



**HAL**  
open science

## DNA Methylation Readers in Plants

Daniel Grimanelli, Mathieu Ingouff

► **To cite this version:**

Daniel Grimanelli, Mathieu Ingouff. DNA Methylation Readers in Plants. *Journal of Molecular Biology*, 2020, 432, pp.1706 - 1717. 10.1016/j.jmb.2019.12.043 . hal-03489574

**HAL Id: hal-03489574**

**<https://hal.science/hal-03489574v1>**

Submitted on 20 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **DNA Methylation Readers in Plants**

Daniel Grimanelli \* & Mathieu Ingouff \*

Institut de Recherche pour le Développement (IRD), Université de Montpellier, 911  
Avenue Agropolis, 34394 Montpellier, France

Email/ Phone number

mathieu.ingouff@ird.fr, +33 (0)4 67 41 64 30

daniel.grimanelli@ird.fr +33 (0)4 67 41 63 76

## HIGHLIGHTS

- Plants have acquired a diverse set of DNA methylation readers to cope with an extended repertoire of methylated sites.
- This diversity is reflected throughout land-plant evolution.
- While there are evidences for some level of conservation between animals and plants, plants have evolved a number of unique features, included dedicated polymerases and plant-specific protein complexes.
- Plant SUVH proteins can have both demethylase and methyltransferase activity
- Maintenance of CG methylation in plants differs from the canonical animal model, with VIM, the homolog of UHRF1, bearing atypical motifs.

## ABSTRACT

In plants, DNA methylation occurs in distinct sequence contexts, including CG, CHG and CHH. Thus, plants have developed a surprisingly diverse set of DNA methylation readers to cope with an extended repertoire of methylated sites. The *Arabidopsis* genome contains twelve Methyl-Binding Domain proteins (MBD), and nine SET and RING finger-associated (SRA) domain containing proteins belonging to the SUVH clade, in addition to 3 homologues of UHRF1, namely VIM1-3, all containing SRA domains. In this review, we will highlight several research questions that remain unresolved with respect to the function of plant DNA methylation readers, which can have both demethylase, *de novo* and maintenance activity. We argue that maintenance of CG methylation in plants likely involved actors not found in their mammalian counterparts, and that new evidence suggesting significant reprogramming of DNA methylation during plant reproduction as an important new development in the field.

## INTRODUCTION

Proteins that recognize DNA methylation can be separated in distinct classes. First are “*readers*” of DNA methylation, i.e. proteins that contain either Methyl Binding domains (MBD), or SET and RING finger-associated (SRA) domain [1] [2]. Recent data also suggests that Transcription Factors (TF) might have a significant role in “*reading*” DNA methylation [3] [4] [5]. The second classes of proteins are defined as “*writers*”, i.e. proteins with DNA methyl-transferase activity, some of which are plant specific [6] [7] [8] [9] [10]. Finally, “*erasers*” are proteins that can actively demethylate cytosine residues on the DNA molecule. While plants do not have homologues of Ten-Eleven Translocation (TET) DNA demethylases [reviewed in 11], they have developed dedicated proteins to perform such function via the Base Excision Repair (BER) pathway (reviewed in [9]).

## READERS OF DE NOVO METHYLATION

In plants, DNA methylation can occur in three sequence contexts: CG, CHG (which are symmetrical sites) and CHH (where H= A, T or C, which is considered asymmetrical) (reviewed in [7] [8] [9] [10]; Figure 1). The first step in the establishment of *de novo* DNA methylation patterns is performed by the RNA-dependent DNA methylation (RdDM) pathway, which relies on specialized, plant-specific RNA polymerases NRPD (or POLIV), NRPE (or POLV), and a recently identified grass-specific NRPF (or POLVI) to establish DNA methylation in all three sequence contexts, all of which evolved from POLII subunits [12] [13] [14]. RNA POLYMERASE IV (POLIV) generates short (26 to 45 nt) single-stranded RNAs (ssRNAs) from its templates, which then serves a substrate for RNA-DEPENDENT RNA POLYMERASE 2 (RDR2) that interacts with POLIV and converts them into

double stranded RNA (dsRNA). Pol IV transcripts were recently shown to be processed following a one precursor - one siRNA model [15] [16]. The dsRNAs are then processed into 24 nucleotides (24-nt) small interfering RNAs (siRNAs) by DICER-LIKE 3 (DCL3), methylated at their 3'-end by HUA ENHANCER 1 (HEN1) and subsequently recruited by ARGONAUTE 4 (AGO4) [17], or other ARGONAUTE members of the RdDM clades, AGO6 and AGO9 [18]. AGO4-siRNA complexes are then targeted to transcripts generated by POLV, via a RNA-RNA pairing interaction between the siRNA present in the complex, and the POLV-dependent transcript. The AGO4-siRNA complexes then recruit DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 1 and 2 (DRM1, DRM2) to the target DNA [19] [20].

The positioning of POLIV and POLV at their target loci is not well understood. POLIV can be recruited to loci harboring chromatin with methylated residues in the Lysine 9 of Histone 3 by interacting with SAWADEE HOMEODOMAIN HOMOLOG 1 (SHH1) [21] [22], which binds H3K9me2. Recent data in *Arabidopsis* also indicates that all members of SNF2-related, putative chromatin remodeling factors CLASSY (CLSY1-4) are necessary for the association between SHH1 and POLIV, and for the production of most 24-nt siRNAs [23]. Interestingly, members of the CLSY family can have a dual function of methylation and demethylation activity at specific loci where DNA methylation depends on RdDM [24]. POLV also interacts with DEFECTIVE IN RNA DIRECTED DNA METHYLATION 1 (DRD1), DEFECTIVE IN MERISTEM SILENCING 3 (DMS3), and RNA-DIRECTED DNA METHYLATION 1 (RDM1), to produce long noncoding scaffold transcripts [25] [26] [27]. This complex in turn recruits two histone methyltransferases of the SUVH family, SUVH2 and SUVH9, both of which contain SRA domains and thus can bind DNA, but have lost

methyltransferase activity [28]. Thus, SUVH2 and 9 act as *bona fide* “readers” of *de novo* methylation. Interestingly, both enzymes have lost their post-SET domain, suggesting that their mode of action relies on indirect interactions with chromatin. Two-hybrid screens indeed suggest that the Microrchidia (MORC) adenosine triphosphatase (MORC6), which is thought to act downstream of DNA methylation [29] [30], acts as a bridge between SUVH9 and the INVOLVED IN DE NOVO 2 (IDN2) [31] [32].

## **READERS OF MAINTENANCE METHYLATION**

Once established, DNA methylation patterns are maintained and transmitted across mitotic divisions by distinct mechanisms. Members of the family VARIANT IN METHYLATION 1-6 (VIM1-6, but only VIM1-3 appear required for maintenance of DNA methylation [33] [34]) ensure the maintenance of methylation at CG sites by binding hemi-methylated CG on the parental strand and recruiting METHYLTRANSFERASE 1 (MET1) to methylate the complementary CG sites on the daughter strand. This model is based on functional evidences obtained from the rather well-conserved mammalian homologues, the DNA methyltransferase DNMT1 and the SRA-domain containing protein UHRF1 (Figure 2). Interestingly, the VARIANT IN METHYLATION (VIM) family of protein is rather atypical, containing two Really Interesting New Gene domains (RING), one Plant Homeodomain, and a SRA domain. All VIM members show ubiquitin ligase activity *in vitro*, similar to UHRF1, via intermediates of the UBIQUITIN CONJUGATING ENZYME (UBC) family [35], but do not contain the Tandem TUDOR domains found in UHRF1, which ensures binding to H3K9me<sub>2/3</sub>, while the PHD domain mostly recognizes H3R2 [36] [37] [38]. Its PHD domain, however, appears sufficient to promote association with several of the core

histones, including H2B, H3 and H4 [39]. In *Arabidopsis*, H3K9me2 is mostly restricted to peri-centromeric heterochromatin; thus, VIM proteins do not necessarily require a direct interaction with histones to ensure faithful maintenance of hemimethylated CG, as the PHD domain might prove sufficient for such function. Yet, identifying a putative TUDOR domain associating with VIM proteins, using either yeast 2-hybrid screens or co-precipitation experiments should be a key priority in field. This would provide strong evidence for an alternative model, where VIM proteins recruit a TUDOR domain, capable of binding H3K9me2, the classical mark associated with heterochromatin in plants. A recent paper [40] indeed suggests that both MET1 and CMT3 are independently required for the establishment of CHH methylation, representing a roughly 20% overlap with a *cmt2* null mutant, and up to 10% in a *cmt3* null background. Similar results had been previously reported [41], thus adding credence to the concept that MET1 might have significant function outside of CG maintenance. Three additional MET1 homologs are present in *Arabidopsis* and are expressed in reproductive tissues [42]. Their contribution to CG methylation maintenance pathway is unclear. Taken together, the evidence thus suggests critical differences in the mechanisms of CG maintenance between plants and animals.

