



Epigenetics and Epigenomics of “complex genomes”

ProCoGen 2nd Training Workshop Alcalá de Henares
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Célia Miguel



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Framework Programme

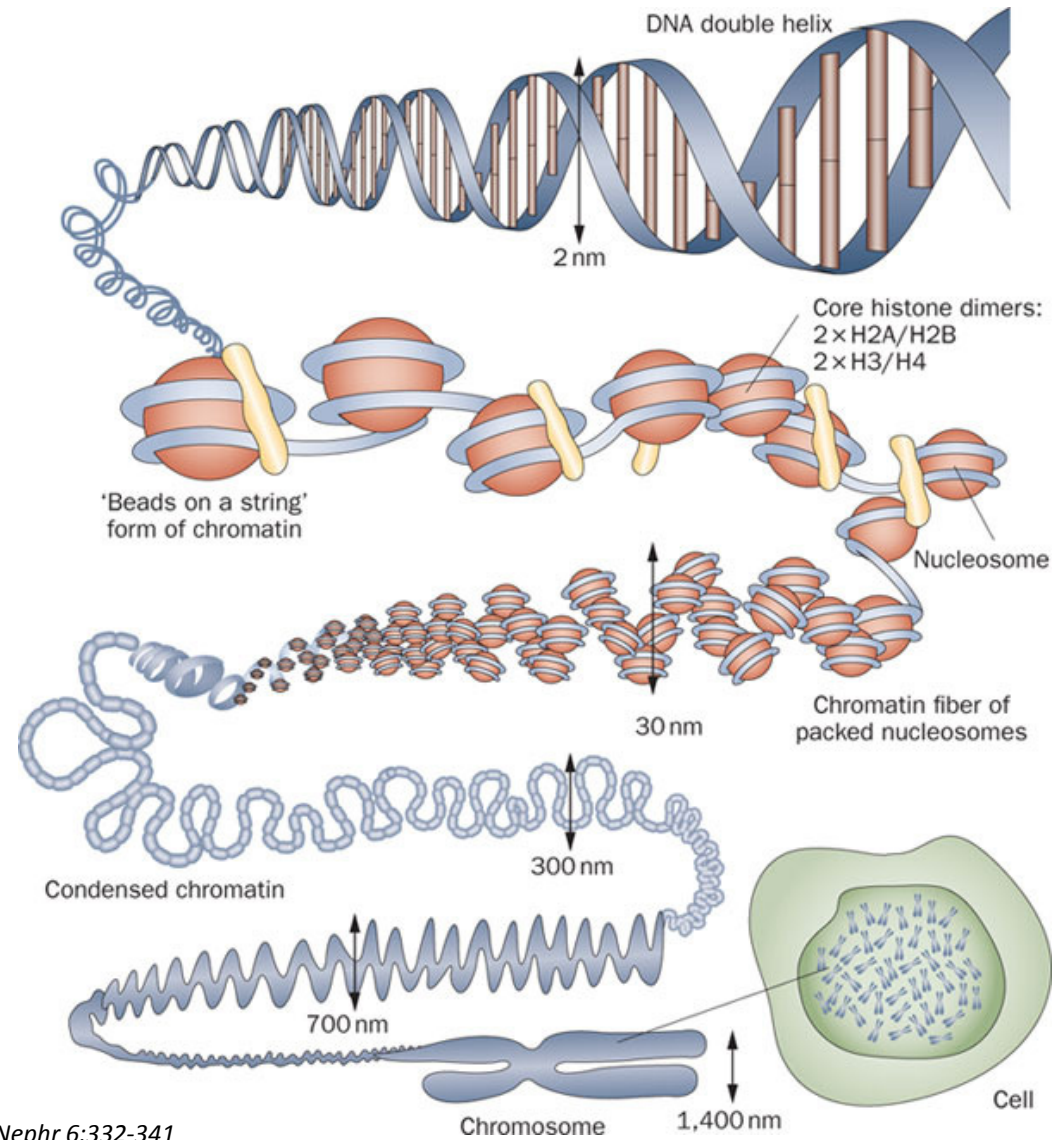
Epigenetics and Epigenomics of complex genomes

Outline

- ▶ Concepts
- ▶ Epigenetic pathways
 - ▶ DNA methylation
 - ▶ Histones
 - ▶ sRNAs
- ▶ Genome-wide methodologies
- ▶ Epigenome landscape in plants
- ▶ Examples



DNA is heavily packed



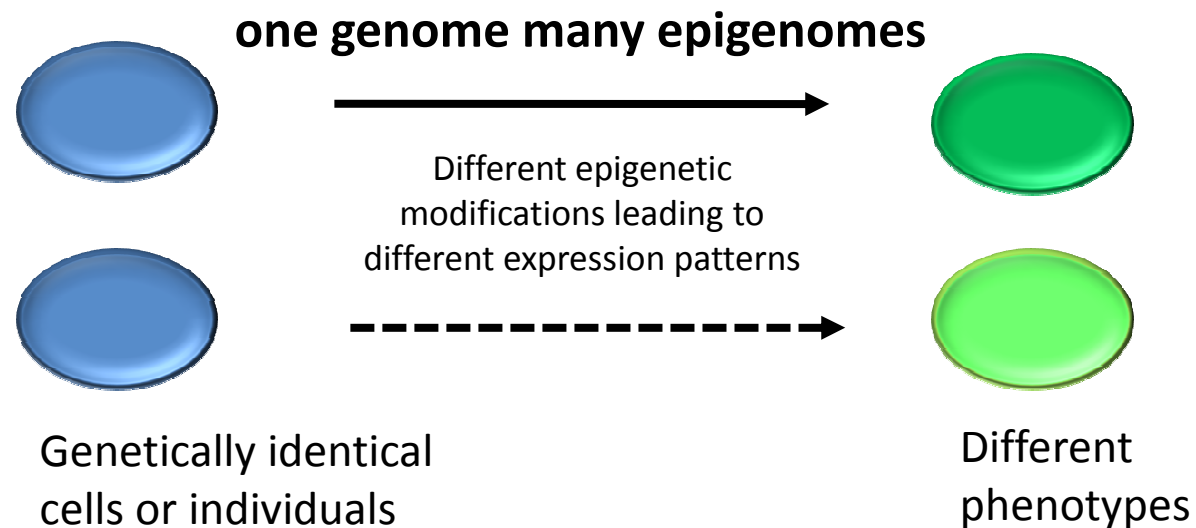
Epigenetics & Epigenomics

Epigenetics refers to the processes that lead to heritable changes in gene expression (during development or across generations) without changes in the DNA sequence itself

"the interactions of genes with their environment which bring the phenotype into being"

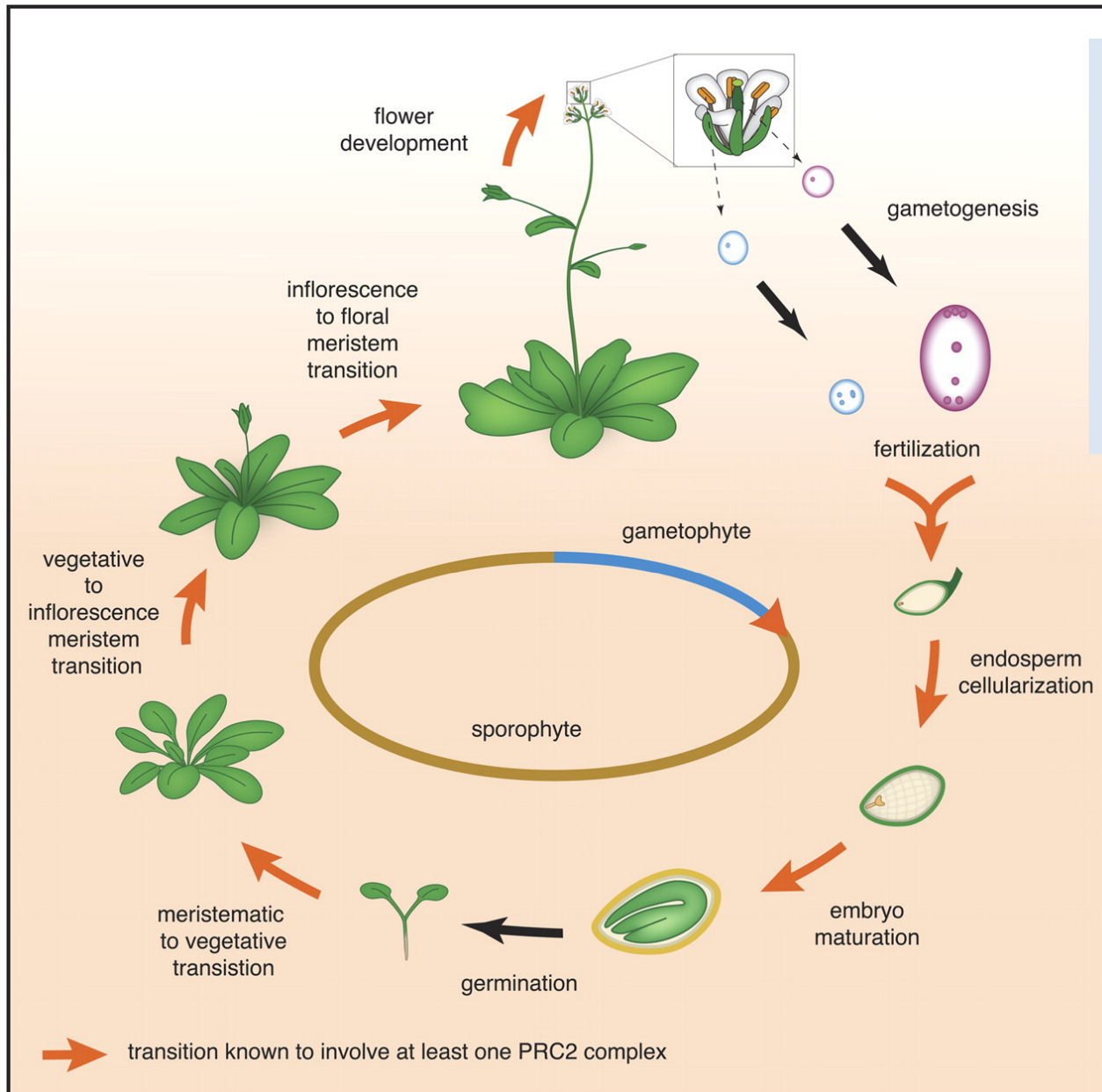
Conrad Waddington (1940)

Epigenomics refers to the study of epigenetic modifications at the whole genome level



Adapted from TTPB (Plant Cell)

