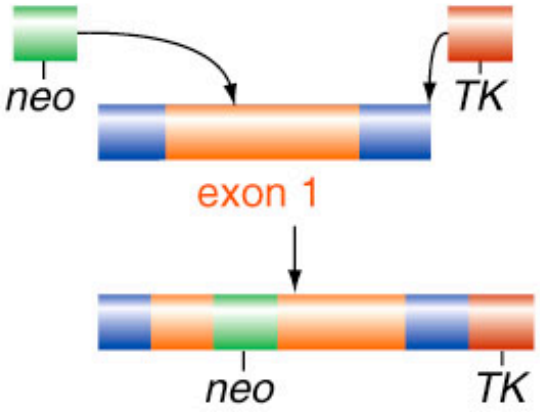
# Using transgenic technology

## (4) Gene knockouts to create mouse model for human diseases

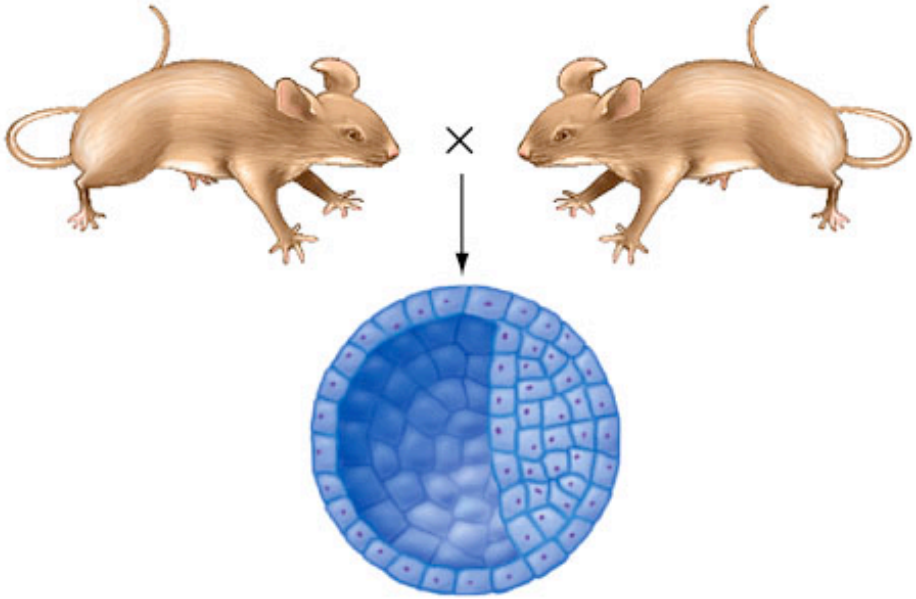


**(a)** Plasmid clone containing portion of mouse *CFTR* locus with first exon


**(b)** Develop DNA construct by adding selectable marker (*neo*) and *TK* gene to *CFTR* restriction fragments



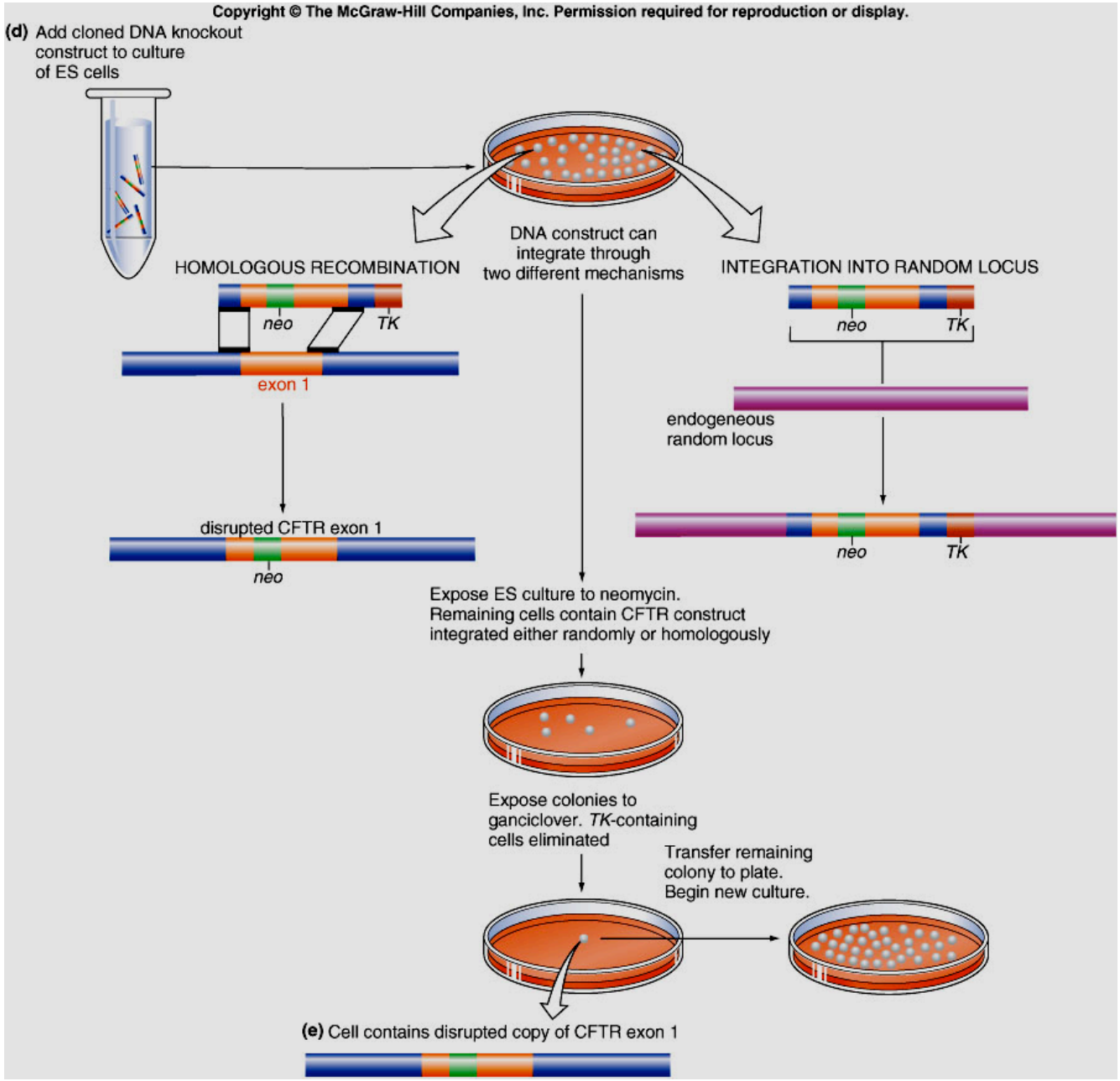

**(c)** Early blastocyst recovered from mating between two agouti parents of the 129/SvJ strain



Develop ES cell culture by placing blastocysts in petri dish to undergo many cell divisions without differentiation

ES culture

