

ISA

Genética Quantitativa e  
Melhoramento de Plantas

Epigenetics in Plant Breeding  
2021

ULISBOA

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DE LISBOA



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AGRONOMIA

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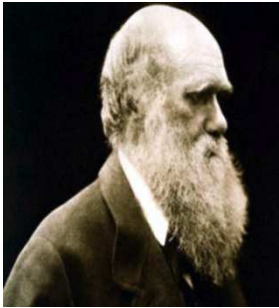
# ISA

## *1<sup>st</sup> session*

- The gene centered view
- Epigenetic marks and transmission
- Plant development and gene silencing
- Genomic Imprinting

## *2nd session*

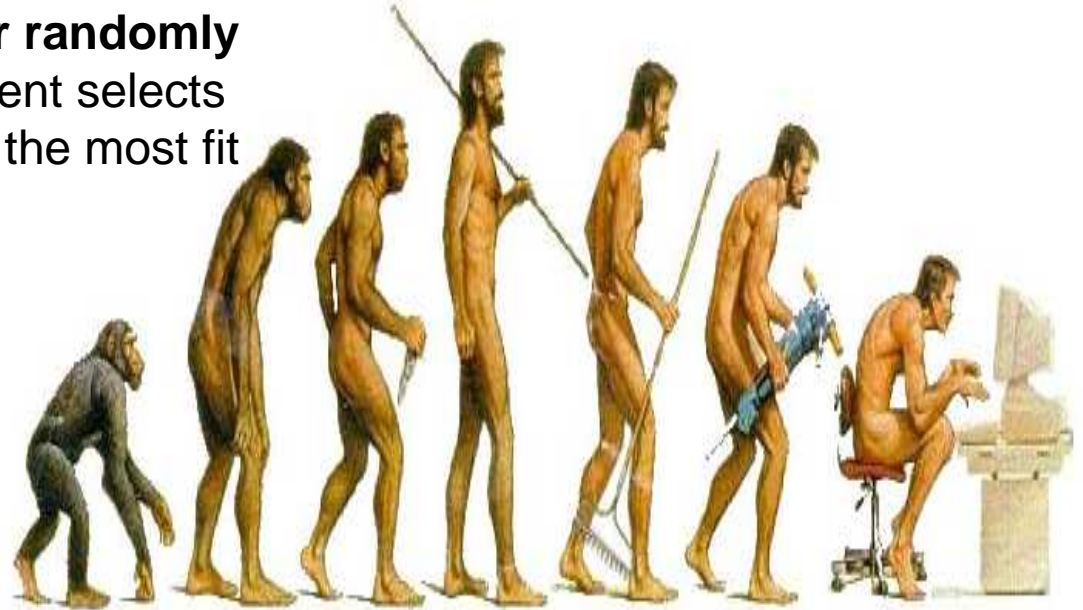
- Environmental stress and plant adaptation
- Epigenetically mediated novel phenotypes
- Epigenetic changes driving genetic mutations



**Charles Darwin**  
**Theory of Natural Selection**

**changes occur randomly**  
and the environment selects  
the most fit

**19th century controversies**
















**Jean-Baptiste Lamarck**  
**Theory of Inheritance of Acquired Characteristics**

**the environment induces changes**  
through use/disuse of characteristics

Charles Darwin could not account for how traits were passed from generation to generation



In 1865 Gregor Mendel found that traits were inherited in a predictable manner

Character	Dominant trait	Recessive trait	Character	Dominant trait	Recessive trait
Seed shape	 Spherical	 Wrinkled	Flower position	 Axial	 Terminal
Seed color	 Yellow	 Green		Stem height	 Tall
Flower color	 Purple	 White			
Pod shape	 Inflated	 Constricted			
Pod color	 Green	 Yellow			

## Genetics and Plant Breeding – the struggle of the 30's

Nikolai Vavilov

**Theory on the centers of origin**  
of cultivated plants

Regions with the highest diversity of each species are very important to plant breeding, in order to avoid genetic erosion and loss of germplasm.

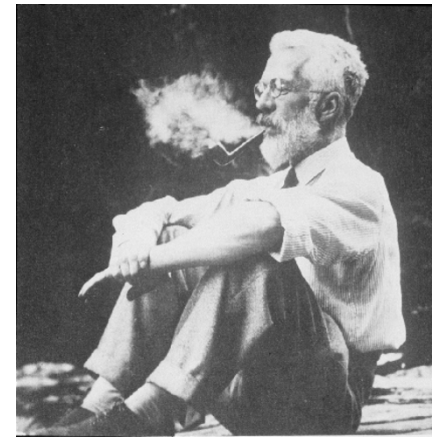
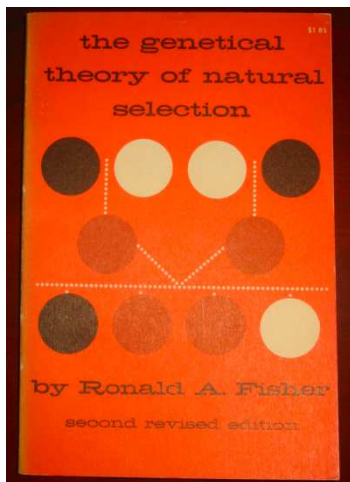
Trofim Lysenko

Heredity had only a limited role in development

Practiced a form of Lamarckism, insisting in acquired characteristics

1930s – Darwinian natural selection became understood in combination with Mendelian inheritance, which connected the units of evolution (genes) and the mechanism of evolution (natural selection).

A substantial part of the variation in phenotypes in a population is caused by differences between their genotypes



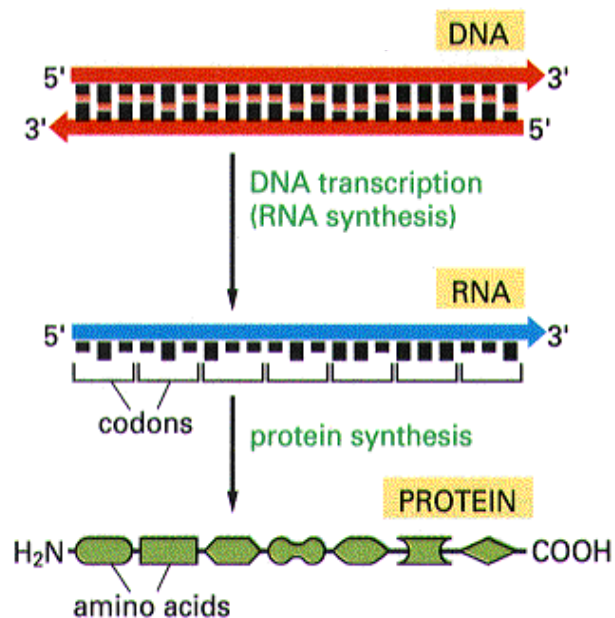
1890-1962

Ronald Fisher, set the foundations for the establishment of the field of Population Genetics.

An individual organism's phenotype results from both its genotype and the influence from the environment it has lived in..

## 50s – The gene, centered view of evolution

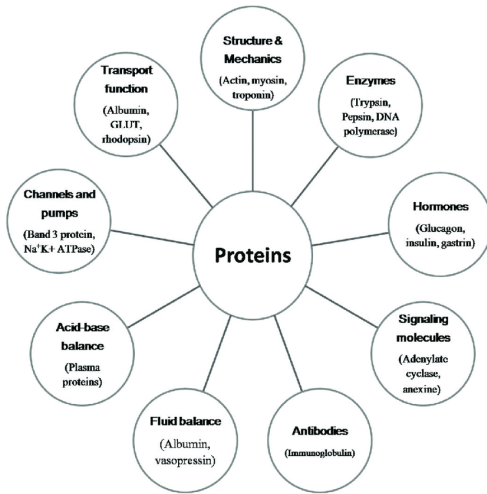
James Watson and Francis Crick in 1953, demonstrated the physical basis for inheritance. Genetics and molecular biology have become core parts of evolutionary biology



The formulation of the central dogma of molecular biology made it clear that the inheritance of acquired characters was not an evolutionary factor in a physical sense and identified genes as lasting entities that survive through many generations

Natural variation among ecotypes, defined as strains or races of a species that are adapted to specific niches, reflect naturally occurring **nucleotide sequence changes (mutations)**, due to recombination, gains or losses of DNA sequences, or hybridization events

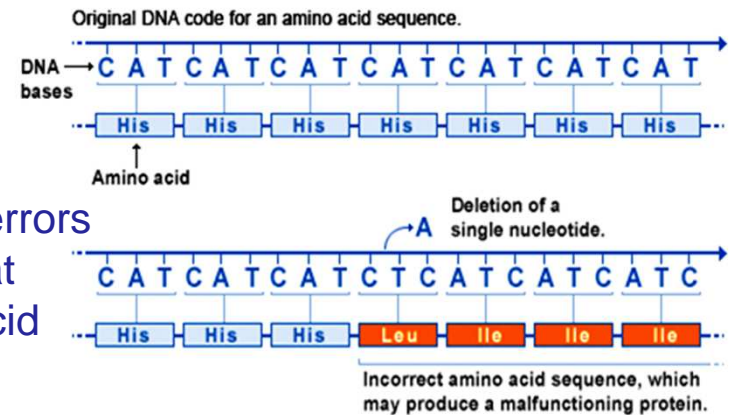
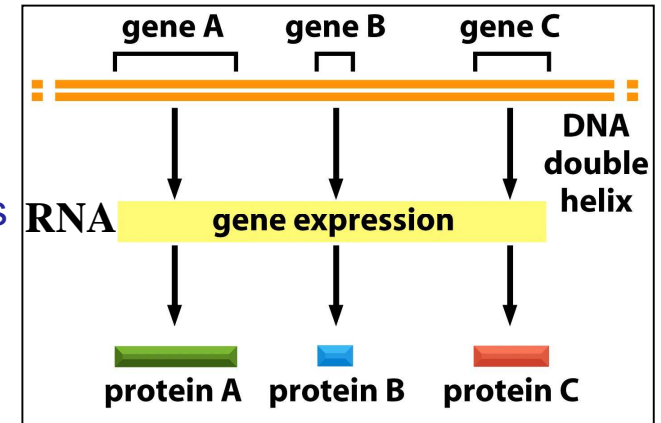
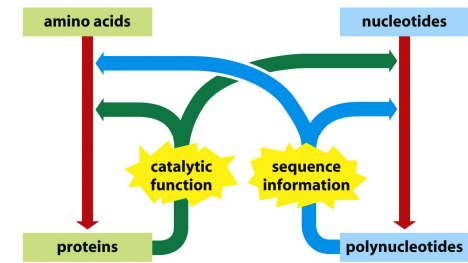
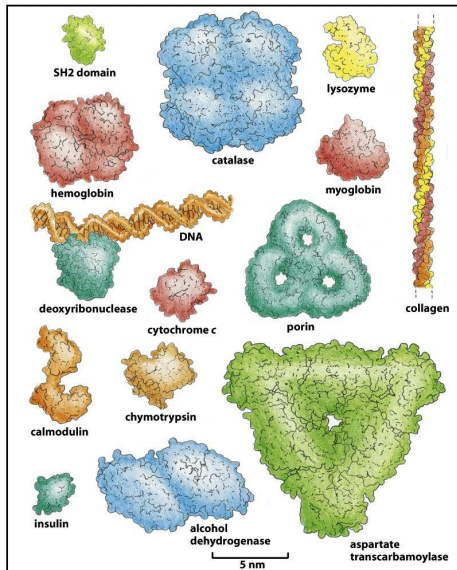
**Remember that....** proteins are responsible for most metabolic processes in cells being required for the structure, function, and regulation of organisms



**Gene diversity** results from differences in nucleotide sequences

**Genetic Code** - relationships between triplets of nucleotides (codons) and amino acids

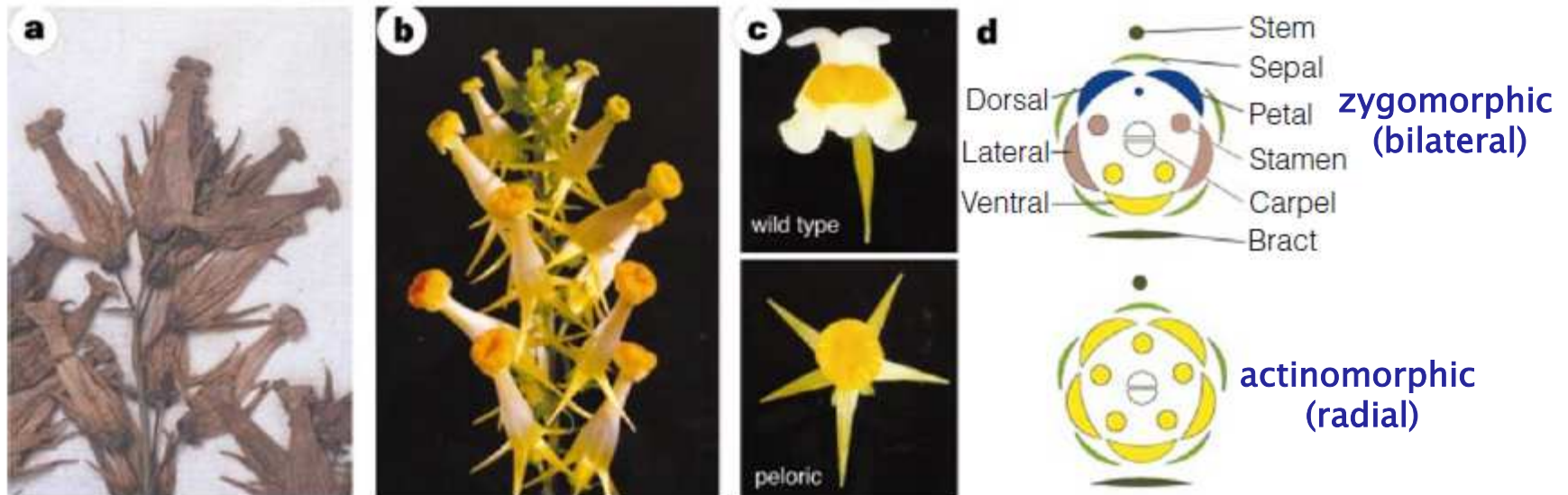
**Protein diversity** results from different amino acid sequences



**Mutations** – spontaneous errors in nucleotide sequences that lead to changes in amino acid sequences

Figure 4-7 *Molecular Biology of the Cell* (© Garland Science 2008)

## The gene centered view of phenotype differences



Cubas, P. et al. 1999 *Nature* **401**: 157–161

- Original herbarium specimen of peloric *Linaria vulgaris* inflorescence collected 250 years ago by Linnaeus and currently housed in the Linnean Society, London.
- Peloric\* *Linaria* inflorescence from a living specimen.
- Wild-type *Linaria* flower compared to a peloric mutant.
- Floral diagrams of wild-type (top) and peloric (bottom) flowers showing the relative positions of different organs: blue (dorsal) brown (lateral) yellow (ventral).

**wild-type flower** has a dorsoventral axis orientated asymmetry (upper part near the stem and lower part near the subtending leaf).

