

Pbre 141

# Plant Molecular Biology and Biotechnology

**Instructor:**

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# Learning Outcomes



To determine the factors contributing to variation in gene expression



Differentiate genetic and epigenetic changes that causes variation in gene expression



To identify the sources of variation in gene expression



How do you differentiate a Variant  
and a Mutant?



## Scenario 1:

A farmer notices that one of his tomato plants produces **yellow fruits**, while all others produce red fruits. He collects seeds from the yellow-fruited plant and grows them. **Some offspring also produce yellow fruits, while others are red.**

## Scenario 2:

A researcher is studying a population of rice seedlings and notices that **some seedlings appear completely white (albino) instead of green**. These albino seedlings **fail to survive past the early growth stages**.

To investigate further, the researcher **sequences the DNA of both albino and normal green seedlings** and discovers that there is a change of sequence in a **gene responsible for chlorophyll production**.

## Scenario 3:

A researcher finds that certain group of rice in the field **survive pest infestations**, while others die. The researcher observes that **these resistance is consistently observed** over time.

To understand why, the researcher conducts a **gene expression analysis** and discovers that **the susceptibility gene is methylated**, which could explain why the herbicide is no longer effective.

## *Variant*

- An individual or organism that differs from the parent in phenotype, but alterations at the DNA sequence level have not been confirmed through molecular (sequencing) or genetic (segregation analysis) methods.

## *Mutant*

- An individual or organism with a proven DNA sequence change, confirmed through molecular or genetic analysis.



# Epigenetic and genetic Modifications

## Epigenetic

- Describes the DNA **state**
- Often inducible
- Relatively frequent
- Reversible
- Sometimes transmitted through meiosis, but no general rule
- Variants are called **epi-alleles**  
(or changes in the epigenome)

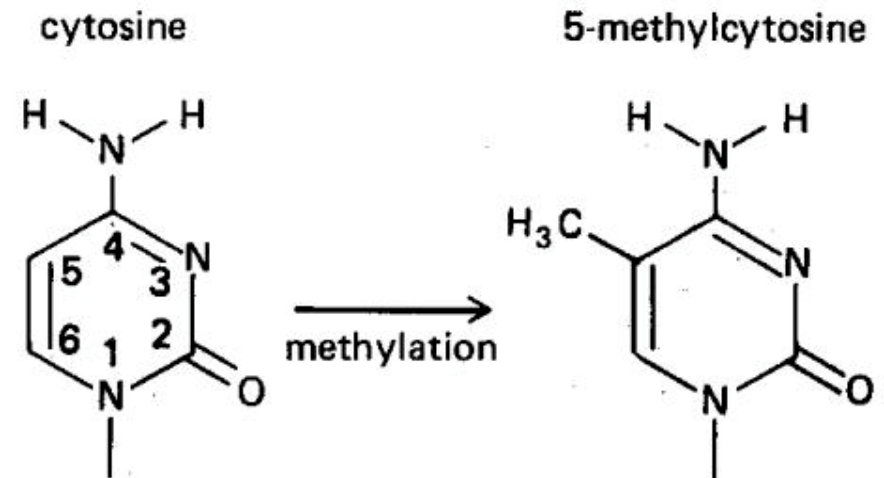
## Genetic

- Describes the DNA **sequence**
- Random
- Relatively rare
- Not reversible
- Transmitted through meiosis following “Mendel’s laws”
- Variants are **alleles**

