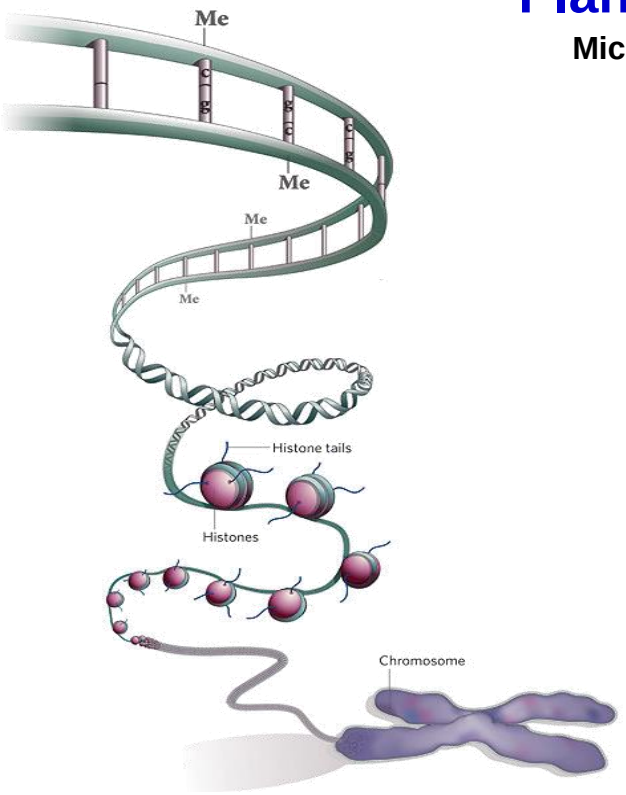
AIPlanta
Institute for Plant Research

Gesellschaft für Pflanzenbiotechnologie e.V.

Workshop on Molecular Breeding

Geisenheim University of Applied Sciences, 05.-06. Sept. 2019



Plant epigenetics and plant breeding

Michael Wassenegger

Breitenweg 71, 67435 Neustadt

RLP AgroScience GmbH

AIPlanta
Institute for Plant Research

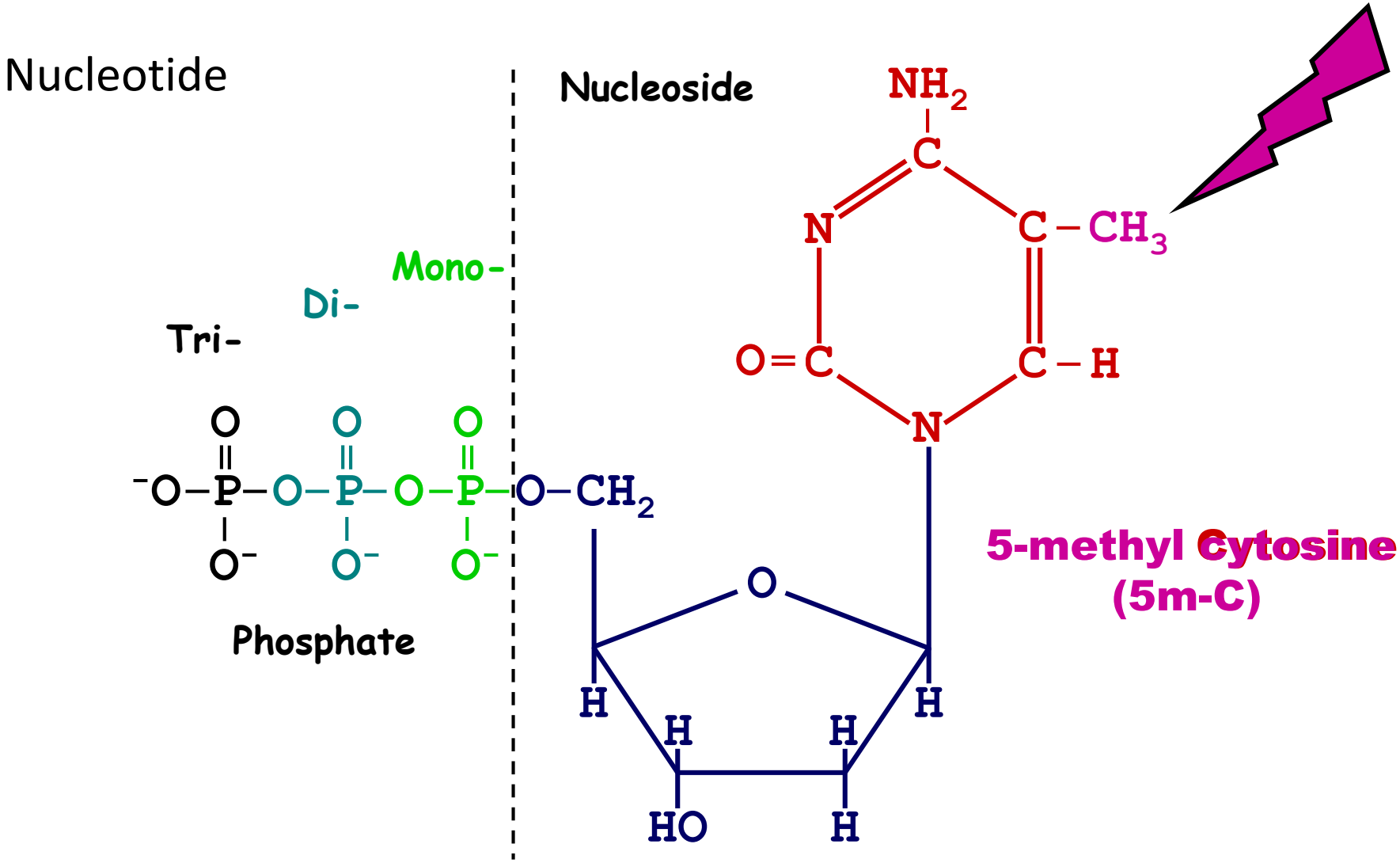
Epigenetics in plants:

- DNA methylation and chromatin modifications
- Function of epigenetic gene regulation
- Induction of epigenetic changes

Epigenetics as a tool for plant breeding:

- RNA-directed DNA methylation
- Genome editing tools for induction of epigenetic modifications
- Direct/indirect epigenetic gene regulations
- Mutagenesis by transposon activation
- A public view of the significance of epigenetics in plant breeding

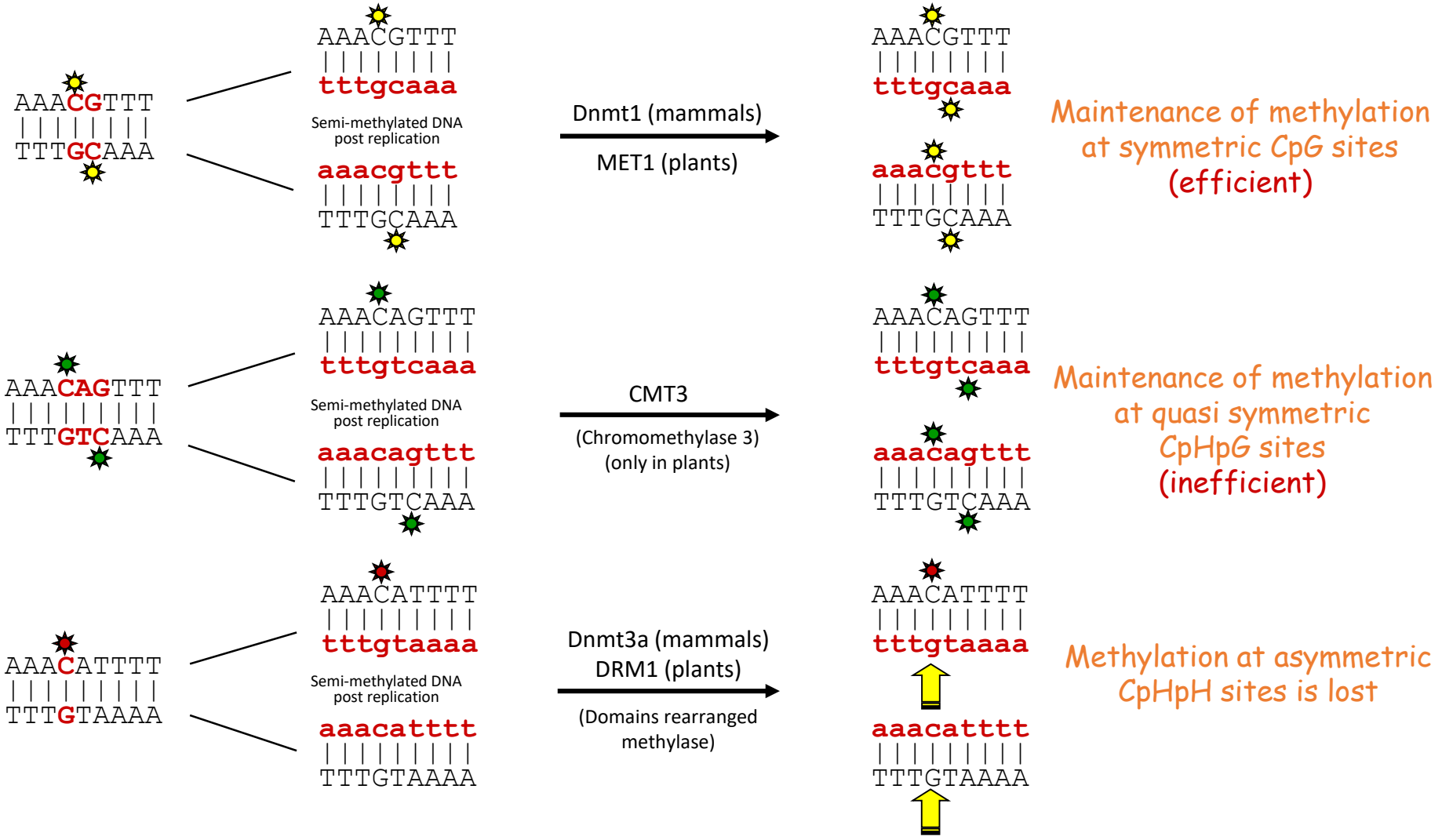
DNA methylation and chromatin modifications



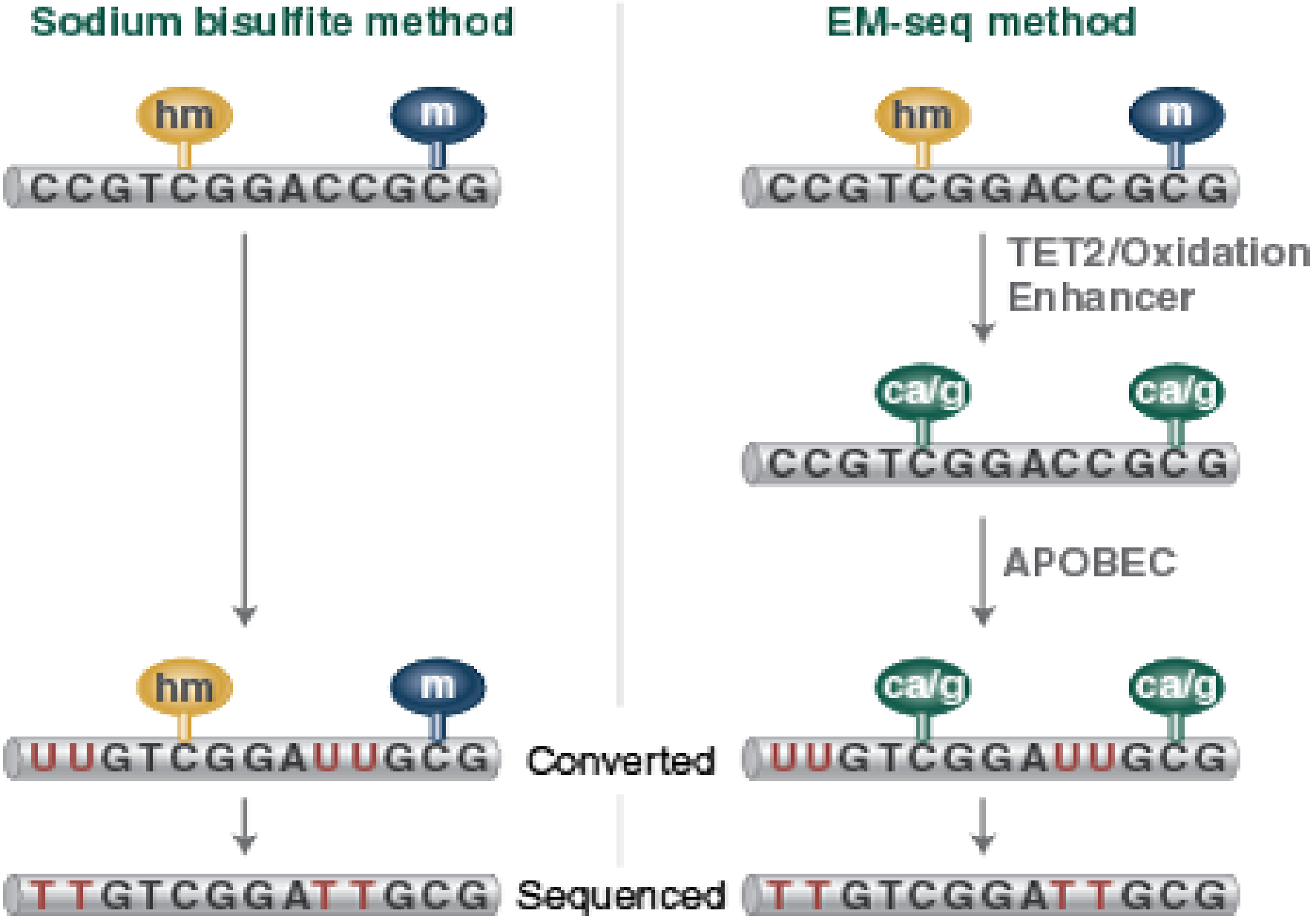
In addition to 5mC, N6-adenine (6mA) methylation has been detected. In Arabidopsis, 6mA is a DNA mark associated with actively expressed genes, suggesting that 6mA serves as a hitherto unknown epigenetic mark in land plants.
(Liang et al., Dev Cell, 2018)

