Lecture 1: Mutagen

Lecture 2: Mutagenesis and Mutant Types

Lecture 3: Mutant Characterization

I. Mutation

Changes in DNA, heritable, mostly devastating, few good

1. Substitution-1 base --> one of the three other bases

   Transition: purine --> purine or pyrimidine --> pyrimidine
   A--> G or G--> A   T--> C or C--> T

   Transvertion: purine --> pyrimidine or *vice versa*
   A--> T, C; G -->T,C; T-->A, G; C-->A,G

   causes missense, nonsense, silent, neutral or splicing mutational effects

2. Deletion or insertion-often causes frameshift mutation

3. Chromosomal rearrangement
   inversion or translocation can change multiple genes

Amino acid effects of point mutations

tyrosine TAT, TAC

Codon change	Amino acid change	Effect
TAT -> CAT	tyr -> his	misense
TAT -> TAA	tyr -> stop	nonsense
TAT -> TTT	tyr -> phe	neutral in many cases
TAT -> TAC	tyr-> tyr	silent

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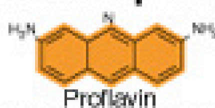
Frameshift mutations

(a) The mutagen proflavin can insert between two base pairs



Molecule of proflavin inserted between stacked base pairs

(b.1) Consequences of exposure to proflavin

rIIB+ Wildtype

Exposure to proflavin

FC0

rIIB−

Exposure to proflavin

FC0 FC7

rIIB+ revertant

Original mutation

Second mutation

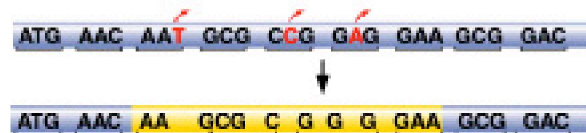
(b.2) Crossing *rIIB*+ revertant with wildtype yields *rIIB*− recombinants

FC0 FC7 *rIIB*− FC0 *rIIB*− FC7

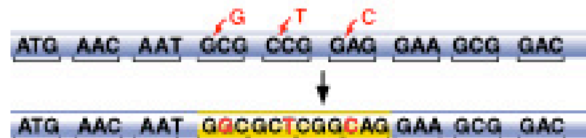
(c) Different sets of mutations generate either a mutant or a normal phenotype

Proflavin-induced mutations (+) insertion (−) deletion	Phenotype
− or +	Mutant
− − or + +	Mutant
− − − − or − − − − −	Mutant
− +	Wildtype
− − − or − − − − − − or + + + or + + + + + +	Wildtype

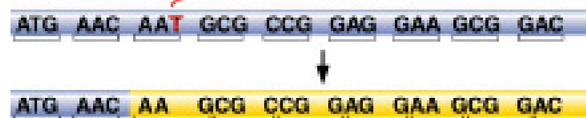
(d) Three single base deletions (− − −)



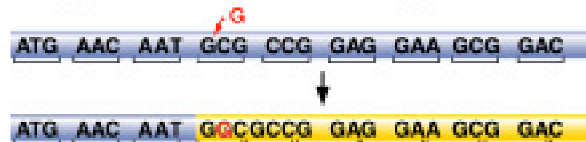
Three single base insertions (+ + +)



(e) Single base deletion (−)



Single base insertion (+)



correct triplet

incorrect triplet

II. Mutagen and Mutagenesis

1. Spontaneous mutation is rare: 2-12X 10^{-6} (per generation per gene)

Spontaneous mutations can be caused by

a. mistakes made during DNA replication (error rate 10^{-9})

b. environmrntal effect:

UV light: thymidine dimer

X-ray: break sugar-phosphate DNA back bone

Oxidative damages: G --> 8-oxodG (pair with A)

c. chemical changes (hydrolysis):

depurination; A,G --> O

deamination: C--> U

2. 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)