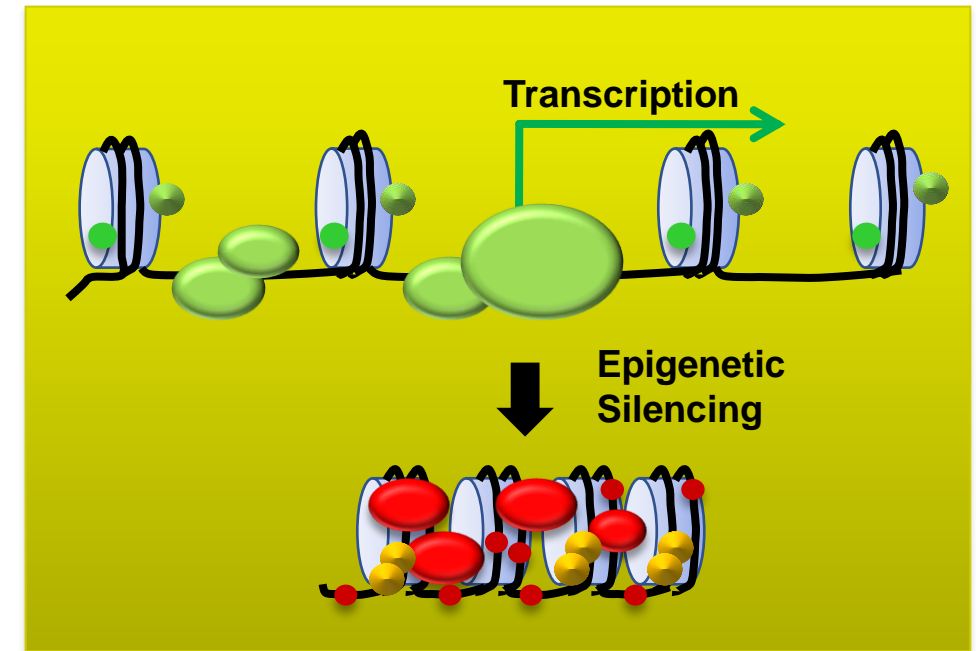
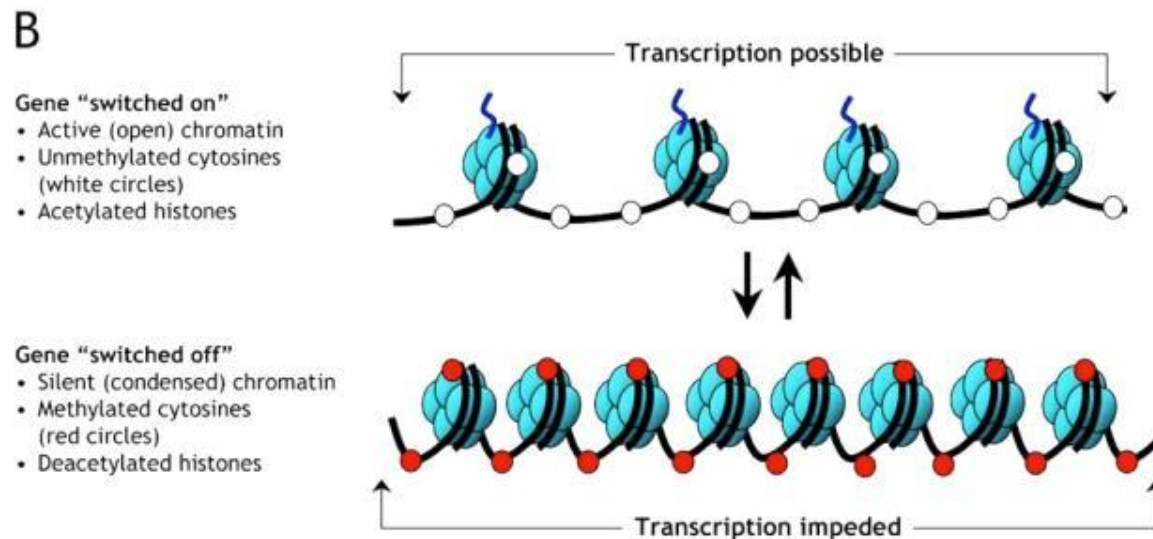
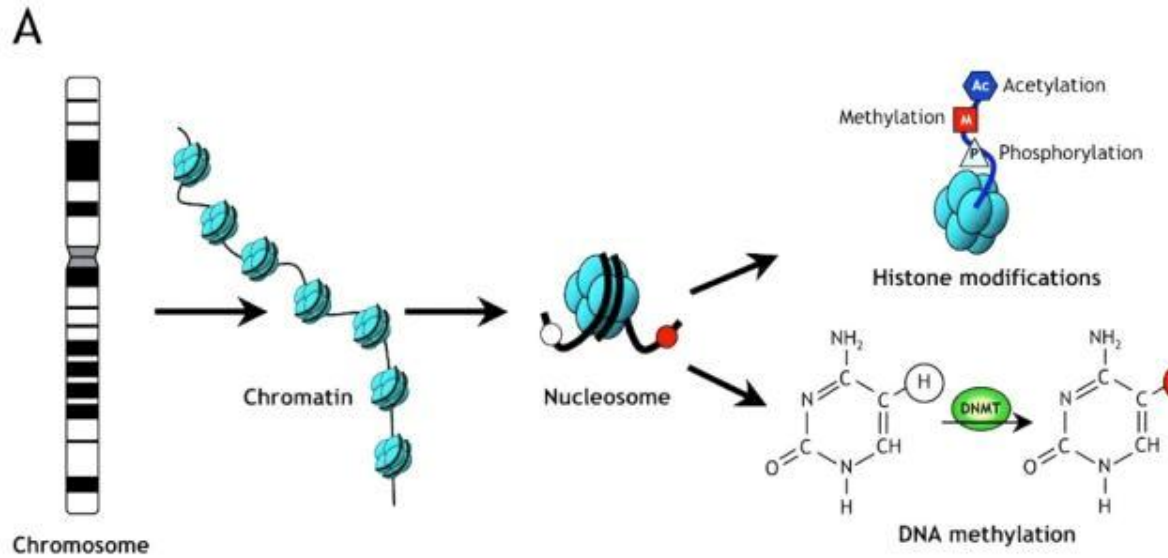


# DNA methylation

- DNA methylation is stable but may disappear (due to stress) and then leads to changes in the expression of genes
- It often occurs in non-transcribed heterochromatic regions of chromosomes
- Needed to silence and suppress transposable elements
- Methylation is mainly at C's next to G.

