Lecture 3 Mutagens and Mutagenesis

1. Mutagens

A. Physical and Chemical mutagens

B. Transposons and retrotransposons

C. T-DNA

2. Mutagenesis

A. Screen

B. Selection

C. Lethal mutations

Read: 606-612

Figs: 14.10-13

Transposon (transposable element) as a mutagen

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

(1) Retrotransposons

Copia	Drosophila (LTR-type)
Ty1	Yeast (LTR-type)
LINEs	Human (non-LTR-type)
SINEs (Alu)	Human (non-LTR-type)

(2) Transposons

Ac/Ds	Maize
P-element	Drosophila

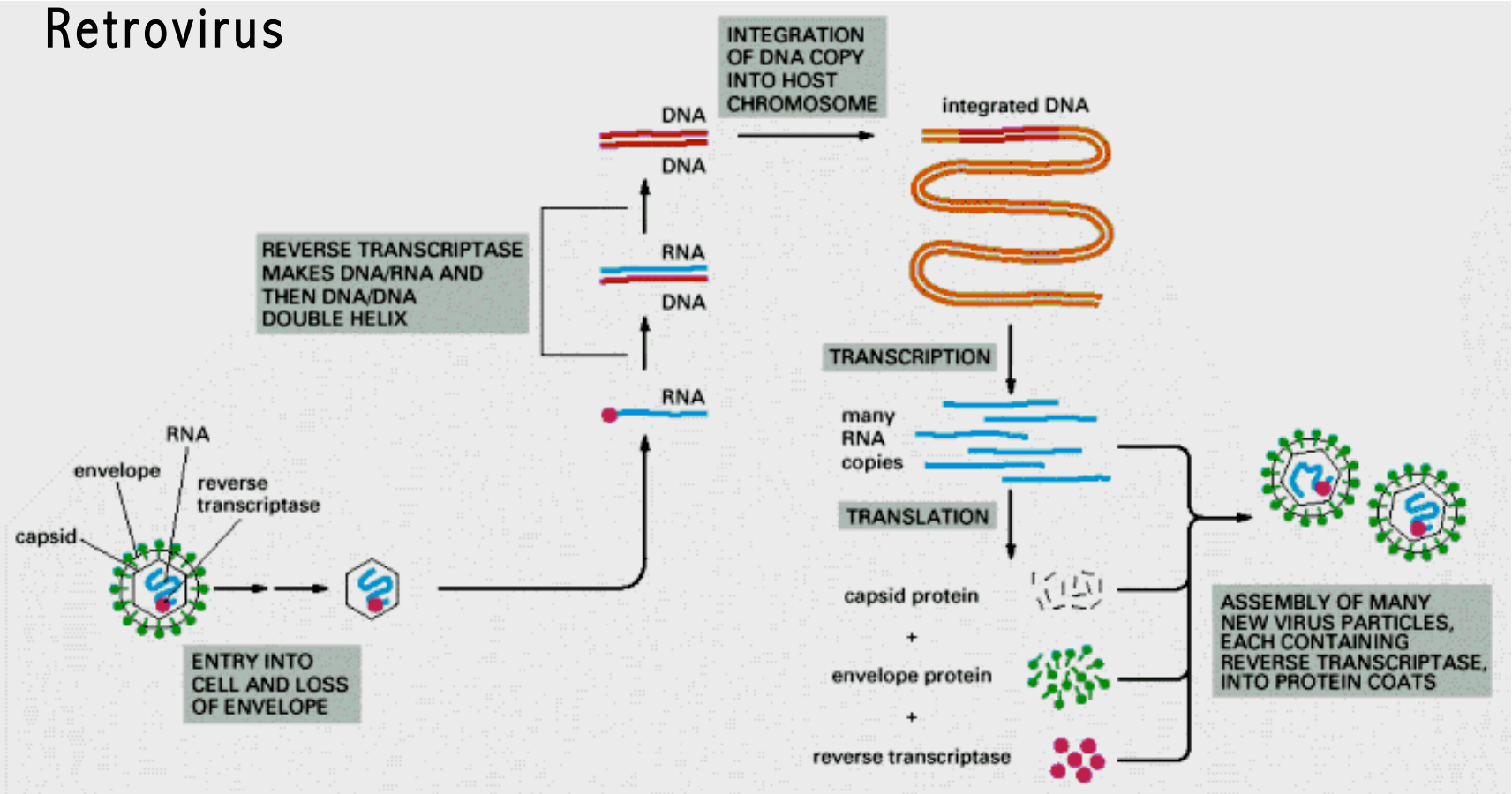
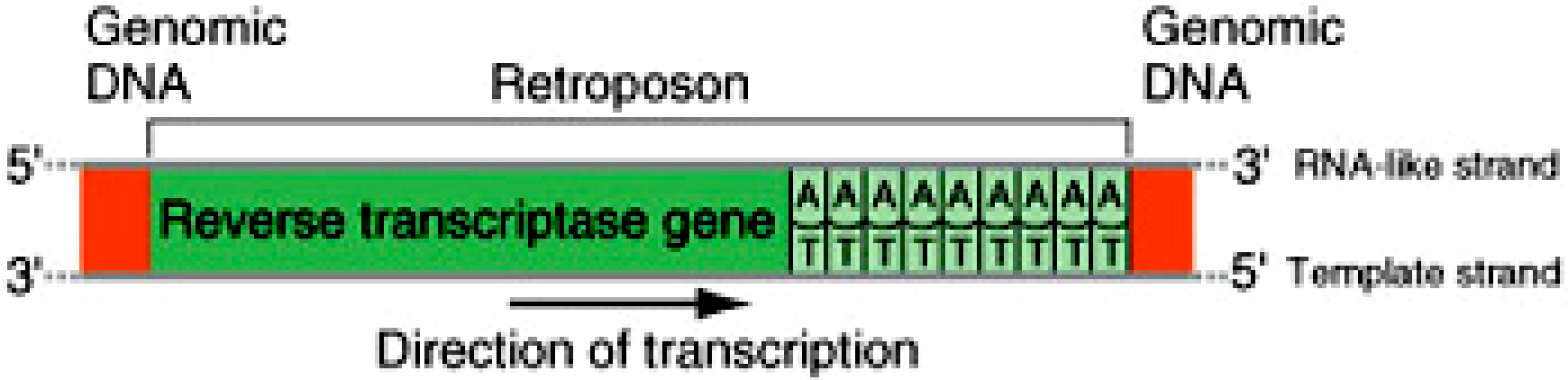
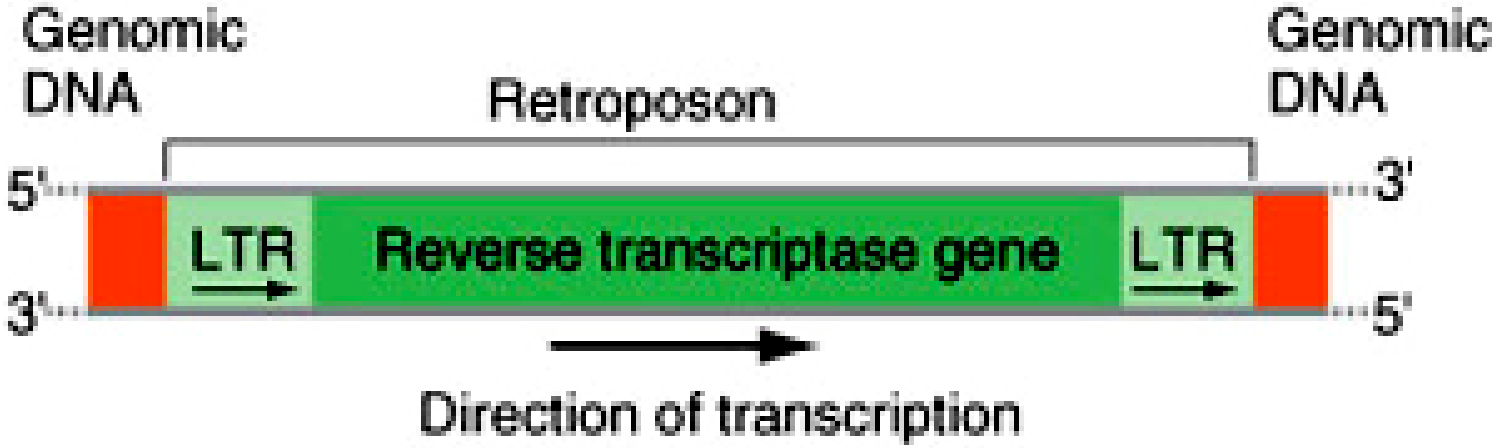
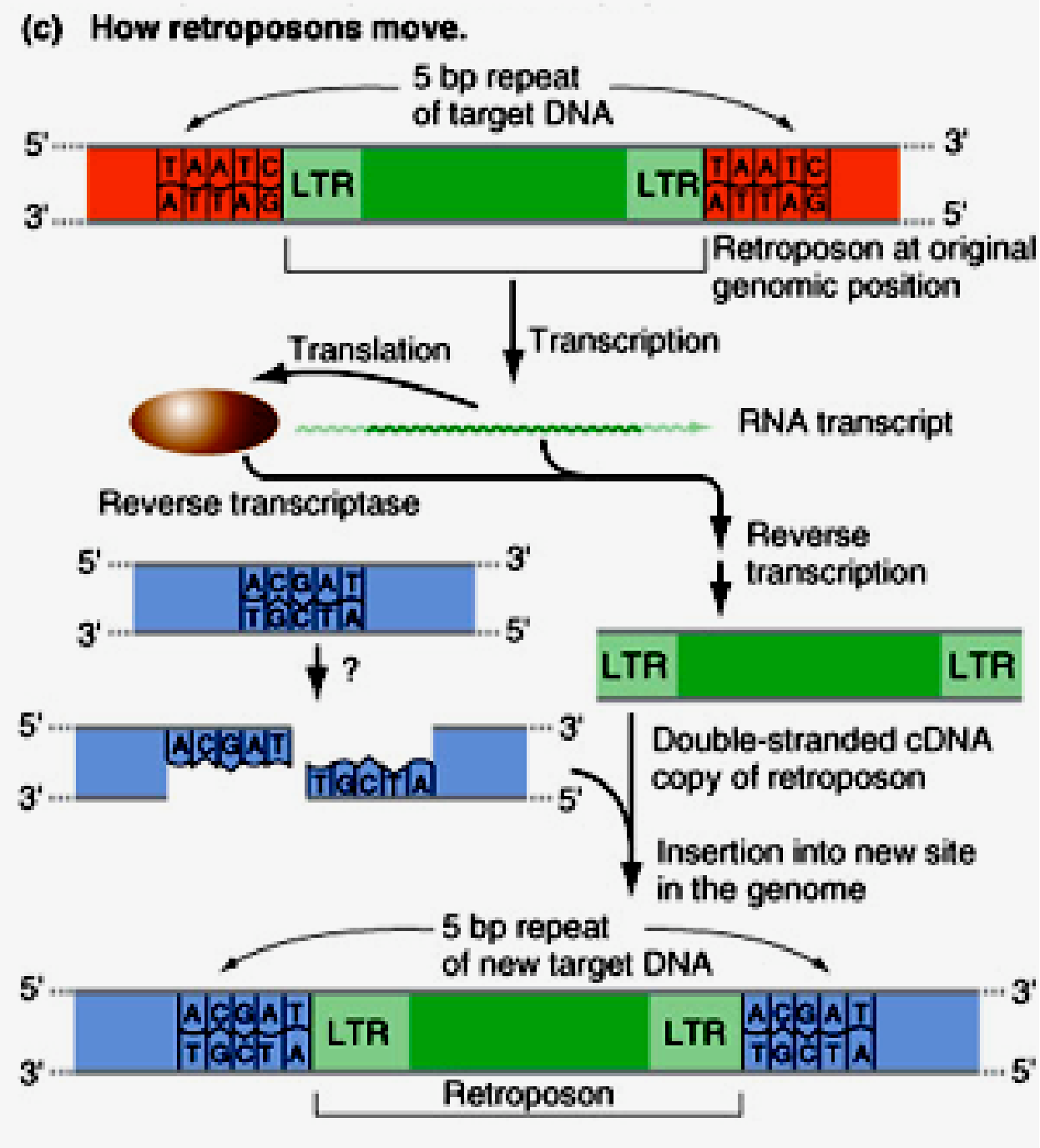


Figure 5-73. The life cycle of a retrovirus. The retrovirus genome consists of an RNA molecule of about 8500 nucleotides; two such molecules are packaged into each viral particle. The enzyme reverse transcriptase first makes a DNA copy of the viral RNA molecule and then a second DNA strand, generating a double-stranded DNA copy of the RNA genome. The integration of this DNA double helix into the host chromosome is then catalyzed by a virus-encoded integrase enzyme. This integration is required for the synthesis of new viral RNA molecules by the host cell RNA polymerase, the enzyme that transcribes DNA into RNA. (From Molecular Biology of the Cell by Alberts et al.)