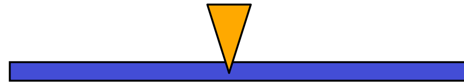
deaminating agent such as nitrous acid

intercalating agent such as Acridine Orange

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

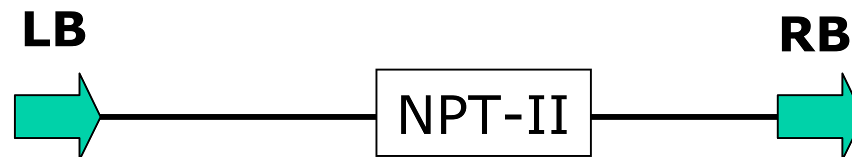
Plant transposon-tagging

Transposon mutagenesis facilitates gene cloning

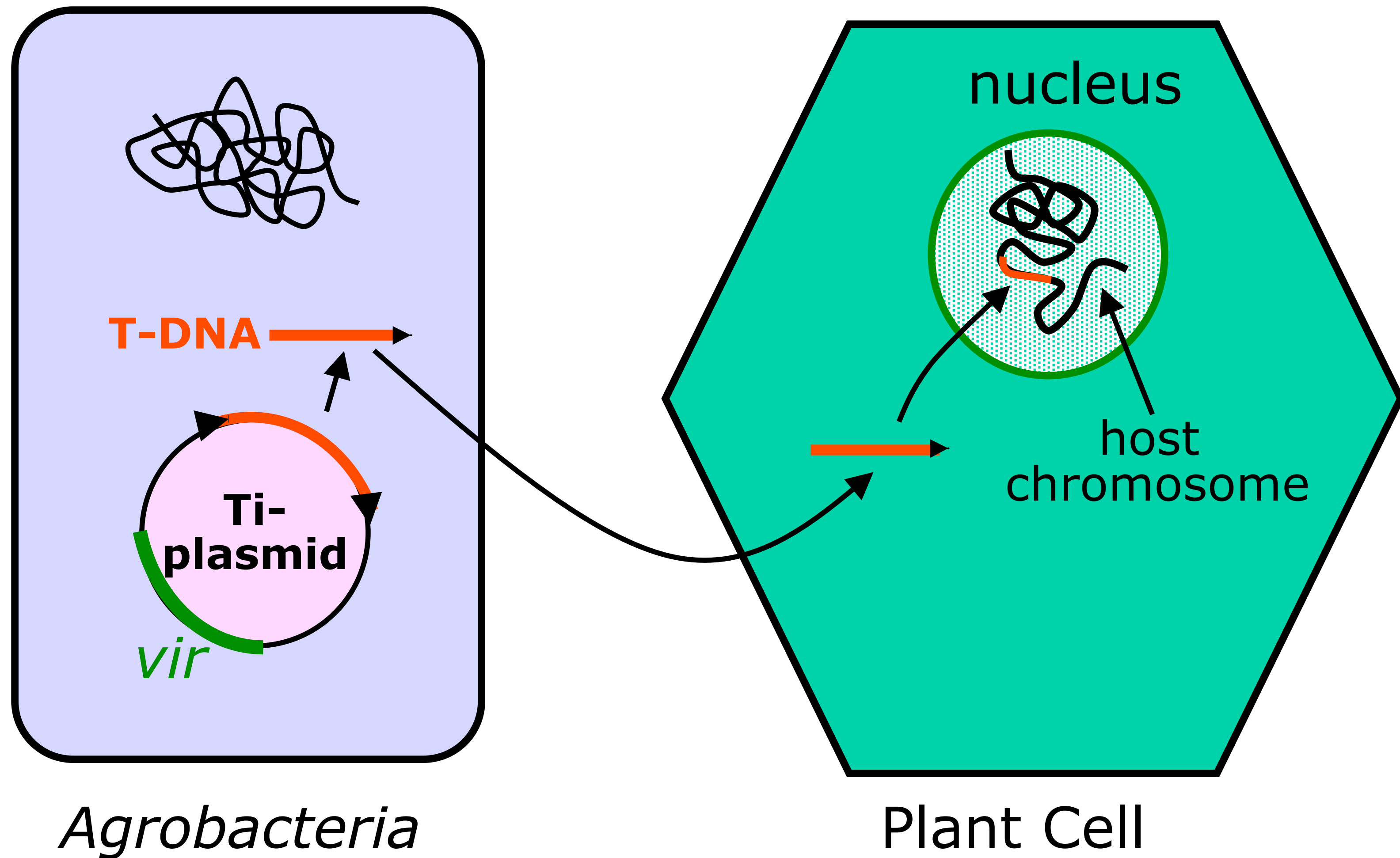


1. T-DNA (transfer DNA) from *Agrobacterium tumefaciens*

Agrobacterium causes crown-gall diseases in plants (page 877 Buchanan book).
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

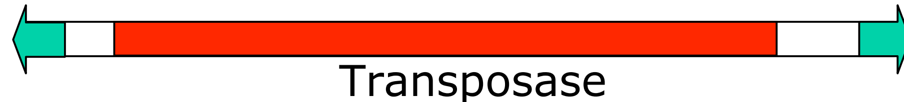


vir: ***vir*** **region (vir = virulence)**
Ti: tumor-inducing plasmid

2. *Ac/Ds:* Maize transposons. Ac/Ds can function in other plants

(chapter 7 page 334-335)