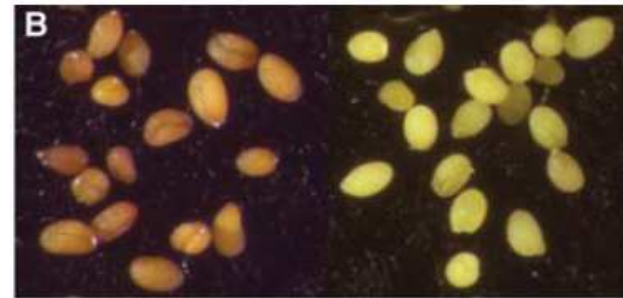
**\*peloric flower** is radially symmetrical, with all petals resembling the ventral petal of the wild type. *\*Pelorism is the term, said to be first used by Charles Darwin, for the formation of 'peloric flowers' which botanically is the abnormal production of actinomorphic flowers in a species that usually produces zygomorphic flowers.* <https://en.wikipedia.org/wiki/Pelorism>



Classically, it was believed that evolution only requires mutations in DNA sequences, and that resulting phenotypic variation reflects the strength of selection upon phenotypic variability



Expression of the dihydroflavonol reductase gene is required for dark purple petunia flowers



Seeds from Arabidopsis expressing the chalcone synthase gene have dark seed coats

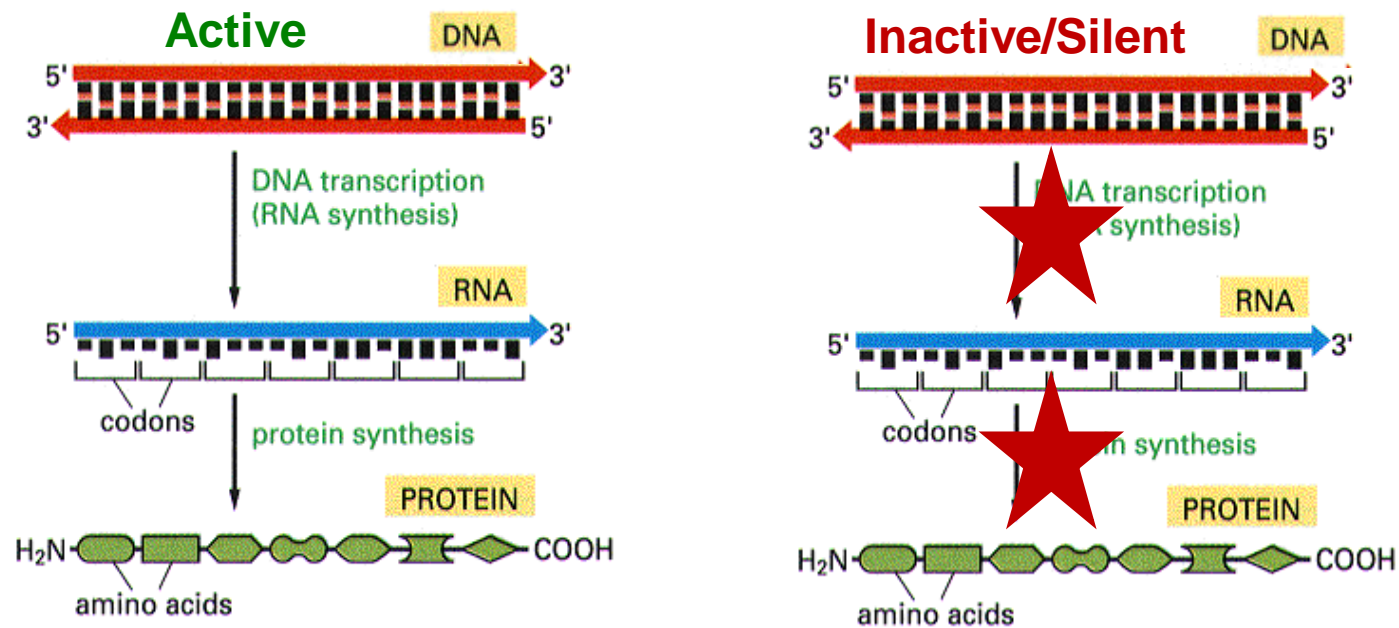


Soybeans colours in cultivated varieties

C.S. Pikaard and O. Mittelsten Scheid Cold Spring Harb Perspect Biol 2014;6:a019315

**but.....  
the unique effect of genomic variation on evolutionary  
changes start to be discussed in the present century**

Researches on epigenetic phenomena and the discovery of **transgenerational epigenetic inheritance**, challenged the view that only changes of genetic sequences undergird the phenotypic variation upon which natural selection can act



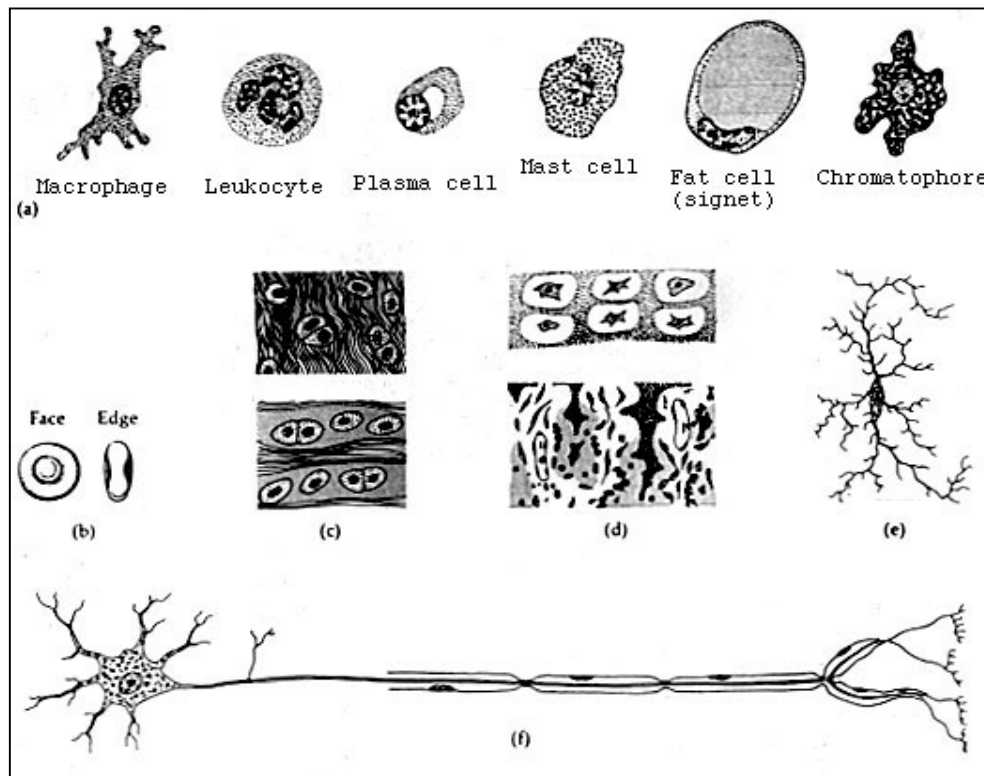
since....  
a new layer of information above the genes emerged  
– **Epigenetics (*epi-above*)** –  
identical genes with distinct expression patterns

# Gene Memory: transmission of expression patterns

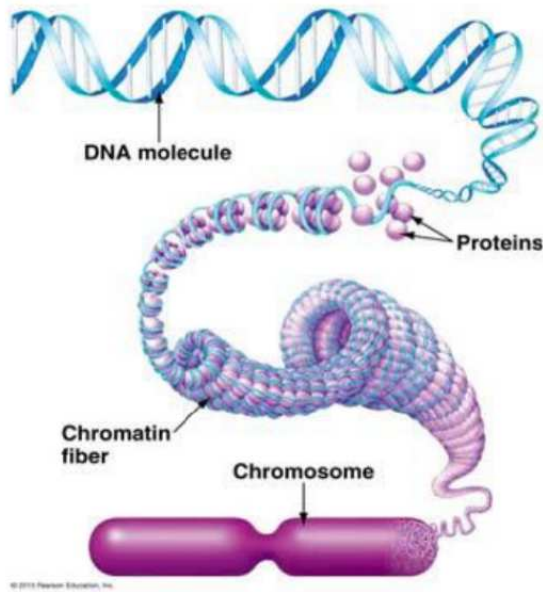
Different cell types have distinct active genes but an identical genome.



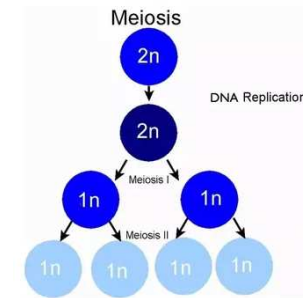
Waddington 1942 first used the term **epigenetics**:  
“the causal mechanisms by which genes of the genotype bring about phenotypic effects”.



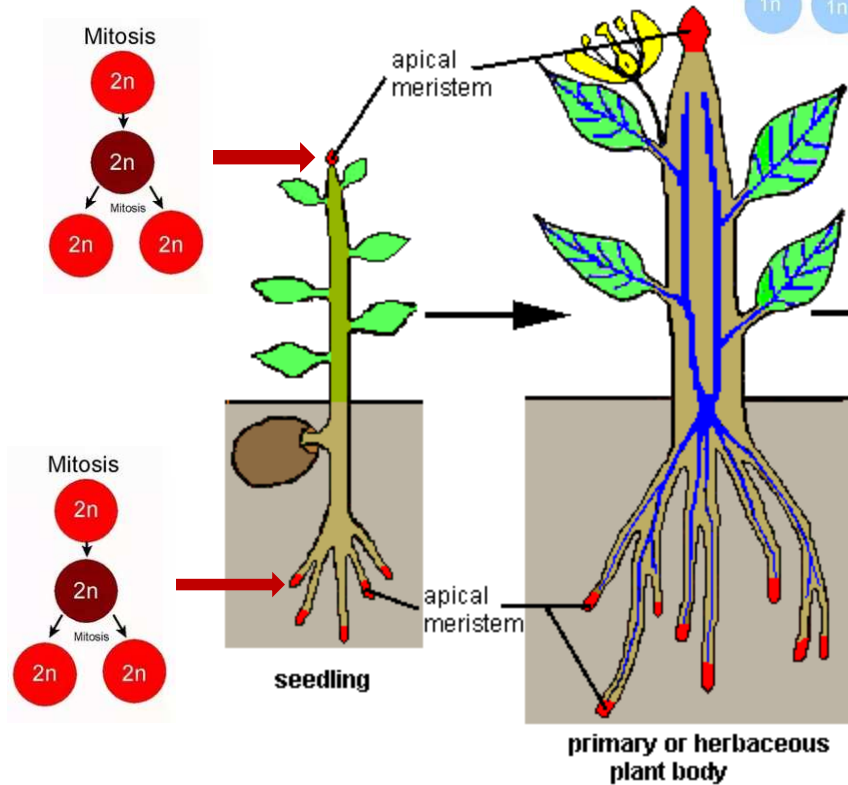
**Chromosomes are made of Chromatin = DNA + proteins**



Chromosomes are made of chromatin and are the vehicles of genes transmitted through mitosis during cell multiplication and between generations through germ cells produced by meiosis



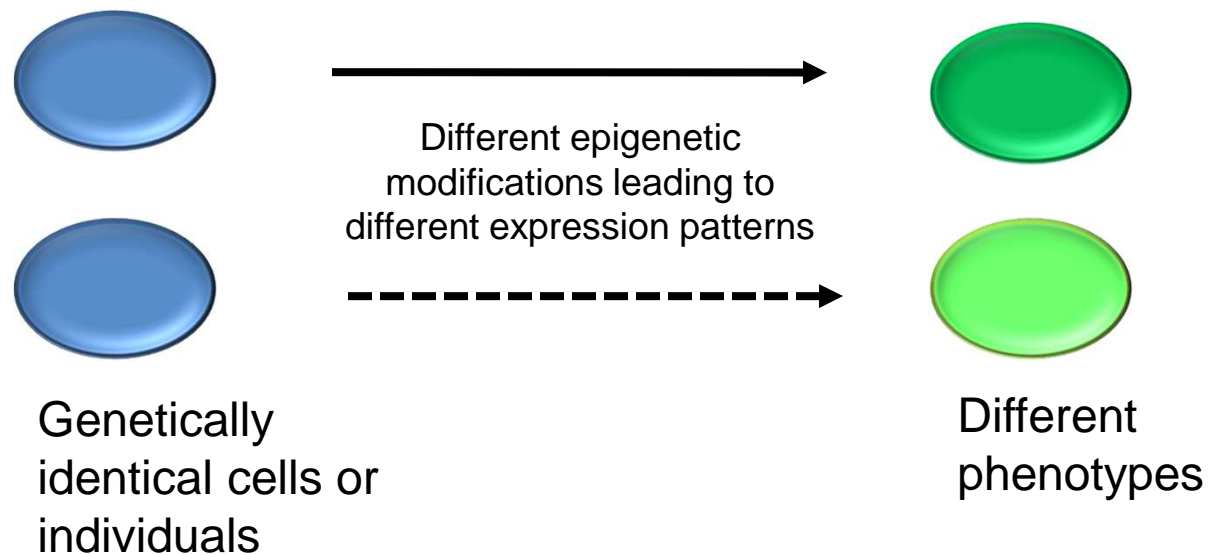
All somatic cells in an organism have identical chromosomes/genomes



Only germ cells have chromosomes with new allelic combinations resulting from recombination events during meiosis

## What does “epigenetics” mean?

**Practically**, epigenetics describes phenomena in which genetically identical cells or organisms express their genomes differently, causing phenotypic differences.



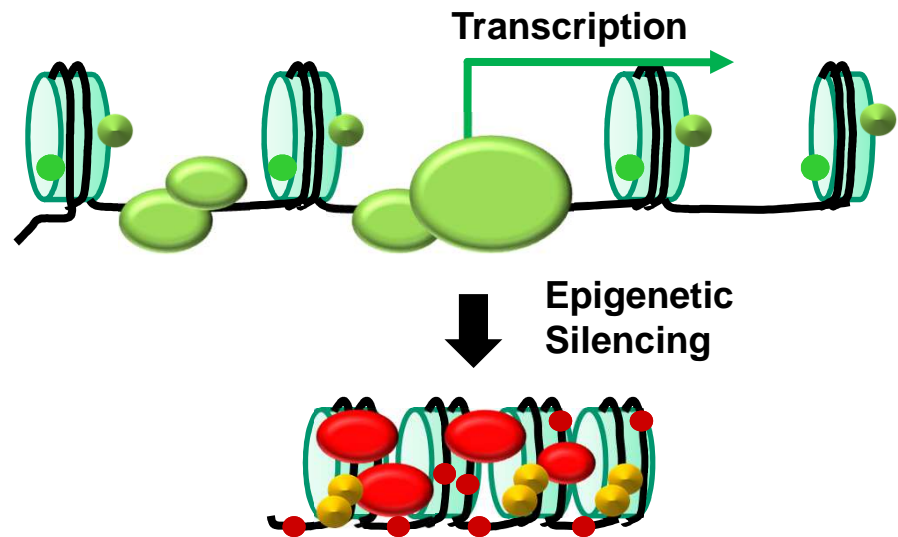
## Epigenetic modifications

**Literally**, epigenetics means above, or on top of, genetics.

Usually this means information coded beyond the DNA sequence, such as in covalent modifications to the DNA or modifications to the chromatin structure.