AIPlanta
Institute for Plant Research

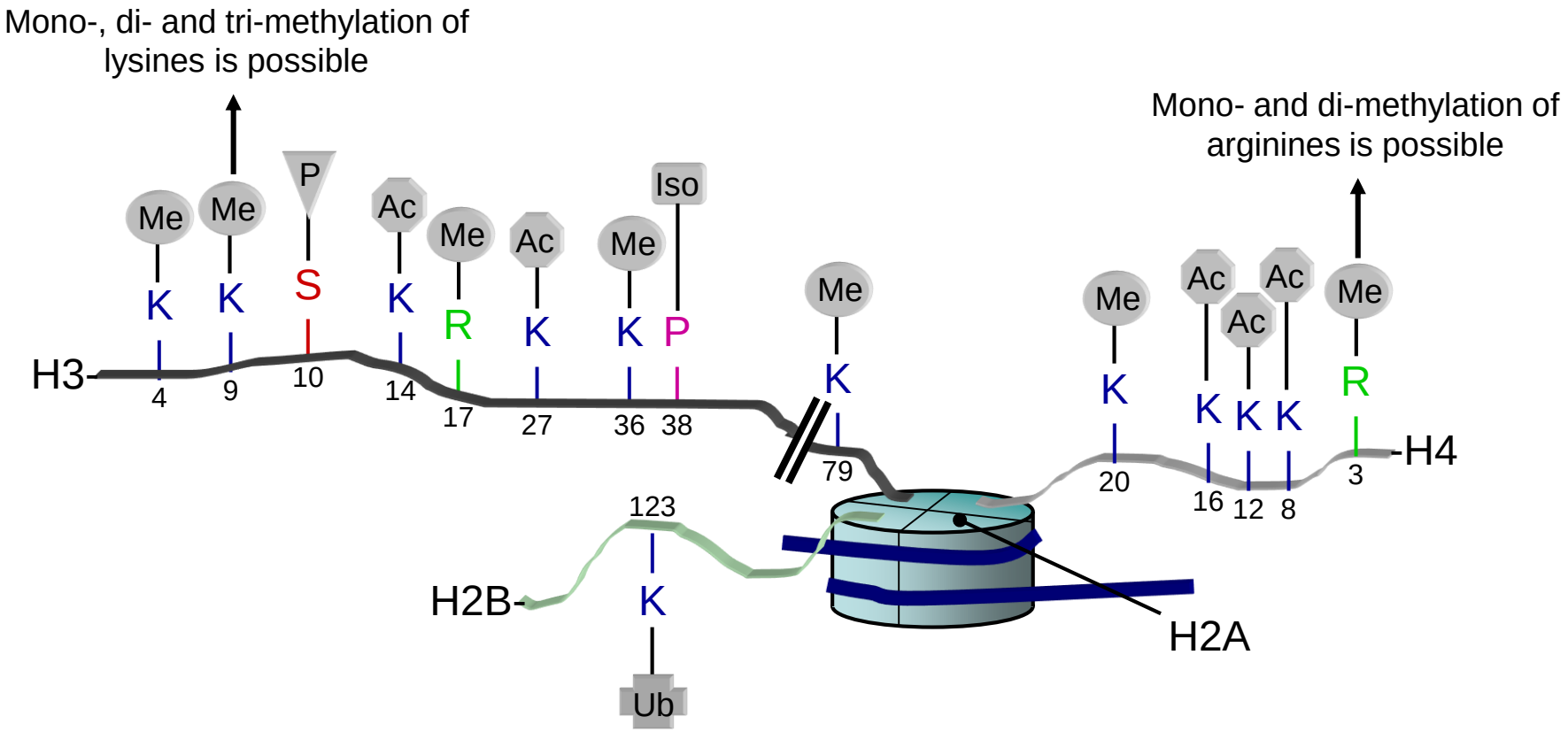
Significance of methylation patterns in view of inheritance



Only symmetric methylation is maintained!


Sodium bisulfite method
EM-seq method
hm
m
CCGTCGGACCGCG
TET2/Oxidation
Enhancer
ca/g
APOBEC
UUGTCGGAUUGCG
Converted
TTGTCGGATTGCG
Sequenced

The most prominent positions of the 60 known residues on histones that are modified



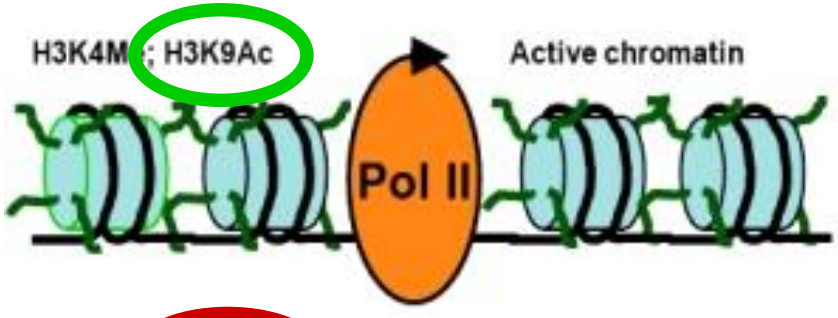
Histone modifications are highly dynamic. At the chromatin, acetylation, methylation, phosphorylation and deimination can appear and disappear within minutes!

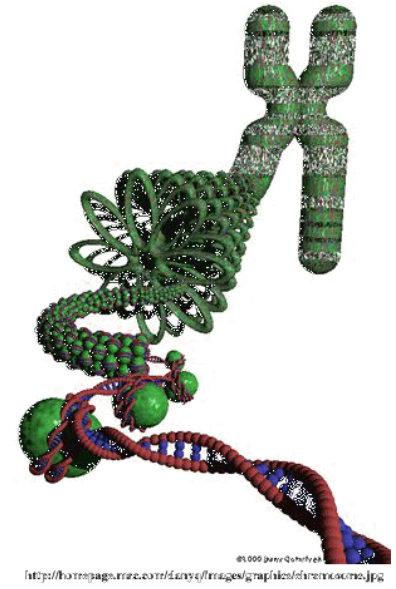
K = Lysine; S = Serine; R = Arginine; P = Proline