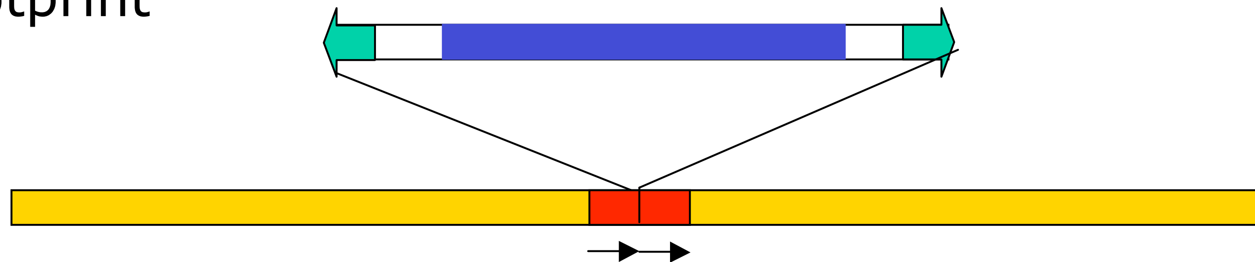
Ac: activator, autonomous, 4.6 kb long, enocdes a 3.5 kb transcript of transposase



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



Type of mutations:

a) null mutation- complete absence of activity

b) loss of function - loss of most of activity

c) gain of function- new function of gene

d) suppressors- compensate for other mutations

e) enhancer- enhances phenotype of a mutation

Type of mutational effects

Recessive

hypomorph: reduced level or a protein with a weak function

Null: complete loss of function

Dominant

hypermorph: increased level or more effective activity

neomorphic: new function

dominant-negative: poisonous effect

haploid-insufficient

semi-dominant/incomplete dominance

Mutagenesis scheme in Arabidopsis

Part II

Mutagenesis

Screen:

Visual: trichome, flower morphology

Biochemical pathway mutant (trp- auxotrophy)

Reporter gene expression (luc bioluminescent protein from firefly)

Selection:

trp1

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 (ts)

morphological mutant

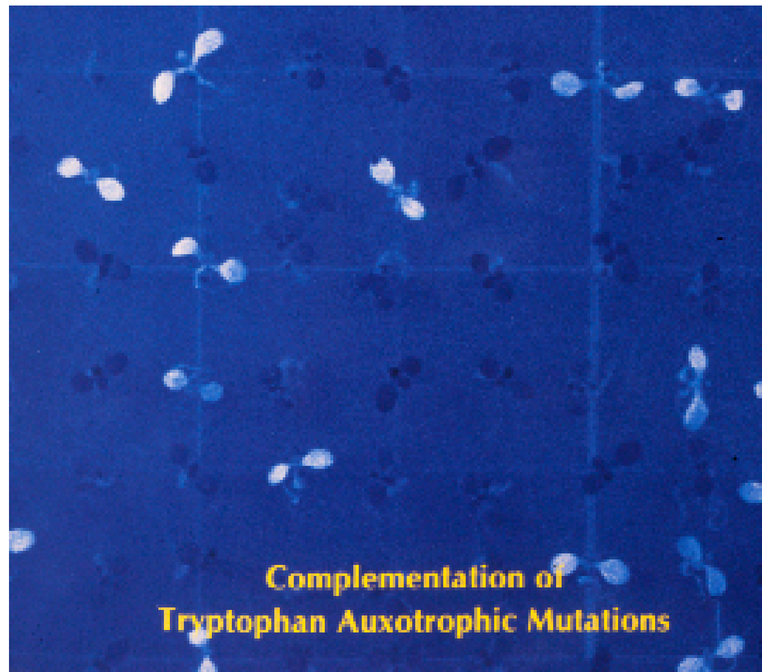
wt

gl (GLABRA) 1

WT *Arabidopsis* flower

***ap2-2* floral mutant**

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



Buchanan textbook
Fig. 8.33
Box 8.5

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)