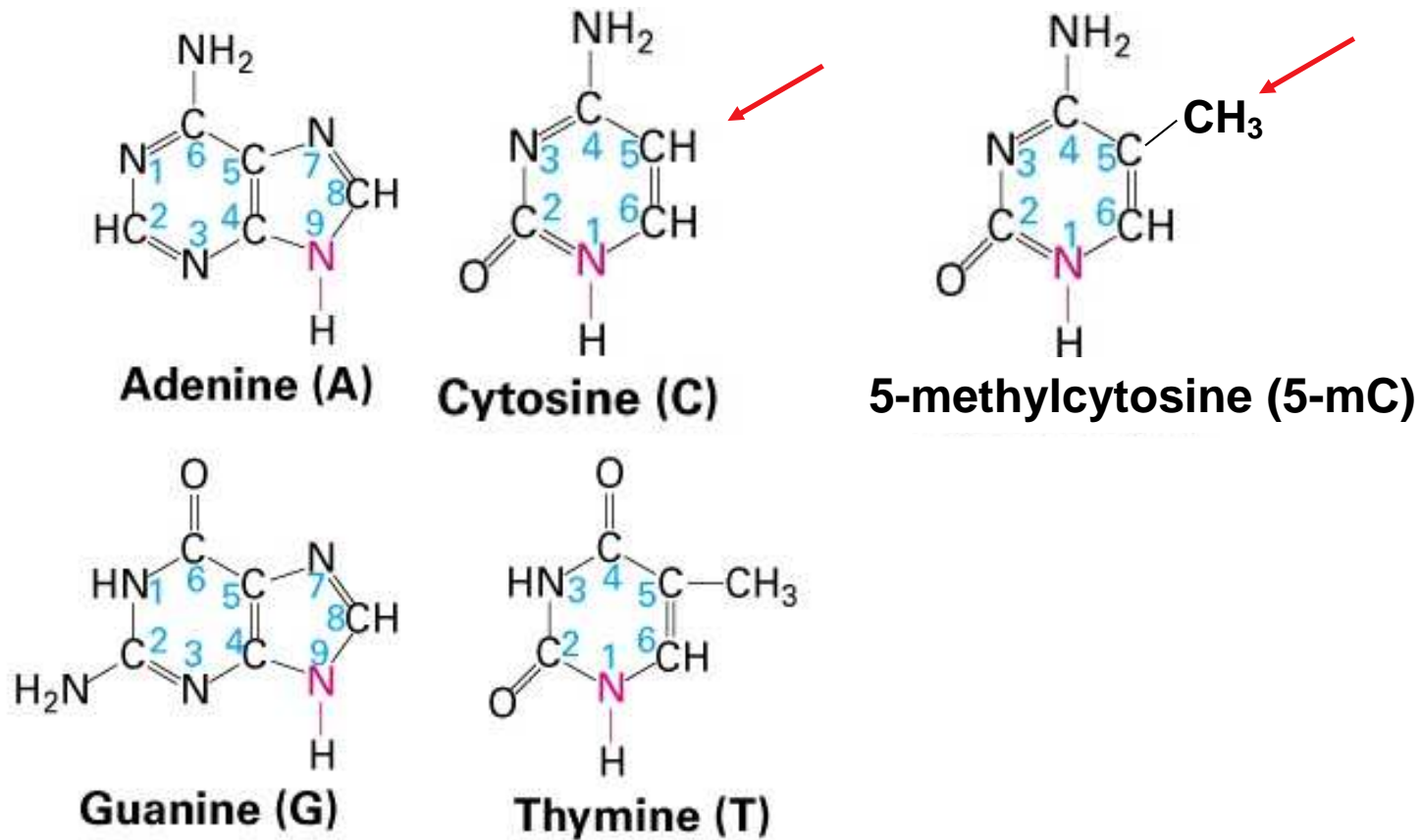
**Epigenetic modifications include:**

- **Histone modifications**
- **Cytosine methylation of DNA**

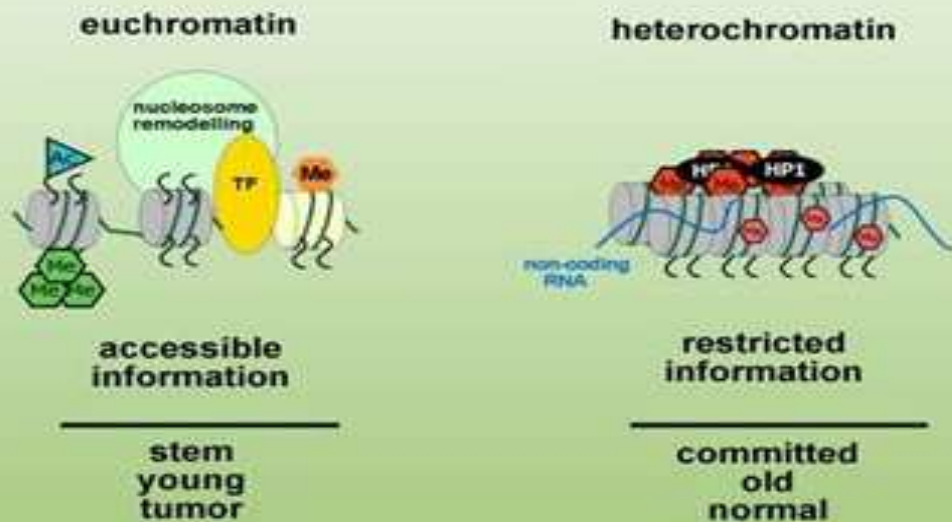
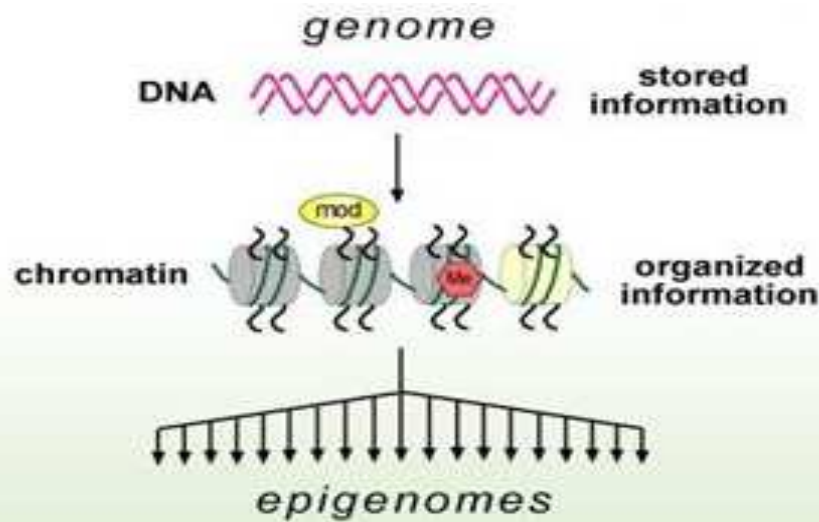


Collectively, these changes contribute to the distribution of DNA into silent, heterochromatin and active euchromatin

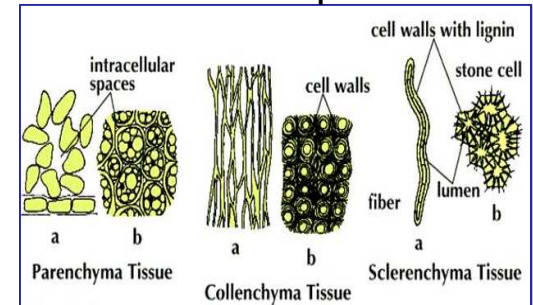
## Epigenetic marks – DNA methylation as the “fifth” nucleotide base



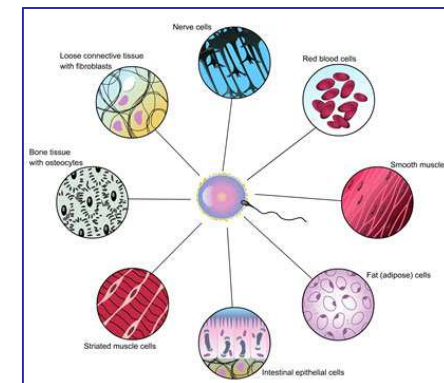
# Cell differentiation represent multiple epigenomes



## Differentiated plant cells



**One genome, but several dynamic epigenomes**



Differentiated animal cells



# Phenotypic discordance in monozygotic twins



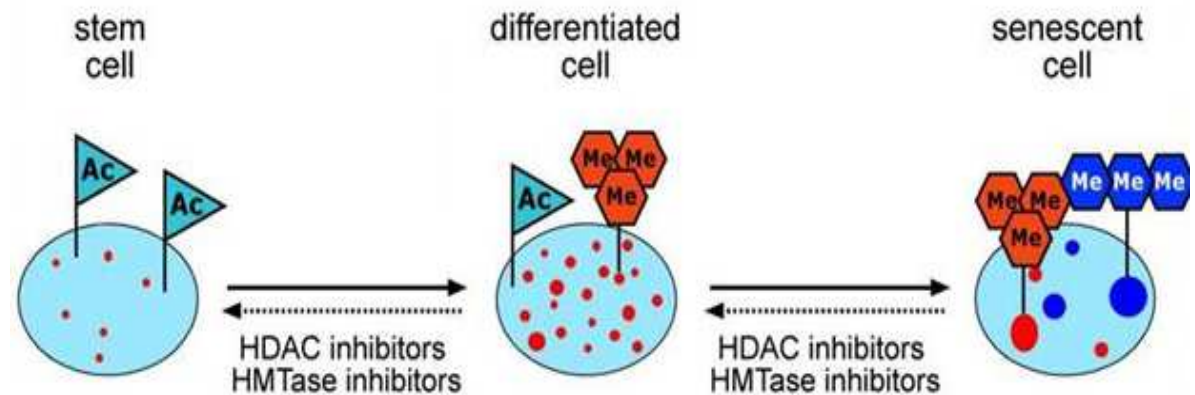
Fraga & Esteller 2007 TIG 23 413

Ageing reveals differences in levels of DNA methylation and histone acetylation with ~ 50% of changes at gene promoter regions.

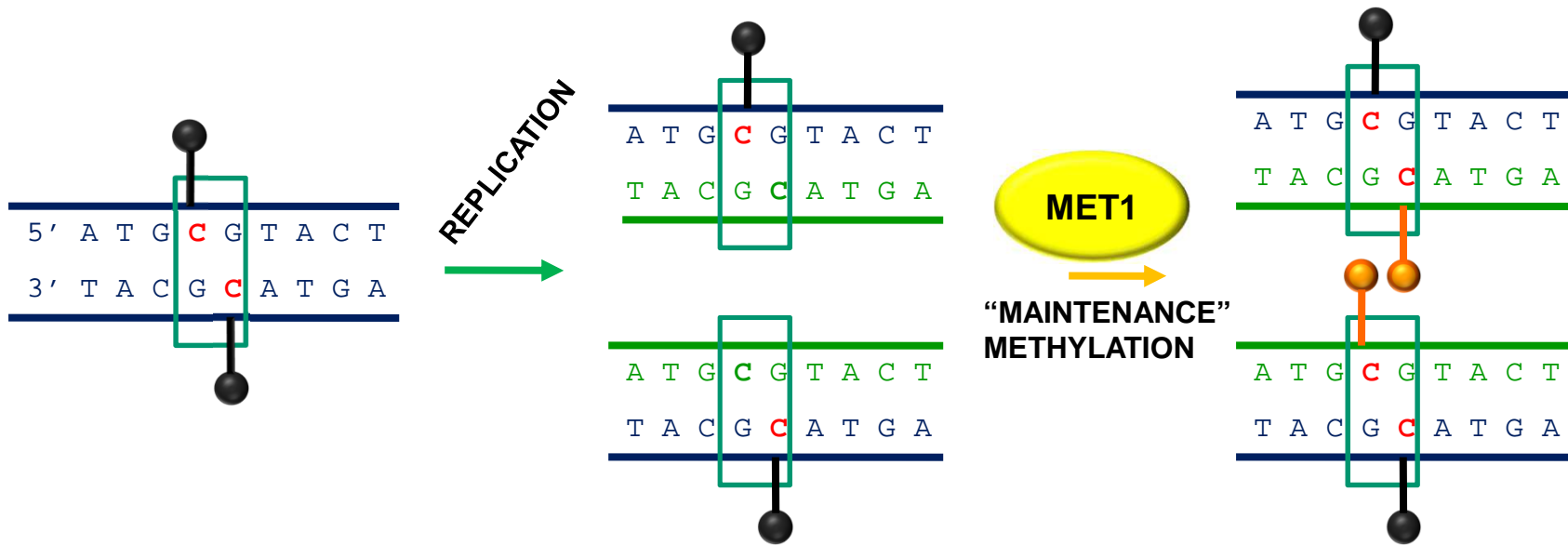
Changes in phenotype accumulate over time, or are influenced by environmental factors as diet

Ageing induces:

- ❑ DNA methylation drift
- ❑ Histone modifications
- ❑ Methylation of tumour-suppressing genes



# Gene memory epigenetic marks are transmitted in each cell cycle

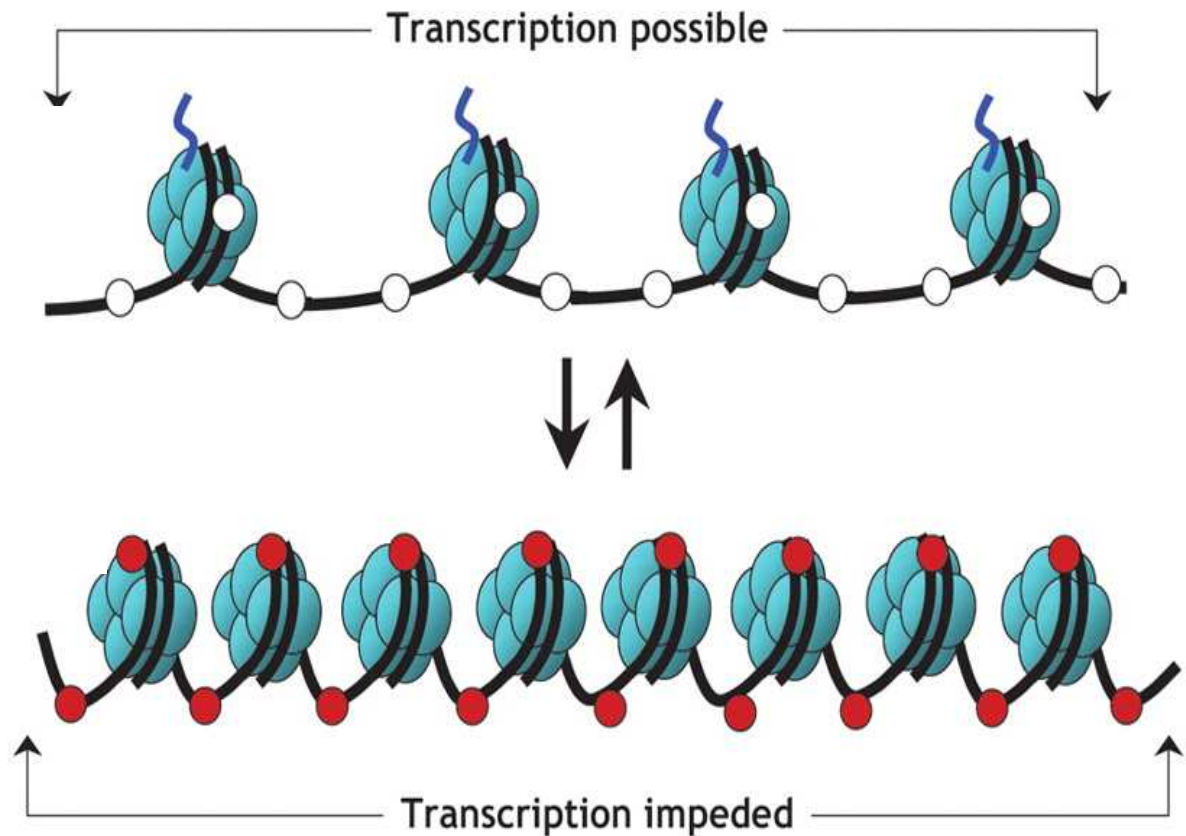


DNA methylation patterns are inherited through DNA methylases

# Open and condensed chromatin

## DNA methylation + histone modifications

- Gene “switched on”
- Euchromatin
  - unmethylated cytosines
  - acetylated histones



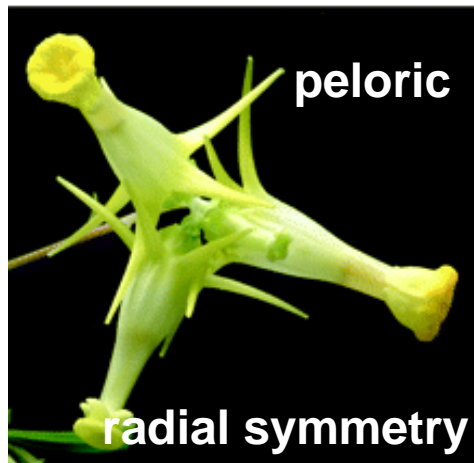
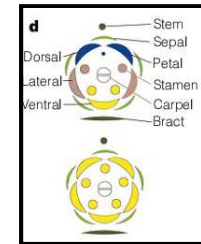
- Gene “switched off”
- Heterochromatin
  - methylated cytosines
  - deacetylated histones

# Epialleles result from changes in DNA methylation

*Linaria vulgaris* sequence of *Lcyc*

gene is identical in both forms

**DNA methylation** of *Lcyc* gene induces silencing in the “mutant”



Sequencing of *Lcyc* in several peloric and wild-type F2 segregants showed that all peloric individuals analysed were homozygous for the *Lcyc* **epiallele** from the peloric parent, whereas the wild types were either homozygous or heterozygous for the allele from the wild-type parent. Thus, the phenotypes in the F2 appeared to reflect transmission of a modified epiallele of *Lcyc*.

