Lecture 3 Mutagens and Mutagenesis

1. Mutagens

A. Physical and Chemical mutagens

B. Transposons

C. T-DNA

2. Mutagenesis

A. Screen

B. Selection

C. Lethal mutations

Read: 460-464

Figs: 7.12; 7.14; 7.20; 5.21; C8; 1.11; 13.20; 13.22; 13.23; 13.24; 13.25

1. Mutagens

Mutagen treatment greatly increases the mutation rate

Exposure to X-ray, UV light

Chemical treatment: base analogs 5'-bromouracil (=T or rarely C)

hydroxylating agent (add OH-group to C)

alkylating agent such as EMS (ethylmethane sulfonate)

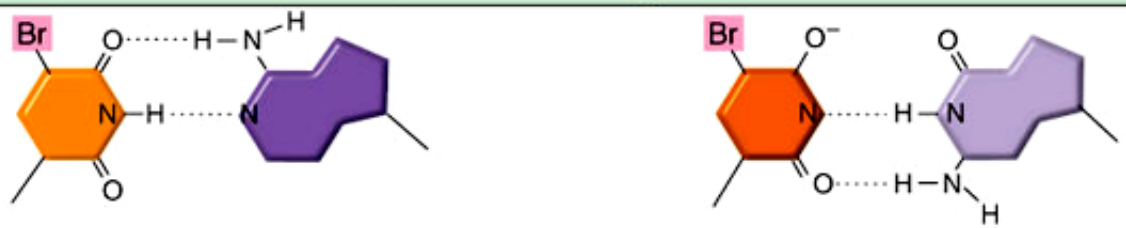
deaminating agent such as nitrous acid

intercalating agent such as Acridine Orange

Transposons that insert into a gene and disrupt the normal reading frame

Chemical Mutagens

Fig. 7.12a1

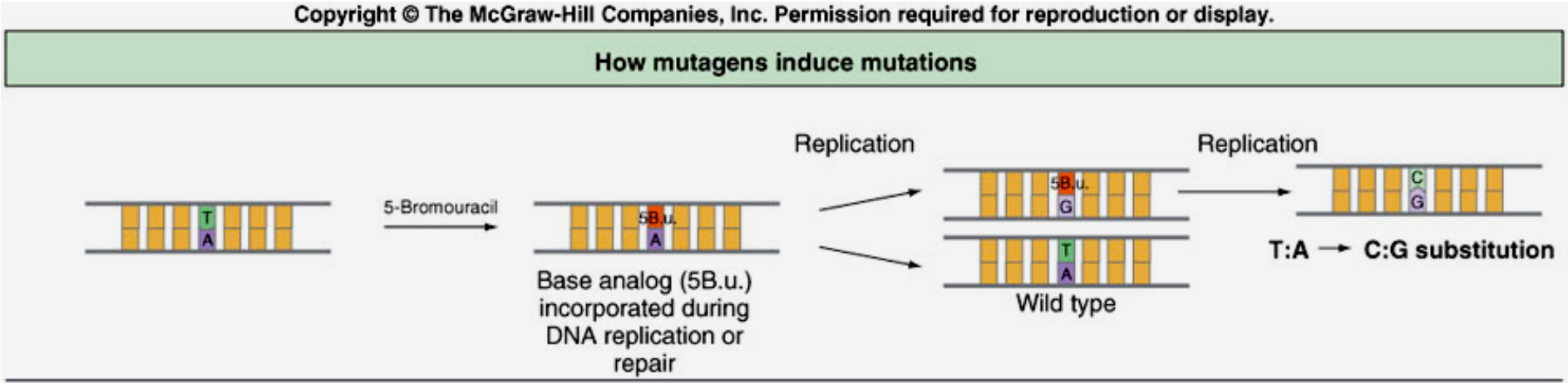
Type of mutagen	Chemical action of mutagen
(a) Replace a base: Base analogs have a chemical structure almost identical to that of a DNA base.	

5-Bromouracil–normal state, behaves like thymine — Adenine

5-Bromouracil–rare state, behaves like cytosine — Guanine

5-Bromouracil: almost identical to thymine. Normally pairs with A; in transient state, pairs with G.

Fig. 7.12a2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How mutagens induce mutations
T
A
5-Bromouracil
5B.u.
A
Base analog (5B.u.) incorporated during DNA replication or repair
Replication
5B.u.
G
T
A
Wild type
Replication
C
G
T:A → C:G substitution

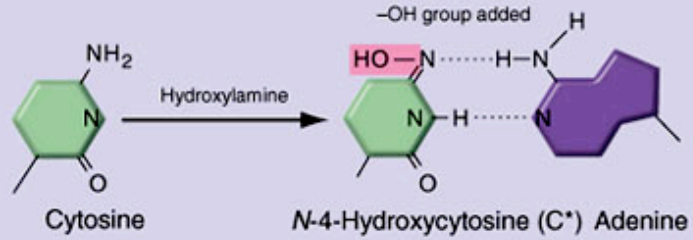
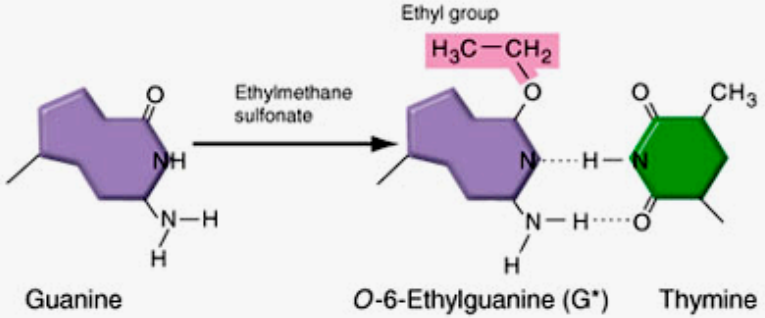
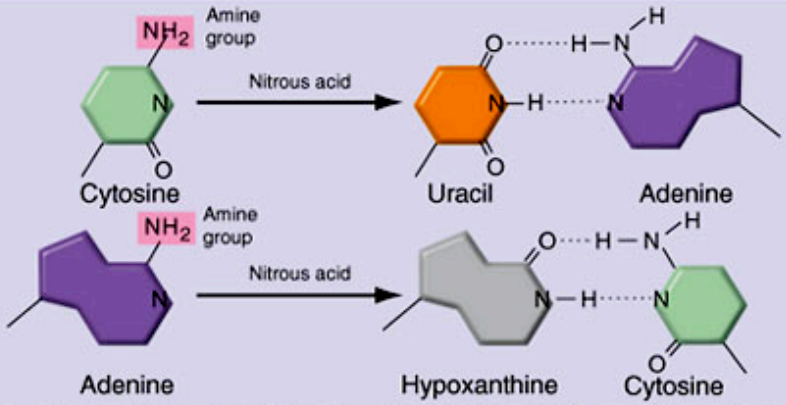
Type of mutagen	Chemical action of mutagen
(b) Alter base structure and properties: *Hydroxylating agents*: add a hydroxyl (–OH) group	 Hydroxylamine adds –OH to cytosine; with the –OH, hydroxylated C now pairs with A instead of G.
Alkylating agents: add ethyl ($-CH_2-CH_3$) or methyl ($-CH_3$) groups	 Ethylmethane sulfonate adds an ethyl group to guanine or thymine. Modified G pairs with T above, and modified T pairs with G (not shown).
Deaminating agents: remove amine ($-NH_2$) groups	 Nitrous acid modifies cytosine to uracil, which pairs with A instead of G; modifies adenine to hypoxanthine, a base that pairs with C instead of T.

