



Epigenetics and Agro-Traits



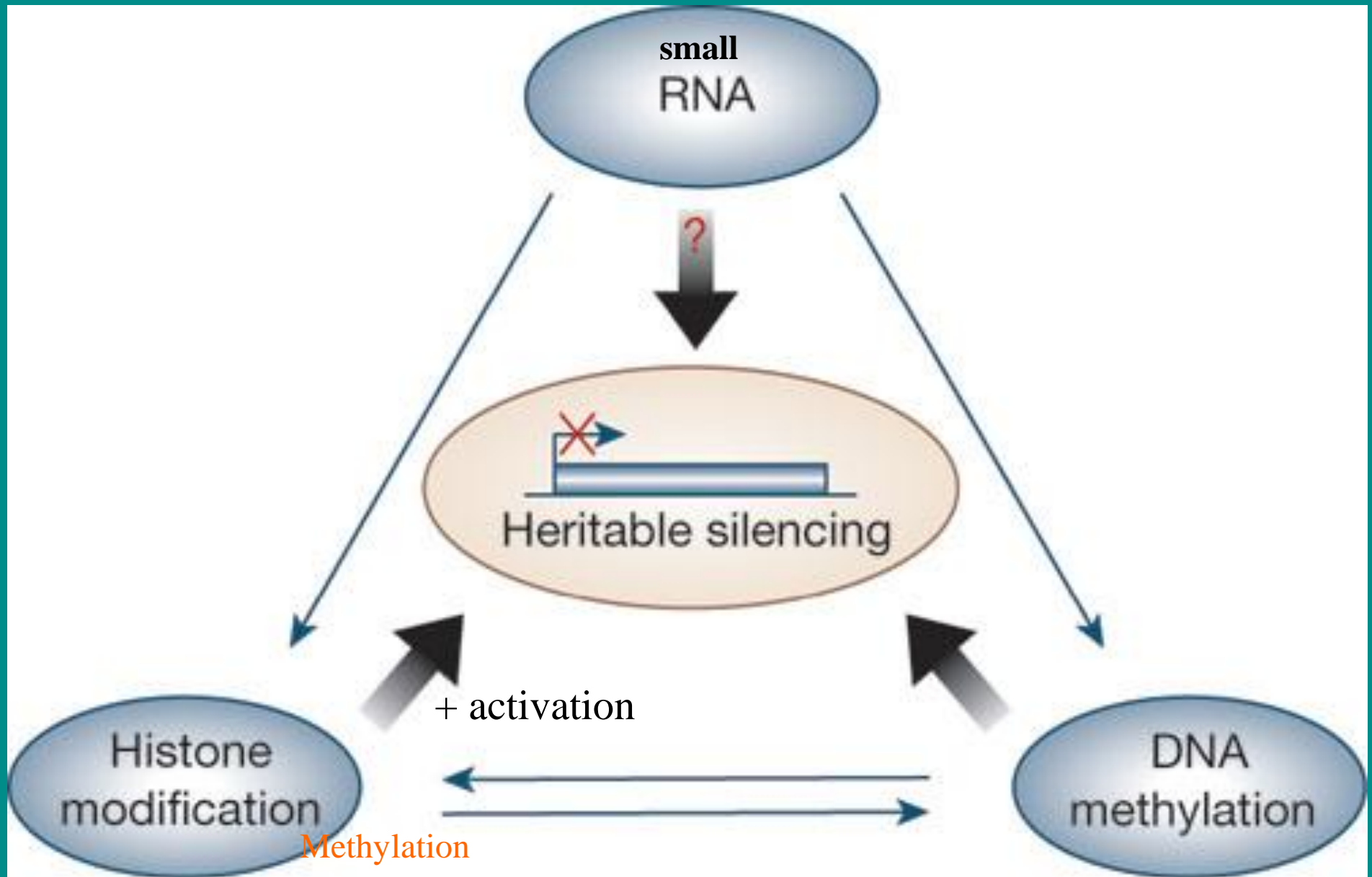
Dr Aliki Kapazoglou

Institute of Applied Biosciences/ CERTH, GREECE

EPIGENETICS

The study of stable and heritable changes in gene expression or cellular phenotype that do not entail changes in the DNA sequence

Epigenetic Mechanisms

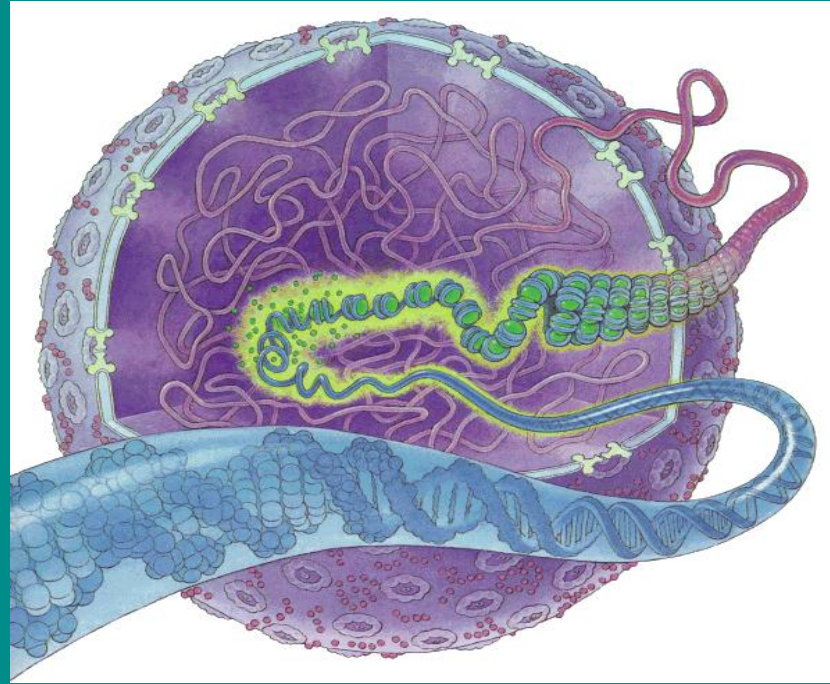


Methylation

Acetylation

Phosphorylation

Chromatin States



Closed compact chromatin



Gene silencing

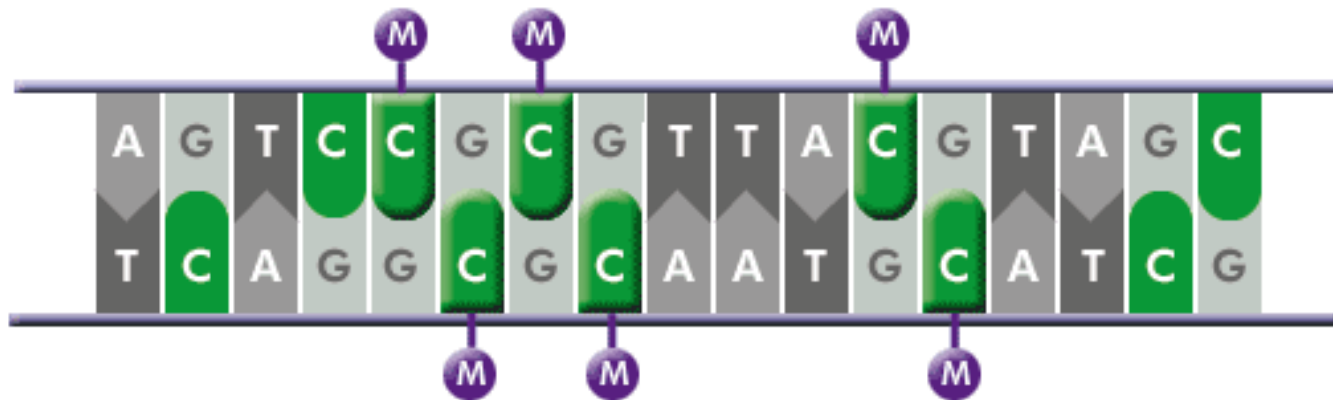
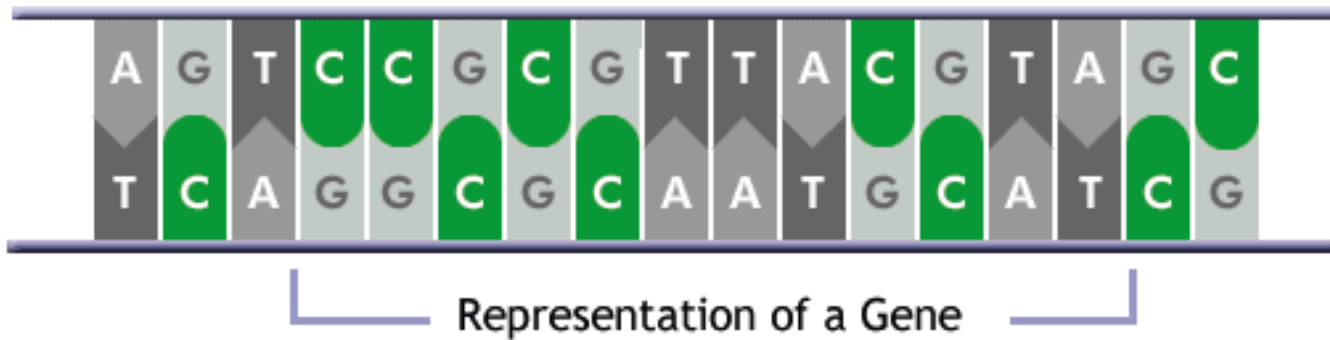


Open chromatin



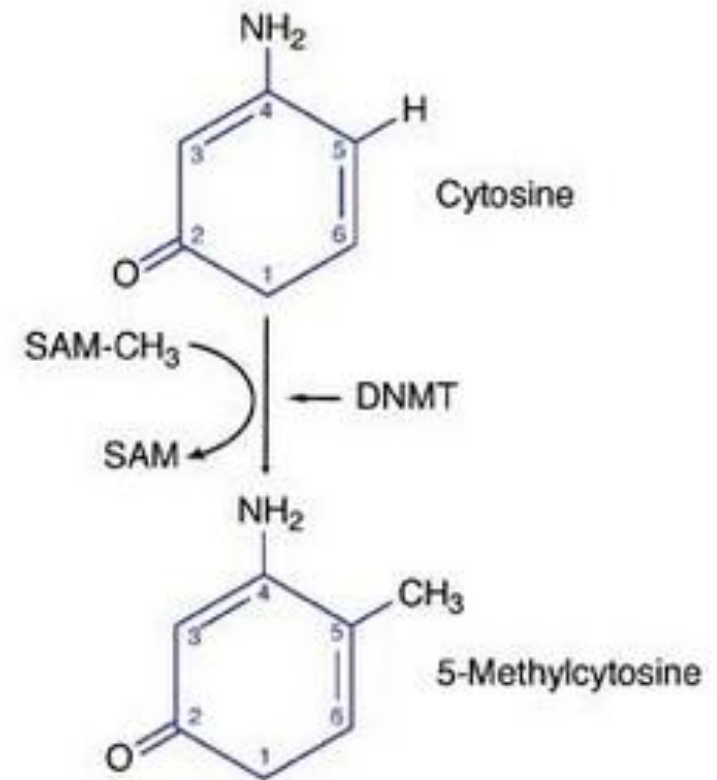
Gene expression

DNA METHYLATION



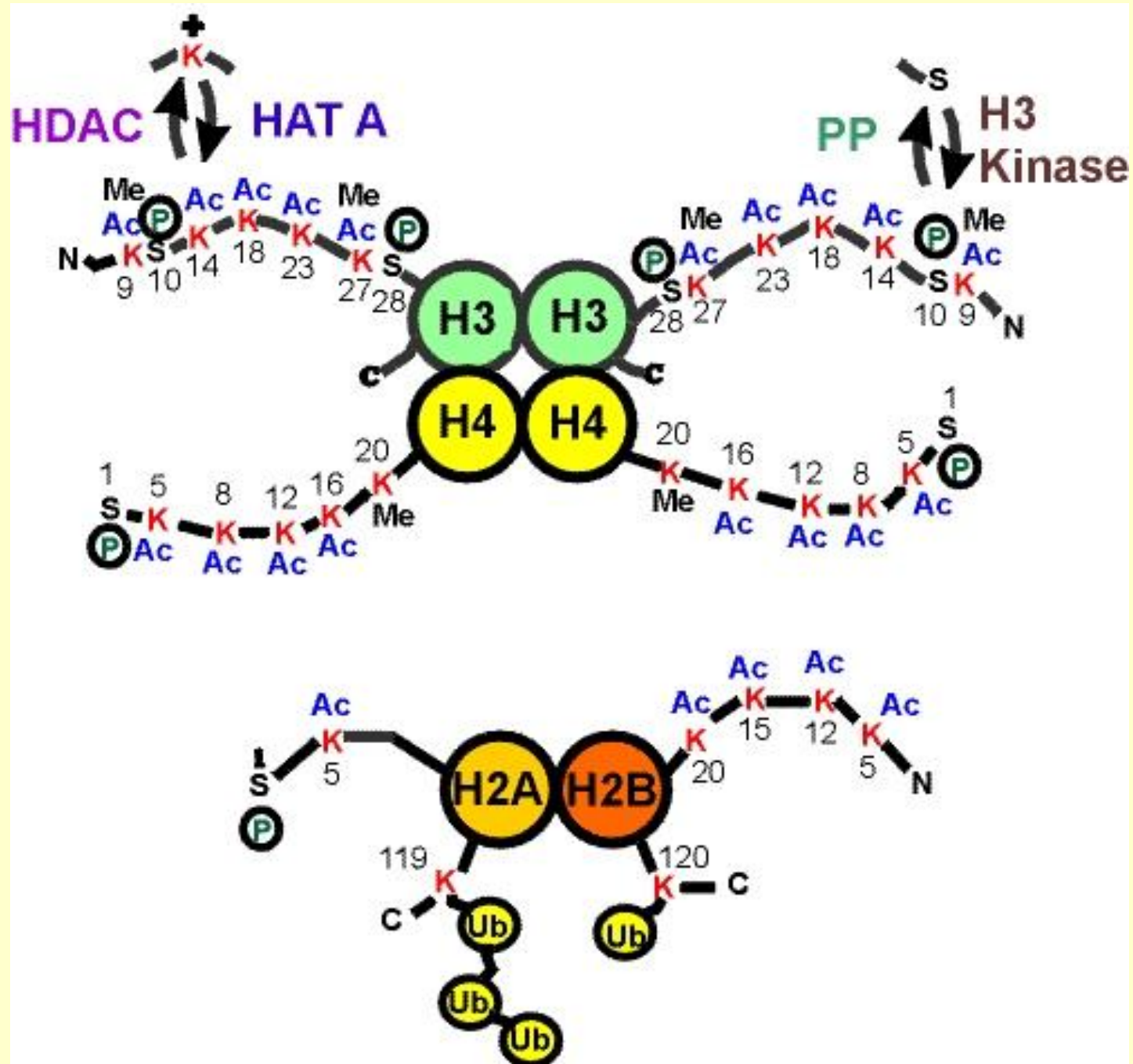
Cytosine methylation

- Transposon silencing
- Epigenetic regulation of endogenous genes
- Critical for normal development

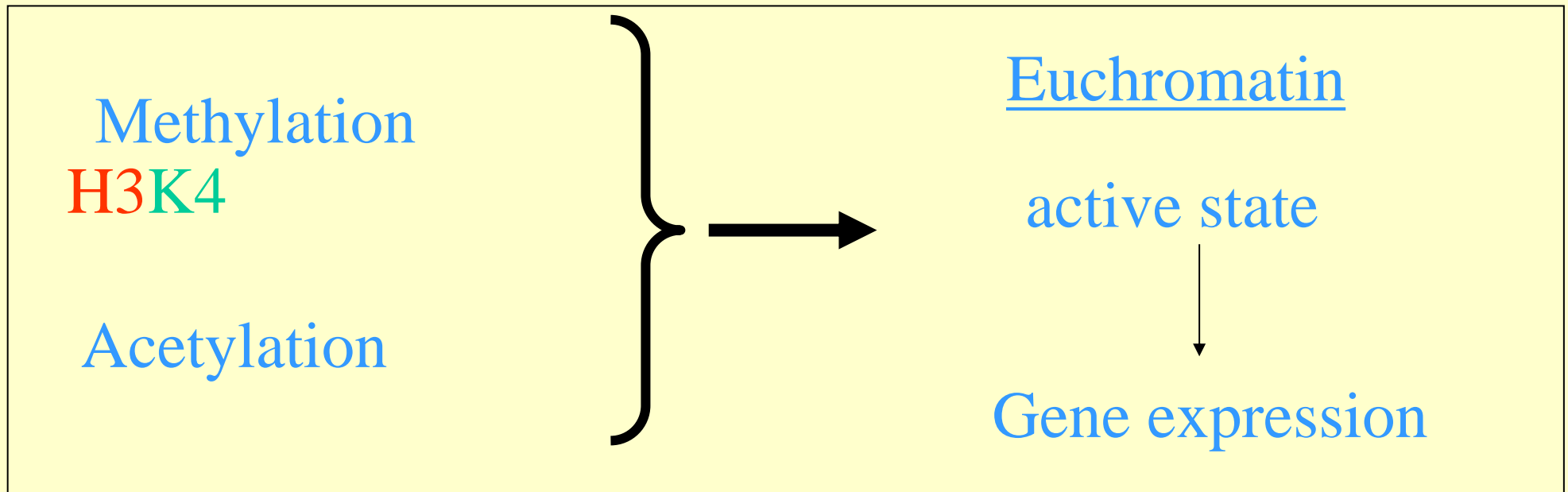
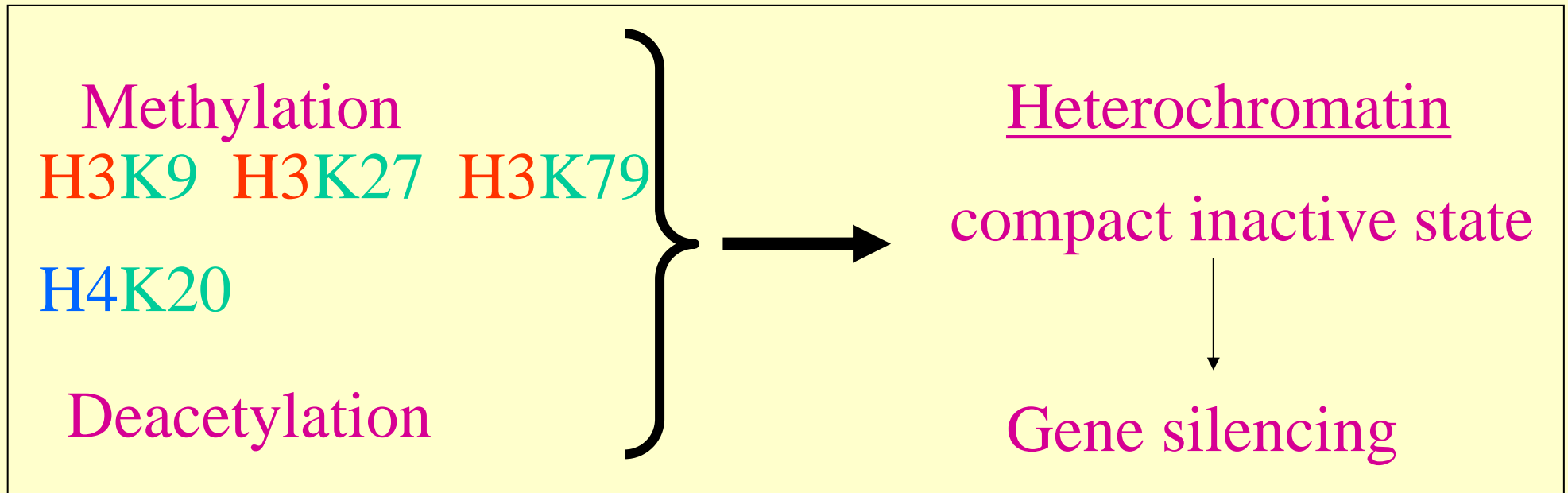


