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INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN
BORDEAUX AQUITAINE

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UMR 1287: Ecophysiologie et génomique fonctionnelle de la vigne

DNA methylation in plants: a general introduction

P Gallusci

The new science of epigenetics reveals how the choices you make can change your genes - and those of your kids

From, Zaidi S K et al. Mol. Cell. Biol. 2010;30:4758-4766

DNA methylation in plants

1. Introduction: epigenetic
2. **Overview on DNA methylation**
3. Mechanisms of DNA methylation in plants
4. Dynamics and functions of DNA methylation
5. Conclusions

Epigenetics

The new science of epigenetics reveals how the choices you make can change your genes - and those of your kids

From, Zaidi S K et al. Mol. Cell. Biol. 2010;30:4758-4766

«Genetics proposes, Epigenetics disposes»

(Medawar and Medawar, 1983)

Epigenetics?

"Epi-genetic", from ancient Grec 'ἐπί' et Genetique
« Above genetic »

- *"An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence"*
  - ***Berger et al (2009)*** *Genes Dev*

- Some natural plant and animal ***"variants"*** are not due to mutations but to epigenetic variations

Sauvage *cnr*

Manning, Seymour et al, Nat Genet 2006

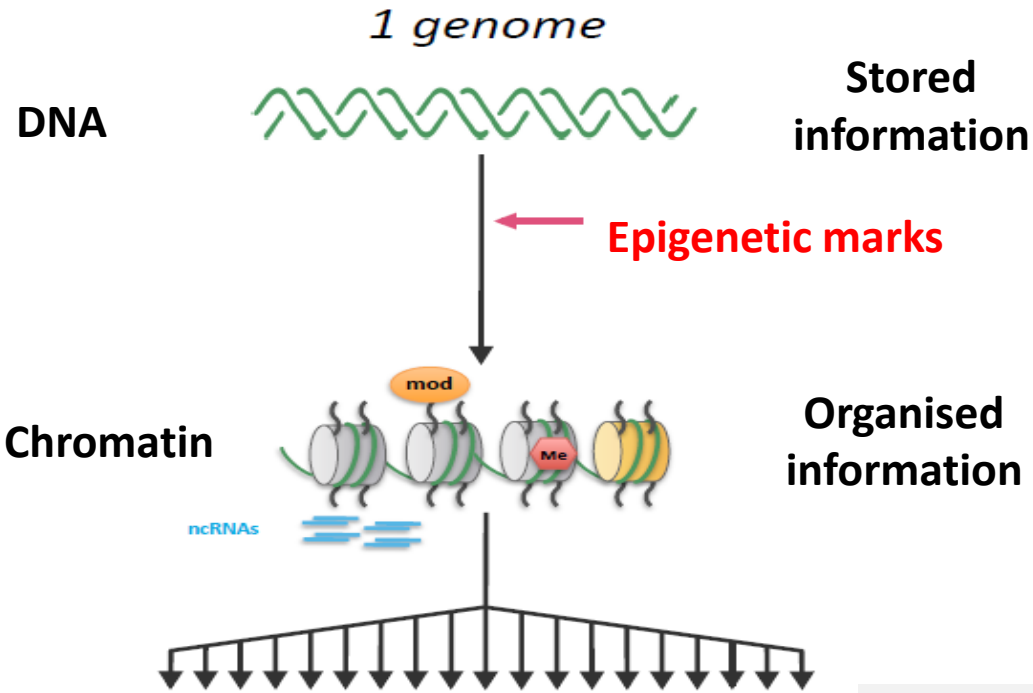
Sauvage *peloria*

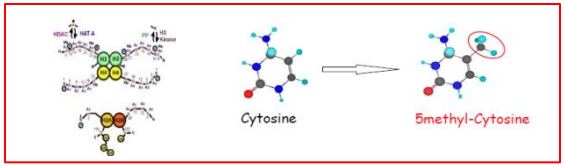
Cubas et al, Nat 1999

Sauvage *agouti*

Morgan et al, Nat 1999

Epigenetics?

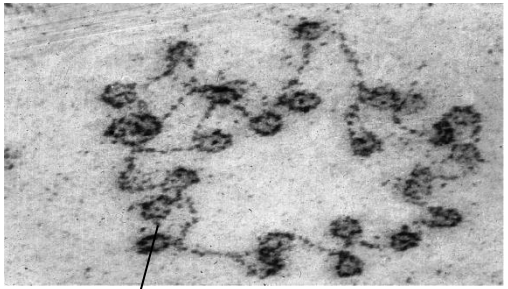


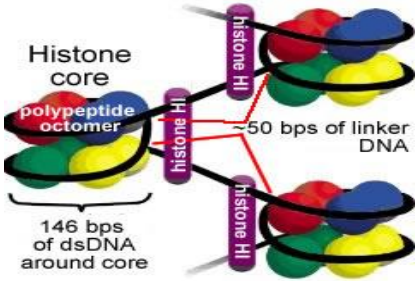


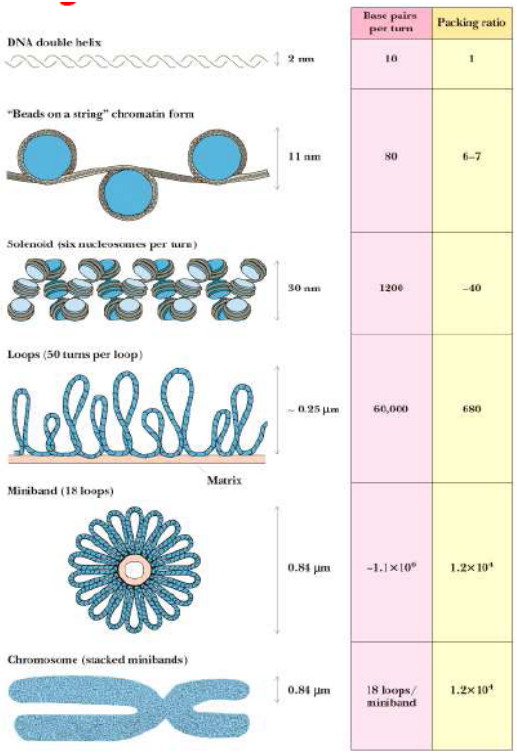
Heritable traits

Adapted from *Epigenetics*, 2006

Epigenetics?







DNA packing ratio

packing ratio

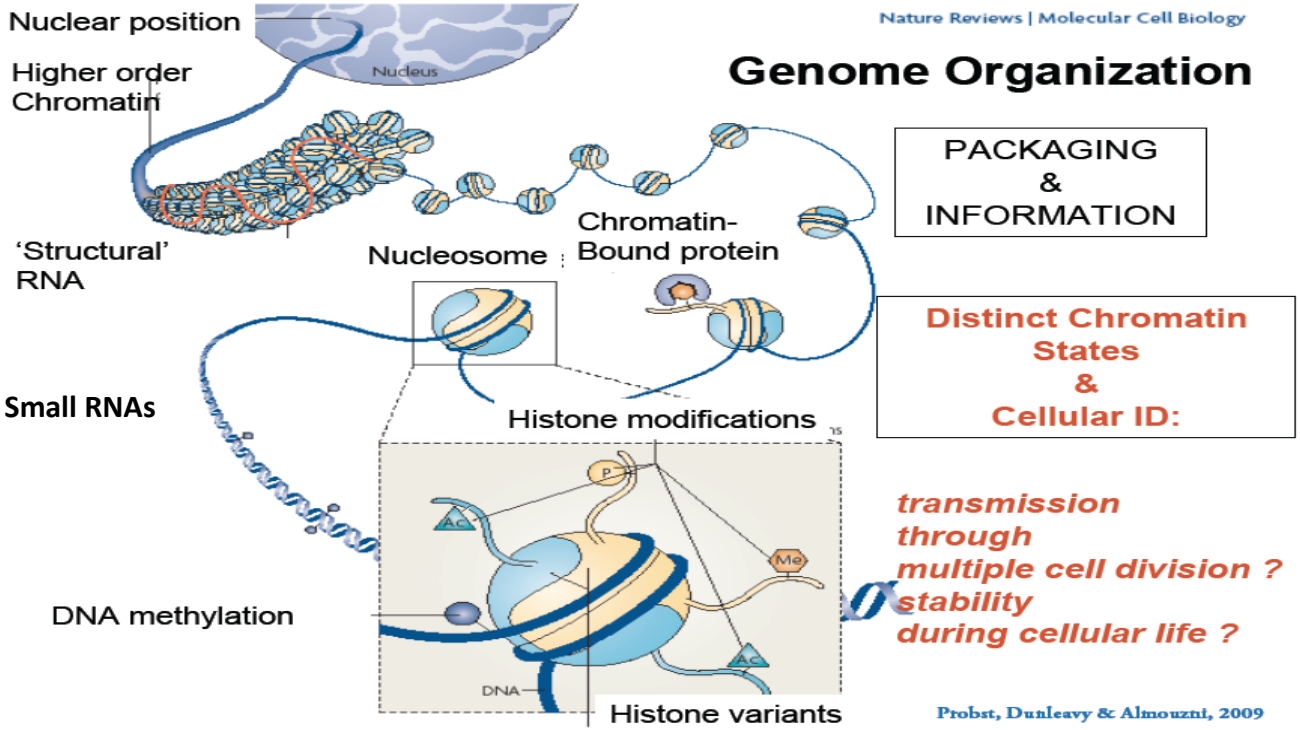
~6

~40

1000x euchromatin

10,000x heterochromatin

Epigenetics?



DNA methylation in plants

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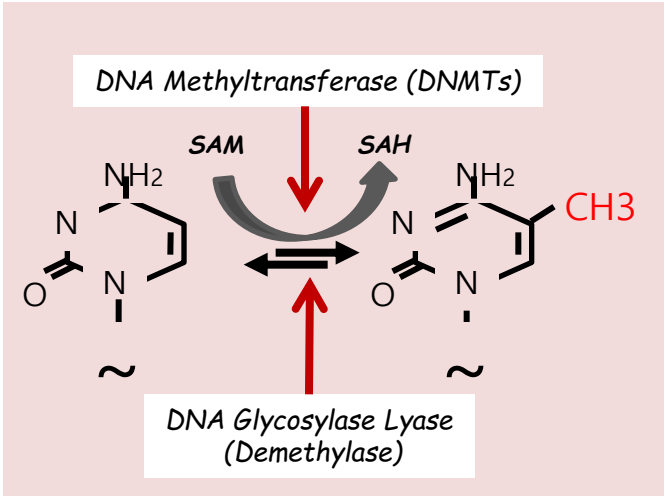
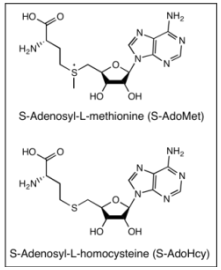
2. Overview of DNA methylation in plants

