Last time:

- Pre-genetics studies of development
- Advantages of genetic approach
- Goals of a developmental geneticist
    1. Which genes
    2. Where and when are they expressed?
    3. How are they controlled?
    4. What do they do?
- Drosophila:
    - Hox genes
    - Segmentation genes

Today:

1. Genetic screens and genetic analysis of development (Drosophila techniques)
2. Regulatory networks in development & cis-regulatory control of expression
3. Post-transcriptional regulation, pleiotropy & disease in development

The goal of a genetic screen: Identify genes specifically involved in a certain biological process.

Developmental genetic screens:
- Loss of function mutations are helpful because they often have a mutant phenotype in the cells where the WT gene is functioning. These are usually recessive.
- Gain of function mutations often hard to interpret but can be distinguished from loss of function mutations because they are usually dominant.

Components of a screen:

1. Saturation mutagenesis
2. Isolate mutants and make stable lines
3. Perform complementation analysis on different lines with similar phenotypes
4. Further characterize

Q: After you mutagenize an individual, how do you isolate the specific kind of mutants you want and make stable lines of them?

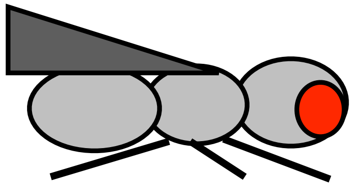
Ans: By being a clever geneticist or doing a lot of work and often both.

Each organism has its own set of visible and selectable markers as well as a host of other genetic tools to help one design a good screen.

Balancer chromosomes in Drosophila

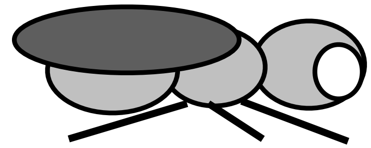
- been made for all chromosomes
- lots of large overlapping inversions prevent them from producing viable offspring if crossing over with a homologous chromosome occurs
- dominant visible marker (e.g. *Cy* curly wings)
- recessive lethal (e.g. *Cy*)