Fig. E.14d-e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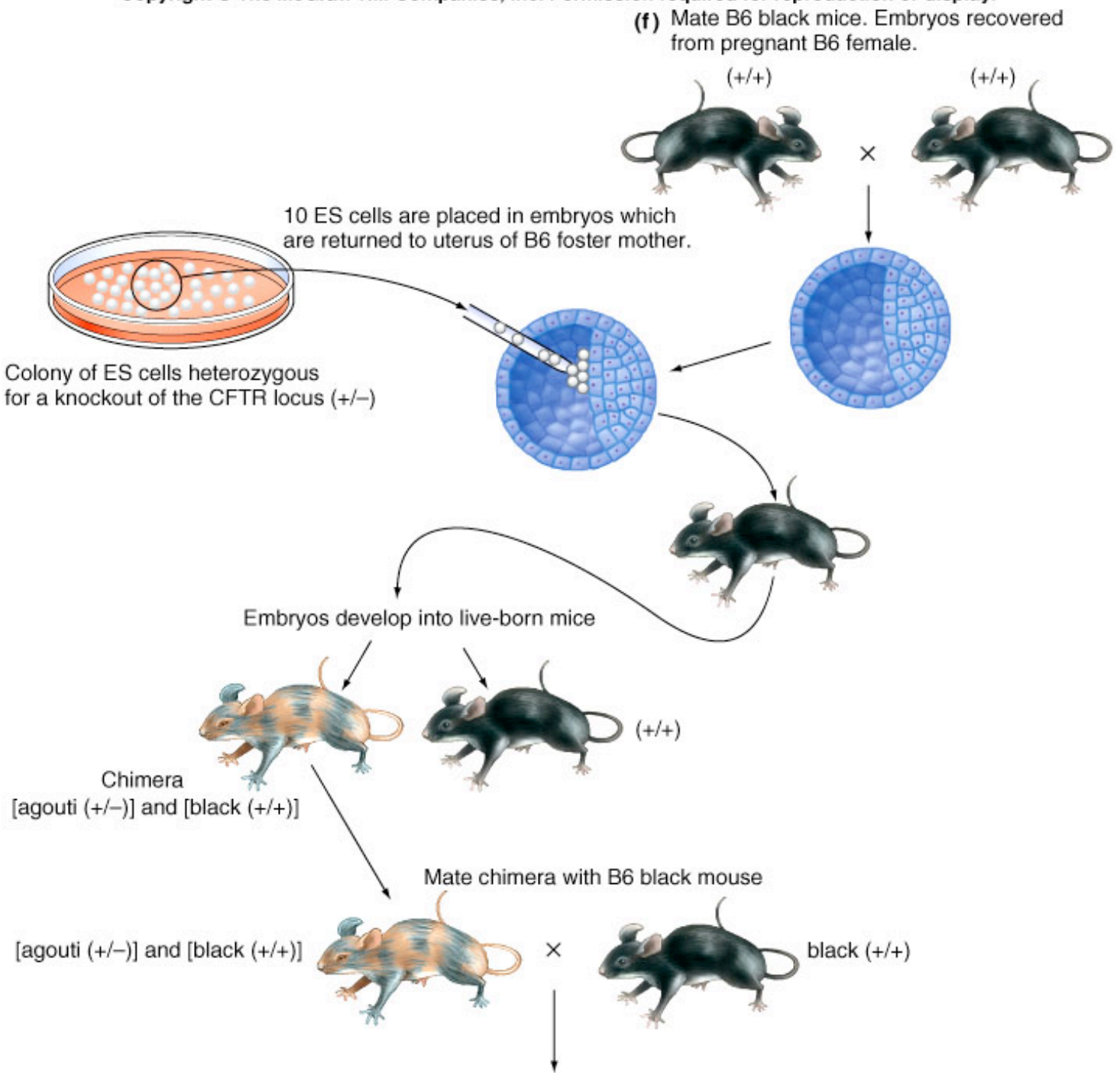
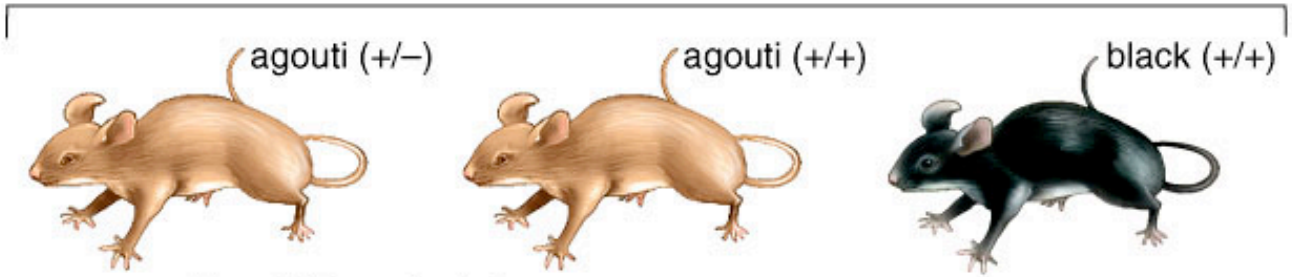
(d) Add cloned DNA knockout construct to culture of ES cells
DNA construct can integrate through two different mechanisms
HOMOLOGOUS RECOMBINATION
neo
TK
exon 1
disrupted CFTR exon 1
neo
INTEGRATION INTO RANDOM LOCUS
neo
TK
endogeneous random locus
neo
TK
Expose ES culture to neomycin. Remaining cells contain CFTR construct integrated either randomly or homologously
Expose colonies to ganciclover. TK-containing cells eliminated
Transfer remaining colony to plate. Begin new culture.
(e) Cell contains disrupted copy of CFTR exon 1