**An issue for taxonomists  
– what is a mutant ?**

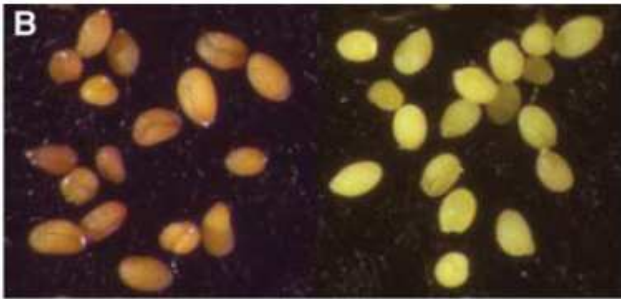
Cubas, P. et al. 1999 *Nature* 401: 157-161

Occasionally the mutant reverts phenotypically during somatic development, correlating with demethylation of *Lcyc* and restoration of gene expression. It is surprising that the first natural morphological mutant to be characterized should trace to methylation. This indicates that epigenetic mutations may play a more significant role in evolution than has hitherto been suspected.

## The epigenetics view of some phenotype differences



Expression of the dihydroflavonol reductase gene (DFR) is required for dark purple petunia flowers, whereas **silencing** of the DFR promoter gives rise to variegated, light coloration



Seeds from Arabidopsis expressing the chalcone synthase gene (CHS) have dark seed coats whereas **silencing** of CHS upon expression of a homologous transgene results in yellow seeds

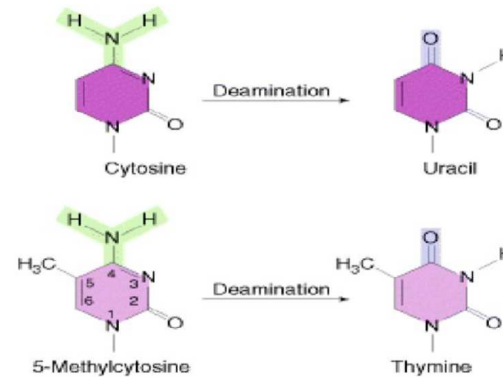


Soybeans colours in cultivated varieties  
The dark color of soybeans (middle) is extinguished in cultivated varieties (left) because of natural posttranscriptional **silencing** of the CHS gene and can be partially reversed by a suppressor protein, producing a mottled pattern (right).

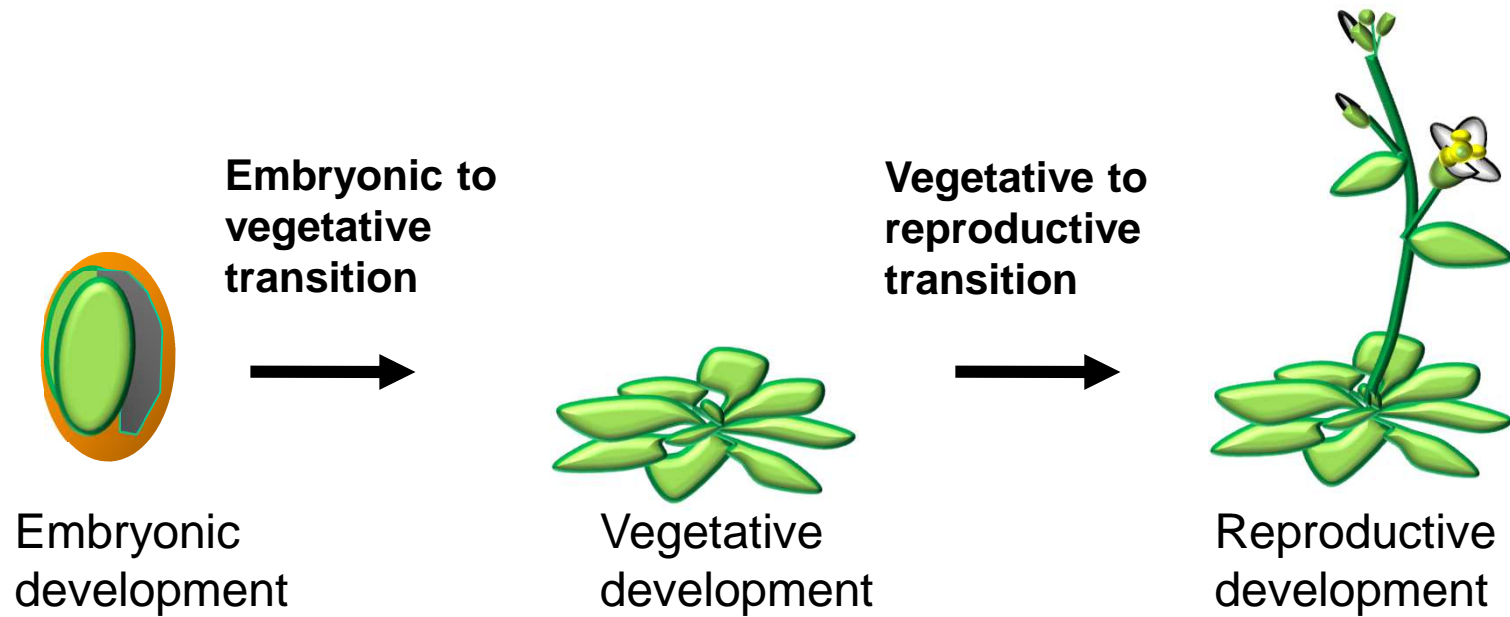
Epimutations appear to be less stable than DNA-sequence alterations, as illustrated by the various degrees of somatic reversion in *Linaria*. Nevertheless, they may have longer-term consequences, depending on how the variation they cause interacts with variation generated by DNA-sequence changes. Furthermore, methylated DNA is more prone to genetic mutations and may therefore have both a short- and long-term significance for plant evolution.



Whether epigenetic phenotypic changes are advantageous in a given environment, and whether they are subject to natural selection is of great interest, and their roles in adaptation and evolution are an area of active research

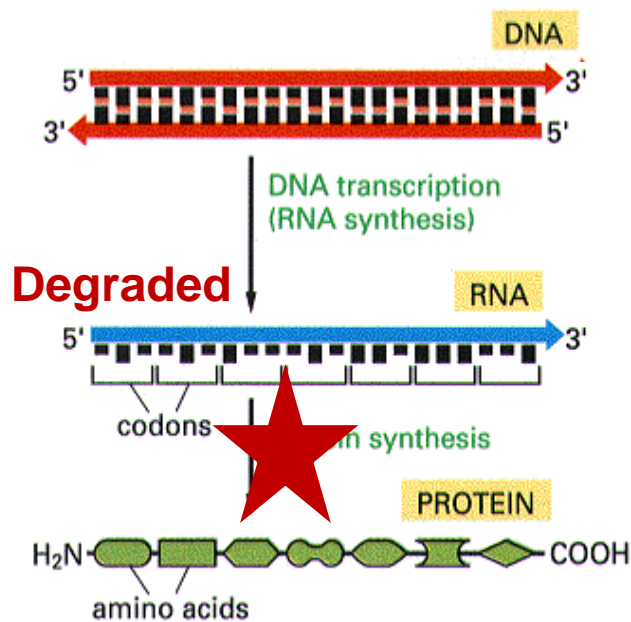


# Epigenetic programming controls developmental transitions

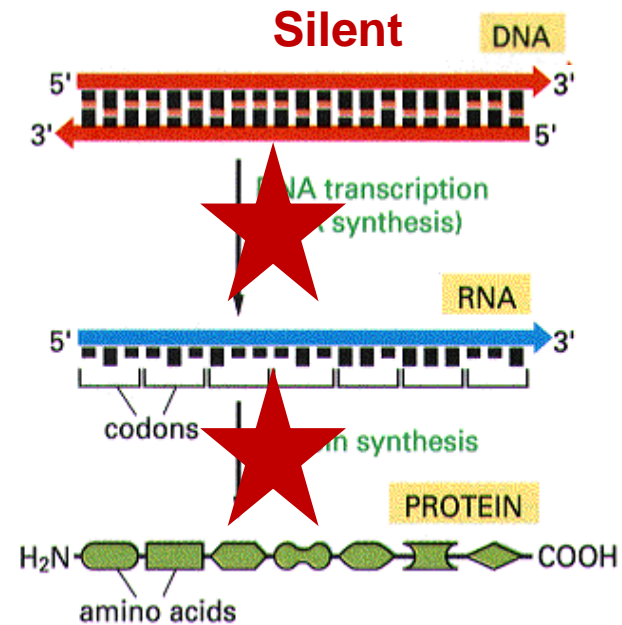


## But how gene silencing can be established?

A new world emerge with discoveries on **interference RNAs** which can bring about posttranscriptional or transcriptional silencing in neighboring cells or even in cells in distant organs



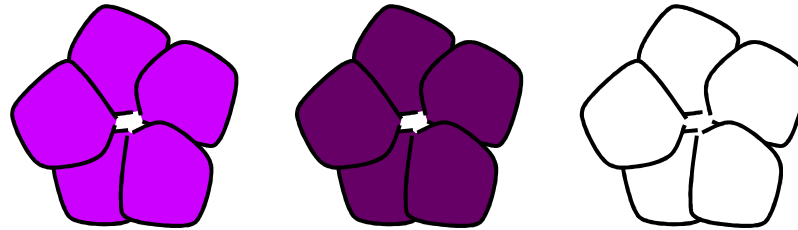
Gene silencing at the translation level



Gene silencing at the transcription level



## Disclosure of siRNAs through the discovery of transgene-induced post-transcriptional silencing



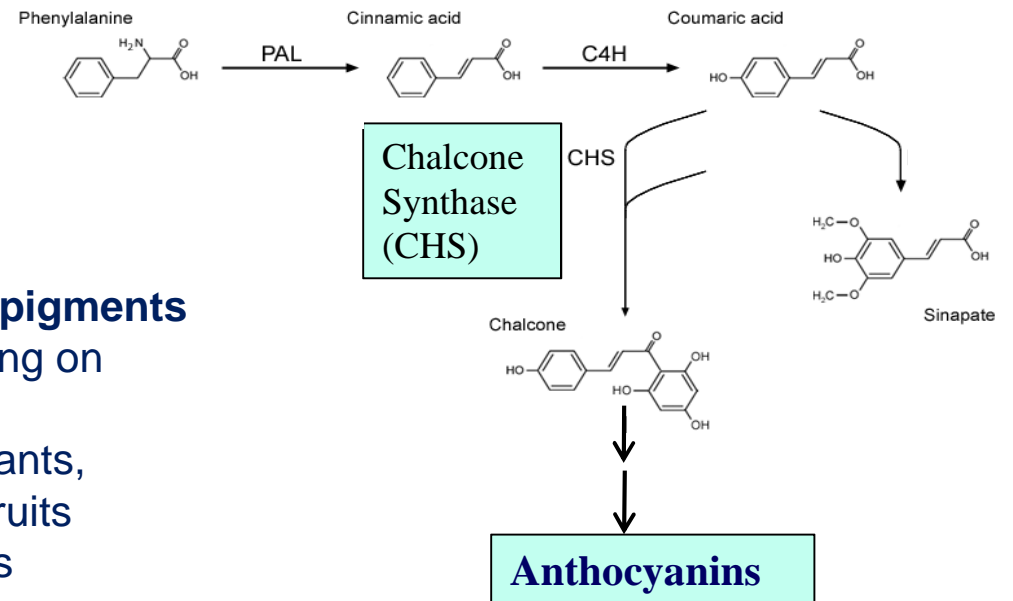
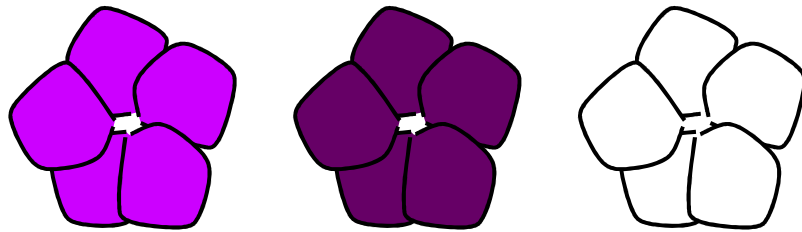
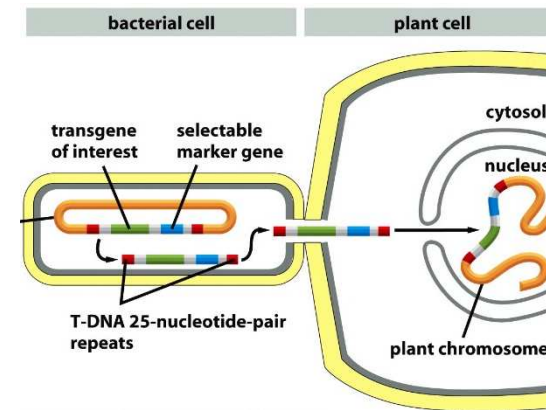
Experiments to modify flower color in petunia gave early evidence of RNA silencing.



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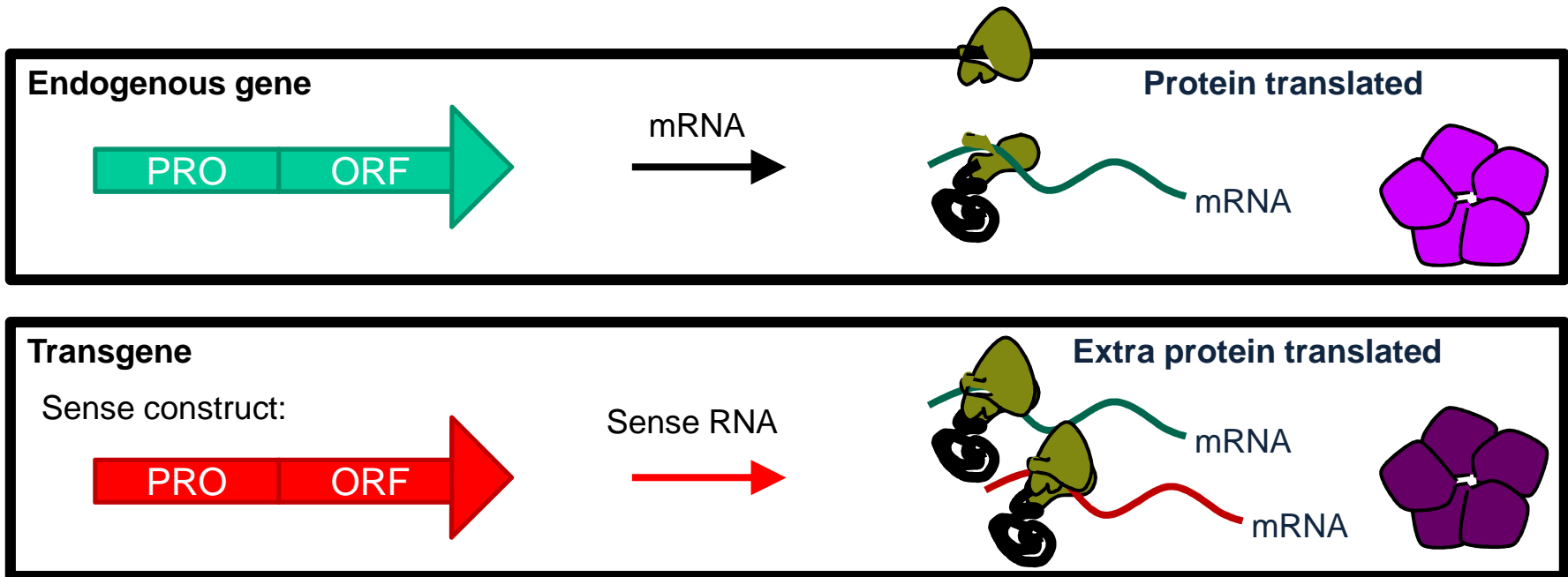
Plants carrying CHS transgene

In an attempt to alter flower colors in petunias, researchers introduced additional copies of a gene encoding **chalcone Synthase (CHS)**, a key enzyme for flower pigmentation into petunia plants