(a) **Two kinds of retroposons.**






(c) How retroposons move.
5 bp repeat of target DNA
5'
3'
T A A T C
A T T A G
LTR
LTR
T A A T C
A T T A G
3'
5'
Retroposon at original genomic position
Transcription
Translation
RNA transcript
Reverse transcriptase
Reverse transcription
5'
3'
A C G A T
T G C T A
3'
5'
?
LTR
LTR
5'
3'
A C G A T
T G C T A
3'
5'
Double-stranded cDNA copy of retroposon
Insertion into new site in the genome
5 bp repeat of new target DNA
5'
3'
A C G A T
T G C T A
LTR
LTR
A C G A T
T G C T A
3'
5'
Retroposon

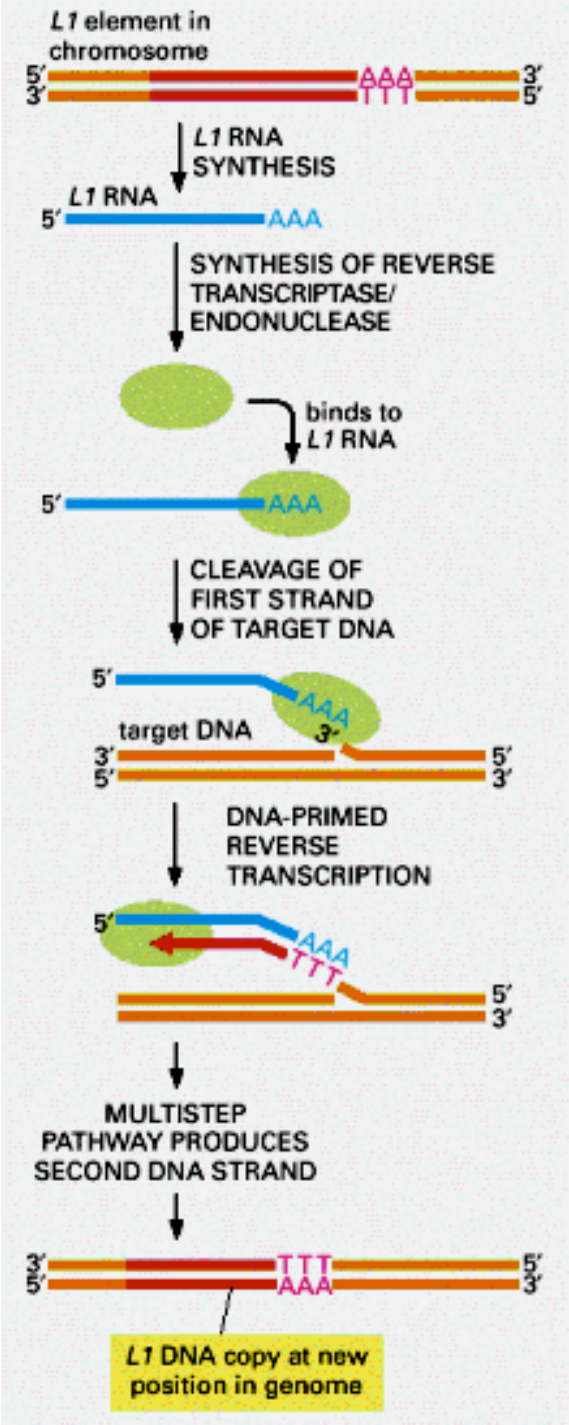


Figure 5-76. Transpositional site-specific recombination by a nonretroviral retrotransposon. Transposition by the L1 element (red) begins when an endonuclease attached to the L1 reverse transcriptase and the L1 RNA (blue) makes a nick in the target DNA at the point at which insertion will occur. This cleavage releases a 3′ -OH DNA end in the target DNA, which is then used as a primer for the reverse transcription step shown. This generates a single-stranded DNA copy of the element that is directly linked to the target DNA. In subsequent reactions, not yet understood in detail, further processing of the single-stranded DNA copy results in the generation of a new double-stranded DNA copy of the L1 element that is inserted at the site where the initial nick was made.

(Based on Molecular Biology of the Cell by Alberts et al.)