P



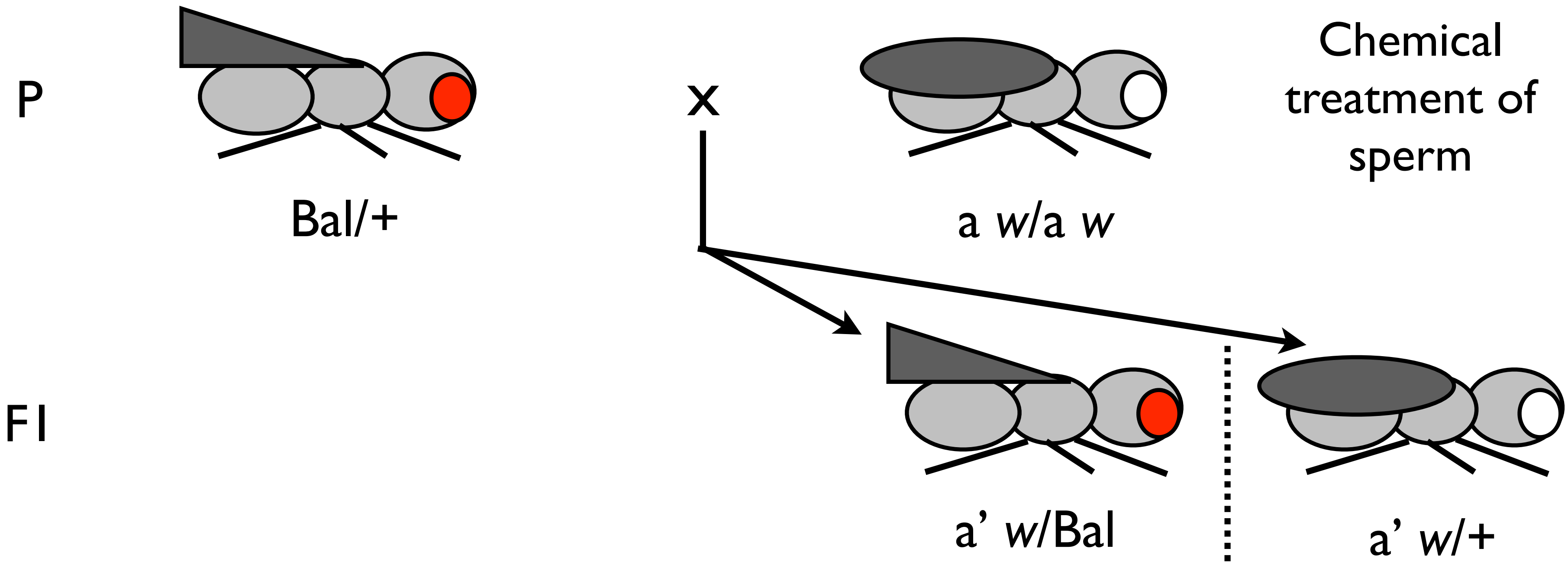
Bal/+

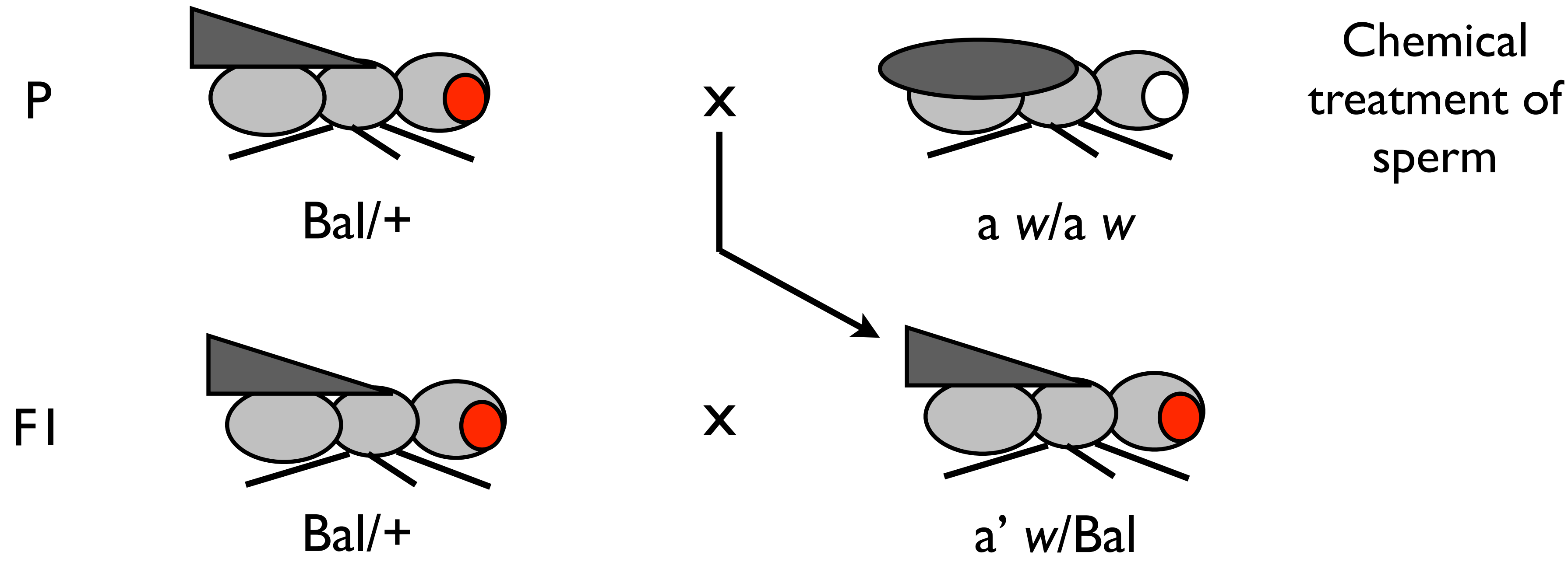
x



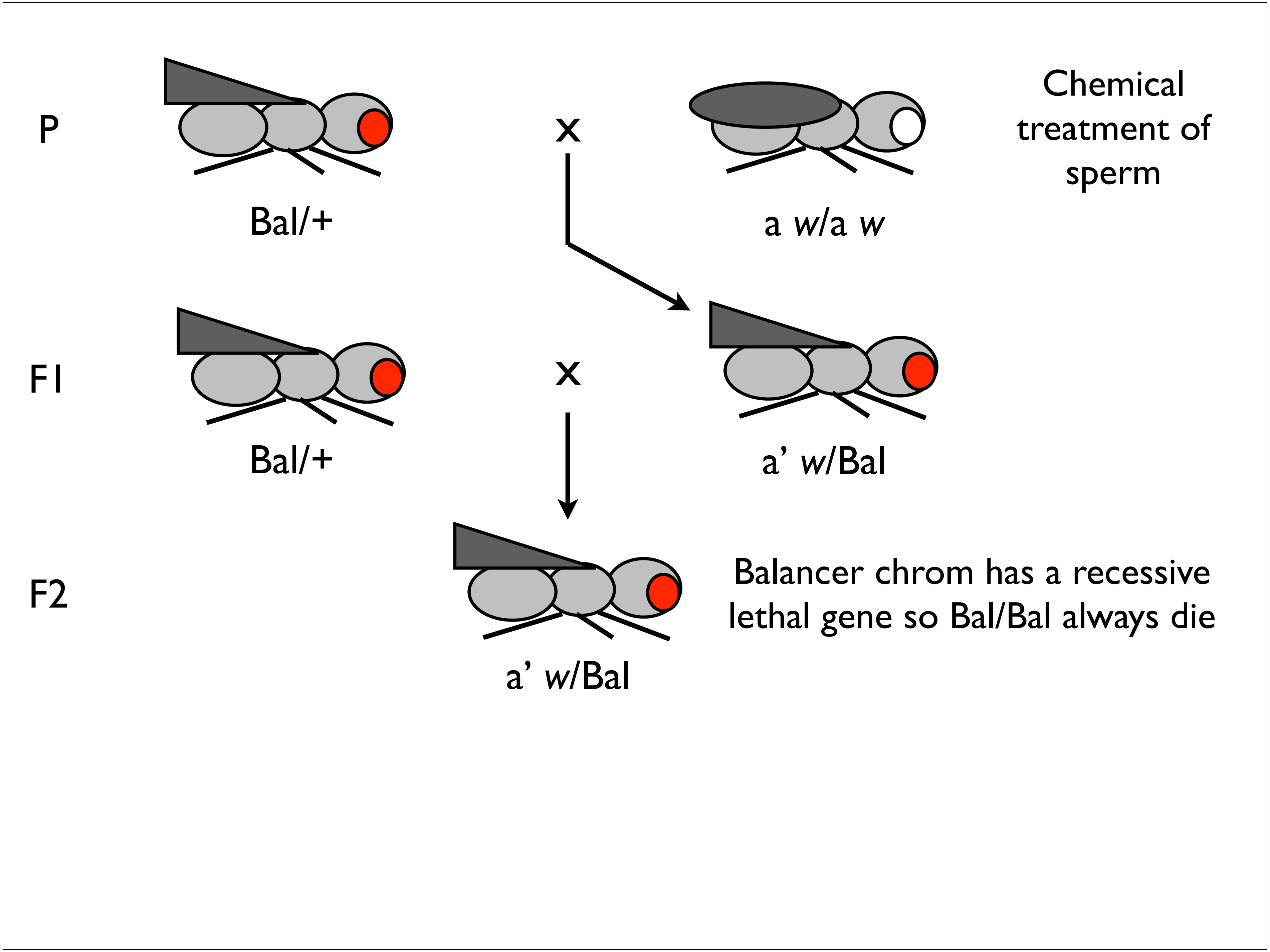
a *w*/a *w*

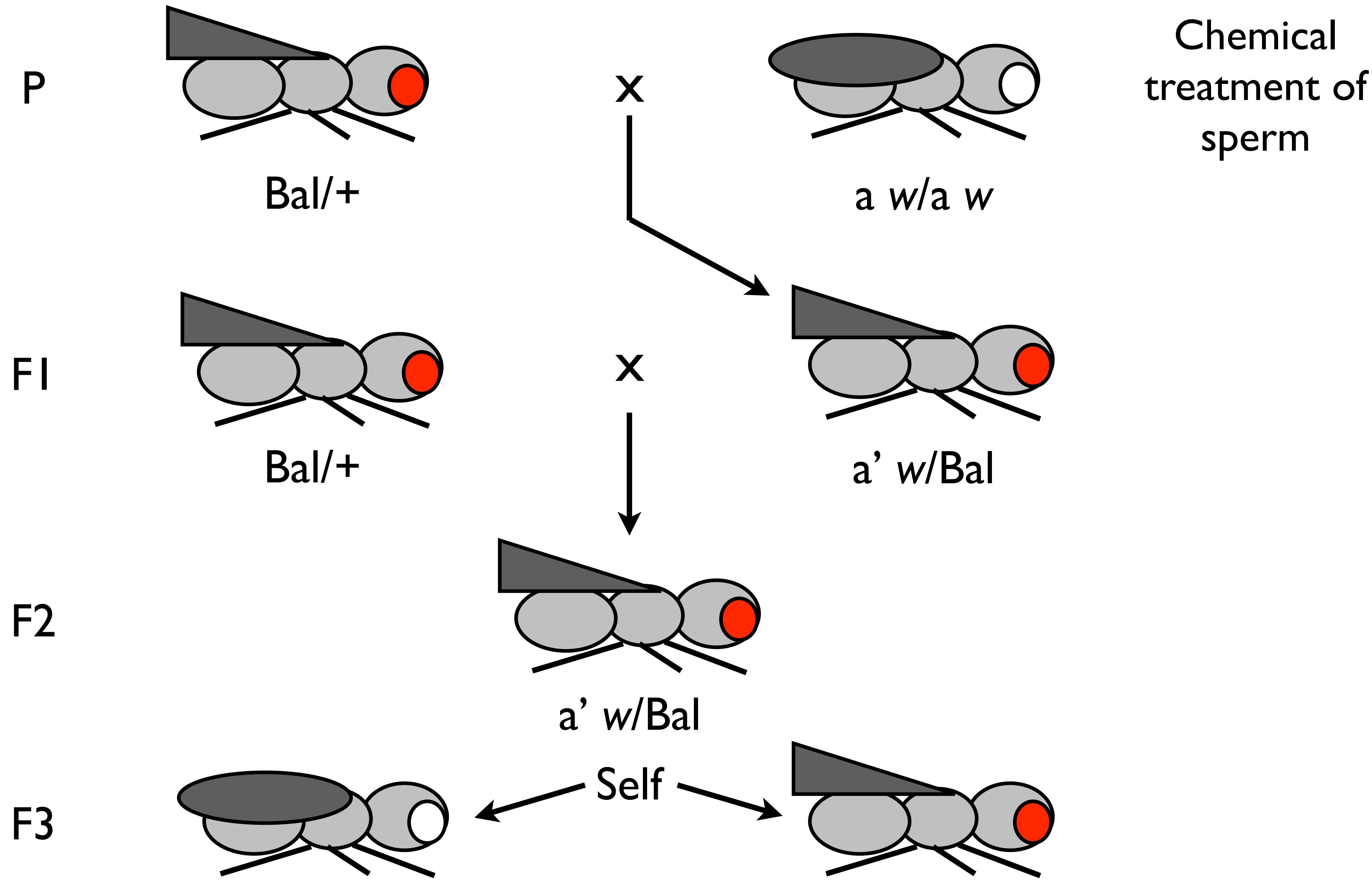
Chemical treatment of sperm

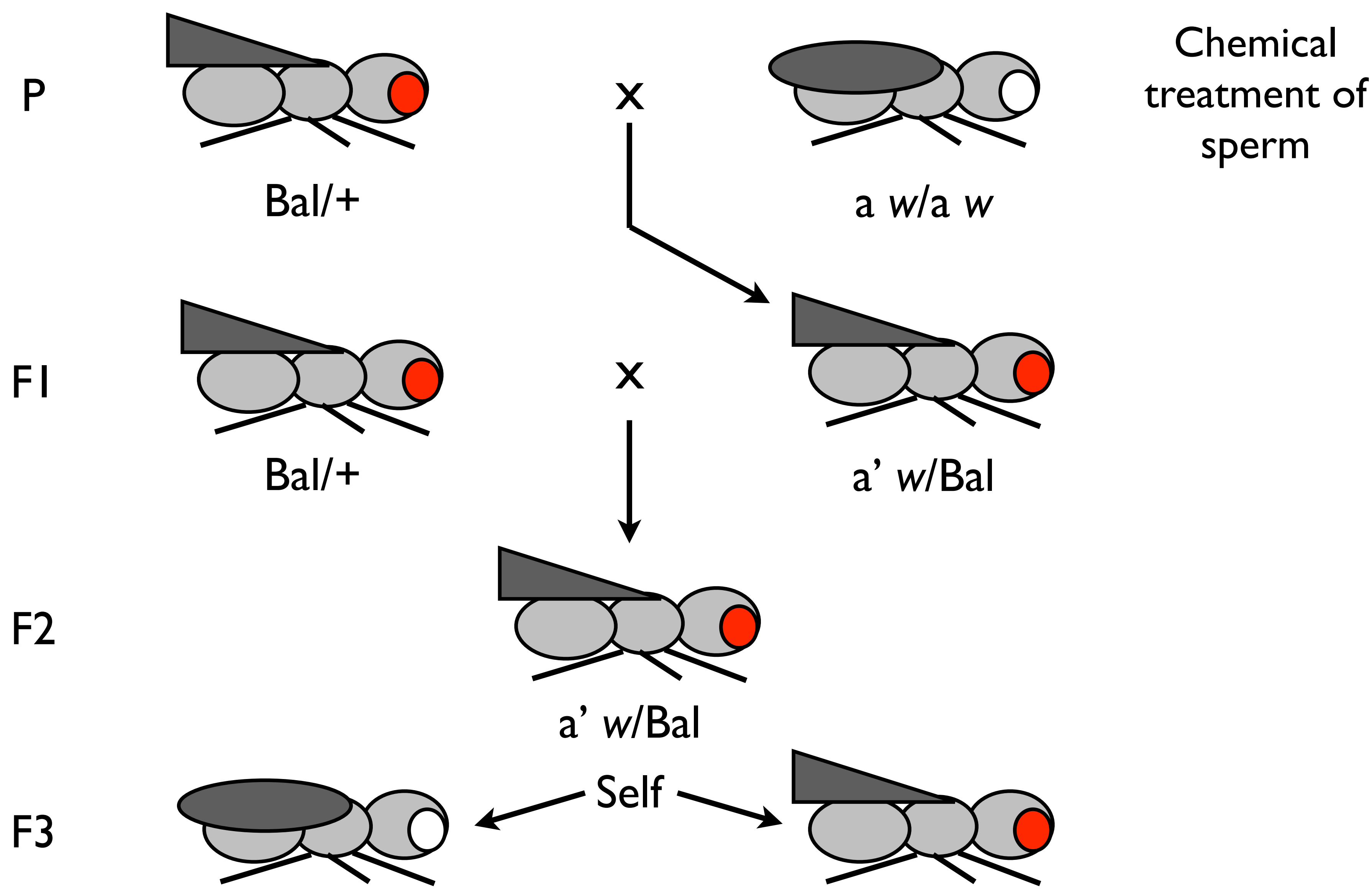

P
Bal/+
x
a *w*/a *w*
Chemical treatment of sperm
F1
a’ *w*/Bal
a’ *w*/+



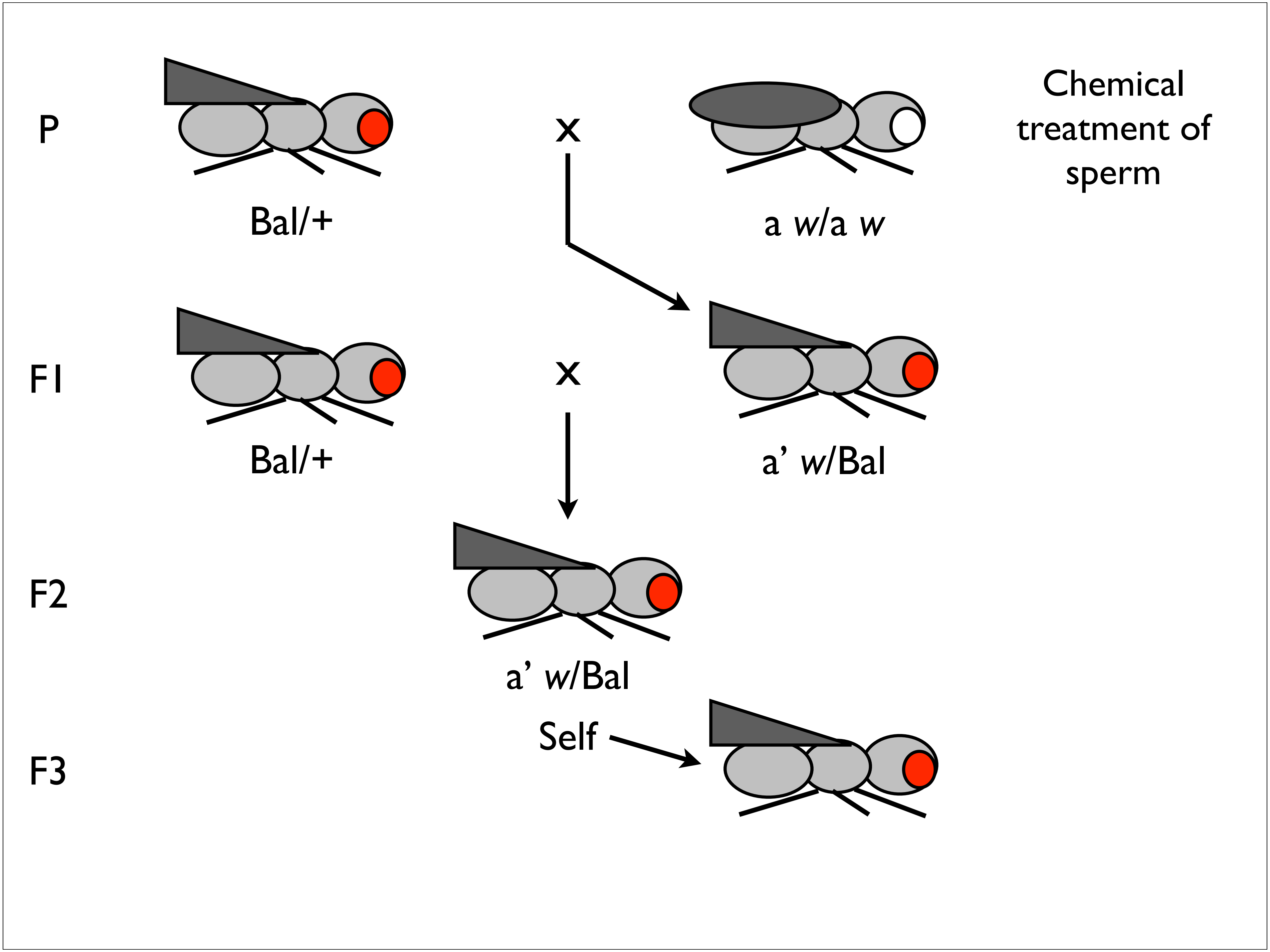
P
Bal/+
x
a *w*/a *w*
Chemical treatment of sperm
F1
Bal/+
x
a' *w*/Bal

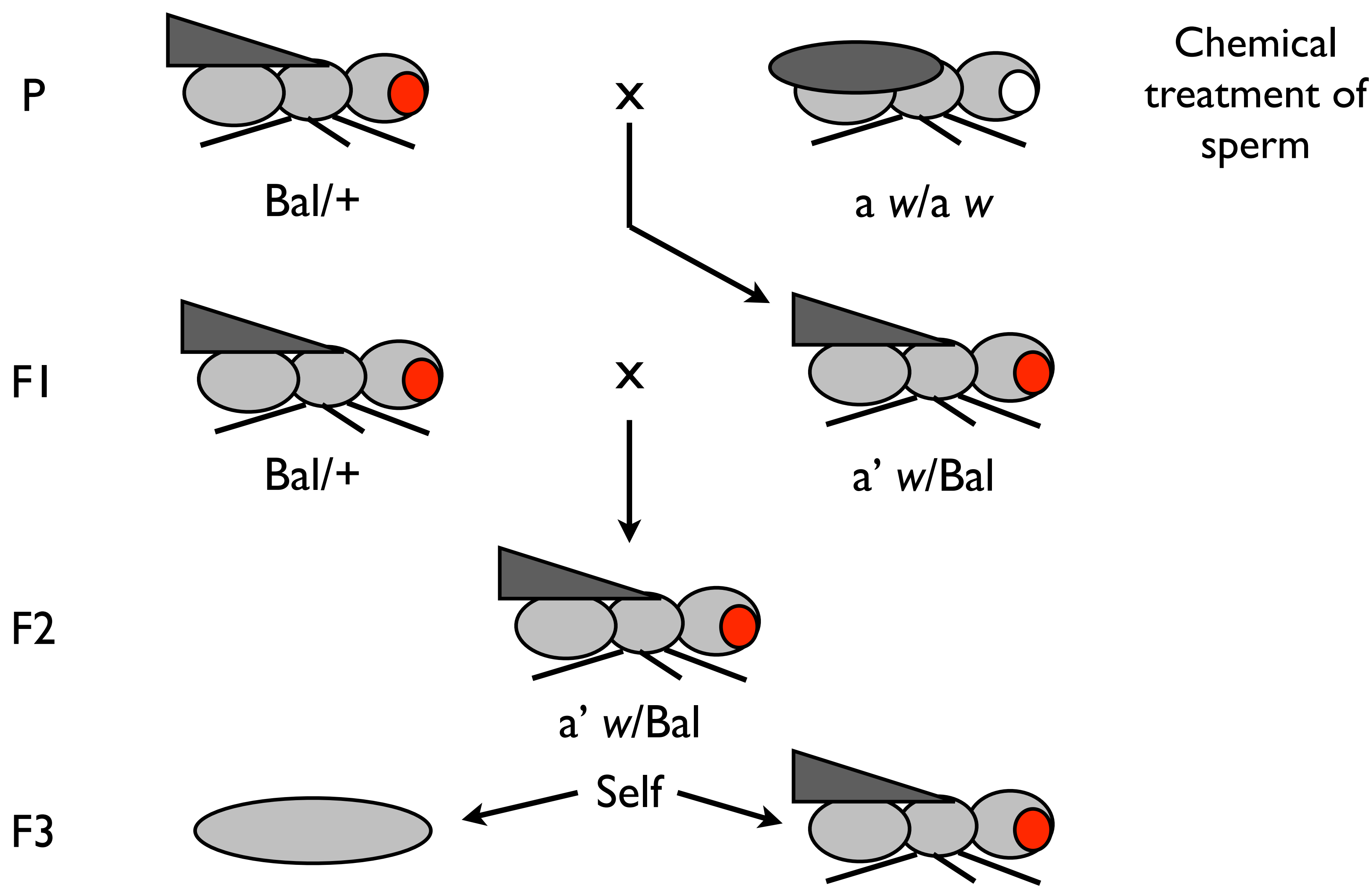

P
Bal/+
x
a w/a w
Chemical treatment of sperm
F1
Bal/+
x
a' w/Bal
F2
a' w/Bal
Balancer chrom has a recessive lethal gene so Bal/Bal always die