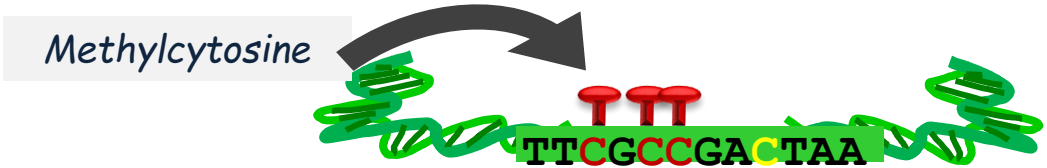
cytosine



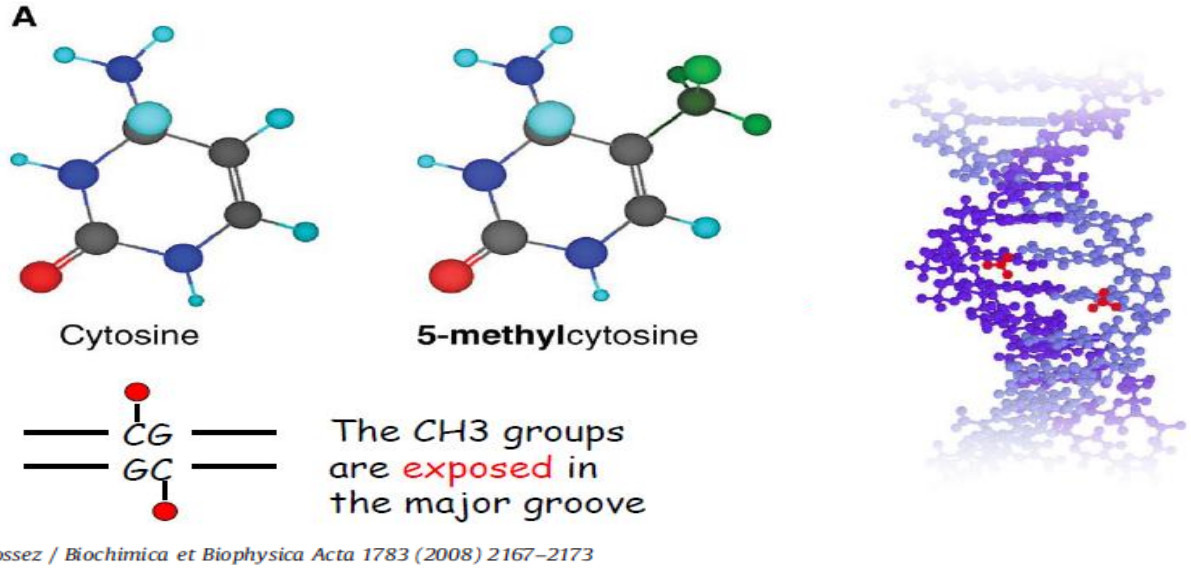
5-methylcytosine

SAM: S Adénosyl methionine
SAH: S Adénosyl Homocystéine





2. Overview of DNA methylation in plants

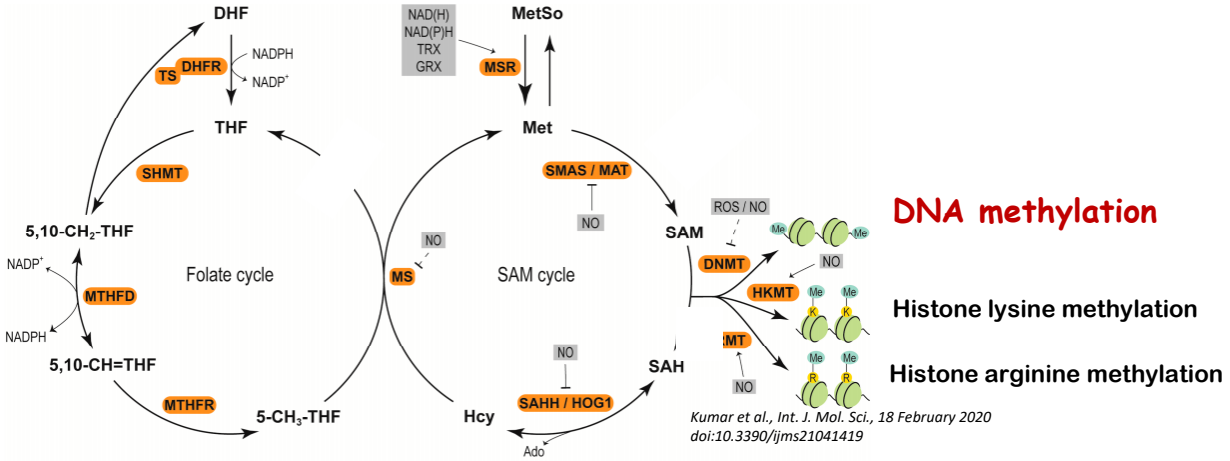


E. Prokhortchouk, P.-A. Defossez / Biochimica et Biophysica Acta 1783 (2008) 2167–2173

Proteins like transcription factors that can bind to specific sequences in double-stranded DNA usually make contacts to the sides of the bases exposed in the major groove.

DNA methylation is closely linked to the cell metabolism

One carbon metabolism :the link between metabolome and epigenome



SAM is a precursor for all Epigenetic modifications requiring a methyl donor

DNA methylation is closely linked to the cell metabolism

Early evidence comes from animals: the agouti phenotype

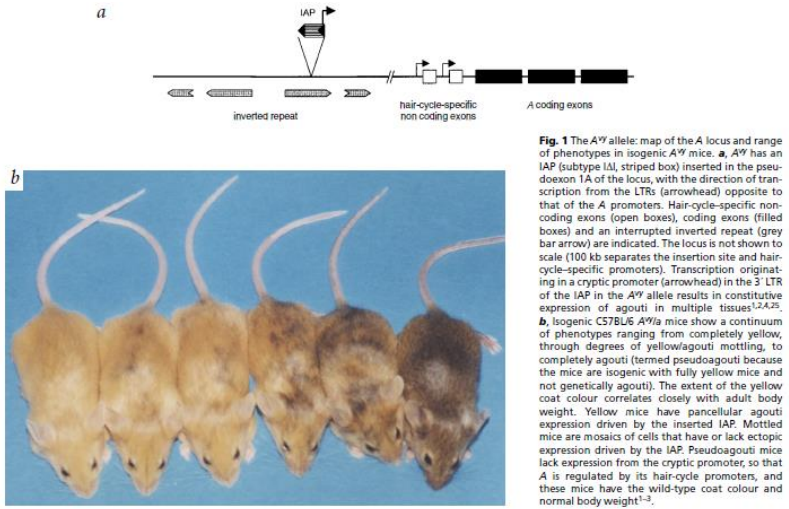


Fig. 1 The A^{vy} allele: map of the *A* locus and range of phenotypes in isogenic A^{vy} mice. ***a***, A^{vy} has an IAP (subtype IAI, striped box) inserted in the pseudoexon 1A of the locus, with the direction of transcription from the LTRs (arrowhead) opposite to that of the *A* promoters. Hair-cycle–specific noncoding exons (open boxes), coding exons (filled boxes) and an interrupted inverted repeat (grey bar arrow) are indicated. The locus is not shown to scale (100 kb separates the insertion site and hair-cycle–specific promoters). Transcription originating in a cryptic promoter (arrowhead) in the 3′ LTR of the IAP in the A^{vy} allele results in constitutive expression of agouti in multiple tissues[1,2,4,25]. ***b***, Isogenic C57BL/6 A^{vy}/a mice show a continuum of phenotypes ranging from completely yellow, through degrees of yellow/agouti mottling, to completely agouti (termed pseudoagouti because the mice are isogenic with fully yellow mice and not genetically agouti). The extent of the yellow coat colour correlates closely with adult body weight. Yellow mice have pancellular agouti expression driven by the inserted IAP. Mottled mice are mosaics of cells that have or lack ectopic expression driven by the IAP. Pseudoagouti mice lack expression from the cryptic promoter, so that *A* is regulated by its hair-cycle promoters, and these mice have the wild-type coat colour and normal body weight[1–3].

[1]*Department of Biochemistry, University of Sydney, NSW, 2006, Australia.* [2]*MRC Human Genetics Unit, Edinburgh, Scotland, UK.* [3]*Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. Correspondence should be addressed to E.W. (e-mail: e.whitelaw@biochem.usyd.edu.au).*

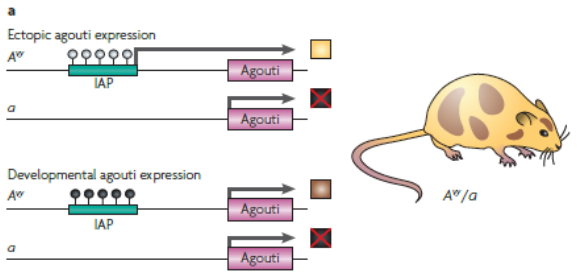
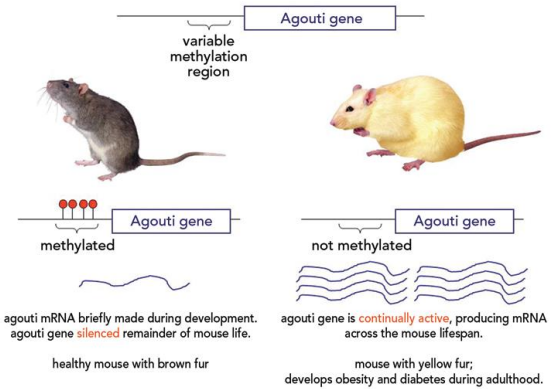
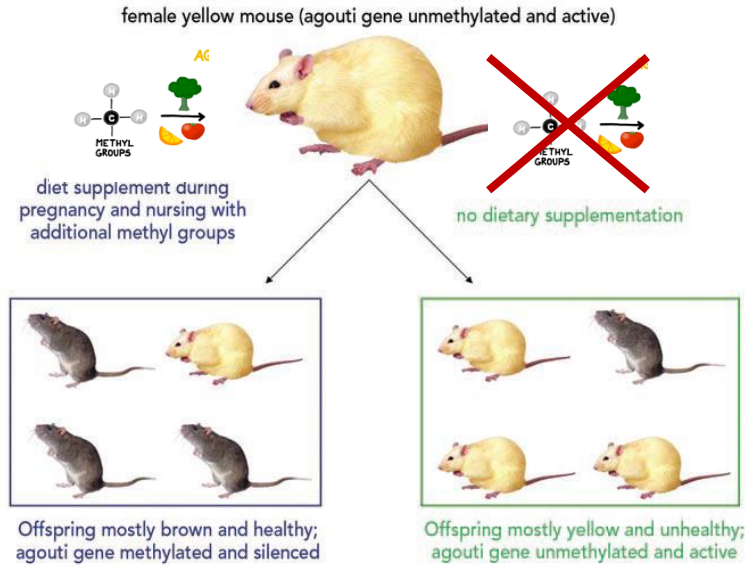


Figure 1 | **Epigenetic regulation of metastable epialleles. a** | Epigenetic regulation of the agouti gene in A^{vy}/a mice. White-filled circles indicate unmethylated CpG sites and black-filled circles indicate methylated CpG sites. Phaeomelanin (the product of the agouti gene) is not produced from the *a* allele because the agouti gene is mutated (shown as a box marked with a red cross). Two potential epigenetic states of the A^{vy} allele can occur within cells of A^{vy}/a mice. The IAP (intracisternal A particle) that lies upstream of the agouti gene can remain unmethylated, allowing ectopic expression of the gene from the IAP and resulting in a yellow coat colour (top). Alternatively, the IAP can be methylated, so that the gene is expressed under its normal developmental controls, leading to a brown coat colour (bottom). If the IAP methylation event occurs later in development and does not affect all embryonic cells, the offspring will have a mottled appearance (illustrated on the right). **b** | Genetically identical week 15 A^{vy}/a mouse littermates are shown, representing five coat-colour phenotypes. Mice that are predominately yellow are also clearly more obese than the brown mice. Part **b** reproduced with permission from REF. 20 © (2006) National Institute of Environmental Health Sciences.

2. Overview of DNA methylation in plants

DNA methylation is closely linked to the cell metabolism



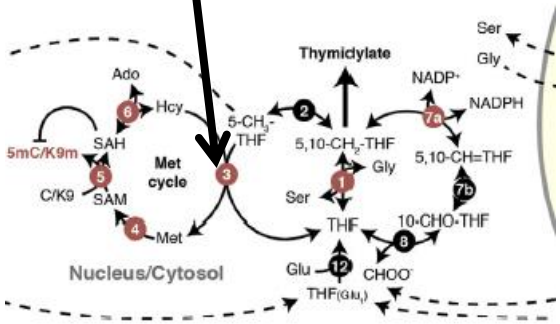


from – Dr. Neil Lamb
director of educational outreach
HudsonAlpha Institute for Biotechnology

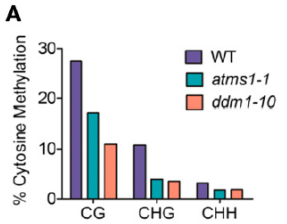
2. Overview of DNA methylation in plants

DNA methylation is closely linked to the cell metabolism

ATMS1 encodes a cytosolic cobalamin-independent Met synthase and is involved in Met regeneration via the activated one-carbon metabolism methyl cycle (SAM cycle), which provides methyl groups for most methylation reactions (Hesse and Hoefgen, 2003; Ravanel et al., 2004). To assess the effects of



Impact on DNA methylation



METHIONINE SYNTHASE1 Is Involved in Chromatin Silencing by Maintaining DNA and Histone Methylation[1]

Xiaojing Yan,[a,b,2] Liang Ma,[c] Hongying Pang,[a,b] Ping Wang,[c] Lei Liu,[c] Yanxia Cheng,[a,b] Jinkui Cheng,[c] Yan Guo,[c] and Quanzi Li[a,b,2,3]

Figure 3. Effect of ATMS1 on genome-wide DNA methylation. A, Whole-genome DNA methylation levels at CG, CHG, and CHH sites in the wild type (WT) and *atms1-1* and *ddm1-10* mutants. B, DNA methylation patterns (CG, CHG, and CHH) of genes and their 2-kb upstream and downstream regions in the wild type and the *atms1-1* mutant. The blue lines in the diagrams represent DNA methylation level in the wild type, while the red lines represent DNA methylation level in *atms1-1*. C, DNA methylation patterns (CG, CHG, and CHH) of TEs and their 2-kb upstream and downstream regions in the wild type and the *atms1-1* mutant. The blue lines in the diagrams represent DNA methylation level in the wild type, while the red lines represent DNA methylation level in *atms1-1*.

2. Overview of DNA methylation in plants: link with cell metabolism

DNA methylation is closely linked to the cell metabolism

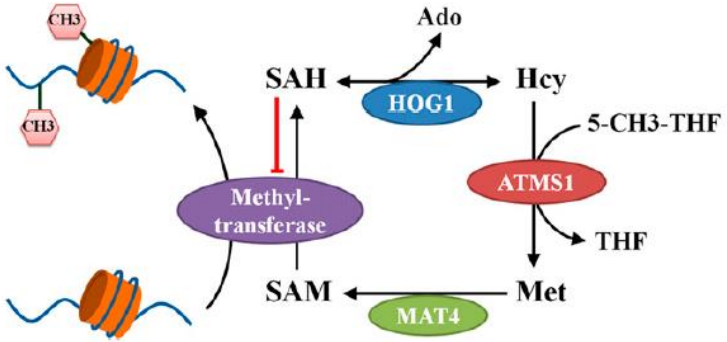


Figure 8. Model for the function of ATMS1 on methyl-group supply in one-carbon metabolism and DNA/histone methylation in Arabidopsis. ATMS1 catalyzes the formation of Met by the transfer of a methyl group from 5-CH3-THF to Hcy. MAT4 synthesizes SAM using Met and ATP as substrates. After supplying the methyl group to DNA or histone by methyltransferases, SAM is changed into SAH, which is an inhibitor of methyltransferases. SAH is converted to Hcy and adenosine by HOG1.