Fig. 7.12b1

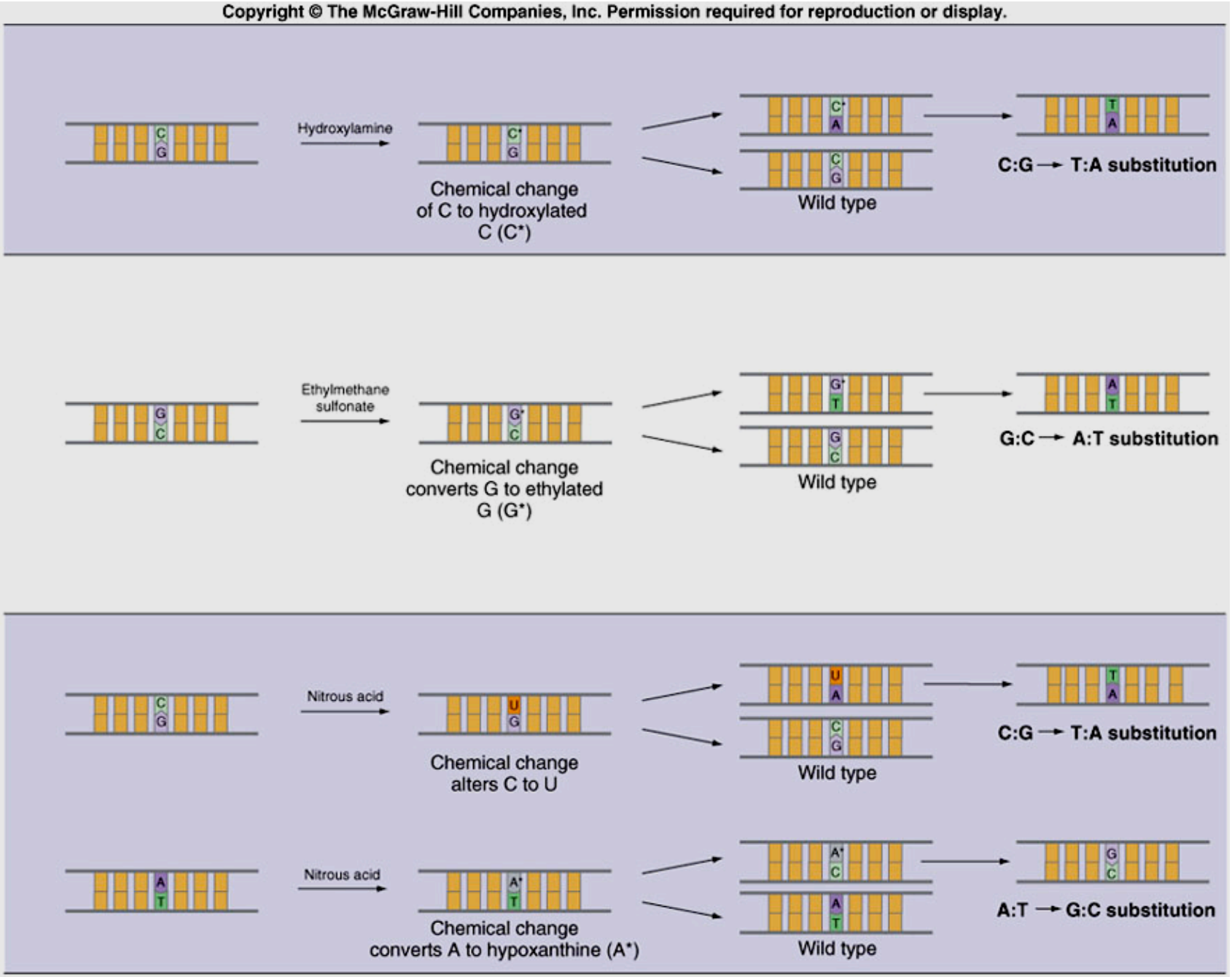


Fig. 7.12c1

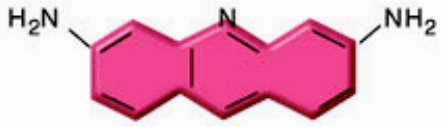
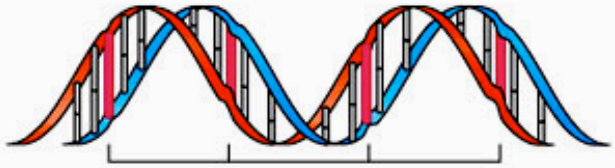
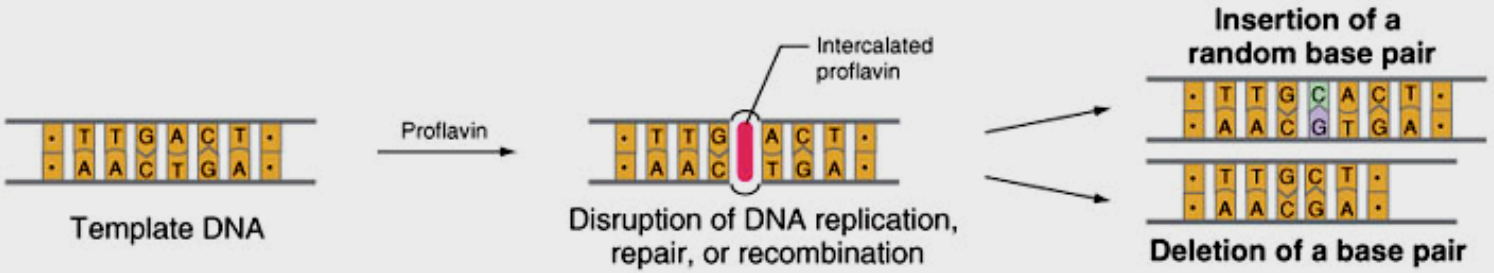
Type of mutagen	Chemical action of mutagen
(c) Insert between bases: Intercalating agents	 Proflavin  Intercalated proflavin molecules Proflavin intercalates into the double helix. This disrupts DNA metabolism, eventually resulting in deletion or addition of a base pair.