Me = methylation; P = phosphorylation; Ac = acetylation; Iso = isomerization

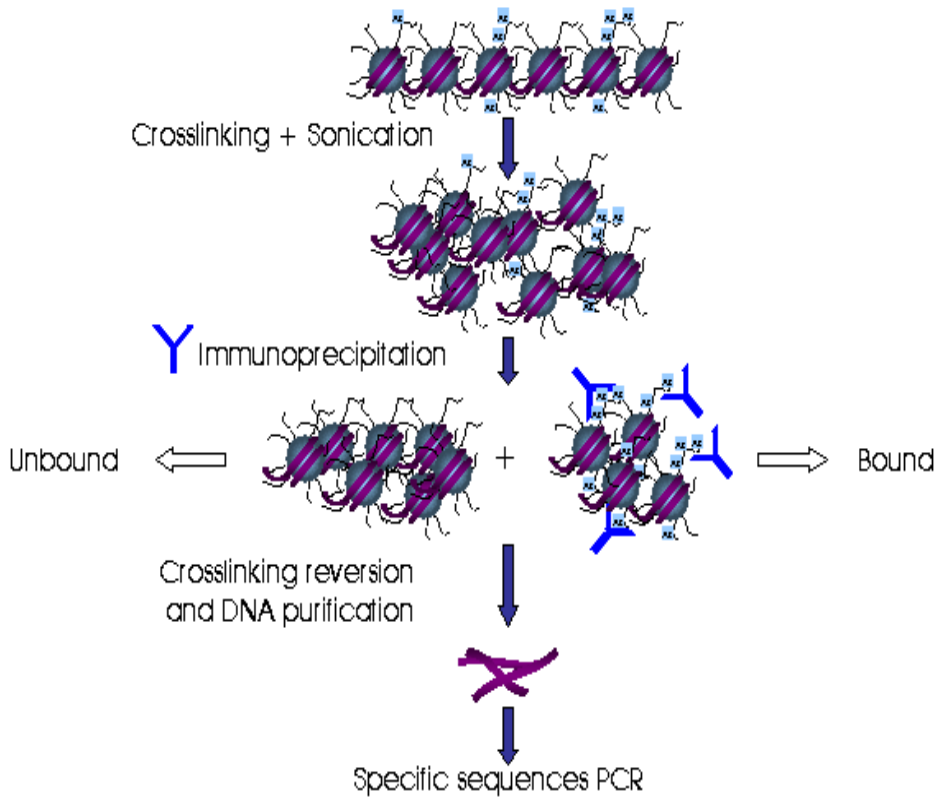
Ub = ubiquitination

Chromatin modifications and chromatin immunoprecipitation procedure



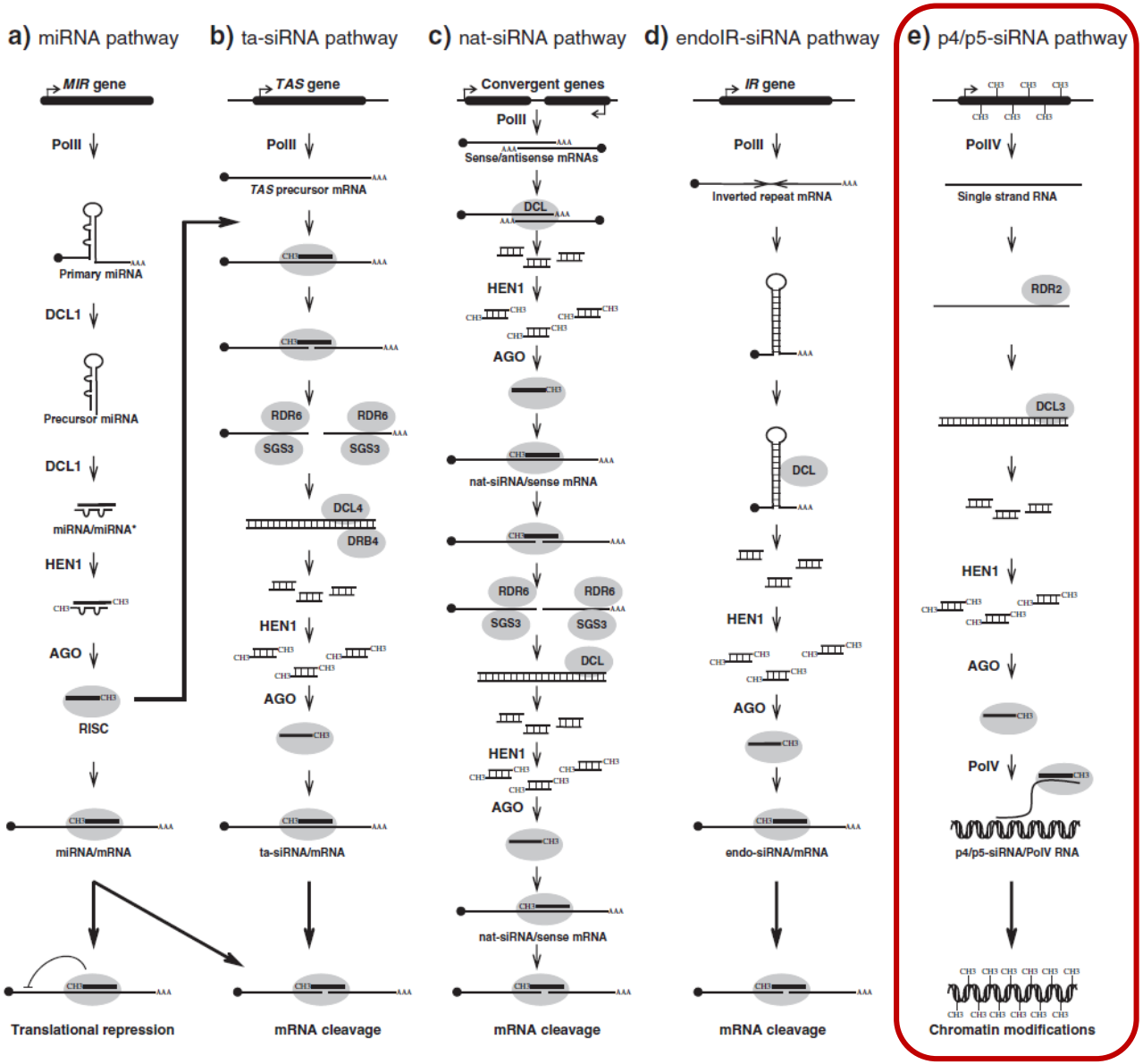


PRODUCT NAME	SPECIES
Ac-Histone H2B (Lys5/12/15/20)	m, r, h, e, c, b, p, a
Ac-Histone H3 (Lys 24)	broad
Ac-Histone H3 (Lys9/14)	m, r, h, C. elegans, Xenopus, Drosphila, e, c, b, p, a
Ac-Histone H3 (AH3-120)	m, r, h, Drosophila, Xenopus, C. elegans
Ac-Histone H4 (E-5)	broad species
Ac-Histone H4 (F-3)	broad species
Ac-Histone H4 (Lys 12)	broad
Ac-Histone H4 (Lys 5)	m, r, h
Ac-Histone H4 (Lys 8)-R	m, r, h, c, e, b
Ac-Histone H4 (Lys16)	m, r, h, b, c
Ac-Histone H4 (Ser 1/Lys 5/Lys 8/Lys 12)	broad, e, c, b, p, a
Histone cluster 1 H1A (N-16)	human
Histone cluster 1 H1B (N-15)	m, r, h
Histone cluster 1 H1C (T-18)	human
Histone cluster 1 H1D (A-20)	human
Histone cluster 1 H1T (C-14)	human
Histone cluster 1 H1T (P-17)	human
Histone cluster 1 H3D (6H8)	m, h
Histone cluster 1 H4G (N-16)	human
Histone cluster 2 H2AA3 (W-26)	m, r, h
Histone cluster 2 H2AC (X-21)	m, r, h
Histone H1 (AE-4)	broad
Histone H10 (F-10)	m, r, h
Histone H1 (C-17)	m, r, h
Histone H1 (FL-219)	broad mammalian, Xenopus, e, b
Histone H1 (N-16)	m, r, h
Histone H1 (N-19)	m, r, h, c, b
Histone H1° (27)	m, r, h, b, Xenopus
Histone H1° (34)	m, r, h, b, Xenopus
Histone H1° (G-12)	m, r, h
Histone H1° (H-85)	m, r, h, e, c, b, p
Histone H1X (G-19)	human
Histone H1° (Y-16)	m, r, h, e, c, b, p, a
Histone H1 (SPM256)	human
Histone H2A (C-19)	m, r, h, e, c, b, p, a, Drosophila, Xenopus, C. elegans
Histone H2A (H-124)	broad mammalian, z, Drosophila, Xenopus and C. elegans, e, c, b, p, a
Histone H2A (N-15)	m, r, h, Xenopus
Histone H2A.Bbd (A-16)	m, r
Histone H2A.Bbd (C-17)	human
Histone H2A.Bbd (E-14)	human
Histone H2A.X (C-20)	h, > m, c, b, p
Histone H2A.Z/H2A.F/Z (FL-128)	m, r, h, c, b, p, a, e
Histone H2A.Z/H2A.F/Z (G-13)	m, r, h, e, c, b, p, a
Histone H2A.Z/H2A.F/Z (N-15)	m, r, h
Histone H2B (N-20)	m, r, h, e, c, b, p, a
Histone H2B (C-19)	m, r, h, e, c, b, p, a
Histone H3 (C-16)	m, r, h, e, c, b, p, a
Histone H3 (FL-136)	broad mammalian, Drosophila, Xenopus and C. elegans, e, c, b, p, a
Dimethyl Histone H3 (Lys 80)	mammalian and yeast
Trimethyl Histone H3 (6F12-H4)	human
Histone H4 (C-20)-R	m, r, h, e, c, b, p, a
Histone H4 (F-9)	m, r, h, e, c, b, p, a
Histone H4 (H-97)	broad mammalian species, Drosophila, Xenopus and C. elegans, e, c, b, p, a
Histone H4 (N-16)	m, r, h, e, c, b, p, a
Monomethyl Histone H4 (5E10-D8)	m, r, h
Trimethyl Histone H4 (6F8-D9)	m, r, h



AIPlanta
Institute for Plant Research

Is double stranded RNA a 3rd layer of epigenetic modification?



miRNA = micro RNA

siRNA = small interfering RNA

ta-siRNA = trans-actin siRNA

nat-siRNA = natural antisense transcript-derived siRNAs

p4/p5-siRNA = DNA-Dependent RNA Polymerase IV (PolIIV)/PolV siRNAs

DCL = Dicer-like

HEN1 = Hua Enhancer 1

AGO = Argonaute

RISC = RNA-induced silencing complex

RDR = RNA-directed RNA polymerase

SGS = suppressor of gene silencing

DRB = dsRNA-binding

Martínez de Alba et al., Biochim Biophys Acta 1829, 2013

Function of epigenetic gene regulation

RNA can initiate DNA methylation

↓

DNA methylation can initiate histone modifications and *vice versa*

↓

DNA methylation and histone modifications are epigenetic marks and result in chromatin remodeling

↓

Chromatin remodeling is associated with heritable processes, including:

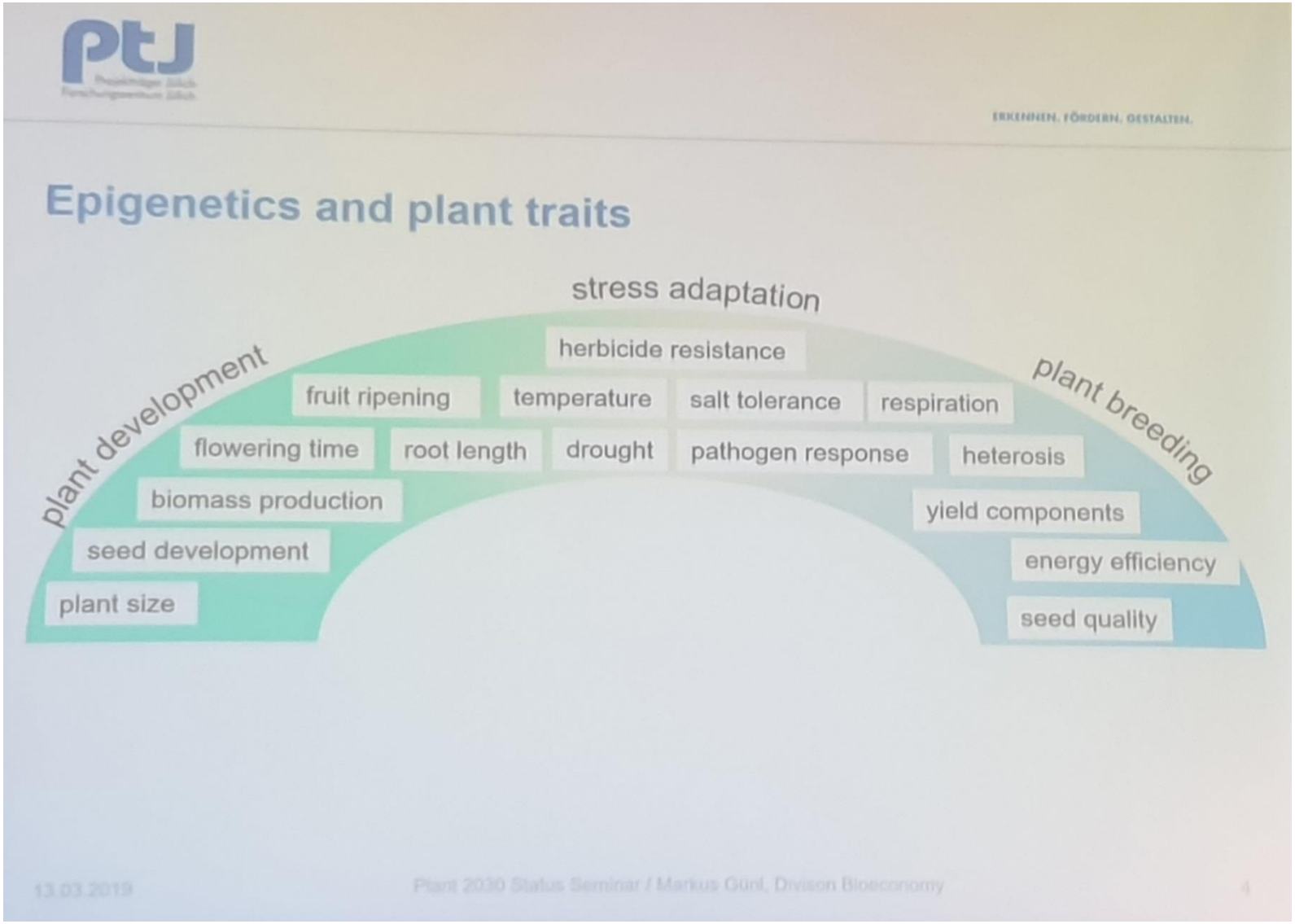
- **Imprinting**
- **Parental chromosome inactivation**
- **Heterochromatin formation**
- **Gene silencing** (transposable elements)
- **Environmentally induced gene regulation**