P
F1
F2
F3
x
x
Bal/+
a w/a w
Chemical treatment of sperm
Bal/+
a' w/Bal
a' w/Bal
Self

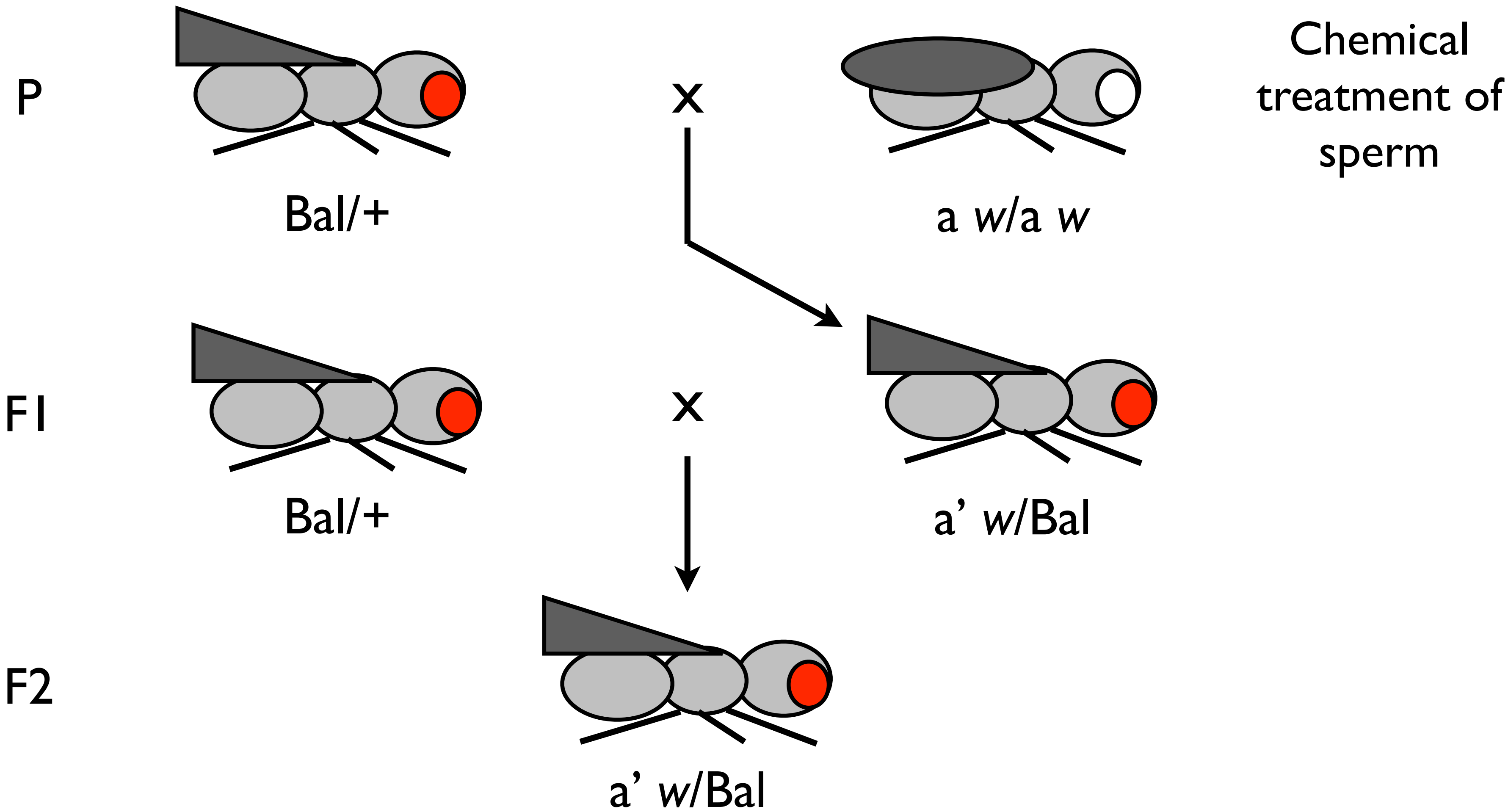


If you get any white eyed F3s it means no mutation or a' not recessive lethal


P
F1
F2
F3
Bal/+
x
a w/a w
Chemical treatment of sperm
Bal/+
x
a' w/Bal
a' w/Bal
Self



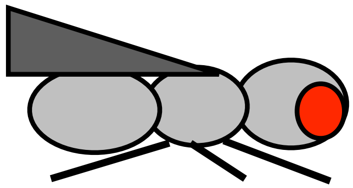
Only red eyed flies means you have a recessive lethal.
Next step is to examine embryos for phenotype.



Bonus is that a’ *w*/Bal can be maintained as a stable non-recombining stock that will immediately reveal contamination and is suitable for many useful crosses.

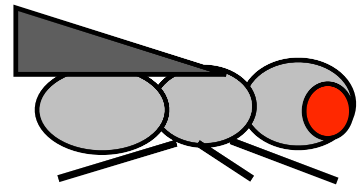
Complementation test with balancers

P



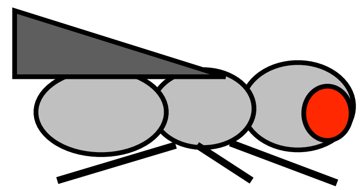
a’ *w*/Bal

x



b’ *w*/Bal

a’ w/b’ w?

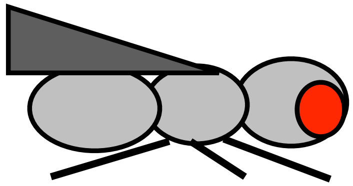
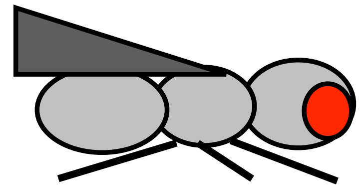


a’ *w*/Bal or b’ *w*/Bal

Dead

Bal/Bal

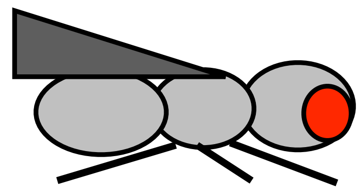
Complementation test with balancers

P  x 

a’ *w*/Bal b’ *w*/Bal

a’ w/b’ w?

If allelic would die

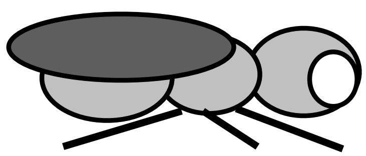


a’ *w*/Bal or b’ *w*/Bal

Dead

Bal/Bal

If complement:



Q: Why might a genetic screen fail to find some genes involved in a given biological process?

Q: Why might a genetic screen fail to find some genes involved in a given biological process?

1. Lethality before time of selection (Christine Nusslein-Volhard & Eric Wieschaus' screen was really cool because it was 'looking' for embryonic lethality)

2. Redundancy masking mutant phenotype

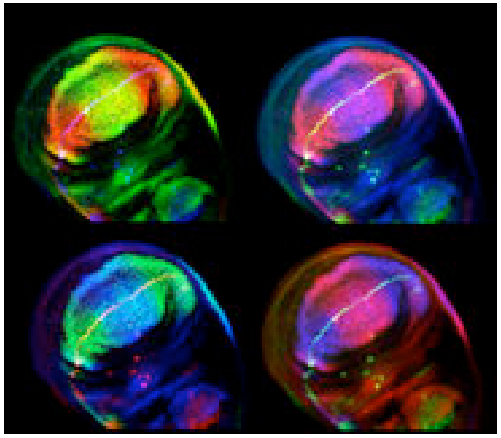
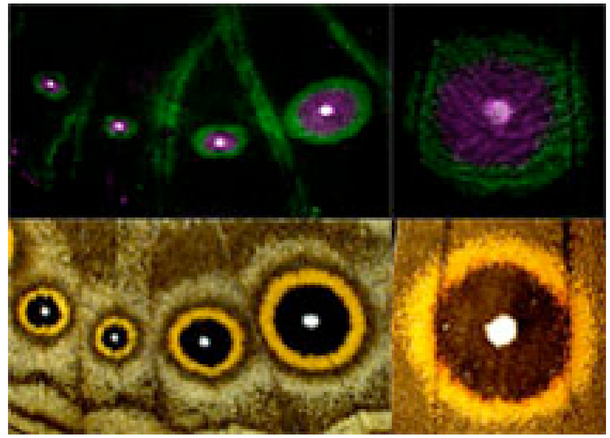
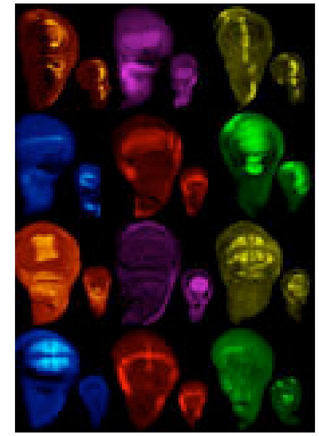
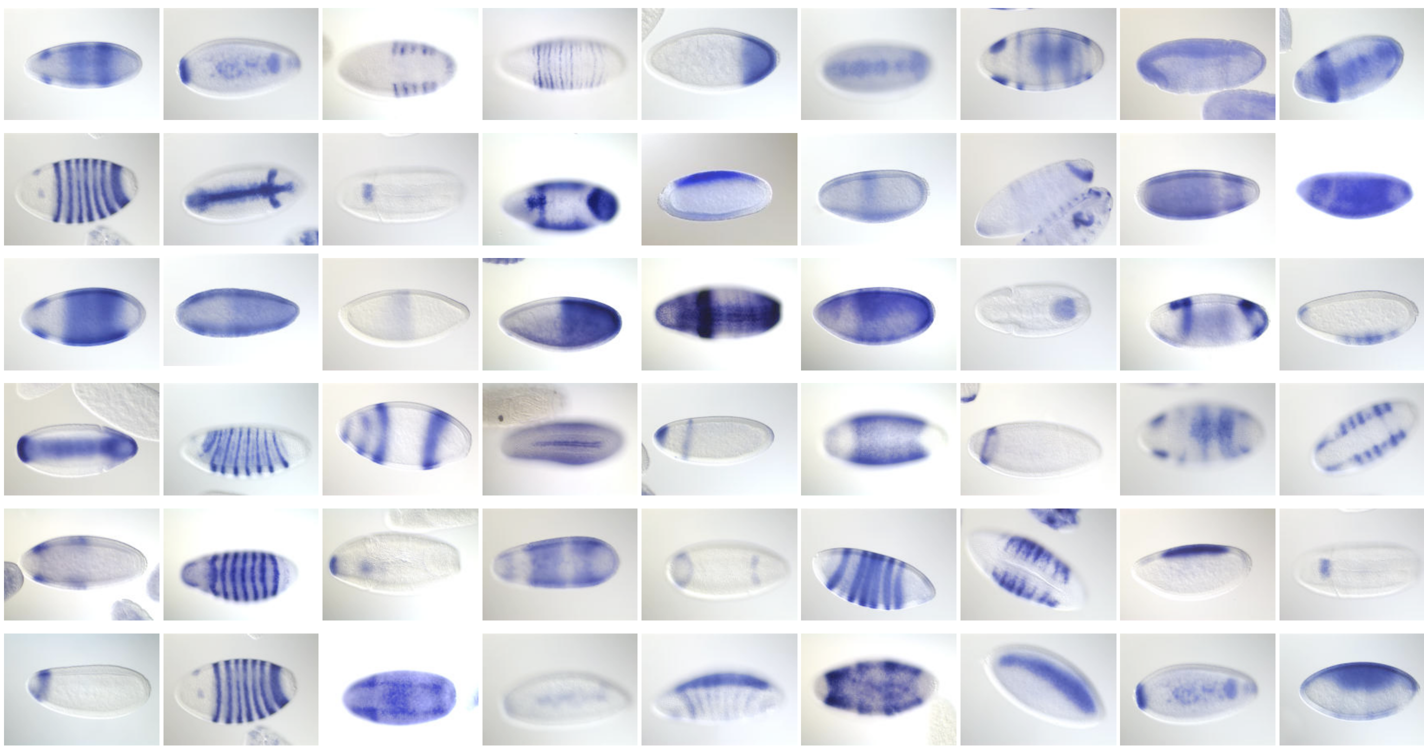
Today:
1. Genetic screens and genetic analysis of development (Drosophila techniques)
2. Regulatory networks in development & cis-regulatory control of expression
3. Post-transcriptional regulation, pleiotropy & disease in development

Review question: How do you assay which cells in a developing organism are expressing a gene of interest?