1C metabolism is critical for DNA methylation in plants

DNA methylation in plants

1. Introduction: epigenetic
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3. Mechanisms of DNA methylation

DNA methylation needs to be established and maintained

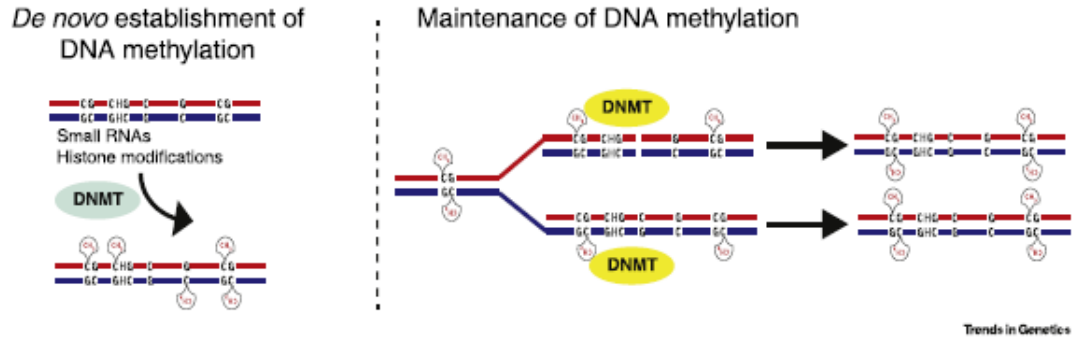
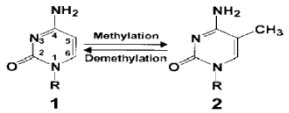


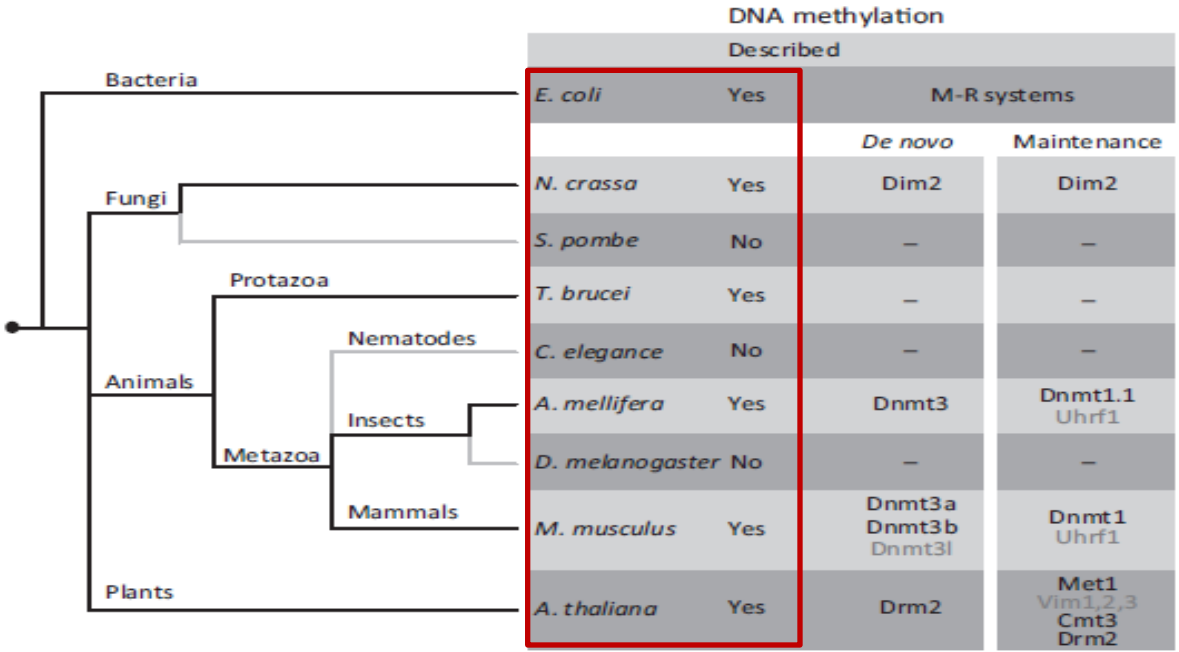
Figure 2. Schematic Diagram of *De Novo* and Maintenance DNA Methylation.
Methylation of cytosines *de novo* is independent of existing 5-methylcytosine (5mC) and can be targeted to cytosines in CpG and non-CpG contexts. Recruitment of *de novo* cytosine methyltransferases is regulated by small RNAs and/or specific chromatin modifications. Maintenance methylation involves recognition of hemi-methylated CpG sites generated during DNA replication. Maintenance methyltransferases target CpG sequences on the newly synthesized strand to generate a fully methylated site.

Trends in Genetics, November, Vol. 35, No. 11 821

3. Mechanisms of DNA methylation



Methylation of cytosine differs between eukaryotic genomes

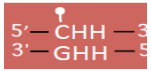


Trends in Cell Biology, February 2014, Vol. 24, No. 2

In plants DNA methylation can occur in different sequence context

- *In plants Cytosine Methylation occurs in all sequence context*
  - ❑ *symmetrical CG , CHG*
  - ❑ *non symmetrical : CHH.*

(H= A, T or C)



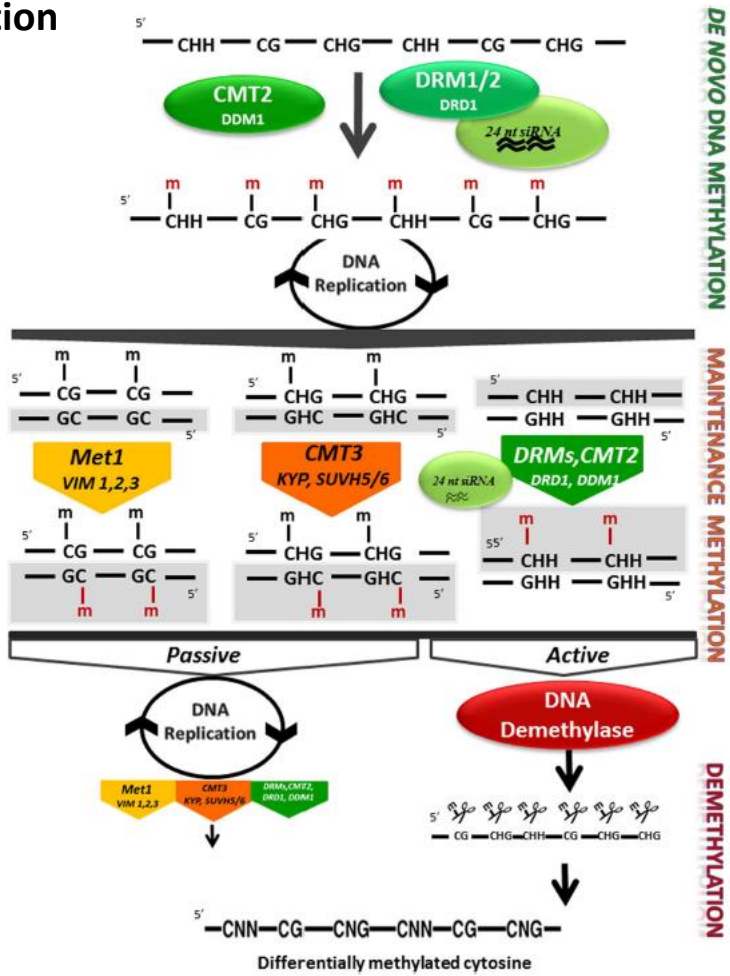
- *Mammalians: essentially CG methylation*

3. Mechanisms of DNA methylation

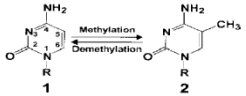
DNA Methylation and Chromatin Regulation during Fleshy Fruit Development and Ripening

Citation:

Gallusci P, Hodgman C, Teyssier E and Seymour GB (2016) DNA Methylation and Chromatin Regulation during Fleshy Fruit Development and Ripening. Front. Plant Sci. 7:807. doi: 10.3389/fpls.2016.00807



3. Mechanisms of DNA methylation



ARTICLE

OPEN

RdDM-independent de novo and heterochromatin DNA methylation by plant CMT and DNMT3 orthologs

Rafael Yaari[1], Aviva Katz[1], Katherine Domb[1], Keith D. Harris[1], Assaf Zemach[1] & Nir Ohad[1,2]

DNMTs vary between EK

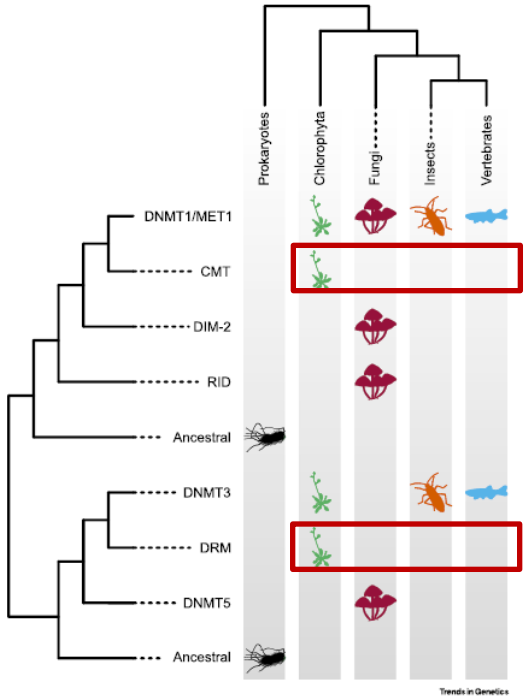
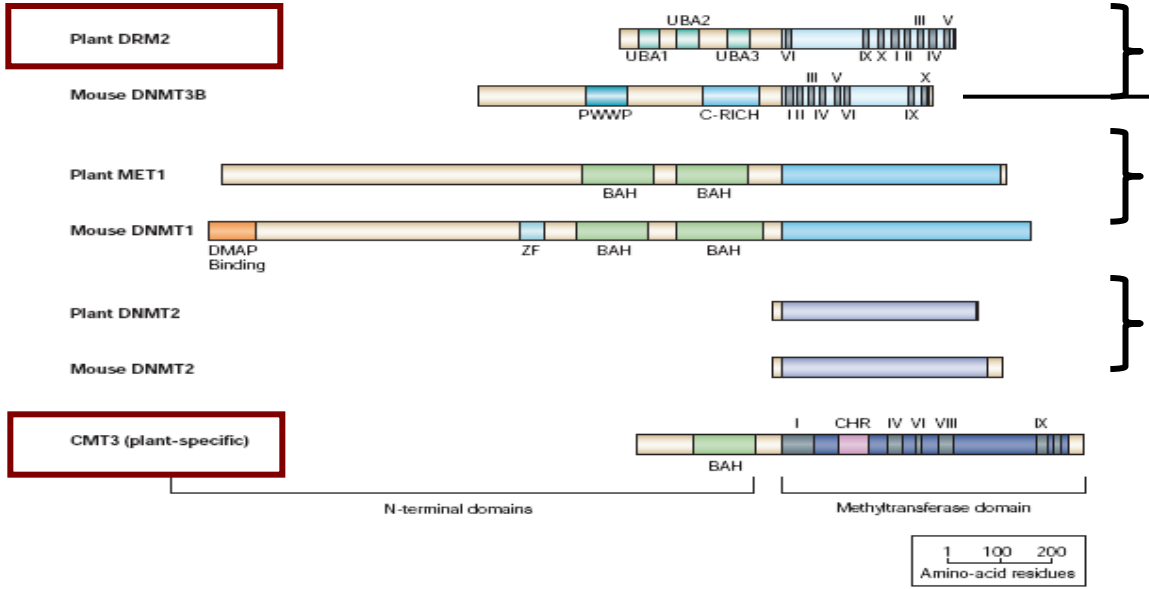


Figure 1. Evolutionary Relationship of Eukaryotic DNA Methyltransferases.
DNMT1 homologs are found in essentially all eukaryotes that utilize 5-methylcytosine (5mC), whereas lineage-specific losses and gains of DNA methyltransferases (DNMTs) are found in specific taxa. This phylogeny is a representation and is not applicable to all species within each lineage owing to recurrent loss of the DNA methylation machinery. Figure courtesy of Adam Bewick.