↓

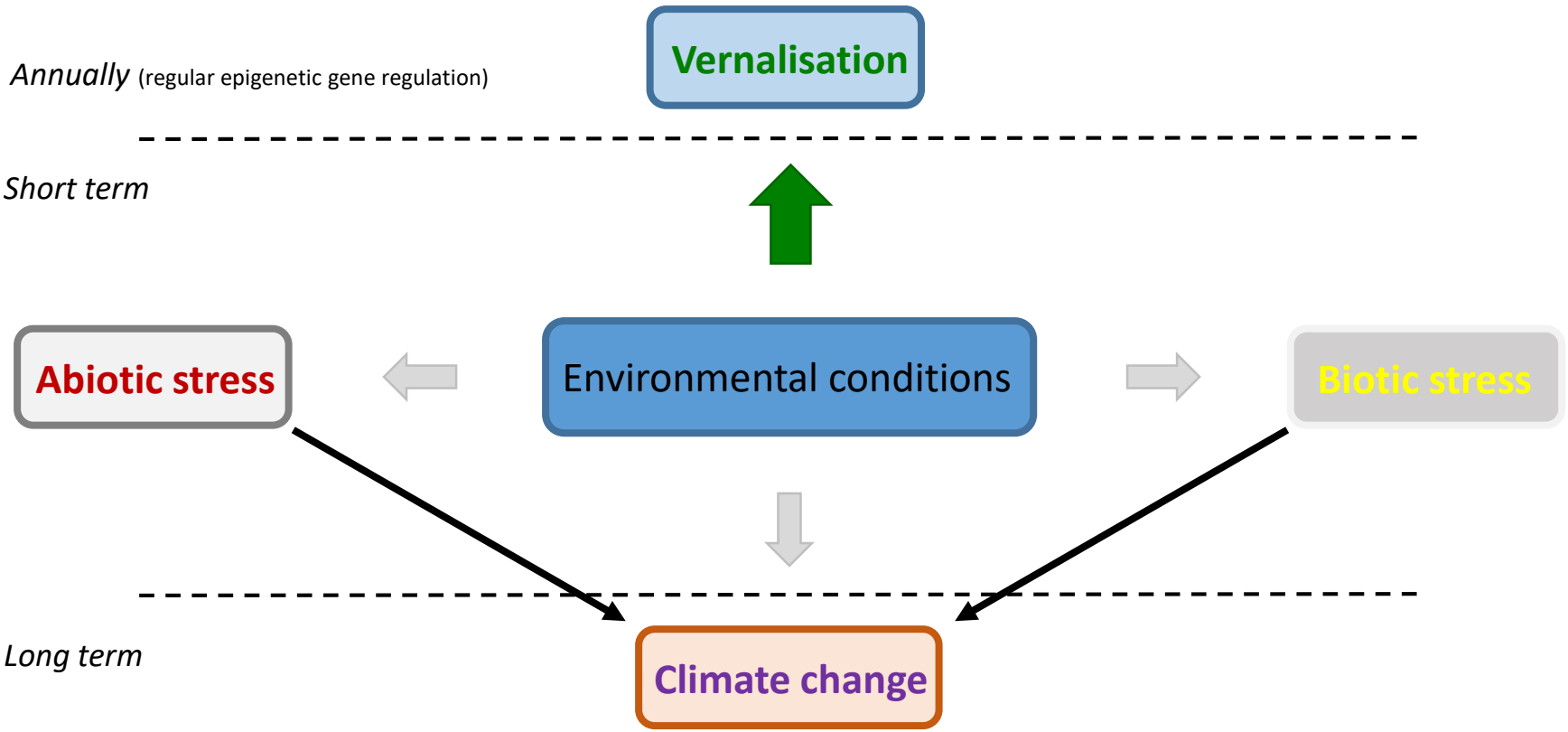
Maintenance of genome integrity

DNA methylation is a key process to maintain the epigenetic architecture of eukaryotic genomes

An expert group elaborated the following table of major epigenetic effects in plants
(Fachgespräch „Epigenetik in den Pflanzenwissenschaften“, BMBF, Sept. 2018)



Induction of epigenetic changes



Epigenetic modifications are reversible (transient) and most of them are not inherited

Examples of natural induction of epigenetic modifications that are well known

"Harden off" plants by a mild exposure to low temperature so that they are then protected against later freezing temperatures.

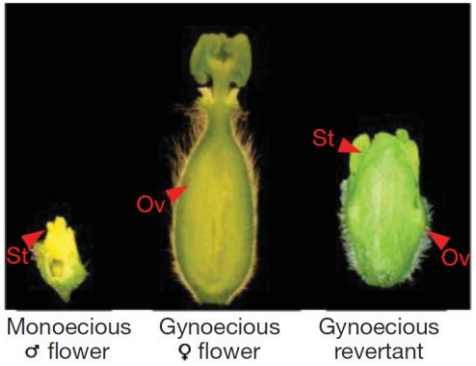
Deficit irrigation, in which long-term drought tolerance can be induced by transient or partial drying.

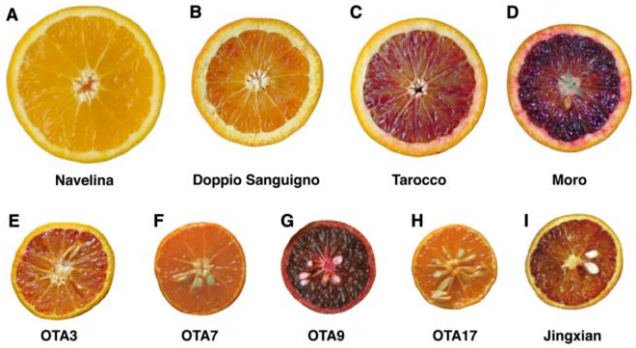
Less well known examples

In Melon, transition from male to female flowers results from epigenetic changes in the promoter of a transcription factor.
(Martin et al., Nature 461, 2009)

A naturally occurring epigenetic mutation in an SBP-box transcription factor gene inhibits tomato fruit ripening.
(Manning et al., Nat Genet 38, 2006)

Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges.
(Butelli et al., Plant Cell 24, 2012)





These processes need to be repeated in each generation (reversibility of epigenetics).
However, could be an useful procedure for vegetatively propagated plants.

AIPlanta
Institute for Plant Research

RNA-directed DNA methylation

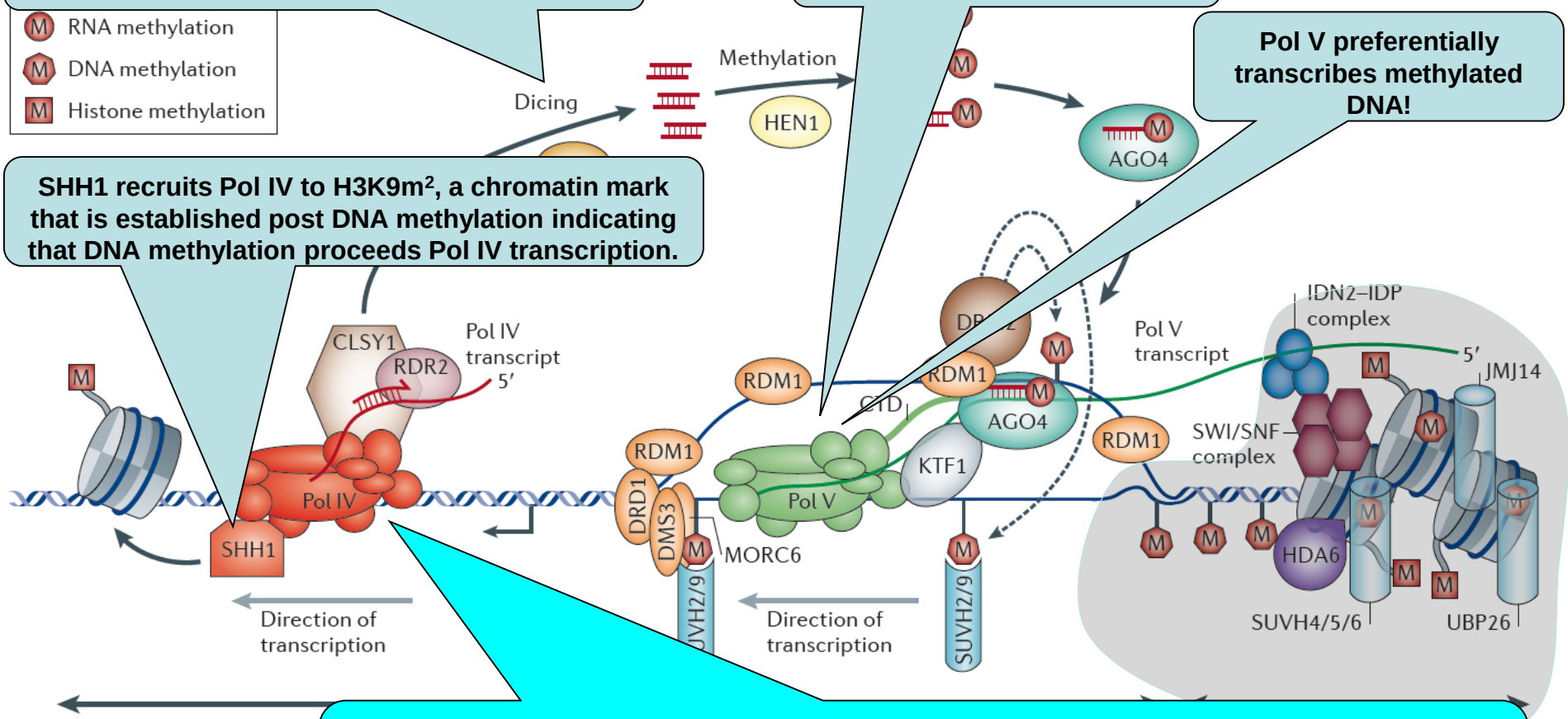
Critical view on the current RdDM models

***De novo* methylation is only WEAKLY reduced in *dcl2/dcl3/dcl4* mutants!**

What signals Pol V to start and stop transcription?

Pol V preferentially transcribes methylated DNA!

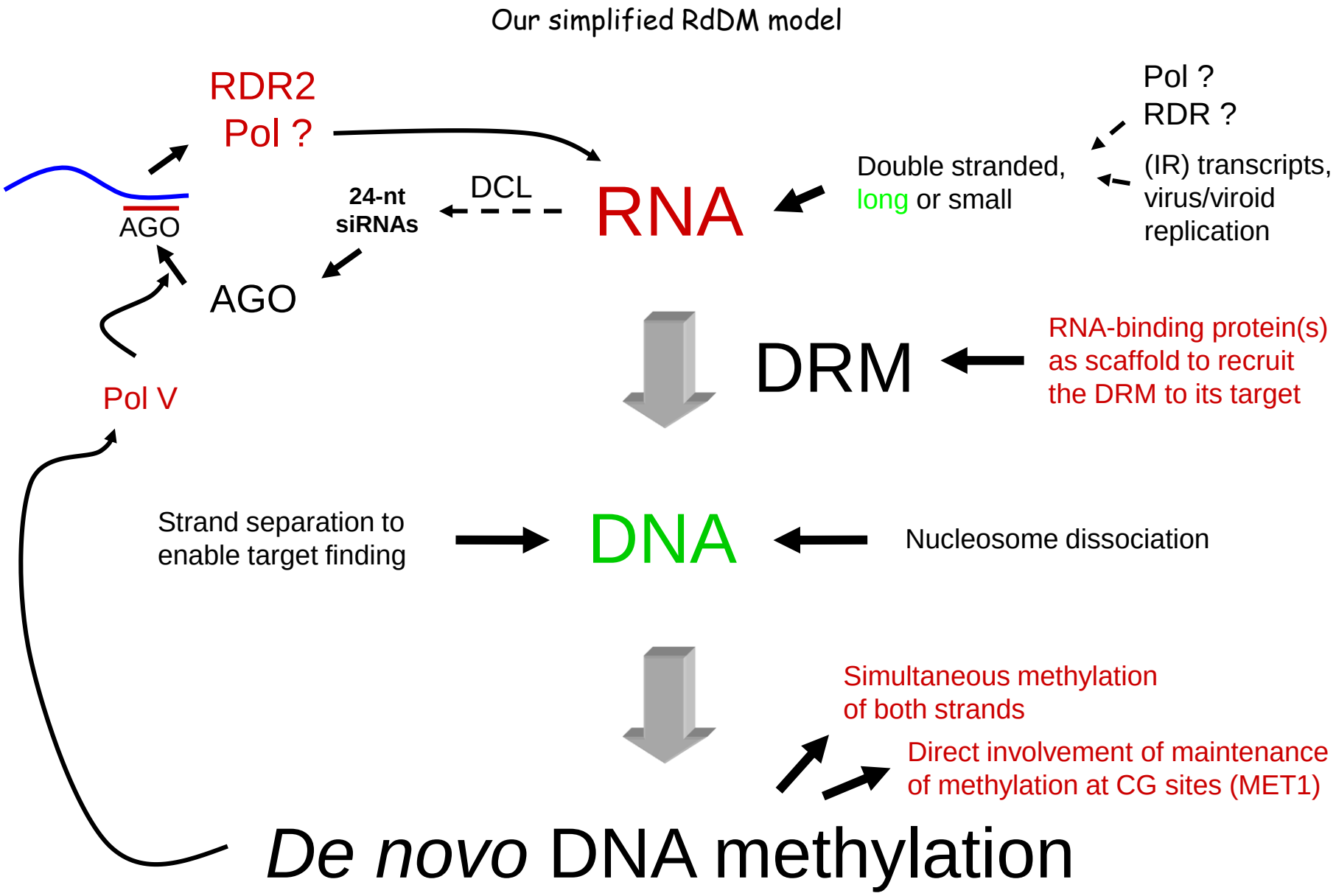
SHH1 recruits Pol IV to H3K9m², a chromatin mark that is established post DNA methylation indicating that DNA methylation proceeds Pol IV transcription.