Visualize using microscopy:

For mRNA use in situ hybridization

For protein use immunohistochemistry

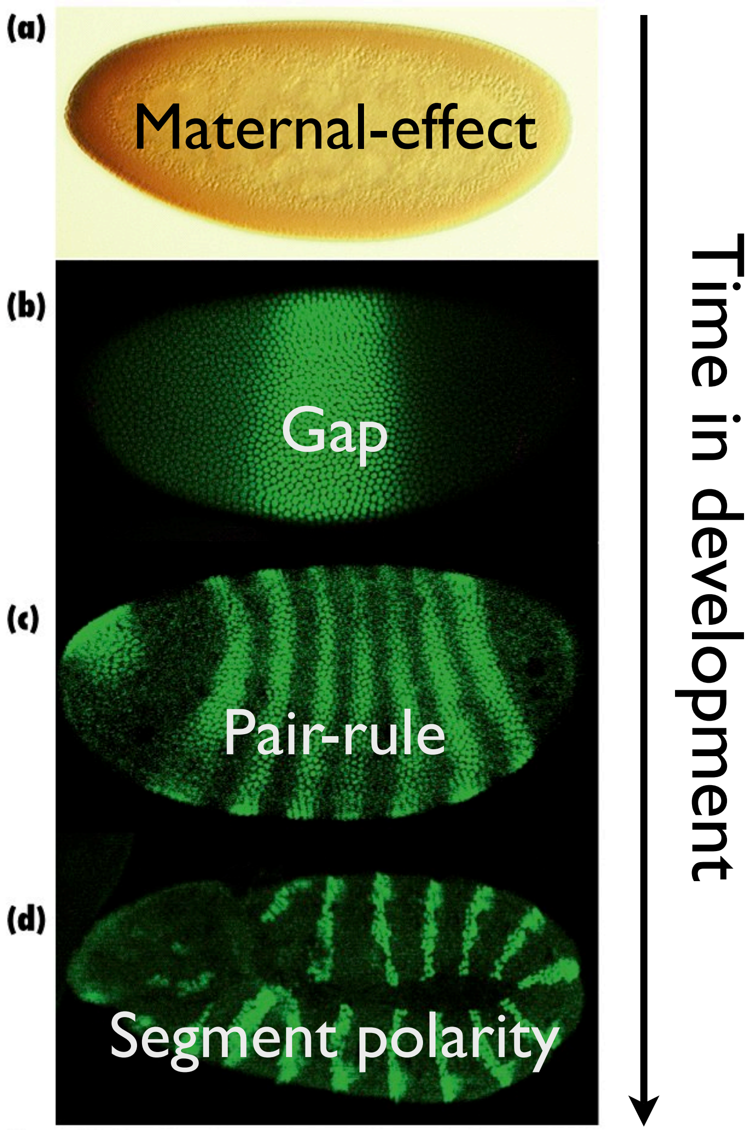


Figure 12-15
Introduction to Genetic Analysis, Ninth Edition

From last time:
Expression patterns of four classes of genes found in screen for genes affecting segmentation

Most of these genes were found to be transcription factors

Appears to be a network where earlier genes affect later genes

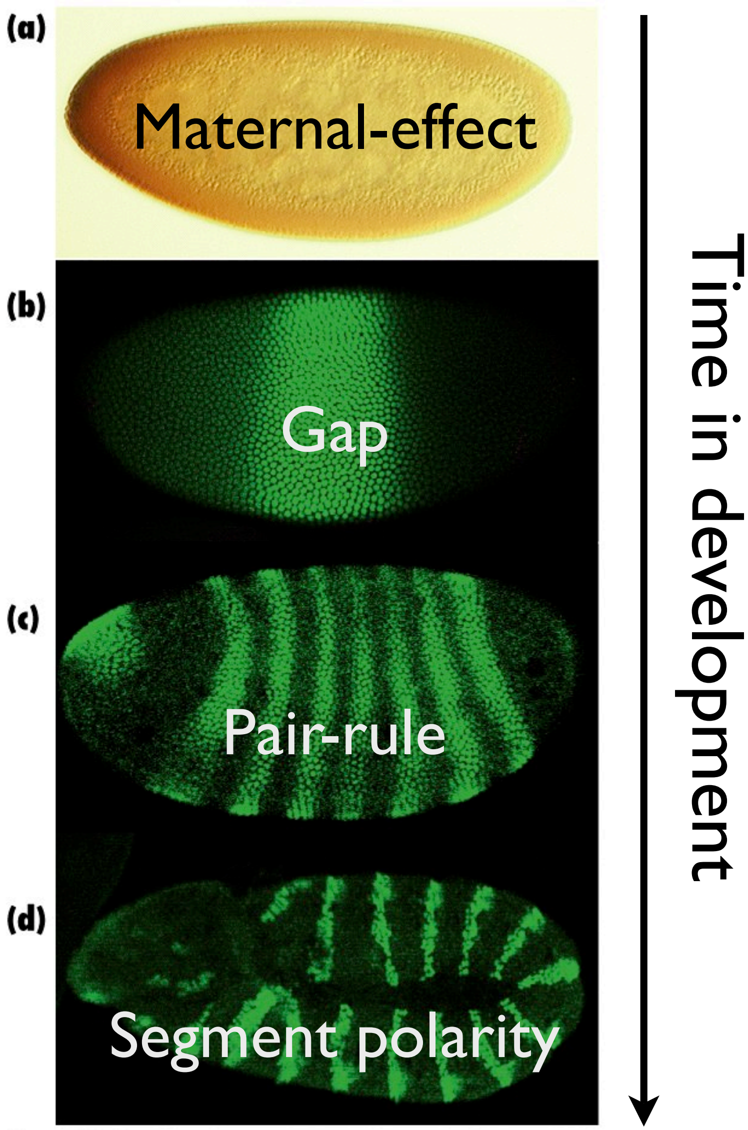


Figure 12-15
Introduction to Genetic Analysis, Ninth Edition

How does a simple gradient lead to more complex expression patterns through transcriptional regulation?

Bicoid is the first expression pattern to appear with a gradient of high anterior to low posterior expression.

How can a downstream gene 'read' the bicoid gradient?

Hunchback 'reads' the Bicoid gradient

The concentration dependent response of hunchback to the graded input of Bicoid gradient is achieved through multiple Bicoid binding sites in a 5' cis-acting regulatory element.