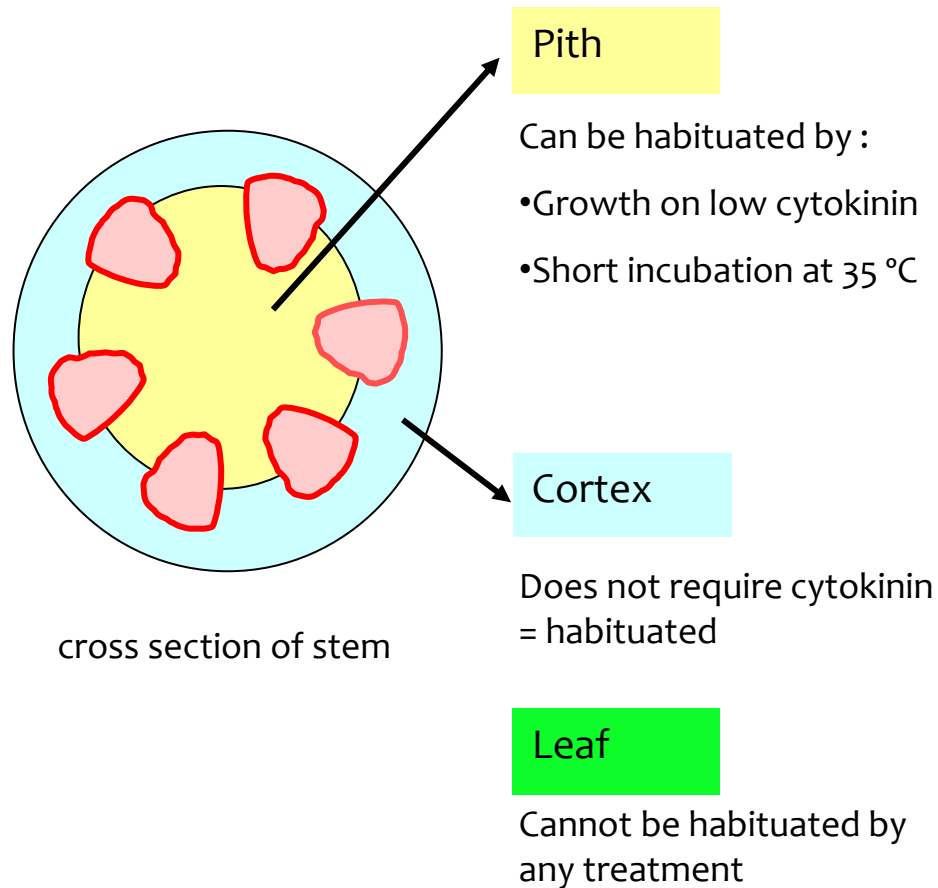


# A model of epigenetic modifications and their effect on transcription



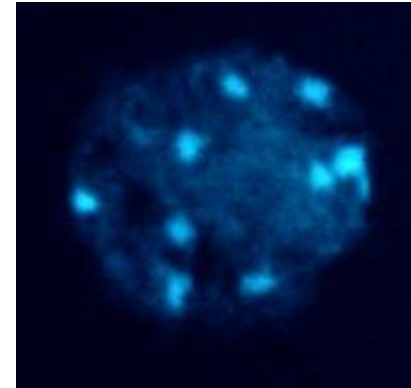
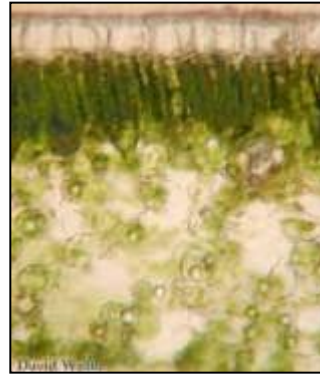
# Examples of epigenetic changes

Cytokinin habituation = growth of callus on media without cytokinins



Callus induced on red beet tissue may or may not express all genes for pigment production

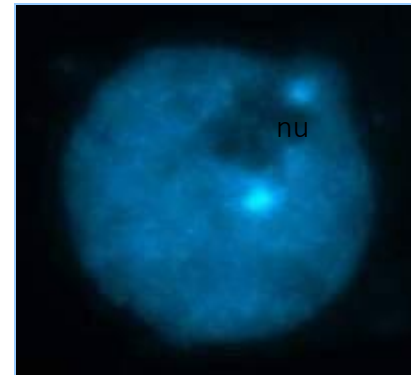
# Chromocentres disappear under stress



> 70% cell division

plant regeneration

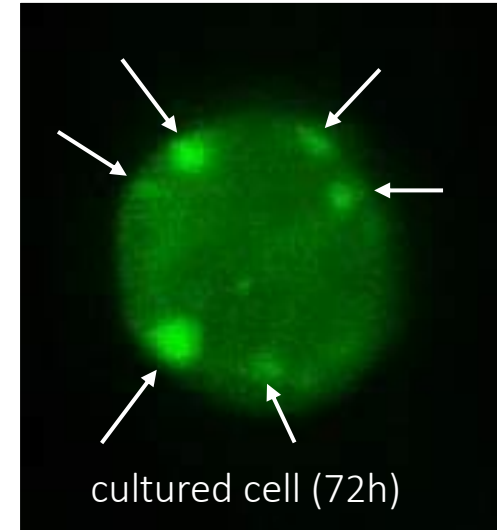
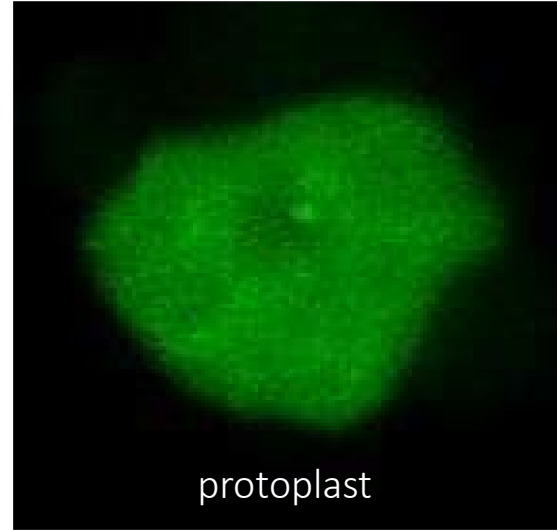
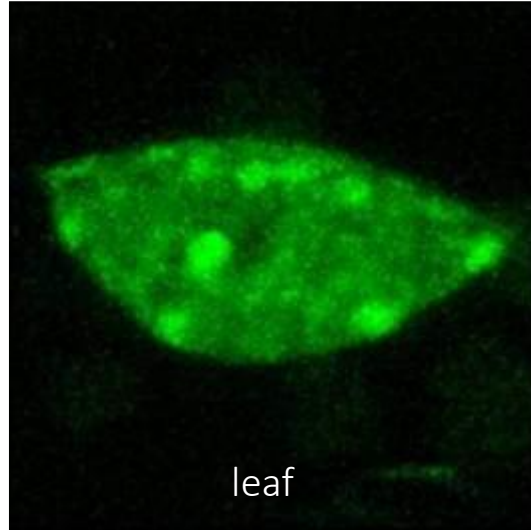
Chromocenters are regions of heterochromatin, with repeats and highly methylated DNA