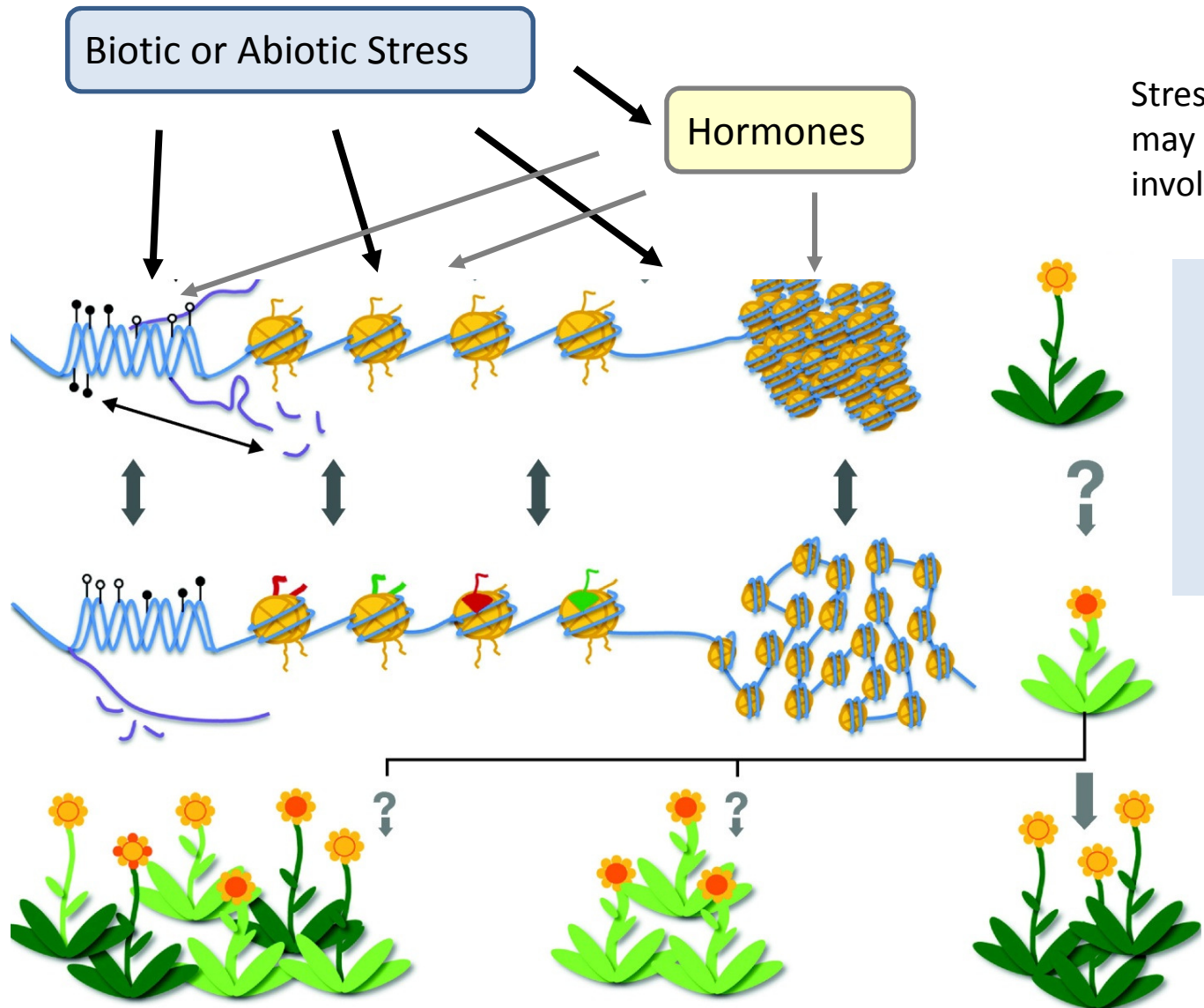
Williams ME (2013) The Plant Cell (online), doi/10.1105/tpc.110.tt0110



Many developmental genes and switches are epigenetically regulated

- Imprinting
- Vernalization
- Heterosis
- Bud burst
- ...

Holec and Berger (2012). *Plant Physiol.* 158: 35-43
 Adapted from *TTPB (Plant Cell)*
 Williams ME (2013) *The Plant Cell* (online), doi/10.1105/tpc.110.tt0110



Stress signalling may or may not involve hormones

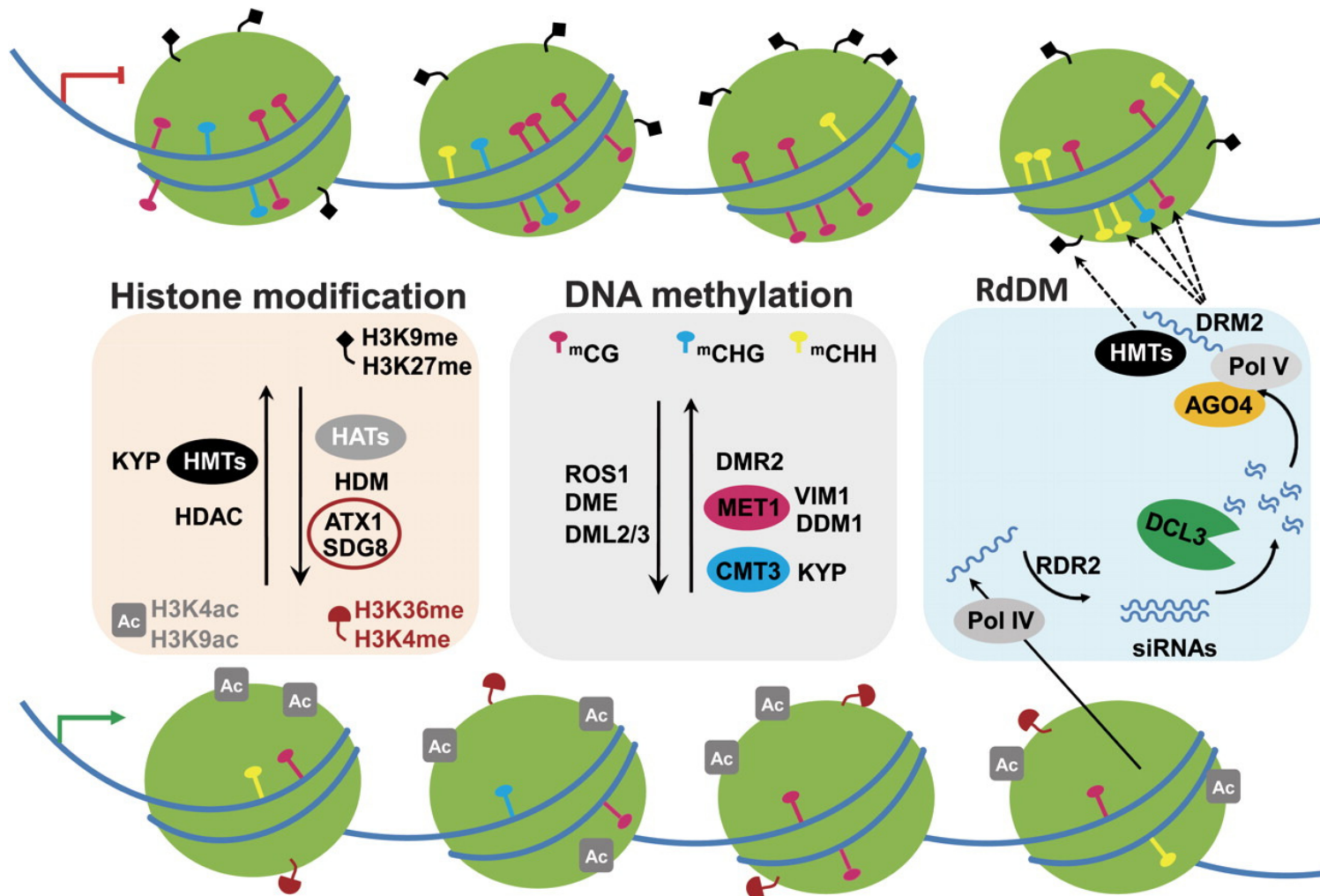
Many stress-responsive genes are epigenetically regulated

The stress-responsive phenotype is usually not transmitted to progeny, but chromatin changes can be heritable

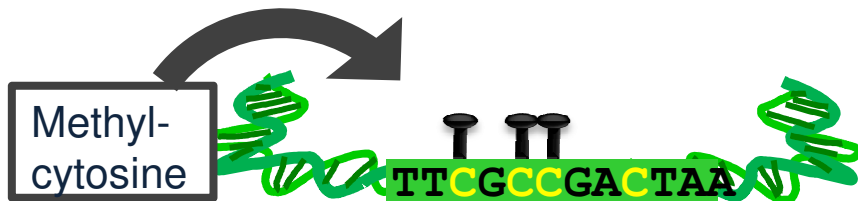
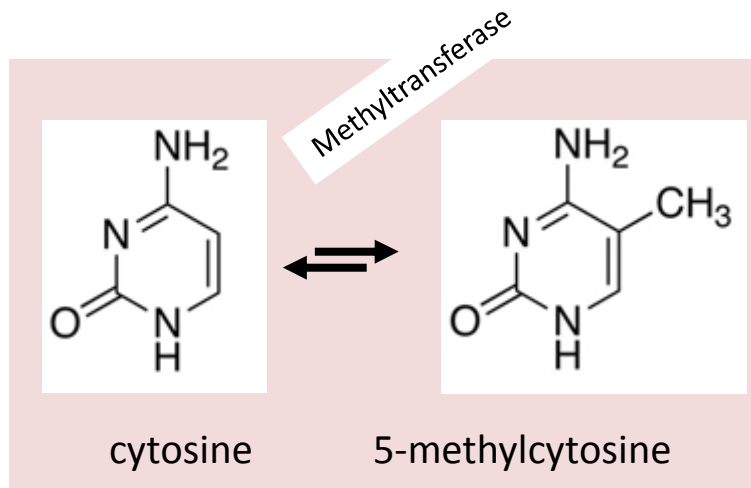
Current Opinion in Plant Biology

Gutzat and Mittelsten Scheid (2012) *Curr Opin Plant Biol* 15: 568-573
 Adapted from *TTPB (Plant Cell)*
 Williams ME (2013) *The Plant Cell* (online), doi/10.1105/tpc.110.tt0110

Changes in chromatin structure: DNA methylation, histone modification, small RNA-directed DNA methylation



DNA methylation



DNA methyltransferases in Arabidopsis

MET1 (METHYLTRANSFERASE1)

- 5'-CG-3' sites
- Silencing of transposons, repetitive elements, some imprinted genes

CMT3 (CHROMOMETHYLASE3)

- 5'-CHG-3' sites
- (H= A, C or T)
- Interacts with histone mark

DRM 1, DRM 2 (DOMAINS REARRANGED 1 and 2)

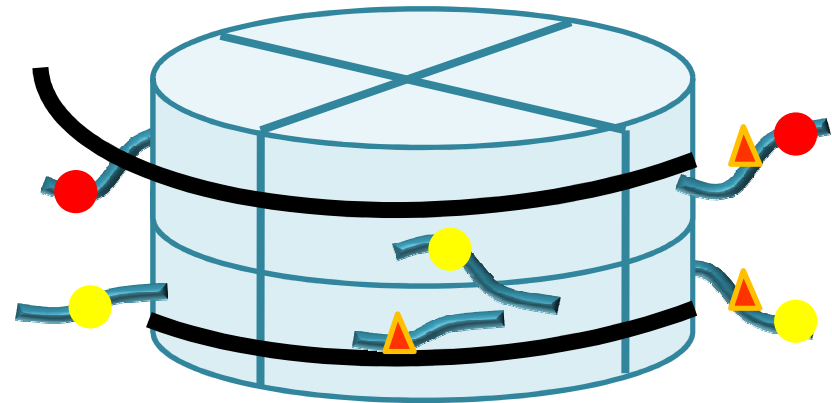
- 5'-CHH-3' sites
- Primarily targets repetitive elements
- Requires the active targeting of siRNAs

Adapted from TTPB (Plant Cell)

Williams ME (2013) The Plant Cell (online), doi/10.1105/tpc.110.tt0110

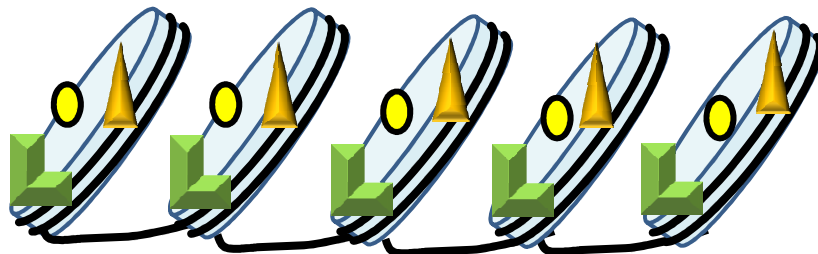
Histone modification affects chromatin structure

- Acetylation (Ac)
- Ubiquitination (Ub)
- Methylation (Me)
- Phosphorylation (P)
- Sumoylation (Su)