Maintenance of DNA methylation at CHG site requires the activity of CHROMOMETHYLASE 3 (CMT3) [43]. At CHH sites, this maintenance activity is controlled by CHROMOMETHYLASE 2 (CMT2) [44] [44]. CHROMOMETHYLASES are plant-specific, and are thus at the same time “readers” of histone methylation and “writers” of DNA methylation. They contain both a chromodomain and a DNA methyltransferase domain, and interact with several members of the SUVH clade, namely SUVH4, 5 and 6, all of which contain a SRA domain and histone



methyltransferase activity, to generate a feedback loop that ensure the proper deposition of both H3K9me2 and either CHG or CHH methylation at Transposable Elements (TEs). SUVH4 (KYP) [46], is responsible for the bulk of H3K9me2 deposition, but only in a triple mutant *suvh4suvh5suvh6* is the majority of H3K9me2 erased from somatic cells in *Arabidopsis* [47].

### **MDB PROTEINS AS READER OF DNA METHYLATION IN PLANTS.**

*Arabidopsis* contains twelve proteins with MBD domains (Figure 3), with INCREASE IN DNA METHYLATION 1 (IDM1) bearing a non-canonical MBD domain [48]. Most of them remain poorly characterized, but *in vitro* data suggest that at least three of them (MBD5, 6 and 7) can bind symmetrically methylated DNA [49] [50] [51]. Both MBD5 and 6 are thus considered “readers” of DNA methylation, or functional MBD proteins, and have been shown to be involved in rDNA silencing [52].

MBD7 exists as part of a large protein complex containing conserved  $\alpha$ -crystallin domain (ACD), and associate with INCREASED DNA METHYLATION 3 (IDM3), INCREASED DNA METHYLATION 2-LIKE 1 (IDL1), IDM1/ROS4, ROS5/IDM2, and HARBINGER TRANSPOSON-DERIVED PROTEIN 1 (HDP1) and HDP2 *in planta*, and acts as an anti-silencing factor in *Arabidopsis* [53] [54] [55] [56] [57]. Whether the complex acts upstream or downstream of DNA methylation remains controversial, and recent data suggest that MBD7 in fact has limited effect on DNA methylation overall [58]. Interestingly, MBD5 interacts with a distinct subset of ACD-containing proteins, and weakly with MBD6, suggesting that they likely perform distinct function [58]. MBD9, via its Bromo Domain, is also an active DNA demethylase, interacting with both PIE1, ARP6, IDM1 and ROS1 to facilitate H2A.Z deposition at IDM1 targets

[59]. Thus, MBD proteins in plants can act both as *bona fine* “readers”, but also as “erasers” of DNA methylation [10].

There are 14 MBD genes in maize (*Zea mays* L.) [61], and 17 in rice (*Oryza sativa* ssp. *Japonica*) [62]. A phylogenetic analysis [61] showed that the atMBD5, 6 and 7 are specific to dicots, and not found in grass species, raising some questions as to whether grasses have retained functional MBD proteins. Possibly, these proteins might be involved in either binding to unmethylated DNA [59], RNA [60] or in mediating protein–protein interactions [52]. Since none of grass MBD proteins have been tested so far with respect to their ability to bind either symmetrical or hemimethylated DNA, it remains an open question as whether they are functionally active proteins. Again, this would indicate that significant divergence occurred during speciation and a clear dichotomy between dicots and grasses [63].

## **SUVH PROTEINS CAN HAVE BOTH HISTONE METHYLTRANSFERASE ACTIVITY AND DEMETHYLASE ACTIVITY IN PLANTS**

The SUPPRESSOR OF VARIATION [SU(VAR)] HOMOLOG (SUVH) family of proteins form a rather complex clade in plants (Figure 4), all of which contain SRA domains. Rice contains twelve SUVH homologues [62], and maize eleven [63]. While most of our knowledge on SUVH proteins is currently derived from *Arabidopsis*, which contains 9 SUVH members, several trends have emerged. First, SUVH members can have both repressive and activating effects [43] [44] [45] [46] [47] [65] [66] [67]. A recent paper showed that SUVH1 and SUVH3 co-localize perfectly with targets of the RNA directed DNA methylation pathway (RdDM), acting as anti-silencing factor in complex with two DNAJ domain-containing homologs, DNAJ1 and DNAJ2, to counteract the potential negative effect of TE insertion near genes [66].

Another recently published paper provided further proof that indeed, SUVH1 and SUVH3 act as anti-silencing factors [67]. In both scenarios, those proteins act downstream of RdDM, have limited impact on maintenance methylation, but are essential to ensure the accessibility of the regulatory machinery to genes located in close proximity to proliferating Transposable Elements (TEs) [68] [69] [70] [71].

On the other hand, SUVH proteins also have strong repressive effects on TEs [46] [64] [65]. An interesting recent paper [72], dissecting more precisely the contribution of the various SUVH and CMT proteins to the tri-nucleotides specific for CGN, CHG and CHH found that in *Arabidopsis*, which contains three members of the CHROMOMETHYLASE (CMT) family (CMT1, whose function remains undetermined), CMT3 [43] and CMT2 [44] [45]). CMT3 in association with SUVH4, is mostly responsible for maintenance of methylation at CCG/CGG and CAG/CTG sites, while SUVH5 and 6 collaborate to maintain methylation at sites carrying CTA or CAA methylation. As maize does not have a CMT2 homologue [74], two CMT-like proteins, ZMET1 and ZMET2 contribute to ensure faithful transmission of CHG and CHH methylation in most contexts, with a clear preference for the CAA and CTA contexts, similar to *Arabidopsis*.

Surprisingly, CMT members, and in particular CMT3, seem to have an important role in determining gene body methylation (gbM) in plants [73] [74]. This role was recently confirmed by artificially inducing gain-of-function CMT3 expression in *Eutrema salsugineum*, a plant species devoid of gbM [75]. The data indicates that ectopic gbM methylation at CG sites was preferentially maintained relative to genic CHG or CHH methylation following the loss of AtCMT3 expression, suggesting that CMT3 is active irrespective of the presence of either H3K9me2 or DNA methylation in the body of genes [75].

Similar experiments using gain-of-function induction of DNA methylation at the FLOWERING IN WAGENINGEN (*FWA*) locus, which displays a classical “early flowering phenotype”, suggest a clear hierarchy in the RdDM pathway [76]. Interestingly, the results confirmed that targeting MORC6 to the *FWA* locus using an artificial Zinc Finger (ZF) approach can induce DNA methylation even in a background deficient for the *suvh2suvh9* proteins. This indicates that MORC6 can act independently of SUVH9 and 2 [31] [32], suggesting that they might be dispensable as “readers”. More surprisingly, DMS3-ZF is capable of targeting DNA methylation at *FWA* even in the absence of siRNAs. The authors also showed that DMS3-ZF requires the bulk of the RdDM dependent AGO proteins, as the induction of DNA methylation is severely compromised in both an *ago4* background, and an *ago469* triple mutant, suggesting that at least one ARGONAUTE of the AGO4/6/9 clade is essential for DMS3-targeted methylation. Thus, DMS3-ZF might act in an AGO-dependent, siRNA independent manner. How these ARGONAUTE proteins actually “read” DNA methylation remains to be determined. Lastly, targeting both POLIV and POLV (via DMS3-ZF) to chromatin results in several phenotypic abnormalities, and significant increases in DNA methylation in proximity of the transcription start sites [76]. So what can we learn from such experiments? First, because the targeted proteins include a ZF motif, which would facilitate access to chromatin, they are likely ‘self-sufficient’ in their potential to target chromatin. They are also gain-of function experiments, and as such might bypass some of the key enzymes involved in RdDM.