Mechanism of DNA methylation

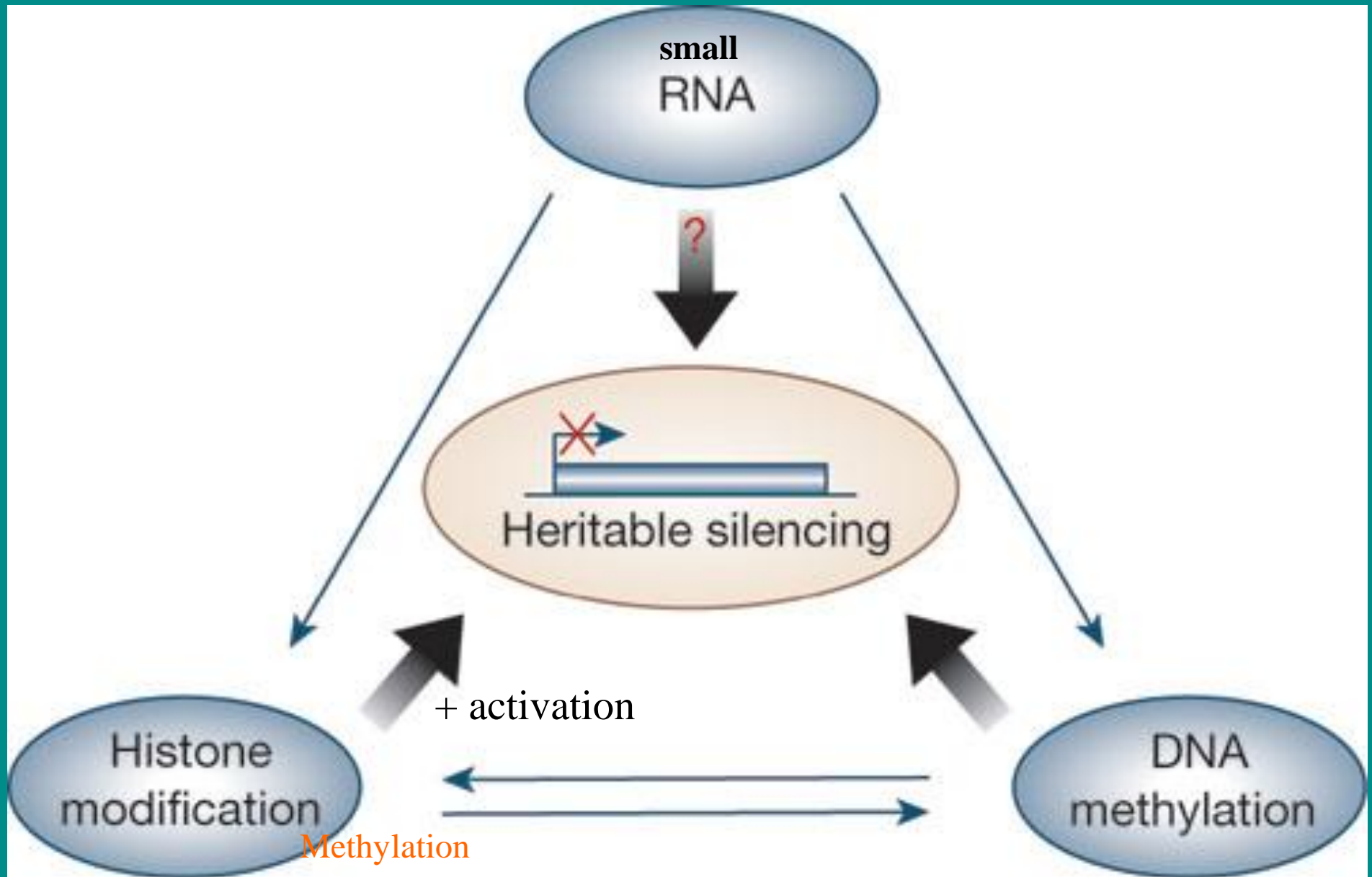
Histone modifications



Histone modifications



Epigenetic Mechanisms



Methylation

Acetylation

Phosphorylation

Enzymes involved in chromatin modifications

➤ DNA methyltransferase

-mainly silencers

➤ Histone methyltransferases

-silencers and activators (site specific)

➤ Histone demethylases (Jumonjii)

-silencer and activator (site specific)

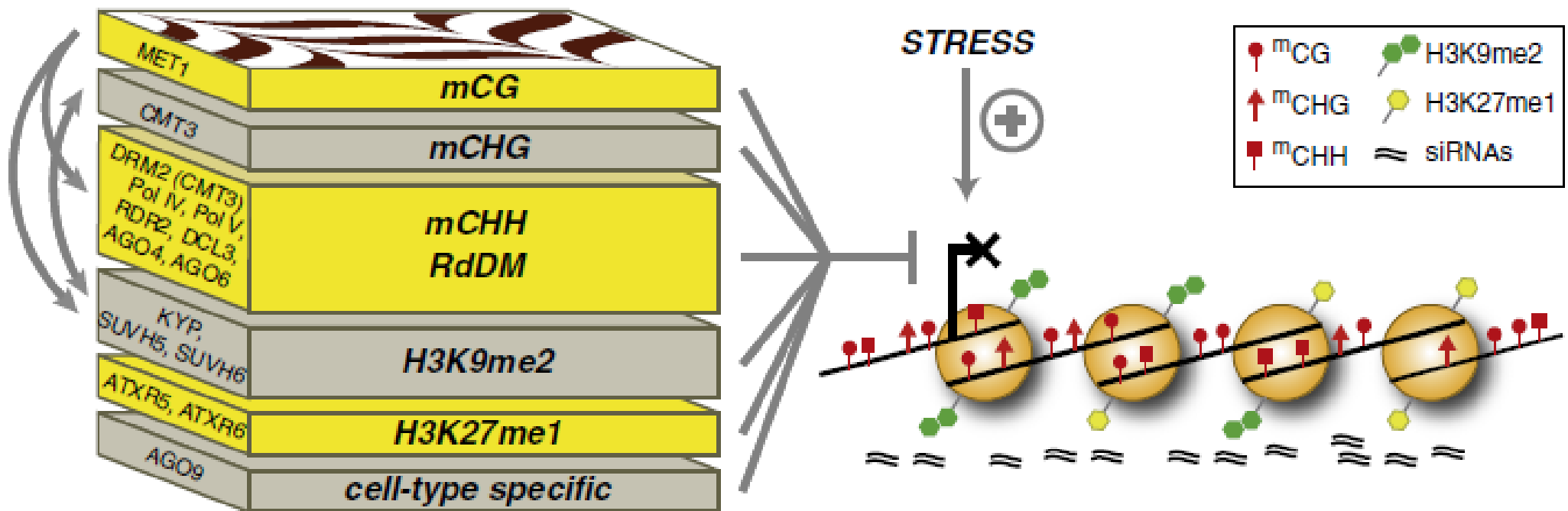
➤ Histone acetyltransferases

-mainly activators

➤ Histone deacetylases

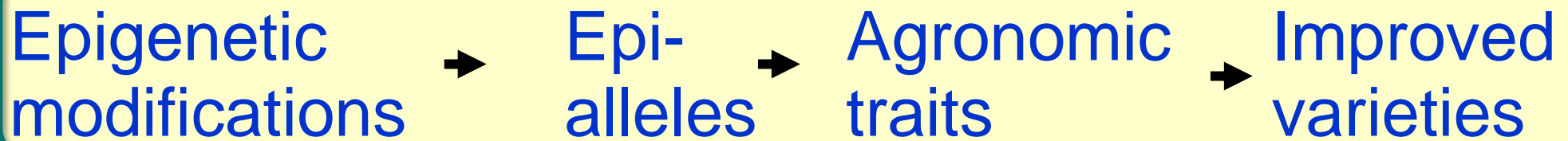
-mainly silencers

A 'mille-feuille' of Epigenetic mechanisms

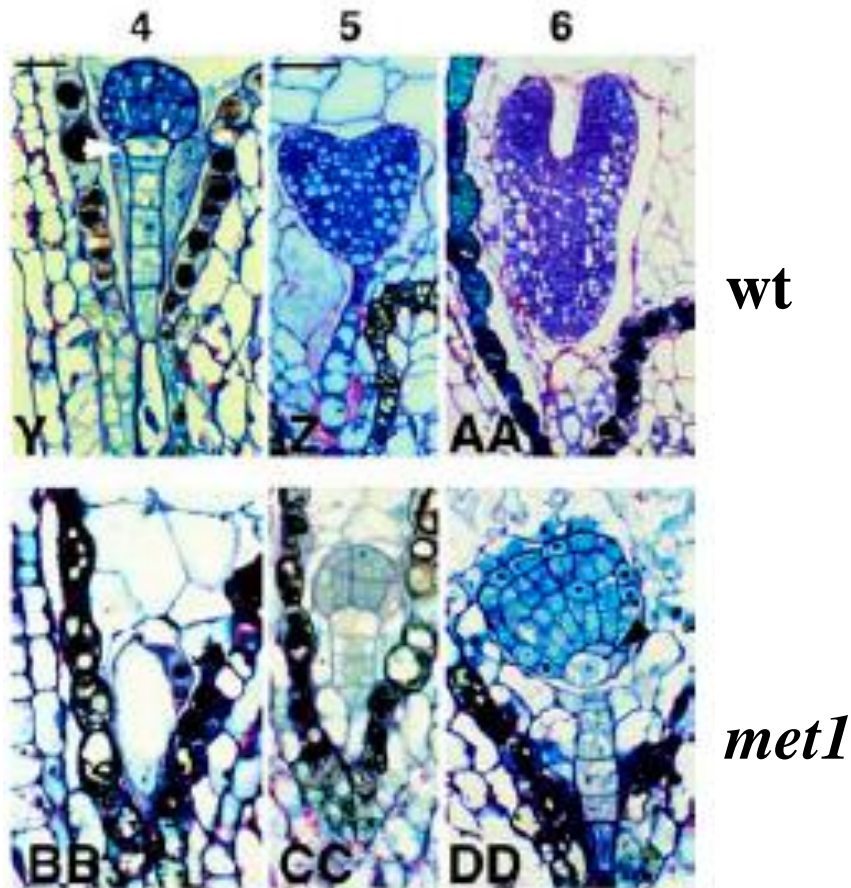


EPIGENETIC PHENOMENA

- **Vegetative development**
- **Flower Development**
- **Parental imprinting in Seed Development**
- **Stress response**
- **Transposon silencing**



DNA Methylation is critical for *Arabidopsis* normal development



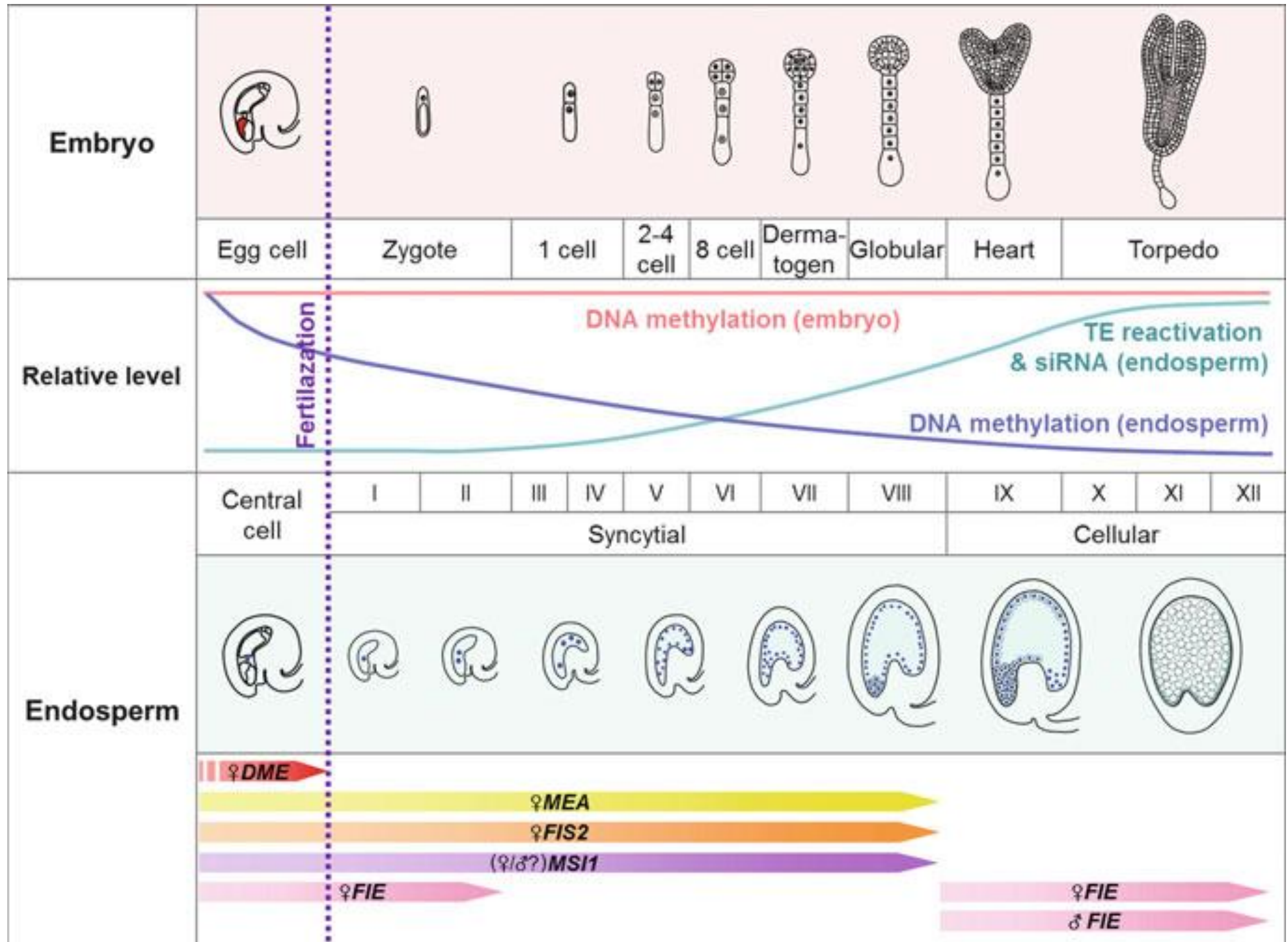
Embryo development at 4, 5, 6 DAF
(Days after Fertilization)