Fig. 13.24a

P-element in Drosophila

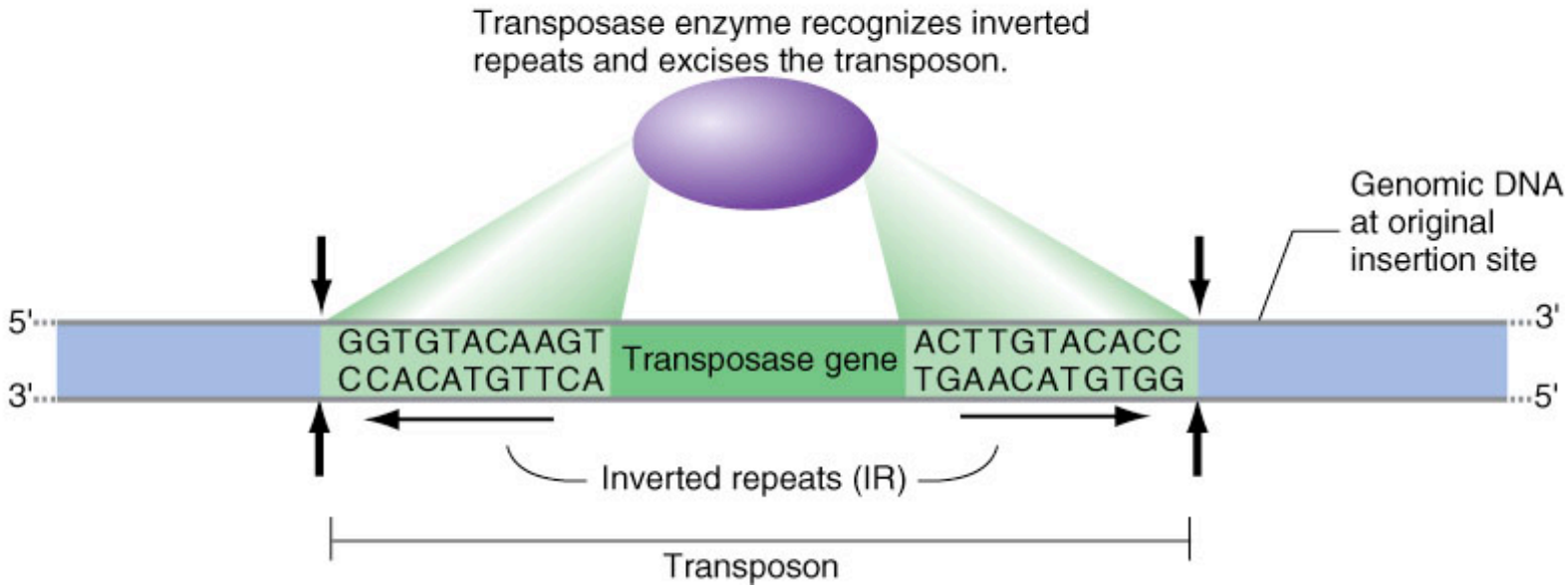
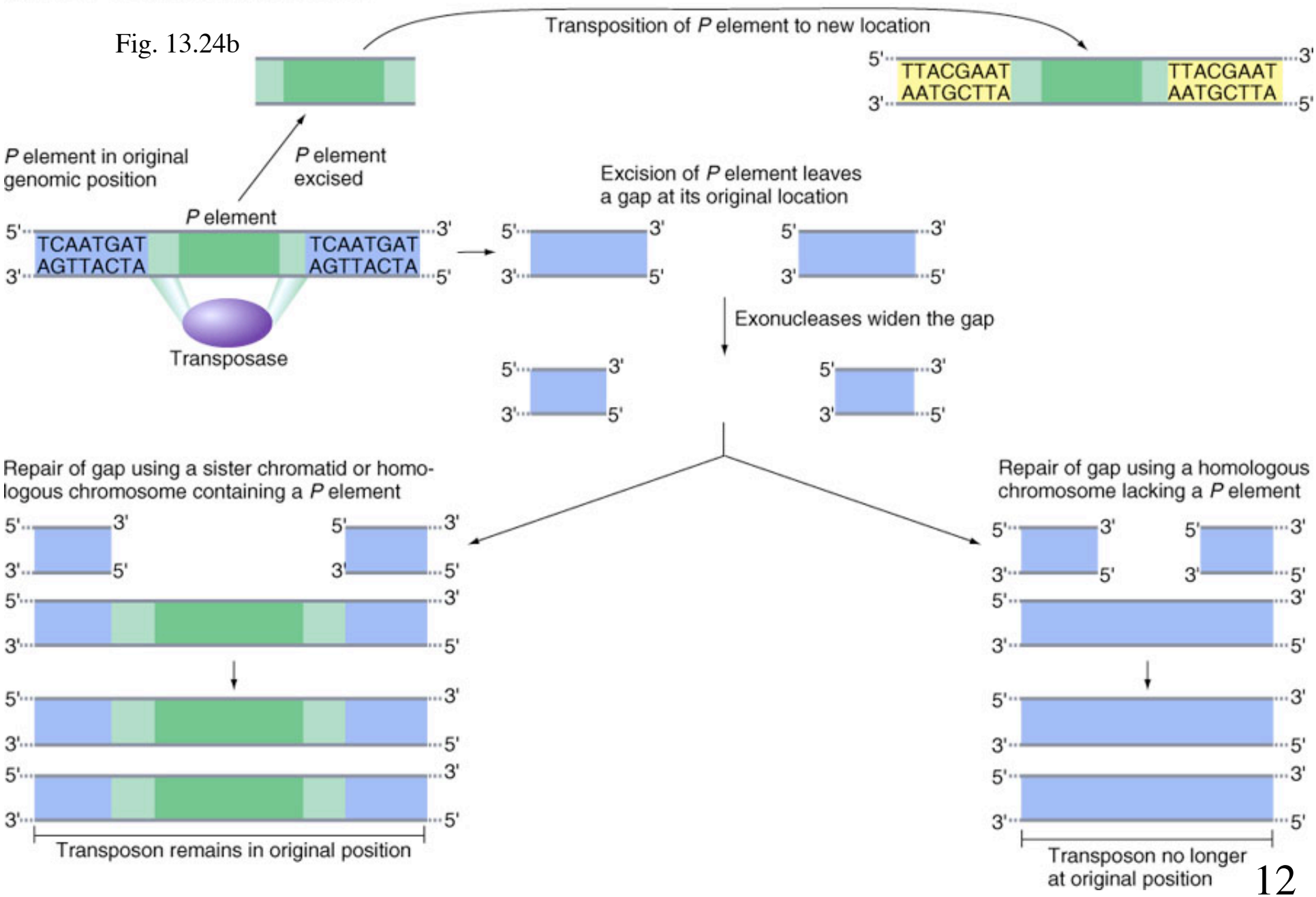
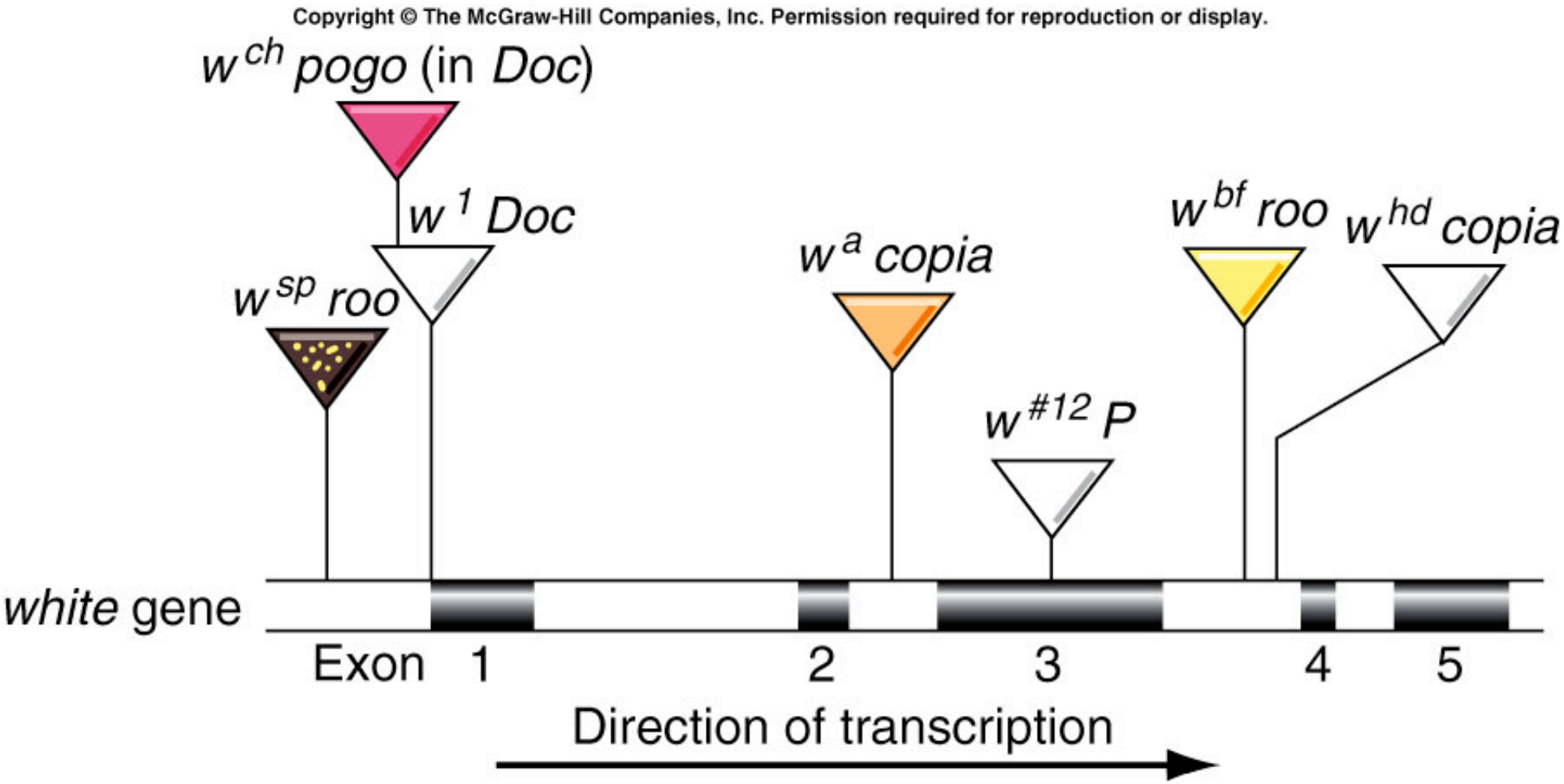