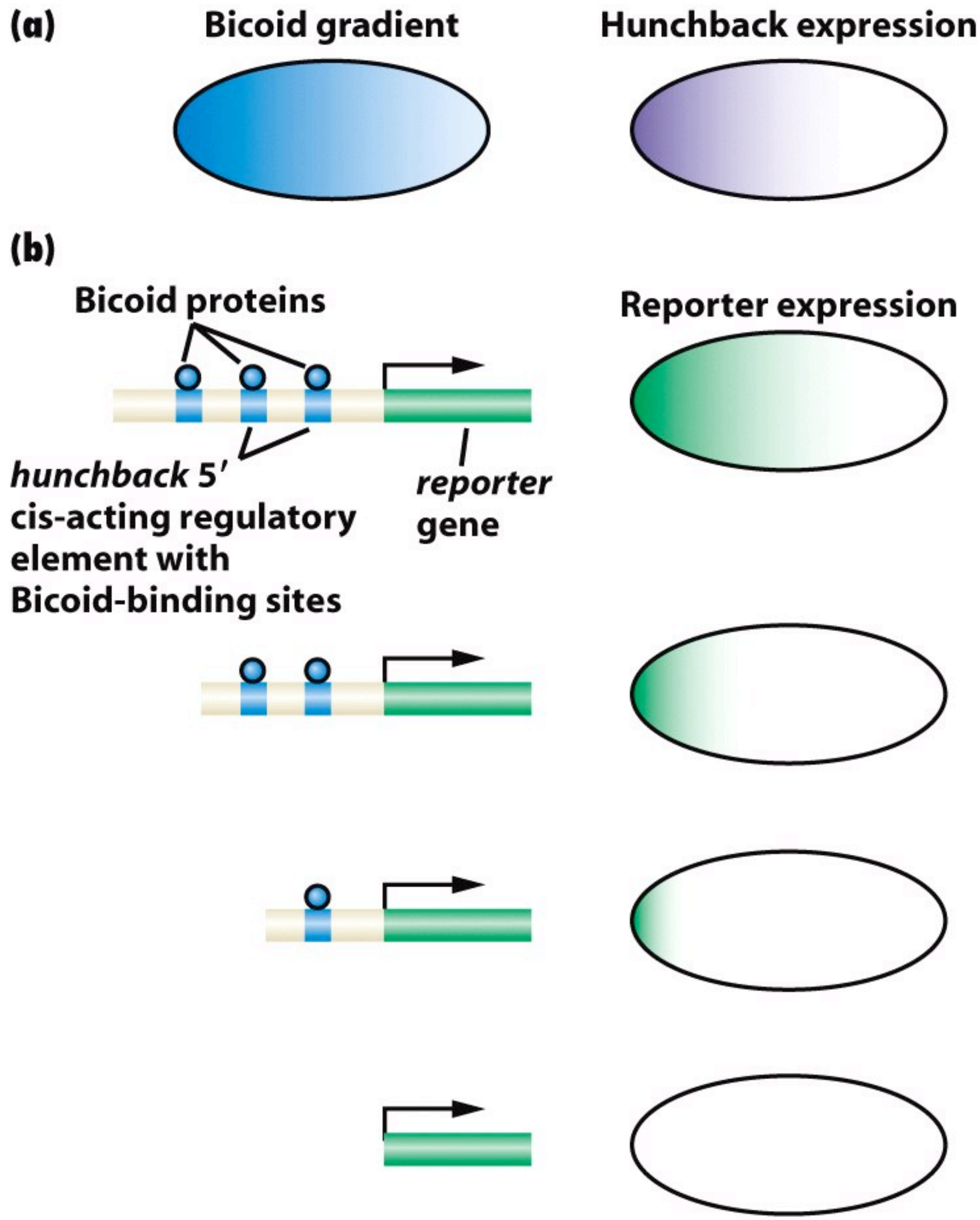


Figure 12-18
Introduction to Genetic Analysis, Ninth Edition

Reporter gene assays allow dissection of independent regulator elements

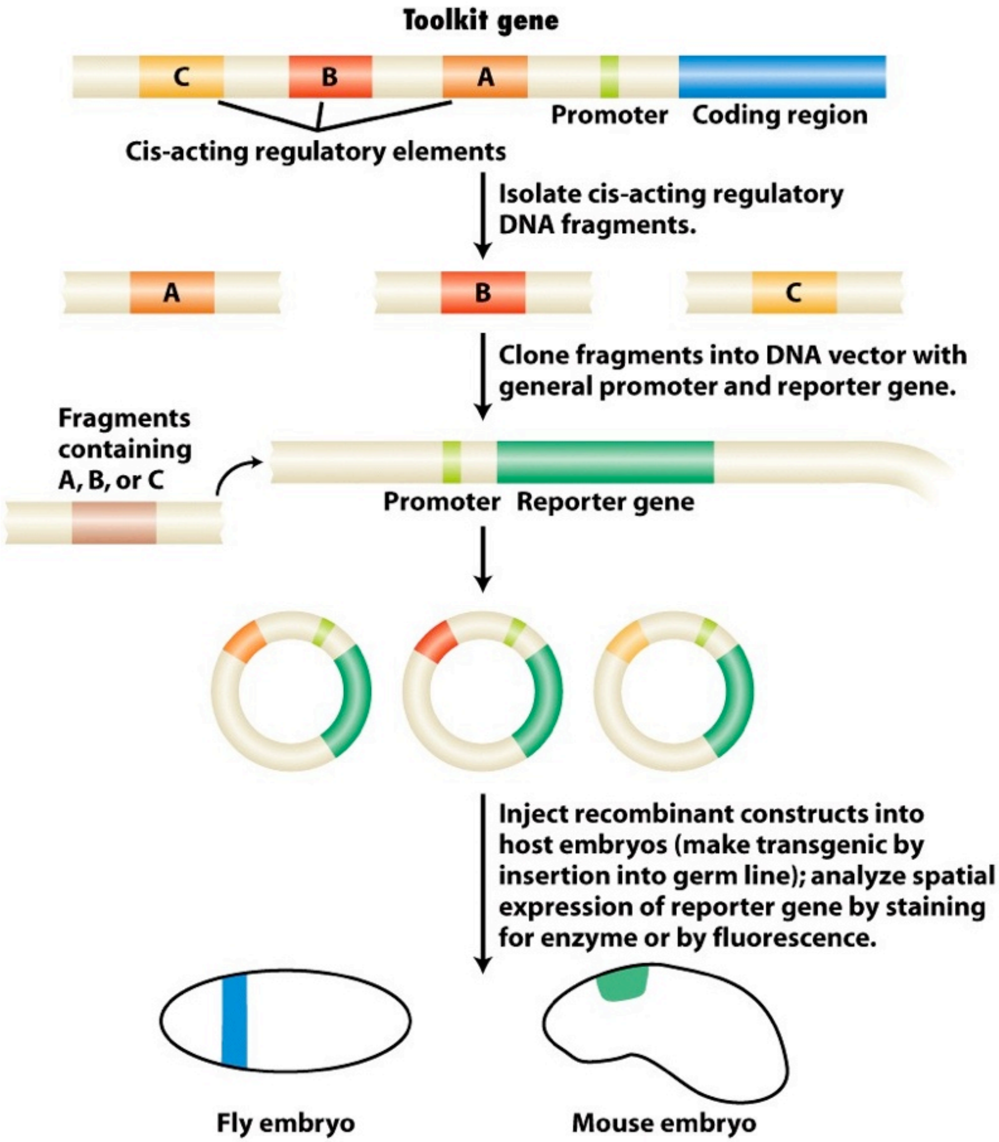
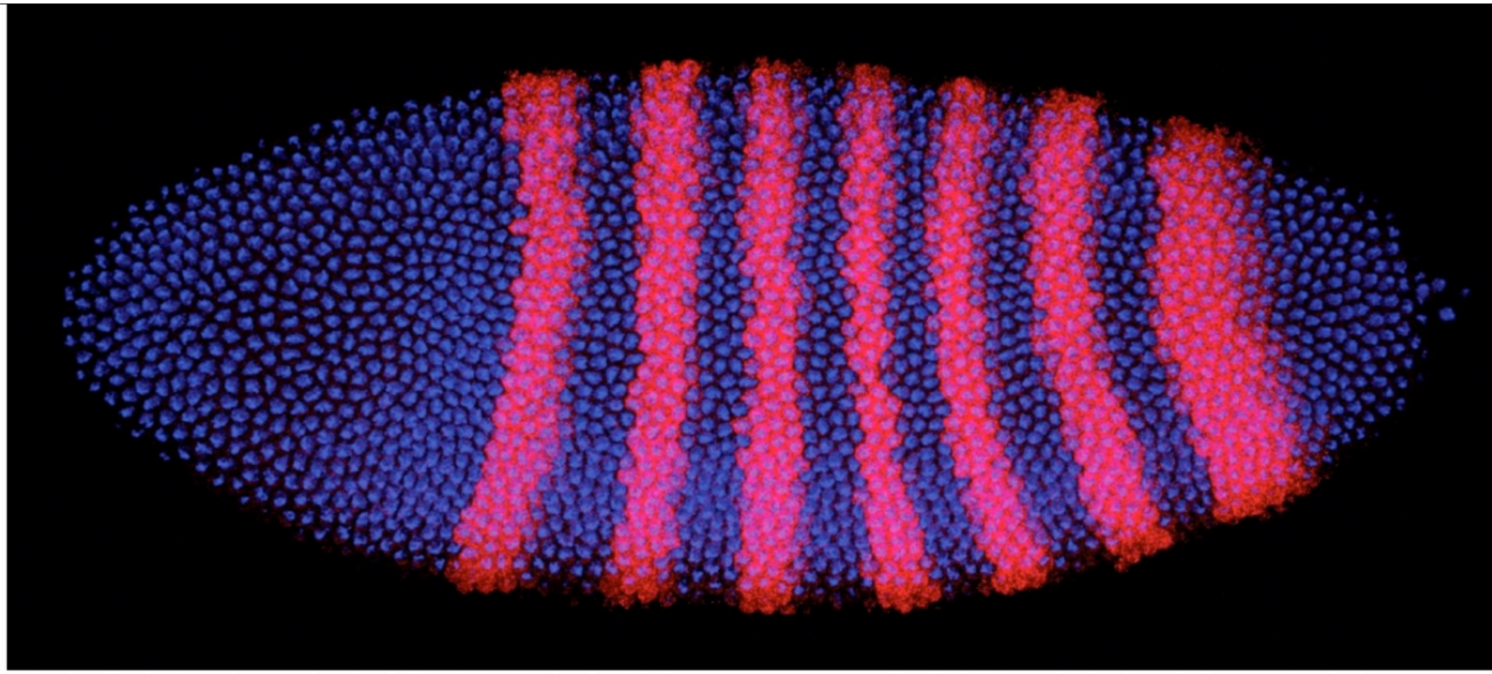


Figure 12-19
Introduction to Genetic Analysis, Ninth Edition

How do you find cis-regulatory sequences?

1. ‘Promoter bashing’. Break up and alter 5’ upstream and test expression patterns in reporter gene assays.
2. Binding assays. Measure binding of a transcription factor to 5’ upstream region. Various kinds with different resolutions and accuracies (DNase I footprinting, SELEX +bioinformatics & Chromatin IP).