RdDM and PTGS are both triggered by dsRNA. However, PTGS is not dependent on Pol IV and RDR2 (transient transgene expression and virus/viroid infections). Thus, primary dsRNA production does not necessarily involve Pol IV/RDR2. It is likely that PTGS-derived components (siRNAs) activate Pol IV/RDR2-mediated dsRNA production.

Pols = RNA polymerases; SHH1 = SAWADEE HOMEO… …NG
TRANSCRIPTION FACTOR 1; RDM1 = RNA-DIRECTED
MORC6 = MICRORCHIDIA 6; IDN2 = INVOLVED IN D

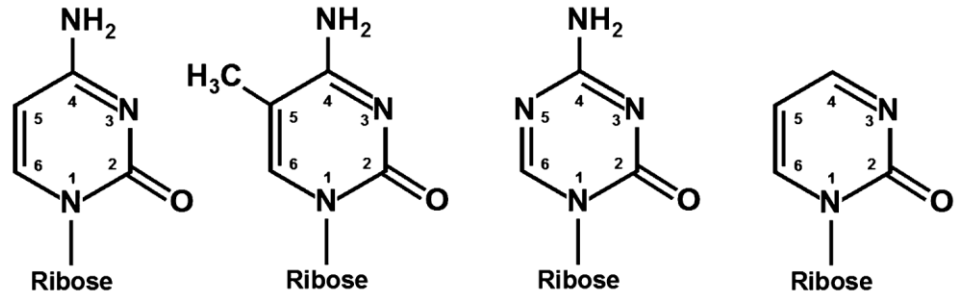
Matzke and Mosher, Nat Rev Genet 15, 2014.


Our simplified RdDM model
RDR2
Pol ?
Pol ?
RDR ?
AGO
24-nt
siRNAs
DCL
RNA
Double stranded,
long or small
(IR) transcripts,
virus/viroid
replication
AGO
DRM
RNA-binding protein(s)
as scaffold to recruit
the DRM to its target
Pol V
Strand separation to
enable target finding
DNA
Nucleosome dissociation
Simultaneous methylation
of both strands
Direct involvement of maintenance
of methylation at CG sites (MET1)
De novo DNA methylation

AIPlanta Institute for Plant Research

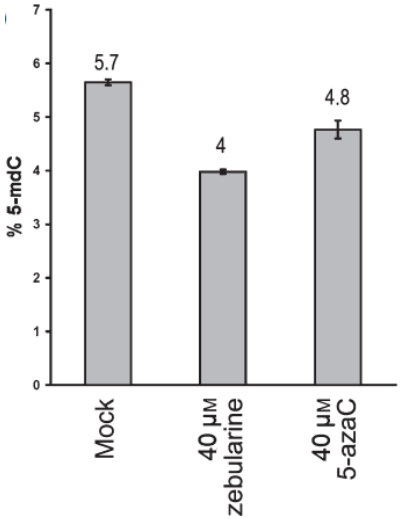
Tools for induction of epigenetic modifications

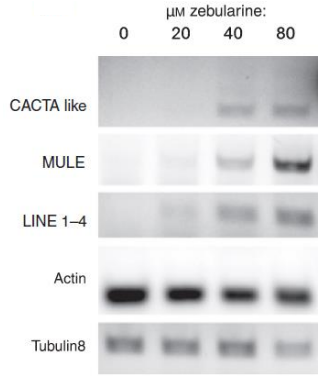
1) Chemical compounds



Cytidine | 5-Methylcytidine | 5-Azacytidine **(AZA)** | Zebularine **(ZEB)**

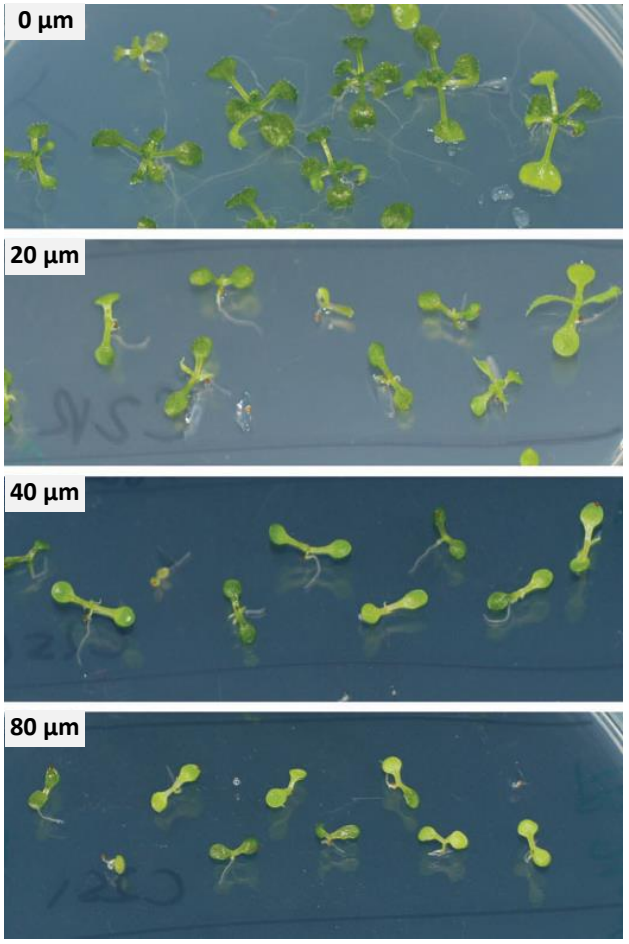
Nonmethylable cytosine analogs





Activation of gene transcription upon ZEB treatment

Zebularine treatment affects plant growth and development.



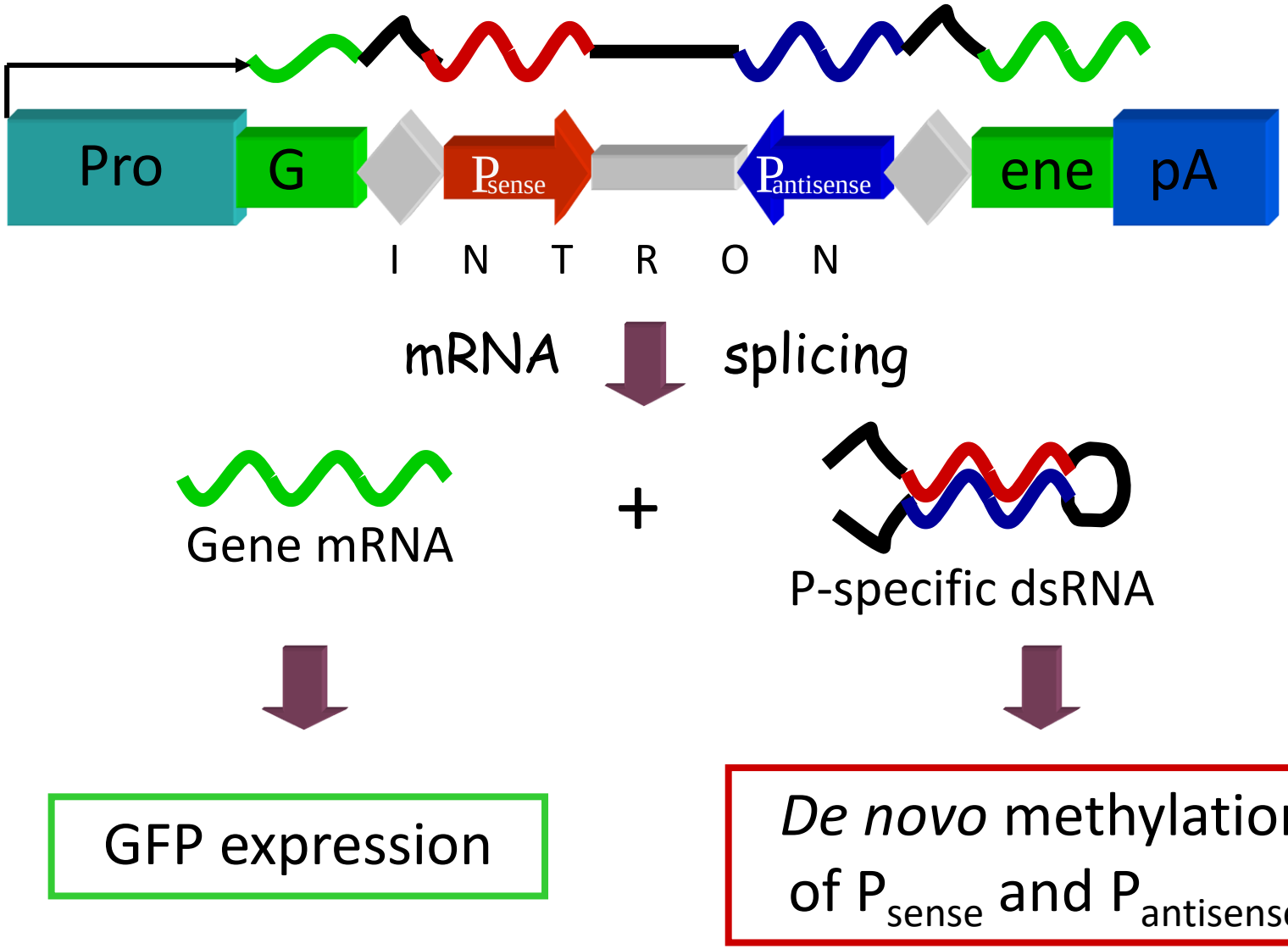
14 days after sowing

AZA/ZEB treatments induce unspecific DNA demethylation!