Open configuration

H3	Me	P	Ac
	K4	S10	K14



Closed configuration

H3	Me	Me P
	K9	K27 S28

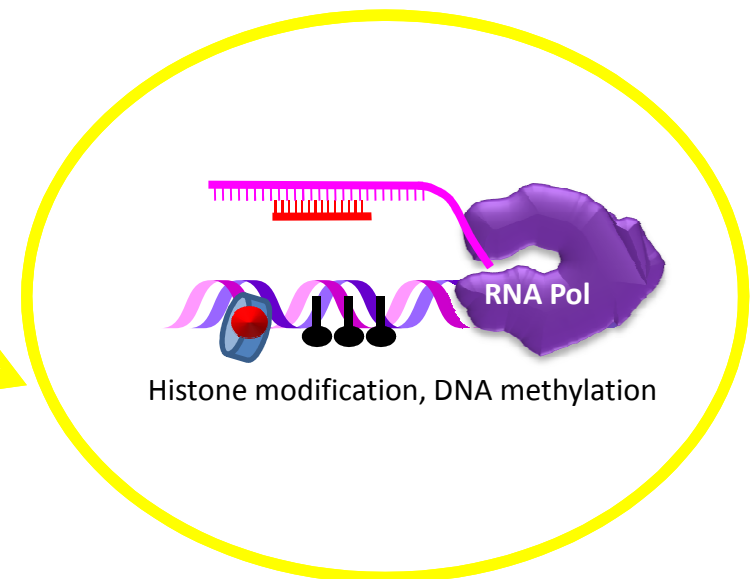


Adapted from TTPB (Plant Cell)

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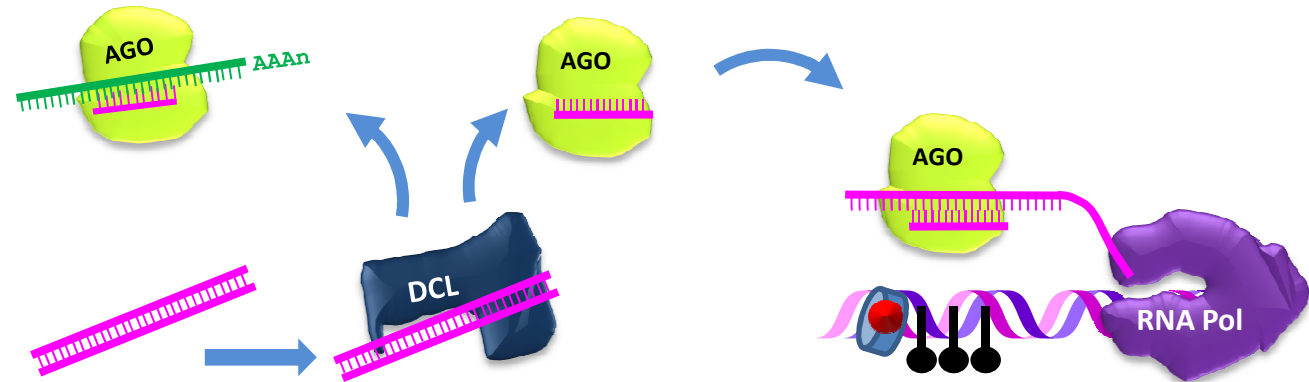
Small RNAs are key actors of epigenetic processes

- Small RNAs are a pool of 21 to 24 nt RNAs that generally function in **gene silencing**
- Small RNAs contribute to **post-transcriptional gene silencing** by affecting mRNA stability or translation
- Small RNAs contribute to **transcriptional gene silencing** through epigenetic modifications to chromatin

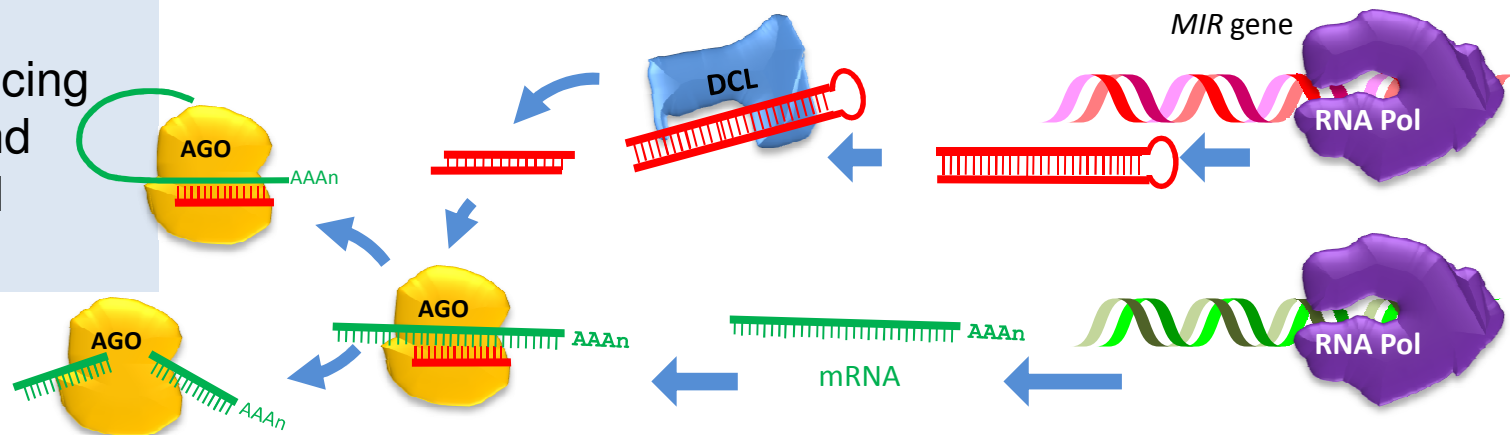


RNA silencing - overview

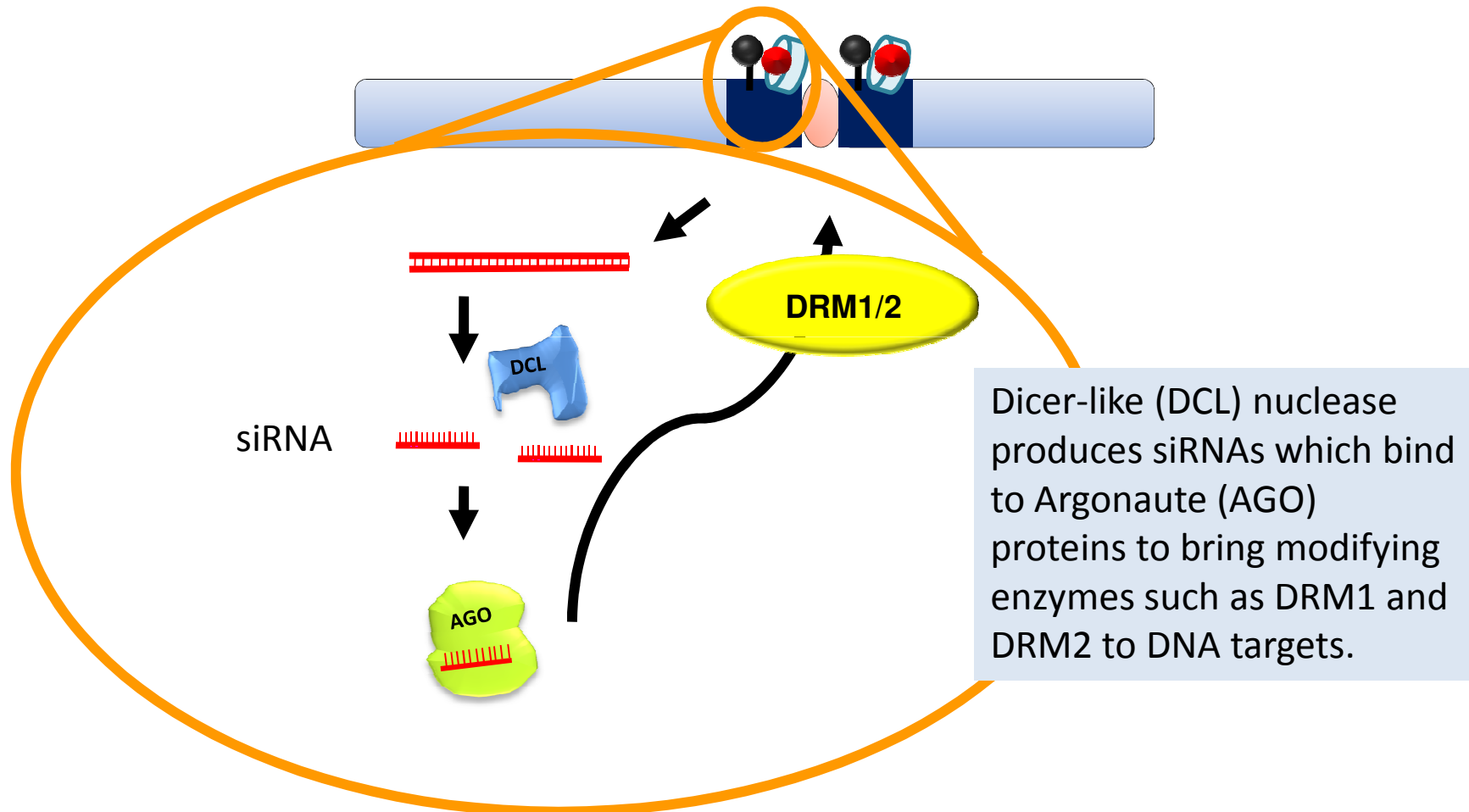
siRNA-mediated silencing via post-transcriptional and transcriptional gene silencing



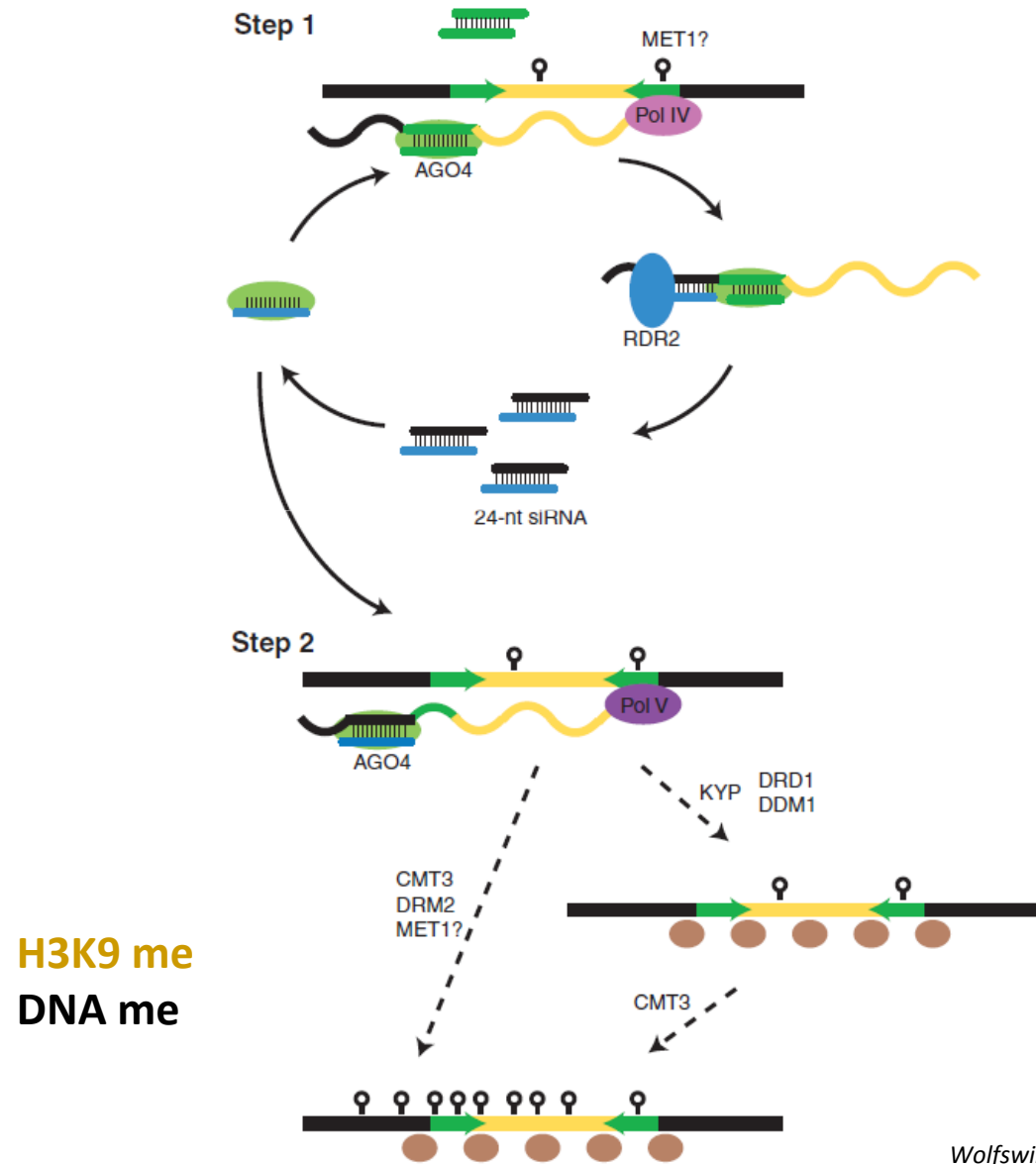
miRNA - mediated slicing of mRNA and translational repression