3. Mechanisms of DNA methylation

Specificity of Plant DNA methyl-transferases



Physcomitrella patens

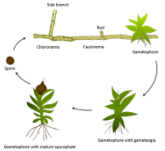


Figure 1 | **Four classes of DNA methyltransferase in *Arabidopsis thaliana*.** The *Arabidopsis thaliana* genome encodes four classes of DNA cytosine methyltransferases. The first class, represented by DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), is orthologous to the mammalian DNA (cytosine-5-)-methyltransferase 3 (DNMT3) (such as the mouse DNMT3B shown in the diagram) and both function to control *de novo* methylation. DRM2 differs from DNMT3 proteins in that it has a unique N terminus and that its catalytic domains are rearranged in the linear sequence. METHYLTRANSFERASE 1 (MET1) is orthologous to the mammalian DNMT1 enzyme and both function to maintain CG methylation. Both MET1 and DNMT1 possess large N termini that contain bromo adjacent homology (BAH) domains. The DNMT2 class of methyltransferase is conserved in many eukaryote genomes but its function is unknown. Plant genomes are distinguished from mammalian genomes by the CHROMOMETHYLASE (CMT) class of methyltransferases, which function to control the maintenance of non-CG methylation. CHR, chromodomain; C-rich, cysteine rich; DMAP binding, DNMT1-associated protein binding; PWWP, Pro-Trp-Trp-Pro domain; UBA, ubiquitin associated domain; ZF, zinc finger.

DNA methylation landscapes: provocative insights from epigenomics
Miho M. Suzuki &
Adrian Bird
Nature Reviews Genetics **volume 9**, pages 465–476 (2008)

3. Mechanisms of DNA methylation

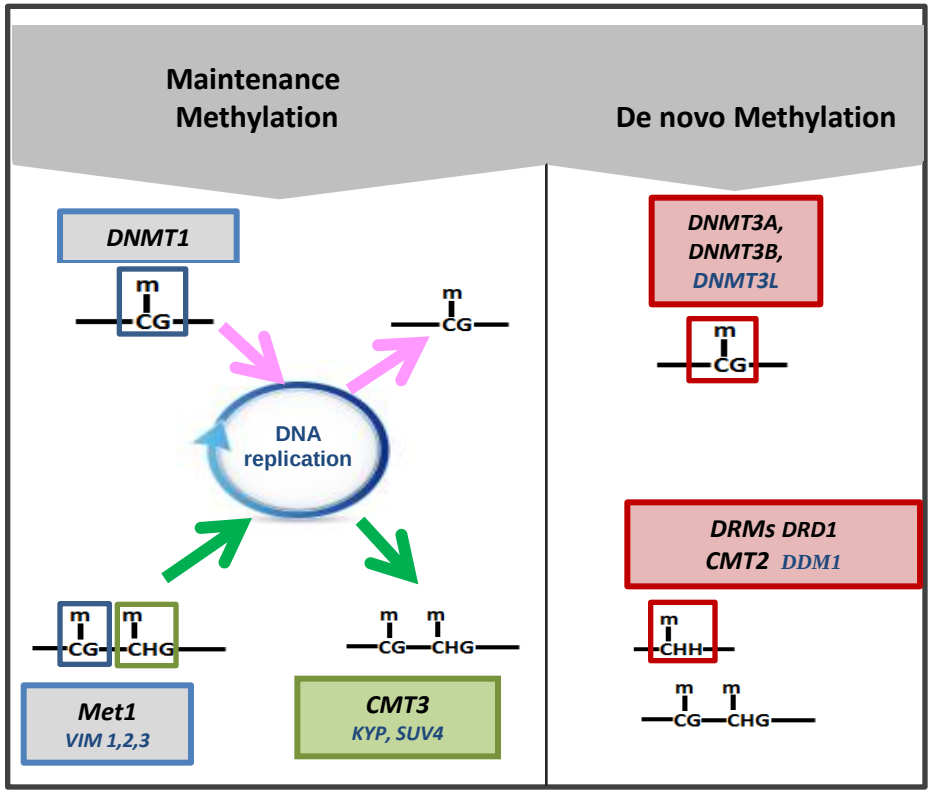
Maintenance of DNA methylation

Mammal

Symmetrical methylation: CG

Plant

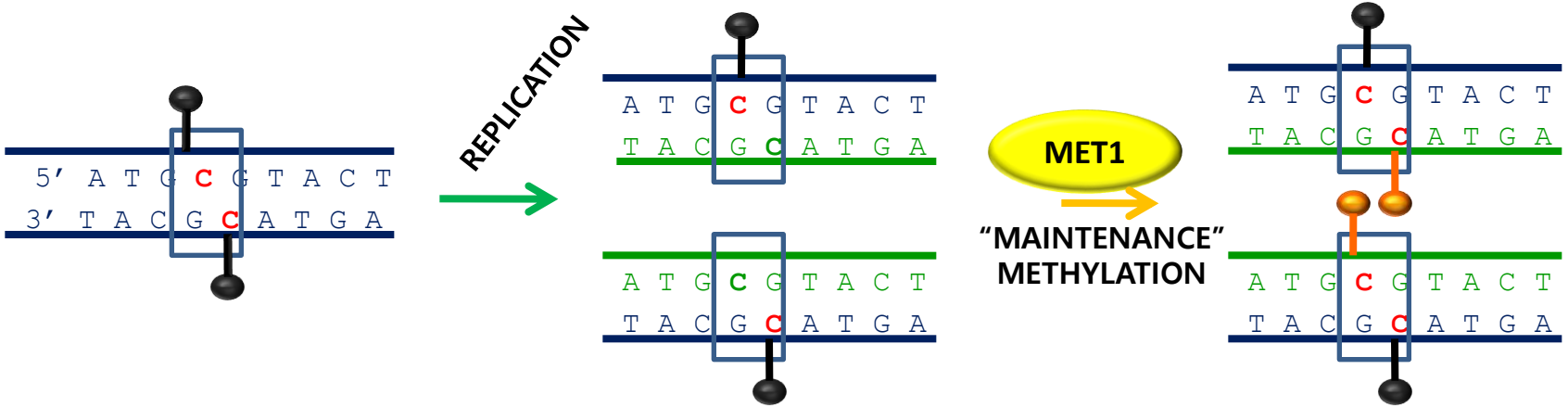
Symmetrical methylation: CG, CHG
Non symmetrical methylation: CHH



Adapted from Teyssier , Gallusci et al , 2015, Applied Plant Genomics and Biotechnology , Chap 8

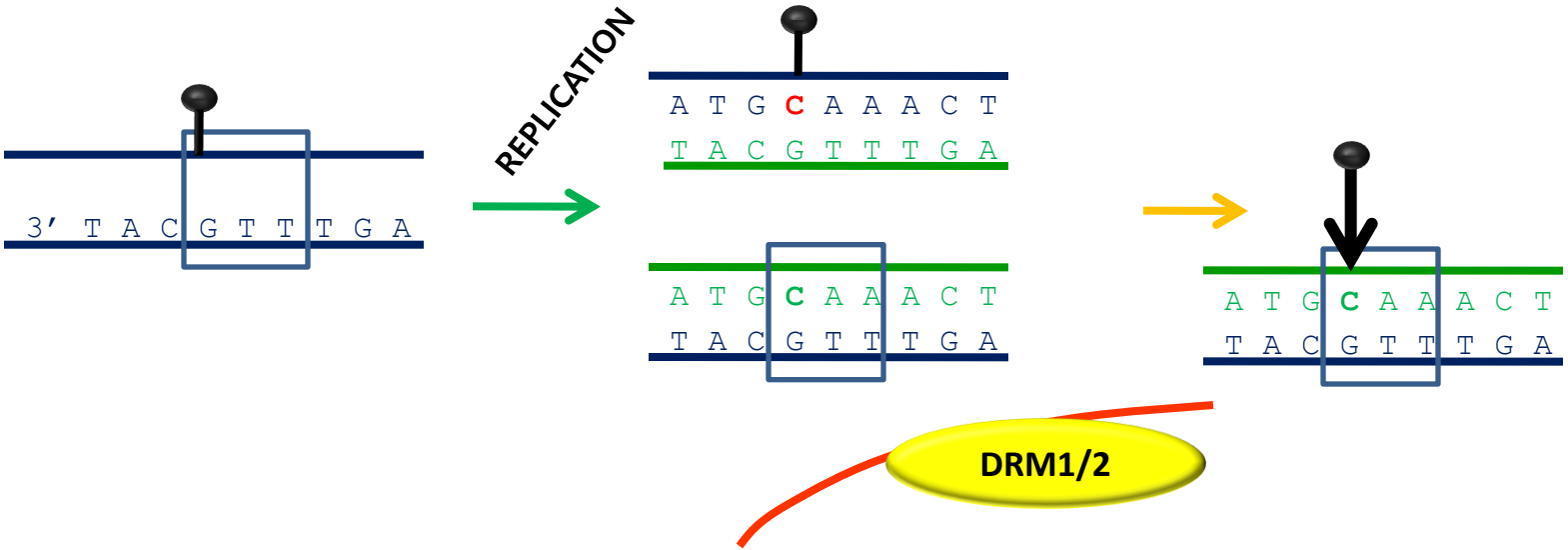
Maintenance of DNA methylation

CG methylation can be propagated during DNA replication



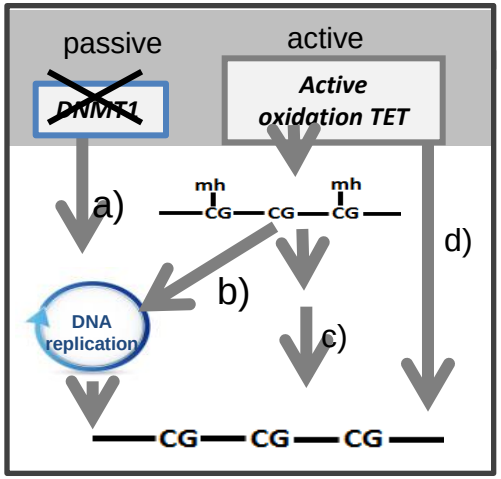
Maintenance of DNA methylation

Non symmetrical are maintained by small interfering RNAs (siRNAs)



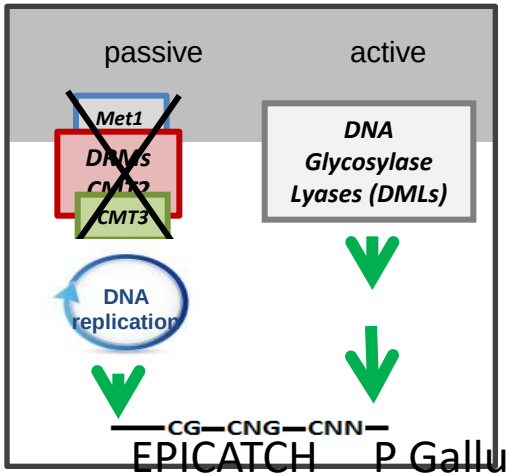
2. Overview on DNA methylation in plants

Mammals



- a) Passive DNA demethylation
- b)Tet-assisted passive DNA demethylation
- c) and d) Tet-assisted active DNA demethylation mechanisms involving Thymine DNA glycosylase

Plants



- Active demethylation is mediated by bi-functionnal DNA glycosylase-lyase, that create abasic sites

From Teyssier , Gallusci et al , 2015, Applied Plant Genomics and Biotechnology , Chap 8

3. Mechanisms of DNA methylation

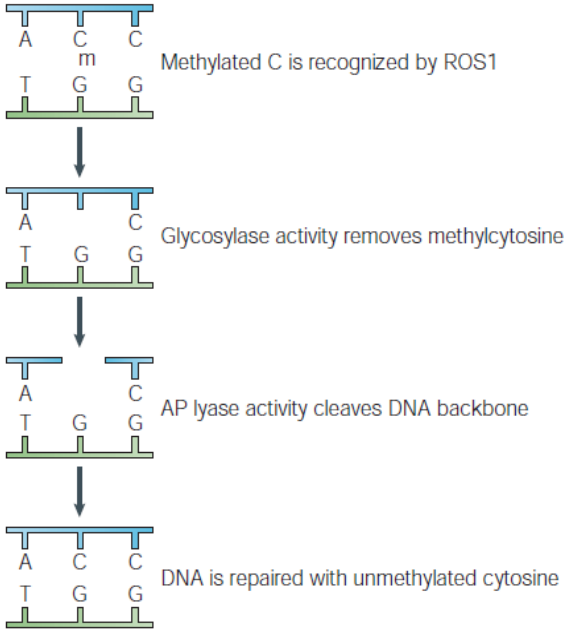
Active demethylation is mediated by bi-functionnal DNA glycosylase-lyase, that create abasic sites

REVIEW

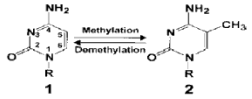
doi:10.1038/nature12750

TET enzymes, TDG and the dynamics of DNA demethylation

Rahul M. Kohli[1,2] & Yi Zhang[3,4,5,6,7]

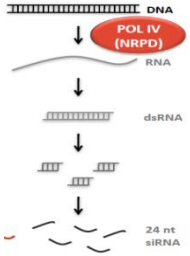


3. Mechanisms of DNA methylation



« Maintenance »
Methylation

« De novo »
Methylation (RdM)



Met1

CMT3

DRM

CMT2

5′— CG —3′
3′— GC —5′

5′— CHG —3′
3′— GHC —5′

5′— CHH —3′
3′— GHH —5′

H=T, A, C

Passive DNA Demethylation

ROS1

DME

Active DNA Demethylation

5′— CG —3′
3′— GC —5′

5′— CHG —3′
3′— GHC —5′

5′— CHH —3′
3′— GHH —5′

3. Mechanisms of DNA methylation

ARTICLE

OPEN

RdDM-independent de novo and heterochromatin DNA methylation by plant CMT and DNMT3 orthologs