Finally, RdDM can also be achieved through alternative routes. While they all depend extensively on the RdDM pathway, and always require associated small RNAs, they need both DRM2 and POLV to direct siRNA to chromatin [77], and can be divided in

a number of independent pathways, including: (i) the RDR6-RdDM pathway that targets transcriptionally active TEs and rely on POLII-generated transcripts (reviewed in [10] [78]), the (ii) the RDR6-DCL3-RdDM pathway that targets high-copy TEs and transgenes [79], the (iii) the POLIV-NERD pathway where the NEEDED FOR RDR2 INDEPENDENT METHYLATION (NERD)-mediated interaction of AGO2 with POLIV and POLV, requires siRNAs whose biogenesis depends on canonical and non-canonical components, RDR1, RDR6, DCL2, DCL3 and DCL4 [80] and (iv) the DICER independent RdDM pathway that targets TEs and relies on the generation of both 21 and 24 nt siRNAs from dsRNA bound to AGO4 [81] [82].

## **REPROGRAMMING OF DNA METHYLATION DURING PLANT REPRODUCTION**

Plants do not possess homologues of Ten-Eleven Translocation (TET) DNA demethylases [11], but there is substantial evidence that DNA demethylation occurs through a plant-specific family of DNA glycosylases (DEMETER, DME and DEMETER-like (DML)) that play an important role during plant reproductive development (reviewed in [83] [84]). In particular, the vegetative nucleus in the male gametophyte and the central cell are the target of the DEMETER DNA glycosylase (DME), and other homologs of demethylase family, including ROS1 (DML1), DML2 and DML3 [85] [86] [87] [88] [89] [90] [91]. The vegetative cell, the companion cell of sperms, undergoes DEMETER-dependent demethylation. Consequently, lack of DME activity in the vegetative cell causes a reduction of small RNA-directed DNA methylation of transposons in the sperms. There is also substantial evidence that CHH methylation, while lost from TEs in microspores, are restored by de novo DNA methyltransferase activity, guided by 24-nt small interfering RNA in the embryo after fertilization. This was recently confirmed in *Arabidopsis* using whole genome bisulfite

sequencing (WGBS) of embryos at different stages of their live cycle [93] [94], and recently in both rice and *Arabidopsis* by comparing non-CG methylation patterns in sperm cells, egg cells and early embryos [92]. Live cell imaging during reproduction further suggests that reprogramming relies on a non-canonical form of RdDM [95]. While the mechanisms underlying such reprogramming remains unclear, due the difficulty in accessing reproductive cells in *Arabidopsis*, there is a growing consensus that plant gametes are indeed capable of reprogramming, similar to mammalian germ cells [83] [84].

## **LAND PLANTS HAVE REPEATEDLY EVOLVED DIFFERENT READERS TO ENSURE MAINTENANCE OF DNA METHYLATION IN ALL THREE SEQUENCE CONTEXTS**

Land plants show significant levels of variation in their methylation patterns [96] [97], including many species lacking gbM. This suggests that different mechanisms are likely acting to ensure proper propagation and/or maintenance of DNA methylation across species. In tomato, for example, siRNA essentially target genic regions, but not pericentromeric heterochromatin [98], and gets redistributed in a DECREASE IN DNA METHYLATION (DDM1) double mutant [99], indicative of significant differences in either the role of these small RNAs, or the relative importance of RdDM in different species. Similarly, *Marchantia polymorpha*, a very basal plant harbor several AGO proteins, but also proteins typically detected in animal reproduction, including two PIWI proteins, PIWIa and PIWIb that lack a discernable PAZ domain but contain a conserved PIWI domain, and are highly enriched in antheridiophores [100] [101], but also protamines, similar to animals [102]. *M. polymorpha* contains an unique homolog of MET1, which shows a methylation phenotype similar to the *Arabidopsis*

mutant [103]. Interestingly, *M. polymorpha* undergoes an extensive reprogramming of DNA methylation, suggesting once more that the process occurred long before the emergence of angiosperms [104]. *P. patens* also harbors two homologs of the DNMT3 methyltransferase found in most animals. These can induce *de novo* methylation of cytosines within heterochromatin, in association with the single CMT found in the *P. patens* genome, while the RdDM pathway in moss is restricted to the euchromatic compartment [105]. Again, these data suggest a surprising diversification of methylation maintenance among land plants, consistent with highly divergent modes of either *de novo* establishment, maintenance, or demethylation.

## **PERSPECTIVES**

Much remains to be determined regarding the function of DNA methylation readers in plants. A classical review, published in 2010 by Law and Jacobsen [7], suggested a significant amount of conservation in the mechanisms of maintenance and establishment between plants and animal models. While several of the conclusions in the review have indeed been confirmed in recent years, including the importance of siRNA for targeting methylation in plants via either the RdDM pathway or alternative routes, and the importance of methyl-binding proteins in this process, a number of key questions remain unsolved. Among these, the fact that VIM/UHRF1 likely play different roles in the maintenance of DNA methylation at CG sites. Second, it is becoming clear that land plants show significant levels of variation in their methylation patterns [95] [96], again indicating that different mechanisms are likely acting to ensure proper propagation and/or maintenance of DNA methylation across species. Two independent papers, published in 2010, compared profiles of DNA methylation in eukaryotes [106] [107]. Yet, the function of DNA methylation readers in

plants remains rather elusive. The presence of non-CG methylation is a hallmark of plant DNA methylation, as shown by both papers. Interestingly, similar patterns have been recently reported in the brain [108] [109], and using single-cell methylomes in oocytes [110]. Thus, while there is some evidence for partial conservation of the core mechanisms of DNA establishment and maintenance, it still remains an open question whether identical actors are acting in both animals and plants. Future directions for research will likely focus on determining the core elements that differentiate plants and animals with respect to maintenance of CG methylation, the role of siRNAs in *de novo* targeting, and the role of epigenetic reprogramming within plant germ cells.

## REFERENCES

1. I.O. Torres, D.G. Fujimori, Functional coupling between writers, erasers and readers of histone and DNA methylation, *Curr. Opin. Struct. Biol.* (2015) 68–75. doi:10.1016/j.sbi.2015.09.00
2. Q. Du, P.L. Luu, C. Stirzaker, S.J. Clark, Methyl-CpG-binding domain proteins: Readers of the epigenome, *Epigenomics.* (2015). doi:10.2217/epi.15.39.
3. H. Zhu, G. Wang, J. Qian, Transcription factors as readers and effectors of DNA methylation, *Nat. Rev. Genet.* (2016) 551–565. doi:10.1038/nrg.2016.83.
4. B.H. Kribelbauer JF, Lu XJ, Rohs R, Mann RS, Towards a mechanistic understanding of DNA methylation readout by transcription factors, *J Mol Biol.* (2019) 30617–5.
5. R.C. O'Malley, S.S.C. Huang, L. Song, M.G. Lewsey, A. Bartlett, J.R. Nery, M. Galli, A. Gallavotti, J.R. Ecker, Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape, *Cell.* (2016) 1280–1292. doi:10.1016/j.cell.2016.04.038.
6. F. Lyko, The DNA methyltransferase family: A versatile toolkit for epigenetic regulation, *Nat. Rev. Genet.* (2018) 81–92. doi:10.1038/nrg.2017.80.