wt

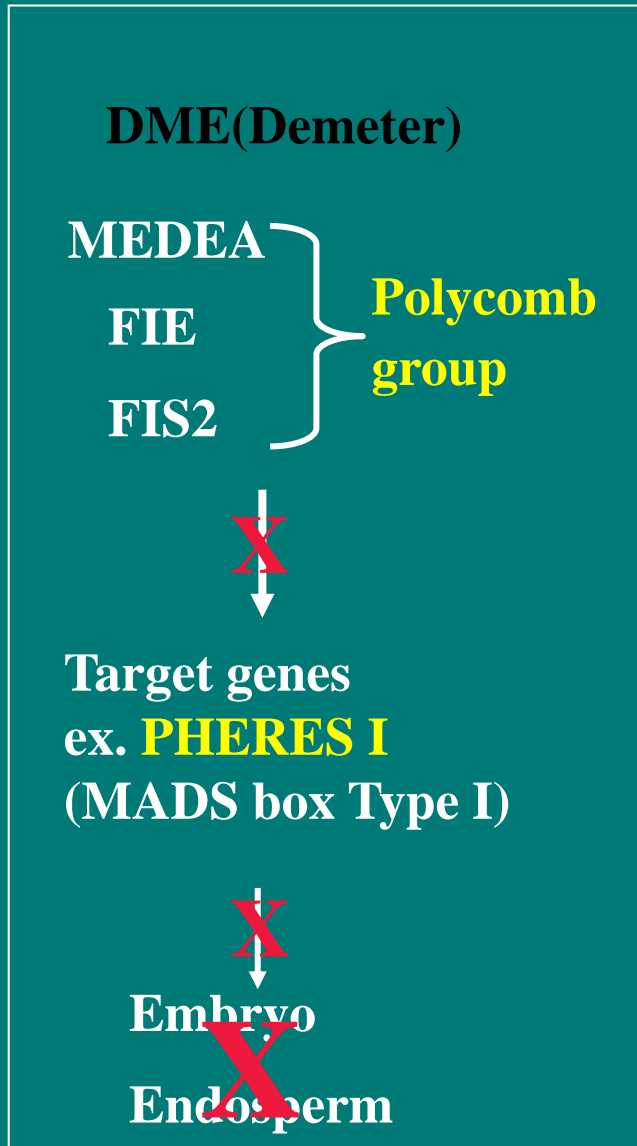
cmt3 met1

Developing seed

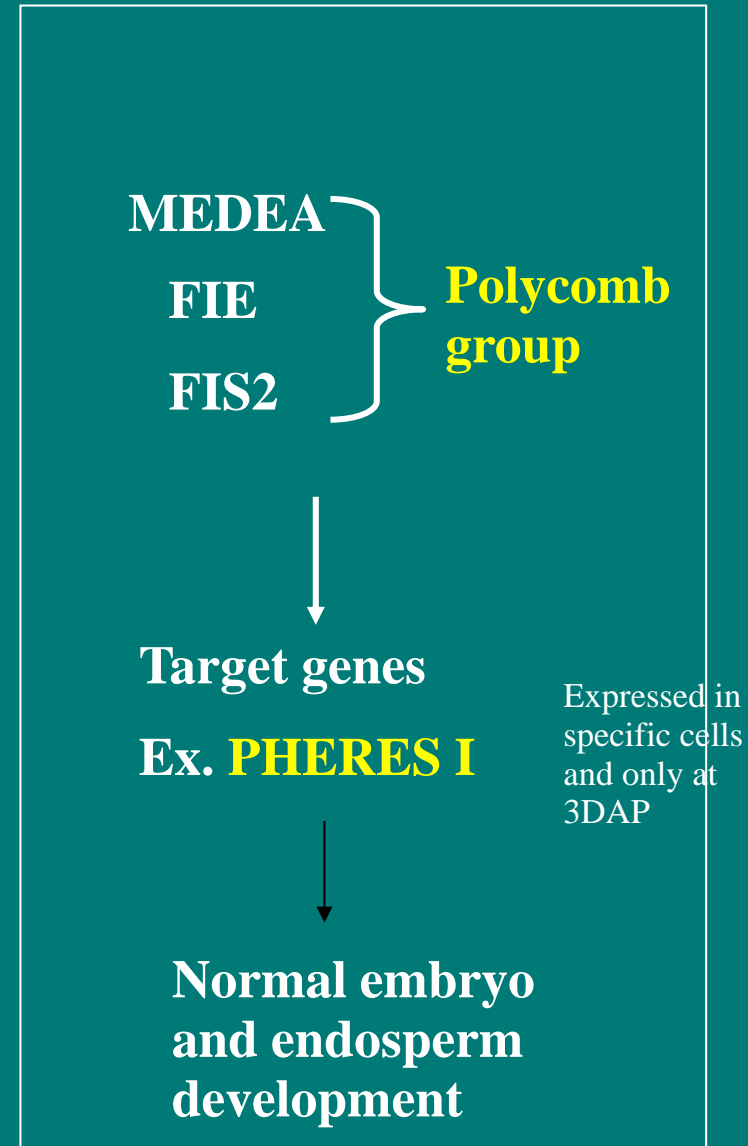


Genes that control seed development

Before fertilization



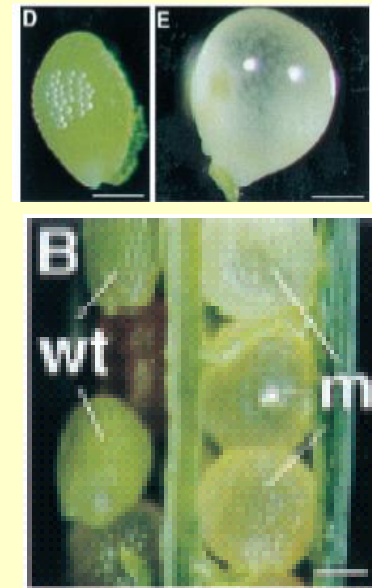
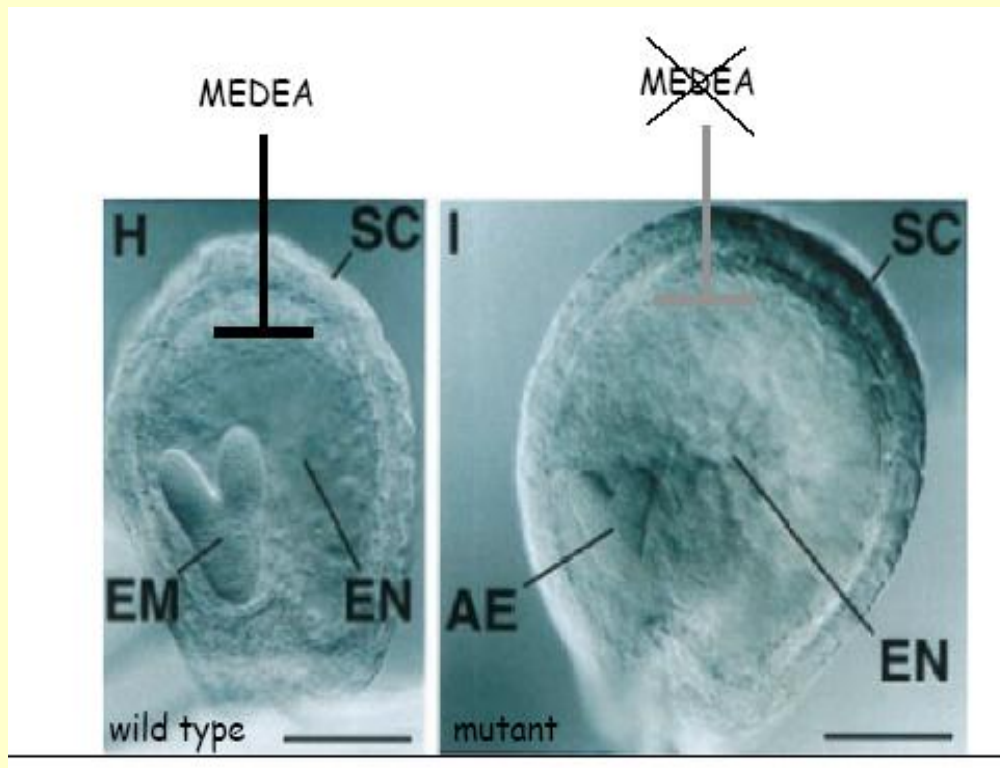
After fertilization



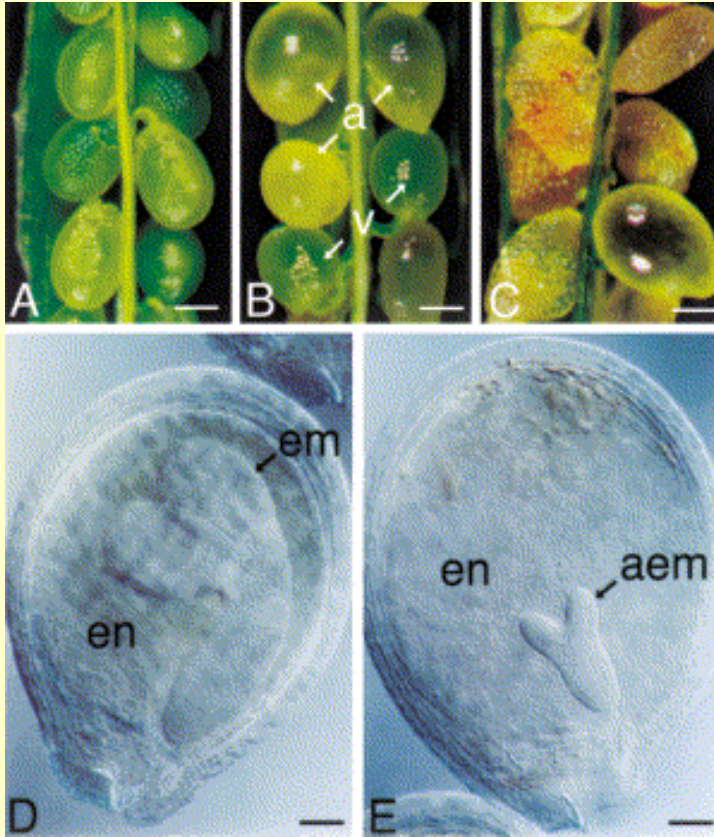
Parental Imprinting regulates seed development

Mutations in the female ♀MEDEA gene lead to abnormal seed development

Wt M



♀DME mutants in Arabidopsis



A: wild type silique

B: Heterozygous DME/dme-1 silique

C: Homozygous dme-1 silique

D: Viable seed

E: Aborted seed

DME wt

Normal
embryo and
endosperm
development

DME mutant

Enlarged
endosperm
and aborted
embryo

PARENTAL IMPRINTING



Medea



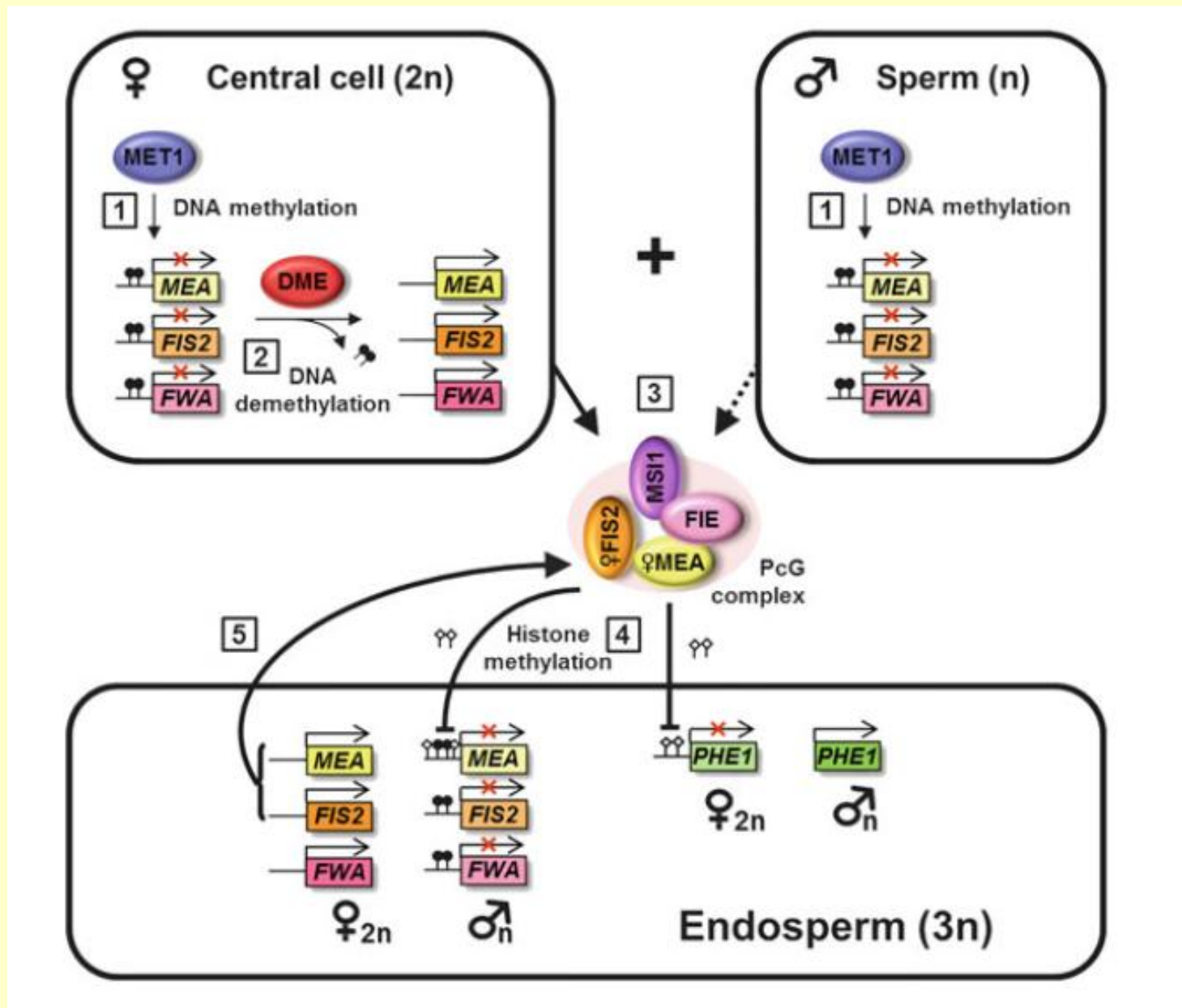
~~Ph~~res and ~~Me~~dos



Μικρός καρπός

Μεγάλος καρπός

Epigenetic modifications in the endosperm



Small RNAs (miRNAs) regulate Leaf Development

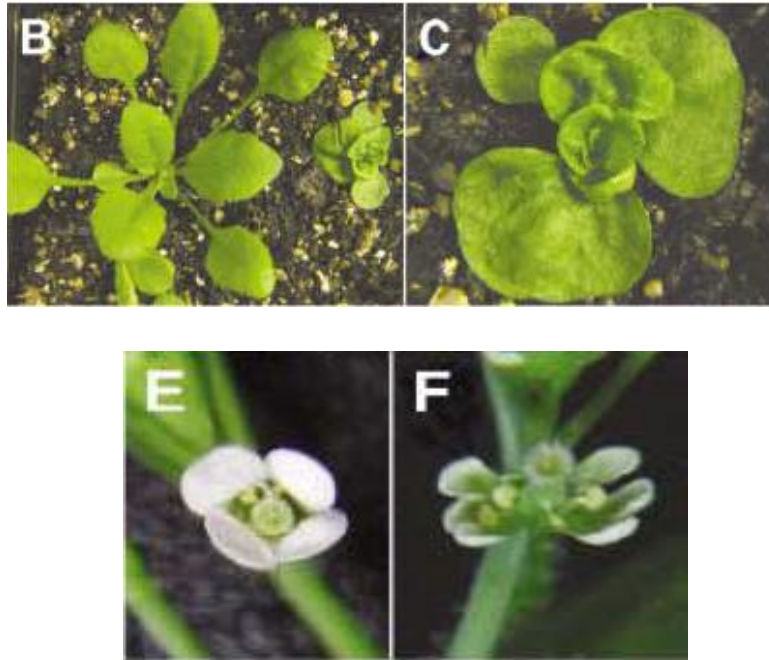
miR- JAW → **TCP** → Cell cycle control



Control of leaf morphogenesis by microRNAs

Javier F. Palatnik, Edwards Allen, Xuelin Wu, Carla Schommer, Rebecca Schwab, James C. Carrington & Detlef Weigel NATURE September 2003

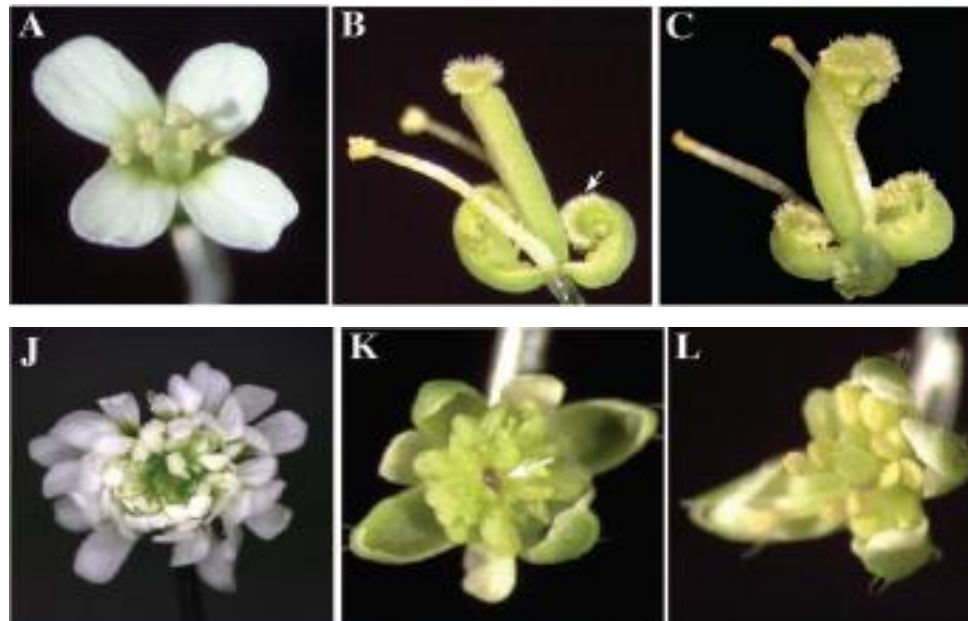
Disruption of miRNA164-regulation of NAC-domain protein in Arabidopsis



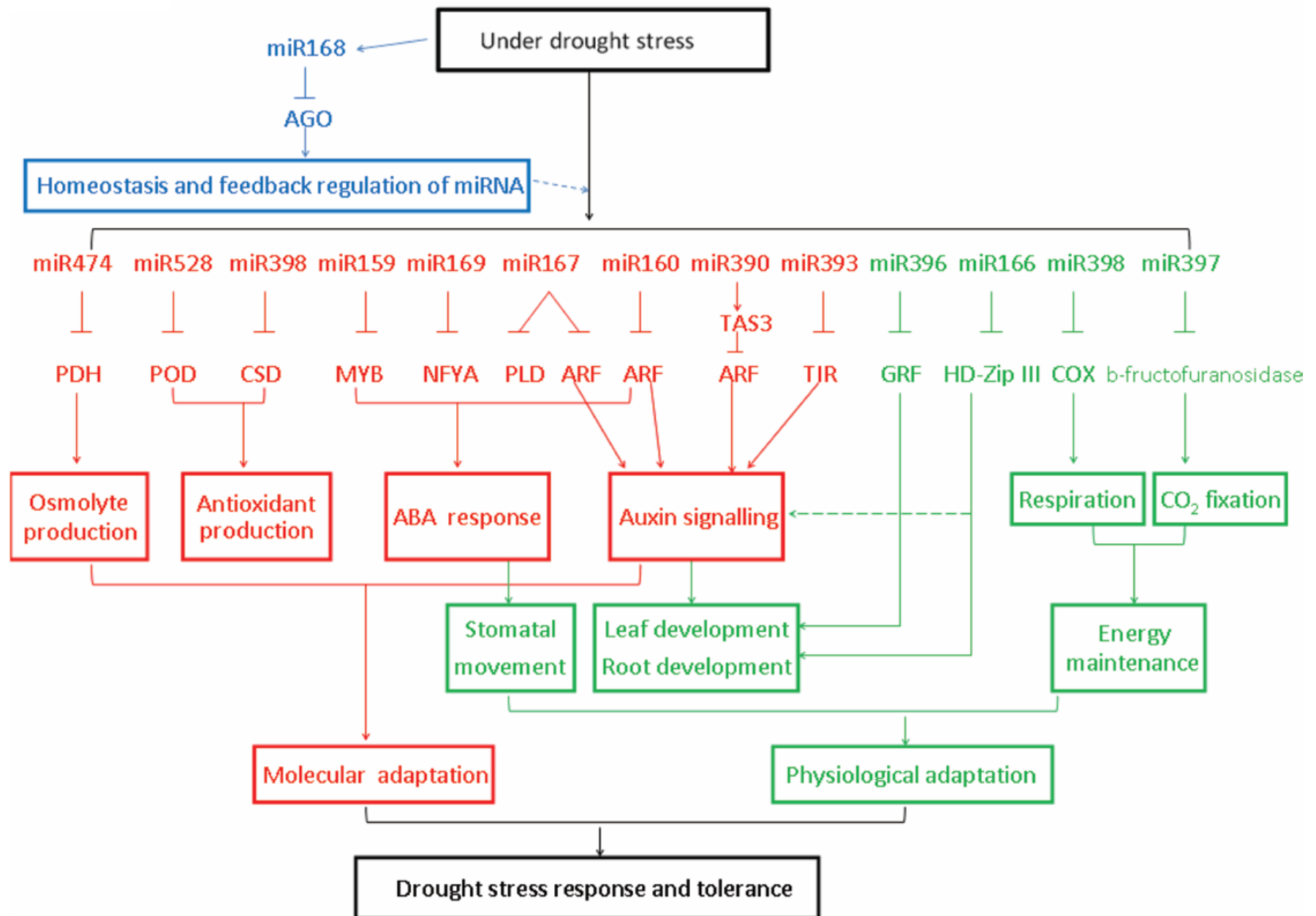
Affects proper formation and separation of adjacent embryonic, vegetative, and floral organs