Fig. E.14f



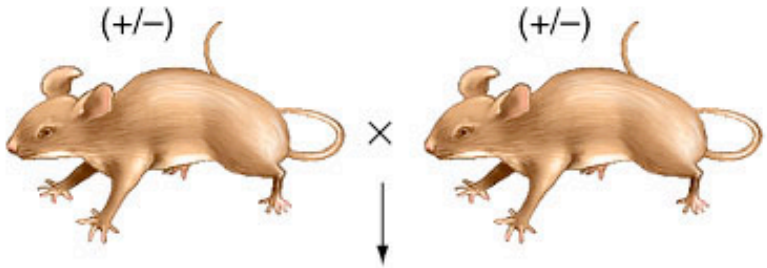
**(g)**

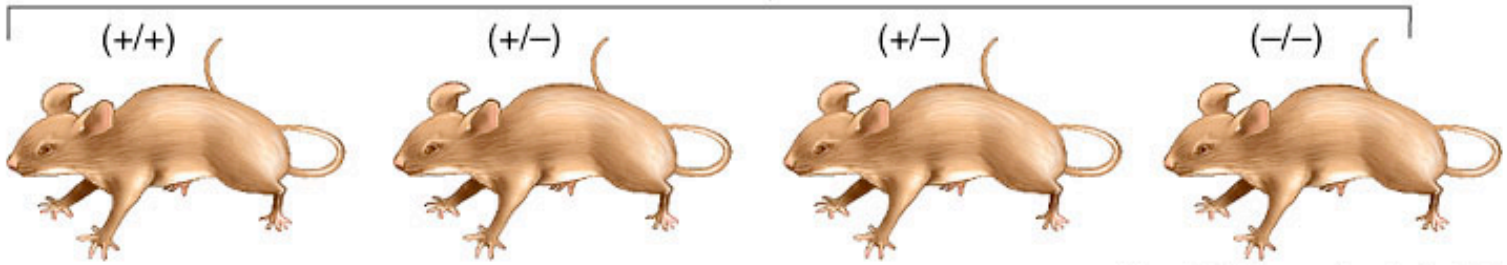
Three types of offspring




**(h)**

Use DNA analysis to identify male and female agouti animals that are heterozygous for the knockout allele of CFTR (+/–) and breed them together




Offspring homozygous for mutant allele serve as models for CF disease state.

Use DNA analysis to identify offspring homozygous for knockout allele to serve as models for cystic fibrosis disease state