7. J.A. Law, S.E. Jacobsen, Establishing, maintaining and modifying DNA methylation patterns in plants and animals, *Nat. Rev. Genet.* (2010). doi:10.1038/nrg2719.
8. M. A. Matzke, R. A. Mosher, RNA-directed DNA methylation: An epigenetic pathway of increasing complexity, *Nat. Rev. Genet.* 15 (2014) 394–408. doi:10.1038/nrg3683
9. M.A. Matzke, T. Kanno, A.J.M. Matzke, RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants, *Annu. Rev. Plant Biol.* 66 (2015) 243–267. doi:10.1146/annurev-arplant-043014-114633.
10. H. Zhang, Z. Lang, J.K. Zhu, Dynamics and function of DNA methylation in plants, *Nat. Rev. Mol. Cell Biol.* (2018) pages489–506. doi:10.1038/s41580-018-0016-z
11. X. Wu, Y. Zhang, TET-mediated active DNA demethylation: Mechanism, function and beyond, *Nat. Rev. Genet.* (2017) 517–534. doi:10.1038/nrg.2017.33.
12. J.R. Haag & C.S. Pikaard, Multisubunit RNA polymerases IV and V: purveyors of non-coding RNA for plant gene silencing, *Nature Reviews Molecular Cell Biology.* 12 (2011) 483–492.
13. M. Zhou, J.A. Law, RNA Pol IV and V in gene silencing: Rebel polymerases evolving away from Pol II's rules, *Curr. Opin. Plant Biol.* 27 (2015) 154–164. doi:10.1016/j.pbi.2015.07.005.
14. J.T. Trujillo, A.S. Seetharam, M.B. Hufford, M.A. Beilstein, R.A. Mosher, M.R. Trujillo JT, Seetharam AS, Hufford MB, Beilstein MA, Evidence for a Unique DNA-Dependent RNA Polymerase in Cereal Crops, *Mol. Biol. Evol.* 35 (2018) 2454–2462. doi:10.1093/molbev/msy146.
15. T. Blevins, R. Podicheti, V. Mishra, M. Marasco, J. Wang, D. Rusch, H. Tang, C.S. Pikaard, Identification of pol IV and RDR2-dependent precursors of 24 nt siRNAs guiding de novo DNA methylation in arabidopsis, *Elife.* 4 (2015). doi:10.7554/eLife.09591.
16. J. Zhai, S. Bischof, H. Wang, S. Feng, T.F. Lee, C. Teng, X. Chen, S.Y. Park, L. Liu, J. Gallego-Bartolome, W. Liu, I.R. Henderson, B.C. Meyers, I. Ausin, S.E. Jacobsen, A one precursor one siRNA model for pol IV-dependent siRNA biogenesis, *Cell.* 163 (2015) 445-455. doi:10.1016/j.cell.2015.09.032.
17. D. Zilberman, X. Cao, S.E. Jacobsen, ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation, *Science.* 299 (2003) 716-719. doi:10.1126/science.1079695.

18. E.R. Havecker, L.M. Wallbridge, T.J. Hardcastle, M.S. Bush, K.A. Kelly, R.M. Dunn, F. Schwach, J.H. Doonan, D.C. Baulcombe, The arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci, *Plant Cell*. 22 (2010) 321–334. doi:10.1105/tpc.109.072199.
19. X. Cao, S. E. Jacobsen, Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. *Proc. Natl. Acad. Sci. U.S.A.* 99 (Suppl 4), 16491–16498 (2002). doi:10.1073/pnas.162371599.
20. Z. Gao, H.L. Liu, L. Daxinger, O. Pontes, X. He, W. Qian, H. Lin, M. Xie, Z.J. Lorkovic, S. Zhang, D. Miki, X. Zhan, D. Pontier, T. Lagrange, H. Jin, A.J.M. Matzke, M. Matzke, C.S. Pikaard, J.K. Zhu, An RNA polymerase II-and AGO4-associated protein acts in RNA-directed DNA methylation, *Nature*. 465 (2010) 106–109. doi:10.1038/nature09025.
21. J.A. Law, J. Du, C.J. Hale, S. Feng, K. Krajewski, A.M.S. Palanca, B.D. Strahl, D.J. Patel, S.E. Jacobsen, Polymerase IV occupancy at RNA-directed DNA methylation sites requires SHH1, *Nature*. 498 (2013) 385–389. doi:10.1038/nature12178.
22. H. Zhang, Z.Y. Ma, L. Zeng, K. Tanaka, C.J. Zhang, J. Ma, G. Bai, P. Wang, S.W. Zhang, Z.W. Liu, T. Cai, K. Tang, R. Liu, X. Shi, X.J. He, J.K. Zhu, DTF1 is a core component of RNA-directed DNA methylation and may assist in the recruitment of Pol IV, *Proc. Natl. Acad. Sci. U. S. A.* (2013) 8290–8295. doi:10.1073/pnas.1300585110.
23. M. Zhou, A.M.S. Palanca, J.A. Law, Locus-specific control of the de novo DNA methylation pathway in Arabidopsis by the CLASSY family, *Nat. Genet.* 50 (2018) 865–873. doi:10.1038/s41588-018-0115-y.
24. D.L. Yang, G. Zhang, L. Wang, J. Li, D. Xu, C. Di, K. Tang, L. Yang, L. Zeng, D. Miki, C.G. Duan, H. Zhang, J.K. Zhu, Four putative SWI2/SNF2 chromatin remodelers have dual roles in regulating DNA methylation in Arabidopsis, *Cell Discov.* (2018). doi:10.1038/s41421-018-0056-8.
25. J.A. Law, I. Ausin, L.M. Johnson, A.A. Vashisht, J.K. Zhu, J.A. Wohlschlegel, S.E. Jacobsen, A Protein Complex Required for Polymerase V Transcripts and RNA- Directed DNA Methylation in Arabidopsis, *Curr. Biol.* 20 (2010) 951–956. doi:10.1016/j.cub.2010.03.062.

26. T. Kanno, M.F. Mette, D.P. Kreil, W. Aufsatz, M. Matzke, A.J.M. Matzke, Involvement of putative SNF2 chromatin remodeling protein DRD1 in RNA-directed DNA methylation, *Curr. Biol.* 54 (2004) 793-804. doi:10.1016/j.cub.2004.04.037.
27. W. Liu, S. H. Duttke, J. Hetzel, M. Groth, S. Feng, J. Gallego-Bartolome, Z. Zhong, H. Y.Kuo, Z. Wang, J. Zhai, J. Chory, S. E. Jacobsen, RNA-directed DNA methylation involves co-transcriptional small-RNA-guided slicing of polymerase V transcripts in Arabidopsis, *Nat. Plants.* 4 (2018) 181–188. doi:10.1038/s41477-017-0100-y Medline
28. L.M. Johnson, J. Du, C.J. Hale, S. Bischof, S. Feng, R.K. Chodavarapu, X. Zhong, G. Marson, M. Pellegrini, D.J. Segal, D.J. Patel, S.E. Jacobsen, SRA-and SET-domain-containing proteins link RNA polymerase v occupancy to DNA methylation, *Nature.* 507 (2014) 124–128. doi:10.1038/nature12931.
29. G. Moissiard, S.J. Cokus, J. Cary, S. Feng, A.C. Billi, H. Stroud, D. Husmann, Y. Zhan, B.R. Lajoie, R.P. McCord, C.J. Hale, W. Feng, S.D. Michaels, A.R. Frand, M. Pellegrini, J. Dekker, J.K. Kim, S.E. Jacobsen, MORC family ATPases required for heterochromatin condensation and gene silencing, *Science.* 336 (2012) 1448–1451. doi:10.1126/science.1221472.
30. T.R. Brabbs, Z. He, K. Hogg, A. Kamenski, Y. Li, K.H. Paszkiewicz, K.A. Moore, P. O'Toole, I.A. Graham, L. Jones, The stochastic silencing phenotype of Arabidopsis morc6 mutants reveals a role in efficient RNA -directed DNA methylation, *Plant J.* 75 (2013) 836-846. doi:10.1111/tpj.12246.
31. Z.W. Liu, C.R. Shao, C.J. Zhang, J.X. Zhou, S.W. Zhang, L. Li, S. Chen, H.W. Huang, T. Cai, X.J. He, The SET Domain Proteins SUVH2 and SUVH9 Are Required for Pol V Occupancy at RNA-Directed DNA Methylation Loci, *PLoS Genet.* 10 (2014). doi:10.1371/journal.pgen.1003948.
32. Y. Jing, H. Sun, W. Yuan, Y. Wang, Q. Li, Y. Liu, Y. Li, W. Qian, SUVH2 and SUVH9 Couple Two Essential Steps for Transcriptional Gene Silencing in Arabidopsis, *Mol. Plant.* 9 (2016) 1156–1167. doi:10.1016/j.molp.2016.05.006
33. H.R. Woo, O. Pontes, C.S. Pikaard, E.J. Richards, protein required for centromeric heterochromatinization, *Genes Dev.* 1 (2007) 267–277. doi:10.1101/gad.1512007.Bestor.