Flower Development

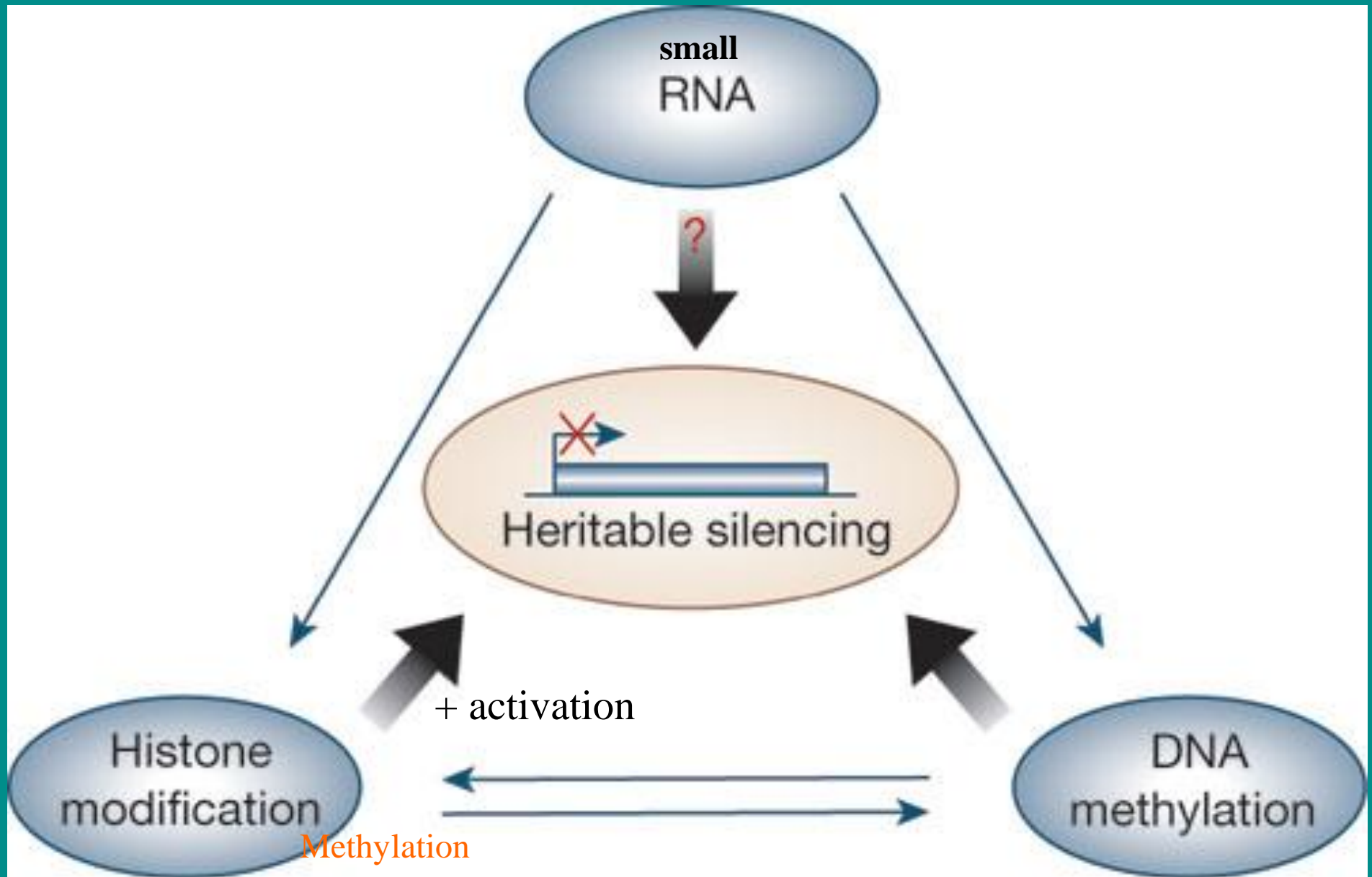
Regulation of APETALA 2 like genes by miR172



Epigenetic Mechanisms regulate the response to environmental changes



Epigenetic Mechanisms



Methylation

Acetylation

Phosphorylation

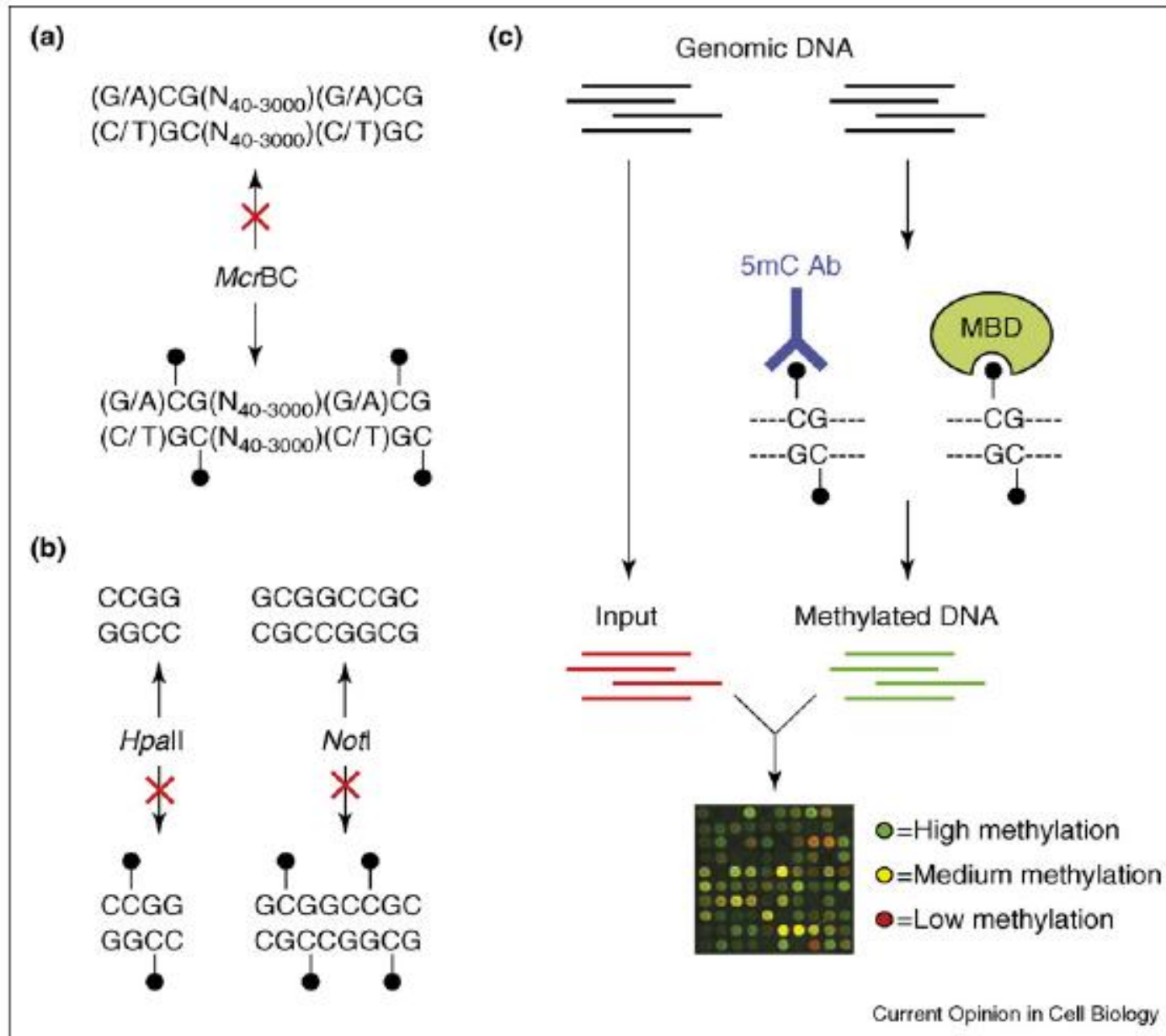
EPIGENOMES

**Locus-specific
epigenetic
modifications**



**Large-scale
genome wide
epigenetic
landscapes**

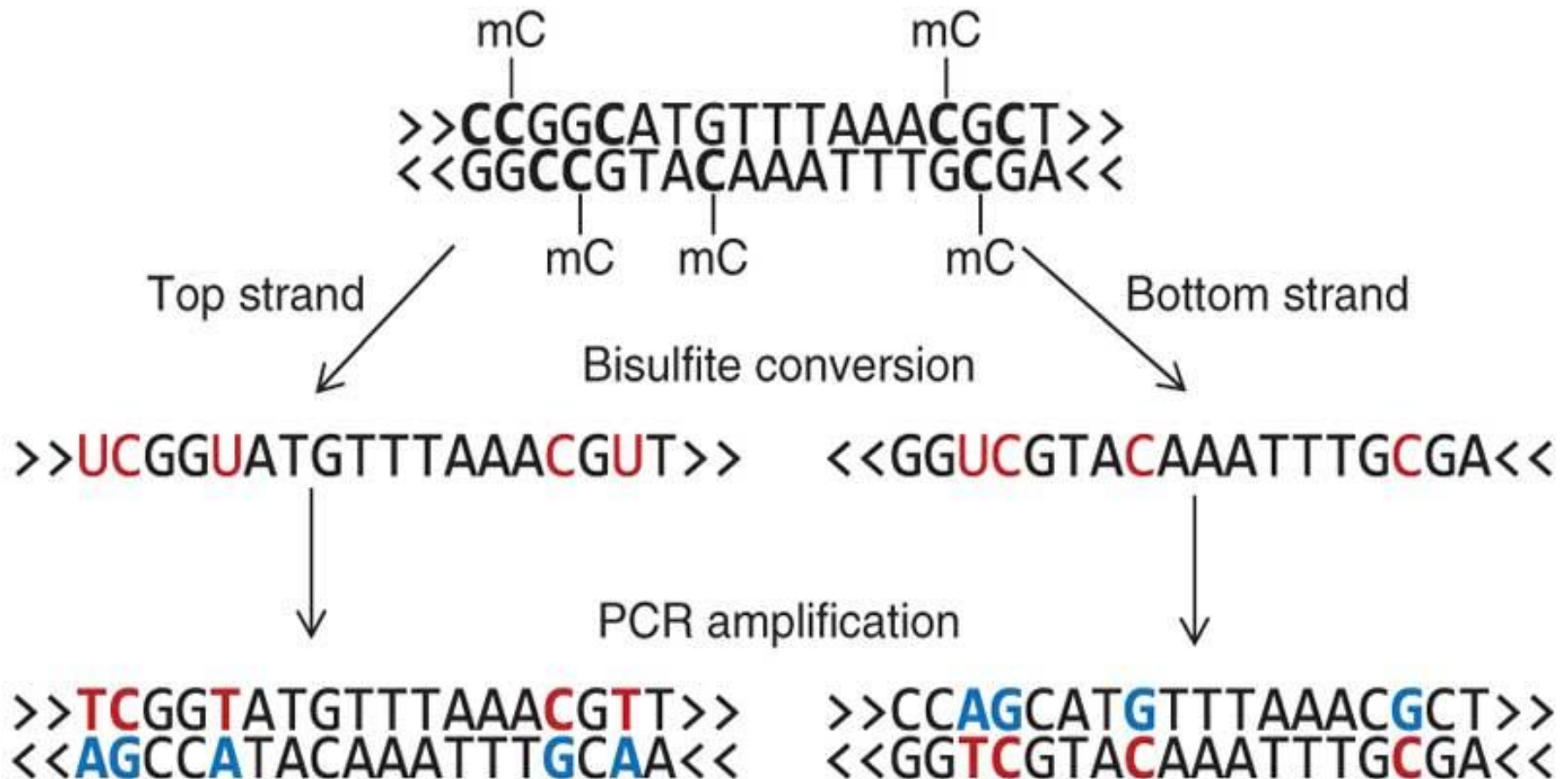
Genome-wide DNA methylation techniques



Technologies to map DNA methylation genome-wide. Classical approaches to study DNA methylation use restriction enzymes that cut only (a) methylated (*MspI*) or (b) unmethylated (*HpaII*, *NotI*) DNA; however, these methods limit the analysis to particular sequence motifs. (c) Alternative methods use isolation of methylated DNA with antibodies or MBD proteins. The methylated DNA (labeled in green) can be used for cohybridization with input DNA (labeled in red) on any existing microarray. Lollipop shapes denote methyl groups.

Genome Bisulfite Sequence (BS-seq)

•Combination of Bisulfite treatment with Deep Sequencing



HUMAN METHYLOME

- Different cell and tissue types
- Normal and disease conditions
- Different Methylation Pattern in cancer cells

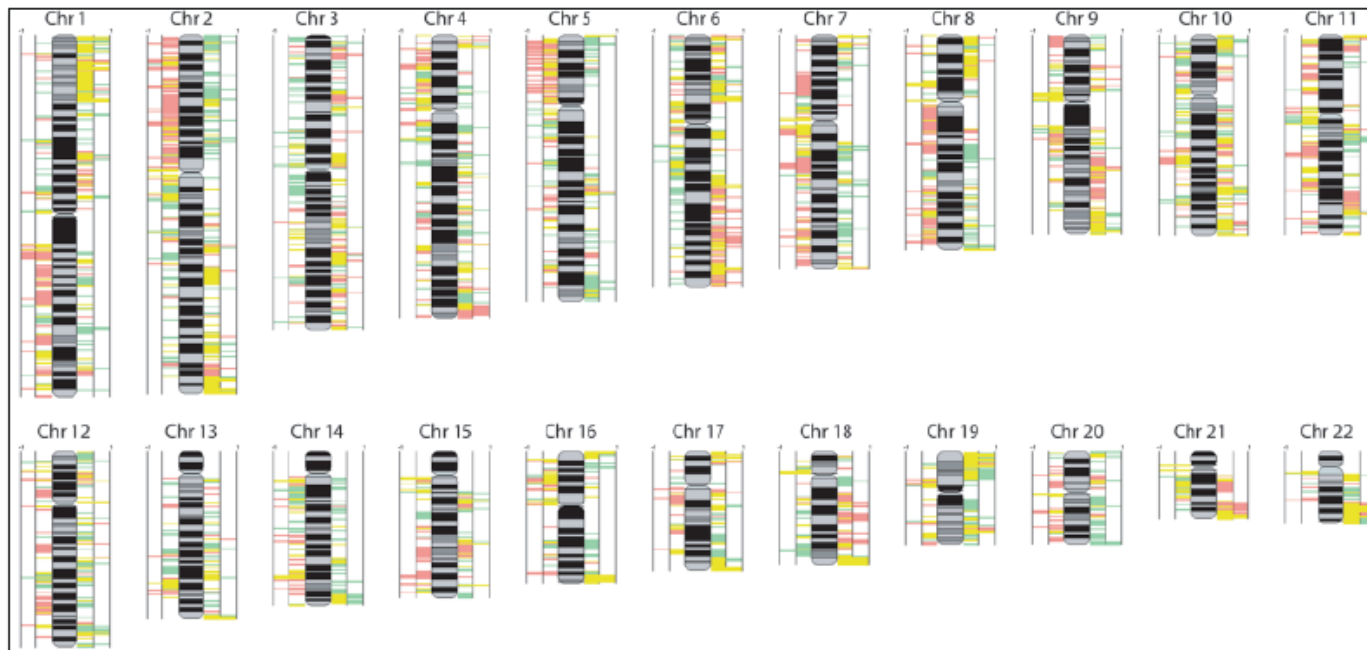


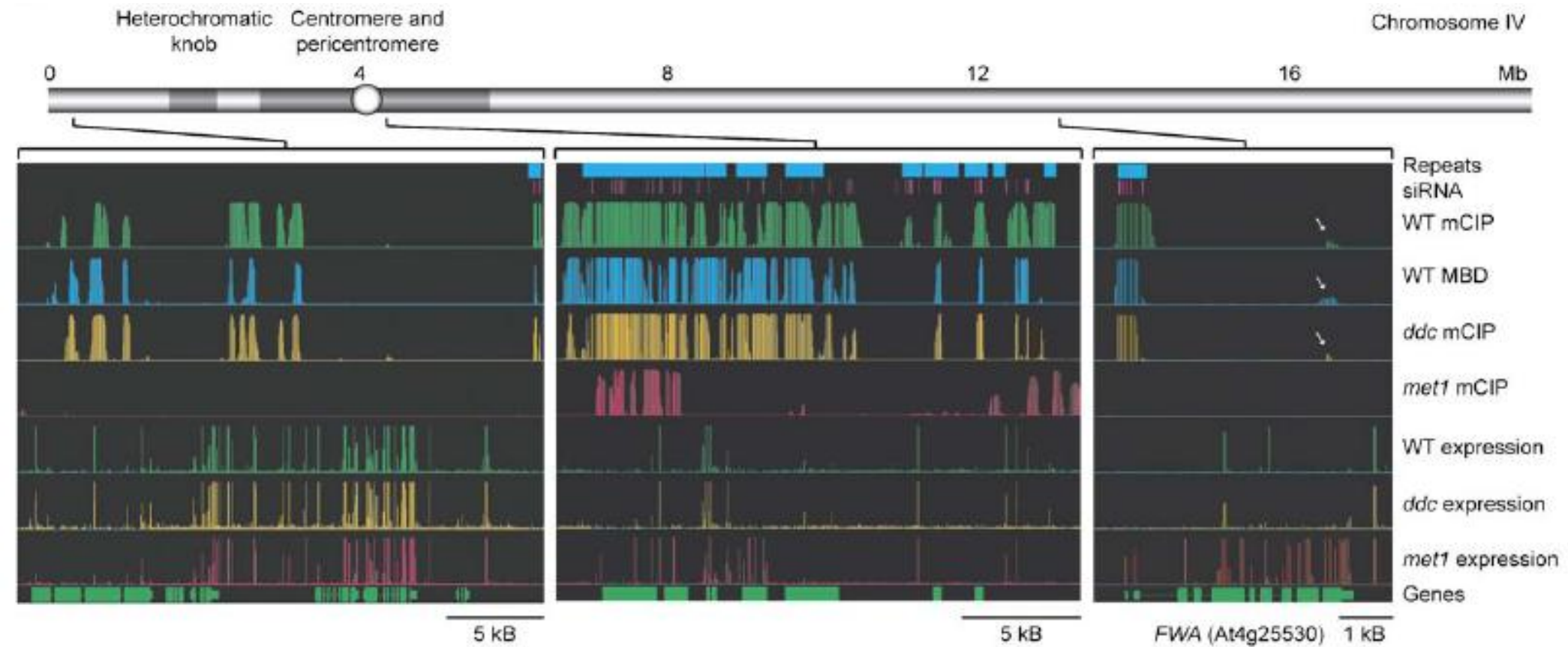
Figure 2. Epigenomic instability in lung cancer. This plot summarizes the frequency of methylated regions in two adenocarcinoma and matched lymphoblast samples. A large number of differentially methylated regions exist between tumor and lymphoblast sample. Although tissue-specific patterns need to be identified, we predict that a large number of these alterations are involved in or are a consequence of oncogenesis. Hypermethylated regions are right of the chromosome and hypomethylated regions are to the left. Red indicates adenocarcinoma, green denotes lymphoblast, yellow is common to both.