Chapter 12 Opener
Introduction to Ge

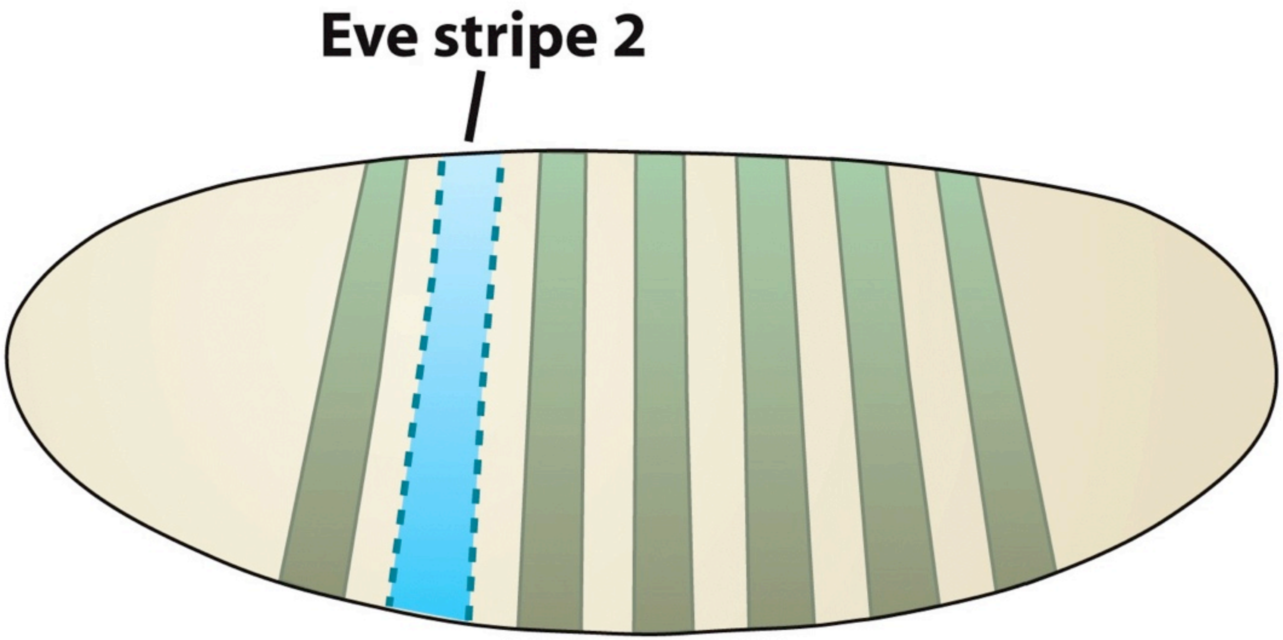


Figure 12-20a
Introduction to Genetic Analysis, Ninth Edition

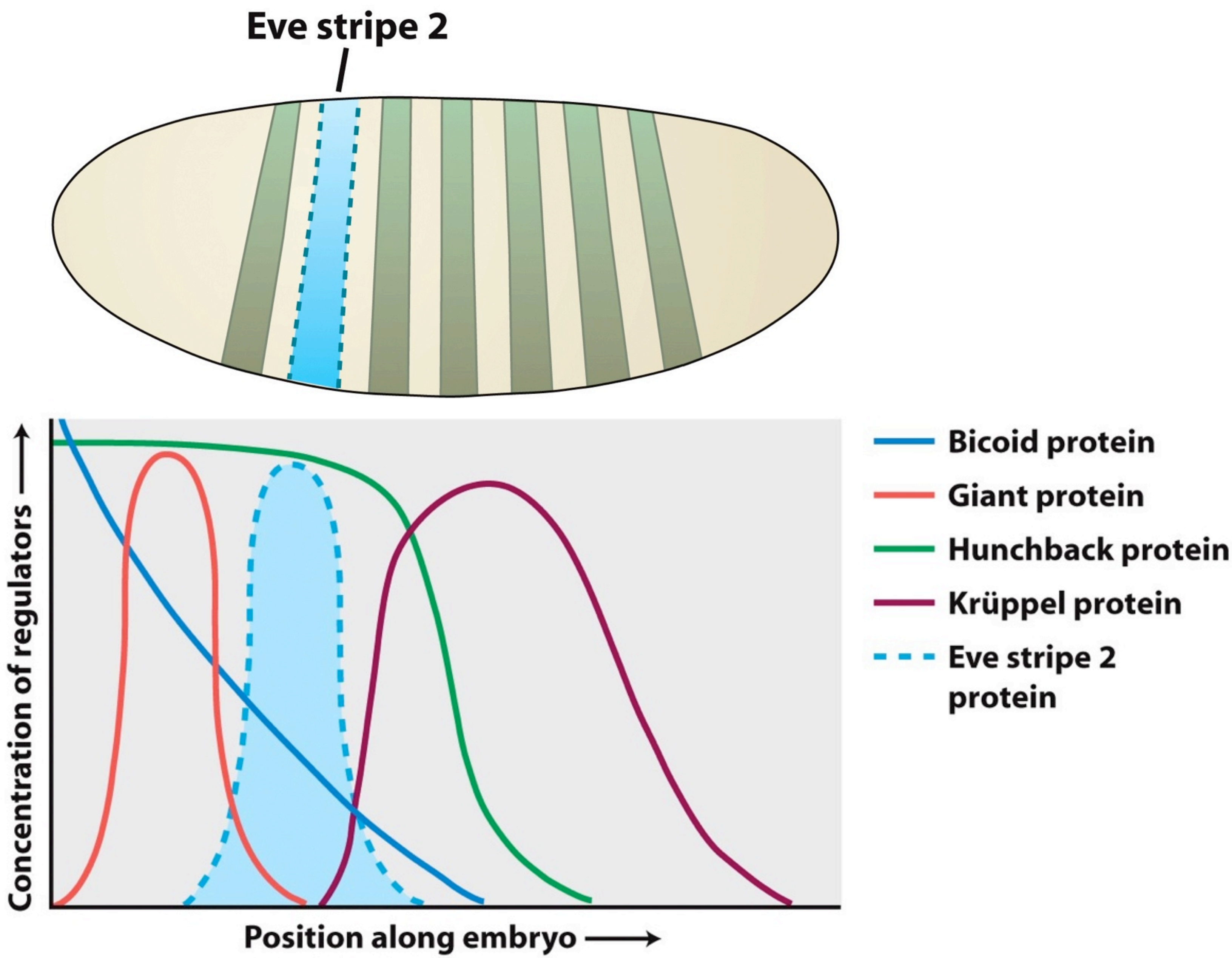


Figure 12-20b
Introduction to Genetic Analysis, Ninth Edition

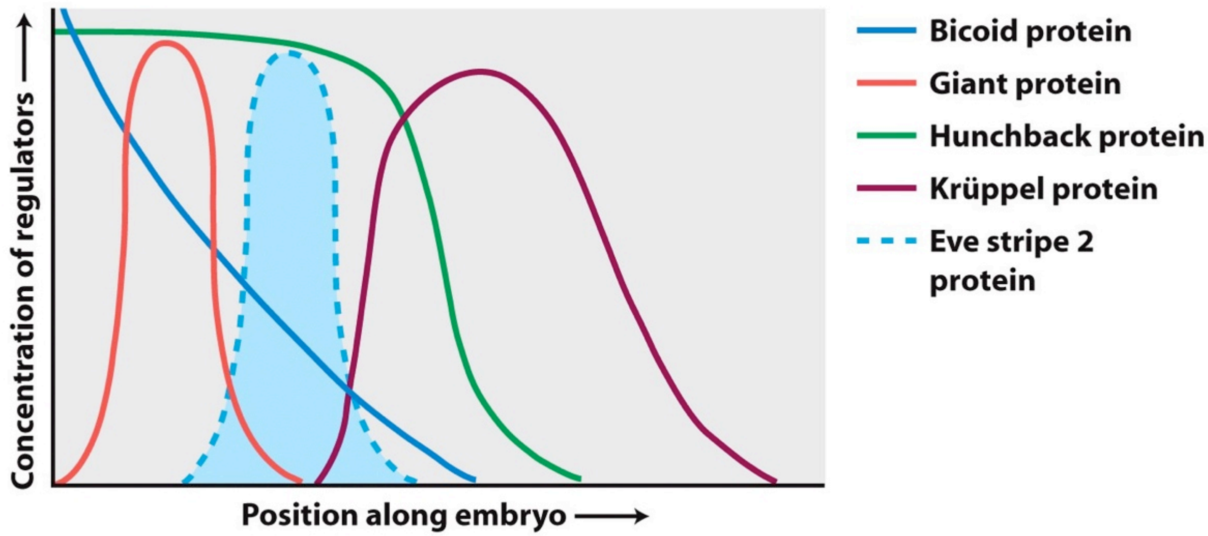


Figure 12-20b
Introduction to Genetic Analysis, Ninth Edition

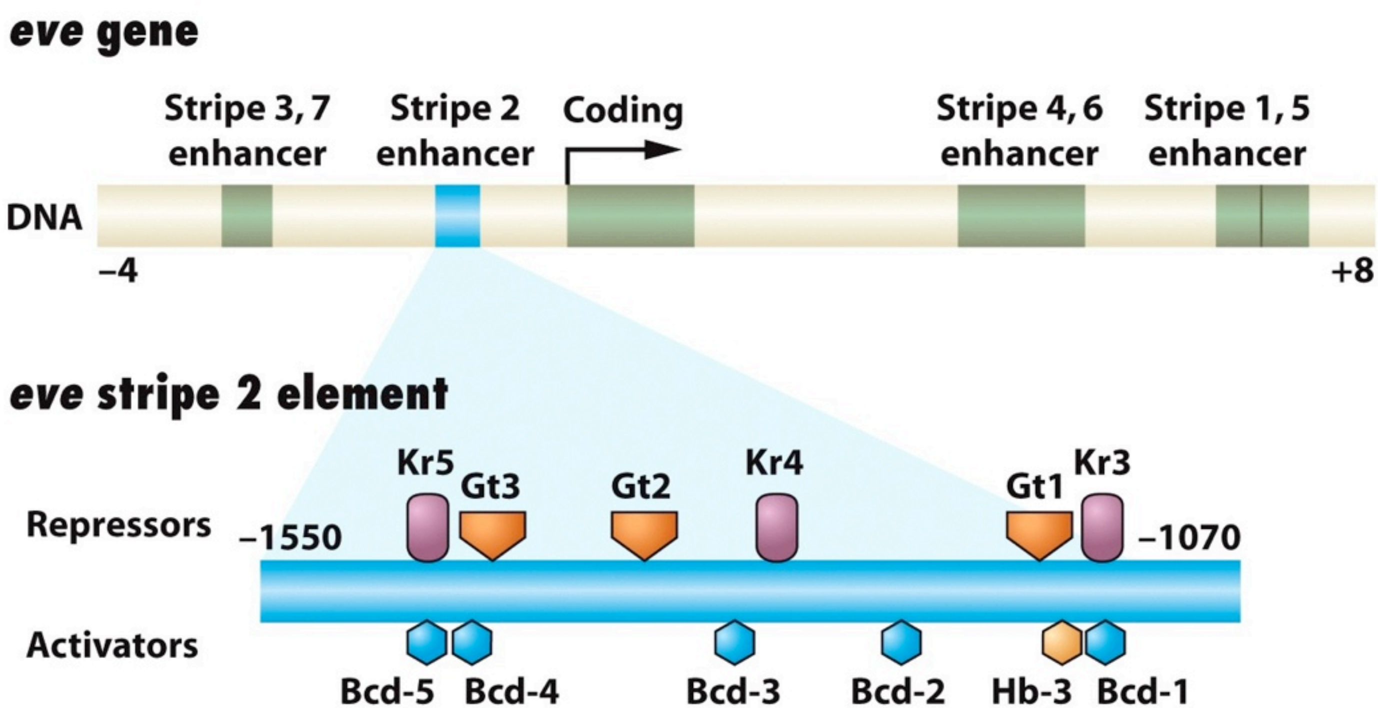


Figure 12-20cd
Introduction to Genetic Analysis, Ninth Edition

Some principles:

1. Cis-regulatory elements integrate combinations of activators and repressors in the spatial regulation of gene expression.
2. Complex patterns of inputs are integrated into simpler patterns through a single cis-regulatory element.
3. The full expression pattern of a gene is often derived from the patterns of many independent cis-regulatory elements.

Back to Hox genes: how do you make segments different?

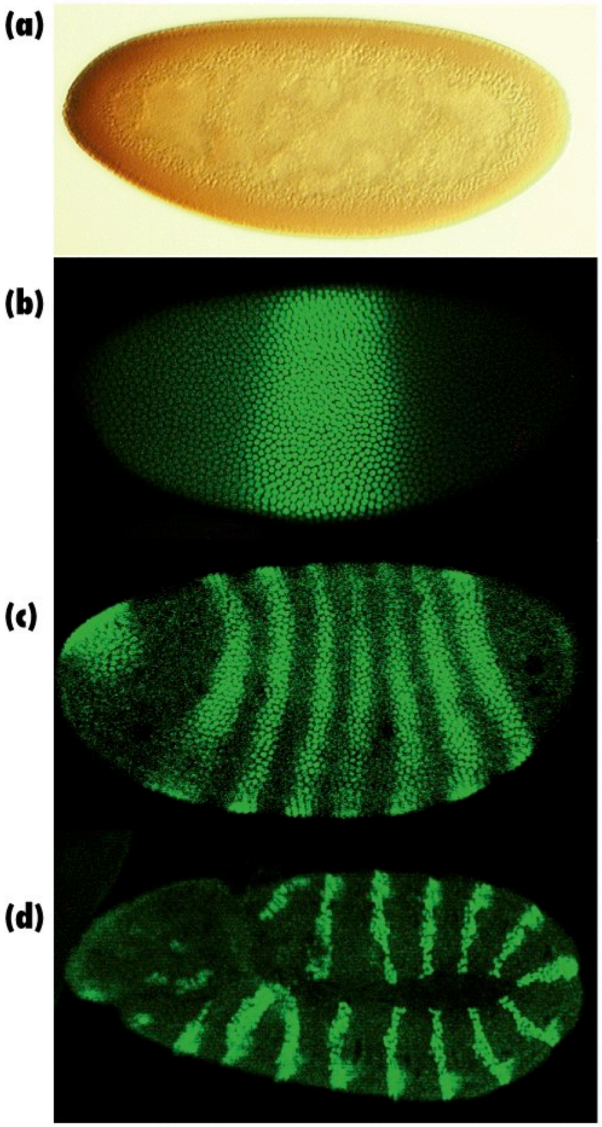
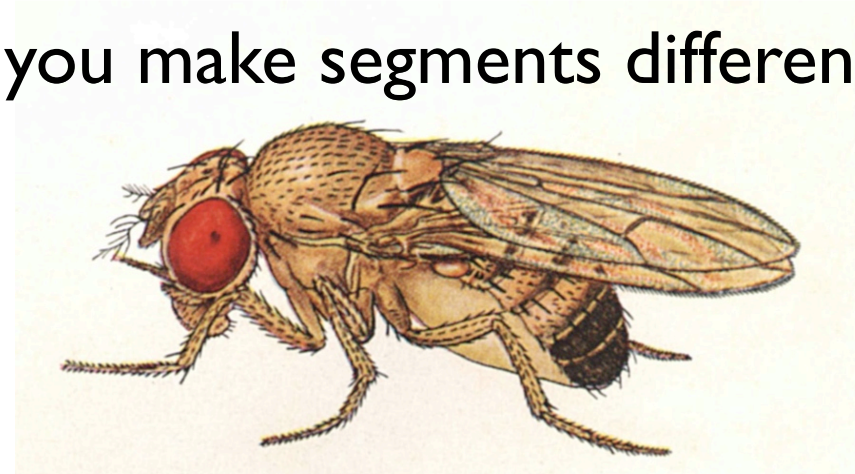


Figure 12-15
Introduction to Genetic Analysis, Ninth Edition



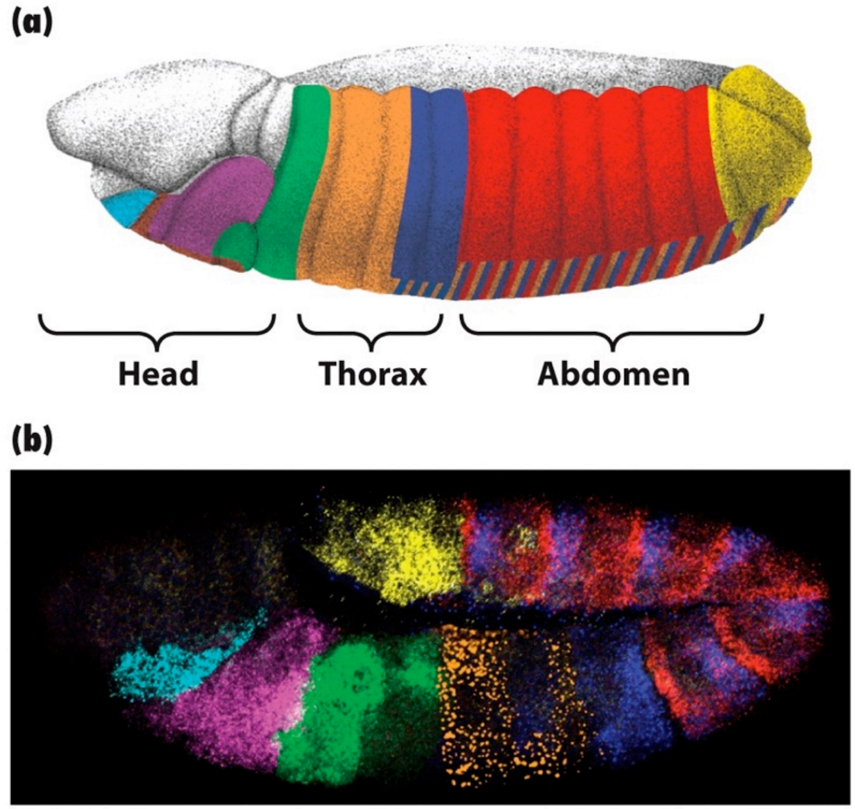
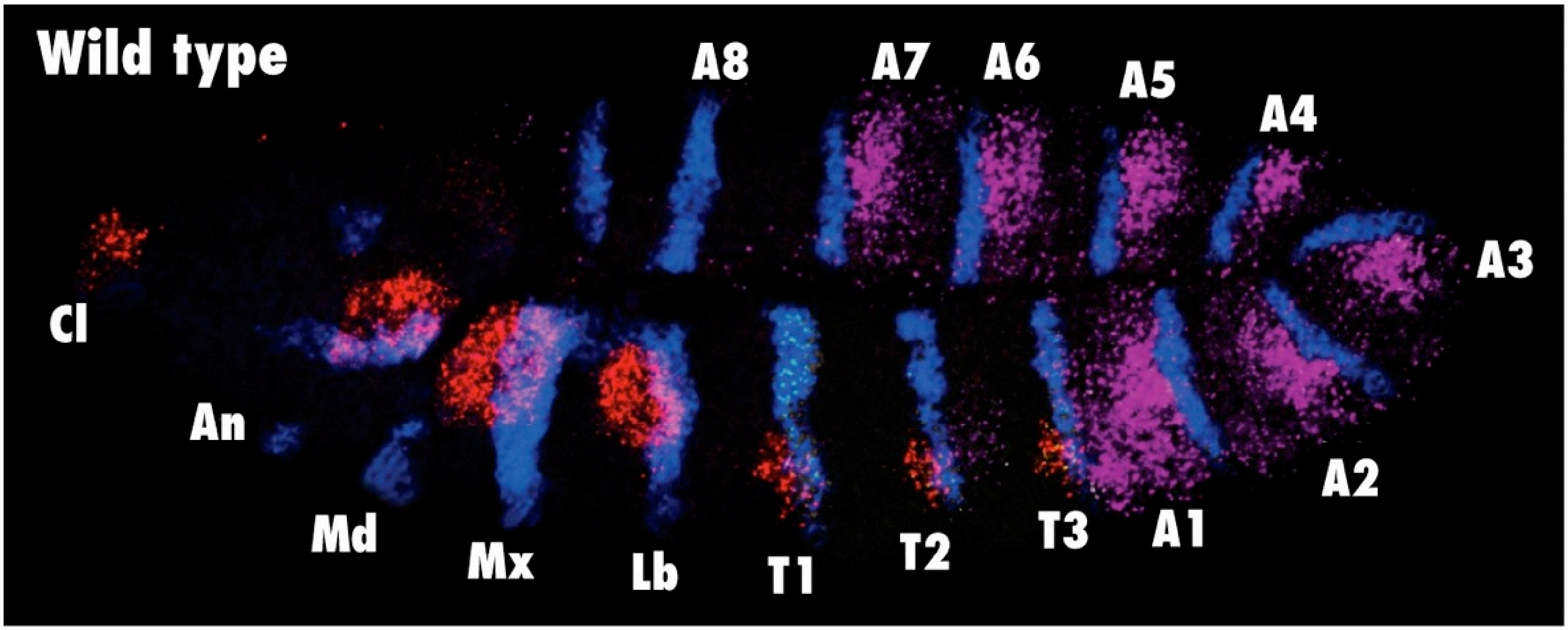


Figure 12-6
Introduction to Genetic Analysis, Ninth Edition

Distal-less (Dll) expression marks appendage primordia & is essential for limb development. How does the fly not develop limbs on its abdomen?