Rafael Yaari[1], Aviva Katz[1], Katherine Domb[1], Keith D. Harris[1], Assaf Zemach[1] & Nir Ohad[1,2]

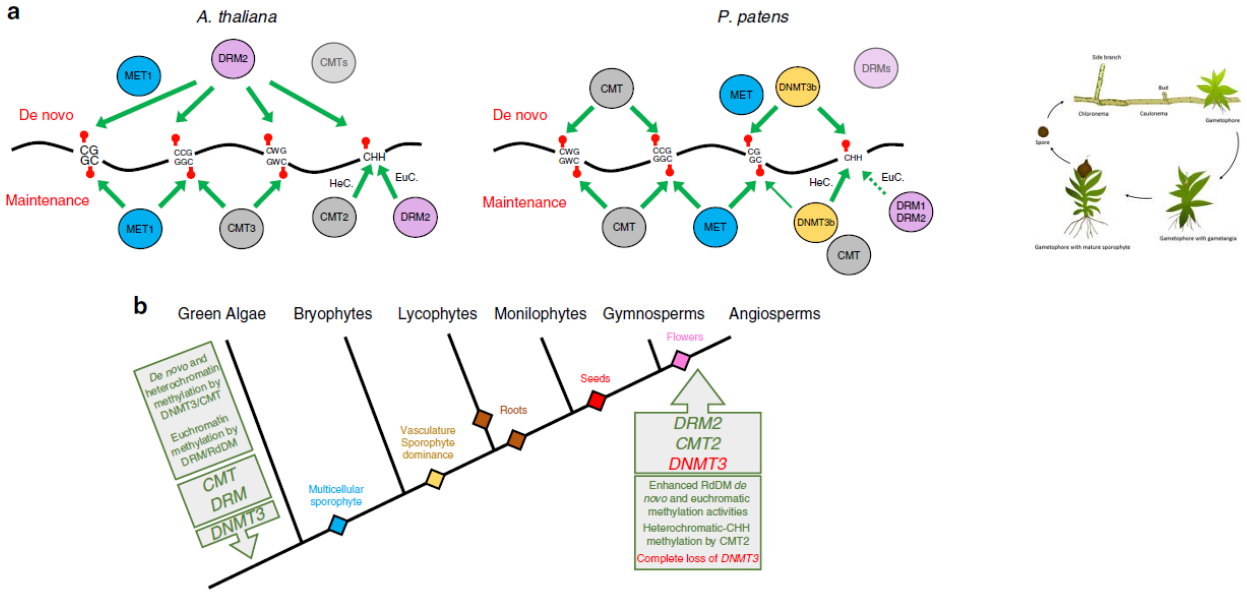


Fig. 5 Mechanisms and evolution of plant DNMTs. **a** DNMT methylation mechanisms are illustrated based on current knowledge. Black line represents the DNA with different cytosine subcontexts embedded in it. Lollipops represent methylation. Arrows width is corresponding qualitatively to the relative level of methylation mediated by indicated DNMTs. HeC. = heterochromatin, EuC. = euchromatin. De novo and maintenance methylation activities are shown above and below the DNA, respectively. De novo methylation in *P. patens* is based on our RPS transgene results. Future studies would need to check the de novo methylation activity of CMTs and DRMs (masked ovals) in *Arabidopsis* (angiosperms) and *P. patens* (basal/DNMT3-encoding plants), respectively. **b** Schematic illustration of the evolution of plant DNMTs and their function based on previous and our studies. Backbone of phylogenetic tree is inspired by https://langdalelab.com/

DNA methylation in plants

1. Introduction: epigenetic
2. Overview on DNA methylation
3. Mechanisms of DNA methylation in plants
4. **Dynamics and functions of DNA methylation**
5. Conclusions

4. DNA methylation dynamics and functions in plants

Review
DNA Methylation Analysis: Choosing the Right Method
Sergey Kurdyukov [1,*] and Martyn Bullock [2]
Biology **2016**, *5*, 3; doi:10.3390/biology5010003

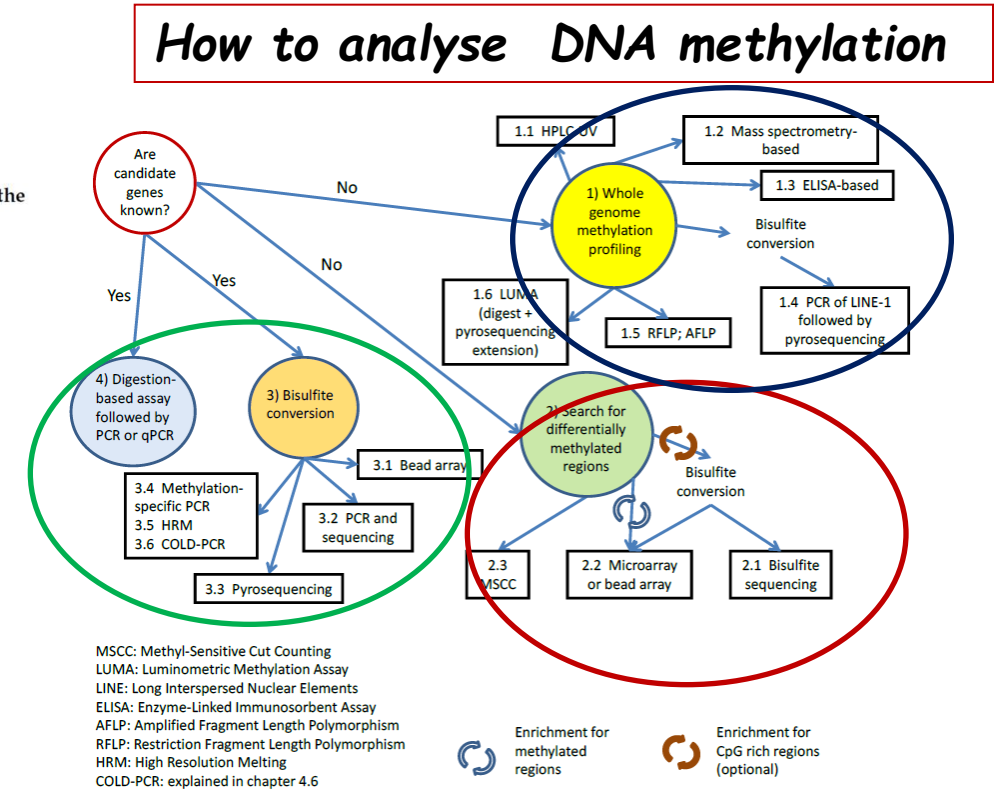
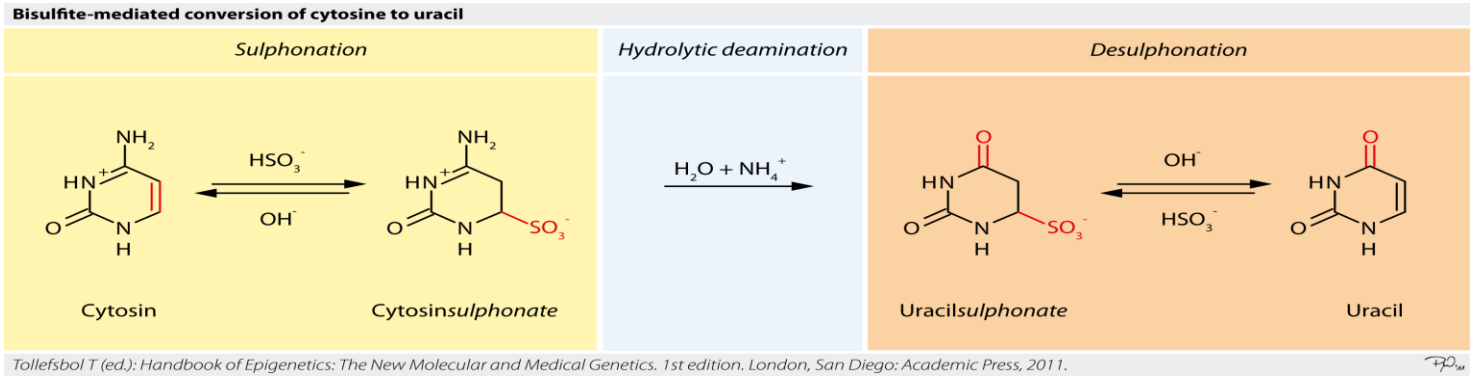


Figure 1. Algorithm for choosing a suitable method for DNA methylation analysis.

Methods for studying DNA methylation

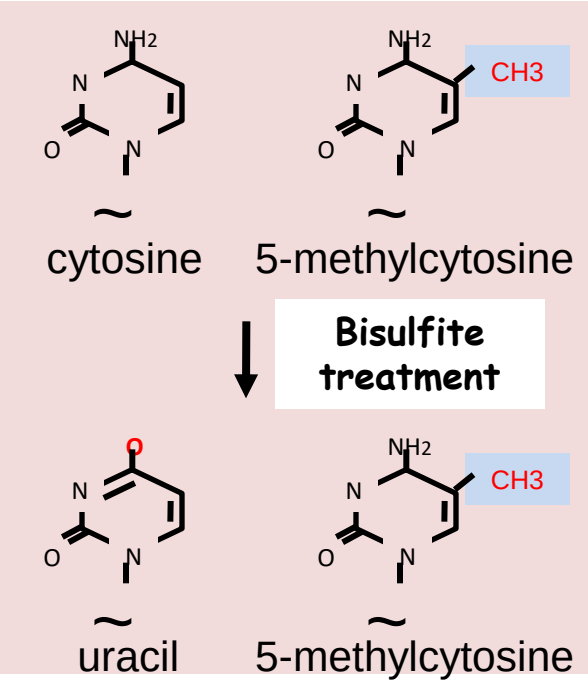
DNA methylation- bisulfite sequencing

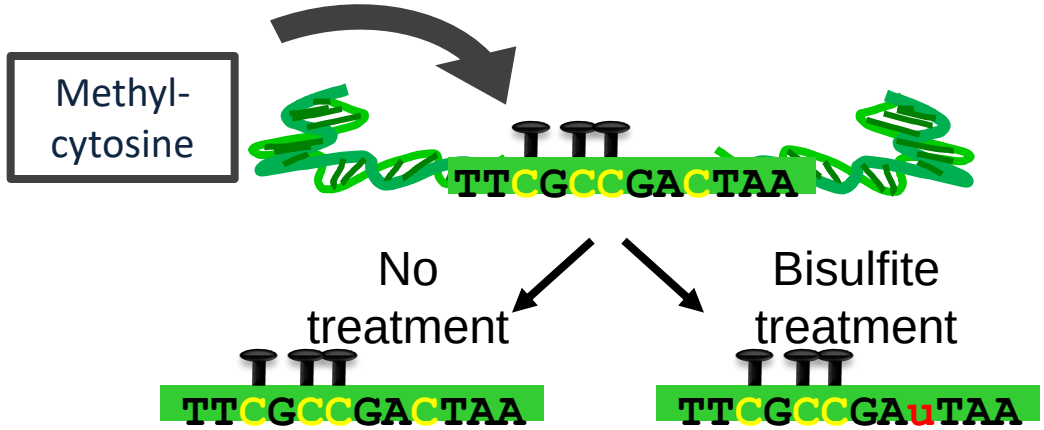
Bisulfite transforms cytosine into uracil, except when cytosine is methylated



Adapted from

Bisulfite treatment differentiates cytosine and methylcytosine





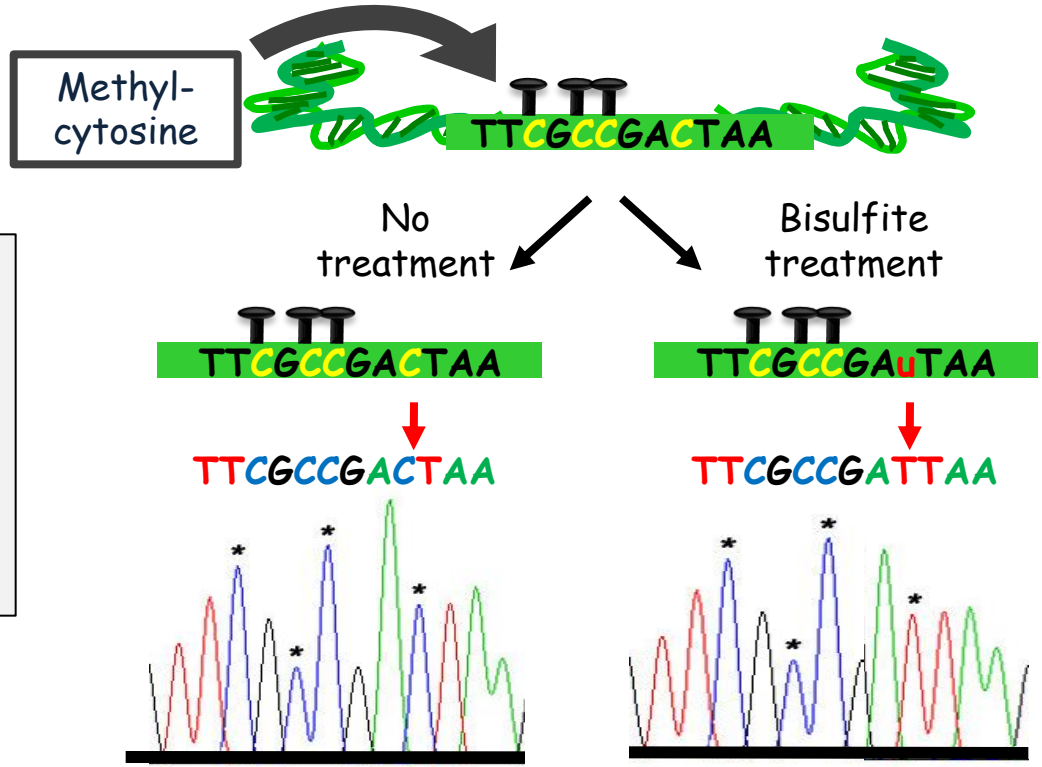
When DNA is bisulfite treated, unmethylated cytosine is converted to uracil. Methylcytosine is not affected.