DNA METHYLATION LANDSCAPE OF THE ARABIDOPSIS GENOME

.Wt

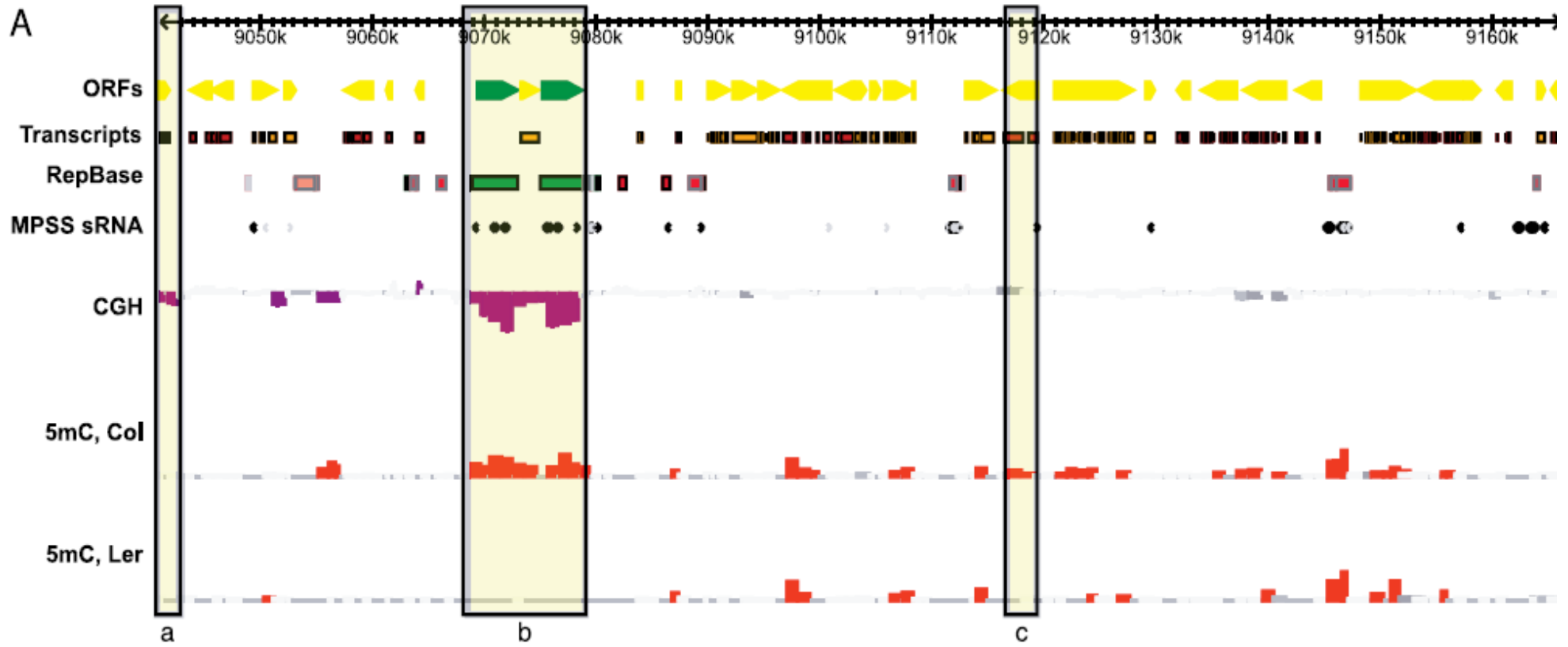
.*met-1* mutant

.*drm1/drm2/cmt3* triple mutant (*ddc*)



‘Genome-wide High-Resolution Mapping and Functional Analysis of DNA Methylation in Arabidopsis’

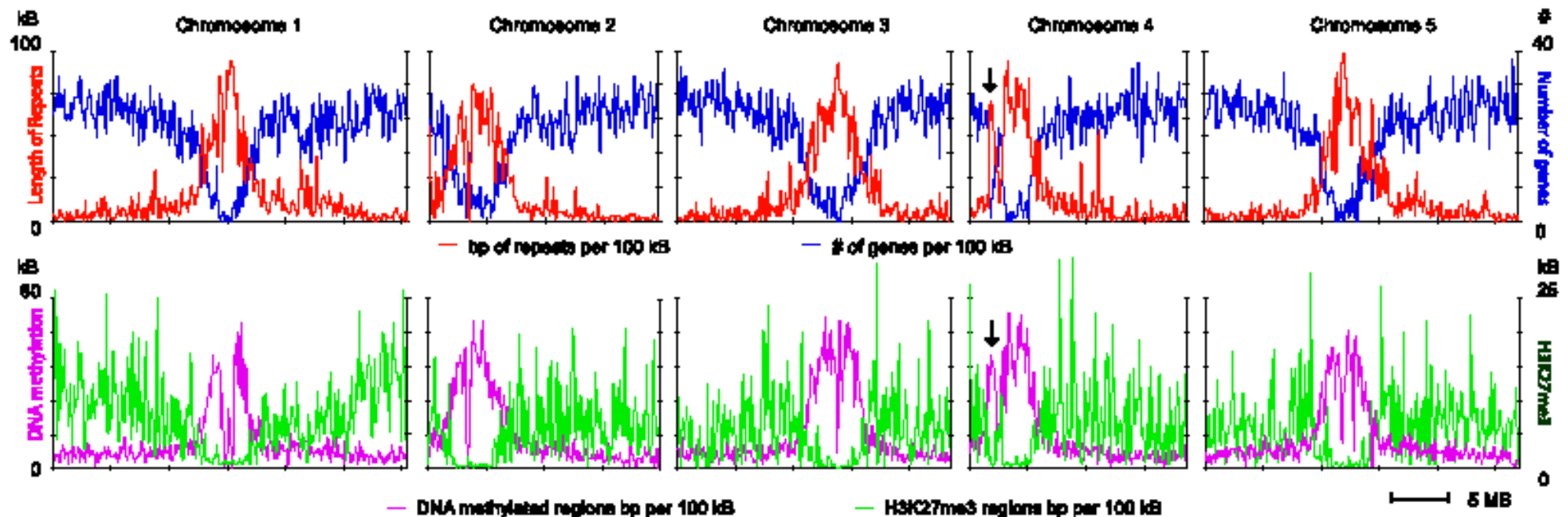
Methylation Profiles for Columbia and Landsberg *Arabidopsis* ecotypes



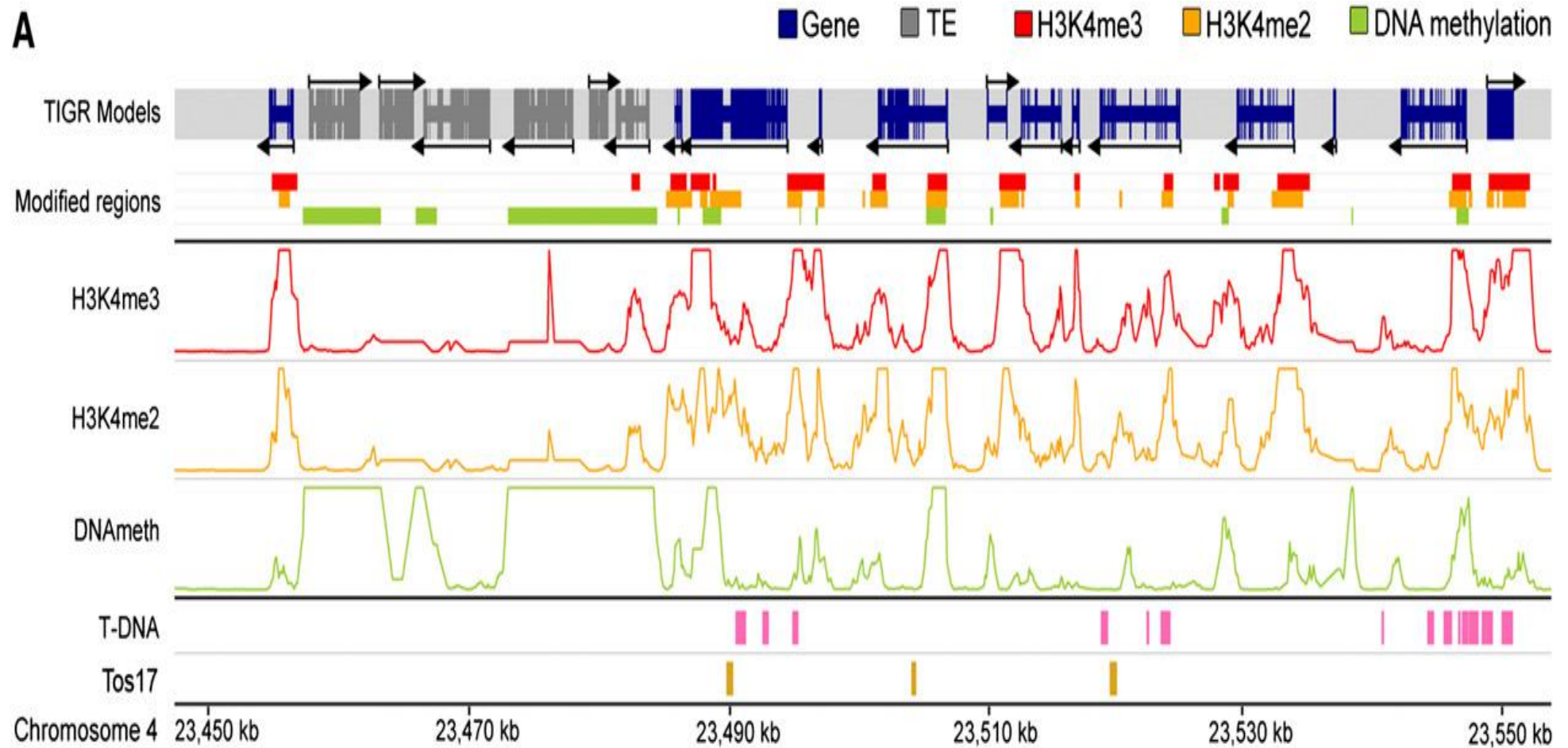
Genome-wide analysis in Arabidopsis

❖ DNA methylation

❖ Histone H3 Lysine 27 Trimethylation



LARGE-SCALE EPIGENETIC LANDSCAPE IN RICE



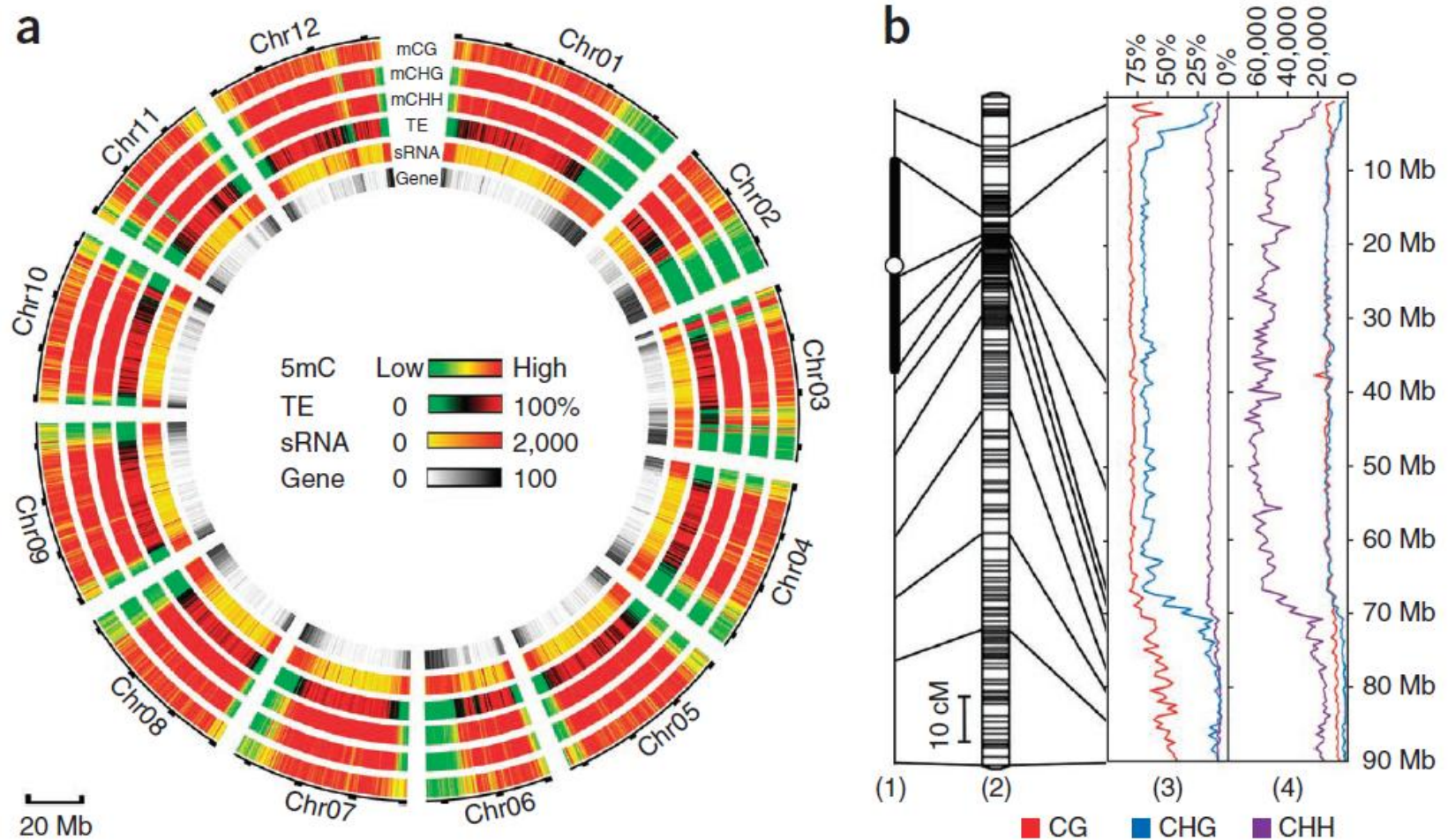
RESEARCH ARTICLE

Open Access

Single-base resolution maps of cultivated and wild rice methylomes and regulatory roles of DNA methylation in plant gene expression

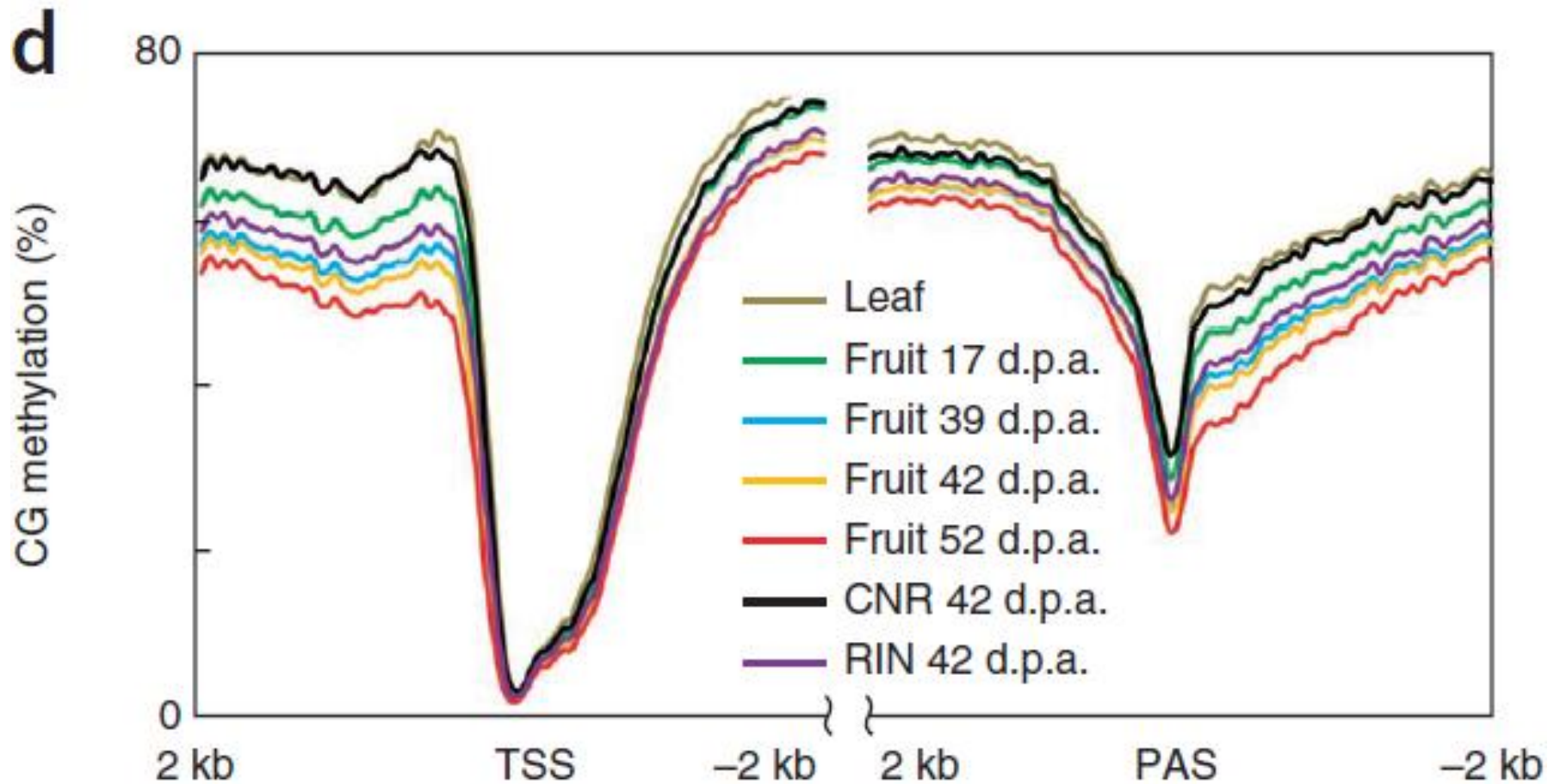
Xin Li^{1,2†}, Jingde Zhu^{3,4†}, Fengyi Hu^{5†}, Song Ge^{6†}, Mingzhi Ye², Hui Xiang¹, Guojie Zhang^{1,2}, Xiaoming Zheng⁶, Hongyu Zhang³, Shilai Zhang⁵, Qiong Li⁵, Ruibang Luo^{2,7}, Chang Yu², Jian Yu³, Jingfeng Sun³, Xiaoyu Zou³, Xiaofeng Cao⁸, Xianfa Xie^{9*}, Jun Wang^{2,10*} and Wen Wang^{1*}