Fig. 13.24b



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

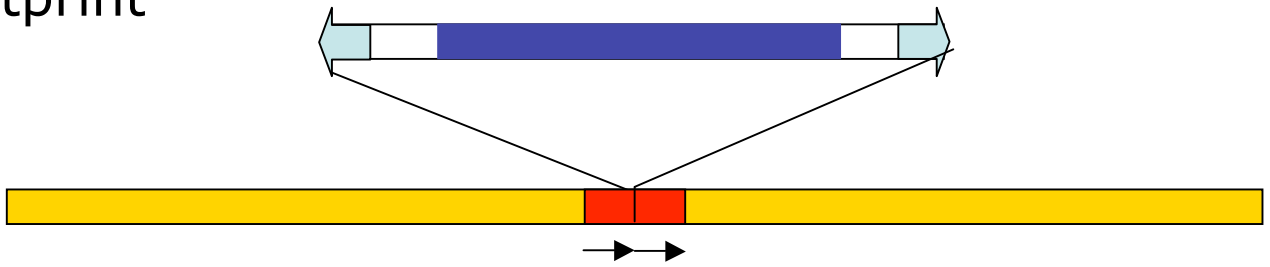


Fig. 13.22

(b) TEs cause mottling in corn.

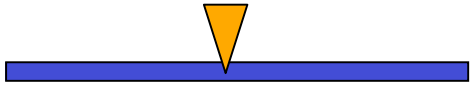
Ac/Ds in Corn Kernels

Fig. 13.20

Barbara McClintock

T-DNA for Plant transposon-tagging

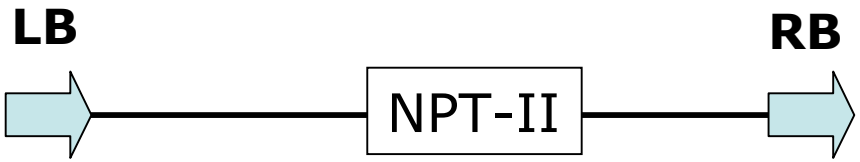
Transposon mutagenesis facilitates gene cloning



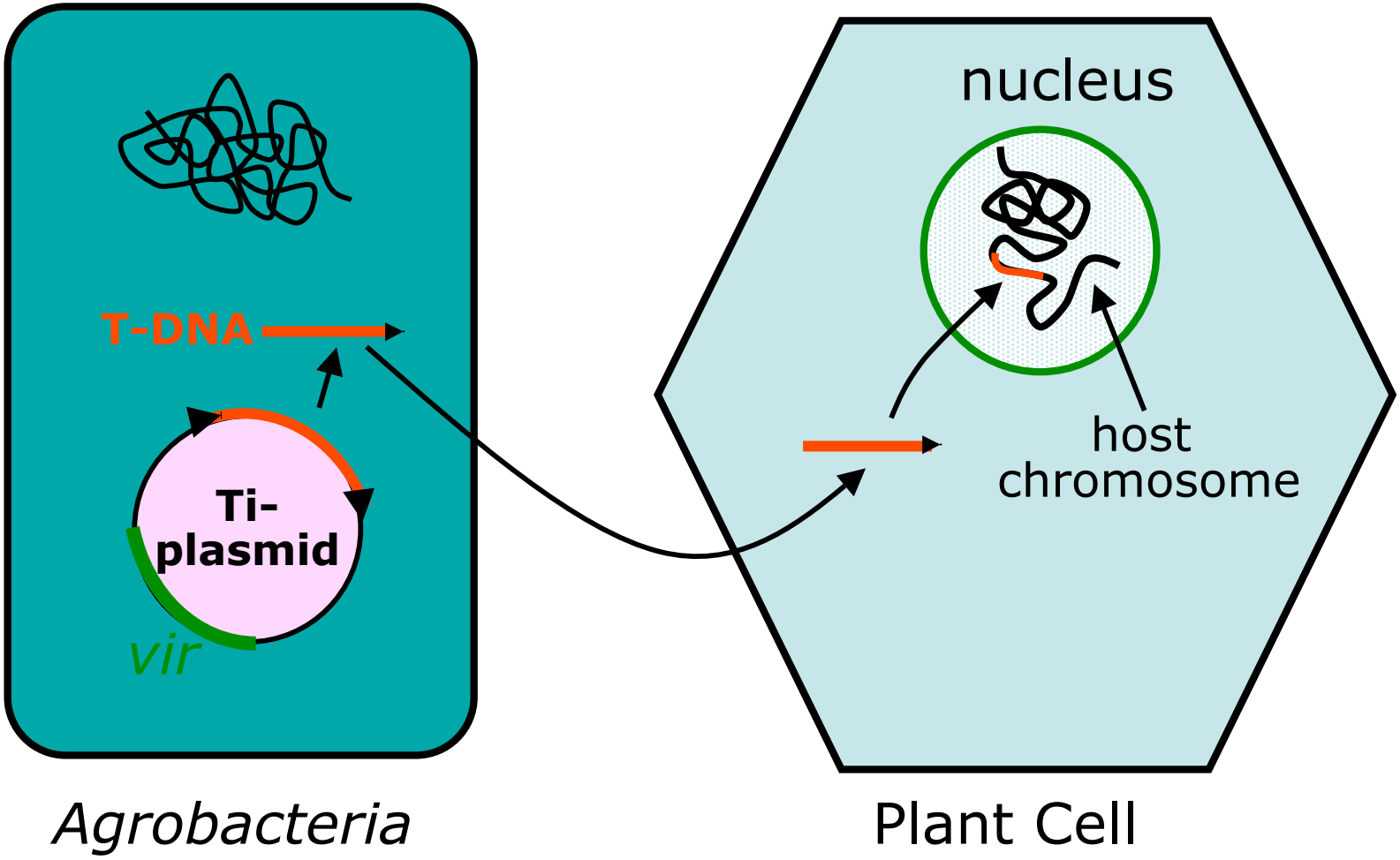
1. T-DNA (transfer DNA) from *Agrobacterium tumefaciens*