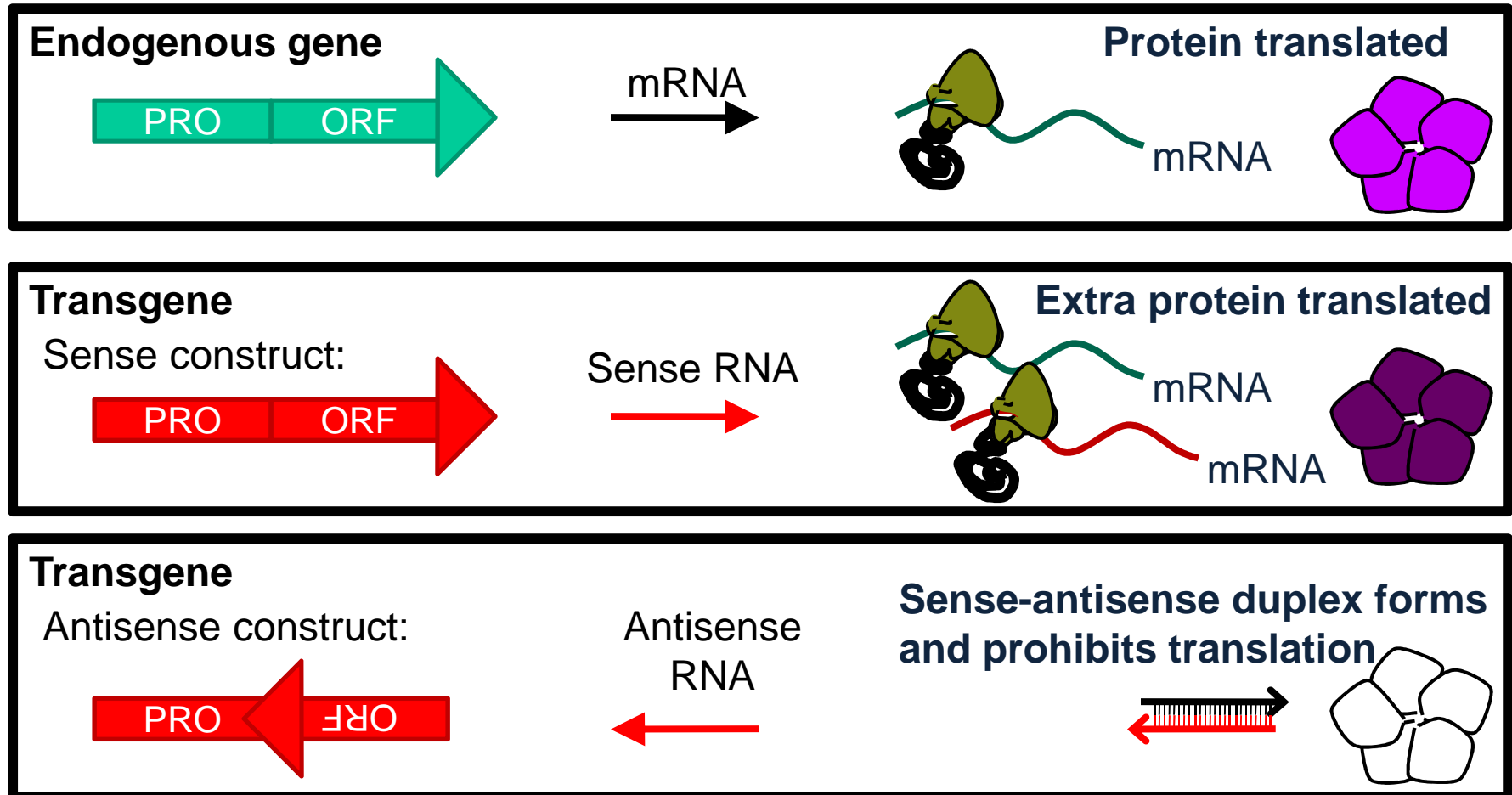


**Anthocyanins are water-soluble vacuolar pigments** that may appear red, purple, or blue depending on the pH. Food plants are rich in anthocyanins. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits and also some of the colors of autumn leaves come from anthocyanins.

Expectation – sense RNA production would enhance pigmentation...



# But.... antisense RNA production block pigmentation in some flowers





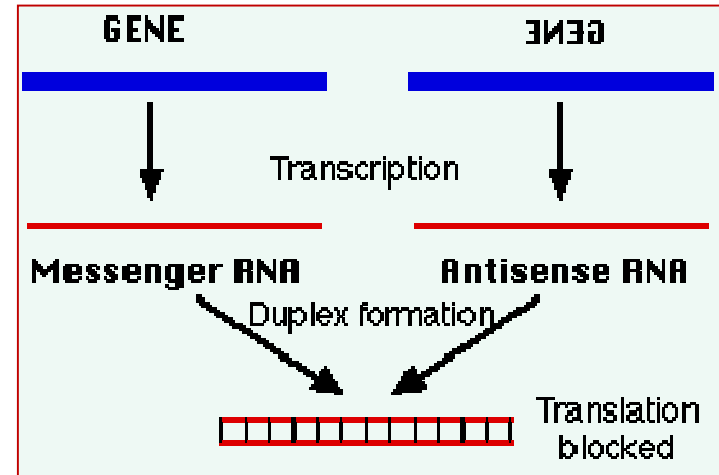
The Nobel Prize in Physiology or Medicine 2006  
"for their discovery of RNA interference –  
gene silencing by double-stranded RNA"



Andrew Z. Fire



Craig C. Mello



In 1998, Fire and Mello published a study of RNA interference in *C. elegans* showing that the optimal trigger for silencing was dsRNA

## siRNAs as Genomic Defenders



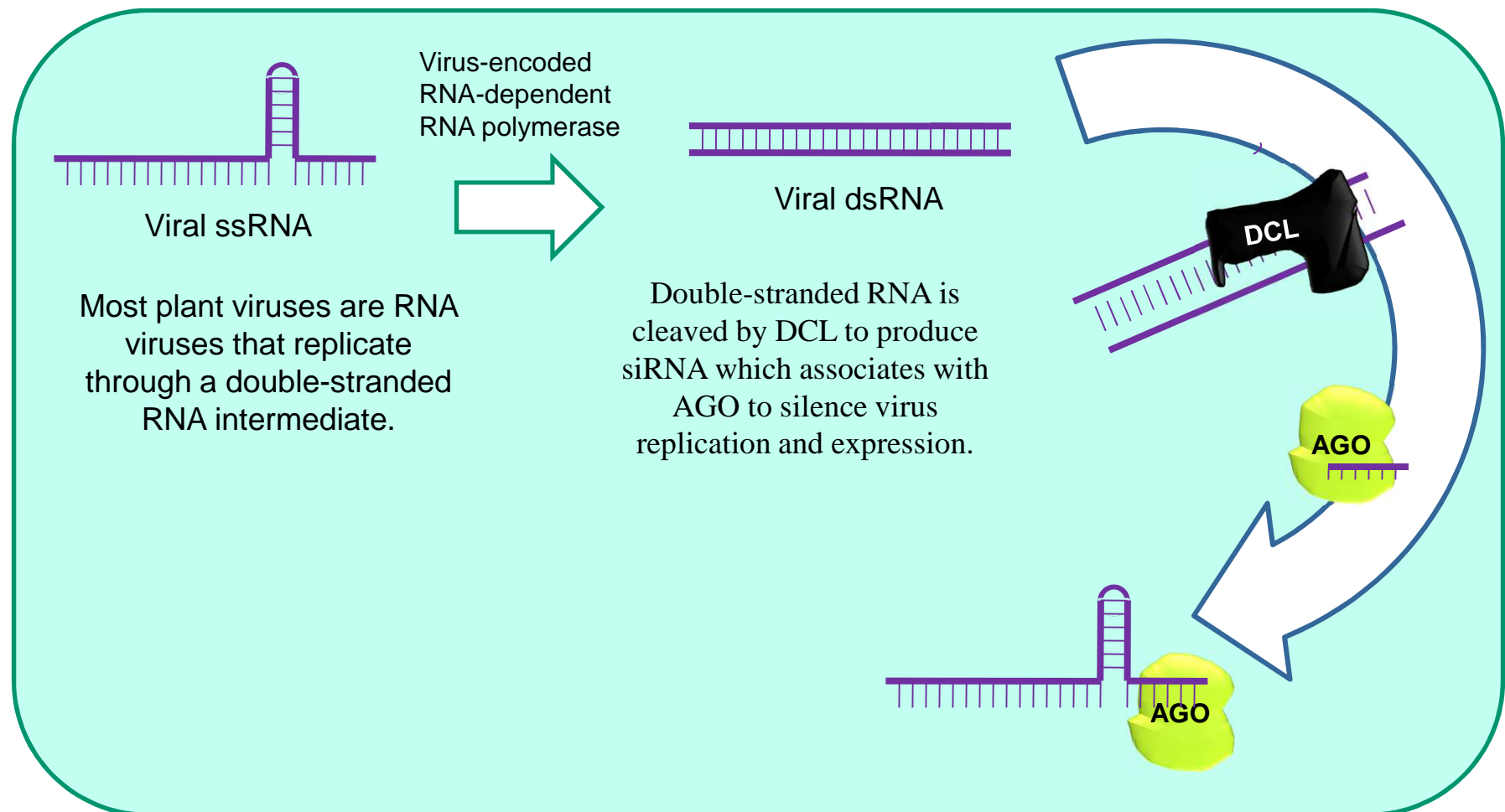
siRNAs maintain some genes in an epigenetically silent state

siRNAs also protect the genome by

- suppressing invading viruses
- silencing sources of aberrant transcripts
- silencing transposons and repetitive elements

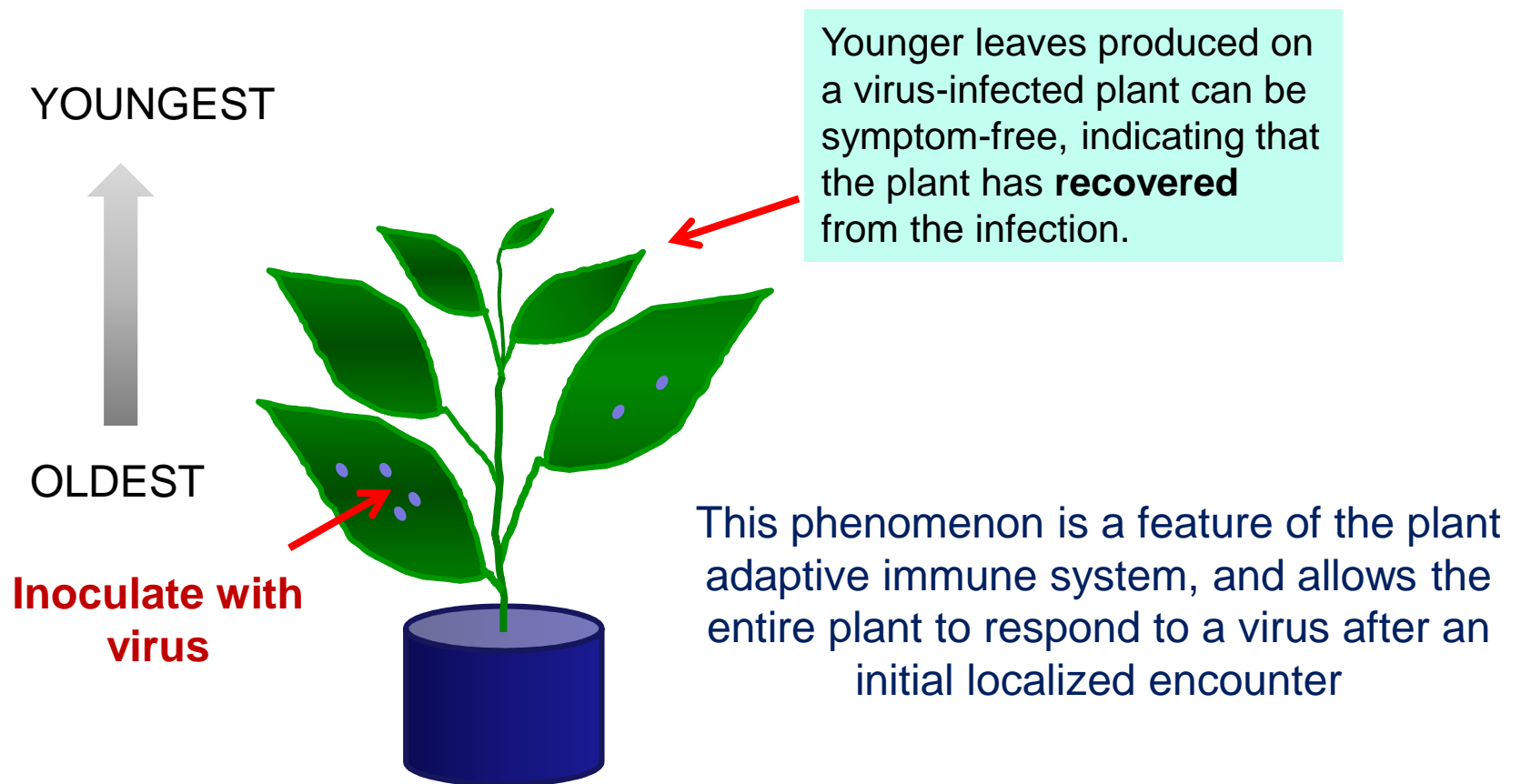
## Viral induced gene silencing – overview

RNA interference is a vital part of the immune response to viruses and other foreign genetic material, especially in plants where it may also prevent the self-propagation of transposons (mobile DNA sequences)



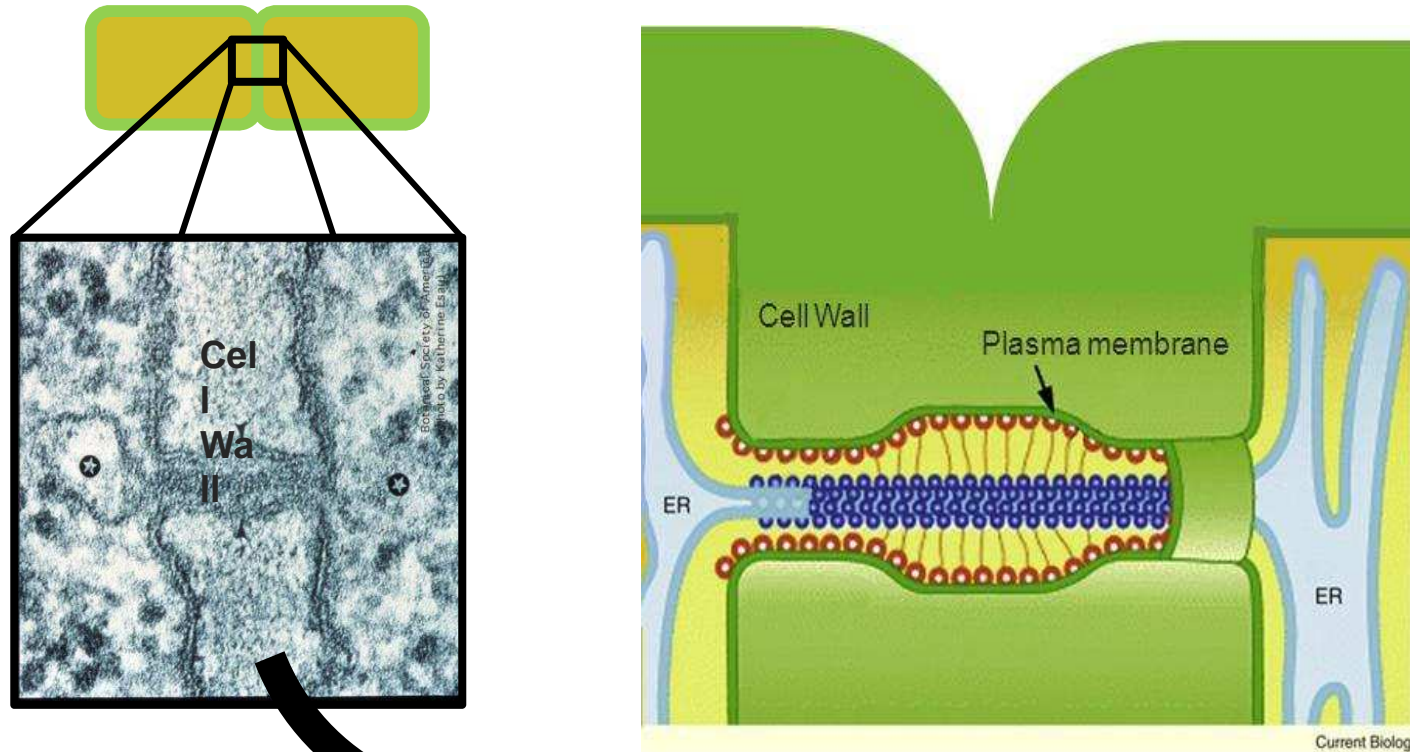
## Plants can recover from viral infection and become resistant

Even before the RNAi pathway was fully understood, it was known that induced gene silencing in plants could spread throughout the plant in a systemic effect.