The tomato methylome

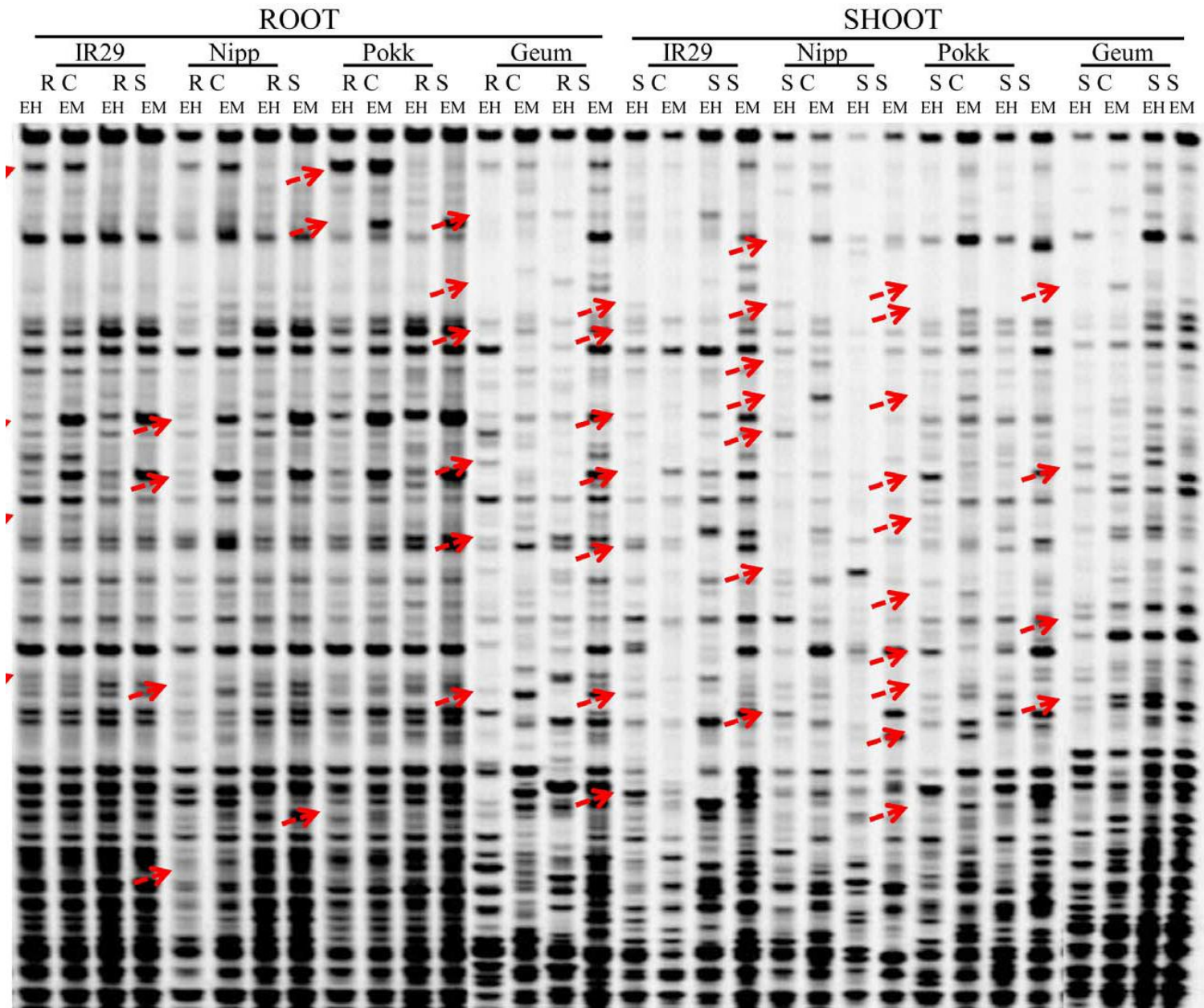


Density of methylated DNA and other features in chromosomes of the tomato fruit

Tissue and developmental specific epigenetic variation in tomato



MSAP: Methylation Sensitive Amplified Polymorphism



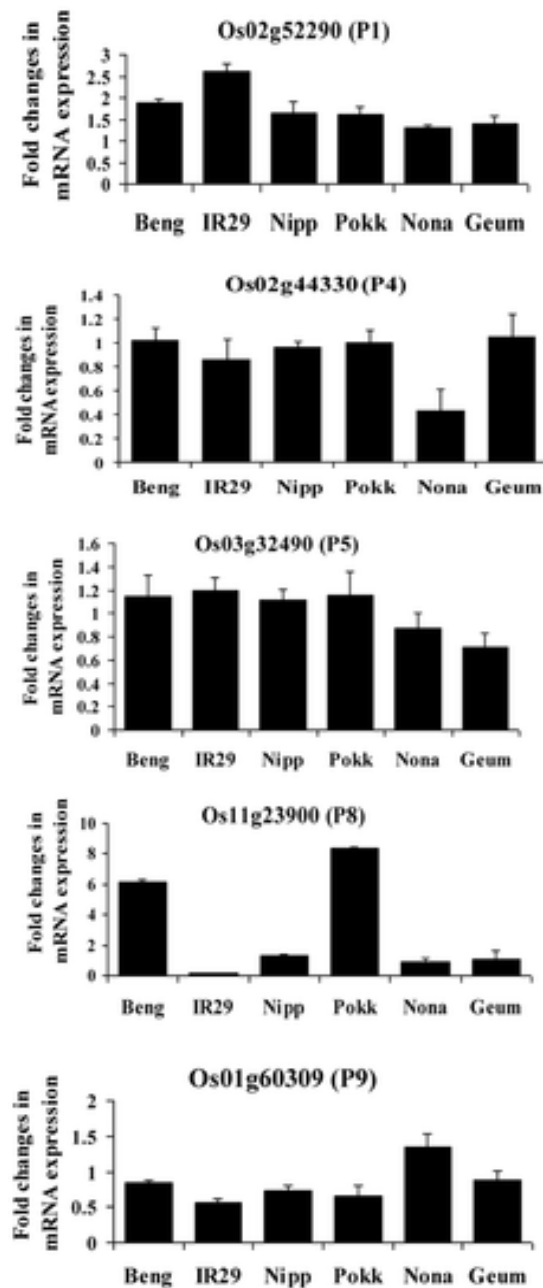
DNA methylation changes under non-stress and salinity conditions

Table 1. DNA methylation changes in shoot and root at seedling stage under non-stress and salinity stress conditions.

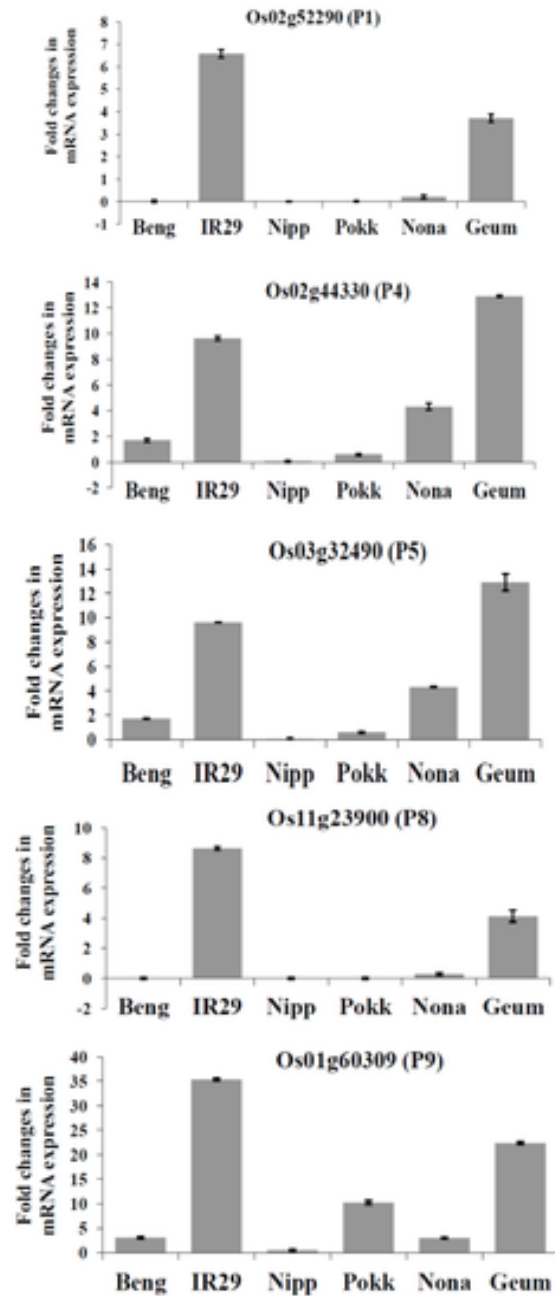
	IR29		Nipponbare		Pokkali		Geumgangbyeon	
MSAP band type	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
A. Shoot								
I	665	789	877	692	698	566	838	1067
II	201	113	246	290	293	317	143	119
III	322	425	205	235	171	158	160	93
IV	446	308	306	418	471	593	489	350
Total amplified bands	1634	1635	1634	1635	1633	1634	1630	1629
MSAP (%) ^a	59.3	51.7	46.4	57.6	57.2	65.3	48.6	34.5
Fully methylated band (%) ^b	47.0	44.8	31.3	39.9	39.3	45.9	39.8	27.2
Hemi-methylated band (%) ^c	12.3	6.9	15.1	17.7	17.9	19.4	8.8	7.3
B. Root								
I	1276	1314	1103	1310	1231	1232	1311	1319
II	7	23	34	39	32	34	10	19
III	98	84	239	83	91	114	95	97
IV	254	211	259	203	232	202	218	197
Total amplified bands	1635	1632	1635	1635	1586	1582	1634	1632
MSAP (%) ^a	21.9	19.5	32.6	19.9	22.4	22.1	19.8	19.2
Fully methylated band (%) ^b	21.5	18.1	30.5	17.5	20.4	19.9	19.2	18.0
Hemi-methylated band (%) ^c	0.4	1.4	2.1	2.4	2.0	2.2	0.6	1.2

Expression profiles of MSAP loci in rice under salt stress

A. Shoot



B. Root



MSAP: Methylation Sensitive Amplified Polymorphism

MSAP is being widely used to detect epigenetic variation in different species, cultivars, tissues and in different environmental conditions

Poplar

Grapes

Coffee

Tomato

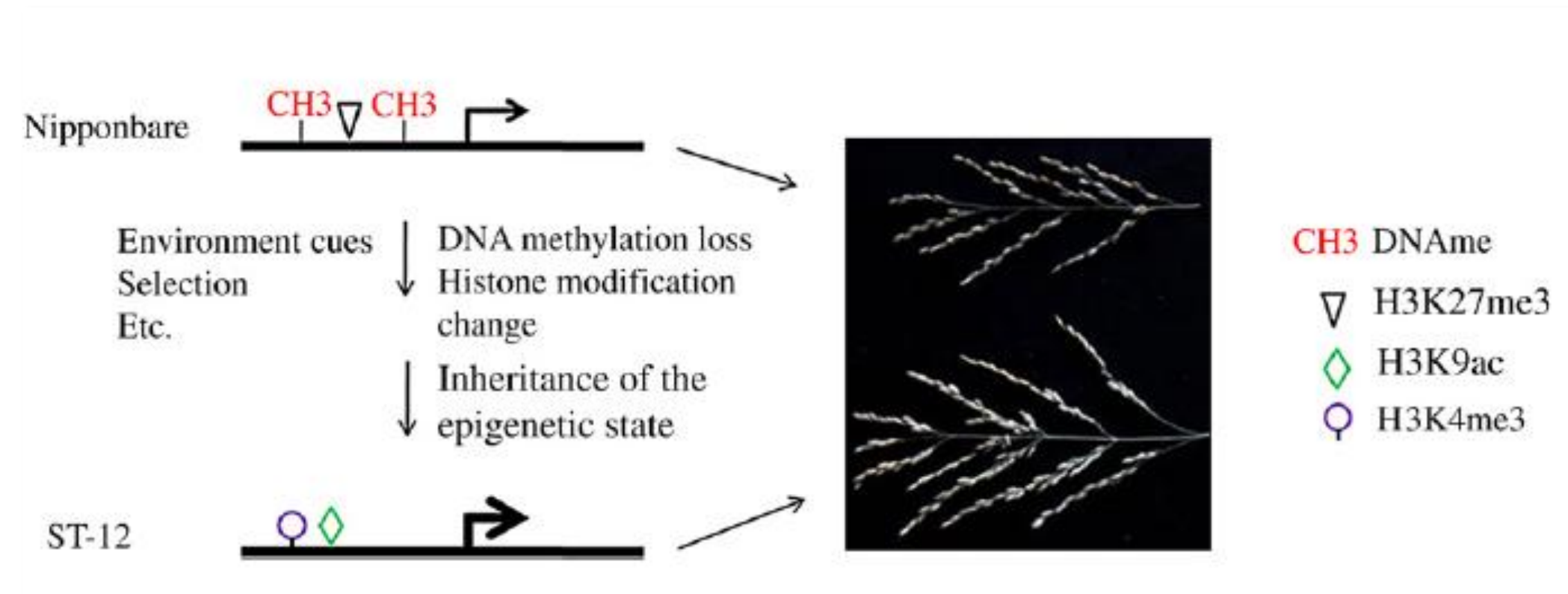
Grafted vegetables

TRAITS

**Association of epigenetic variation
with important agricultural traits**

Inheritable Epigenetic States lead to Agronomic Trait Variants

Squamosa promoter binding protein like14 (SPL14) → panicle branching and higher grain yield in rice



DNA Methylation mapping of rice *Xa21*

✓ *Xa21* is a rice disease resistance gene

✓ Encodes a receptor-like kinase

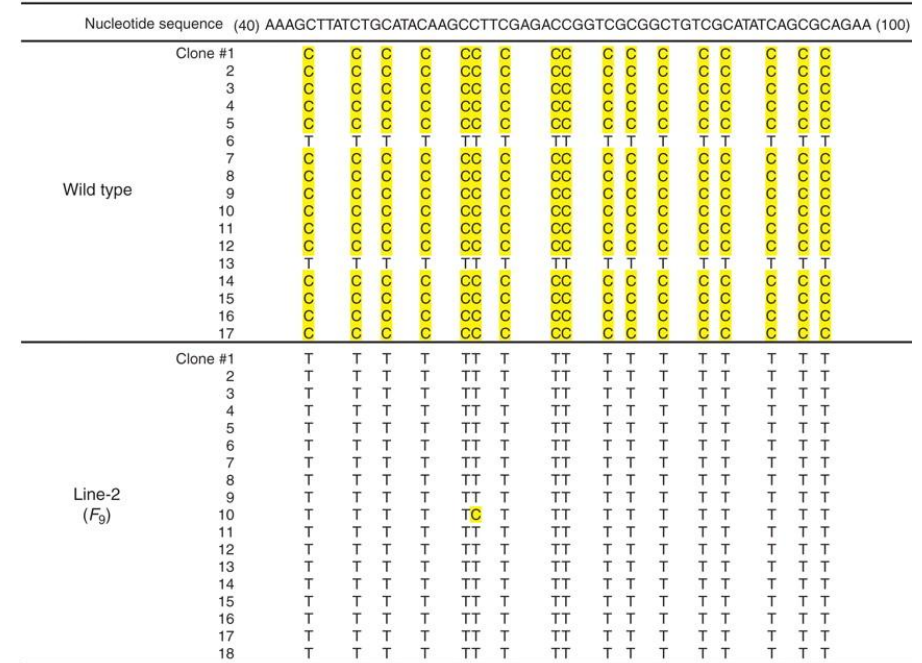
✓ 5' end methylation prevents gene expression

✓ Treatment with 5'-aza (DNA methylation inhibitor) resulted in high levels of expression and resistance to

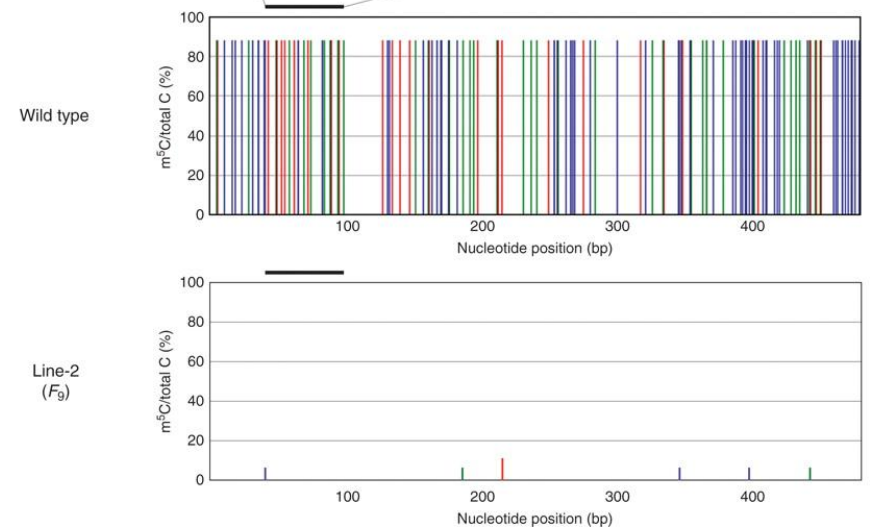
✓ *Xanthomonas oryzae* pv *oryzae*

✓ Hypomethylation and resistant trait were stably inherited in progeny (10 ys)

A

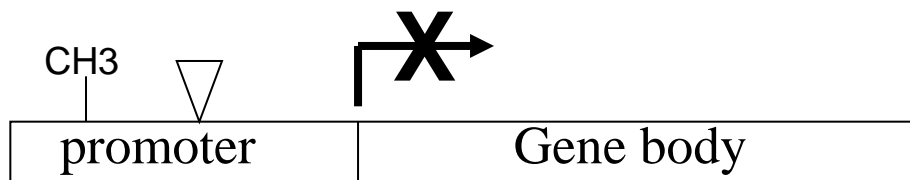
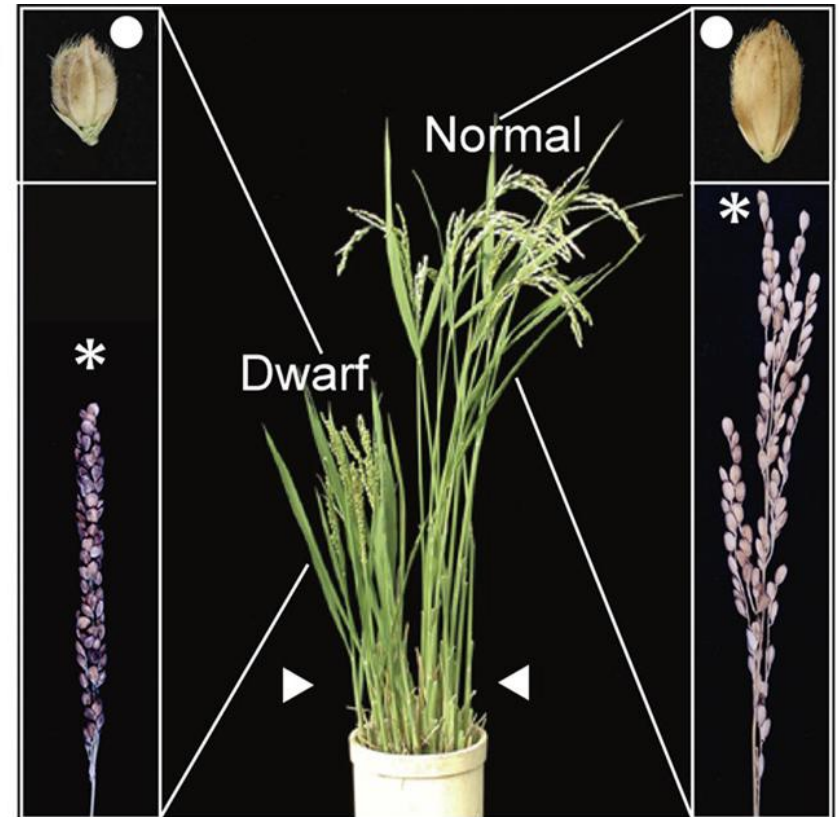