(De-)differentiation of protoplasts

Interesting work from Paul Fransz group (Univ of Amsterdam) on heterochromatin:

[Fransz et al., 2005, Chrom. Res 14:71](#); [Tessadori et al., 2004, Trends Plant Sci 9: 147](#)

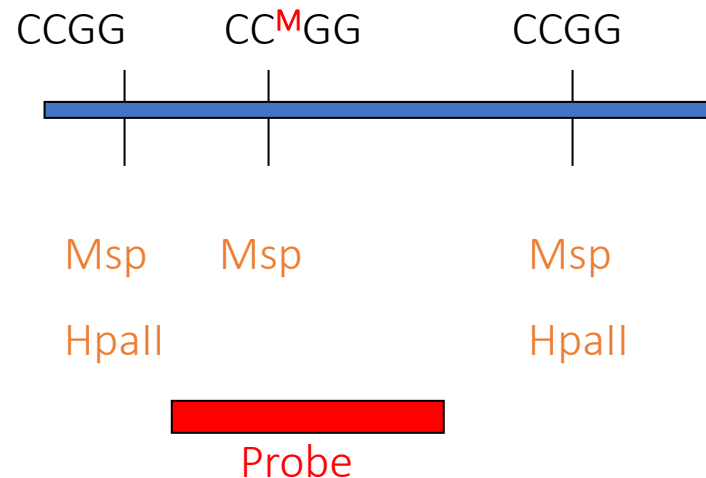




# How to determine DNA methylation

## 1. Use of Restriction Enzymes HpaII and MspI

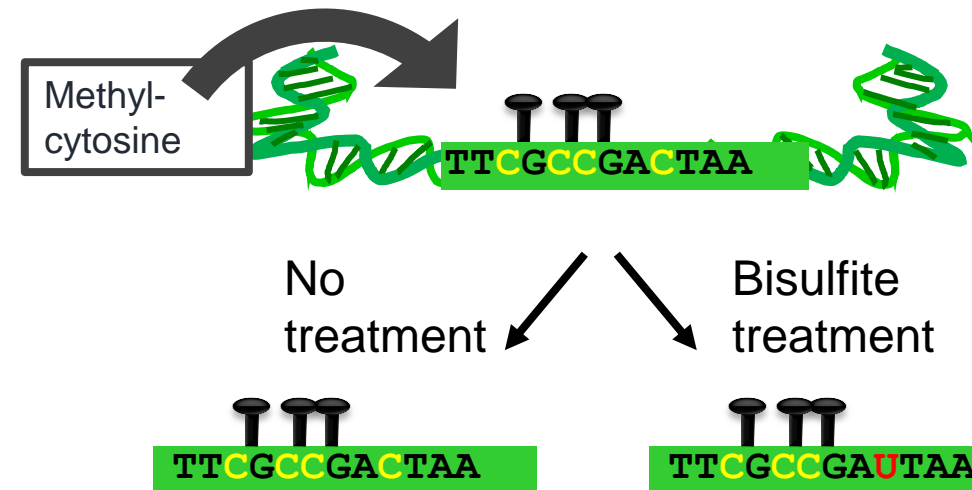
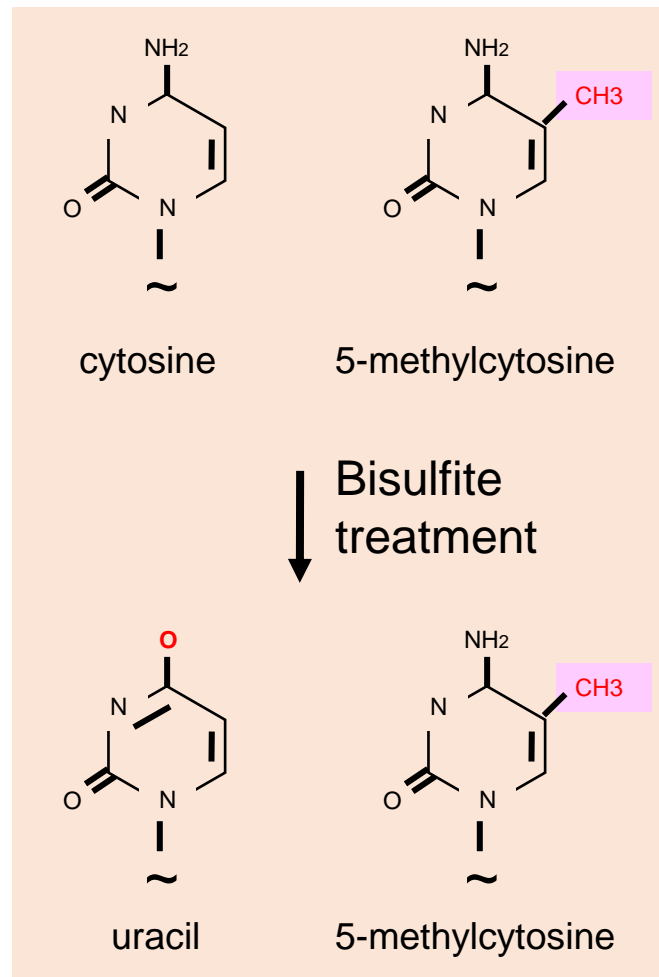
Some restriction enzymes cut the same restriction site but one may not do so when the C is methylated, e.g.,  
*HpaII* cuts CCGG, *not* CC<sup>M</sup>GG  
*MspI* cuts CCGG *and* CC<sup>M</sup>GG