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

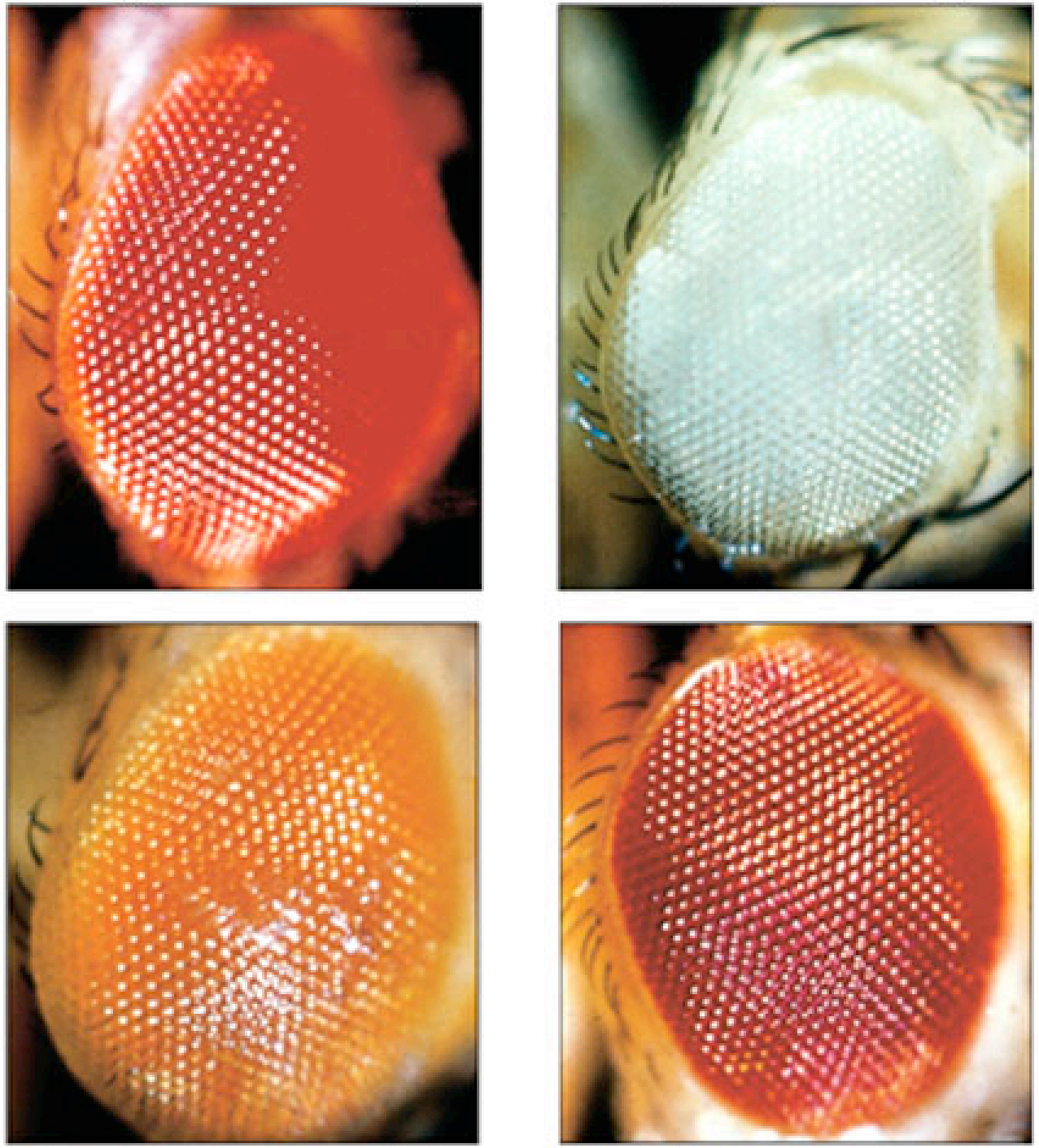
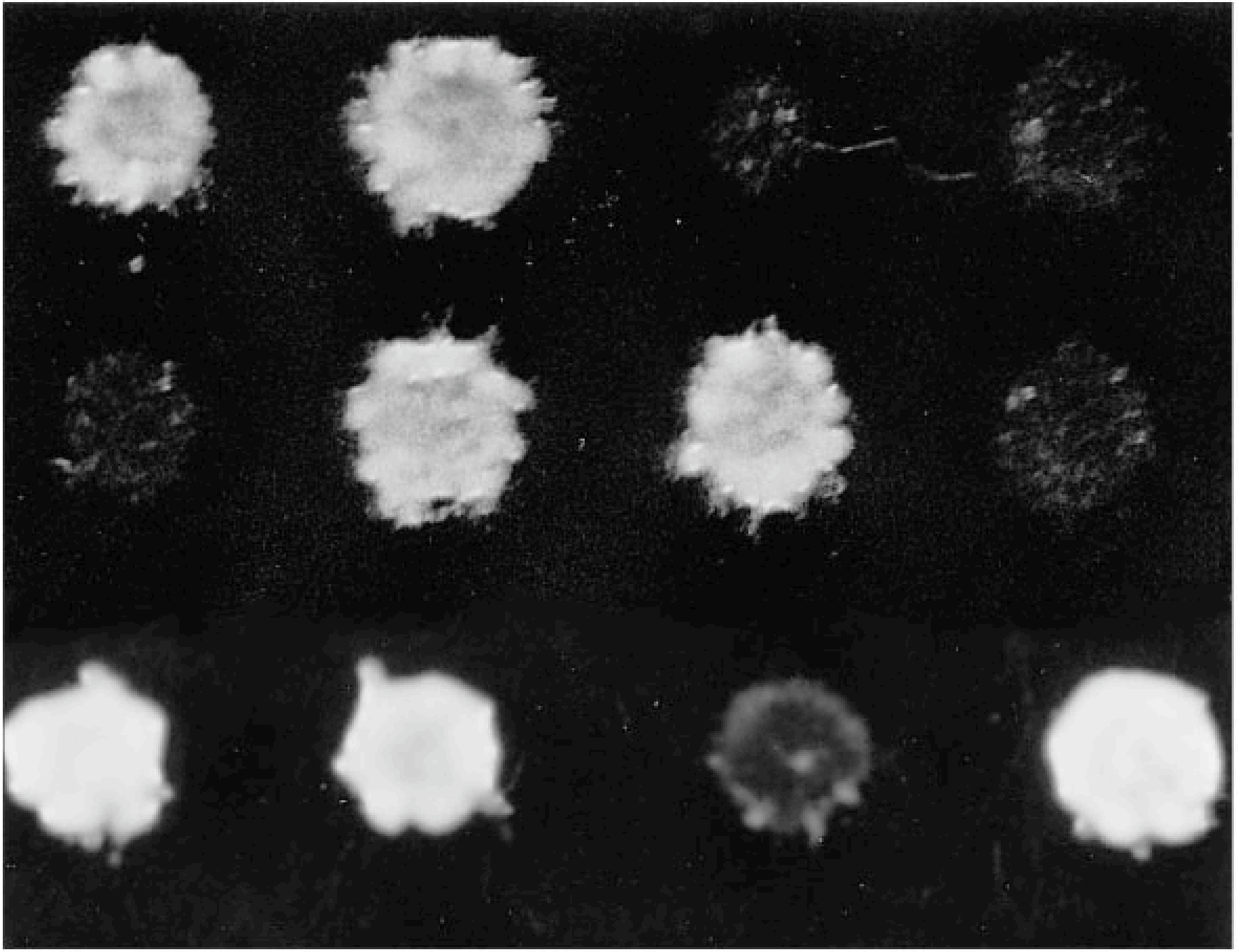