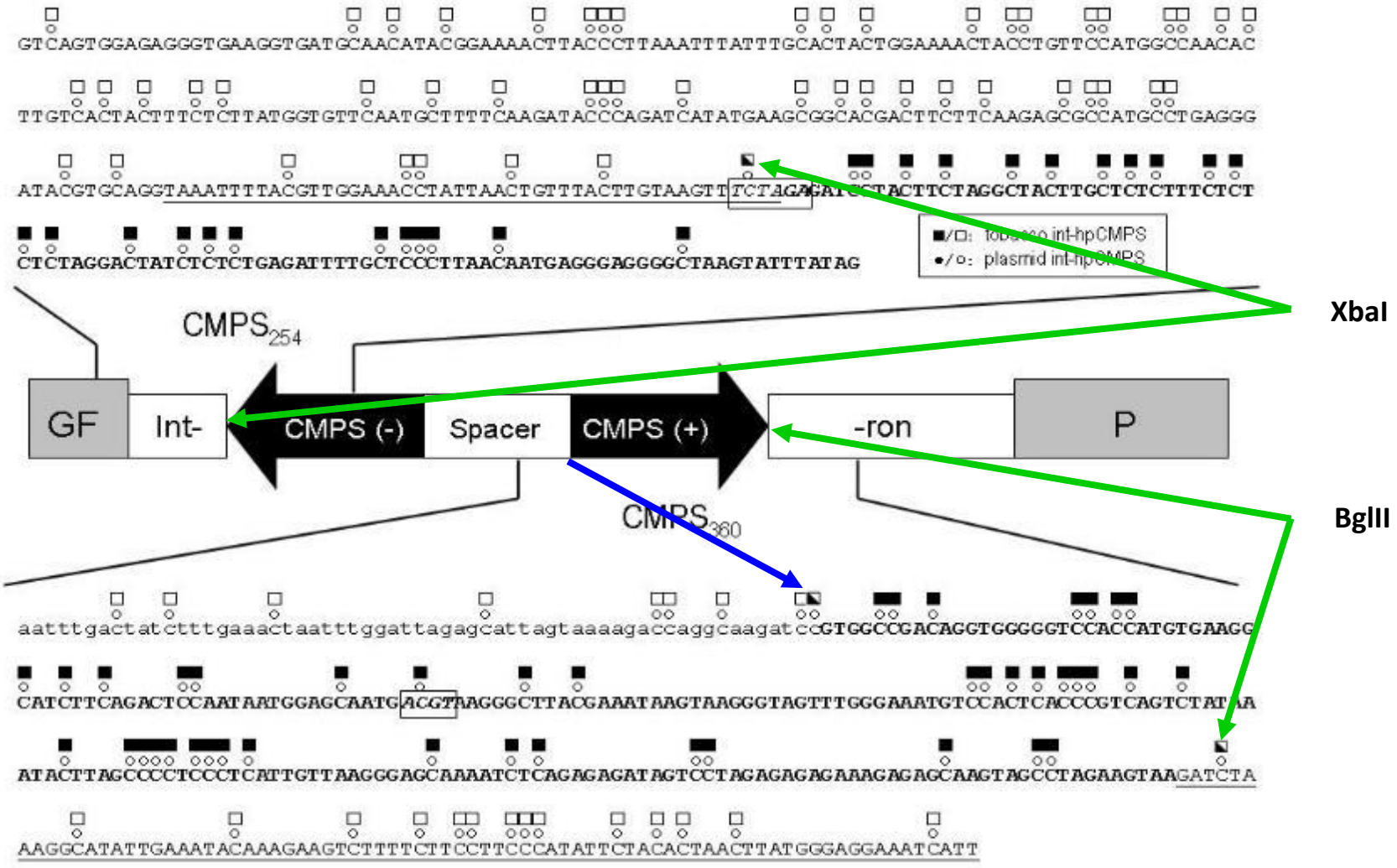
Baubec et al., Plant J 57, 2009

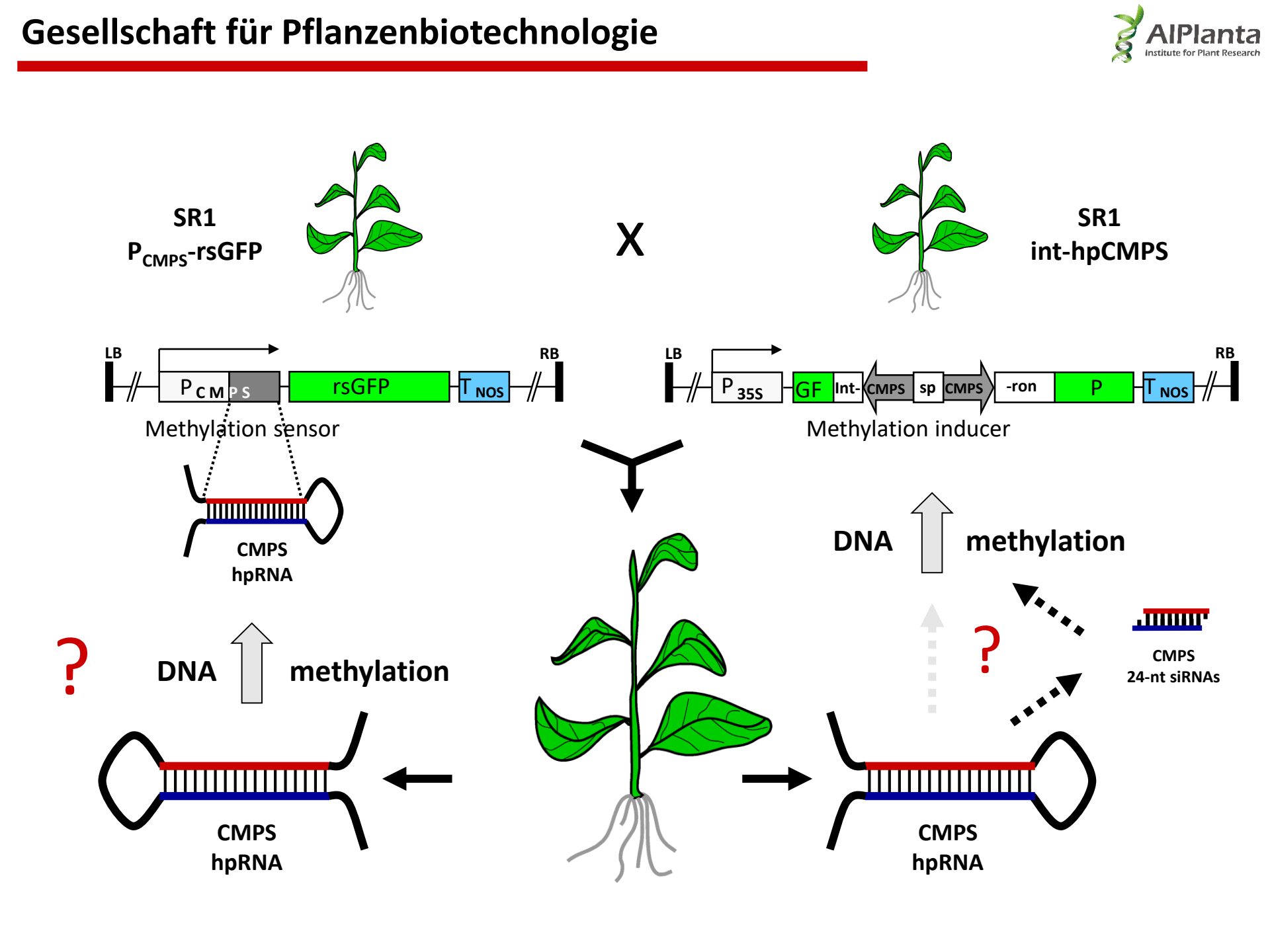
2) RdDM

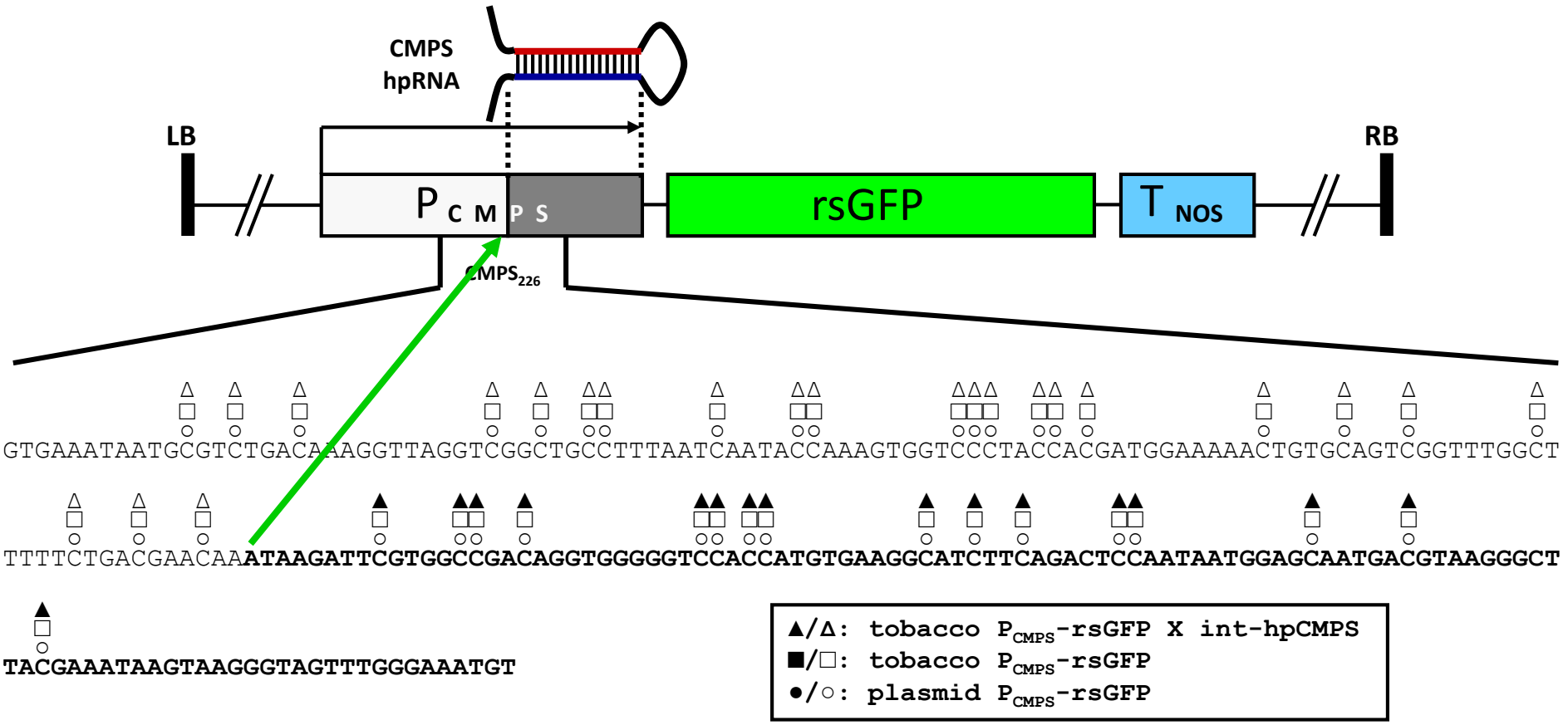
I Transgene-induced RdDM



Dalakouras et al., Plant J 60, 2009.

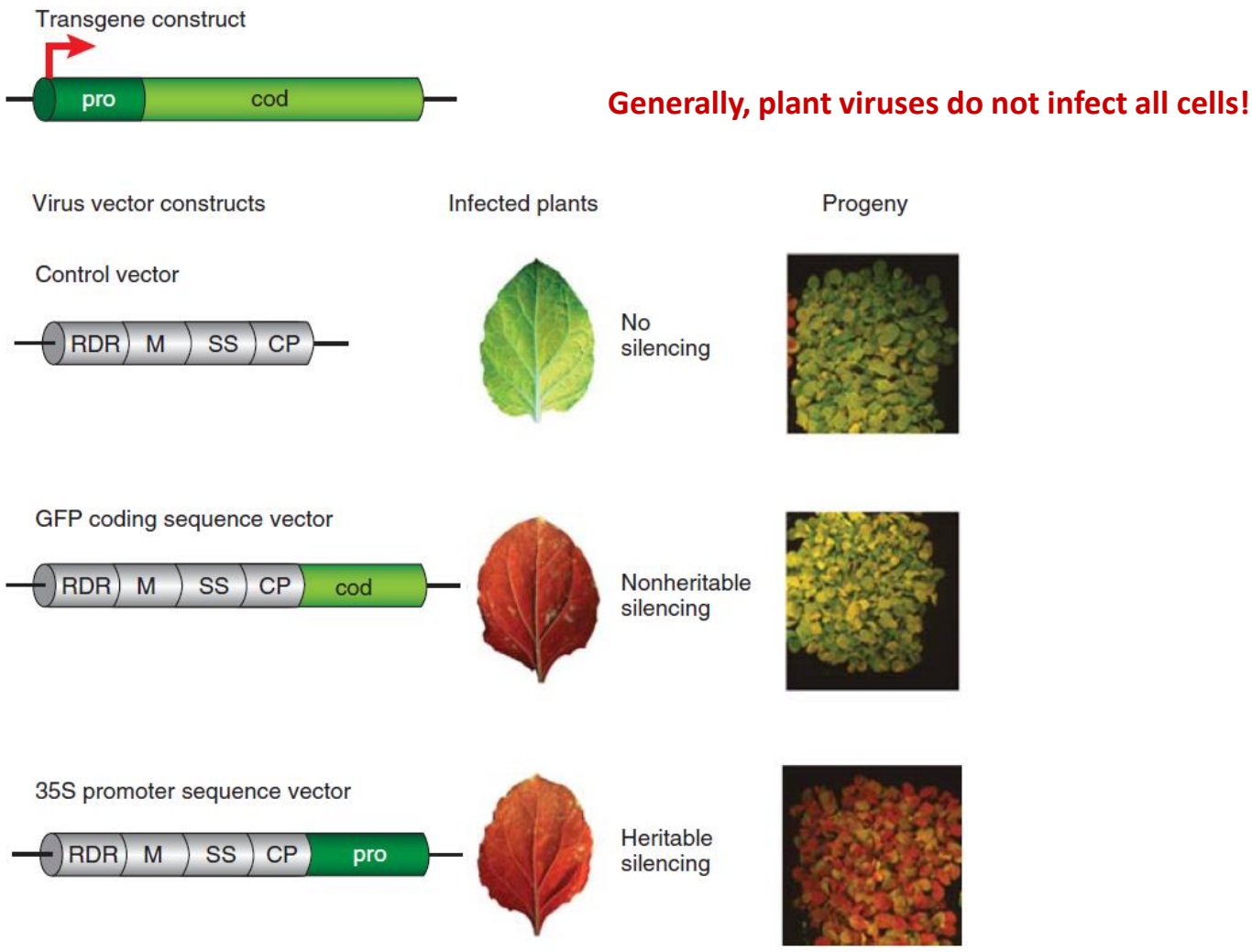

GTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACAC
TTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAGCGGCACGACTTCTTCAAGAGCGCCATGCCTGAGGG
ATACGTGCAGGTAAATTTTACGTTGGAAACCTATTAACTGTTTACTTGTAAGTTTCTAGAGATCCTACTTCTAGGCTACTTGCTCTCTTTCTCT
CTCTAGGACTATCTCTCTGAGATTTTGCTCCCTTAACAATGAGGGAGGGGCTAAGTATTTATAG
■/□: tobacco int-hpCMPS
●/○: plasmid int-hpCMPS
CMPS254
GF
Int-
CMPS (-)
Spacer
CMPS (+)
-ron
P
XbaI
BglII
CMPS360
aatttgactatctttgaaactaatttggattagagcattagtaaaagaccaggcaagatccGTGGCCGACAGGTGGGGGTCCACCATGTGAAGG
CATCTTCAGACTCCAATAATGGAGCAATGACGTAAGGGCTTACGAAATAAGTAAGGGTAGTTTGGGAAATGTCCACTCACCCGTCAGTCTATAA
ATACTTAGCCCCTCCCTCATTGTTAAGGGAGCAAAATCTCAGAGAGATAGTCCTAGAGAGAGAAAGAGAGCAAGTAGCCTAGAAGTAAGATCTA
AAGGCATATTGAAATACAAAGAAGTCTTTTCTTCCTTCCCATATTCTACACTAACTTATGGGAGGAAATCATT