34. H.R. Woo, T.A. Dittmer, E.J. Richards, Three SRA-domain methylcytosine-binding proteins cooperate to maintain global CpG methylation and epigenetic silencing in Arabidopsis, *PLoS Genet.* 4 (2008), 8. doi:10.1371/journal.pgen.1000156.
35. E. Kraft, M. Bostick, S.E. Jacobsen, ORTHO/VIM proteins that regulate DNA methylation are functional ubiquitin E3 ligases. *Plant J.* 56 (2008) 704–715.
36. S. Harrison, E. M. Cornett, D. Goldfarb, P. A. DaRosa, Z. M. Li, F. Yan, B. M. Dickson, A. H. Guo, D. V. Cantu, L. Kaustov, P. J. Brown, C. H. Arrowsmith, D. A. Erie, M. B. Major, R. E. Klevit, K. Krajewski, B. Kuhlman, B. D. Strahl, S. B. Rothbart, Hemi-methylated DNA regulates DNA methylation inheritance through allosteric activation of H3 ubiquitylation by UHRF1, *eLife.* 5 (2016) e17101. doi:10.7554/eLife.17101 Medline
37. L. Ferry, A. Fournier, T. Tsusaka, G. Adelmant, T. Shimazu, S. Matano, O. Kirsh, R. Amouroux, N. Dohmae, T. Suzuki, G.J. Filion, W. Deng, M. de Dieuleveult, L. Fritsch, S. Kudithipudi, A. Jeltsch, H. Leonhardt, P. Hajkova, J.A. Marto, K. Arita, Y. Shinkai, P.A. Defossez, Methylation of DNA Ligase 1 by G9a/GLP Recruits UHRF1 to Replicating DNA and Regulates DNA Methylation, *Mol. Cell.* 67 (2017) 550-565.e5. doi:10.1016/j.molcel.2017.07.012.
38. C. Bronner, M. Alhosin, A. Hamiche, M. Mousli, Coordinated dialogue between UHRF1 and DNMT1 to ensure faithful inheritance of methylated DNA patterns, *Genes (Basel).* 10 (2019) E65. doi:10.3390/genes10010065.
39. J. Kim, E. J. Richards, K. M. Chung, & H. R. Woo, Arabidopsis VIM proteins regulate epigenetic silencing by modulating DNA methylation and histone modification in cooperation with MET1, *Mol. Plant.* 7 (2014) 1470-1485. <https://doi.org/10.1093/mp/ssu079>
40. Y. Zhang, C.J. Harris, Q. Liu, W. Liu, I. Ausin, Y. Long, L. Xiao, L. Feng, X. Chen, Y. Xie, X. Chen, L. Zhan, S. Feng, J.J. Li, H. Wang, J. Zhai, S.E. Jacobsen, Large-scale comparative epigenomics reveals hierarchical regulation of non-CG methylation in Arabidopsis, *Proc. Natl. Acad. Sci. U. S. A.* 115 (2018) E1069–E1074. doi:10.1073/pnas.1716300115.
41. W. Aufsatz, M.F. Mette, A.J.M. Matzke, M. Matzke, The role of MET1 in RNA-directed de novo and maintenance methylation of CG dinucleotides, *Plant Mol. Biol.* 54 (2004) 793–804. doi:10.1007/s11103-004-0179-1.

42. P.E. Jullien, D. Susaki, R. Yelagandula, T. Higashiyama, F. Berger, DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*, *Curr. Biol.* 22 (2012) 1825–1830. doi:10.1016/j.cub.2012.07.061.
43. J.S. Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Requirement of CHROMOMETHYLASE3 for Maintenance of CpXpG Methylation, *Science.* 292 (2001) 2077–2080.
44. A. Zemach, M.Y. Kim, P.H. Hsieh, D. Coleman-Derr, L. Eshed-Williams, K. Thao, S.L. Harmer, D. Zilberman, The arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin, *Cell.* 153 (2013) 193–205. doi:10.1016/j.cell.2013.02.033.
45. H. Stroud, T. Do, J. Du, X. Zhong, S. Feng, L. Johnson, D.J. Patel, S.E. Jacobsen, Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*, *Nat. Struct. Mol. Biol.* 21 (2014) 64–72. doi:10.1038/nsmb.2735.
46. J.P. Jackson, A.M. Lindroth, X. Cao, S.E. Jacobsen, Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase, *Nature.* 416 (2002) 556–560. doi:10.1038/nature731.
47. H. Stroud, M.V.C. Greenberg, S. Feng, Y. V. Bernatavichute, S.E. Jacobsen, Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome, *Cell.* 152 (2013) 352–364. doi:10.1016/j.cell.2012.10.054.
48. W. Qian, D. Miki, H. Zhang, Y. Liu, X. Zhang, K. Tang, Y. Kan, H. La, X. Li, S. Li, X. Zhu, X. Shi, K. Zhang, O. Pontes, X. Chen, R. Liu, Z. Gong, J.K. Zhu, A histone acetyltransferase regulates active DNA demethylation in *Arabidopsis*, *Science.* (2012) 1445–1448. doi:10.1126/science.1219416.
49. A. Zemach & G. Grafi, Characterization of *Arabidopsis thaliana* methyl-CpG-binding domain (MBD) proteins, *Plant J.* 34 (2003) 565–72.
50. A. Zemach, Y. Li, B. Wayburn, H. Ben-Meir, V. Kiss, Y. Avivi, V. Kalchenko, S.E. Jacobsen, G. Grafi, DDM1 Binds *Arabidopsis* Methyl-CpG Binding Domain Proteins and Affects Their Subnuclear Localization, *Plant Cell.* 17 (2005) 1549–1558. doi:10.1105/tpc.105.031567.

51. A. Zemach, L.K. Paul, P. Stambolsky, I. Efroni, V. Rotter, G. Grafi, The C-terminal domain of the Arabidopsis AtMBD7 protein confers strong chromatin binding activity, *Exp. Cell Res.* 315 (2009) 3554–3562. doi:10.1016/j.yexcr.2009.07.022.
52. P.C. Preuss SB, Costa-Nunes P, Tucker S, Pontes O, Lawrence RJ, Mosher R, Kasschau KD, Carrington JC, Baulcombe DC, Viegas W, C.S. Pikaard, Multimegabase silencing in nucleolar dominance involves siRNA-directed DNA methylation and specific methylcytosine-binding proteins, *Mol Cell.* 32 (2008) 673–84.
53. Z. Lang, et al., The methyl-CpG-binding protein MBD7 facilitates active DNA demethylation to limit DNA hyper-methylation and transcriptional gene silencing, *Mol Cell.* 57 (2015) 971–983.
54. Q.W. Li et al., Regulation of Active DNA Demethylation by a Methyl-CpG-Binding Domain Protein in *Arabidopsis thaliana*, *PLoS Genet.* 11 (2015) e1005210.
55. G.Z. Wang et al., Methyl-CpG-binding domain protein MBD7 is required for active DNA demethylation in *Arabidopsis*, *Plant Physiol.* 167 (2015) 905–14.
56. C.-G. Duan, et al., A pair of transposon-derived proteins function in a histone acetyltransferase complex for active DNA demethylation, *Cell Res.* 27 (2017) 226–240. doi:10.1038/cr.2016.147.
57. D. Li, A.M.S. Palanca, S.Y. Won, L. Gao, Y. Feng, A.A. Vashisht, L. Liu, Y. Zhao, X. Liu, X. Wu, S. Li, B. Le, Y.J. Kim, G. Yang, S. Li, J. Liu, J.A. Wohlschlegel, H. Guo, B. Mo, X. Chen, J.A. Law, The MBD7 complex promotes expression of methylated transgenes without significantly altering their methylation status, *Elife.* 6 (2017). doi:10.7554/eLife.19893.
58. W.-F. Nie, et al., Histone acetylation recruits the SWR1 complex to regulate active DNA demethylation in *Arabidopsis*, *Proc. Natl. Acad. Sci.* (2019) 16641–16650. doi:10.1073/pnas.1906023116.
59. A. Zemach, O. Gaspan, G. Grafi, The three methyl-CpG-binding domains of AtMBD7 control its subnuclear localization and mobility, *J. Biol. Chem.* 283 (2008) 8406–8411. doi:10.1074/jbc.M706221200.