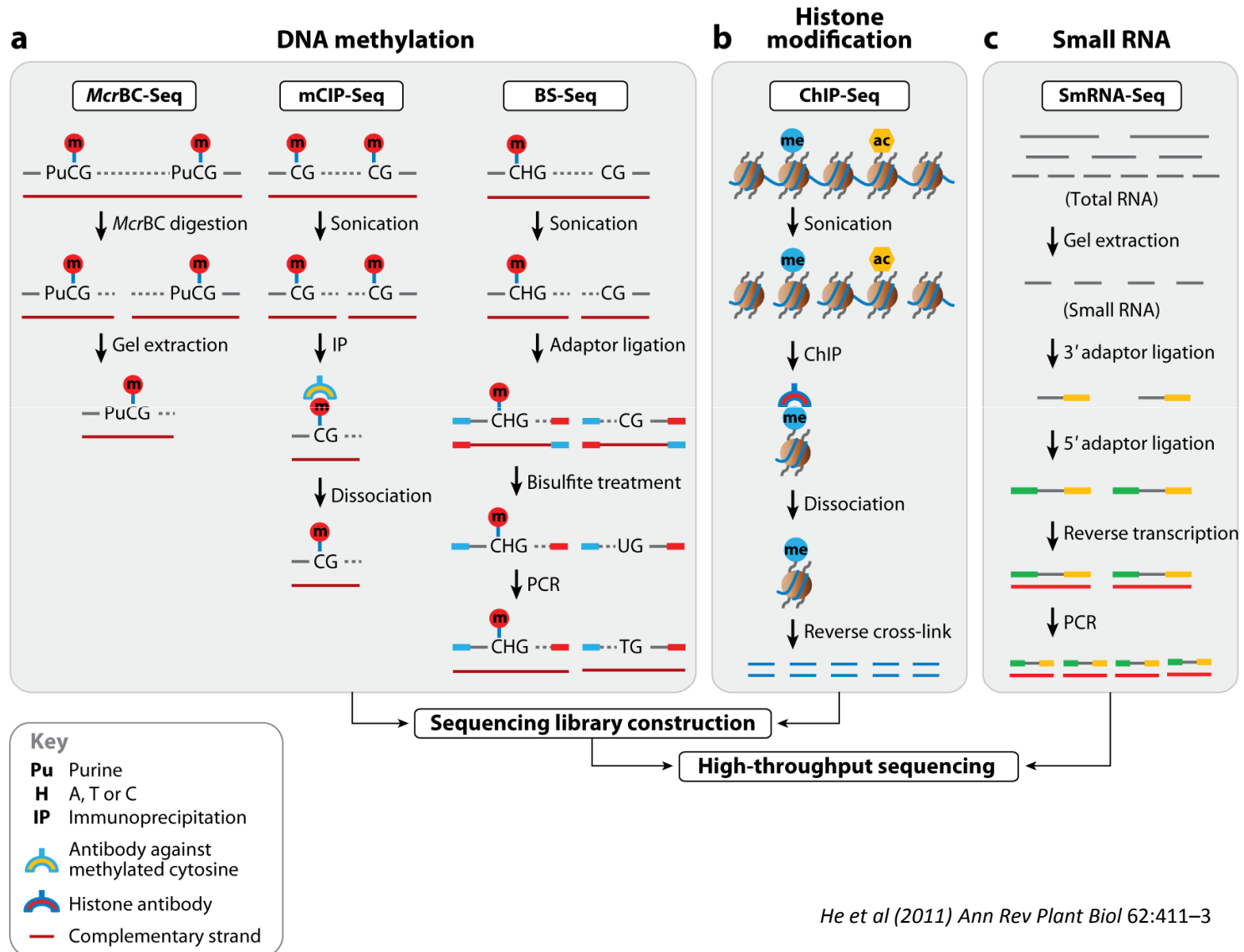
siRNAs recruit DNA methylases and histone-modifying enzymes to targets



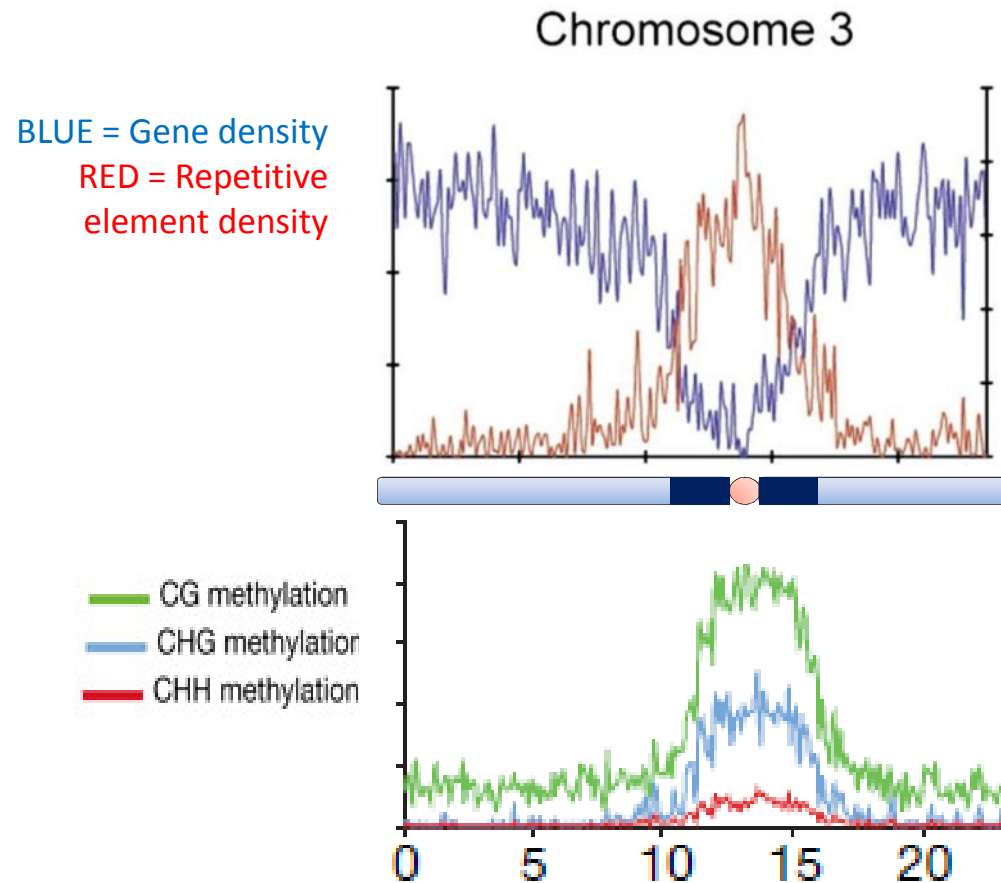
Mechanisms of transposable silencing in plants - siRNAs



Cracking the epigenetic code



Heterochromatin DNA is highly methylated



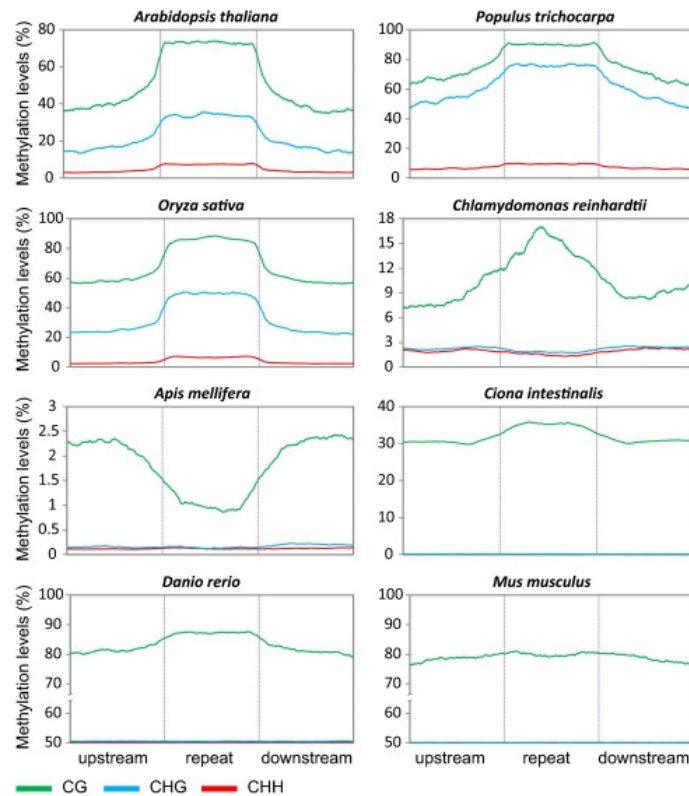
Whereas DNA methylation in all contexts are enriched in pericentromeric regions, CG methylation extends to non-TE genes

Zhang et al (2006) *Cell* 126: 1189–1201
Cokus et al., (2008) *Nature* 452: 215-219

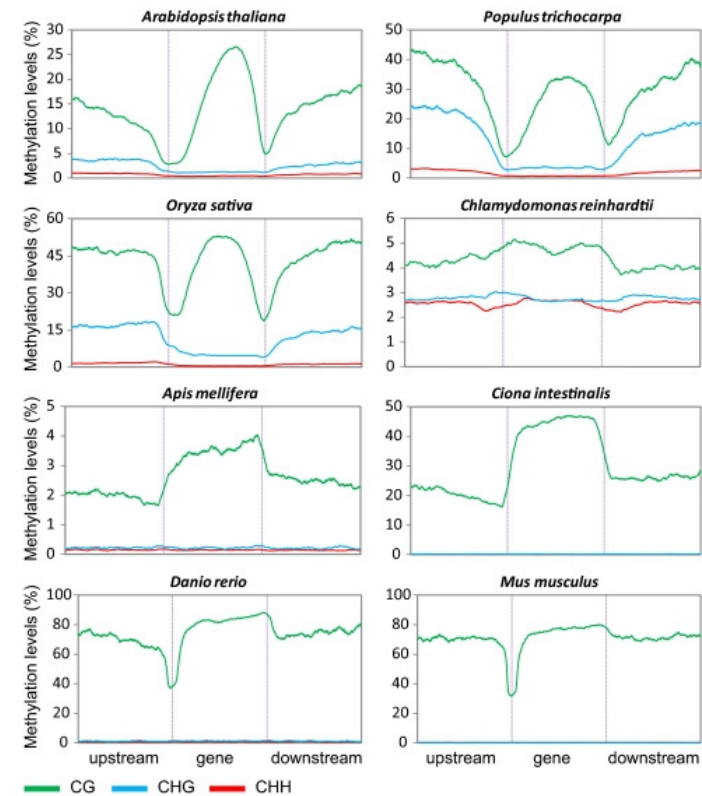
Adapted from TTPB (*Plant Cell*)
Williams ME (2013) *The Plant Cell* (online), doi/10.1105/tpc.110.tt0110