SR1
P CMPS -rsGFP
X
SR1
int-hpCMPS
LB
P CMPS
rsGFP
T NOS
RB
Methylation sensor
LB
P 35S
GF
Int-
CMPS
sp
CMPS
-ron
P
T NOS
RB
Methylation inducer
CMPS
hpRNA
DNA
methylation
?
DNA
methylation
?
CMPS
24-nt siRNAs
CMPS
hpRNA
CMPS
hpRNA


CMPS
hpRNA
LB
RB
P C M P S
rsGFP
T NOS
CMPS226
GTGAAATAATGCGTCTGACAAAGGTTAGGTCGGCTGCCTTTAATCAATACCAAAGTGGTCCCTACCACGATGGAAAAACTGTGCAGTCGGTTTGGCT
TTTTCTGACGAACAAATAAGATTCGTGGCCGACAGGTGGGGGTCCACCATGTGAAGGCATCTTCAGACTCCAATAATGGAGCAATGACGTAAGGGCT
TACGAAATAAGTAAGGGTAGTTTGGGAAATGT
▲/Δ: tobacco P CMPS -rsGFP X int-hpCMPS
■/□: tobacco P CMPS -rsGFP
●/○: plasmid P CMPS -rsGFP

AIPlanta
Institute for Plant Research

II Virus-induced RdDM



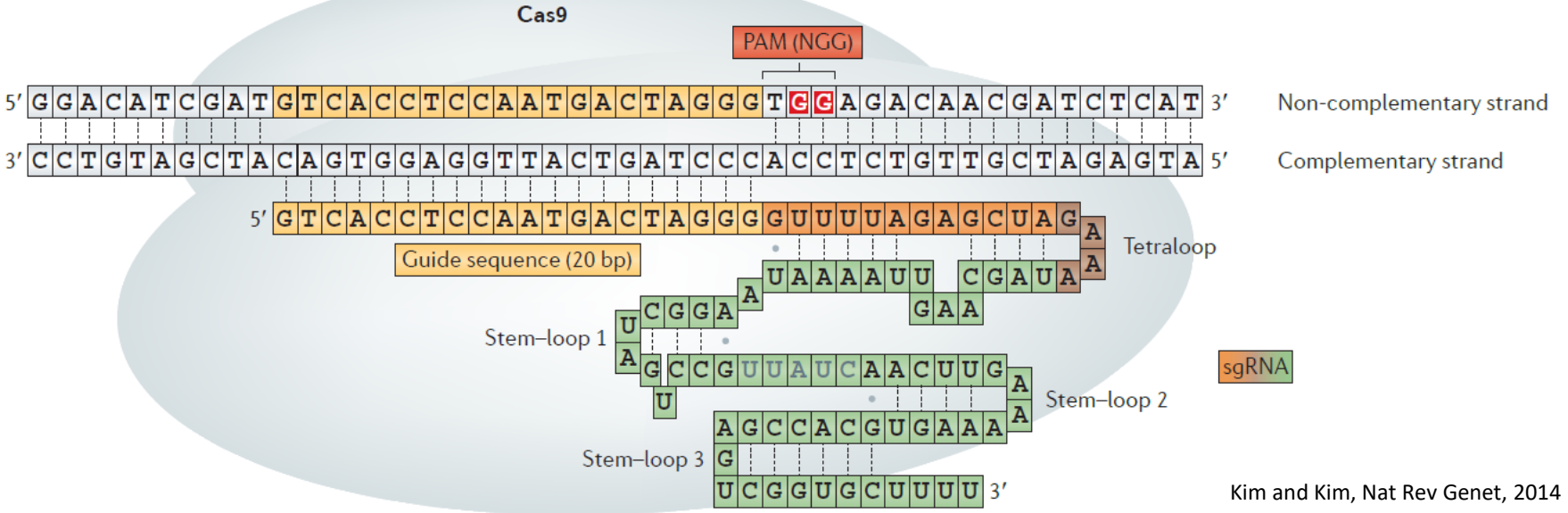
RdDM tools enable specific DNA methylation!

Baulcombe and Dean, Cold Spring Harb Perspect Biol 6, 2014.

3) Genome editing and transgenic tools

I. Knock- **athways**

CRISPR/Cas9 may be replaced by alternative genome editing systems, e.g. Zinc finger nucleases (ZFN) or Transcription activator-like effector nucleases (TALENs)



Kim and Kim, Nat Rev Genet, 2014

Putative examples of knock-out/down applications and the functions of the targeted genes:

DRM genes ⇨ *De novo* DNA methylation

REPRESSOR OF SILENCING (ROS) genes ⇨ DNA demethylation

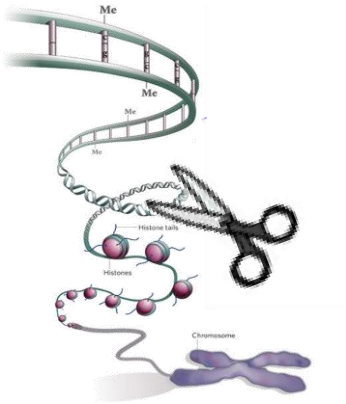
DECREASED DNA METHYLATION 1* (DDM1) genes ⇨ DNA methylation

METHYLTRANSFERASE 1* (MET1) genes ⇨ Maintenance of DNA methylation

SUVH4 genes ⇨ DNA methylation

Pol VI/V genes ⇨ *De novo* DNA methylation

***KO mutants of these genes are not viable!**



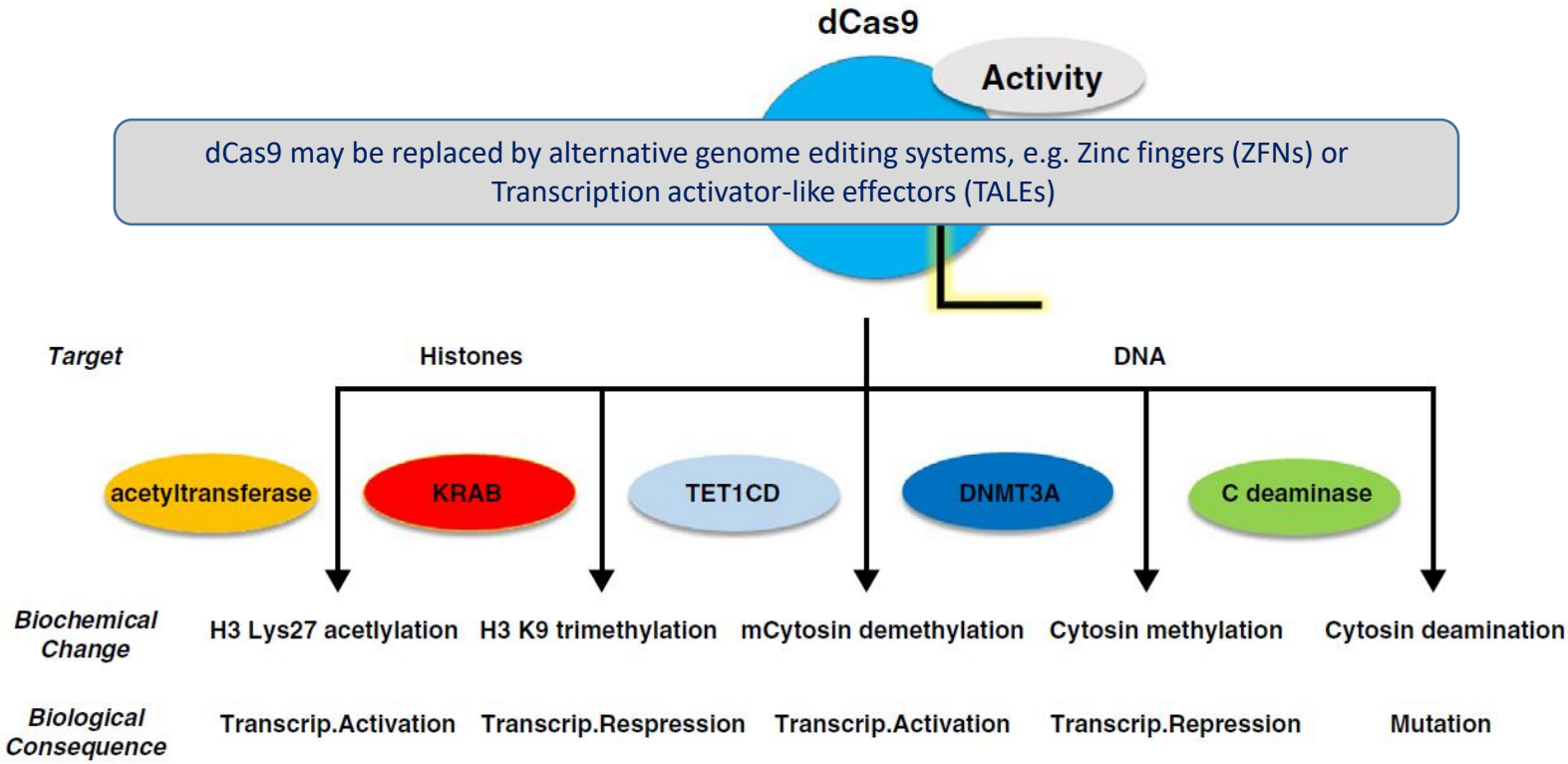
II. Overexpression of key enzymes of the DNA methylation and chromatin modification pathways

Putative examples of transgene-mediated overexpression and the functions of the corresponding genes:

- **DRM genes ⇨ *De novo* DNA methylation**
- **ROS genes ⇨ DNA demethylation**
- **MET1 genes ⇨ Maintenance of DNA methylation**