60. A.P. Parida, A. Sharma, A.K. Sharma, AtMBD6, a methyl CpG binding domain protein, maintains gene silencing in Arabidopsis by interacting with RNA binding proteins, *J. Biosci.* 42 (2017) 57–68.
61. K.S. Springer NM, N.M. Springer, S.M. Kaepler, Evolutionary divergence of monocot and dicot methyl-CpG-binding domain proteins, *Plant Physiol.* 138 (2005) 92–104. doi:10.1104/pp.105.060566.
62. Z.D. Qin, Q.W. Sun, L.M. Huang, X.S. Chen, Rice SUVH histone methyltransferase genes display specific functions in chromatin modification and retrotransposon repression, *Mol Plant.* 3 (2010) 773–82..
63. N.M. Springer, C.A. Napoli, D.A. Selinger, R. Pandey, K.C. Cone, V.L. Chandler, H.F. Kaepler, S.M. Kaepler, Comparative analysis of SET domain proteins in maize and Arabidopsis reveals multiple duplications preceding the divergence of monocots and dicots, *Plant Physiol.* 132 (2003) 907–925. doi:10.1104/pp.102.013722.
64. M.L. Ebbs, J. Bender, Locus-Specific Control of DNA Methylation by the Arabidopsis SUVH5 Histone Methyltransferase, *Plant Cell.* 18 (2006) 1166–1176. doi:10.1105/tpc.106.041400.
65. J. Du, L. M. Johnson, M. Groth, S. Feng, C. J. Hale, S. Li, A. A. Vashisht, J. Gallego-Bartolome, J. A. Wohlschlegel, D. J. Patel, S. E. Jacobsen, Mechanism of DNA methylation-directed histone methylation by KRYPTONITE. *Mol. Cell.* 55 (2014) 495–504. doi:10.1016/j.molcel.2014.06.009 Medline
66. C.J. Harris, S.P. Wongpalee, W. Liu, Y. Xue, Z. Zhong, L. Yen, J. Gallego-Bartolome, M. Groth, S.E. Jacobsen, M. Scheibe, F. Butter, E.M. Cornett, R.M. Vaughan, S.B. Rothbart, X. Li, W. Chen, J. Du, Z. Wang, W.D. Barshop, S. Rayatpisheh, J.A. Wohlschlegel, S.E. Jacobsen, A DNA methylation reader complex that enhances gene transcription, *Science.* 362 (2018) 1182–1186. doi:10.1126/science.aar7854.
67. W.-F. Nie, M. Lei, M. Zhang, K. Tang, H. Huang, C. Zhang, D. Miki, P. Liu, Y. Yang, X. Wang, H. Zhang, Z. Lang, N. Liu, X. Xu, R. Yelagandula, H. Zhang, Z. Wang, X. Chai, A. Andreucci, J.-Q. Yu, F. Berger, R. Lozano-Duran, J.-K. Zhu, Histone acetylation recruits the SWR1 complex to regulate active DNA demethylation in Arabidopsis, *Proc. Natl. Acad. Sci.* (2019) 16641–16650. doi:10.1073/pnas.1906023116.

68. J. D. Hollister, B. S. Gaut, Epigenetic silencing of transposable elements: A trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res.* 19 (2009) 1419–1428. doi:10.1101/gr.091678.109 Medline
69. T. Stuart, S. R. Eichten, J. Cahn, Y. V. Karpievitch, J. O. Borevitz, R. Lister, Population scale mapping of transposable element diversity reveals links to gene regulation and epigenomic variation, *eLife.* 5 (2016) e20777. doi:10.7554/eLife.20777 Medline
70. L. Quadrana, A. Bortolini Silveira, G. F. Mayhew, C. LeBlanc, R. A. Martienssen, J. A. Jeddelloh, V. Colot, The *Arabidopsis thaliana* mobilome and its impact at the species level. *eLife* 5 (2016). e15716 doi:10.7554/eLife.15716
71. X. Wang, D. Wiegell, L. M. Smith, Transposon variants and their effects on gene expression in *Arabidopsis*. *PLOS Genet.* 9 (2013) e1003255. doi:10.1371/journal.pgen.1003255.
72. Q. Gouil, D.C. Baulcombe, DNA Methylation Signatures of the Plant Chromomethyltransferases, *PLoS Genet.* 12 (2016). doi:10.1371/journal.pgen.1006526.
73. A.J. Bewick, L. Ji, C.E. Niederhuth, E.M. Willing, B.T. Hofmeister, X. Shi, L. Wang, Z. Lu, N.A. Rohr, B. Hartwig, C. Kiefer, R.B. Deal, J. Schmutz, J. Grimwood, H. Stroud, S.E. Jacobsen, K. Schneeberger, X. Zhang, R.J. Schmitz, On the origin and evolutionary consequences of gene body DNA methylation, *Proc. Natl. Acad. Sci. U. S. A.* 113 (2016) 9111–9116. doi:10.1073/pnas.1604666113.
74. S.R. Bewick AJ, Niederhuth CE, Ji L, Rohr NA, Griffin PT, Leebens-Mack J, The evolution of CHROMOMETHYLASES and gene body DNA methylation in plants., *Genome Biol.* 18 (2017).
75. J.M. Wendte, Y. Zhang, L. Ji, X. Shi, R.R. Hazarika, Y. Shahryary, F. Johannes, R.J. Schmitz, Epimutations are associated with CHROMOMETHYLASE 3-induced de novo DNA methylation, *Elife* 8 (2019). doi:10.7554/elife.47891.
76. J. Gallego-Bartolomé, W. Liu, P.H. Kuo, S. Feng, B. Ghoshal, J. Gardiner, J.M.C. Zhao, S.Y. Park, J. Chory, S.E. Jacobsen, Co-targeting RNA Polymerases IV and V Promotes Efficient De Novo DNA Methylation in *Arabidopsis*, *Cell.* 176 (2019) 1068-1082.e19. doi:10.1016/j.cell.2019.01.029.



77. D. Cuerda-Gil, R.K. Slotkin, Non-canonical RNA-directed DNA methylation, *Nat. Plants*. 2 (2016) 16163 . doi:10.1038/nplants.2016.163.
78. S. Nuthikattu, A.D. McCue, K. Panda, D. Fultz, C. Defraia, E.N. Thomas, R.K. Slotkin, The Initiation of Epigenetic Silencing of Active Transposable Elements Is Triggered by RDR6, *Plant Physiol.* 162 (2013) 116–131. doi:10.1104/pp.113.216481.
79. K. Panda, L. Ji, D.A. Neumann, J. Daron, R.J. Schmitz, R.K. Slotkin, Full-length autonomous transposable elements are preferentially targeted by expression-dependent forms of RNA-directed DNA methylation, *Genome Biol.* 17 (2016). doi:10.1186/s13059-016-1032-y.
80. D. Pontier, C. Picart, F. Roudier, D. Garcia, S. Lahmy, J. Azevedo, E. Alart, M. Laudié, W.M. Karlowski, R. Cooke, V. Colot, O. Voinnet, T. Lagrange, NERD, a Plant-Specific GW Protein, Defines an Additional RNAi-Dependent Chromatin-Based Pathway in Arabidopsis, *Mol. Cell.* 48 (2012) 121–132. doi:10.1016/j.molcel.2012.07.027.
81. D.L. Yang, G. Zhang, K. Tang, J. Li, L. Yang, H. Huang, H. Zhang, J.K. Zhu, Dicer-independent RNA-directed DNA methylation in Arabidopsis, *Cell Res.* 26 (2016) 66–82. doi:10.1038/cr.2015.145.
82. R. Ye, Z. Chen, B. Lian, M.J. Rowley, N. Xia, J. Chai, Y. Li, X.J. He, A.T. Wierzbicki, Y. Qi, A Dicer-Independent Route for Biogenesis of siRNAs that Direct DNA Methylation in Arabidopsis, *Mol. Cell.* 61 (2016) 222–235. doi:10.1016/j.molcel.2015.11.015.
83. M. Gehring, Epigenetic dynamics during flowering plant reproduction: evidence for reprogramming?, *New Phytol.* 224 (2019) 91–96. doi:10.1111/nph.15856.
84. T. Kawashima, F. Berger, Epigenetic reprogramming in plant sexual reproduction, *Nat. Rev. Genet.* 15 (2014) 103–10. doi:10.1038/nrg3685.
85. J.H. Huh, M.J. Bauer, T.F. Hsieh, R.L. Fischer, Cellular Programming of Plant Gene Imprinting, *Cell.* 132 (2008) 735–44. doi:10.1016/j.cell.2008.02.018.
86. Z. Gong, T. Morales-Ruiz, R.R. Ariza, T. Roldán-Arjona, L. David, J.K. Zhu, ROS1, a repressor of transcriptional gene silencing in Arabidopsis, encodes a DNA glycosylase/lyase, *Cell.* (2002) 803–14. doi:10.1016/S0092-8674(02)01133-9.
87. Y. Choi, M. Gehring, L. Johnson, M. Hannon, J.J. Harada, R.B. Goldberg, S.E. Jacobsen, R.L. Fischer, DEMETER, a DNA glycosylase domain protein, is required for endosperm

gene imprinting and seed viability in Arabidopsis, *Cell*. 110 (2002) 33–42.

doi:10.1016/S0092-8674(02)00807-3.