Genome-wide distribution of DNA methylation in plants and animals

repetitive DNA



protein-coding genes

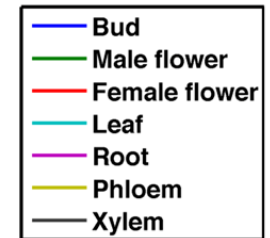
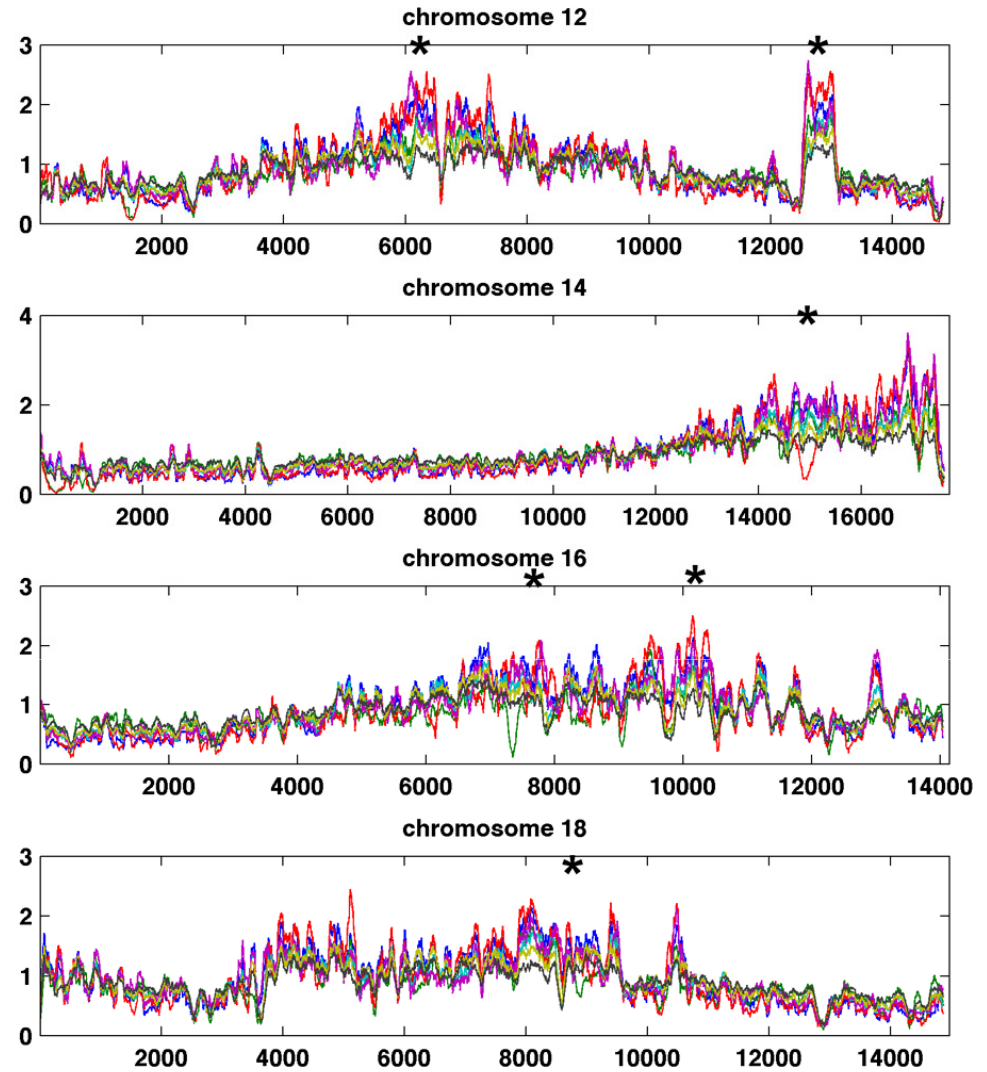
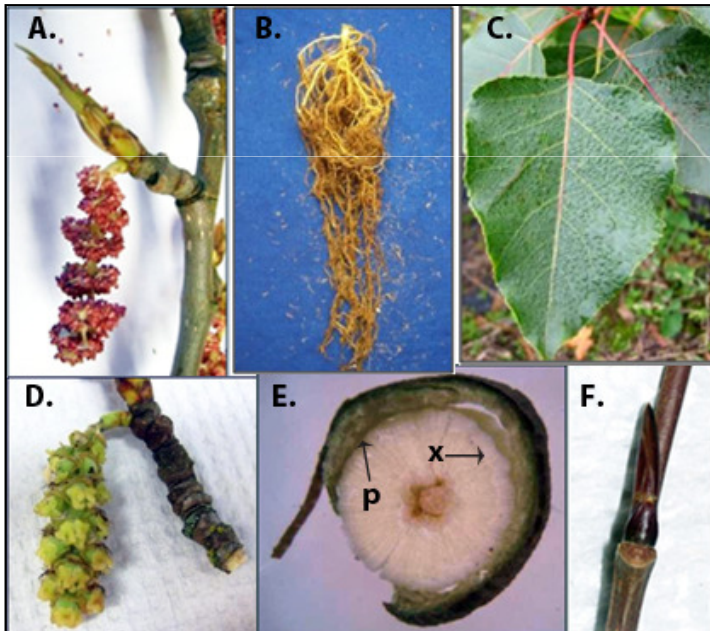


Feng et al (2010) PNAS 107:8689–8694

TTPB (Plant Cell)

Williams ME (2013) The Plant Cell (online), doi/10.1105/tpc.110.tt0110

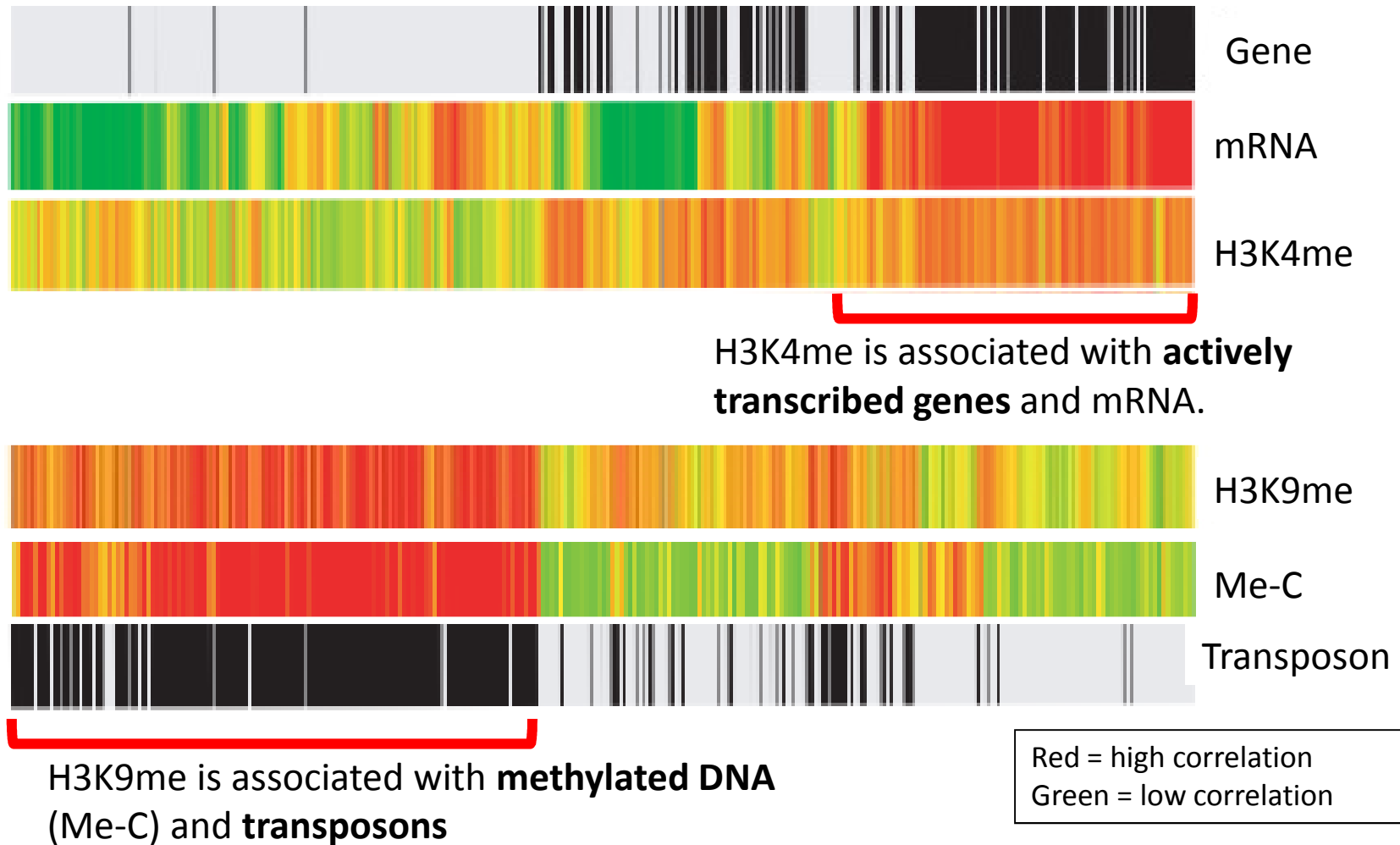
Chromosome-level view of methylation among *Populus* tissues



↑ methylation heterogeneity ↓ gene density

↑ methylation ↑ tissue-associated variation

Different histone modifications are associated with genes and transposons



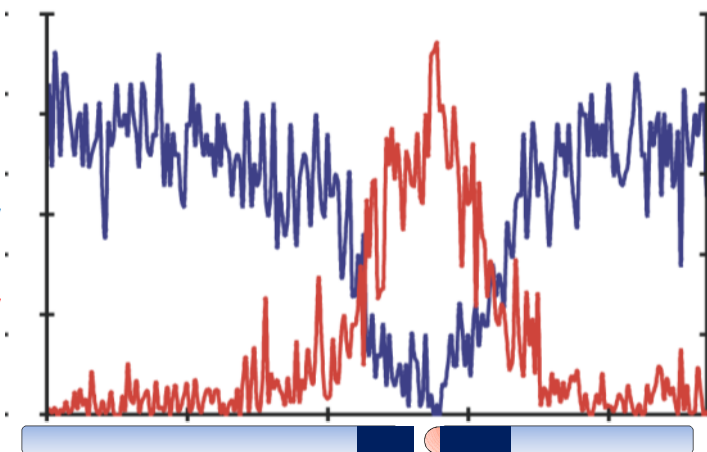
Lippman et al (2004) Nature 430: 471-476

TTPB (Plant Cell)

Williams ME (2013) The Plant Cell (online), doi/10.1105/tpc.110.tt0110

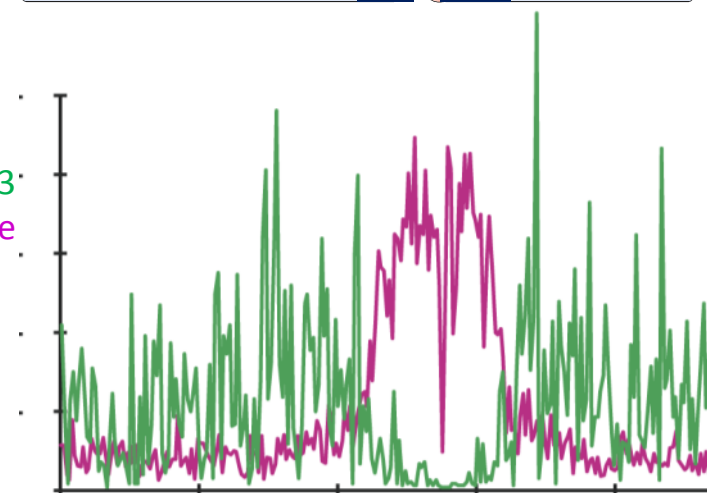
H3K27me3 is associated with genes

BLUE = Gene density
RED = Repetitive
element density



H3K27me3 in Arabidopsis is present within the gene-rich region, not the repeat-rich region.