## Plasmodesmata are regulated connections between plant cells

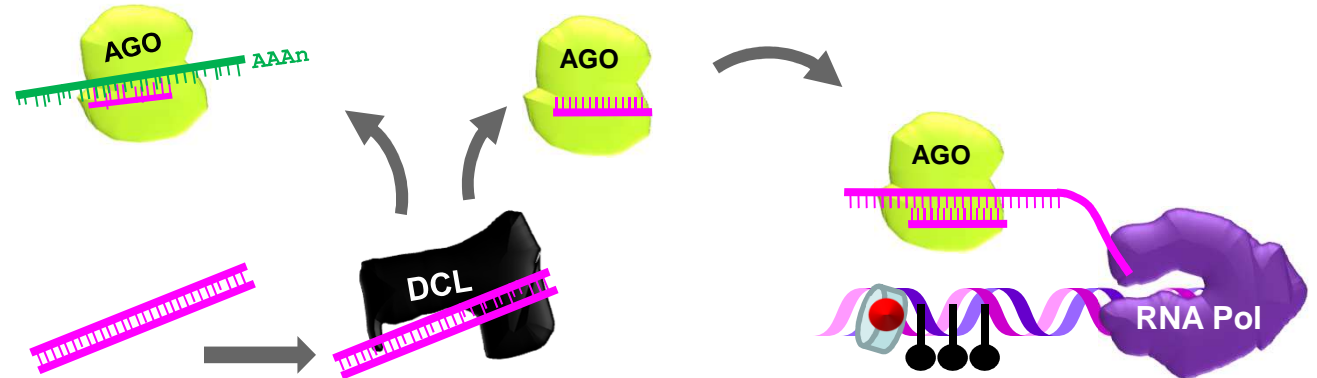


For short-range movement, RNAi travel from their cell of origin into neighboring cells through plasmodesmata.

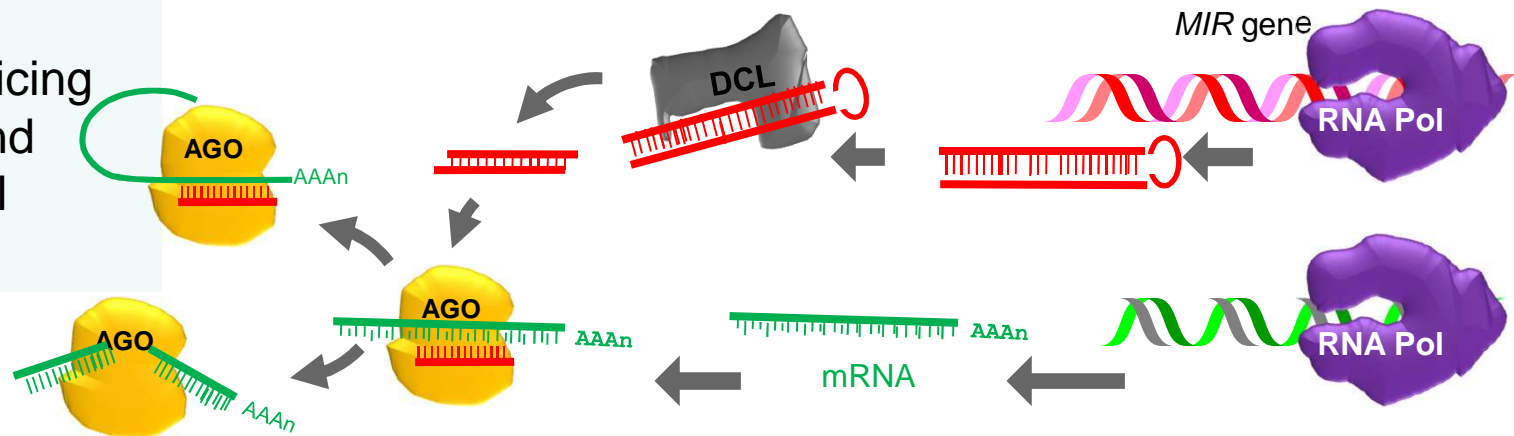
Guard cells, which form the openings (stomata) for gas exchange in leaves, lack plasmodesmata and are excluded from receiving silencing signals from adjacent cells

## RNA silencing - overview

**siRNA**-mediated silencing via post-transcriptional and transcriptional gene silencing



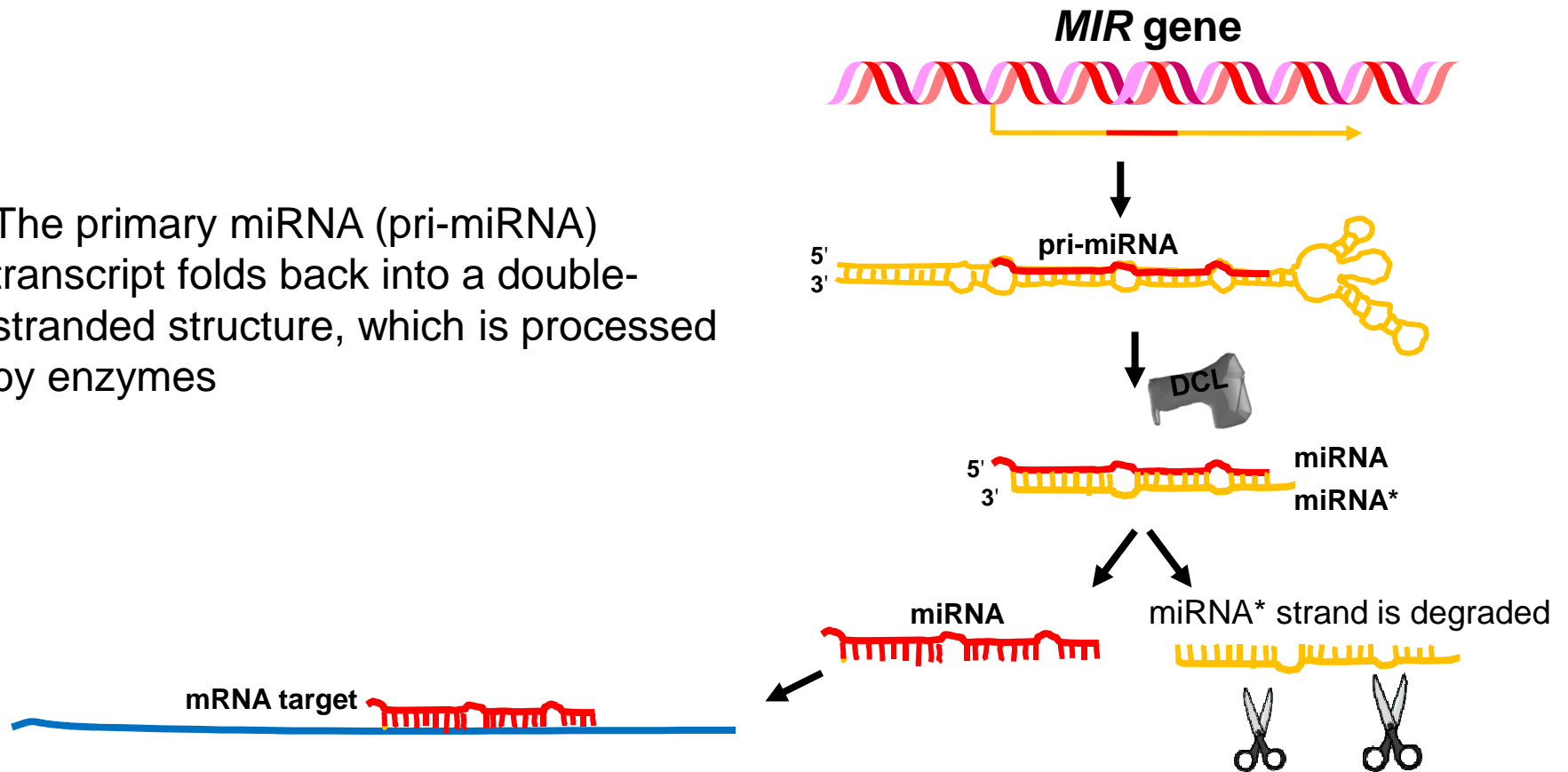
**miRNA** - mediated slicing of mRNA and translational repression



# MIR genes are transcribed into long RNAs that are processed to miRNAs

The primary miRNA (pri-miRNA) transcript folds back into a double-stranded structure, which is processed by enzymes

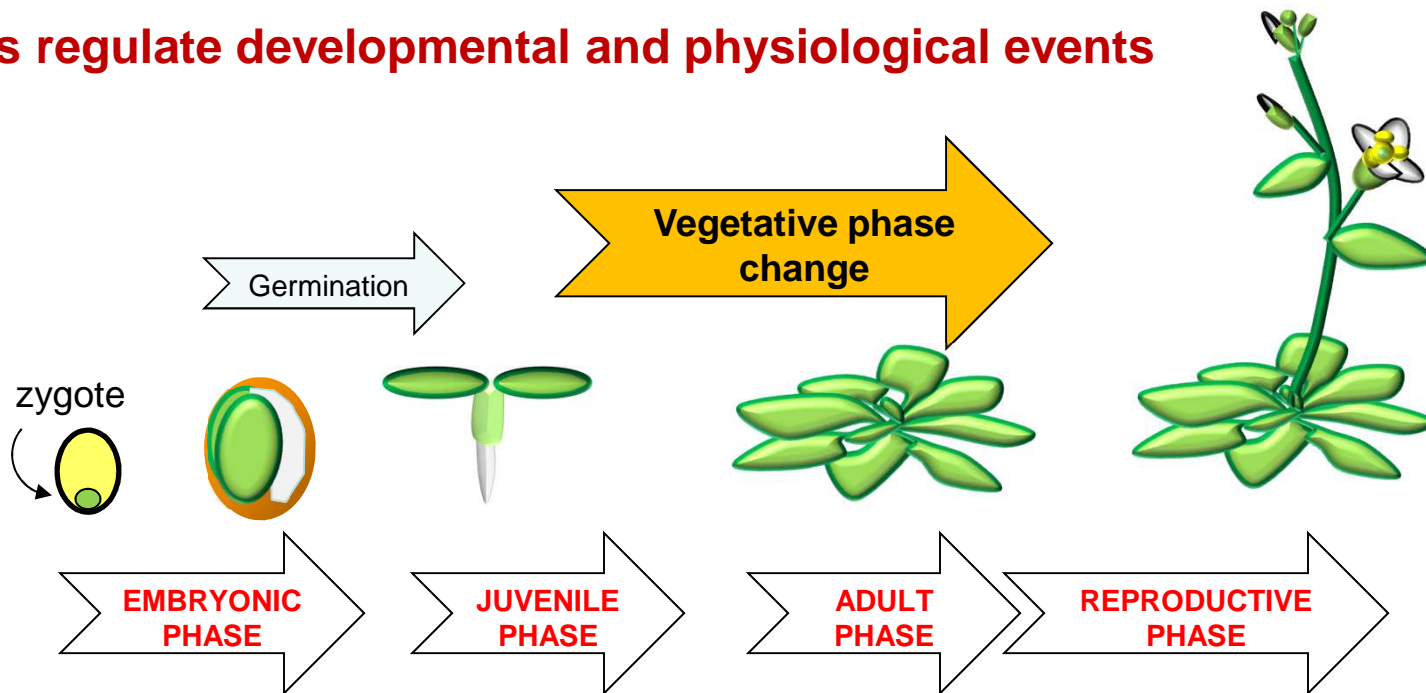
miRNAs are encoded by MIR genes



## miRNAs and vegetative phase change

Vegetative phase change is the transition from juvenile to adult growth in plants.

### miRNAs regulate developmental and physiological events

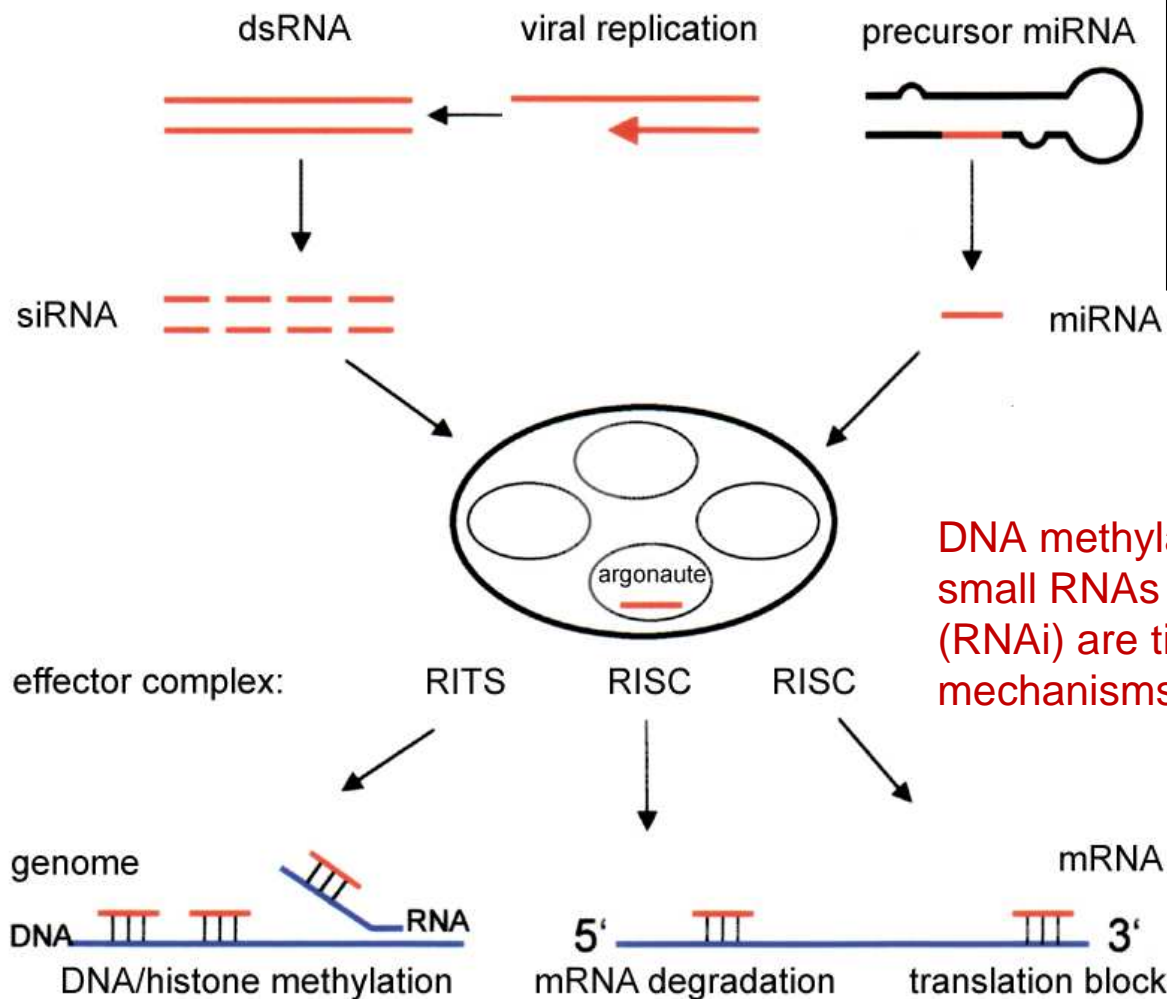


miRNAs are encoded by specific **MIR genes** but act on other genes – **they are trans-acting regulatory factors**

## RNA interference (RNAi)

Two types of small ribonucleic acid (RNA) molecules:

- small interfering RNA (siRNA)
- microRNA (miRNA)



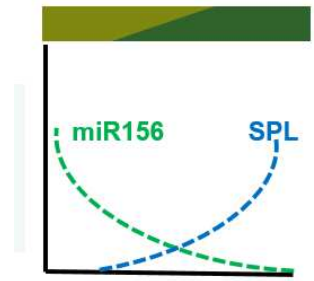
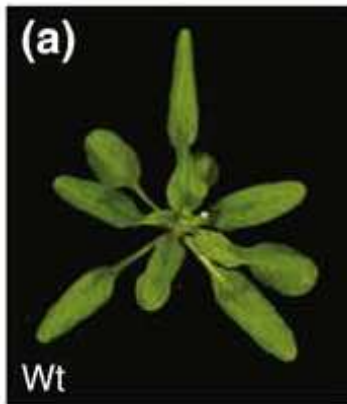
DNA methylation, histone modifications and small RNAs generated by RNA interference (RNAi) are tightly interconnected epigenetic mechanisms in plant genomes

Methylation of DNA can be the major epigenetic mark that is stably inherited for multiple generations through mitoses and meiosis, while histone modifications and small RNA molecules may also contribute to transgenerational transmission of epigenetic memory.