Fig. 5.21



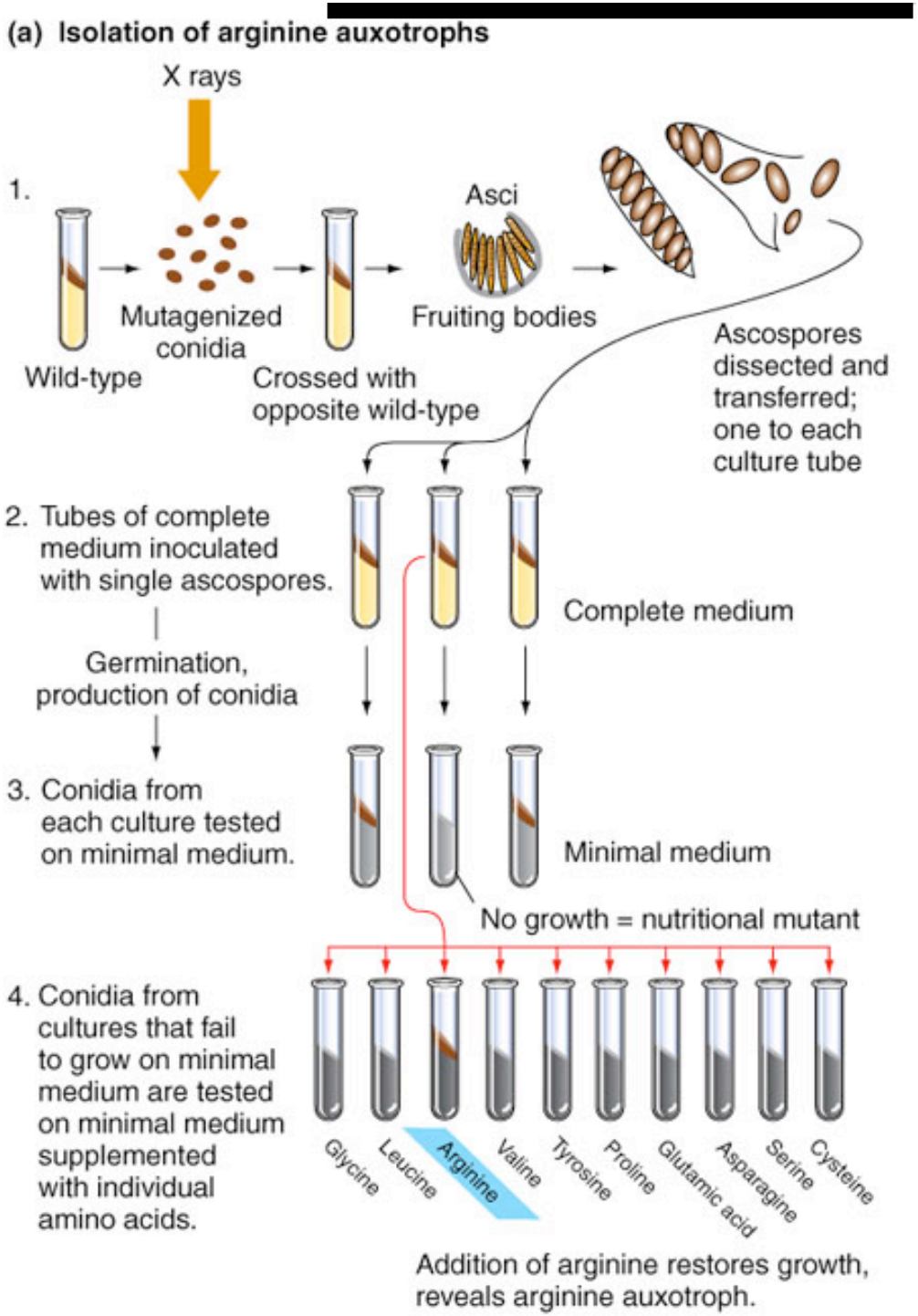
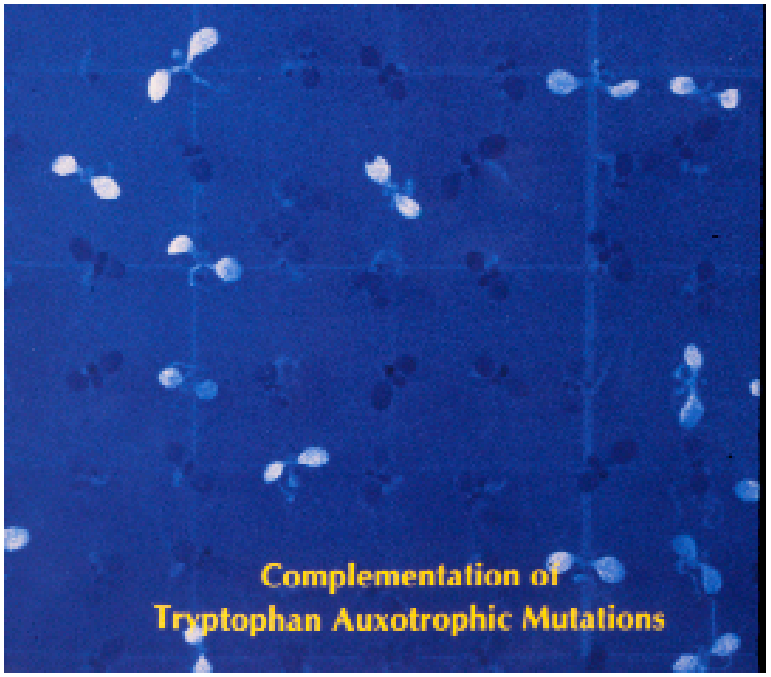