Before stress | After stress

Control

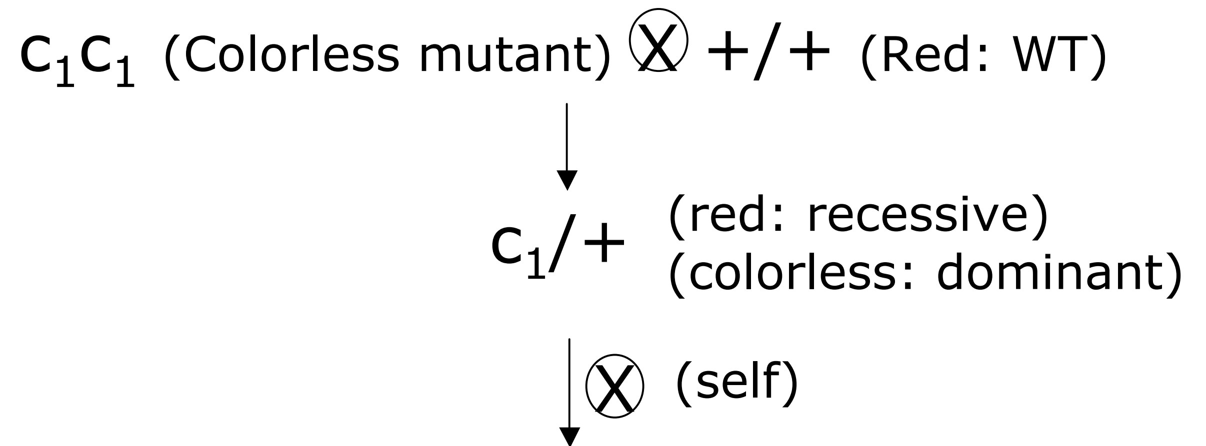
PC-Luc

RD29A-Luc

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

III. Mutant characterization

(1) Determine recessive or dominant nature of the mutation



		Recessive	**dominant**
1/4	c_1/c_1	colorless	colorless
2/4	c_1/+	red	colorless
1/4	+ /+	red	red

(2) determine allelism by complementation tests

pairwise crosses between homozygotes and examine F1 for phenotype
only applicable for recessive mutations

female \ male	c_1c_1	c_2c_2	c_3c_3	c_4c_4	c_5c_5	c_6c_6
c_1c_1	**Colorless**	**red**	**red**	**colorless**	**red**	**red**
c_2c_2		**colorless**	**colorless**	**red**	**red**	**red**
c_3c_3			**colorless**	**red**	**red**	**red**
c_4c_4				**colorless**	**red**	**red**
c_5c_5					**colorless**	**colorless**
c_6c_6						**colorless**

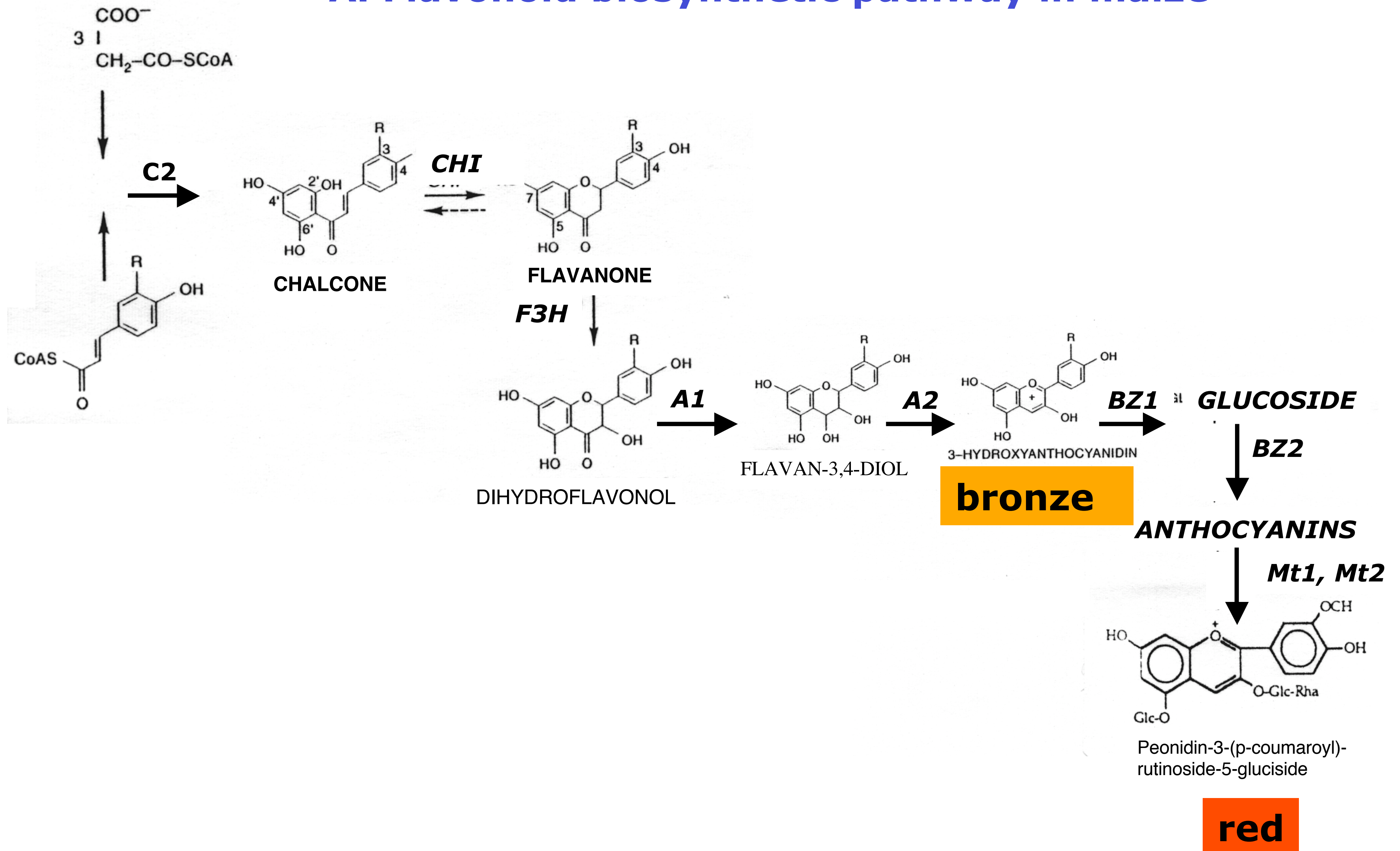
Maize kernel mutants:

c_1-c_6: **colorless, recessive**
wt: red

Three complementation groups:

1. c_1, c_4
2. c_2, c_3
3. c_5, c_6

(3) epistasis analyses (genetic interactions among different mutations)



WT: **Red**
Mutations in c2, a1, a2: **Colorless**
Mutations in bz1, bz2: **bronze**

Double mutants

C2/a1: colourless-but uninformative
bz1/a1: colorless-a1 comes before bz1
bz2/a1: colorless-a1 comes before bz2

For biosynthetic pathways, the phenotype of the earlier gene in the pathway shows in the double mutant.
ie. the earlier-step mutant is epistatic to the late-step mutant

Determine relationship between a1 and c2 by feeding experiment:
add flavanone (naringenin): c2+naringenin = red
a1+naringenin = colorless

B. Regulatory pathways

Signal → A → B → C ⊣ D ⊣ gene expression

→ Positive action-stimulate next step.
Null mutation makes insensitive to signal

⊣ Negative action-represses next step.
Null mutation makes the gene turned on at all time (constitutively

b^-: never turned on even in the presence of the signal

c^-: constitutive on in the absence of signal

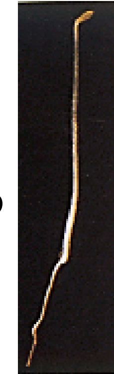
$b^-c^- = c^-$: constitutively on

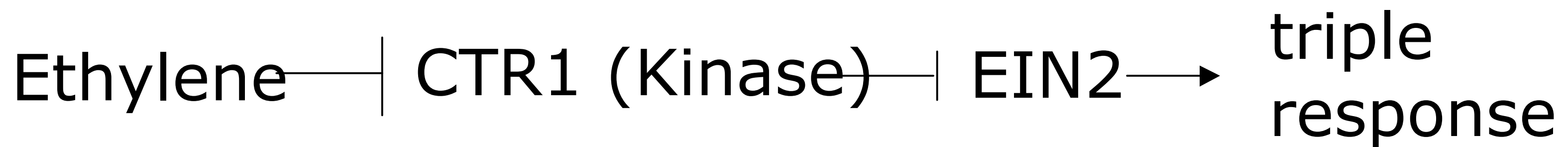
For regulatory pathways, the phenotype of the later-acting genes shows in the double mutant.
ie. the later-acting mutant is epistatic to the earlier-acting mutant

wt *wt* *ctr1* *ein2*

ethylene air air ethylene

ctr ein2 :?





For regulatory pathways, the phenotype of the later-acting genes shows in the double mutant.

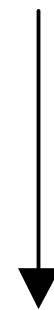
ie. the later-acting mutant is <u>epistatic</u> to the earlier-acting mutant

C. Additive pathways

Double mutants of dissimilar phenotypes produce a combination of both phenotypes

Indicate that the two mutations are in genes acting in separate pathways

ap2-2 (flower abnormal) X *gl* (no trichome)



ap2-2 gl double mutant
abnormal flower and no trichome

Home work assignment: 2001 Springs' mid term exam questions 1-4 (pdf file on the web)