Southern blot of DNA cut with *HpaII* and *MspI* and hybridized with **probe**

	<i>HpaII</i>	<i>MspI</i>
6kb	—	
4kb		—
2kb		—

## 2. Epigenome sequencing: bisulfite treatment

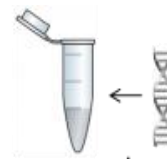


### NOTE!!!

When DNA is bisulfite treated, unmethylated cytosine is converted to uracil. Methylcytosine is not affected.

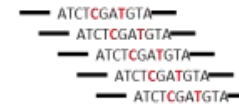
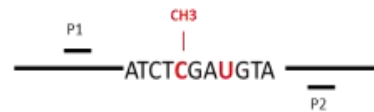
In a PCR, "U" is copied as "T"





Genomic DNA

ATC<sup>m</sup>TC<sup>m</sup>GACGTAAATTCC<sup>m</sup>CGGCGG



BSS primers design & PCR



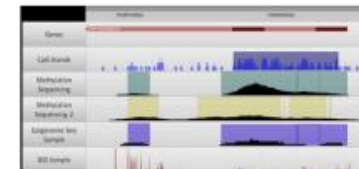
Library Prep & Sequencing

ATCTCGATGTAAATTTCTGGTGG

Cleansing & Mapping

Access to data files

AGTCTCATGTCATCGTGTCTTCGACTCTTAC  
TCTCGTACGACTCTGACTGACTGCGATC



2 months access to proprietary  
H2G2 Genome Browser

Reference sequence

ATGCCGACTCCGATTTAAGCCT

Treated with Bisulfite

ATGCTGATTCCGATTTAAGTCT

Methylated sequence

ATG<sup>m</sup>CGA<sup>m</sup>T<sup>m</sup>C<sup>m</sup>GATTTAAGCC<sup>m</sup>T

# Types of genetic variation in cell cultures

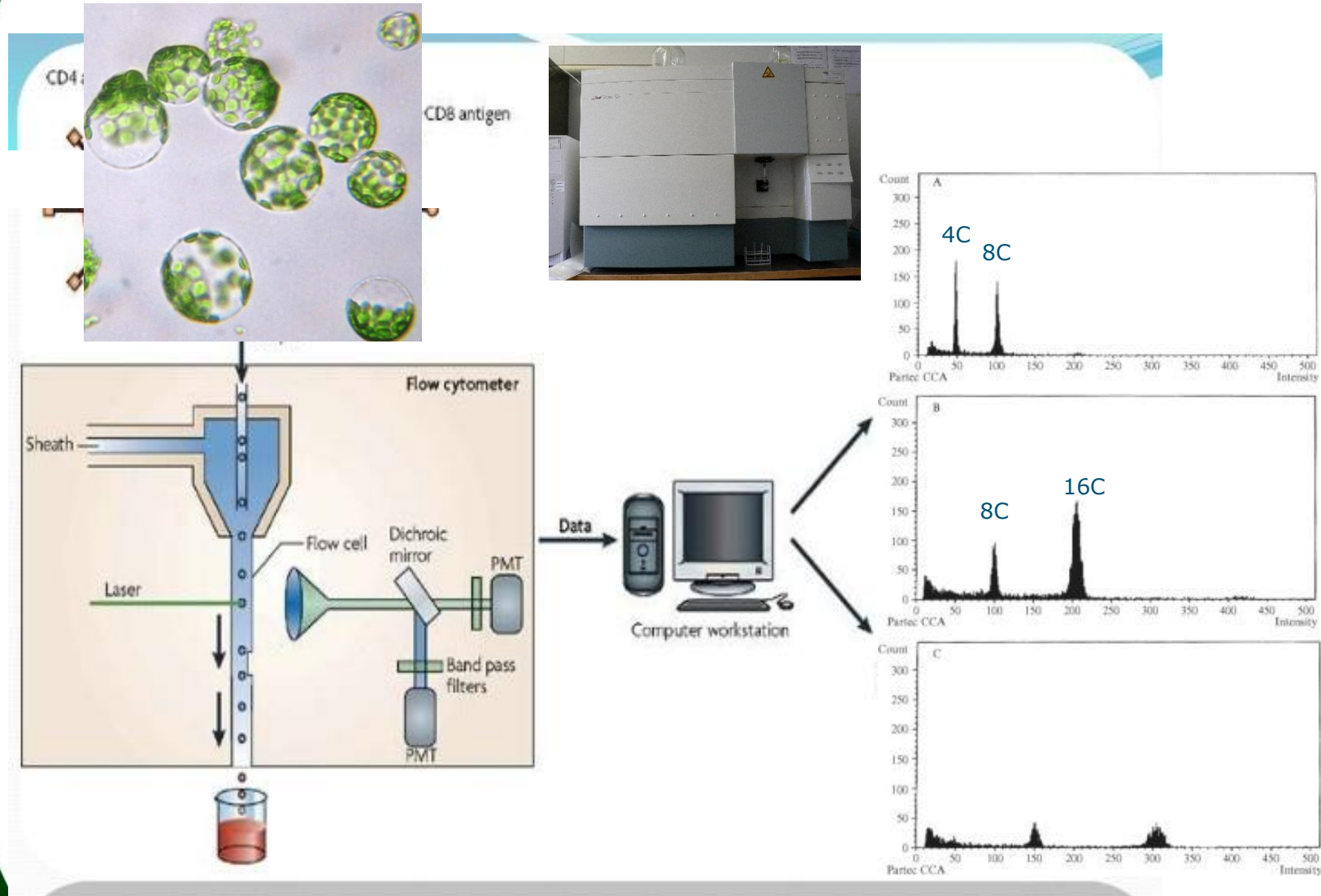
## 1. Chromosomal mutations:

**Ploidy** refers to the number of sets of chromosomes in a cell. When plant tissue experience stress, cells may undergo **polyploidy** (having extra sets of chromosomes) or **haploidy** (having fewer sets).

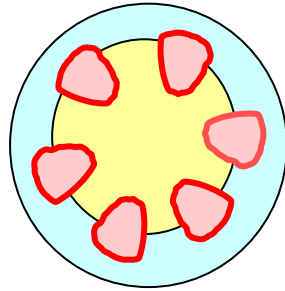
**Aneuploidy** is when cells have **an abnormal number of chromosomes**, which can cause developmental abnormalities or genetic instability.

Example: A diploid plant ( $2n$ ) becoming tetraploid ( $4n$ ) due to prolonged stress.

# Flow cytometry for DNA count

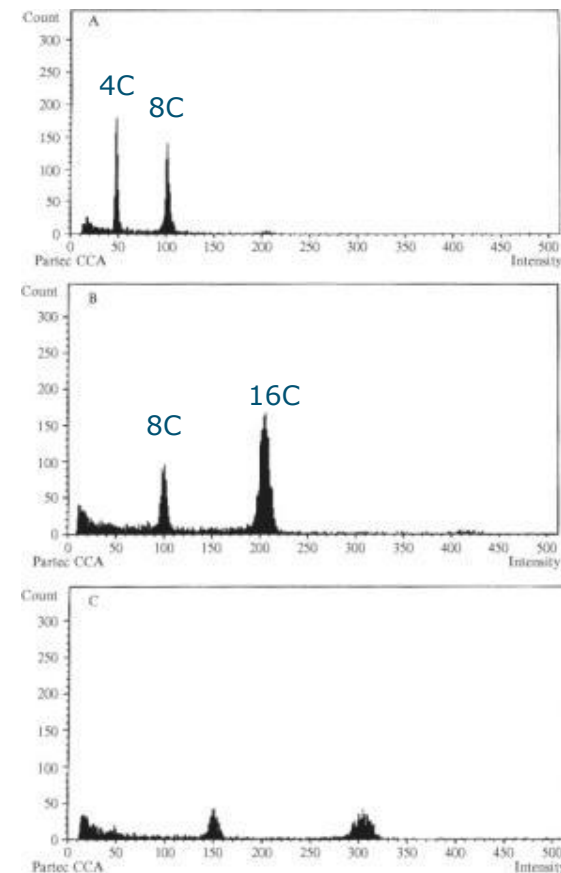


# Ploidy levels in tissue cultures of allo-tetraploid tobacco



origin	4x	8x	16x	an-eupl
pith (top)	47	53		
pith (low)	9	71	16	4
callus 1 yr		27	20	53
callus 6 yrs				100

Tissue culture itself also leads to chromosome number changes (polyploidy and aneuploidy)



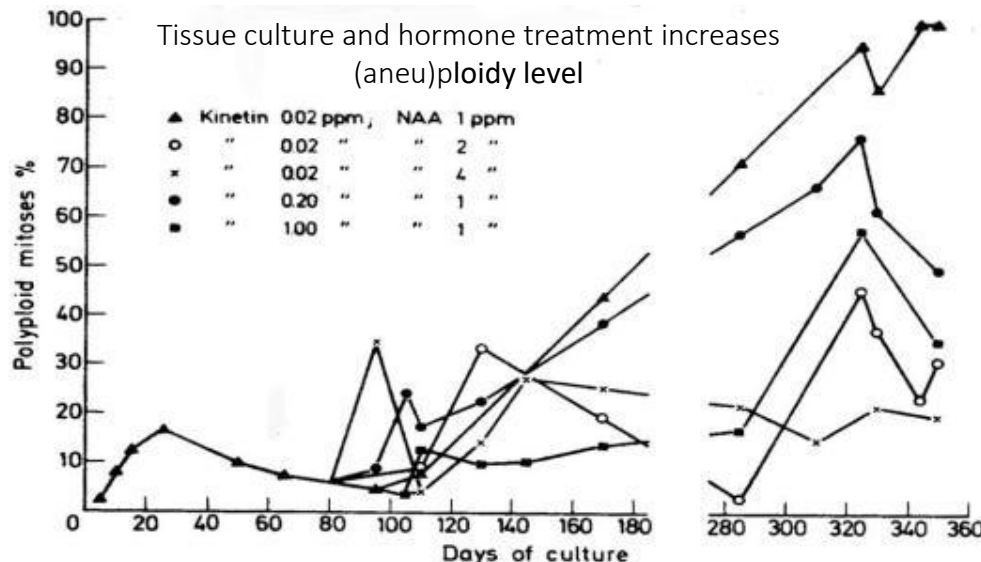
flow cytometry

# Relation between ploidy level of explant and ploidy of regenerated plants

tissues have different ploidy levels



explant	% 2C cells	% diploid plants regenerated
leaf	93	99
cotyledon	60	95
hypocotyledon	22	58



Developmental processes play role in genome stability

In due time callus will be entirely unbalanced and cannot be regenerated into plants anymore



## 2. Nucleotide rearrangement:

**Rearrangements** are structural changes where chromosome segments are **shuffled** or **moved** to a different location.