(a) Isolation of arginine auxotrophs
X rays
1.
Asci
Mutagenized conidia
Fruiting bodies
Wild-type
Crossed with opposite wild-type
Ascospores dissected and transferred; one to each culture tube
2. Tubes of complete medium inoculated with single ascospores.
Complete medium
Germination, production of conidia
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

↓

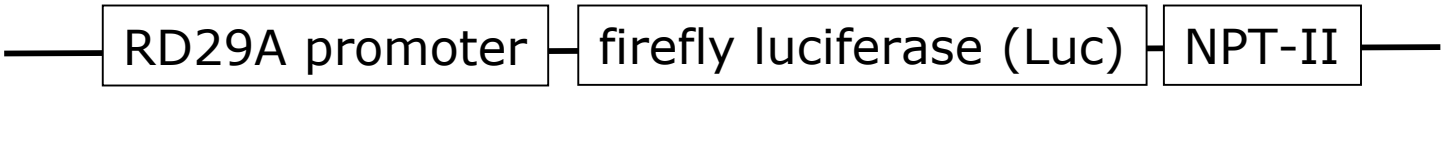
Indole-3-glycerol phosphate

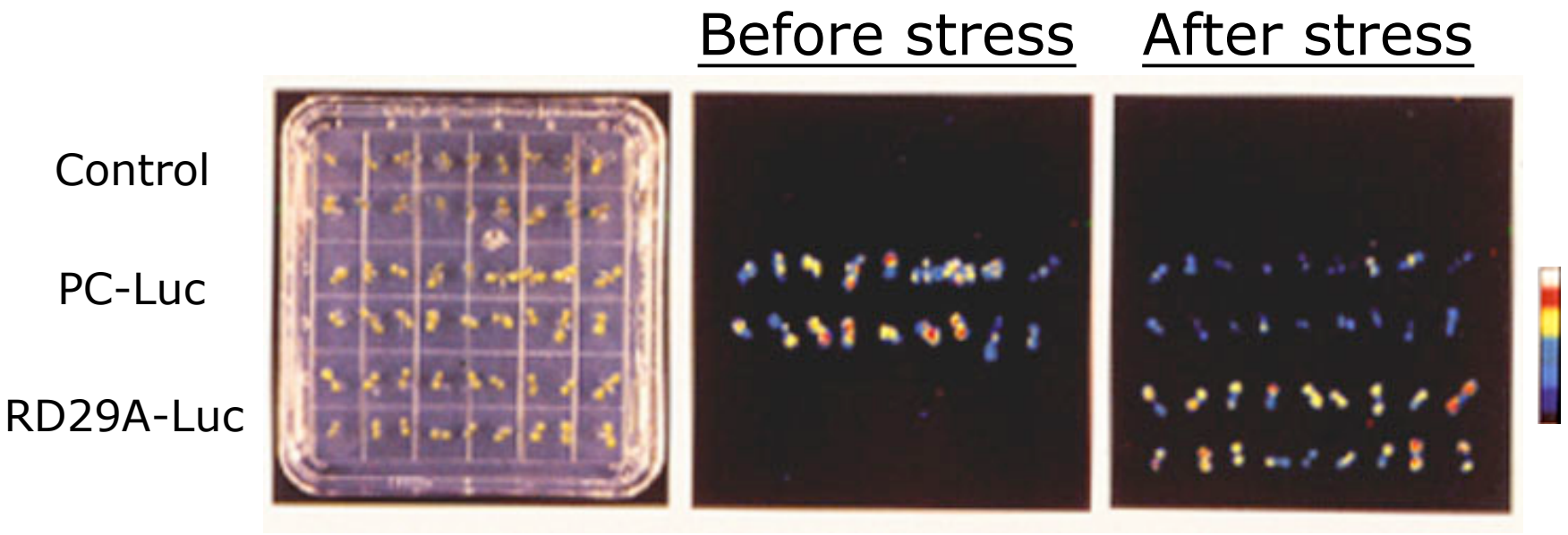
↓

Indole

↓

L-tryptophan (amino acid)





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

Fig. C.8