GREEN = H3K27me3
PURPLE = methylcytosine



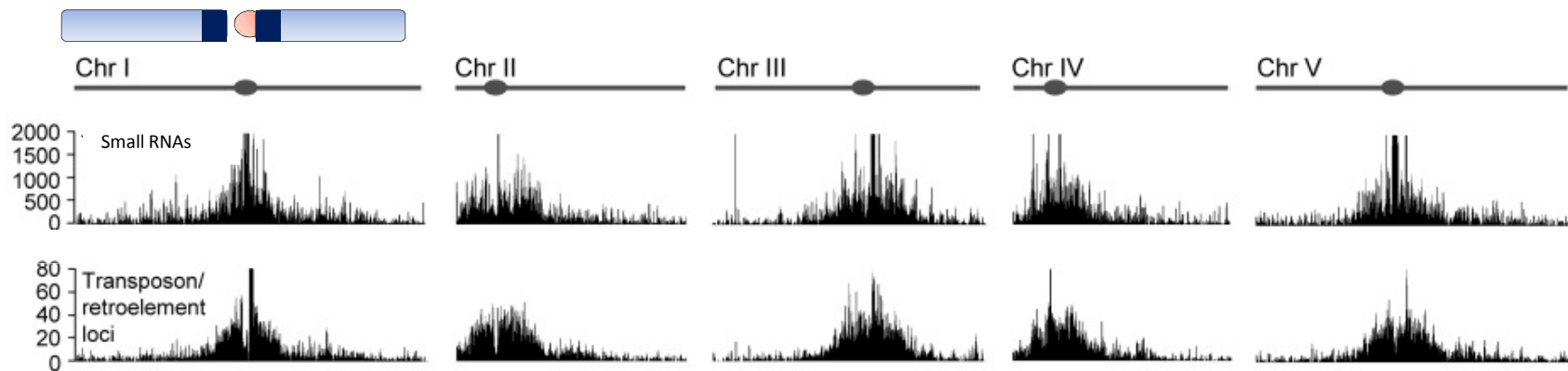
FLOWERING LOCUS C (FLC)
AGAMOUS (AG)
SHOOT MERISTEMLESS (STM)
MEDEA (MEA)
TFs
...

Zhang (2007) *PLoS Biol.* 5: e129

TTPB (Plant Cell)

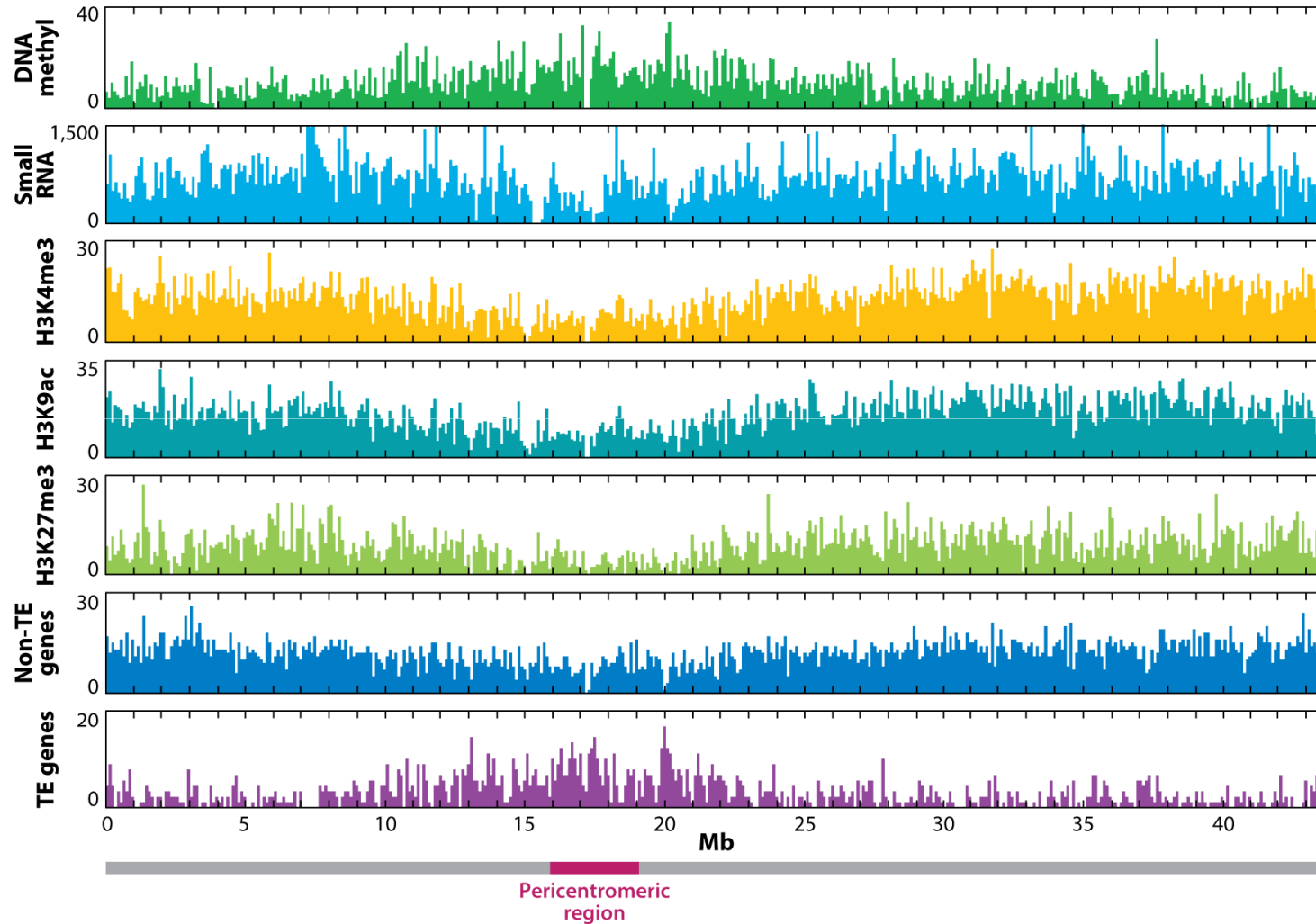
Williams ME (2013) *The Plant Cell* (online), doi/10.1105/tpc.110.tt0110

Small interfering RNAs (siRNAs) are preferentially derived from pericentromeric regions

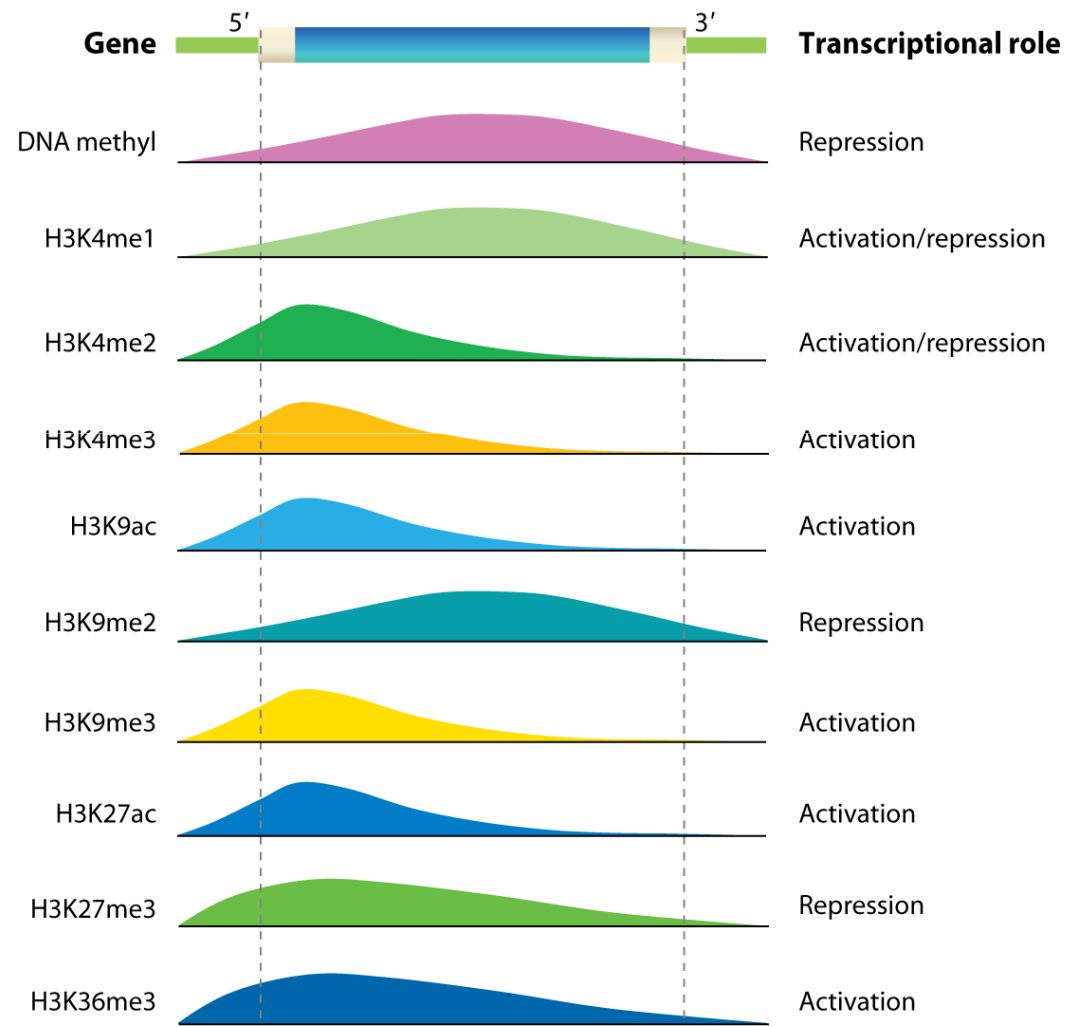


The density of small RNA-homologous loci is highest in the centromeric and pericentromeric regions which contain a high density of repeat sequence classes