Fig. 7.12c2



Transposon (transposable element) as a mutagen

Transposon: DNA segment that can move from one position to another

(1) Retrotransposons

Copia	Drosophila
Ty1	Yeast
LINEs	Human
SINEs (Alu)	Human

(2) Transposons

Ac/Ds	Maize
P-element	Drosophila

Fig. 13.23

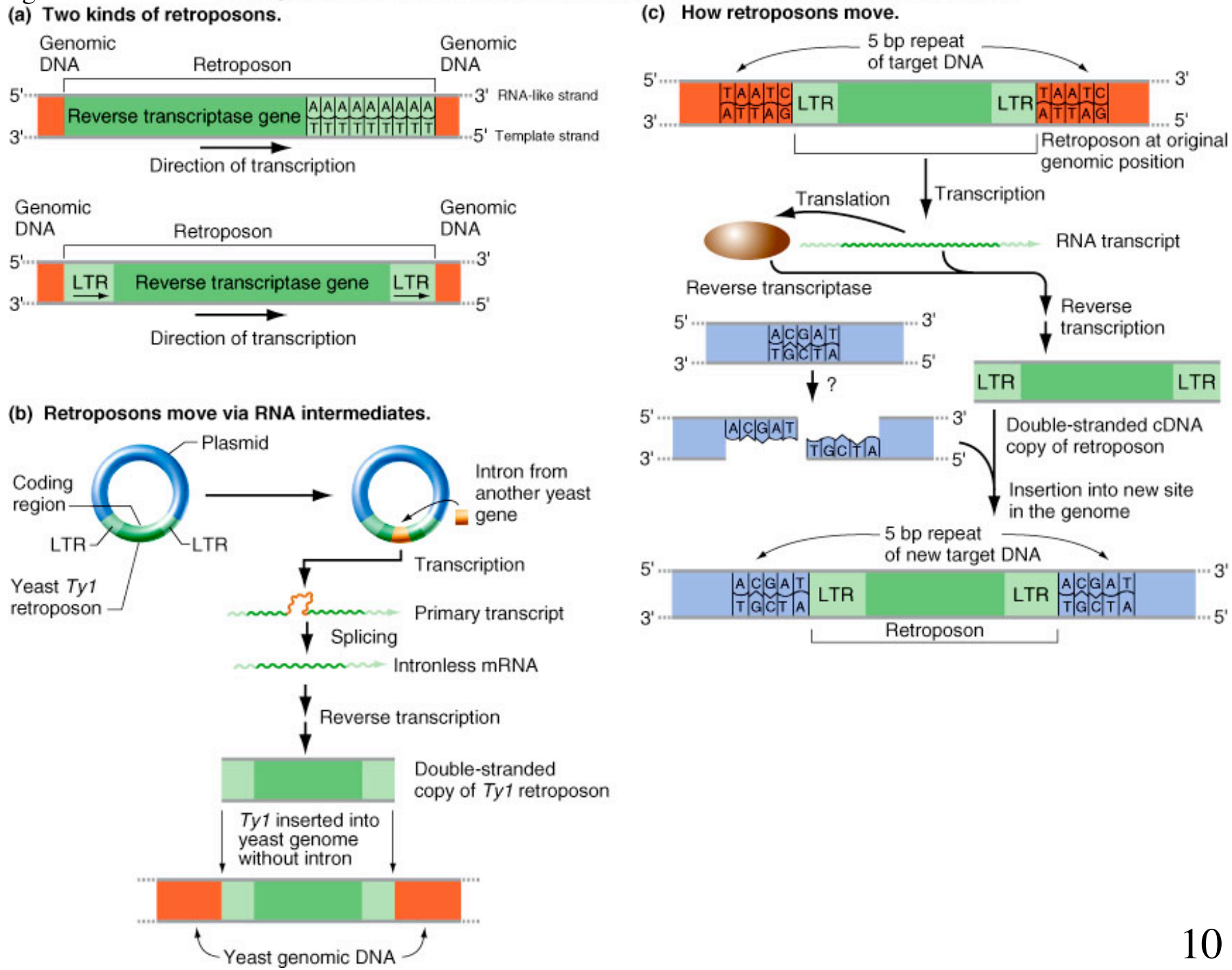
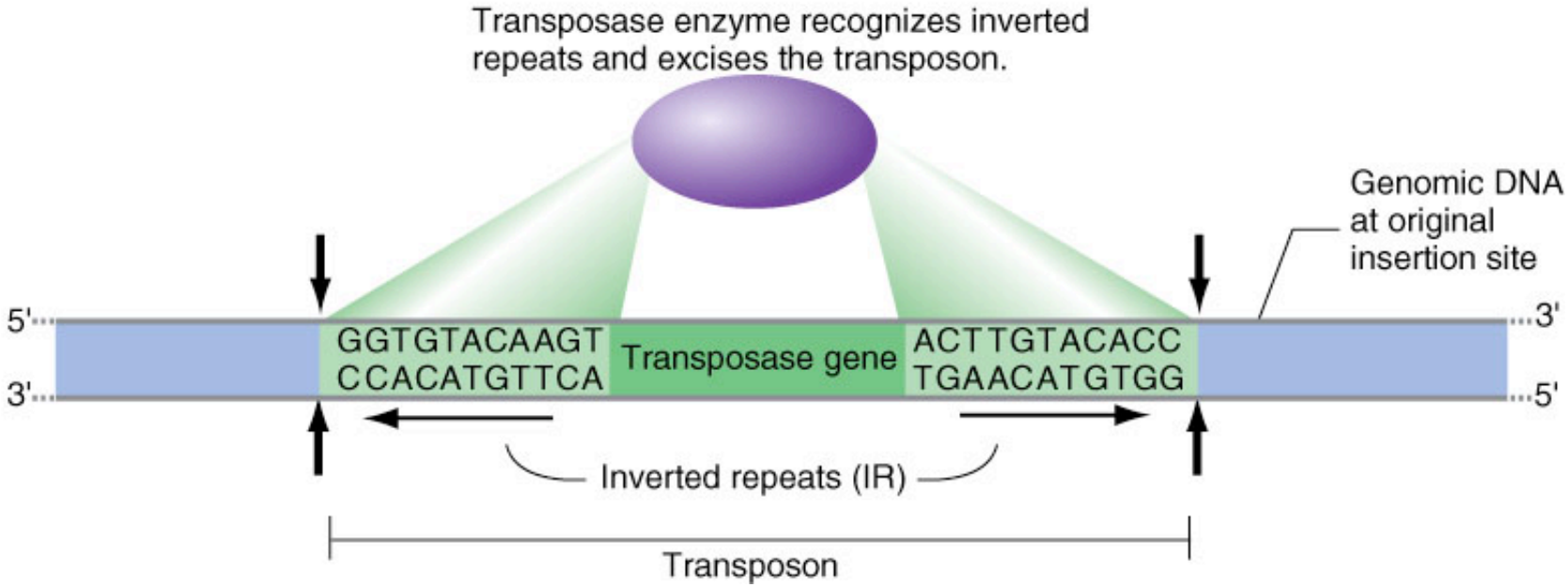