# In Arabidopsis, phase change affects leaf shape and trichome patterning through miRNA regulation

- Juvenile leaves are rounder, less serrated, and have trichomes only on the upper surface
- adult leaves also have trichomes on the lower surface.

Wild type

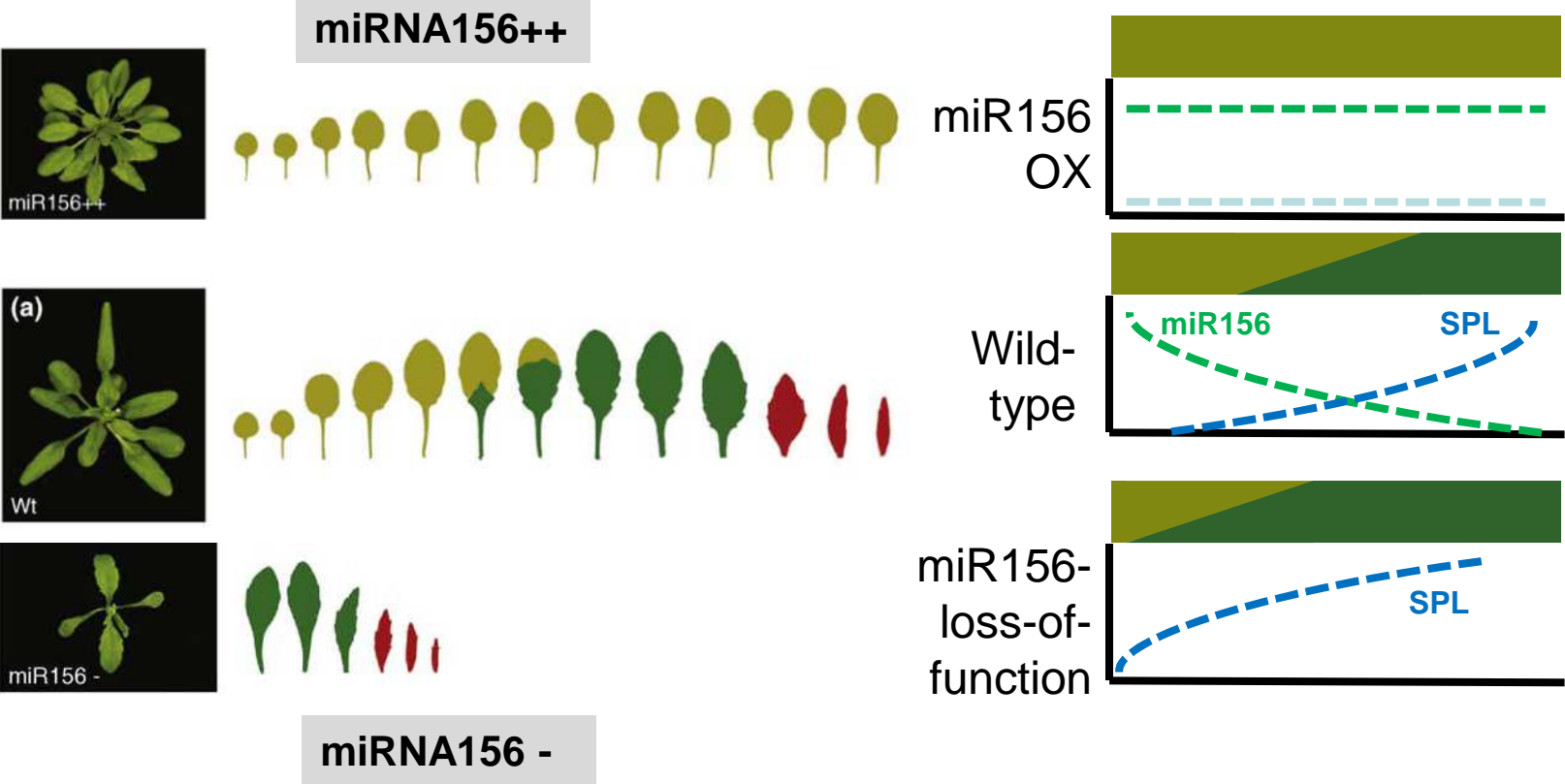


**miR156 targets *SPL* genes, promoters of phase change**

In wild-type plants, miR156 expression decreases with plant age, allowing SPL to accumulate and promote phase change.

using transgene-induced RNAi to knockout or knockdown the expression of candidate genes

**miR156 overexpression prolongs juvenile phase**



**miR156 loss-of-function promotes precocious phase change**

## Vernalization is epigenetically regulated

Environmental conditions such as photoperiod or temperature provoke changes in RNA- or chromatin-based transcriptional regulation.

Some environmental conditions induce altered chromatin and gene expression states that persist even after a return to the original environmental condition, as in the case of vernalization in which plants “remember” their experience of winter to flower the following spring.

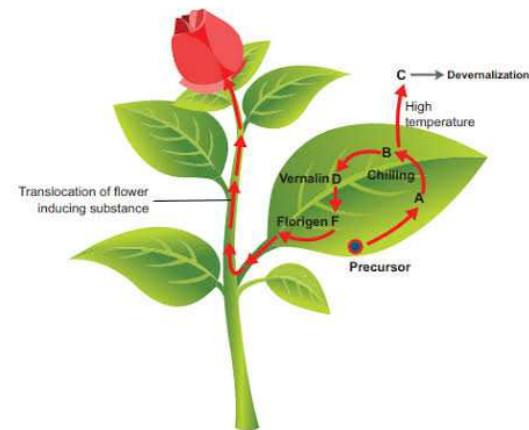
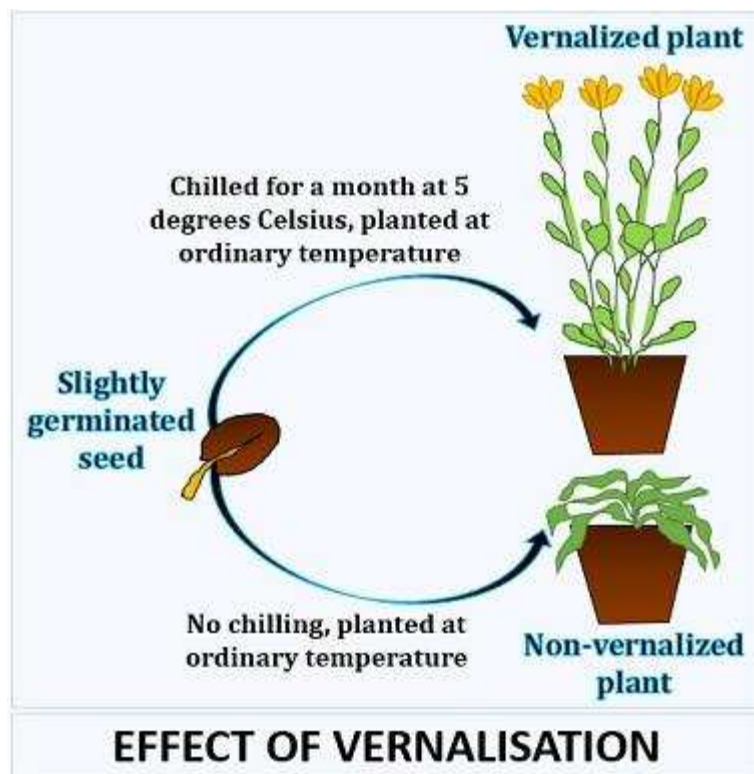
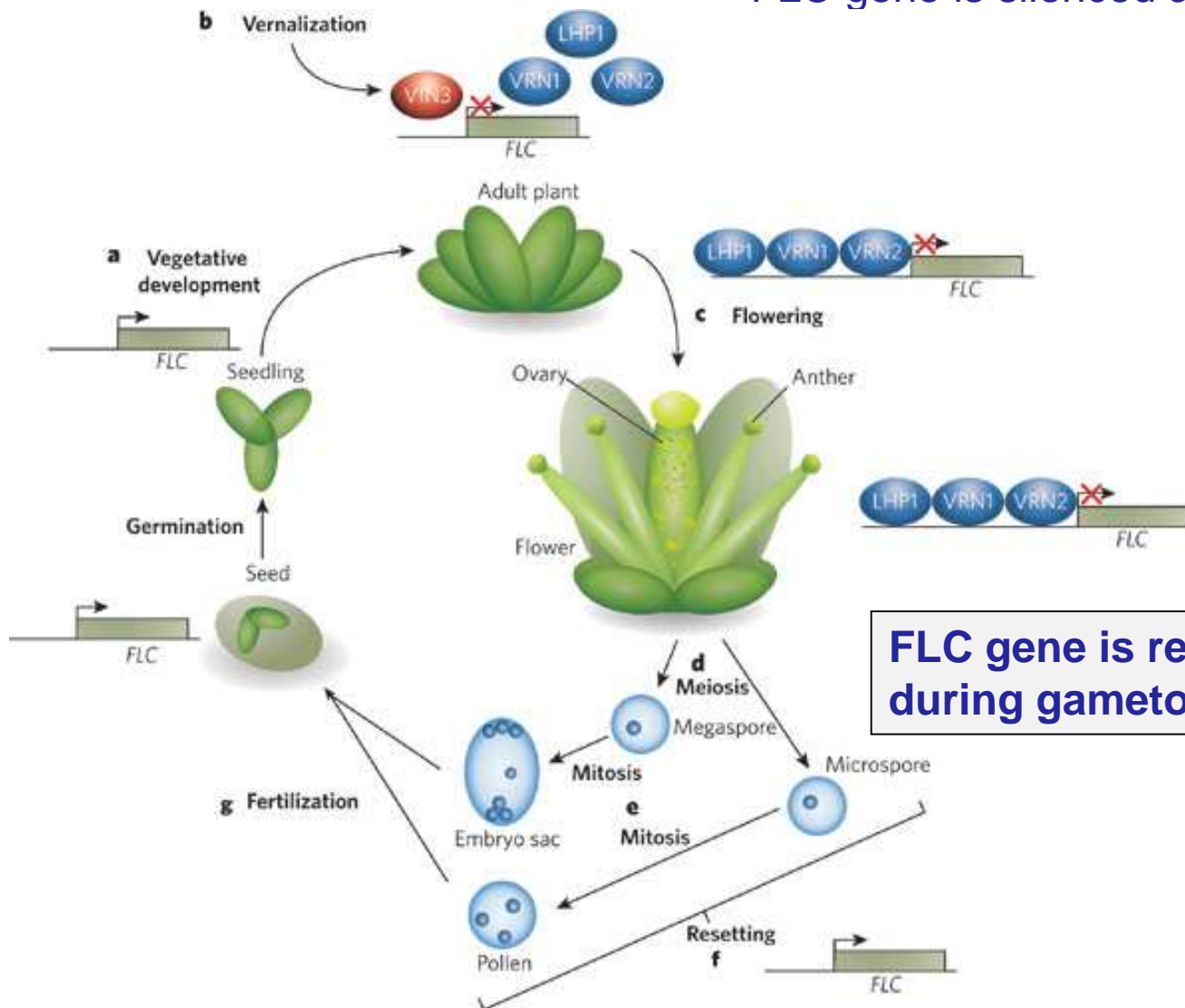


Figure 15.26: Vernalization and Flowering



# Meiosis reprograms epigenetics regulation

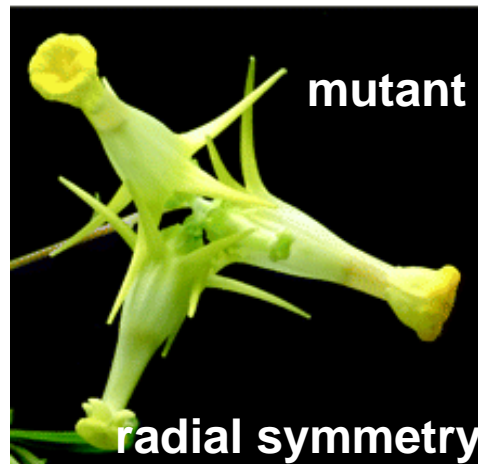
FLC protein repress flowering  
FLC gene is silenced after cold exposure



**FLC gene is reprogrammed during gametogenesis**

# Epialleles are escapees from reprogram and erasure in meiosis?

*Linaria vulgaris*



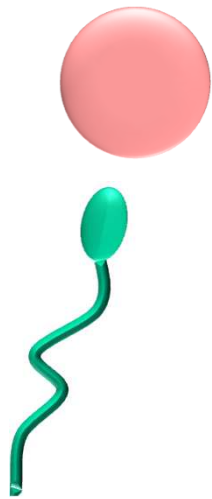
- ❖ Sequence of *Lcyc* gene is identical in both forms
- ❖ DNA methylation of *Lcyc* gene induces silencing in the peloric “mutant”

methyated > 250 years

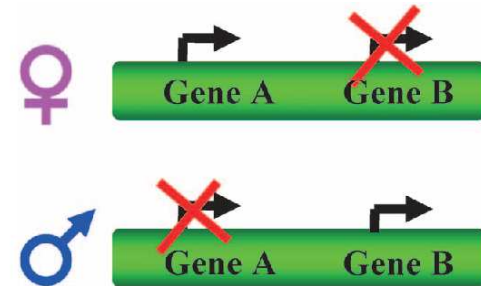
....or a remethylation process?

# Genomic imprinting in animals

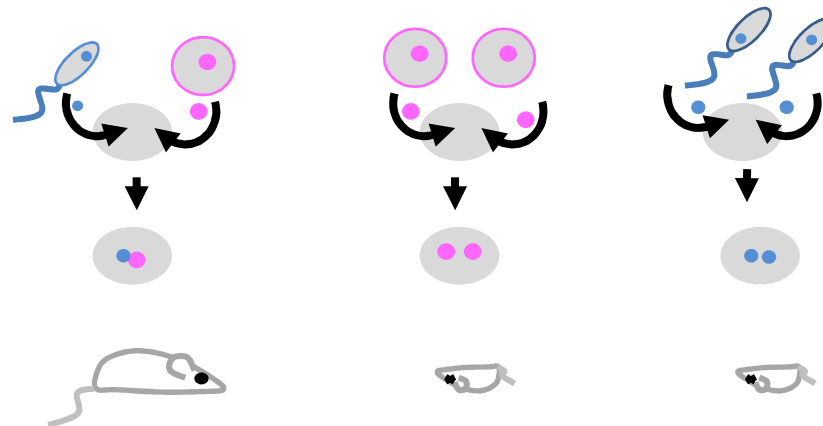
**Imprinting** occurs when the two alleles of a gene are expressed at different levels depending on their parent of origin, and is known to be regulated by epigenetic mechanisms



- The zygote receives two copies of each gene, one from the mother's genome and one from the father's.
- At most loci, both copies are active but some **imprinted** loci, show a "parent of origin effect".
- Expression of these loci is controlled by epigenetic factors.