Adapted from

Bisulfite treatment differentiates cytosine and methylcytosine

After bisulfite treatment, unmethylated **C**s are read as **T** and so differ in the treated and untreated samples.

By contrast, **methyl-C** is read as **C** and is the same as the reference sequence.



Adapted from

Distribution of DNA methylation in plant genomes

First methylome in plants!:

Vol 452|13 March 2008|

Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning

Shawn J. Cokus[1]*, Suhua Feng[1,2]*, Xiaoyu Zhang[1]†, Zugen Chen[3], Barry Merriman[3], Christian D. Haudenschild[4], Sriharsa Pradhan[5], Stanley F. Nelson[3], Matteo Pellegrini[1] & Steven E. Jacobsen[1,2]

- **To generate a DNA methylation map at one nucleotide resolution across the genome.**
- **Strategy: shotgun sequencing of BS treated arabidopsis genomic DNA, using the Solexa Sequencing Technology.**
- **Obtain 2.6 billions nucleotides mapping to unique genomic lcations**
- **Coverage of 93% of all cytosine in the genome**

Distribution of DNA methylation in plant genomes

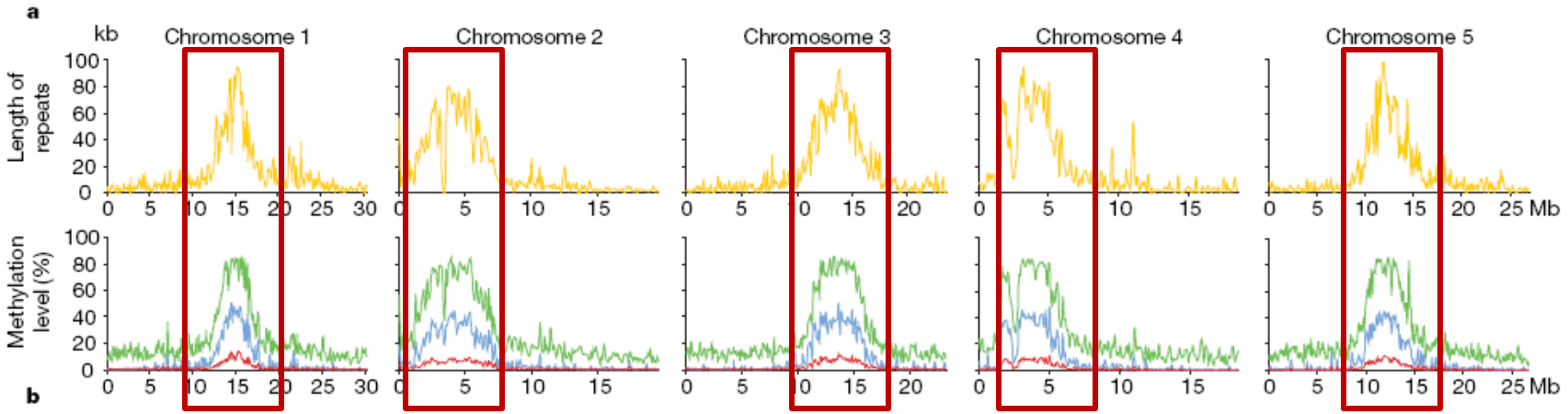
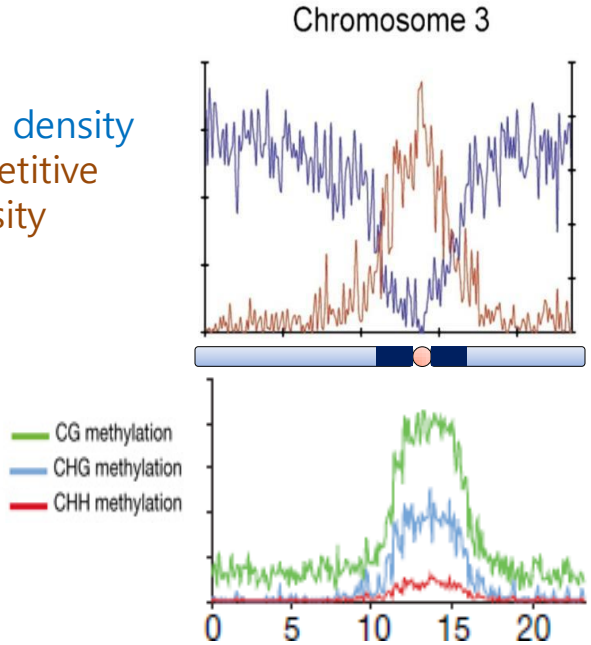


Figure 1 | Methylation of different fractions of the *Arabidopsis* genome. **a**, Chromosome-wide distribution of methylation and correlation with repeats in sliding 100-kb windows. **b**, Methylation levels and siRNA

CG, CHG and CHH methylation were highly correlated, showing enrichment in repeat-rich pericentromeric regions (Fig. 1a),

Distribution of DNA methylation in plant genomes

- BLUE = Gene density
- Brown = Repetitive element density



CG methylation is more abundant in pericentromeric regions, a higher proportion of CHH methylation is found there.

Adapted from American society of Plant Biology, 2016

Distribution of DNA methylation differs in genes and transposons

Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning

Shawn J. Cokus[1*], Suhua Feng[1,2*], Xiaoyu Zhang[1], Zugen Chen[1], Barry Merriman[3], Christian D. Haudenschild[3], Sriharsa Pradhan[3], Stanley F. Nelson[1], Matteo Pellegrini[1] & Steven E. Jacobsen[1,2]

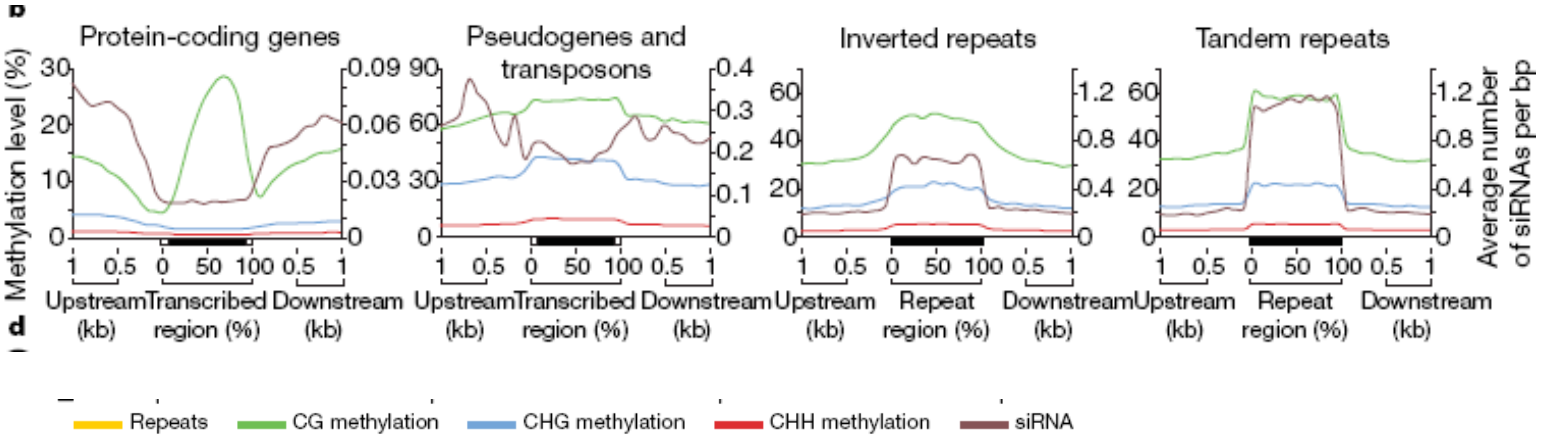
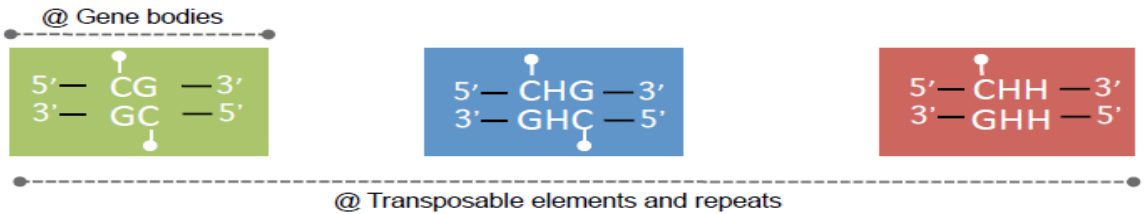
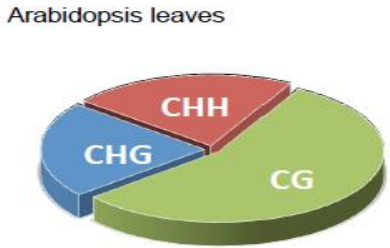


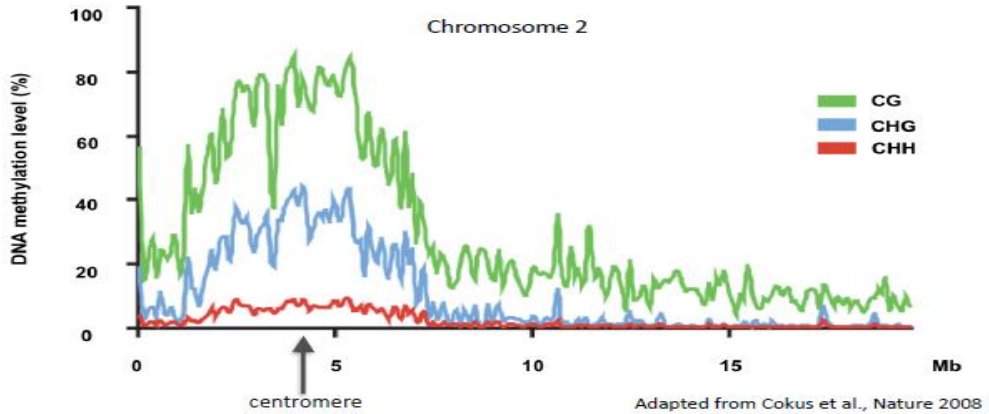
Figure 1 | Methylation of different fractions of the *Arabidopsis* genome. **a**, Chromosome-wide distribution of methylation and correlation with repeats in sliding 100-kb windows. **b**, Methylation levels and siRNA abundance[26] are plotted across different types of repeats and genes. **c**, High levels of methylation are detected at loci corresponding to siRNAs. **d**, Relationship between methylation levels and the length of different types of repeats and genes. **e**, From left to right, methylation levels of the three consecutive cytosines in the $(CCCTAAA)_n$ telomeric repeat unit are calculated in wild type (WT) and the *drm1 drm2 cmt3* mutant, respectively.