88. T.F. Hsieh, C.A. Ibarra, P. Silva, A. Zemach, L. Eshed-Williams, R.L. Fischer, D. Zilberman, Genome-wide demethylation of Arabidopsis endosperm, *Science* 324 (2009) 1451–1454. doi:10.1126/science.1172417.
89. C.A. Ibarra, X. Feng, V.K. Schoft, T.F. Hsieh, R. Uzawa, J.A. Rodrigues, A. Zemach, N. Chumak, A. Machlicova, T. Nishimura, D. Rojas, R.L. Fischer, H. Tamaru, D. Zilberman, Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes, *Science* 337 (2012) 1360–1364. doi:10.1126/science.1224839.
90. R.K. Slotkin, M. Vaughn, F. Borges, M. Tanurdžić, J.D. Becker, J.A. Feijó, R.A. Martienssen, Epigenetic Reprogramming and Small RNA Silencing of Transposable Elements in Pollen, *Cell*. 136 (2009) 461–472. doi:10.1016/j.cell.2008.12.038.
91. C. Zhang, Y.-H. Hung, H.J. Rim, D. Zhang, J.M. Frost, H. Shin, H. Jang, F. Liu, W. Xiao, L.M. Iyer, L. Aravind, X.-Q. Zhang, R.L. Fischer, J.H. Huh, T.-F. Hsieh, The catalytic core of DEMETER guides active DNA demethylation in Arabidopsis, *Proc. Natl. Acad. Sci.* (2019) 17563–17571. doi:10.1073/pnas.1907290116.
92. K. Park, M.Y. Kim, M. Vickers, J.S. Park, Y. Hyun, T. Okamoto, D. Zilberman, R.L. Fischer, X. Feng, Y. Choi, S. Scholten, DNA demethylation is initiated in the central cells of Arabidopsis and rice, *Proc. Natl. Acad. Sci. U. S. A.* 113 (2016) 15138–15143. doi:10.1073/pnas.1619047114.
93. T. Kawakatsu, J.R. Nery, R. Castanon, J.R. Ecker, Dynamic DNA methylation reconfiguration during seed development and germination, *Genome Biol.* 18 (2017). doi:10.1186/s13059-017-1251-x.
94. D. Bouyer, A. Kramdi, M. Kassam, M. Heese, A. Schnittger, F. Roudier, V. Colot, DNA methylation dynamics during early plant life, *Genome Biol.* 18 (2017). doi:10.1186/s13059-017-1313-0
95. M. Ingouff, B. Selles, C. Michaud, T.M. Vu, F. Berger, A.J. Schorn, D. Autran, M. Van Durme, M.K. Nowack, R.A. Martienssen, D. Grimanelli, Live-cell analysis of DNA methylation during sexual reproduction in arabidopsis reveals context and sex-specific dynamics

controlled by noncanonical RdDM, *Genes Dev.* 31 (2017) 72–83.

doi:10.1101/gad.289397.116.

96. S. Takuno, J.H. Ran, B.S. Gaut, Evolutionary patterns of genic DNA methylation vary across land plants, *Nat. Plants.* 2 (2016). doi:10.1038/NPLANTS.2015.222.
97. C.E. Niederhuth, A.J. Bewick, L. Ji, M.S. Alabady, K. Do Kim, Q. Li, N.A. Rohr, A. Rambani, J.M. Burke, J.A. Udall, C. Egesi, J. Schmutz, J. Grimwood, S.A. Jackson, N.M. Springer, R.J. Schmitz, Widespread natural variation of DNA methylation within angiosperms, *Genome Biol.* 17 (2016). doi:10.1186/s13059-016-1059-0.
98. S. Sato, et al., The tomato genome sequence provides insights into fleshy fruit evolution, *Nature.* (2012). doi:10.1038/nature11119.
99. S. Corem, A. Doron-Faigenboim, O. Jouffroy, F. Maumus, T. Arazi, N. Bouché, Redistribution of CHH methylation and small interfering RNAs across the genome of tomato *ddm1* mutants, *Plant Cell* 30 (2018) 1628-1644. doi:10.1105/tpc.18.00167.
100. A. Aguilar-Cruz, D. Grimanelli, J. Haseloff, M.A. Arteaga-Vázquez, DNA methylation in *Marchantia polymorpha*, *New Phytol.* 223 (2019) 575–581. doi:10.1111/nph.15818
101. J.L. Bowman, et al., Insights into Land Plant Evolution Garnered from the *Marchantia polymorpha* Genome, *Cell.* 171 (2017) 287-304.e15. doi:10.1016/j.cell.2017.09.030.
102. H.E. Kasinsky, S. Ellis, G. Martens, J. Ausió, Dynamic aspects of spermiogenic chromatin condensation patterning by phase separation during the histone-to-protamine transition in charalean algae and relation to bryophytes, *Tissue Cell.* 46 (2014) 415–432. doi:10.1016/j.tice.2014.07.003.
103. Y. Ikeda, R. Nishihama, S. Yamaoka, M.A. Arteaga-Vazquez, A. Aguilar-Cruz, D. Grimanelli, R. Pogorelcnik, R.A. Martienssen, K.T. Yamato, T. Kohchi, T. Hirayama, O. Mathieu, Loss of CG Methylation in *Marchantia polymorpha* Causes Disorganization of Cell Division and Reveals Unique DNA Methylation Regulatory Mechanisms of Non-CG Methylation, *Plant Cell Physiol.* 59 (2018) 2421–2431. doi:10.1093/pcp/pcy161.
104. M.W. Schmid, A. Giraldo-Fonseca, M. Rövekamp, D. Smetanin, J.L. Bowman, U. Grossniklaus, Extensive epigenetic reprogramming during the life cycle of *Marchantia polymorpha*, *Genome Biol.* 19 (2018). doi:10.1186/s13059-017-1383-z.

105. R. Yaari, A. Katz, K. Domb, K.D. Harris, A. Zemach, N. Ohad, RdDM-independent de novo and heterochromatin DNA methylation by plant CMT and DNMT3 orthologs, *Nat. Commun.* 10 (2019). doi:10.1038/s41467-019-09496-0.
106. S. Feng, S.J. Cokus, X. Zhang, P.-Y. Chen, M. Bostick, M.G. Goll, J. Hetzel, J. Jain, S.H. Strauss, M.E. Halpern, C. Ukomadu, K.C. Sadler, S. Pradhan, M. Pellegrini, S.E. Jacobsen, Conservation and divergence of methylation patterning in plants and animals, *Proc. Natl. Acad. Sci.* 107 (2010) 8689–8694. doi:10.1073/pnas.1002720107.
107. A. Zemach, I. E. McDaniel, P. Silva, D. Zilberman, Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* 328 (2010) 916–919. doi:10.1126/science.1186366.
108. R. Lister, E.A. Mukamel, J.R. Nery, M. Urich, C.A. Puddifoot, N.D. Johnson, J. Lucero, Y. Huang, A.J. Dwork, M.D. Schultz, M. Yu, J. Tonti-Filippini, H. Heyn, S. Hu, J.C. Wu, A. Rao, M. Esteller, C. He, F.G. Haghghi, T.J. Sejnowski, M.M. Behrens, J.R. Ecker, Global epigenomic reconfiguration during mammalian brain development, *Science* 341 (2013) 1237905. doi:10.1126/science.1237905.
109. H.S. Jang, W.J. Shin, J.E. Lee, J.T. Do, CpG and non-CpG methylation in epigenetic gene regulation and brain function, *Genes (Basel)*. 8 (2017). doi:10.3390/genes8060148.
110. B. Yu, X. Dong, S. Gravina, Ö. Kartal, T. Schimmel, J. Cohen, D. Tortoriello, R. Zody, R.D. Hawkins, J. Vijg, Genome-wide, Single-Cell DNA Methylomics Reveals Increased Non-CpG Methylation during Human Oocyte Maturation, *Stem Cell Reports*. 9 (2017) 397–407. doi:10.1016/j.stemcr.2017.05.026.