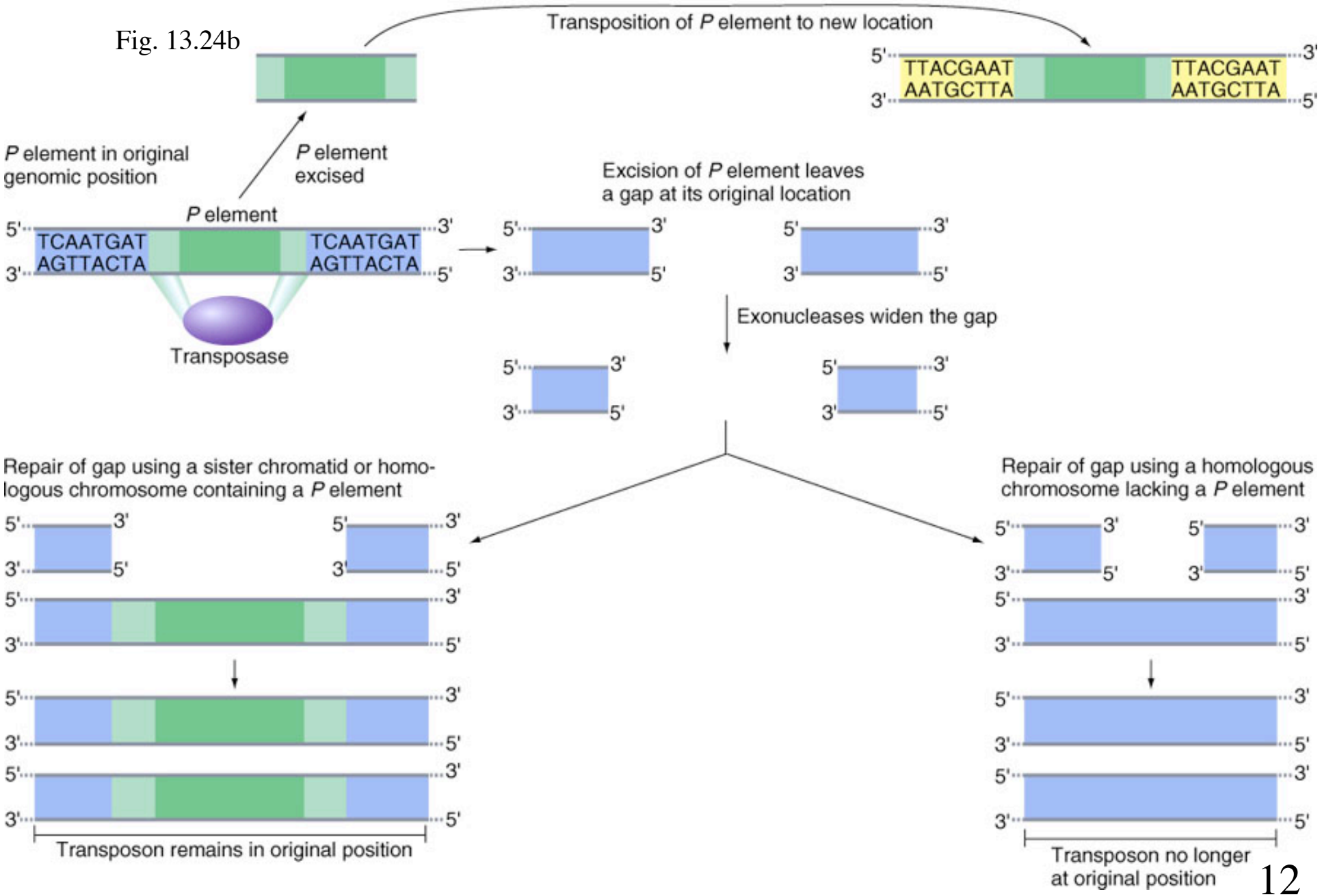
Fig. 13.24a

P-element in Drosophila



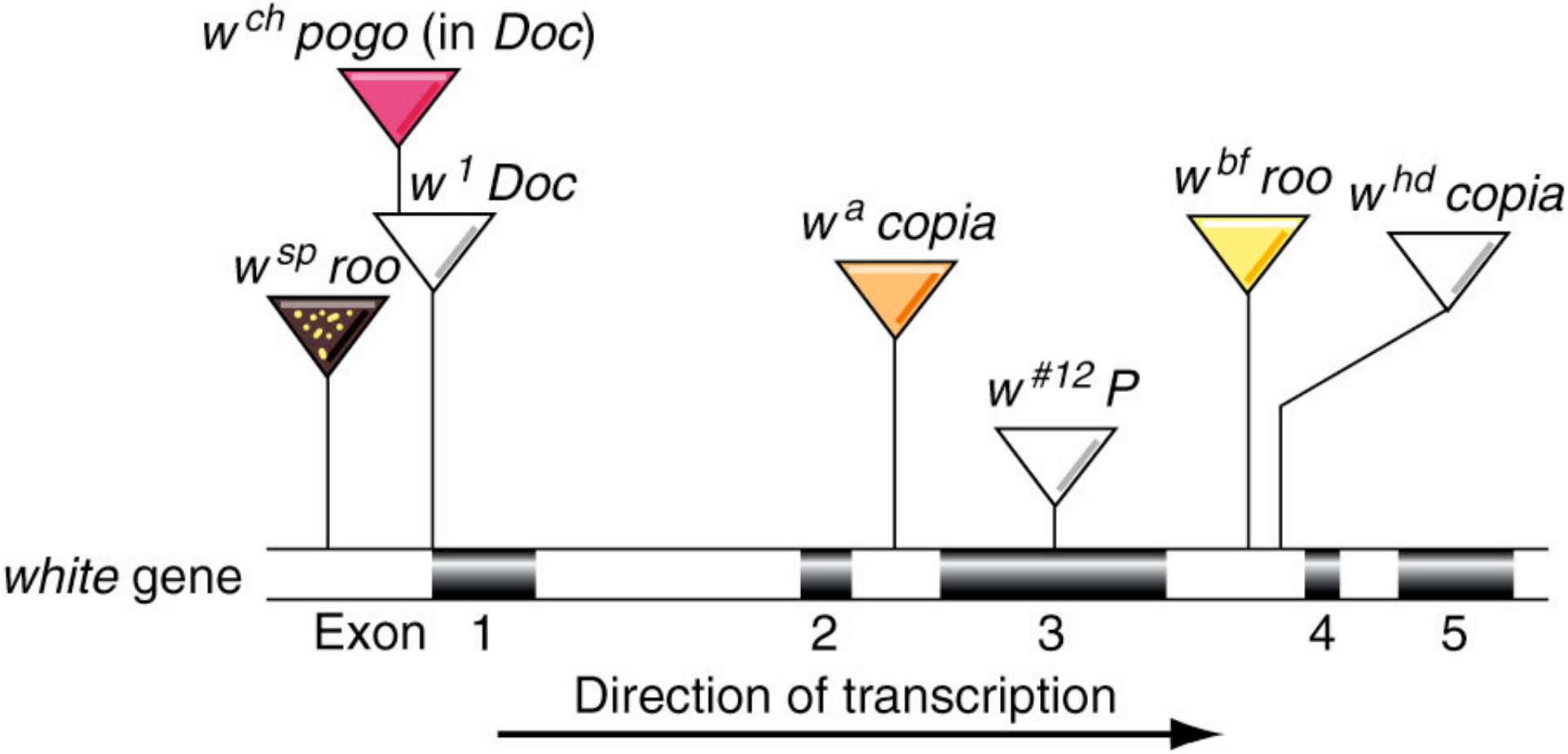
(b) How *P* element transposons move

Fig. 13.24b



Transposons can insert into different regions of a gene

Fig. 13.25



***Ac/Ds* are Maize transposons**

Ac/Ds can function in other plants

Ac: activator, autonomous, 4.6 kb long, enocdes a 3.5 kb transcript of transposase

Ds: dissociation, non autonomous

-both *Ac* and *Ds* have 11 bp inverted repeats at the ends, which function in the transposase recognition