Distribution of DNA methylation differs in genes and transposons

(H = T, A or C)



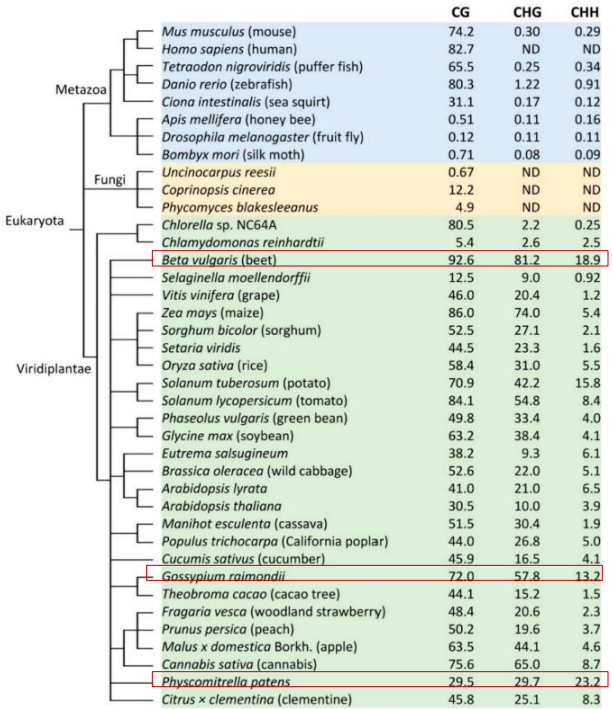




E. Prokhortchouk, P.-A. Defossez / Biochimica et Biophysica Acta 1783 (2008) 2167–2173

4. DNA methylation dynamics and function in plants

DNA methylation levels vary between plant species:
(Whole Genome Bisulfite Sequencing)



	CG	CHG	CHH
Mus musculus (mouse)	74.2	0.30	0.29
Homo sapiens (human)	82.7	ND	ND
Tetraodon nigroviridis (puffer fish)	65.5	0.25	0.34
Danio rerio (zebrafish)	80.3	1.22	0.91
Ciona intestinalis (sea squirt)	31.1	0.17	0.12
Apis mellifera (honey bee)	0.51	0.11	0.16
Drosophila melanogaster (fruit fly)	0.12	0.11	0.11
Bombyx mori (silk moth)	0.71	0.08	0.09
Uncinocarpus reesii	0.67	ND	ND
Coprinopsis cinerea	12.2	ND	ND
Phycomyces blakesleeanus	4.9	ND	ND
Chlorella sp. NC64A	80.5	2.2	0.25
Chlamydomonas reinhardtii	5.4	2.6	2.5
Beta vulgaris (beet)	92.6	81.2	18.9
Selaginella moellendorffii	12.5	9.0	0.92
Vitis vinifera (grape)	46.0	20.4	1.2
Zea mays (maize)	86.0	74.0	5.4
Sorghum bicolor (sorghum)	52.5	27.1	2.1
Setaria viridis	44.5	23.3	1.6
Oryza sativa (rice)	58.4	31.0	5.5
Solanum tuberosum (potato)	70.9	42.2	15.8
Solanum lycopersicum (tomato)	84.1	54.8	8.4
Phaseolus vulgaris (green bean)	49.8	33.4	4.0
Glycine max (soybean)	63.2	38.4	4.1
Eutrema salsugineum	38.2	9.3	6.1
Brassica oleracea (wild cabbage)	52.6	22.0	5.1
Arabidopsis lyrata	41.0	21.0	6.5
Arabidopsis thaliana	30.5	10.0	3.9
Manihot esculenta (cassava)	51.5	30.4	1.9
Populus trichocarpa (California poplar)	44.0	26.8	5.0
Cucumis sativus (cucumber)	45.9	16.5	4.1
Gossypium raimondii	72.0	57.8	13.2
Theobroma cacao (cacao tree)	44.1	15.2	1.5
Fragaria vesca (woodland strawberry)	48.4	20.6	2.3
Prunus persica (peach)	50.2	19.6	3.7
Malus x domestica Borkh. (apple)	63.5	44.1	4.6
Cannabis sativa (cannabis)	75.6	65.0	8.7
Physcomitrella patens	29.5	29.7	23.2
Citrus × clementina (clementine)	45.8	25.1	8.3

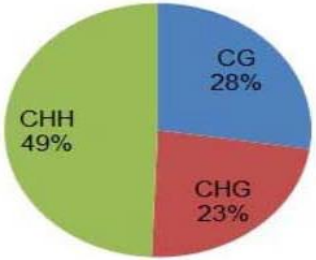
Figure 1. DNA methylation levels in 39 eukaryotic organisms. Although the methylation data were from different studies, and in different organs or tissues that might have some technical variations among different experiments, all of these were from recent methylome research, and procedures and technologies used were similar, including genomic library construction, bisulfite conversion and efficiency, and next-generation sequencing. Taxonomy was obtained from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/taxonomy). Species, materials and methylation data in Figure 1 were from references listed below: *M. musculus*, E13.5 embryos from strain C57BL/6J [45]; *H. sapiens*, H1 human embryonic stem cells [46]; *T. nigroviridis*, whole fish [44]; *D. rerio*, 5-day-old embryos [45]; *C. intestinalis*, *Ciona* animals collected from Half Moon Bay, CA [45]; *A. mellifera*, whole adult workers [44]; *D. melanogaster*, embryo 0–3 h [44]; *B. mori*, whole larvae [44]; *U. reesii*, mycelium [44]; *C. cinereal*, mycelium of strain Okayama 7 [44]; *P. blakesleeanus*, mycelium of strain NRRL 1555 [44]; *Chlorella* sp. NC64A, cells cultured in medium [44]; *C. reinhardtii*, vegetative cells from strain CC503 [45]; *B. vulgaris*, leaf [41]; *S. moellendorffii*, aerial tissues of adult soil plants [44]; *V. vinifera*, leaf [41]; *Z. mays*, kernel [47]; *S. bicolor*, leaf [41]; *S. viridis*, leaf [41]; *O. sativa*, leaf [41]; *S. tuberosum*, tuber tissue [48]; *S. lycopersicum*, leaf [41]; *P. vulgaris*, leaf [41]; *G. max*, fully expanded leaf [39]; *E. salsugineum*, leaf [41]; *B. oleracea*, leaf [41]; *A. lyrate*, leaf [41]; *A. thaliana*, leaf [41]; *M. esculenta*, leaf [41]; *P. trichocarpa*, leaf [41]; *C. sativus*, leaf [41]; *G. raimondii*, leaf [41]; *T. cacao*, leaf [41]; *F. vesca*, leaf [41]; *P. persica*, leaf [41]; *M. domestica*, leaf [41]; *C. sativa*, leaf [41]; *P. patens*, whole plants growing on plates [44]; *C. clementine*, leaf [41]. ND, not determined.


International Journal of Molecular Sciences
MDPI
Review
Dynamic DNA Methylation in Plant Growth and Development

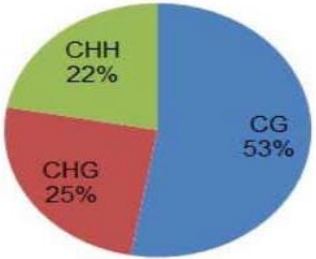
And


Genome Biology
RESEARCH
Open Access
Widespread natural variation of DNA methylation within angiosperms

DNA methylation levels vary between plant species:



Tomato

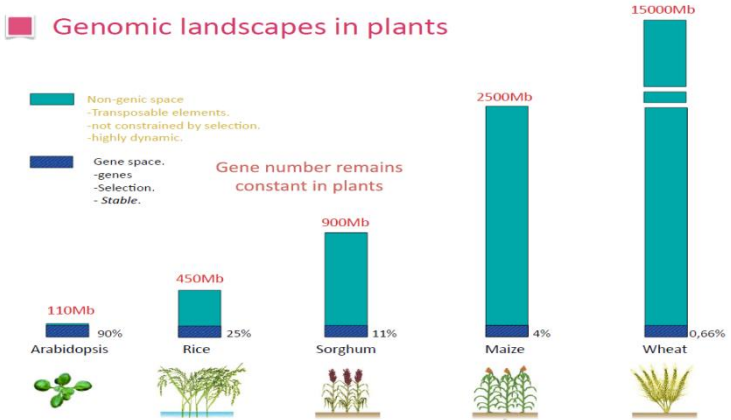


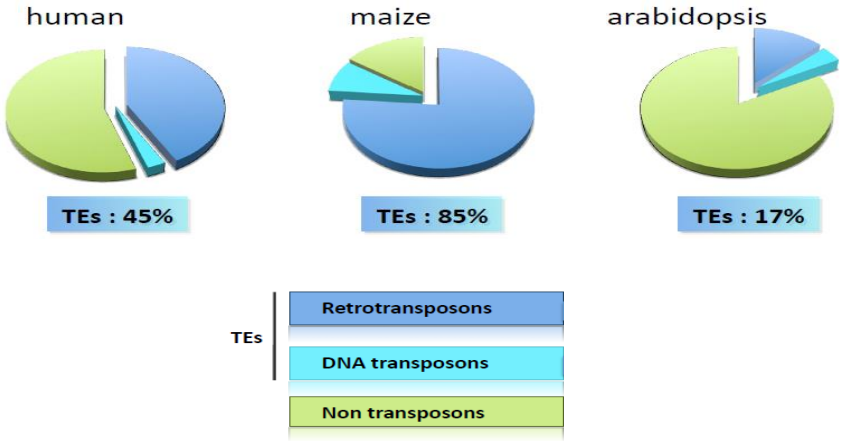
Arabidopsis

Zhong et al, 2013, Nat Biotech

4. DNA methylation dynamics and function in plants

DNA methylation variation between plant species correlates with genome features





After Buisine *et al.*, 2008; Schnable *et al.*, 2009; Cordaux *et* Batzer, 2009

4. DNA methylation dynamics and function in plants

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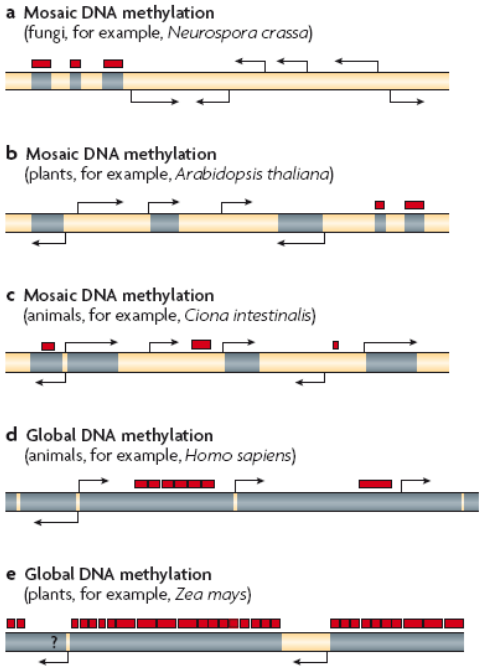


Figure 1 | **DNA methylation landscapes in fungi, animals and plants. a** | Mosaic DNA methylation, whereby stable methylated (grey) and unmethylated (yellow) domains are interspersed, is seen in certain fungi owing to the efficient targeted methylation of transposable elements (red boxes). **b** | The plant *Arabidopsis thaliana* has a small genome and illustrates a mosaic methylation pattern that is due to gene-body methylation, as seen in invertebrates. Unlike animals, transposons and repetitive elements are subject to targeted methylation by an RNA-mediated mechanism of genome defence. **c** | Mosaic methylation is also characteristic of most tested invertebrates, but has only been mapped in detail in the sea squirt *Ciona intestinalis*. Gene-body methylation affects over half of all genes, but the remainder are embedded within unmethylated DNA. Transposable elements are frequently unmethylated and match the methylation status of the surrounding DNA. **d** | Vertebrate genomes are globally methylated, with only CpG islands being unmethylated. Transposable elements are methylated, as are gene bodies and intergenic DNA. **e** | The DNA methylation landscape of plants with large genomes, such as maize, has not been mapped in detail, but it is evident that genes are separated by long tracts of DNA that contain transposable elements and their relics[16]. Genes tend to be unmethylated, but the existence of gene body-targeted methylation has not yet been investigated.

DNA methylation landscapes: provocative insights from epigenomics
Miho M. Suzuki &
Adrian Bird
Nature Reviews Genetics **volume 9**, pages 465–476 (2008)

DNA methylation varies between tissues and developmental stages

Cell Types	CG	CHG	CHH
Vegetative nucleus 1	29.3	15.0	5.4
Vegetative nucleus 2	26.9	13.0	4.0
Sperm cell nuclei 1	31.4	12.3	1.6
Sperm cell nuclei 2	31.2	12.7	2.1
Microspore	28.8	12.5	1.8
Tissues	**CG**	**CHG**	**CHH**
Endosperm	20.9	8.9	2.8
Embryo 1	26.9	10.6	4.4
Embryo 2	27.5	10.7	4.5
Organs	**CG**	**CHG**	**CHH**
Col-0 postmature green seed	25.0	8.5	2.7
Col-0 dry seed	25.9	8.5	2.9
Ws-0 seed at globular stage	23.8	8.0	0.9
Ws-0 postmature green seed	24.3	7.8	1.8
Ws-0 dry seed	25.2	8.4	2.1

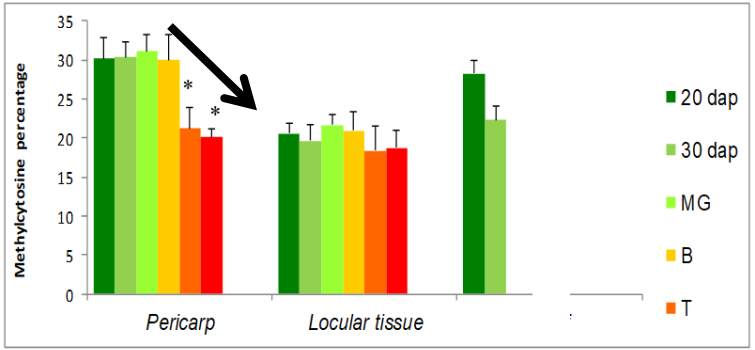
Organs	CG	CHG	CHH
Inflorescence	27.8	12.1	5.8
Shoot 1	22.3	5.9	1.5
Shoot 2 (all tissues of the plant growing above ground)	~21.9	~6.9	~1.8
Leaf 1	30.5	10.0	3.9
Leaf 2	28.5	7.8	1.6
Ws 0 leaf	24.5	5.4	0.8
Rosette leaf 1	32.8	15.7	4.6
Rosette leaf 2	22.1	6.4	2.0
Root (one-month-old)	~21.6	~6.5	~1.8
Whole plant (Five-week-old continuous light-grown)	24.0	6.7	1.7

Figure 2. DNA methylation levels in different organs, tissues, and cells in *Arabidopsis*. Organs, tissues, or cell types were collected from wild type *Col-0* (Columbia) except the organs indicated as *Ws-0*, referring to Wassilewskija. Organs, tissues, cells, and methylation data were from references listed below: vegetative nucleus 1, sperm cell nuclei 1, microspore, embryo 2, and inflorescence [61]; vegetative nucleus 2 and sperm cell nuclei 2 [62]; endosperm and embryo 1 [55]; postmature green seed, dry seed, leaf 2, *Ws-0* seed and leaf [63]; leaf 1 [41]; rosette leaf 1 [64]; Rosette leaf 2 [65]; shoot 1 [45]; shoot 2 and root [53]; whole plant [66]. The methylation data were from different studies that might have some technical variations among different experiments, but procedures and technologies (genomic library construction, bisulfite conversion and efficiency, and next-generation sequencing) used were similar, thus overall results can be compared.