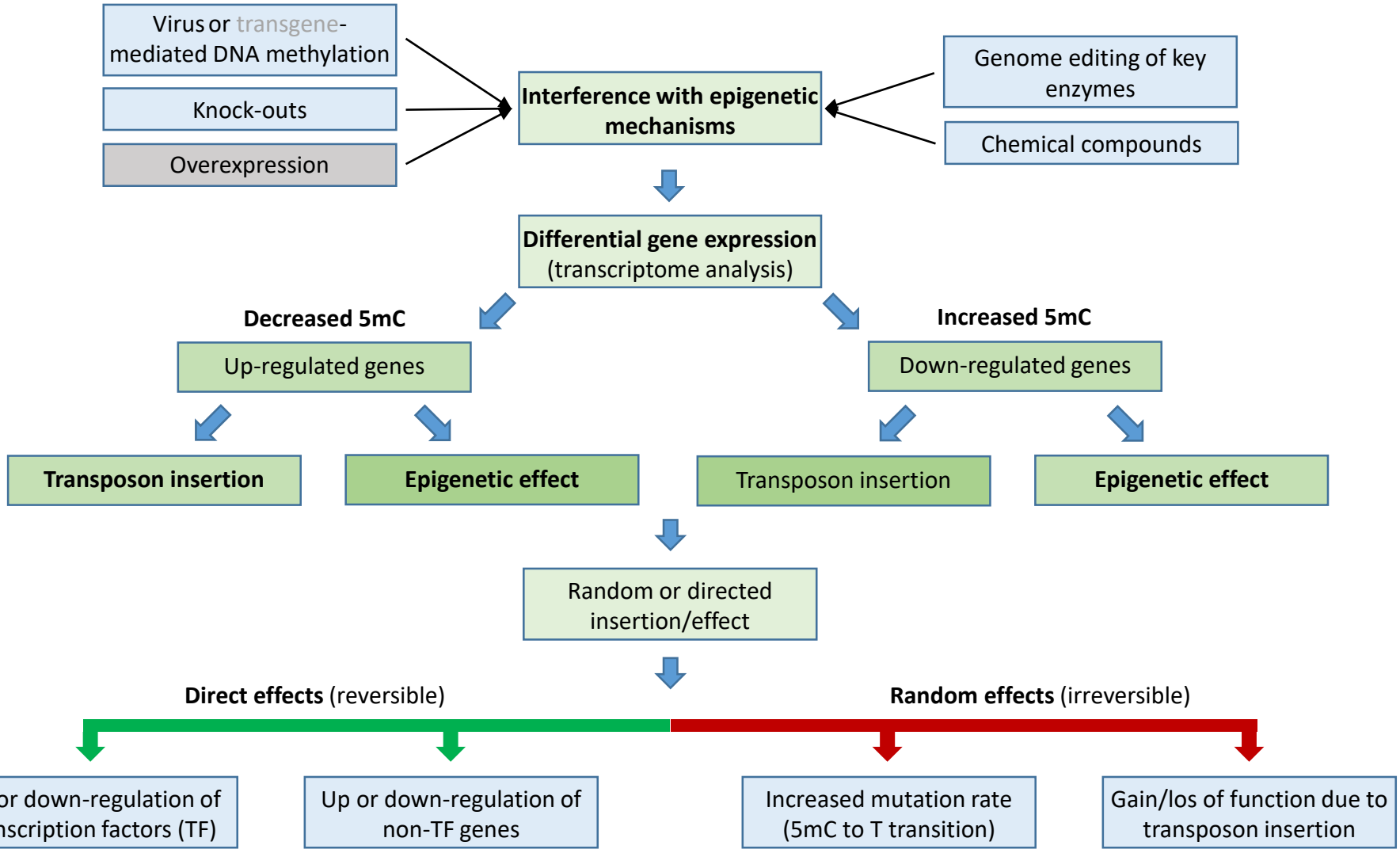
KO mutants and overexpression may be combined, e.g. KO of DRM with overexpression of ROS and *vice versa*!

III. Genome editing tools to target key enzymes of the DNA methylation and chromatin modification pathways



Puchta, Curr Opin Plant Biol 36, 2017

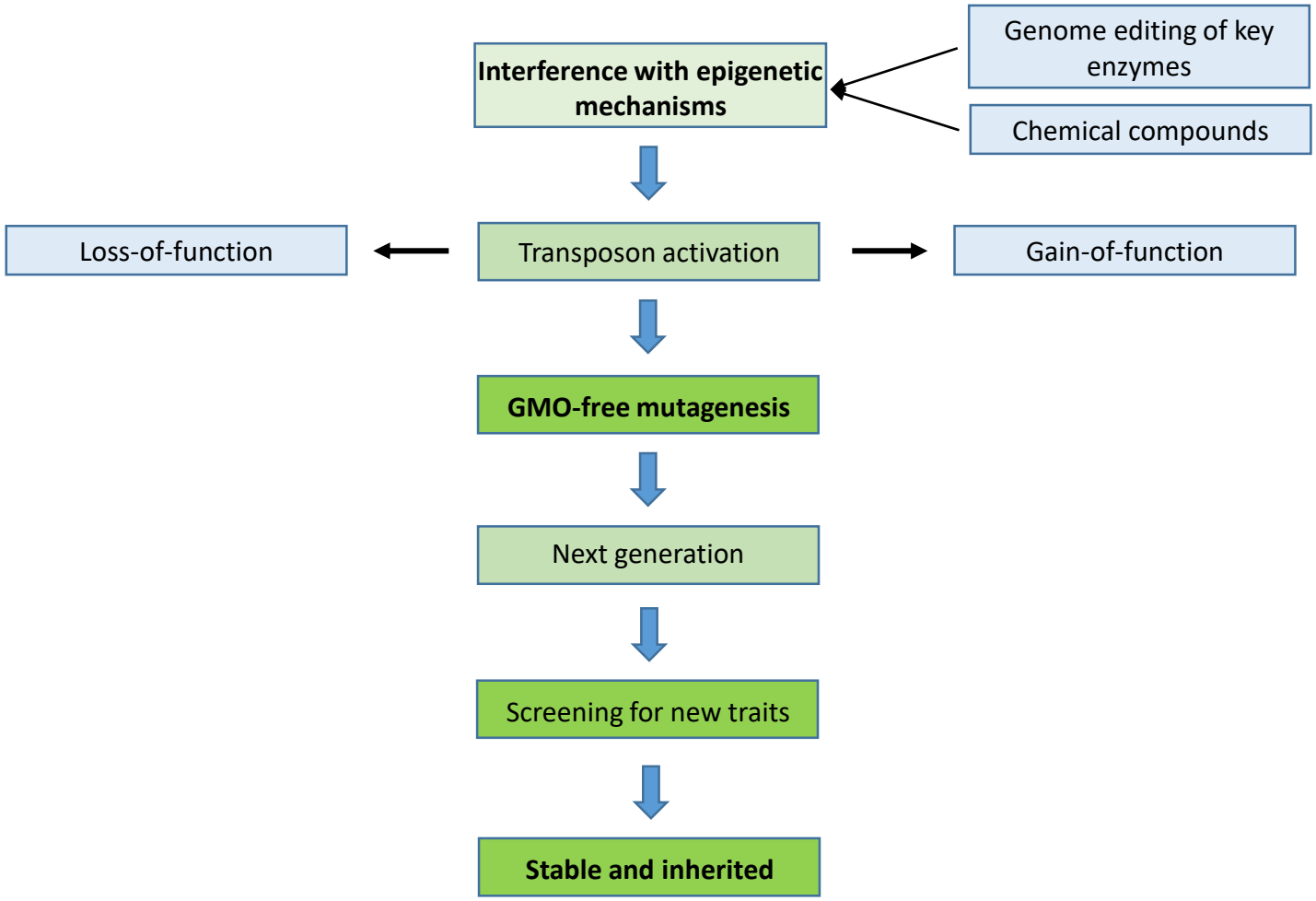
Direct/indirect epigenetic gene regulations



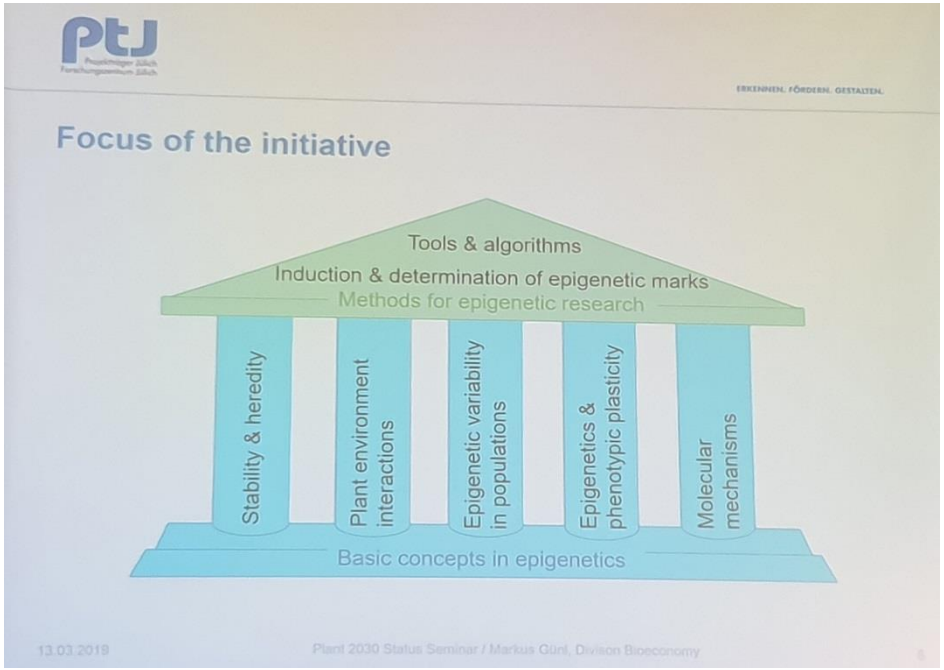
Not all epigenetic gene regulation mechanisms are associated with altered DNA methylation patterns!
However, DNA methylation analysis is more easy than chromatin analysis!

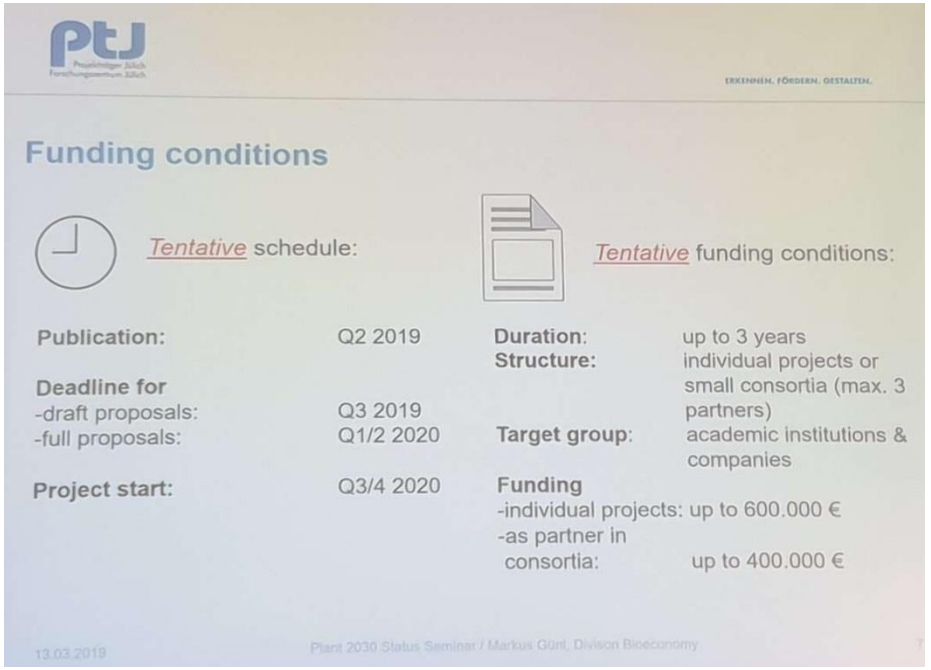
AIPlanta
Institute for Plant Research

Mutagenesis *via* transposon activation



A public view of the significance of epigenetics in plant breeding





Gesellschaft für Pflanzenbiotechnologie

AIPlanta
Institute for Plant Research

The 'RNA-mediated gene silencing' lab

Current lab members

Baßler, Alexandra

Hohenwarter, Linus

Scherer, Petra

Veli Vural Uslu (started in September 2018)

Wassenegger, Michèle

Schwind, Nora (parental leave)

Collaboration

Manfred Heinlein
CNRS, Strasbourg, France

César Llave
CSIC, Madrid, Spain

Ming-Bo Wang
CSIRO, Canberra, Australia

Kriton Kalanditis
FORTH, Heraklion/Crete, Greece

Ricardo Flores Pedauye
UPV-CSIC, University of Valencia, Valencia, Spain

Major funding

DFG

Bundesministerium für Bildung und Forschung

Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz

University of Heidelberg

BASF
We create chemistry

Major consortium projects (Current and past)

DFG
sys-RNAi WA 1019/14-1

Bundesministerium für Bildung und Forschung
FreeEdit 031B532

SIROCCO
SIXTH FRAMEWORK PROGRAMME
LSHG-CT-2006-037900 (IP)

SIXTH FRAMEWORK PROGRAMME
LSH-2003-1.1.0-1 (STRIP)

Bundesministerium für Bildung und Forschung
GAMAVIR 031A324

PLANT-KBBE

Bundesministerium für Bildung und Forschung
BioRN
Biotechnologie-Cluster Rhein-Neckar

ResistVir
SIXTH FRAMEWORK PROGRAMME
FOOD-CT-2005-514048 (Co-ordinated Action)