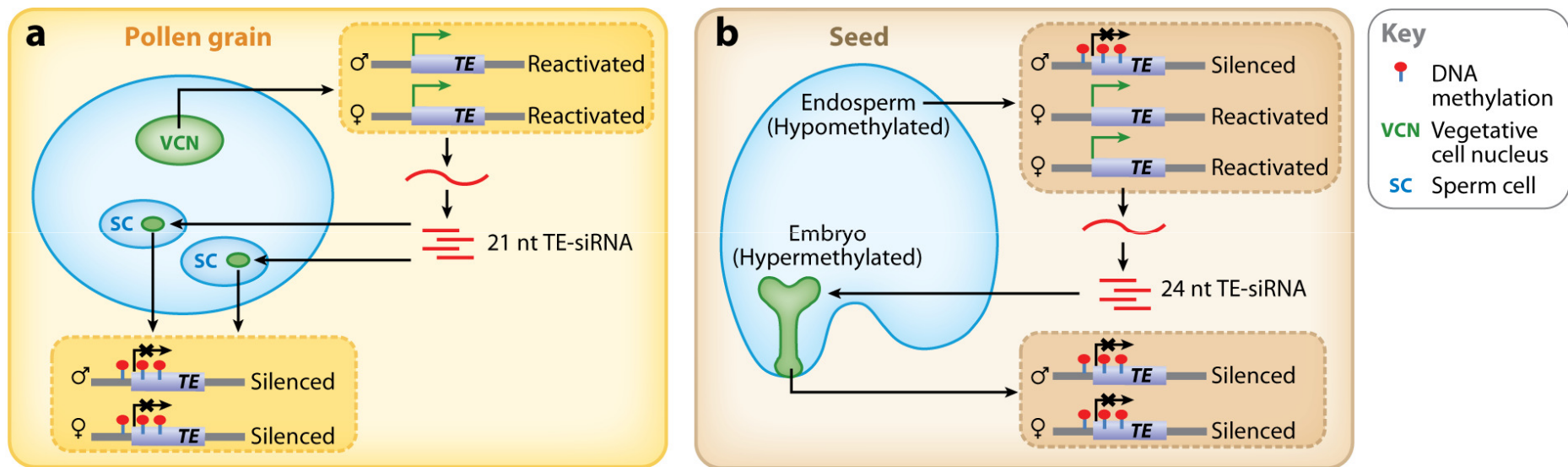
Epigenetic Landscapes in Plants



Distribution of DNA met and H modification in genes and their roles in transcription

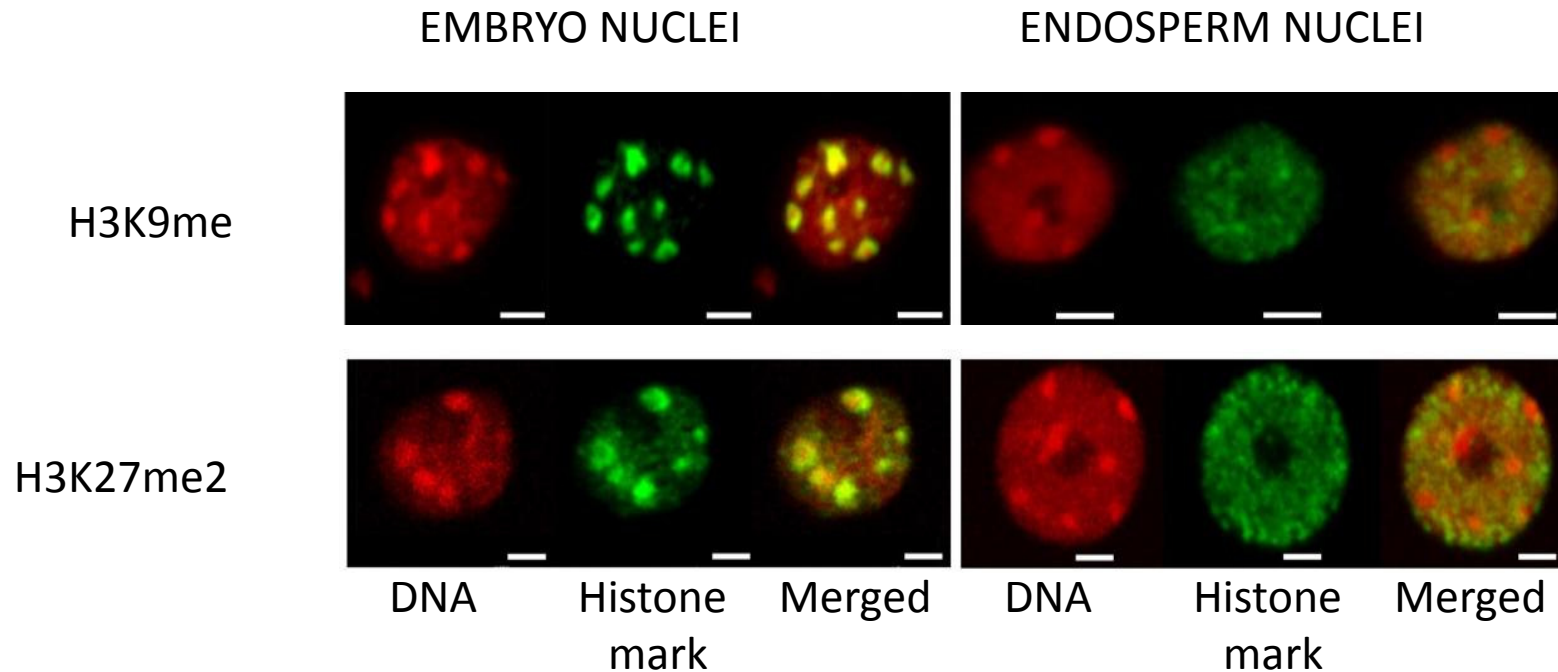


Epigenomic reprogramming during pollen and seed development



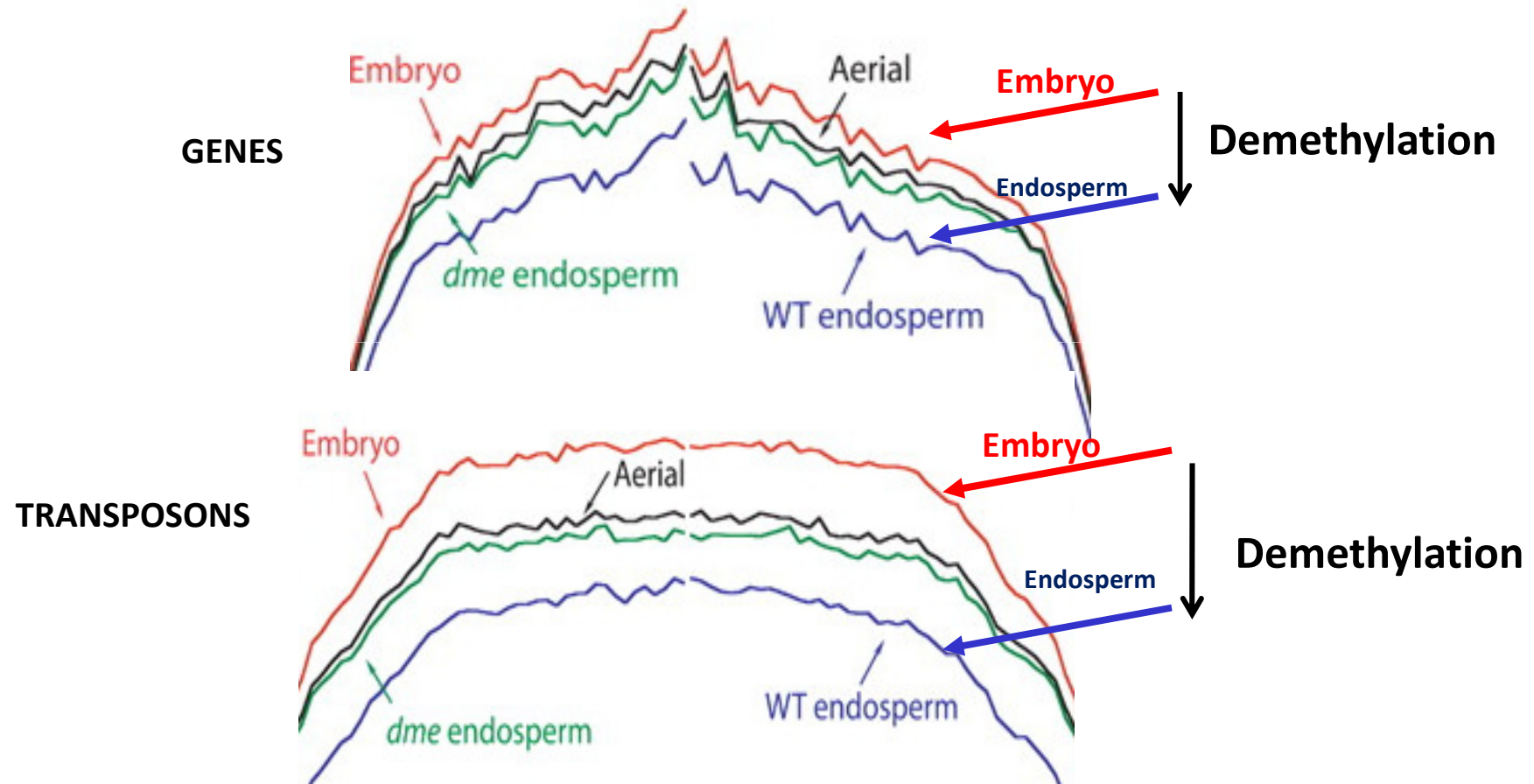
He et al (2011) *Annu Rev Plant Biol* 62: 411-35

The heterochromatin fraction is reduced in endosperm

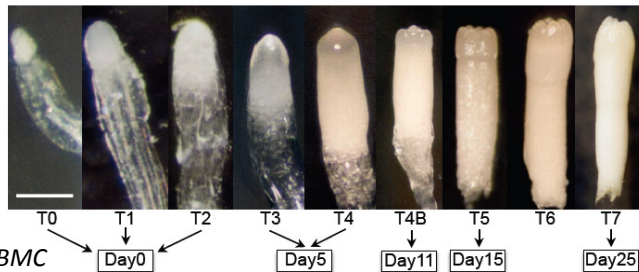
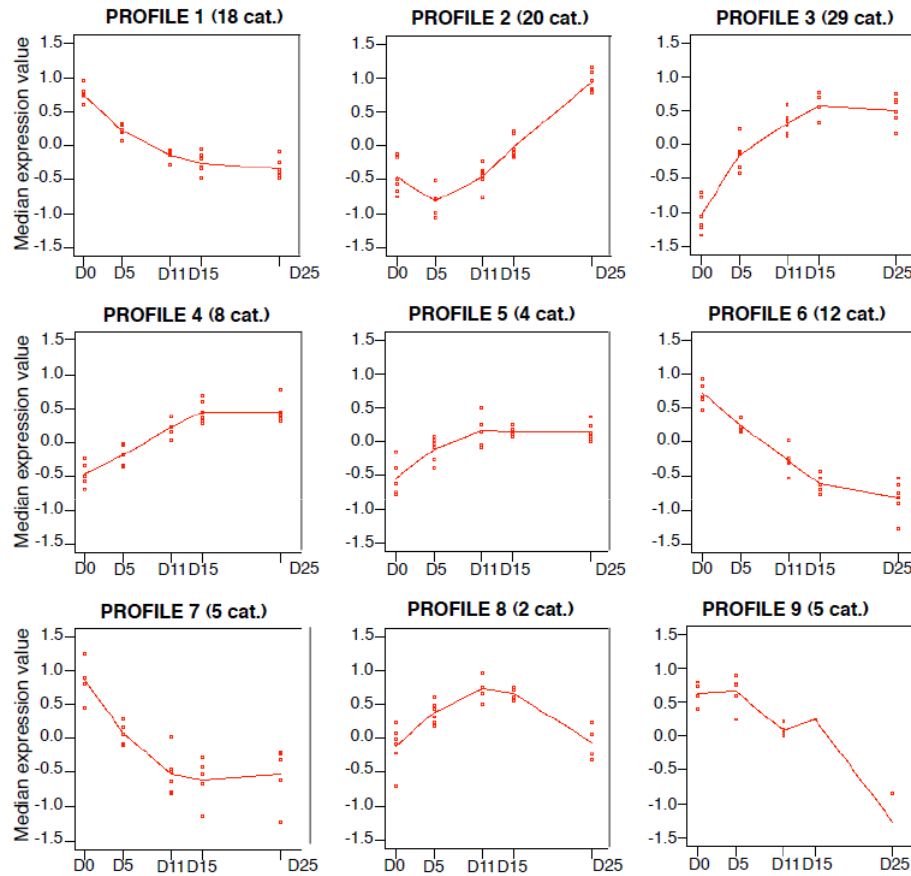


In endosperm nuclei, heterochromatin marks such as H3K9me and K3K27me2 disperse into euchromatin, suggesting genome-wide epigenetic changes.