B

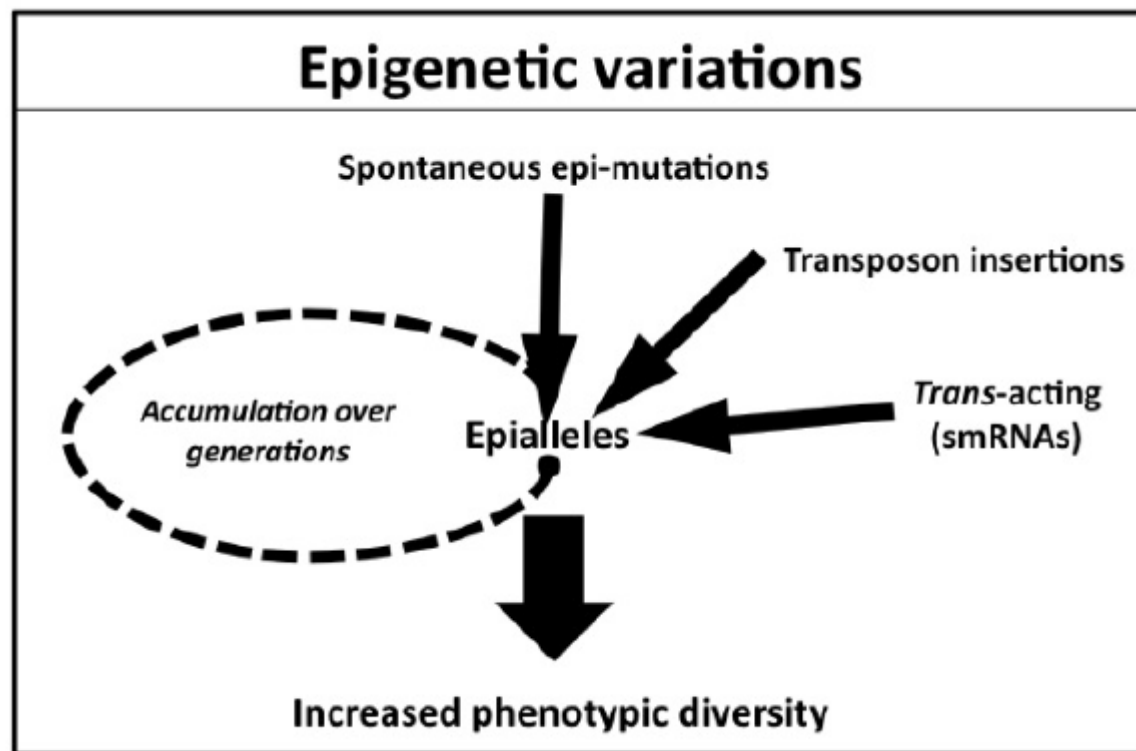


DWARF1 RICE EPIGENETIC MUTANT

- ❖ The epi- allele *DWARF1* (D1), epi-d1,
- ❖ Causes a dwarf phenotype
- ❖ Stably inherited
- ❖ Used as dwarf breeding material in Japan for decades
- ❖ Silenced state associated with repressive histone and DNA methylation marks in the D1 promoter region



Epigenetic changes – Source of natural variation



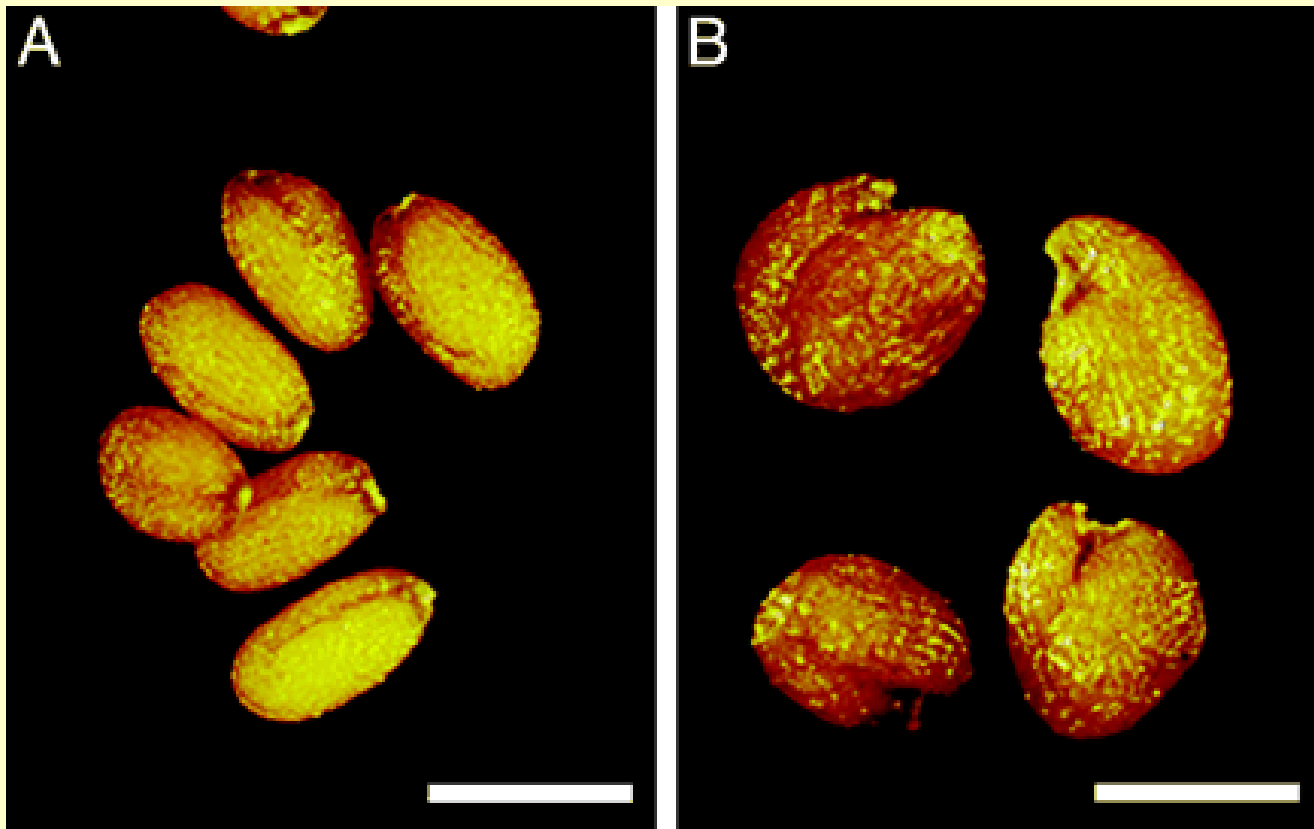
Factors leading to epigenetic mutations in plants

- Spontaneous epimutations
- Transposon insertions
- Transacting small RNAs

Heritable
epi-alleles → accumulate over generations → increases phenotypic diversity

IMPLICATIONS IN BREEDING

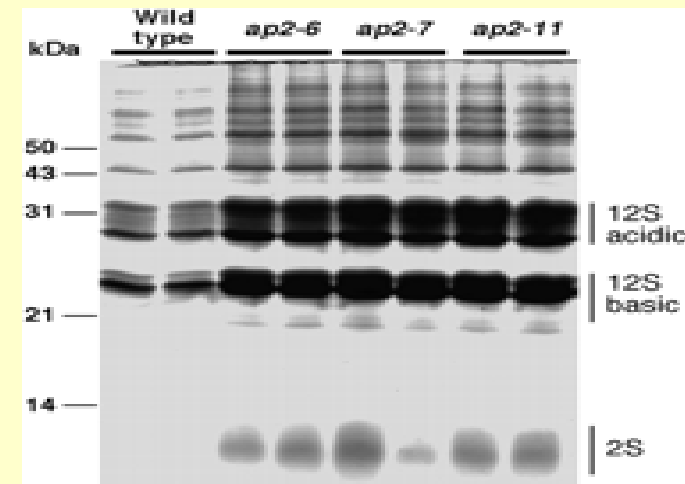
Mutations in *Apetala-2* which is regulated by miRNA172 lead to changes in **SEED SIZE**



Wt

Ap2-11

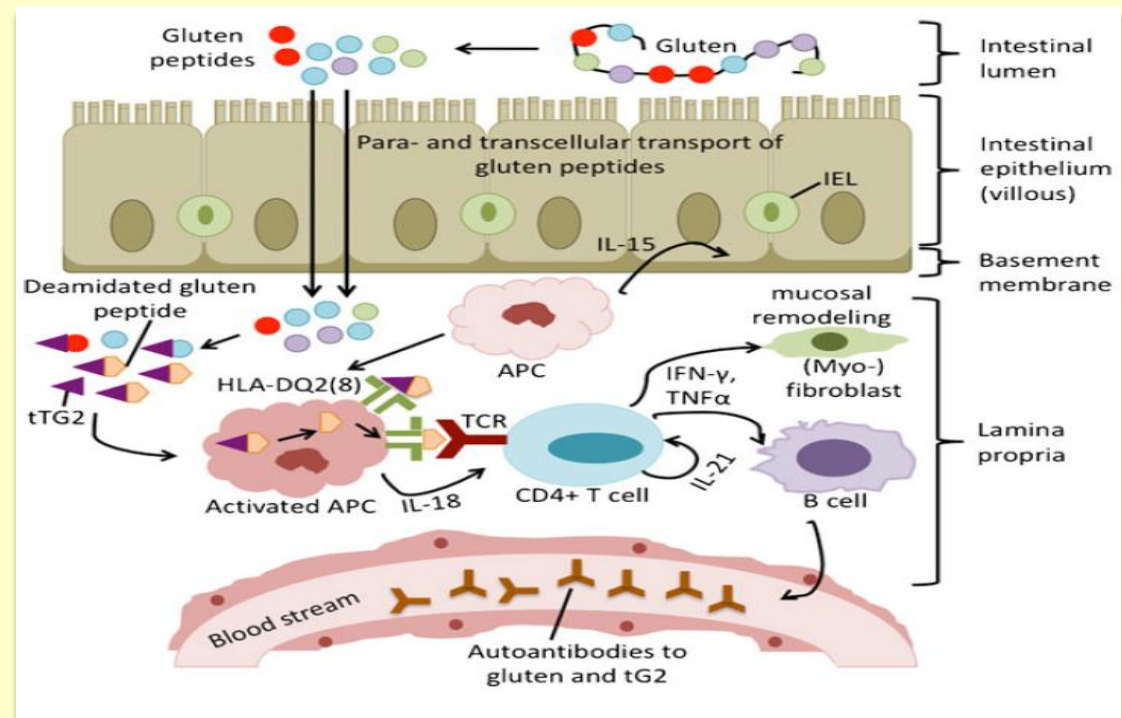
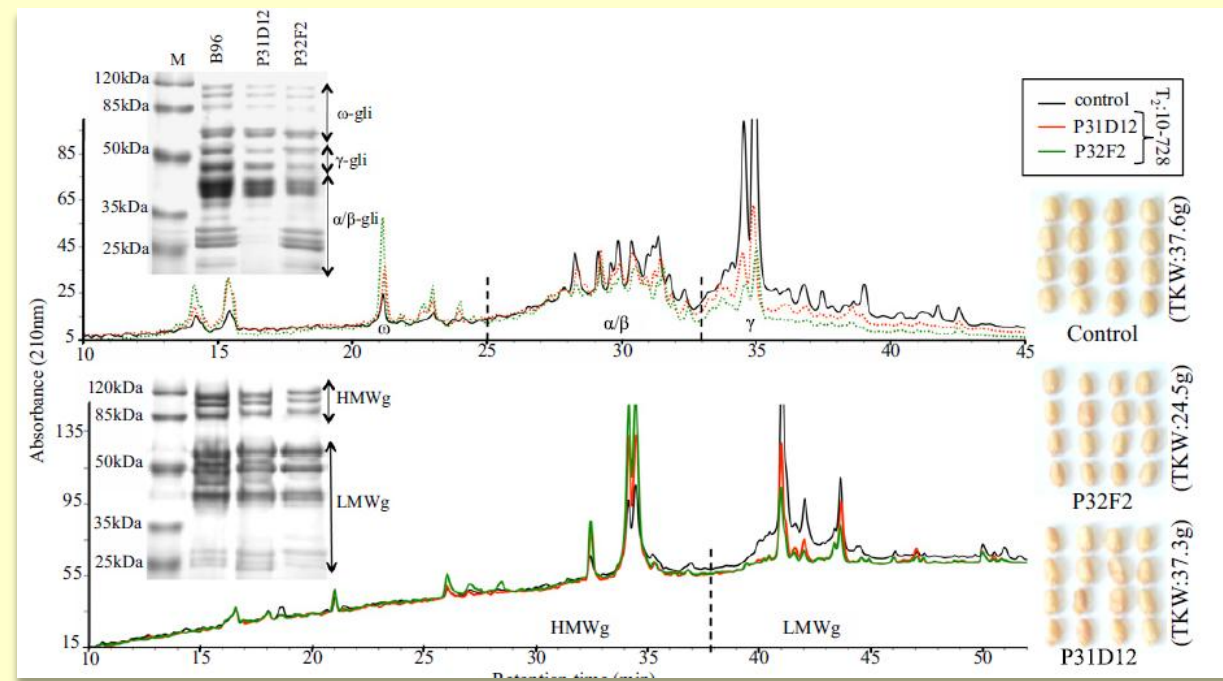
~ 30% increase in seed weight



Silencing of *DEMETERA*



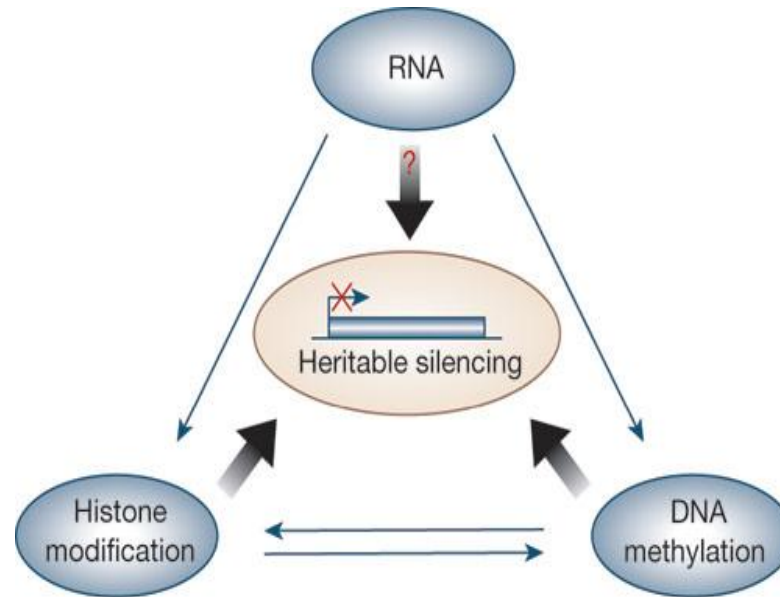
Low-Gluten Wheat



EPIGENETIC MECHANISMS IN CEREALS



- DNA methylation
- Histone modifications
- Small RNAs



Plant processes affected:

- Flowering
- Seed Development
- Stress response

Importance:

Epigenetic modifications ➡ Epi-alleles ➡ Agronomic traits ➡ Improved varieties

Barley cultivars with different seed size

Caresse



Ippolytos

Kos

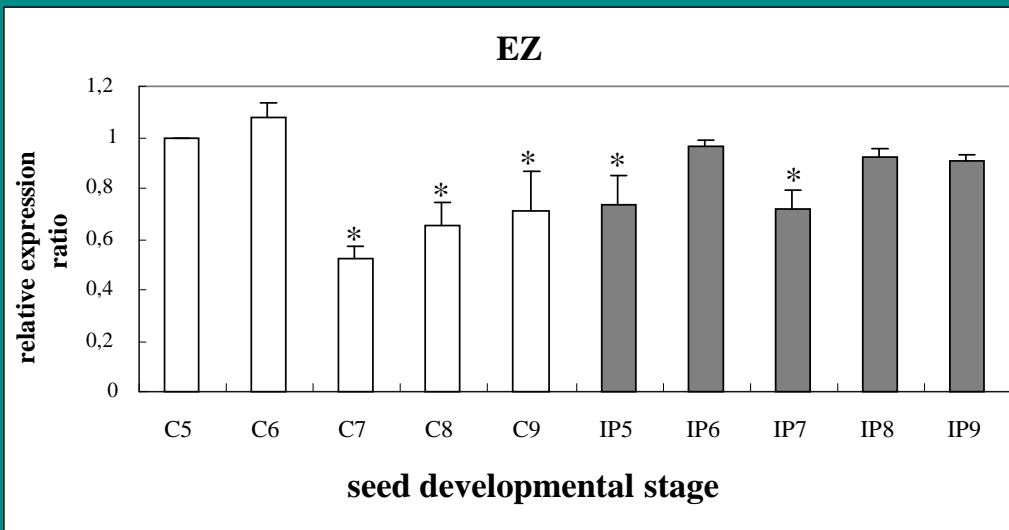
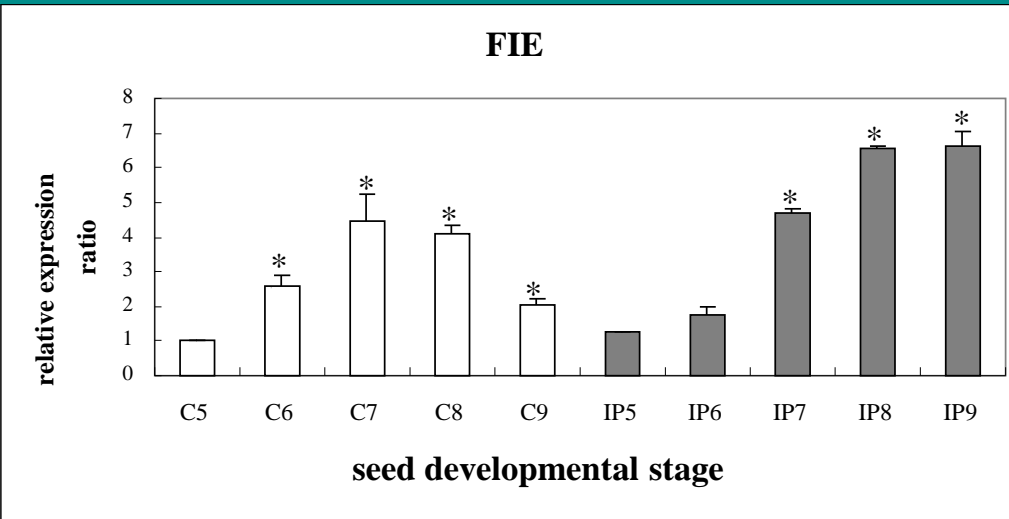
Expression of Polycomb group in barley during seed development