- **Inversions** happen when a chromosome segment **flips** and reinserts in the opposite orientation.
- **Translocations** occur when **part of one chromosome** attaches to a **different chromosome**.

These changes can activate or silence genes, causing variability in plant traits.



### 3. Transposable Elements

Mobile genetic elements that “transpose” through the genome

Two types

- Class I – Retrotransposons: duplication and insertion (copy-and-paste)

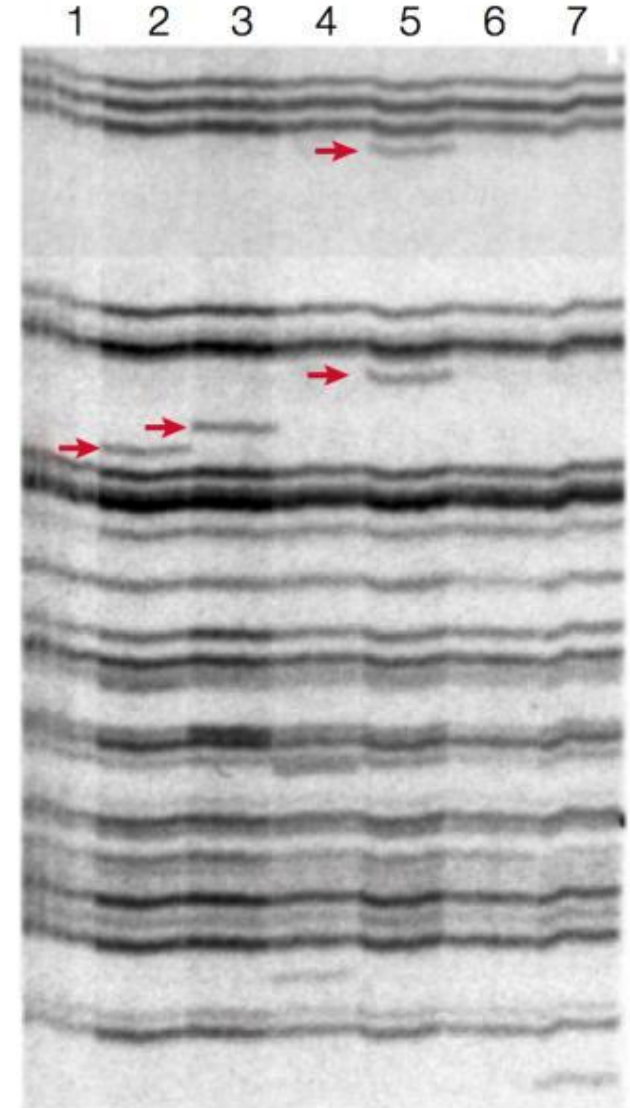
- Class II – True DNA transposons: excision and insertion (cut-and-paste)