DNA in endosperm is demethylated as compared to embryo



Epigenetic regulation during embryo development of *Pinus pinaster*



de Vega-Bartol et al (2013) BMC Plant Biol 13:123

PROFILE 3 (29 cat.)

- F: miRNA binding
- F: siRNA binding
- P: Gene silencing by miRNA
- P: miRNA metabolic process
- F: Plastoquinol/plastocyanin reductase activity
- F: Pyridoxal kinase act.
- F: Specific transcriptional repressor
- P: Adventitious root develop.
- P: Asymmetric cell division
- P: Nonphotoreactive DNA repair
- P: Nucleotide excision repair, preincision complex assembly
- P: Purine base catabolic process
- P: Superoxide anion generation
- EC 1.1.99.1 Choline dehydrogenase
- EC 1.14.13. With NADH or NADPH as one donor
- F: Oxidoreductase activity with NADH or NADPH as a donor
- EC 1.17.1.4 Xanthine dehydrogen.
- EC 1.17.3.2 Xanthine oxidase
- F: Xanthine dehydrogenase act.
- F: Xanthine oxidase act.
- P: Xanthine metabolic process
- F: 2,3-bisphosphoglycerate-depend. phosphoglycerate mutase
- F: 5'-flap endonuclease activity
- P: Somatic stem cell maintenance
- P: Single fertilization
- PO: Hypocotyl epidermis
- PO: Exodermis
- Path: Phytol diphosphate biosynth.
- Path: Guanosine nucleot. degradatio

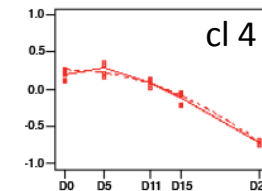
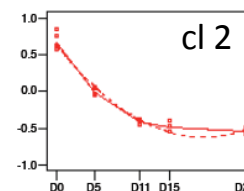
PROFILE 9 (5 cat.)

- P: Maintenance of chromatin silencing
- P: Negative regulation of histone H4 acetylation
- P: Positive regulation of histone H3K9 methylation
- P: Regulation of DNA methylation
- P: Transposition, RNA mediated

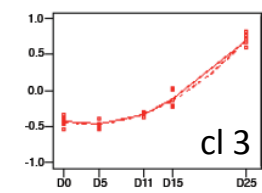
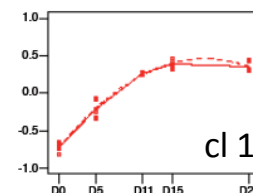
Cluster	At Locus	E-value	Annotation
1	AT1G08460	2E-141	Histone deacetylase 8 (HDA8)
1	AT3G44680	0	Histone deacetylase 9 (HDA9)
1	AT1G09700	2E-43	dsRNA-binding protein 1 (DRB1), HYPONASTIC LEAVES 1 (HYL1)
1	AT1G48410	0	ARGONAUTE 1 (AGO1)
1	AT5G21150	3E-109	ARGONAUTE 9 (AGO9)
1	AT2G23380	2E-151	CURLY LEAF (CLF) , INCURVATA1 (ICU1), SDG1, SET1
1	AT5G22750	2E-155	DNA/RNA helicase protein RAD5
1	AT3G20550	6E-84	SMAD/FHA domain-containing protein DAWDLE, DDL
1	AT5G14170	1E-164	SWIB/MDM2 domain superfamily protein CHC1
2	AT1G01040	0	ABNORMAL SUSPENSOR 1 (ASU1), CARPEL FACTORY (CAF), DICER-LIKE1 (DCL1) , EMBRYO DEFECTIVE 60 / 76 (EMB60 / 76), SHORT INTEGUMENTS 1 (SIN1), SUSPENSOR 1 (SUS1)
3	AT1G77300	5E-27	H3-K4 specific histone methyltransferases, ASH1 HOMOLOG 2 (ASHH2)
3	AT4G38040	7E-150	Exostosin family protein
3	AT3G17590	3E-75	Transcription regulatory protein SNF5 homologue, BUSHY GROWTH (BSH)
4	AT5G03740	4E-19	Histone deacetylase 2C (HD2C)
4	AT1G77540	5E-30	H3/H4 histone acyl-CoA N-acyltransferase
4	AT2G47210	4E-124	myb-like transcription factor family protein
4	AT5G66750	0	CHROMATIN REMODELLING 1 (CHR1), DECREASED DNA METHYLATION 1 (DDM1) , SOMNIFEROUS 1 (SOM1)
4	AT4G16280	9E-73	RNA-mediated chromatin silencing protein, FLOWERING TIME CONTROL PROTEIN FCA
4	AT3G57300	1E-157	INO80 ortholog
5	AT1G57820	0	Zinc C3HC4-type RING finger protein, ORTH2, VARIANT IN METHYLATION 1 (VIM1)
6	AT5G26040	5E-136	Histone deacetylase 2 (HDA2)
6	AT5G04940	5E-123	SU(VAR)3-9 homolog 1 (SUVH1)

Differentially transcribed genes implicated in epigenetic regulation

Gene silencing pathways (TEs) during early embryo development

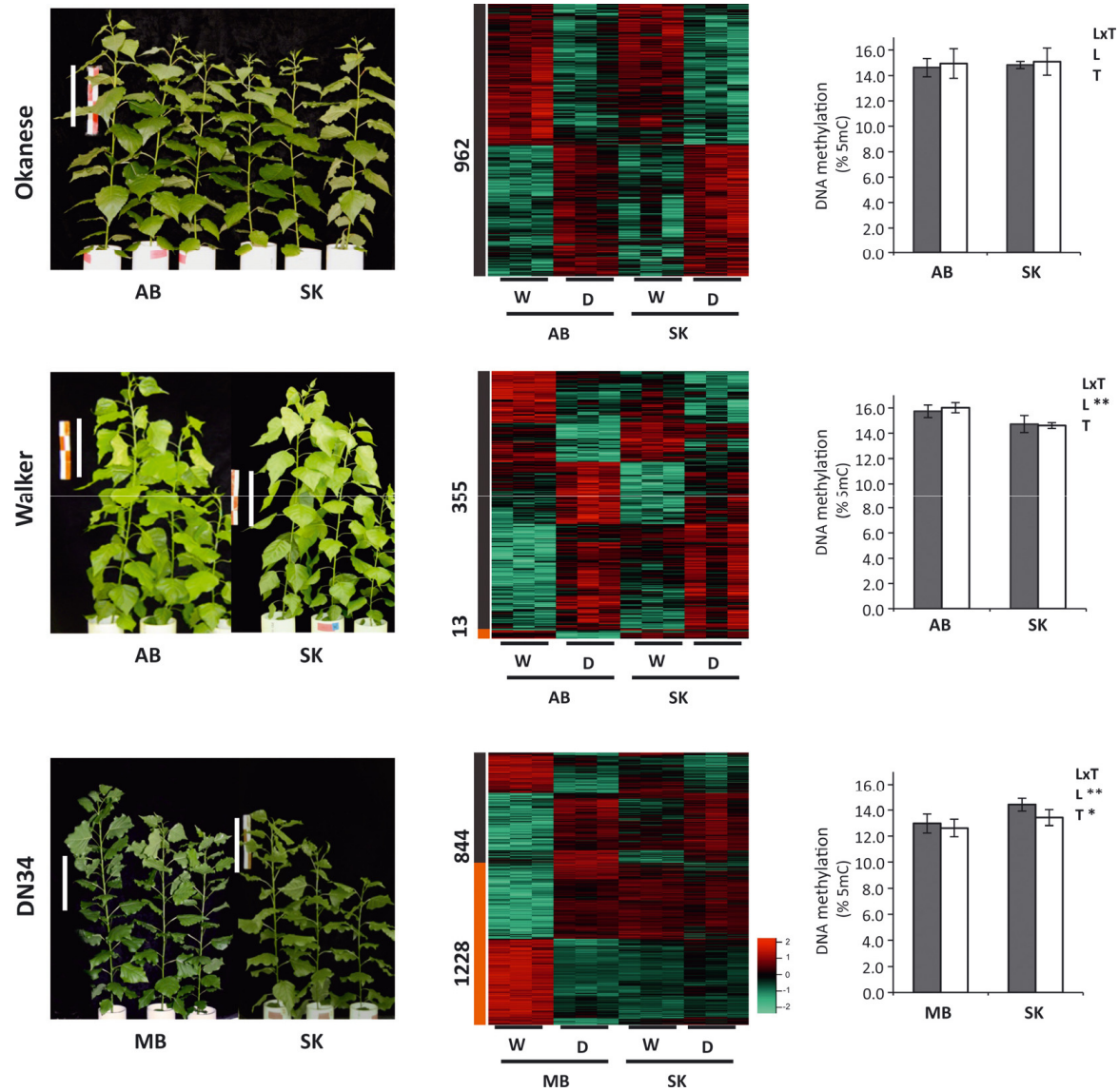


Large chromatin remodelling events during mid-late embryogenesis



Small RNA pathways across all stages of embryo development

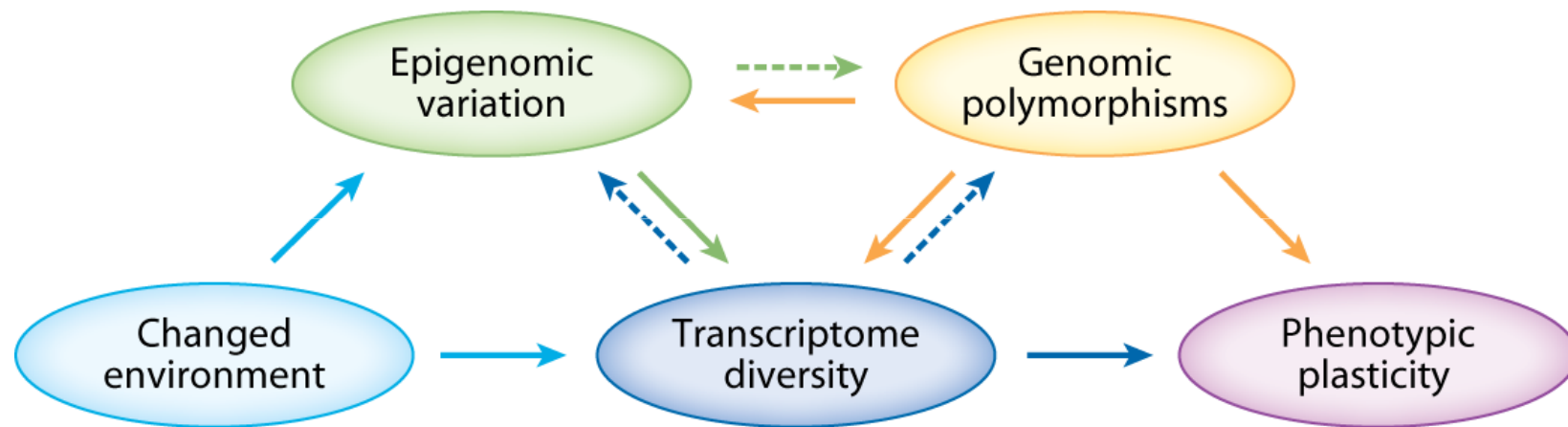
Clone history shapes drought responses in poplar hybrids



K. Brautigam et al. (2012) *Ecol Evol* 399–415

Adapted from Raj et al. (2011) *PNAS* doi: 10.1073/pnas.1103341108

Epigenomic and Genomic variation interactions



He et al (2011) Annu Rev Plant Biol 62: 411-35

whatever you see in one moment will change in the next...