in two cultivars with different seed size



2 4 8 12 18 DAF

HvFIE

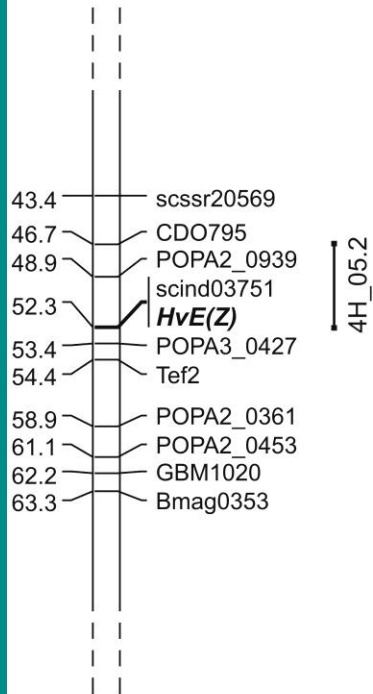


HvEZ

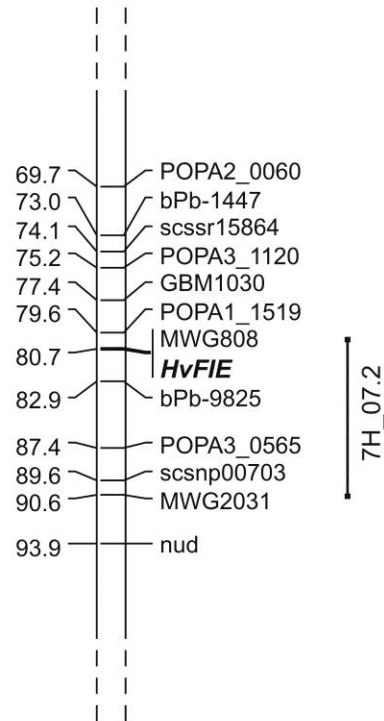
□ Caresse
■ Ippolytos

Mapping of PcG genes on the barley physical map

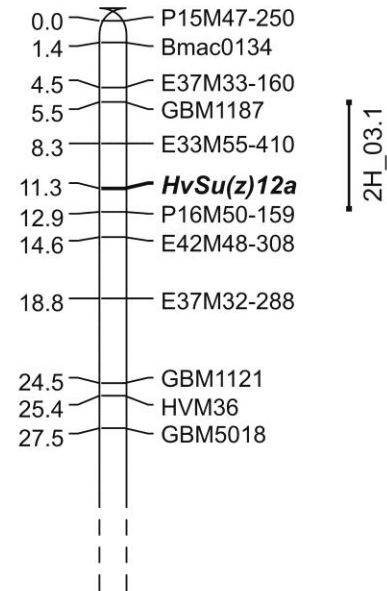
4H-OWB



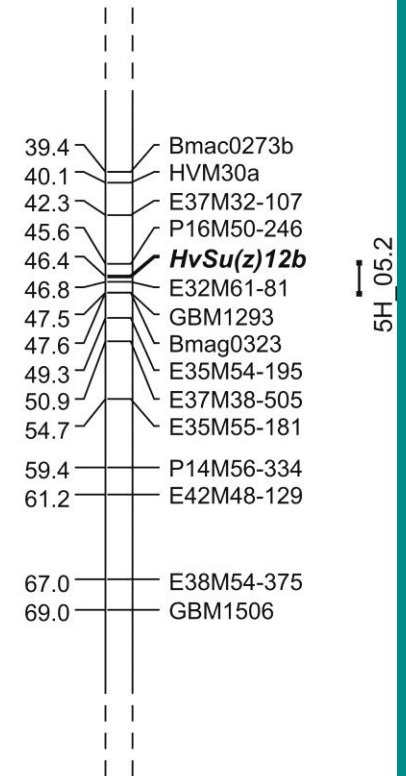
7H-OWB



2H-LxV



5H-LxV



➤ **PcG genes colocalized with regions known to be associated with malting quality traits**

MADS-box Type I genes in Arabidopsis

AGL35 (PHERES I)

AGL61

AGL62 (DIANA)

AGL80

AGL23

Central cell formation

Timing of endosperm
cellularization

Seed development

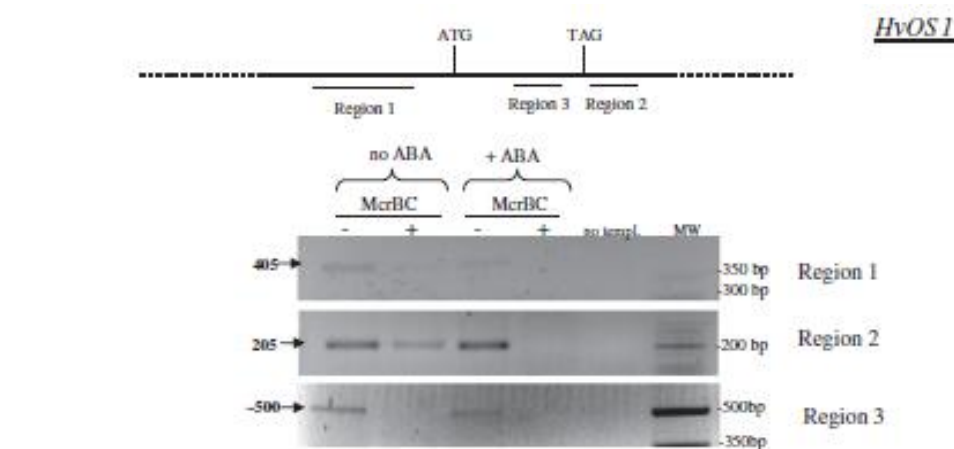
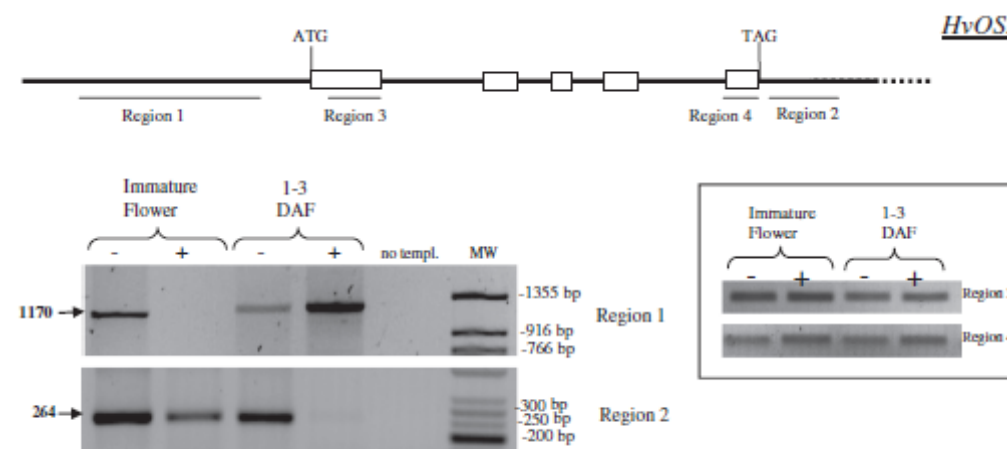
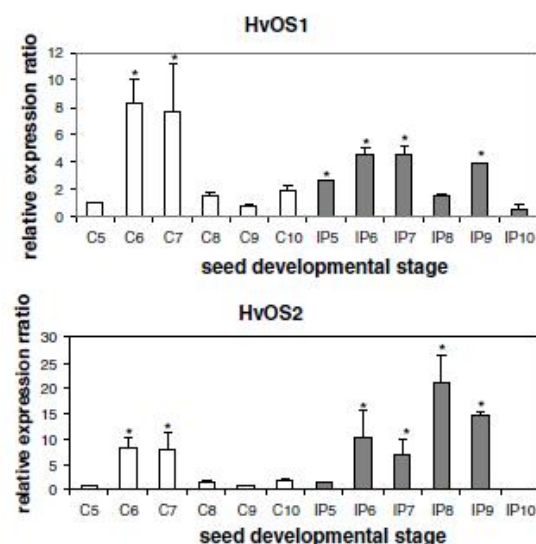
RESEARCH ARTICLE

Open Access

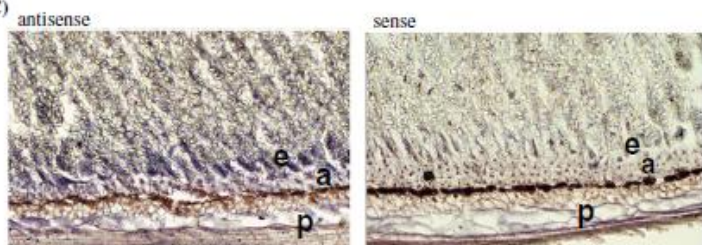
The study of two barley *Type I-like MADS-box* genes as potential targets of epigenetic regulation during seed development

Aliki Kapazoglou¹, Cawas Engineer¹, Vicky Drosou¹, Chrysanthi Kalloniati³, Eleni Tani¹, Aphrodite Tsaballa², Evangelia D Kouri³, Ioannis Ganopoulos², Emmanouil Flemetakis³ and Athanasios S Tsiftaris^{1,2*}

B)



C)



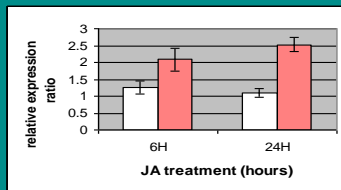
EPIGENETIC MECHANISMS AS MEDIATORS OF ENVIRONMENTAL CHANGES

Responses of barley epigenetic modifier genes to stress-related hormones

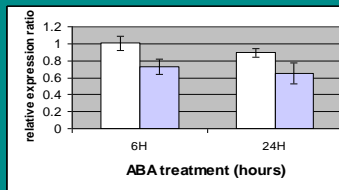
HDACs
family HD2

HvHDAC2-1

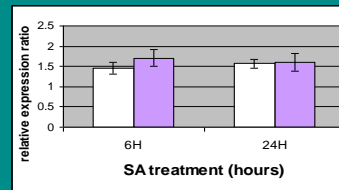
JA



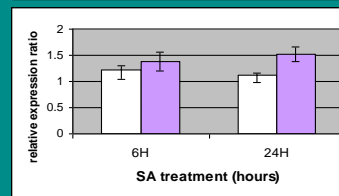
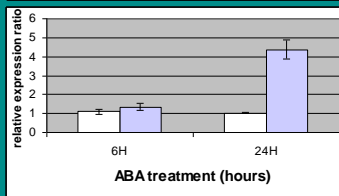
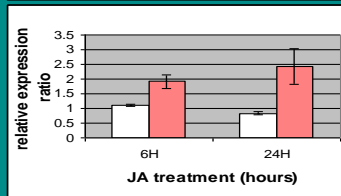
ABA



SA



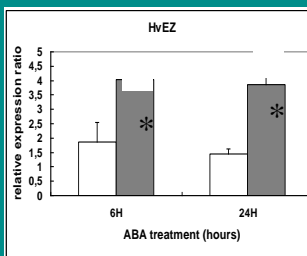
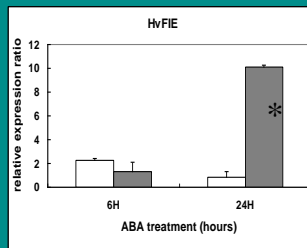
HvHDAC2-2



Demetriou, Kapazoglou, Tondelli, Francia, Stanca, Bladenopoulos, Tsaftaris (2009) *Physiol. Plant.*

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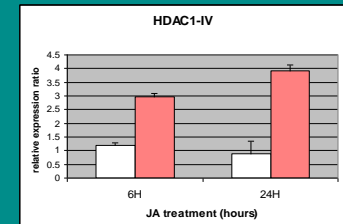
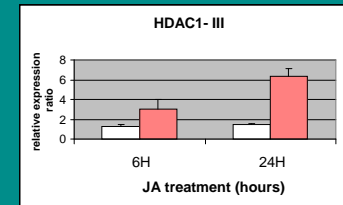
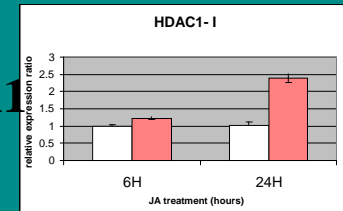
Polycomb



Kapazoglou, Tondelli, Papaefthimiou, Ampatzidou, Francia, Stanca, Bladenopoulos, Tsaftaris (2010) *BMC Plant Biology*

HDACs
Family
RPD3-HDA1

JA

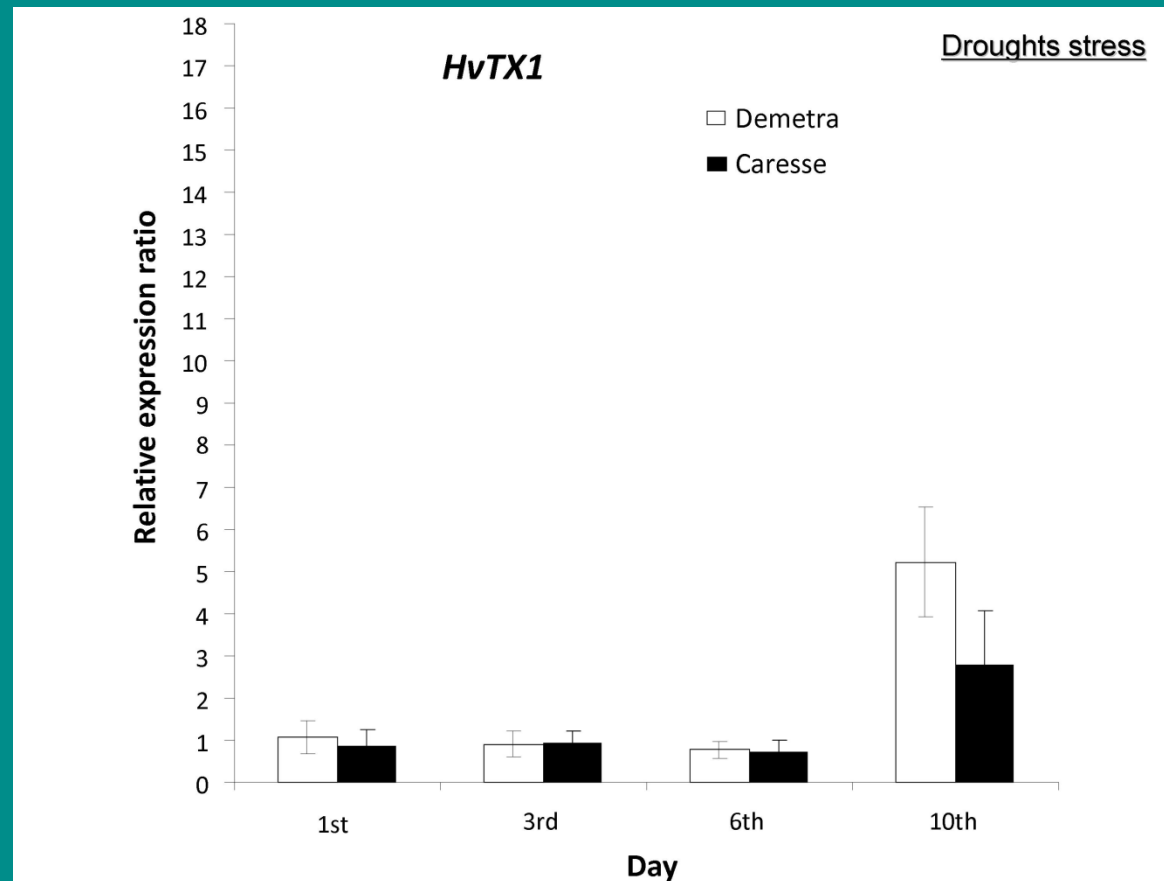


Demetriou, Kapazoglou, Bladenopoulos and Tsaftaris (2010) *Plant Mol Biol Rep*

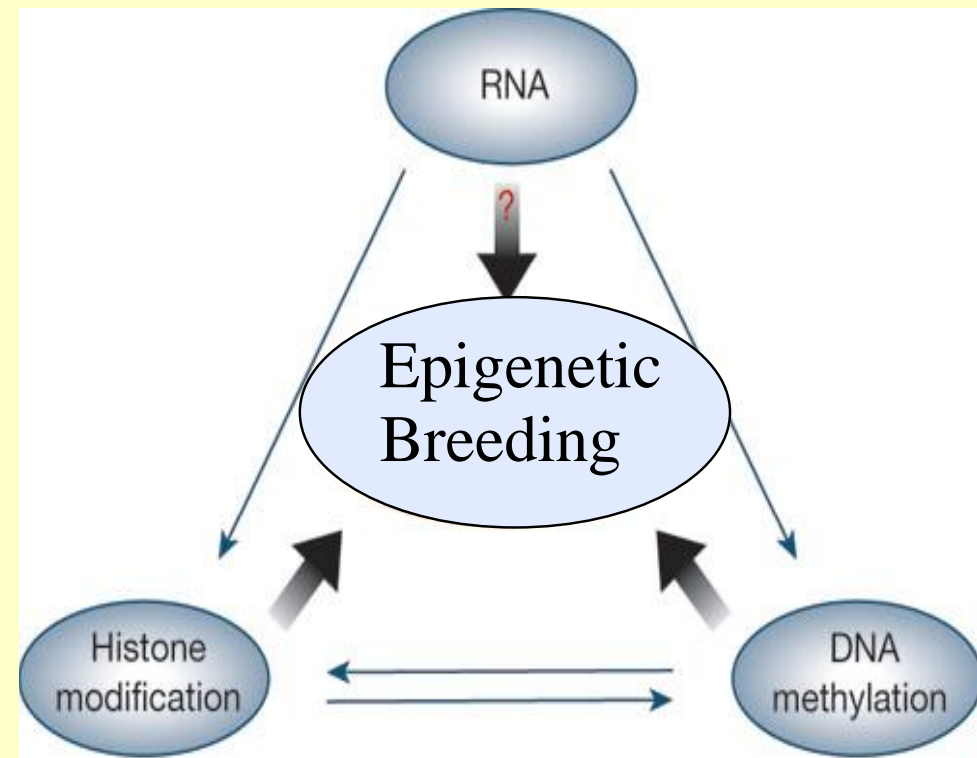
Expression of HvTX1 in response to dehydration

Caresse: drought sensitive

Demetra: drought tolerant



OUTLOOK



To meet the future challenges of

- ❑ **world population growth**
- ❑ **climate change**

➤ **An increased focus on exploring new varieties and novel traits**

➤ **Study and exploit epigenetic variation as well as genetic variation**

➤ **Development of tools and technology to enable easy and rapid selection for improved varieties**

INAB

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GSRT



COST FA0604



Thank you!