-an 8 bp direct repeat generated from the host genome--footprint

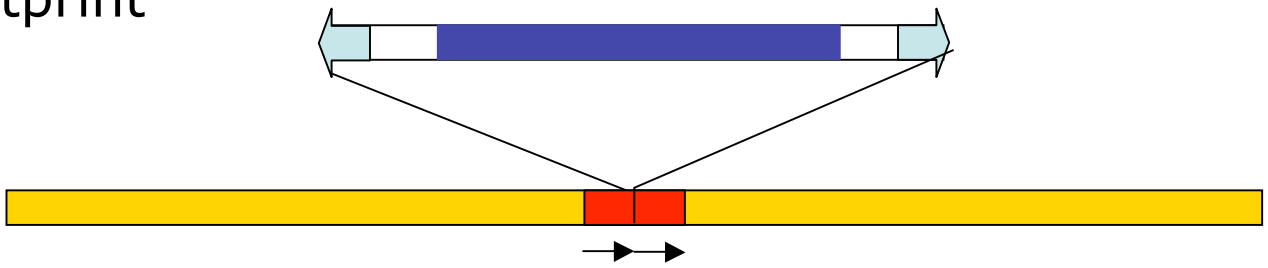
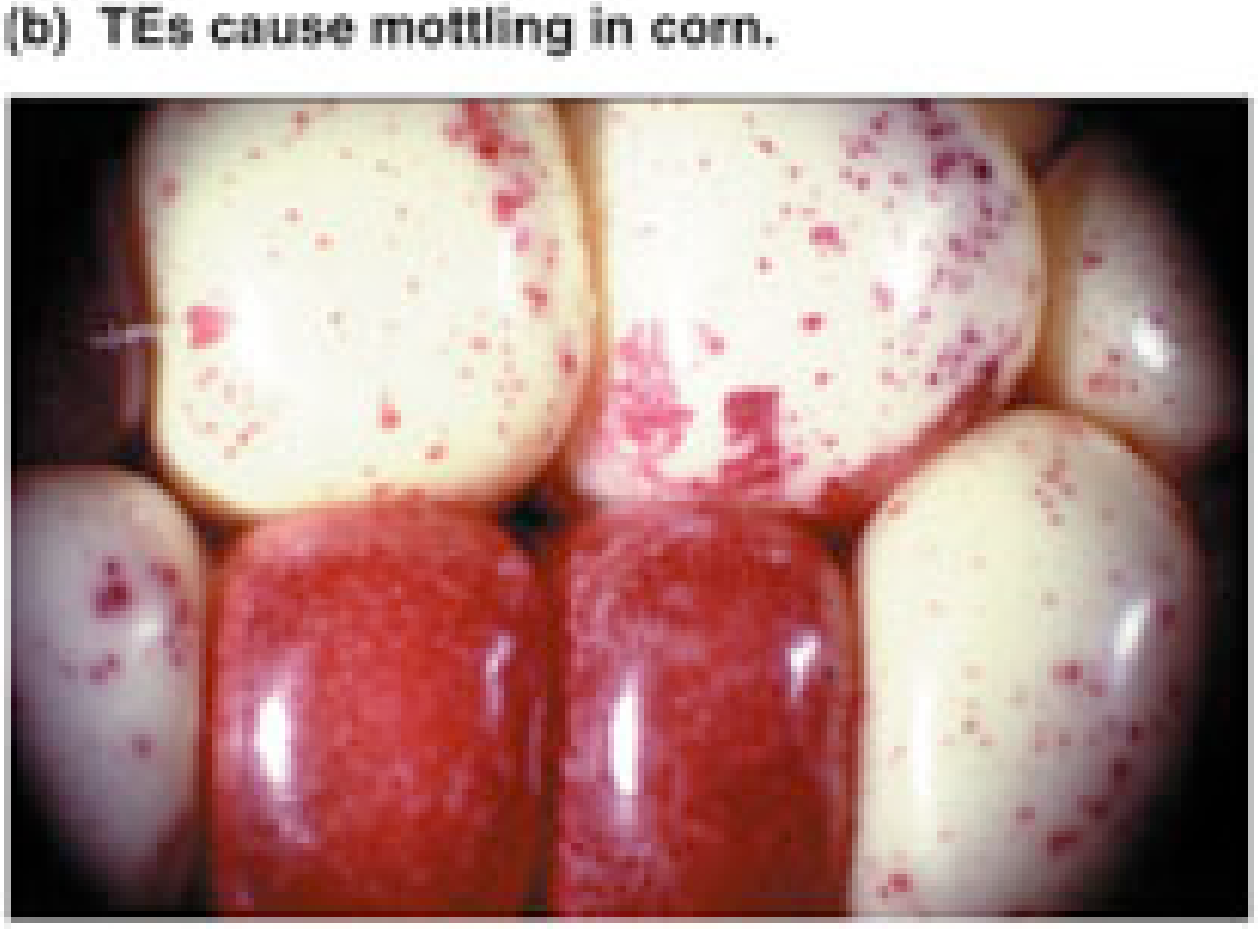


Fig. 13.22



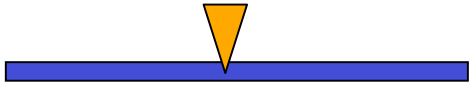
Ac/Ds in Corn Kernels

Fig. 13.20

Barbara McClintock

T-DNA for Plant transposon-tagging

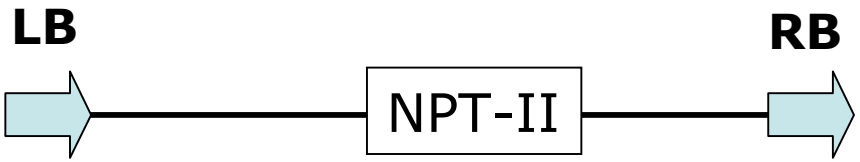
Transposon mutagenesis facilitates gene cloning