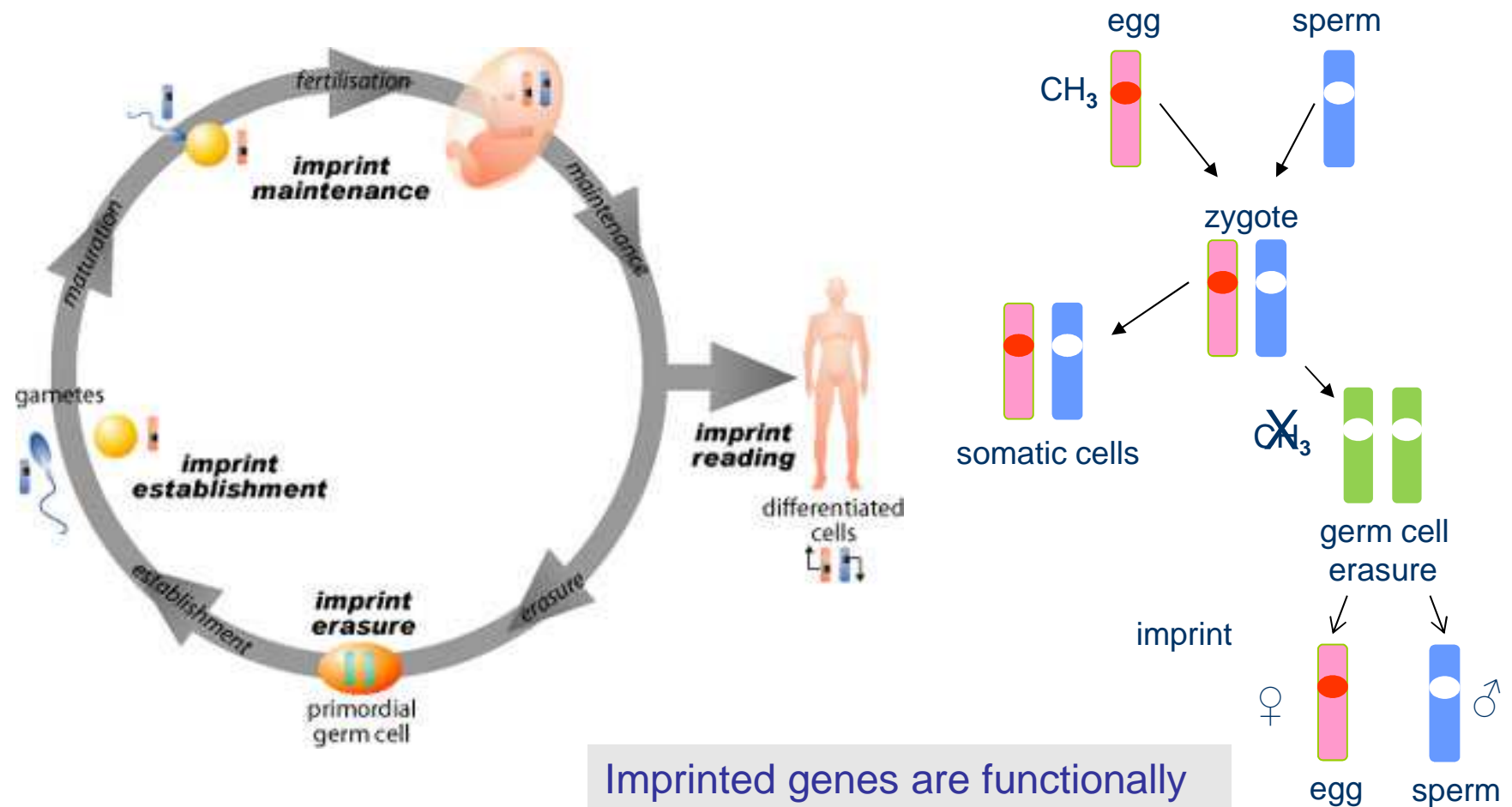
The two parental genomes are not equivalent



Zygotes that receive only maternal or only paternal nuclei do not survive.

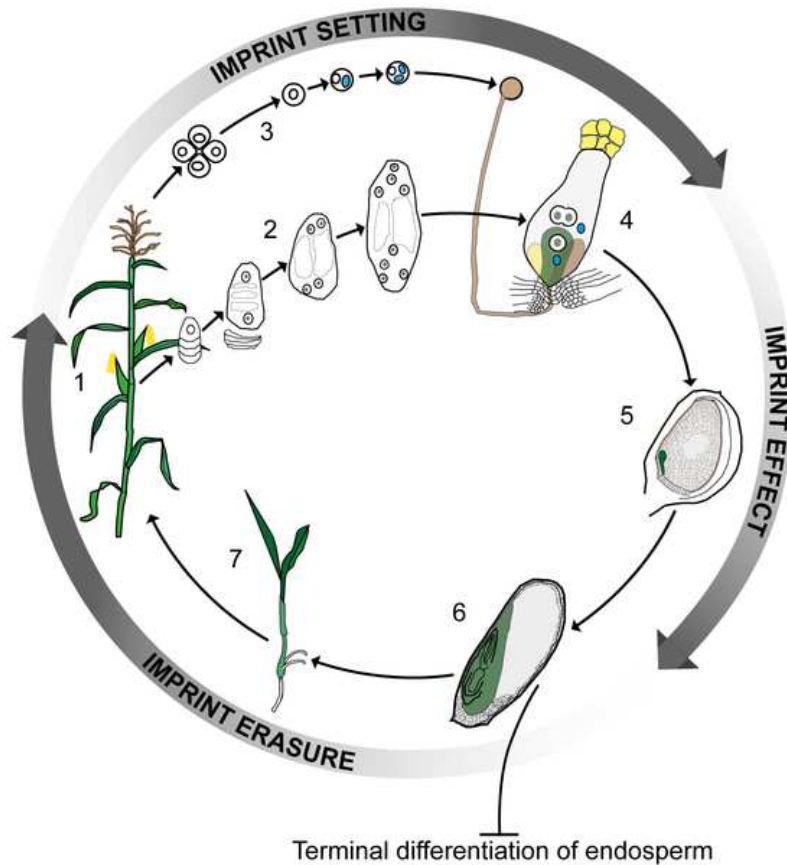
# Imprinting erasure and establishment during gametogenesis

Genomic imprinting results in the preferential expression of alleles after fertilization, depending on their parent of origin.



Imprinted genes are functionally haploid – ca 1-2% human genes

# Genomic imprinting and erasure in plants

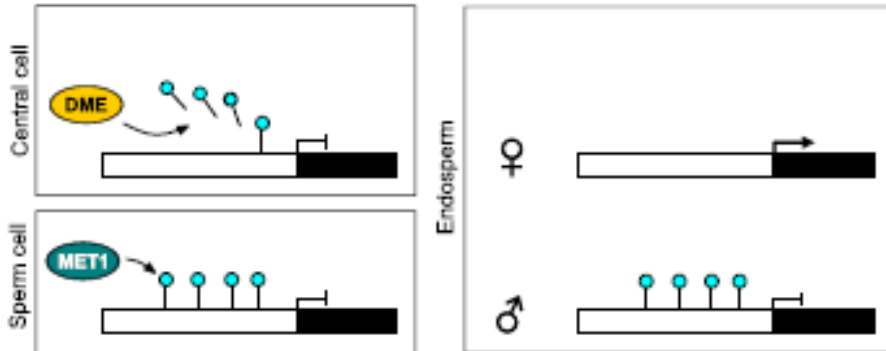


Endosperm imprinting discovery initiated through the detection of unmethylated maternal alleles, whereas identical paternal alleles were methylated and silent

In maize, the imprinted *meg1* gene, which directly regulates maternal provisioning of the embryo, controls ultimate seed composition and size in a strictly gene dosage-dependent manner.

# Maternal DNA demethylation as a primary driver of imprinted expression

- Genes expressed exclusively in the endosperm
- Silenced by DNA methylation in sporophytic tissues
- Maternally activated

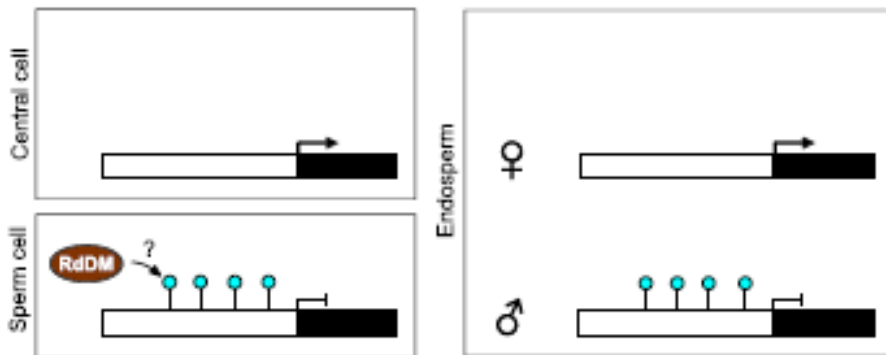


## Maternally expressed genes (MEG)

MEG that are constitutively marked with DNA methylation are silenced in sporophytic tissues.

- maternal expression in the endosperm requires the removal of maternal DNA methylation, as well as maintenance of paternal methylation.

- Genes expressed in endosperm and sporophytic tissues
- No constitutive epigenetic marks
- Paternally repressed

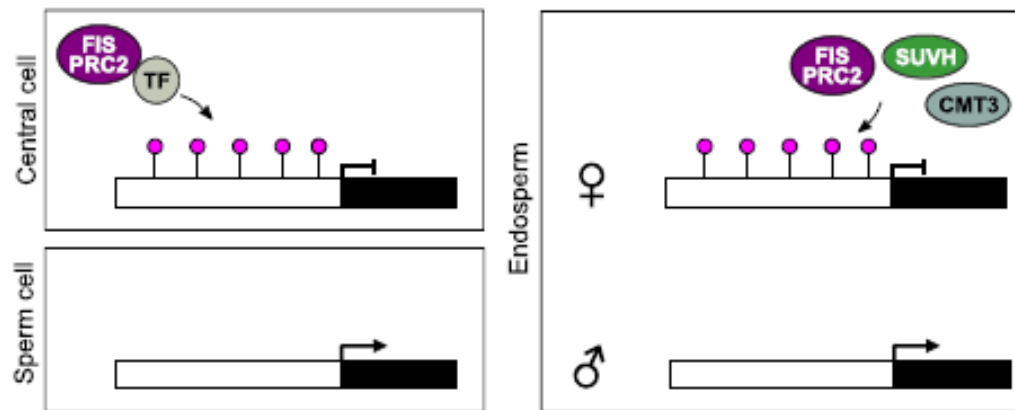


MEG that are expressed both in the endosperm and in sporophytic tissues do not carry any constitutive marks.

- maternal-specific expression is achieved through silencing of the paternal allele in pollen.

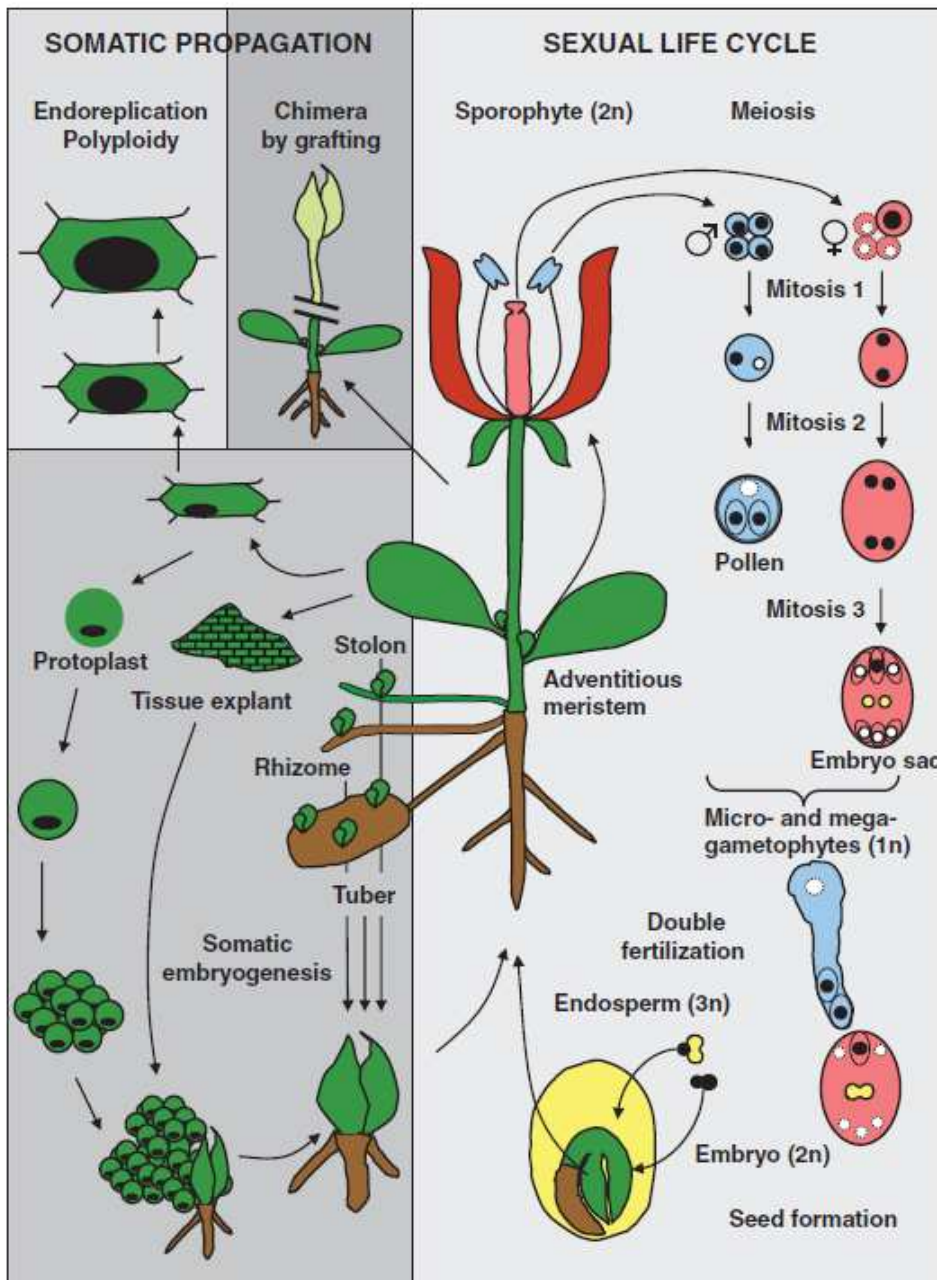
## Paternally expressed genes (PEG) dependent on parental DNA methylation asymmetries and H3K27me3

- Genes expressed in endosperm and sporophytic tissues
- No constitutive epigenetic marks
- Maternally repressed



PEGs that do not show any constitutive epigenetic marks and are expressed in the endosperm as well as in sporophytic tissues.

- Paternal-specific expression in the endosperm can be achieved through silencing of the maternal alleles in the central cell



Overall, a comparison of genome organization and epigenetic regulation in different model systems reveals that there are more common features between plants and mammals than there are within the animal kingdom itself.

Consequently, epigenetic mechanisms discovered in plants or mammals are generally relevant to both systems.

Unlike mammals, there is no evidence for a massive erasure of epigenetic marks during plant gametogenesis. Instead, repressive epigenetic marks in plant sperm and egg cells appear to be reinforced by specific trans-silencing RNAs produced in neighboring nuclei.

This might explain how epigenetic changes are often transmissible through meiosis in plants.



**13 maio 2021**

**Link zoom**

**<https://videoconf-colibri.zoom.us/j/4088210085>**

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