International Journal of Molecular Sciences
MDPI
Review
Dynamic DNA Methylation in Plant Growth and Development
Arthur Bartels [1,†], Qiang Han [1,†], Pooja Nair [1,†], Liam Stacey [1], Hannah Gaynier [1], Matthew Mosley [1], Qi Qing Huang [1], Jacob K. Pearson [1], Tzung-Fu Hsieh [2,], Yong-Qiang Charles An [4] and Wenyan Xiao [1,*]

4. DNA methylation dynamics and functions in plants

DNA methylation varies in a tissue specific manner during organ development in a species dependant manner



Cabernet sauvignon

4. DNA methylation dynamics and functions in plants

Similar organs may present distinct behaviors in plant species: fleshy fruits

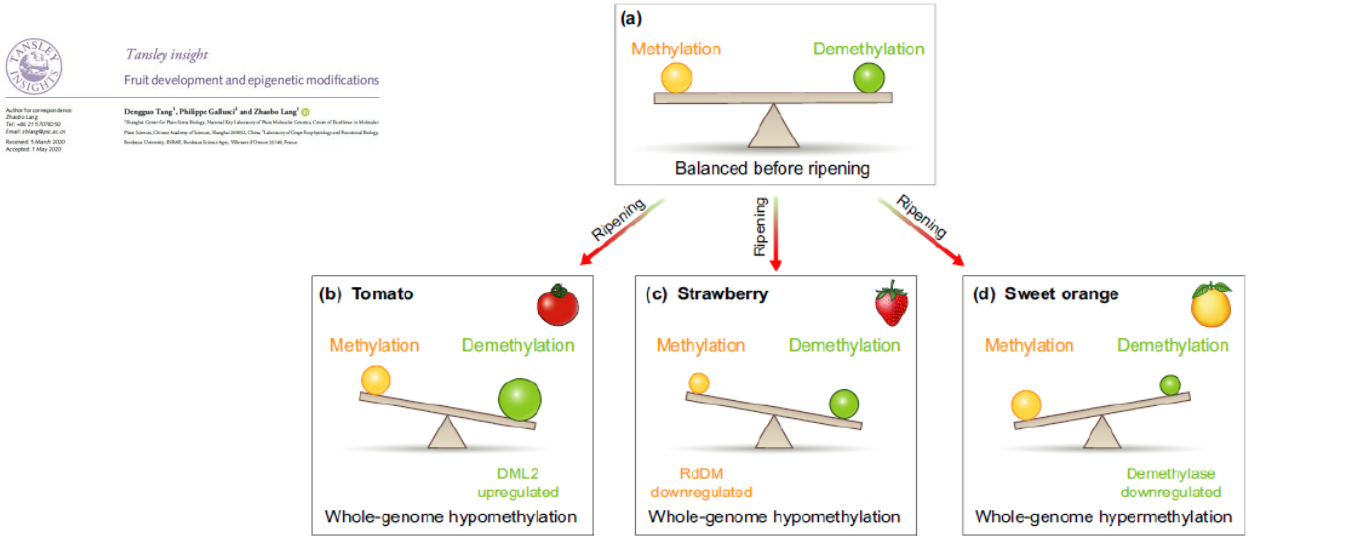
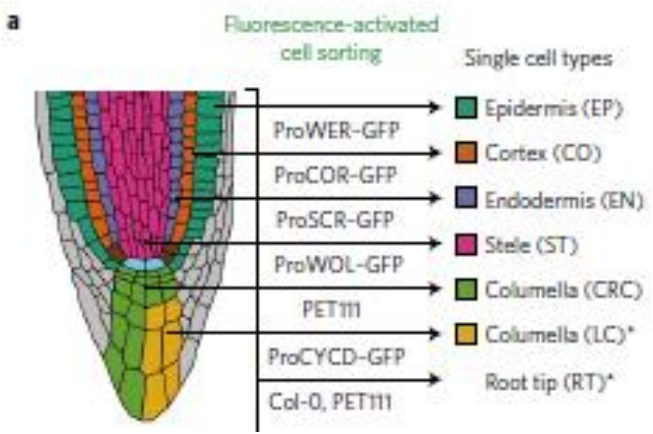


Fig. 1 Changes in the balance between DNA methylation and demethylation during fruit development. (a) The activities of methylation and demethylation reactions are balanced during early fruit development. (b) The balance is disrupted in tomato fruits during ripening. With the increased expression of *SlDML2*, tomato fruits undergo whole-genome hypomethylation, which promotes ripening. (c) Due to the downregulation of RdDM pathway genes during fruit development, strawberry fruits also undergo whole-genome hypomethylation during ripening. (d) Sweet orange fruits undergo whole-genome hypermethylation during development and ripening due to the reduced expression of demethylation genes.

DNA methylation levels vary between cell types in arabidopsis roots



nature plants
ARTICLES
PUBLISHED: 29 APRIL 2016 | ARTICLE NUMBER: 16058 | DOI: 10.1038/NPLANTS.2016.58

Unique cell-type-specific patterns of DNA methylation in the root meristem

Taiji Kawakatsu[1,2,3†], Tim Stuart[4†], Manuel Valdes[5†], Natalie Breakfield[5], Robert J. Schmitz[1,2,6], Joseph R. Nery[2], Mark A. Urich[2], Xinwei Han[5], Ryan Lister[2,4*], Philip N. Benfey[5,7*] and Joseph R. Ecker[1,2,8*]

- Protoplast preparation followed by FACS to isolate cells
- WGBS applied to « enriched cell fractions »

DNA methylation levels vary between cell types in arabidopsis roots

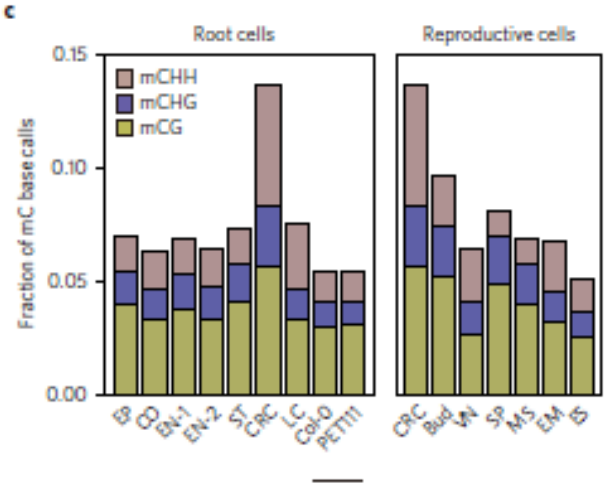


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Figure 1 | Cell-type-specific patterns of DNA methylation in the root meristem. a, Schematic representation of the six root cell types used in this study. *MethylC-seq data only. **b**, A genome browser snapshot showing DNA methylation level, RNA-seq reads and smRNA-seq reads. The endodermis (EN) has two independent replicates (EN-1 and EN-2) for MethylC-seq and RNA-seq. **c**, Global levels of DNA methylation in each context for root and reproductive cells (VN, vegetative nucleus[71]; SP, sperm[71]; MS, microspore[10]; EM, embryo[30]; EN, endosperm[30]). **d**, Heat map showing mC levels within 100 kb bins and genes and TEs within 50 kb bins across the entire genome. Maximum mC levels are 0.91 (mCG), 0.72 (mCHG) and 0.34 (mCHH).

Loss of function of genes encoding DNMTs reveal the importance of DNA methylation in plants

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8449–8454, August 1996
Developmental Biology

Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development

Met1 RNAi plants present numerous phenotypes
- Gene miss-regulation
- TE mobility

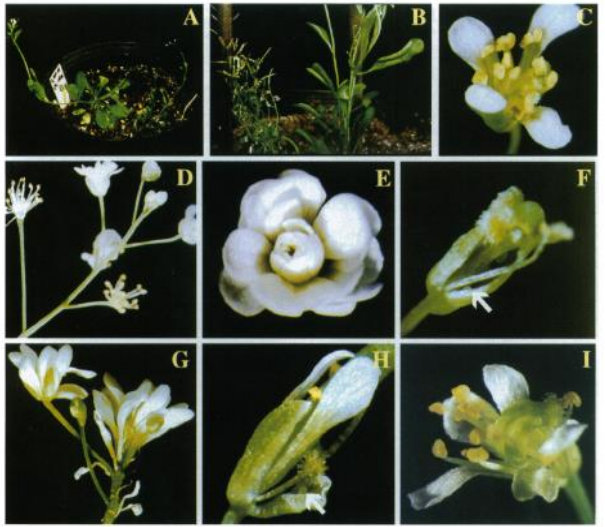


FIG. 3. Phenotypic abnormalities in plants and flowers from methyltransferase antisense plants with reduced levels of DNA methylation. (*A*) Flowering T_2 plants from family 10, both of which carry the antisense construct. The plant on the left has a relatively normal phenotype whereas the plant on the right has decreased apical dominance (is branched), smaller leaves and is greatly reduced in size (reproduced from ref. 30). (*B*) Sibling T_2 plants from family 22–6, both of which carry the methyltransferase antisense. The plant on the left is smaller and bushier than normal, while the plant on the right has leaves which curl to the upper surface. (*C*) A flower from a hemizygous T_3 plant, line 10.1. The organs in the outer two whorls are normal but there are 12 stamens and the carpels of gynoecium (female reproductive organ) are unfused. (*D*) An inflorescence from a homozygous T_4 plant, line 10.5. The early flowers which have extra stamens were followed by flowers with four sepals then petals or staminoid petals in all internal whorls. (*E*) A flower from a hemizygous T_3 plant, line 10.2. This flower has an increased number of organs with four normal sepals then petals or staminoid petals in all the inner whorls. (*F*) A flower from an antisense-null T_4 plant, line 10.2. This flower has no petals and a reduced number of stamens in the third whorl. Two sepals are carpelloid with stigmatic papillae and has ovules on the margin (indicated by an arrow). (*G*) A flower from a homozygous T_4 plant, line 10.5. This flower has an increased number of whorls (note the dried petals, extending down the stem), all organs internal to sepals are petals or staminoid petals, and there are secondary flowers within this flower. (*H*) A flower from a T_3 plant, line 4.1. This flower has a carpelloid sepal with ovules on the margin (indicated by an arrow) and a reduction in the number of organs in whorls two and three. (*I*) A flower from a T_2 plant, line 39.25. This flower has normal organs in the outer two whorls but it has 12 stamens and the carpels of the gynoecium are not fused.

Repression of the tomato DNA demethylase genes SlDML1 and SlDML2 alters multiple aspect of tomato plant development

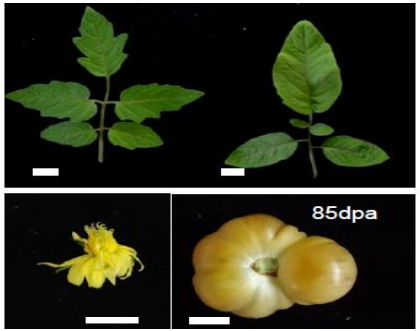
Liu, Teyssier, Gallusci unpublished)

4. DNA methylation dynamics and functions in plants

Repression of the tomato DNA demethylase genes SlDML1 and SlDML2 alters multiple aspect of tomato fruit development

WT

DML



Liu et al, PNAS, 2015

4. DNA methylation dynamics and functions in plants

Natural or induced DNA methylation variations can lead to various developmental phenotypes

Sauvage *cnr*

Manning, Seymour et al, Nat Genet 2006

Sauvage *peloria*

Cubas et al, Nat 1999

And many others

P Kuswandi, L Stammitti, E Teyssier, Y Hong, N Bouché G, Seymour, and P Gallusci, Unpublished

A short summary

- Plant Methylation can occur in various sequence context
  - Symetrical: CG or CHG
  - Non symetrical: CHH

- Plants have high and variable DNA methylation levels that depends on TE content (up to 80%: eg corn, transposon riche genome).

- Methyltransferase are the enzymes seting up DNA methylation; 4 classes of DNA Mtases , only CMTs are plant specific

- Genes can be methylated in their body (CG context; GbM) or in their promoter region (all context).

- Methylation in promoters is in general antagonist to transcription initiation (but not always)

- Transposons are heavily methylated in all sequence context

- Impairment of DNA methylation leads to various plant phenotypes and transposon instability

Can we use DNA methylation variaitons in breeding?