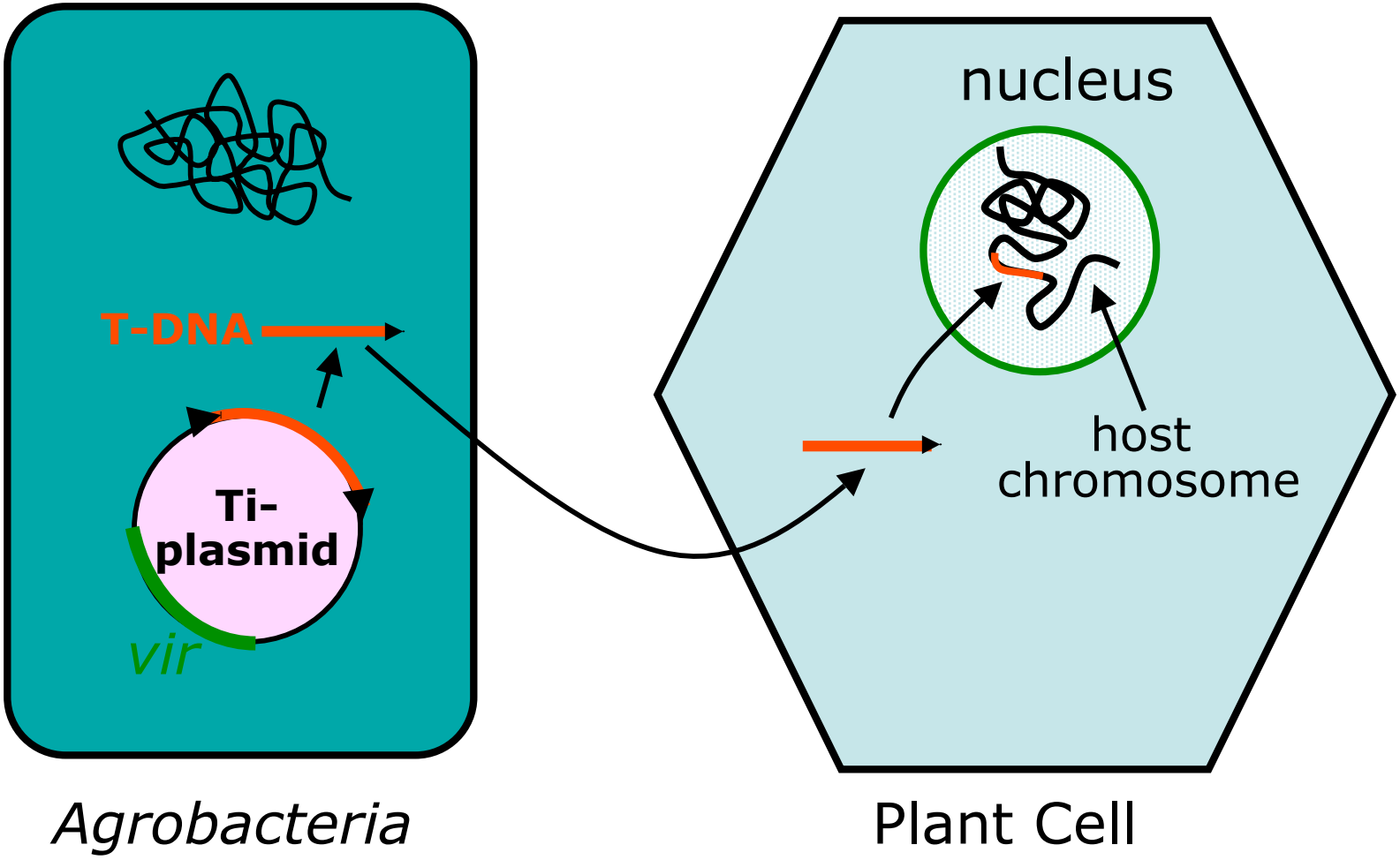
1. T-DNA (transfer DNA) from *Agrobacterium tumefaciens*

Agrobacterium causes crown-gall diseases in plants. This tumor-inducing (Ti) ability is linked to the Ti-plasmid.

During the infection, a segment of the Ti-plasmid, the T-DNA, is transferred into the plant cell and integrated into the plant genome.



Principles of gene transfer from *Agrobacteria* into plant cells



***vir*: *vir* region (vir = virulence)**
Ti: tumor-inducing plasmid

2. Mutagenesis

Screen:

Visual: flower morphology, pigment color,

Biochemical pathway mutant

auxotrophy: $his3^-$ in yeast; arg^- in Neurospora; $trp1^-$ in plant

Reporter gene expression (luc bioluminescent protein from firefly)

Selection:

trp pathway: 5-methylanthranilate ---> 5'methyltrp (toxic)

ADH (alcohol dehydrogenase): Allyl alcohol---> acrolein aldehyde (toxic)

Lethal mutations: (such as house keeping genes)

-maintain as heterozygote

-weak hypomorph

-conditional such as temperature-sensitive mutants in T4 phase (p220-221)

WT *Arabidopsis* flower

***ap2-2* floral mutant**

sex-determination mutant
(*tsl2 =tassel seed 2*)