  - Autonomous: transposition by itself

  - Non-autonomous: transposition controlled by autonomous TE

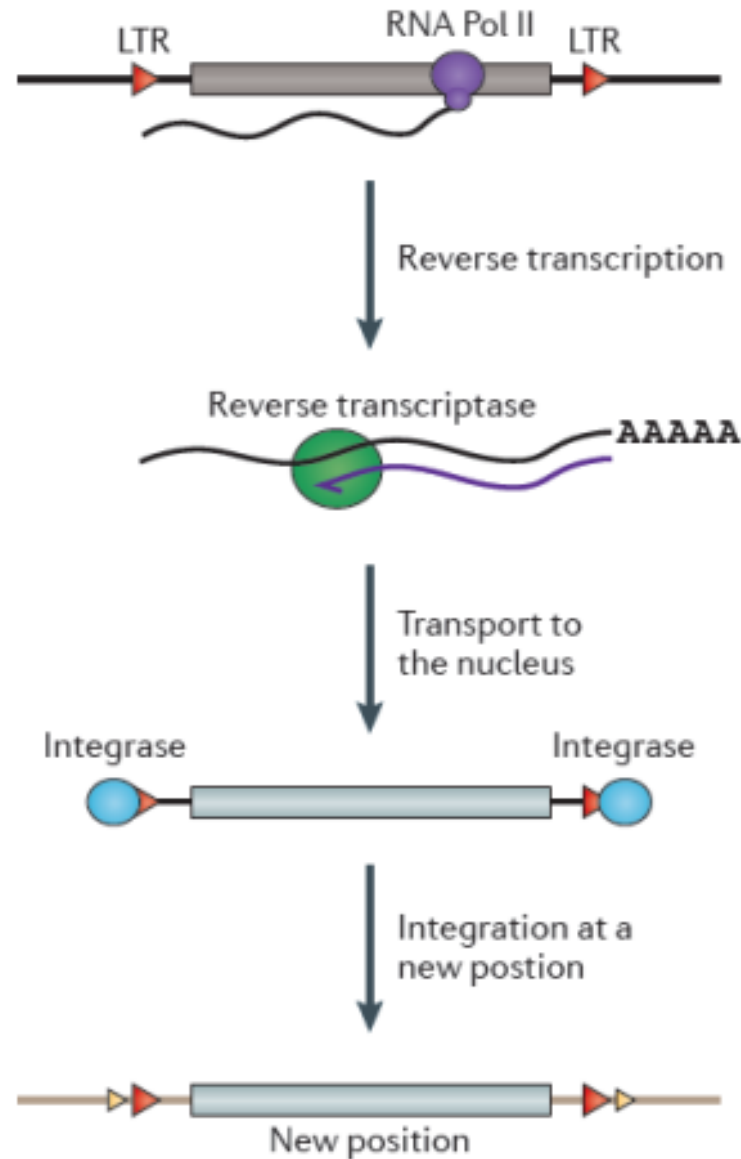
Generally “silenced” by DNA methylation

Can be activated in tissue culture

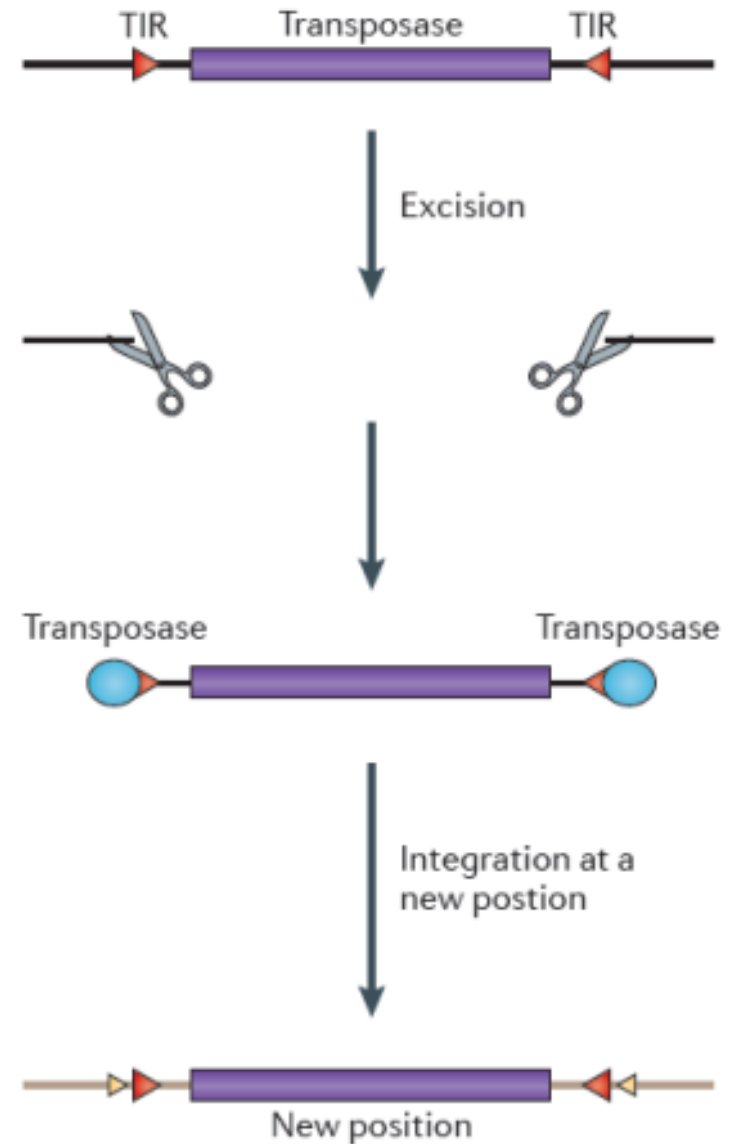


# Two types of Transposable elements

Class I element



Class II element



## Phenotypes produced by transposable elements in corn kernels



**C gene  
(wild type)**

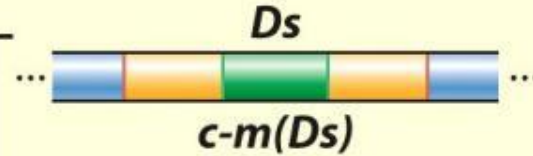


**Phenotypes**

**Pigmented**



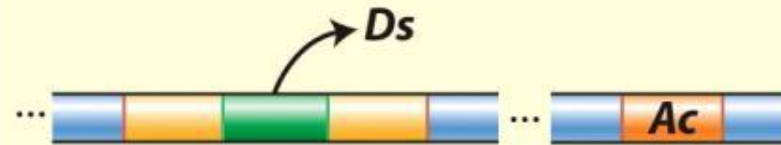
***c-m(Ds)*  
(no Ac)**



**Colorless**



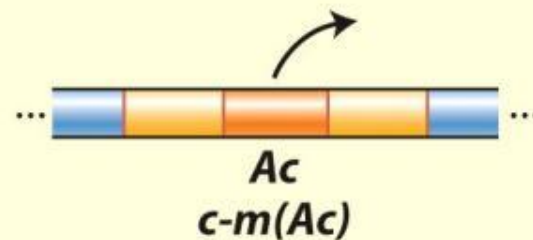
***c-m(Ds)*  
(+Ac)**



**Spotted  
kernels**



***c-m(Ac)***



**Spotted  
kernels**



Figure 14-4 *Introduction to Genetic Analysis*, Ninth Edition, W. H. Freeman and Company

# Development and environment cause (epi)genetic changes in an organism