Dll **(red) repressed in A1–A8**

Figure 12-21a
Introduction to Genetic Analysis, Ninth Edition

Ubx and abd-A (Hox genes) are necessary for repression of Dll in abdominal compartments A1-A7

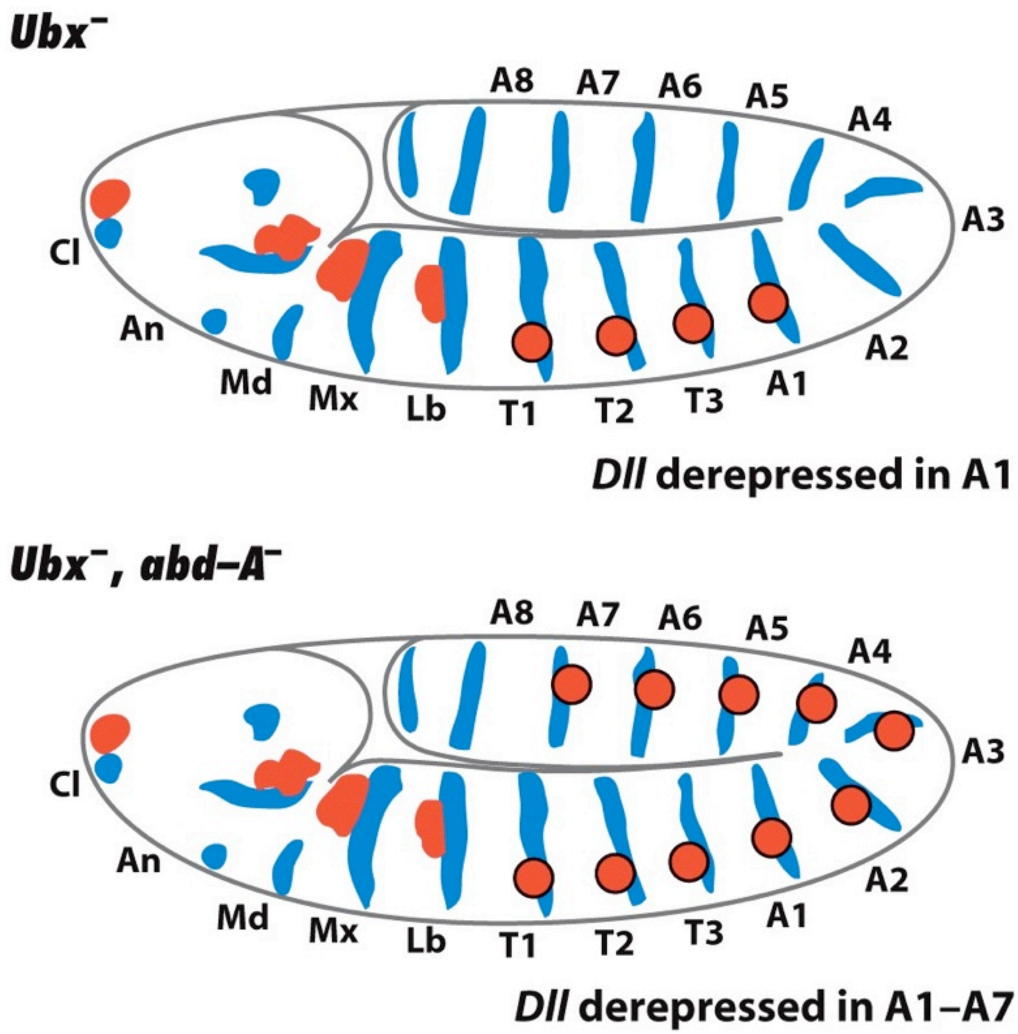


Figure 12-21bc
Introduction to Genetic Analysis, Ninth Edition

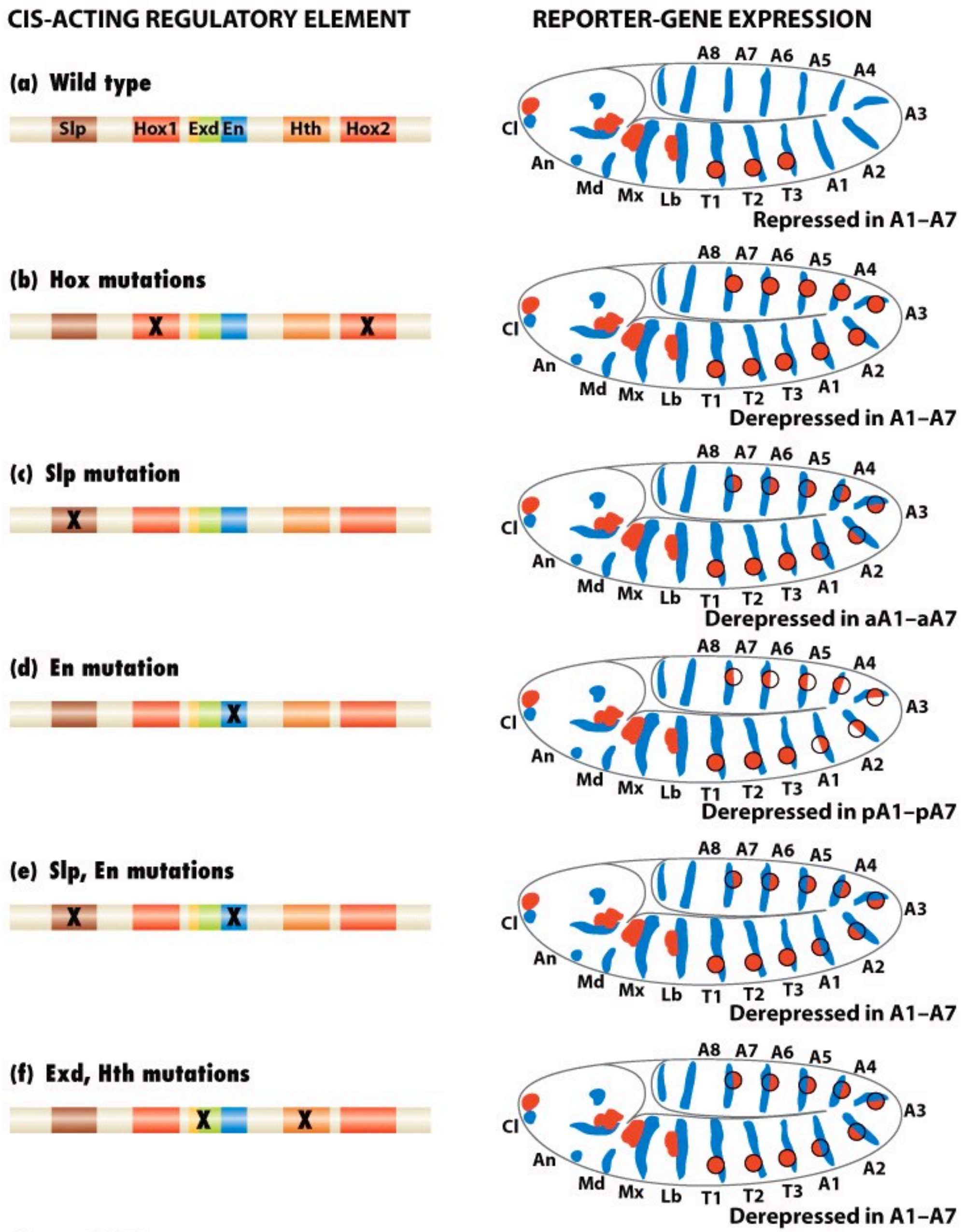


Figure 12-22

Introduction to Genetic Analysis, Ninth Edition

Some principles:

1. Cis-regulatory elements integrate combinations of activators and repressors in the spatial regulation of gene expression.
2. Complex patterns of inputs are integrated into simpler patterns through a single cis-regulatory element.
3. The full expression pattern of a gene is often derived from the patterns of many independent cis-regulatory elements.
4. Combinatorial and cooperative regulation imposes greater specificity on spatial patterns of gene expression.

Today:

1. Genetic screens and genetic analysis of development (Drosophila techniques)
2. Regulatory networks in development & cis-regulatory control of expression
3. Post-transcriptional regulation, pleiotropy & disease in development

Alternative splicing as a means of gene expression regulation

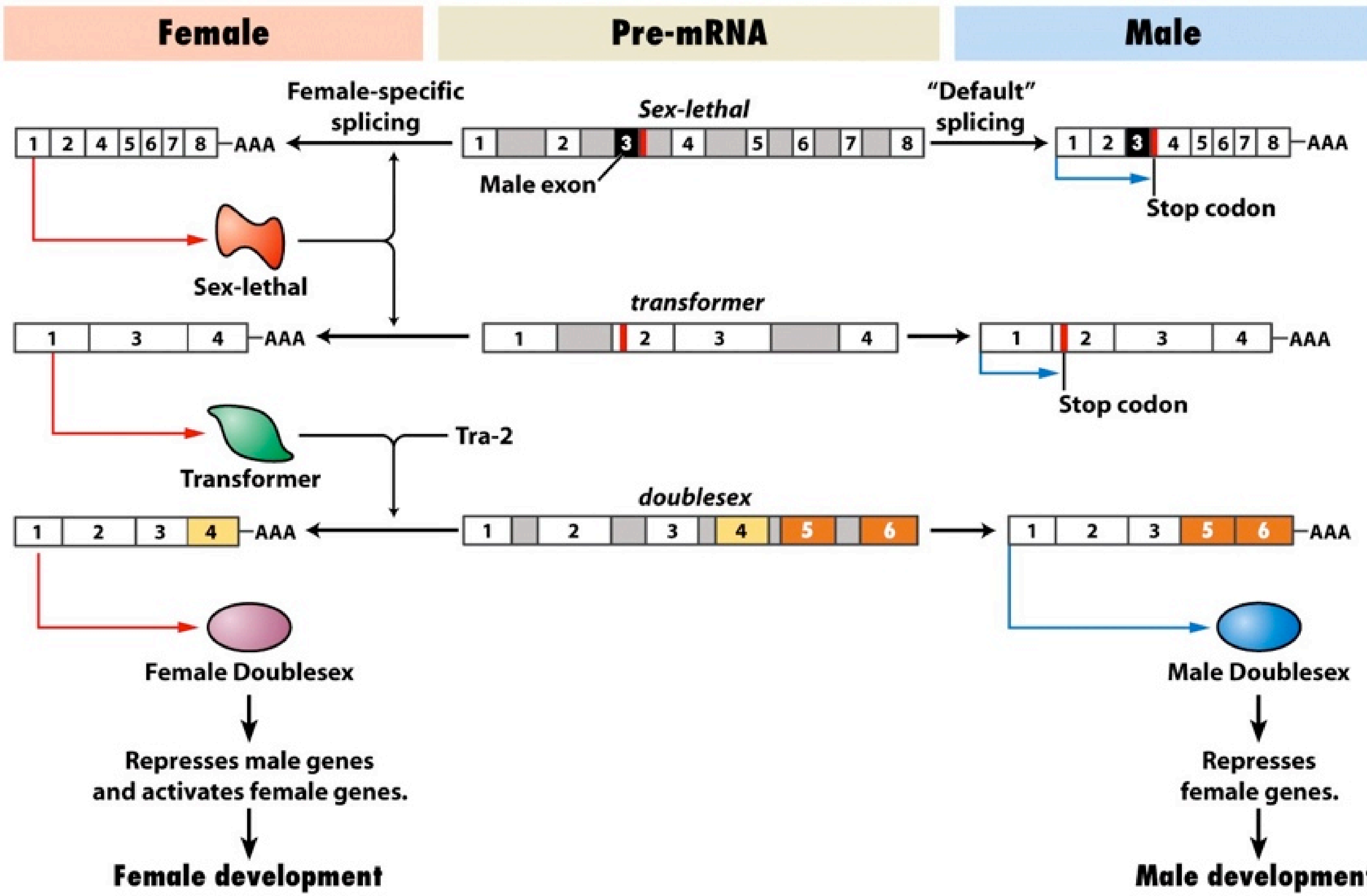
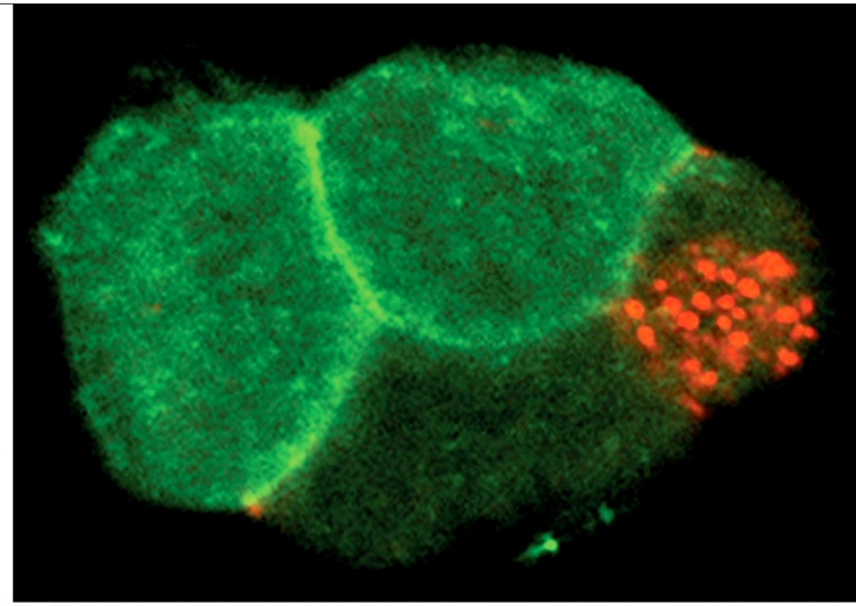