Fig. 1.11

Fig. 7.14

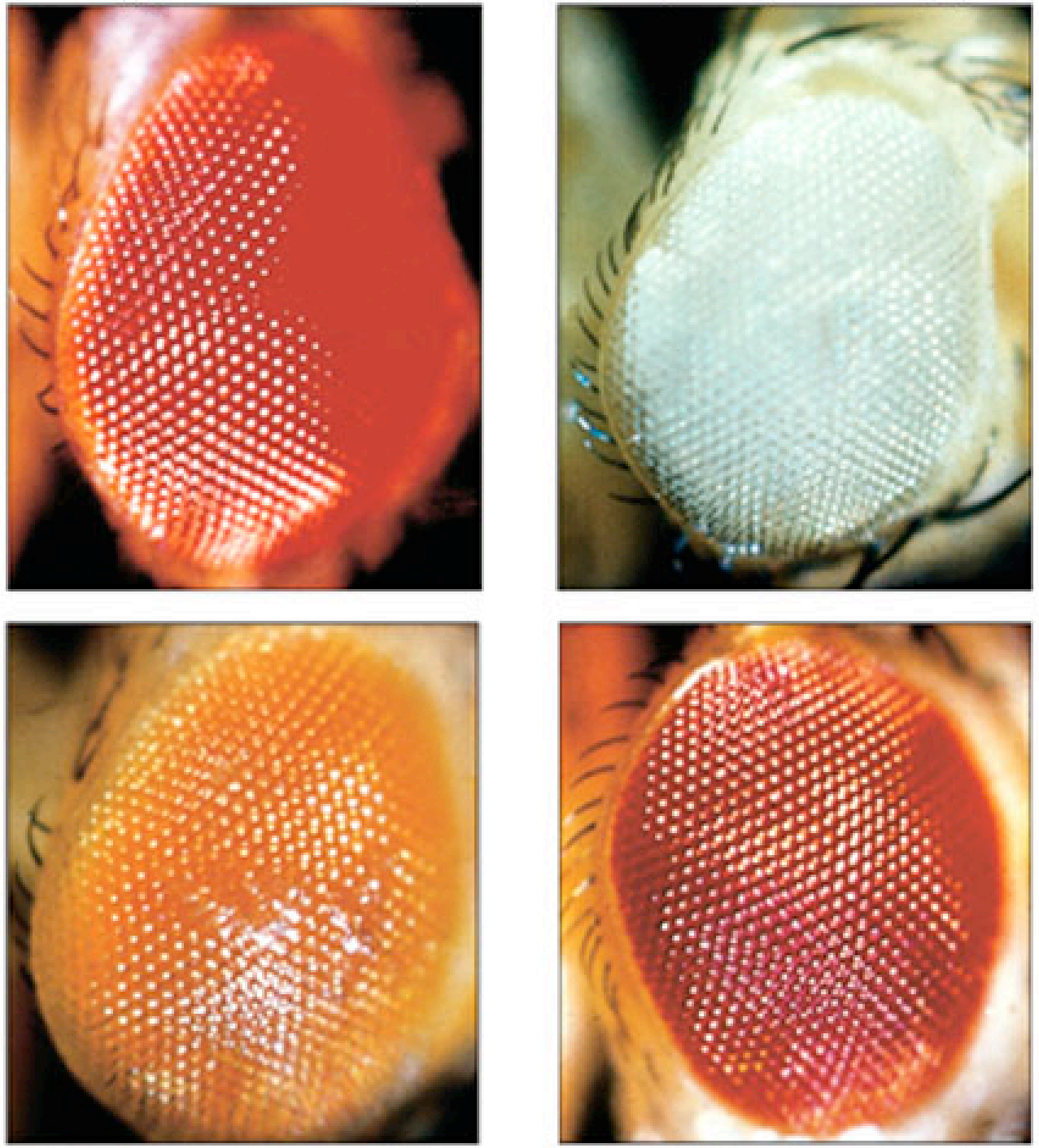
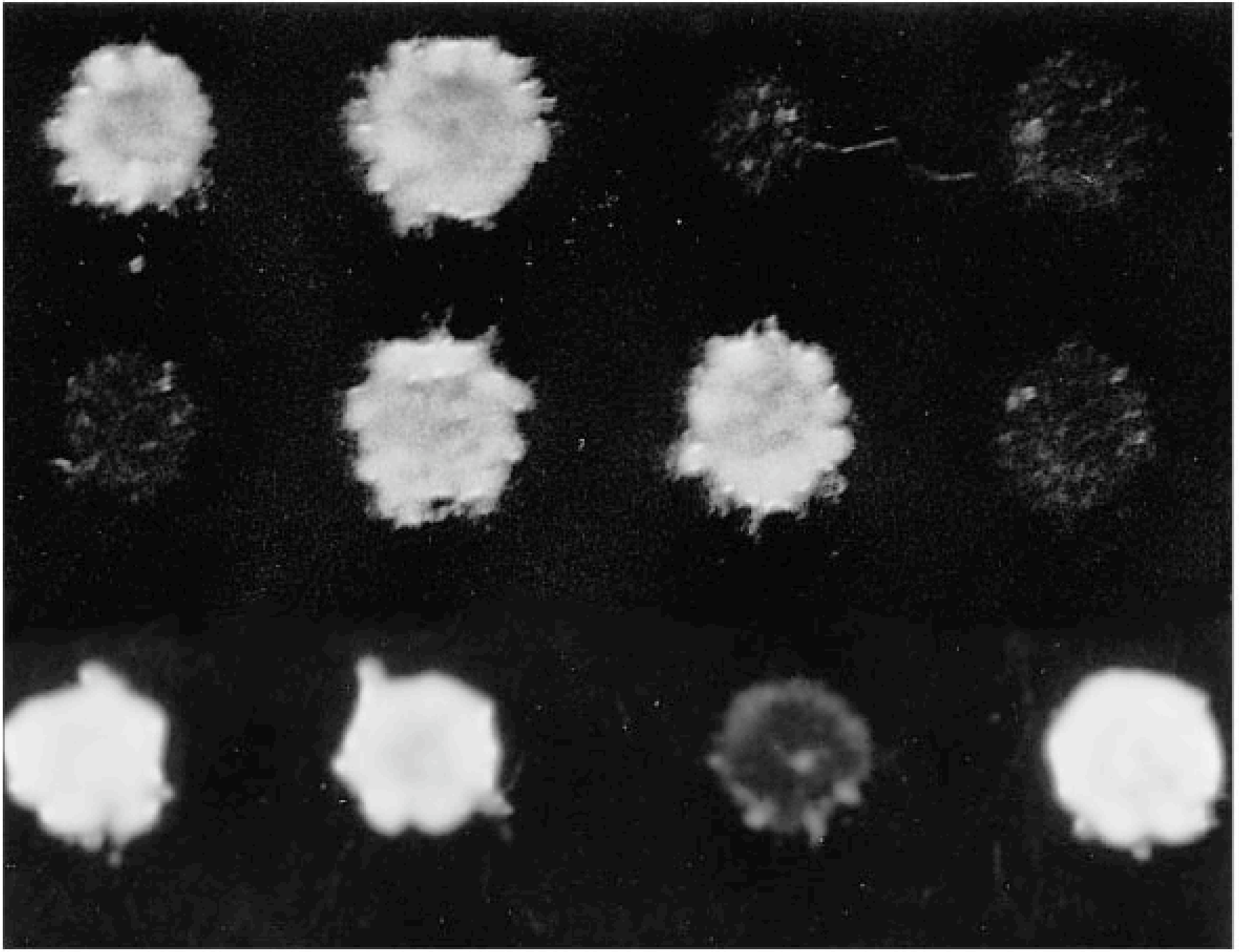


Fig. 5.21



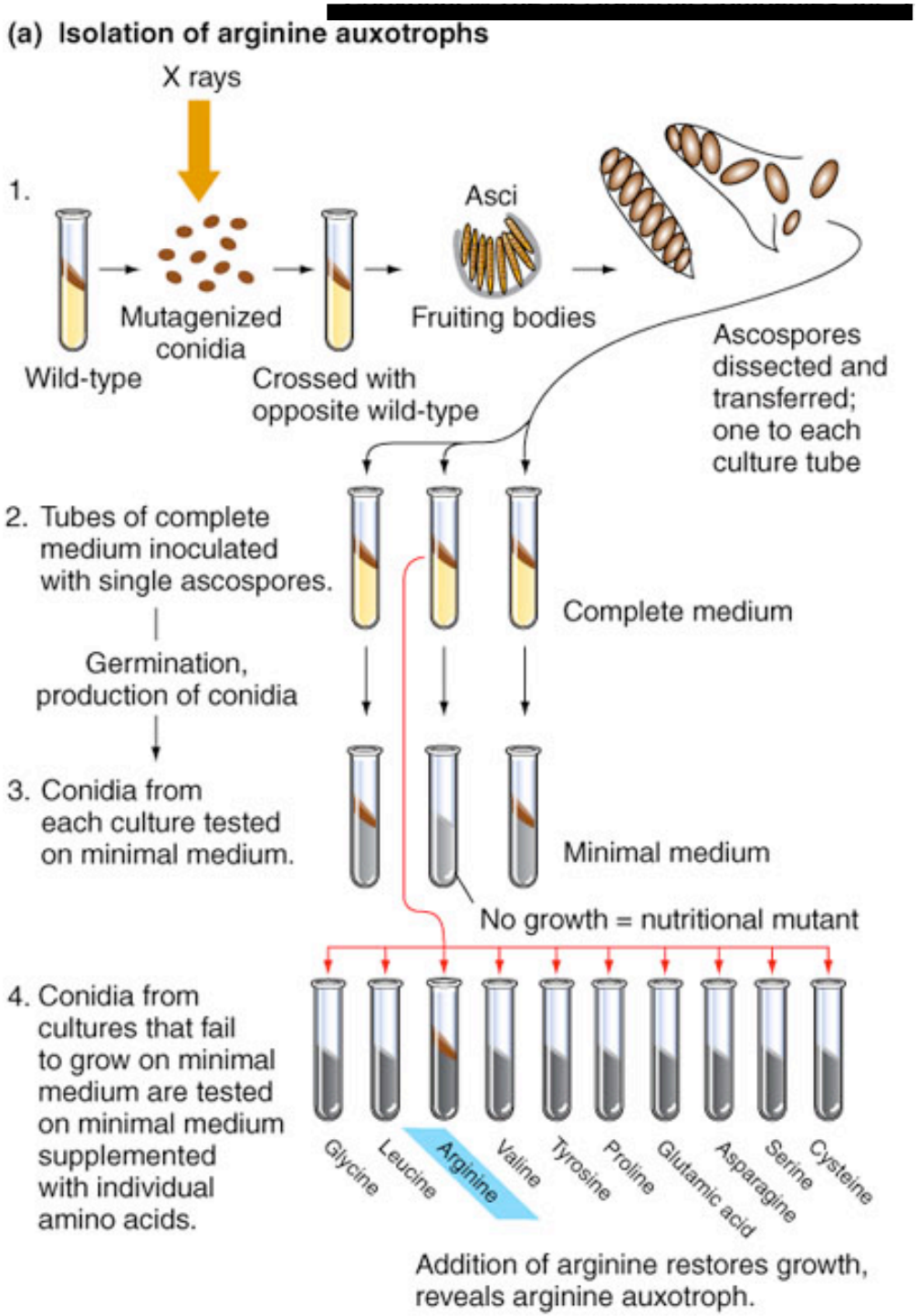
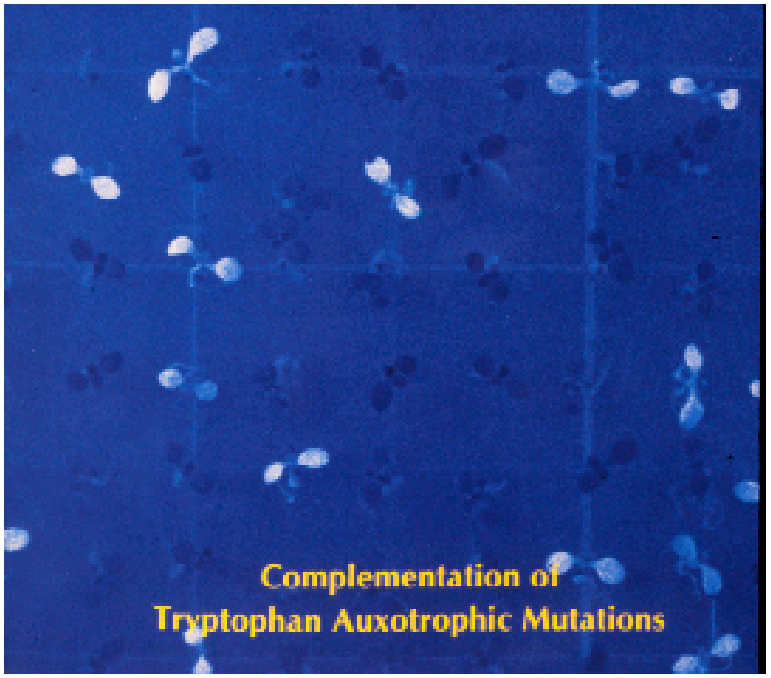