Figure 12-23
Introduction to Genetic Analysis, Ninth Edition

Regulation of mRNA translation in C. elegans:

How is differential expression of GLP achieved at the four cell stage?

GLD-1 binds 3’ UTR and represses translation in posterior cells



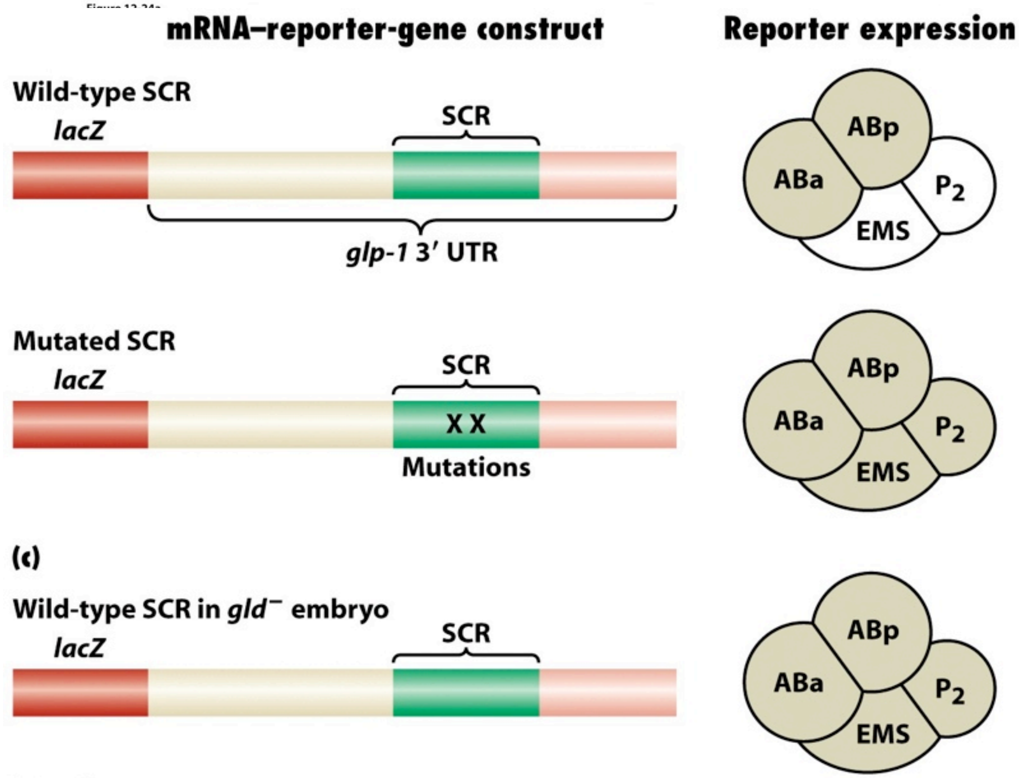


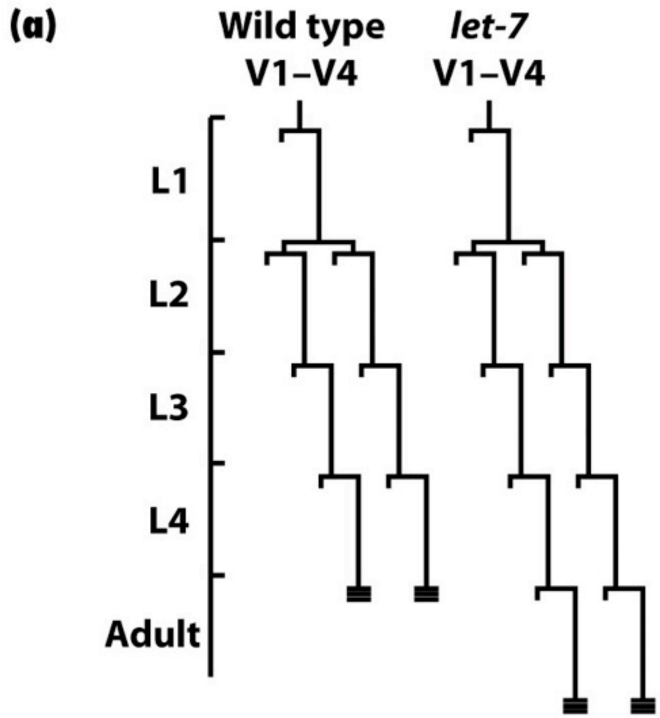
Figure 12-24b
Introduction to Genetic Analysis, Ninth Edition

miRNA control of developmental timing in C. elegans:

let-7 represses translation of target genes leading to adult cell fates

let-7 encodes 70 bp RNA precursor that is processed into a 22 nucleotide miRNA

miRNA binding 3’ UTRs of target genes and repress translation



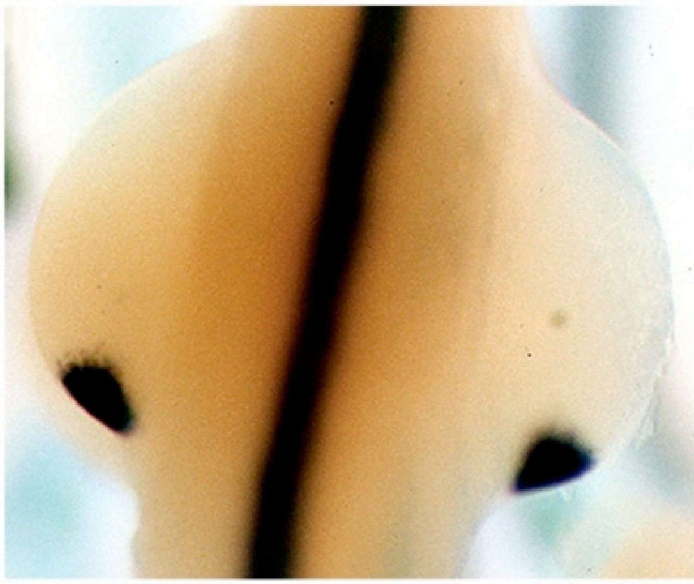
(b)

```
                                         GUU      A
lin–41  3′ UTR   5′-  UUAUACAACC        CUACCUCA-3′
let–7            3′-UGAUAUGUUGG         GAUGGAGU-5′
                                          AU
```

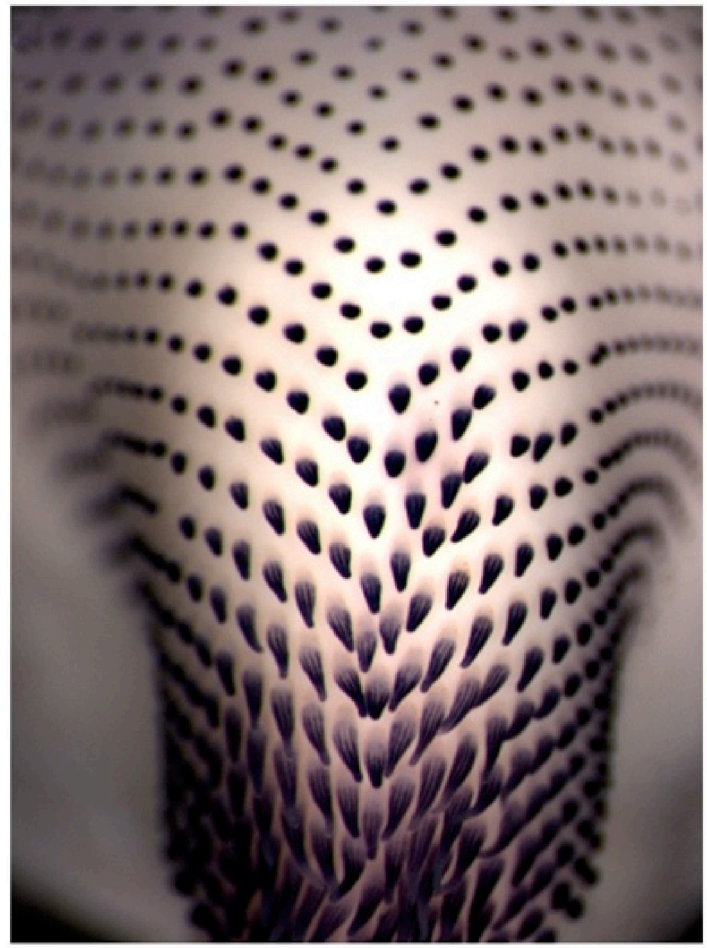
Figure 12-25
Introduction to Genetic Analysis, Ninth Edition

Developmental genes are often used many times throughout development and adult life

(a)



(b)



Sonic hedgehog: segment polarity gene, developing limb bud polarizing zone, neural tube & feather buds

Figure 12-26
Introduction to Genetic Analysis, Ninth Edition

Developmental genes and disease: from model organisms to human health

Polydactyly is caused by mis-expression of Shh in developing limb bud as a result of mutations in cis-regulatory elements.

Figure 12-27
Introduction to Genetic Analysis, Ninth Edition

Note that cis-regulatory mutations are often dominant and will only affect one aspect of a genes function.

Holoprosencephaly is a developmental disease leading to abnormalities in brain and midline development caused by a loss of function mutation in the coding region of Shh. Disease can molecularly be understood because of research in model organisms like Drosophila.

Developmental genes discovered in Drosophila associated with Cancer

Table 12-2 Some Toolkit Genes Having Roles in Cancer

	Fly gene	Mammalian gene	Cancer type
Signaling-Pathway Components			
Wingless	*armadillo*	*β-catenin*	Colon and skin
	D.TCF	*TLF*	Colon
Hedgehog	*cubitus interruptus*	*Gli1*	Basal cell carcinoma
	patched	*patched*	Basal cell carcinoma, medulloblastoma
	smoothened	*smoothened*	Basal cell carcinoma
Notch	*Notch*	*hNotch1*	Leukemia, lymphoma
EGF receptor	*torpedo*	*C-erbB-2*	Breast and colon
Decapentaplegic/TFG-β	*Medea*	*DPC4*	Pancreatic and colon
Toll	*dorsal*	*NF-κB*	Lymphoma
Other	*extradenticle*	*Pbx1*	Acute pre-B-cell leukemia

Table 12-2
Introduction to Genetic Analysis, Ninth Edition