(a) Isolation of arginine auxotrophs
X rays
1.
Asci
Mutagenized conidia
Fruiting bodies
Wild-type
Crossed with opposite wild-type
Ascospores dissected and transferred; one to each culture tube
2. Tubes of complete medium inoculated with single ascospores.
Complete medium
Germination, production of conidia
3. Conidia from each culture tested on minimal medium.
Minimal medium
No growth = nutritional mutant
4. Conidia from cultures that fail to grow on minimal medium are tested on minimal medium supplemented with individual amino acids.
Glycine
Leucine
Arginine
Valine
Tyrosine
Proline
Glutamic acid
Asparagine
Serine
Cysteine
Addition of arginine restores growth, reveals arginine auxotroph.

Fig. 7.20 a



Tryptophan biosynthetic pathway

Chorismate

↓ trp4 (AS)

Anthranilate (blue fluorescent under UV)

↓ trp1(PAT)

Phosphoribosyl (PR) anthranilate (blue fluorescent under UV)

↓ PAI

CDRP

↓

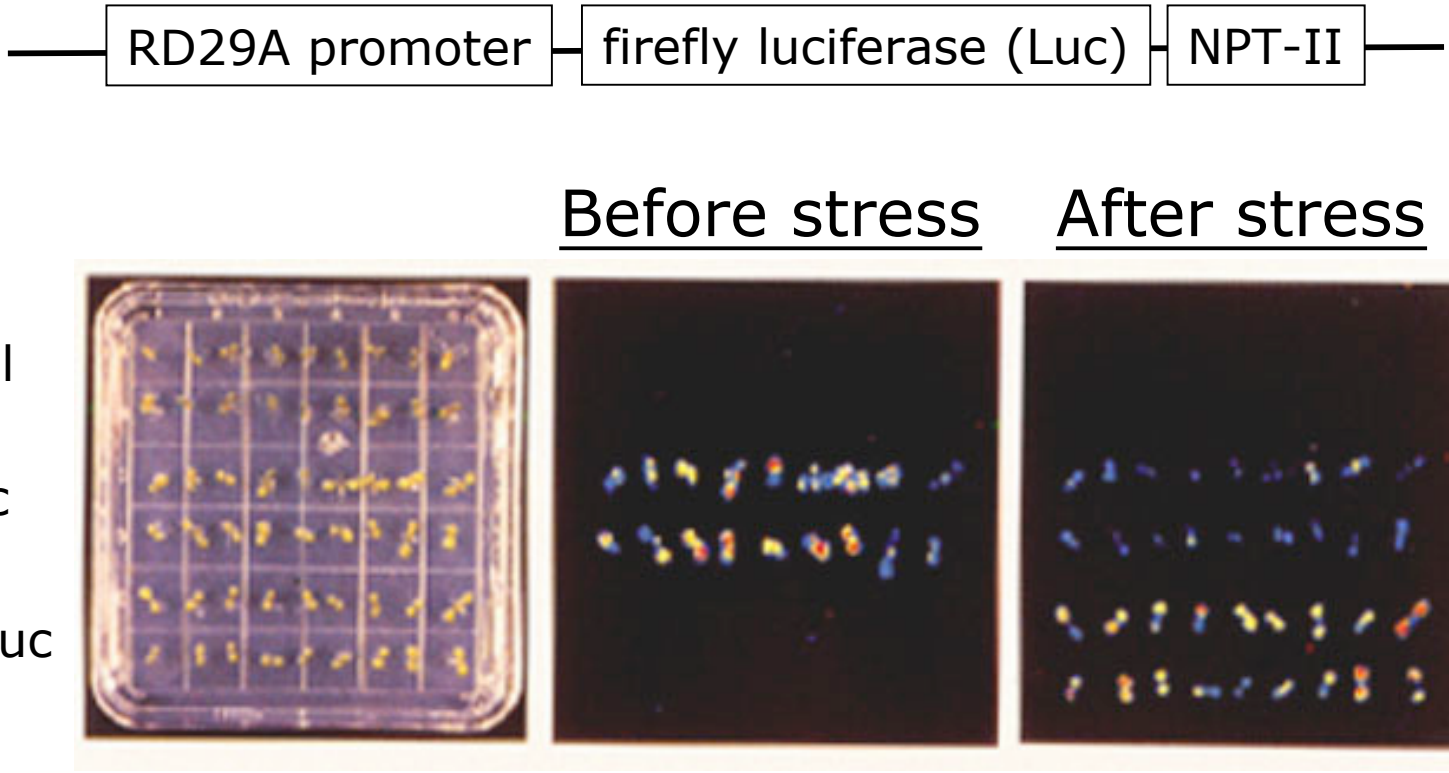
Indole-3-glycerol phosphate

↓

Indole

↓

L-tryptophan (amino acid)



Mutagenizes RD29A-Luc transgenic plants
to look for mutants that stop fluorescence under cold stress

Fig. C.8