### **Figure 1: The RNA Directed DNA Methylation pathway in *Arabidopsis***

The RdDM pathway comprises two modules, involved respectively in the biogenesis of small RNAs (the Pol-IV dependent module), and the targeting of small RNAs to chromatin (Pol-V dependent de novo targeting). The CLSY proteins are required for the generation of the vast majority of 24-nucleotide siRNAs. CLSY1 and CLSY2 are required for the physical association between SHH1 and POLIV, and for the generation of a large fraction of 24-nucleotide siRNAs. POLIV generates short single-stranded RNA (ssRNA), but can also act as dual actors in RdDM. Those are converted into double-stranded RNA (dsRNA) by the activity of RDR2. dsRNA are then processed into 24-nucleotide small interfering RNAs DCL3. These short duplexes are methylated at their 3'-ends by HEN1. Single-stranded, 24-nt siRNAs are subsequently integrated into either AGO4, or other members of the family including AGO6 and AGO9. POLV then recruits DRM2 by generating long ssRNA that interacts with the 24-nucleotide siRNA incorporated in AGO4 via its WG/GW platform, also referred to as the AGO-hook domain. The plant-specific RDM1 protein bind methylated ssDNA to establish the interaction between AGO4 and DRM1/DRM2. DRM1 or DRM2 then methylates DNA in all three sequence contexts. POLV can be recruited to regions with pre-existing DNA methylation through indirect interaction with the histone methyltransferases SU(VAR) HOMOLOG 2 (SUVH2) and SUVH9, which thus as *bona fide* "readers" of *de novo* methylation, via interaction with the DDR complex (DRD1, DMS3, RDM1).

## **Figure 2: VIM proteins contains atypical domains.**

While UHRF1 in mammals contains a TANDEM TUDOR DOMAIN, VIM proteins lack the potential to bind H3K9me2/3. This suggests that significant differences between plants and animals in their strategy for maintenance of CG methylation. Interestingly, VIM proteins contain two REALLY INTERESTING NEW GENE domains (RING). Data suggest that the PHD domain is sufficient to bind the core Histones, but it remains unclear whether they can bind H3K9me2 in plants. An intermediary, yet undefined, TUDOR domain might be thus required to ensure proper maintenance of DNA methylation at CG sites.

**Figure 3: MBD proteins in plants have both anti-silencing and binding potential.**

The MBD family of proteins has been mostly characterized in *Arabidopsis*, but we still have limited knowledge on their role in monocotyledonous plants. Data indicates that the class IV and VI are unique to dicots, raising the possibility that monocots might not have fully functional MBD proteins. Most MBD proteins still lack clearly assigned functions. Class I comprises MBD10, which functions in rDNA silencing and MBD11, of unknown function. Class II comprises MBD1, MBD3 and MBD4, all of which do not have yet assigned function. Class III comprises MBD2 and MBD12, both without defined functions. Class IV includes MBD5 and MBD6, both of which “read” symmetrical CG in vitro. Class V includes MBD9, a very large protein acting in anti-silencing. Class VI includes MBD7, involved similarly in active demethylation. Finally, class VII includes MBD8, which controls flowering time in the C24 ecotype.

**Figure 4: SUVH proteins in plants have both anti-silencing and binding potential.**

SUVH1 and 3 both display anti-silencing function in plants, while SUVH2 and 9 are involved in RdDM. Interestingly, both have lost their post set domains, suggesting that they likely interact with chromatin via a set of intermediary proteins, including IDN2. SUVH4, 5 and 6 show clear evidence for H3K9me2 methyltransferase activity, and interact with CMT3 to ensure a feedback loop for the maintenance of CHG methylation during DNA replication.

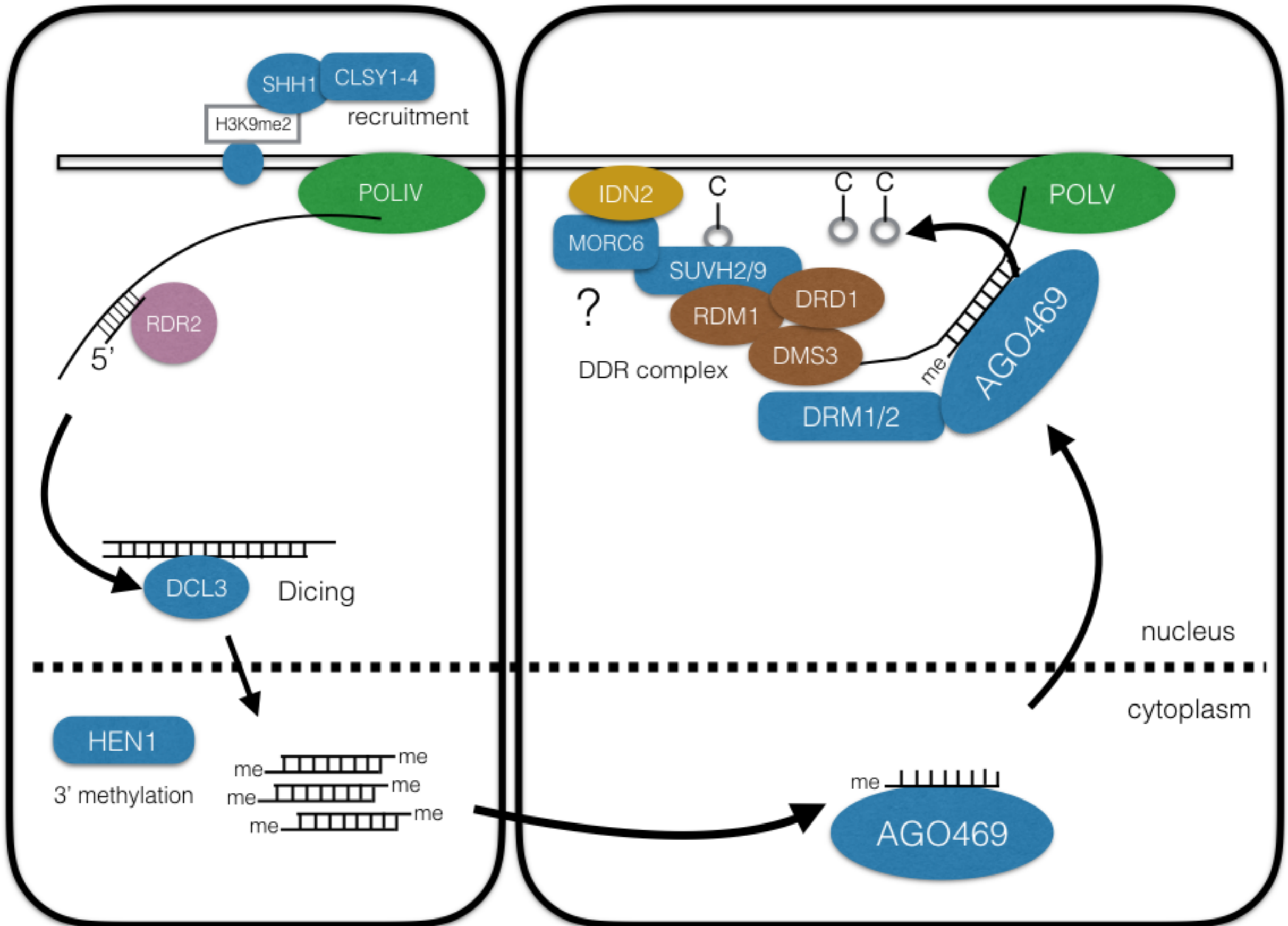


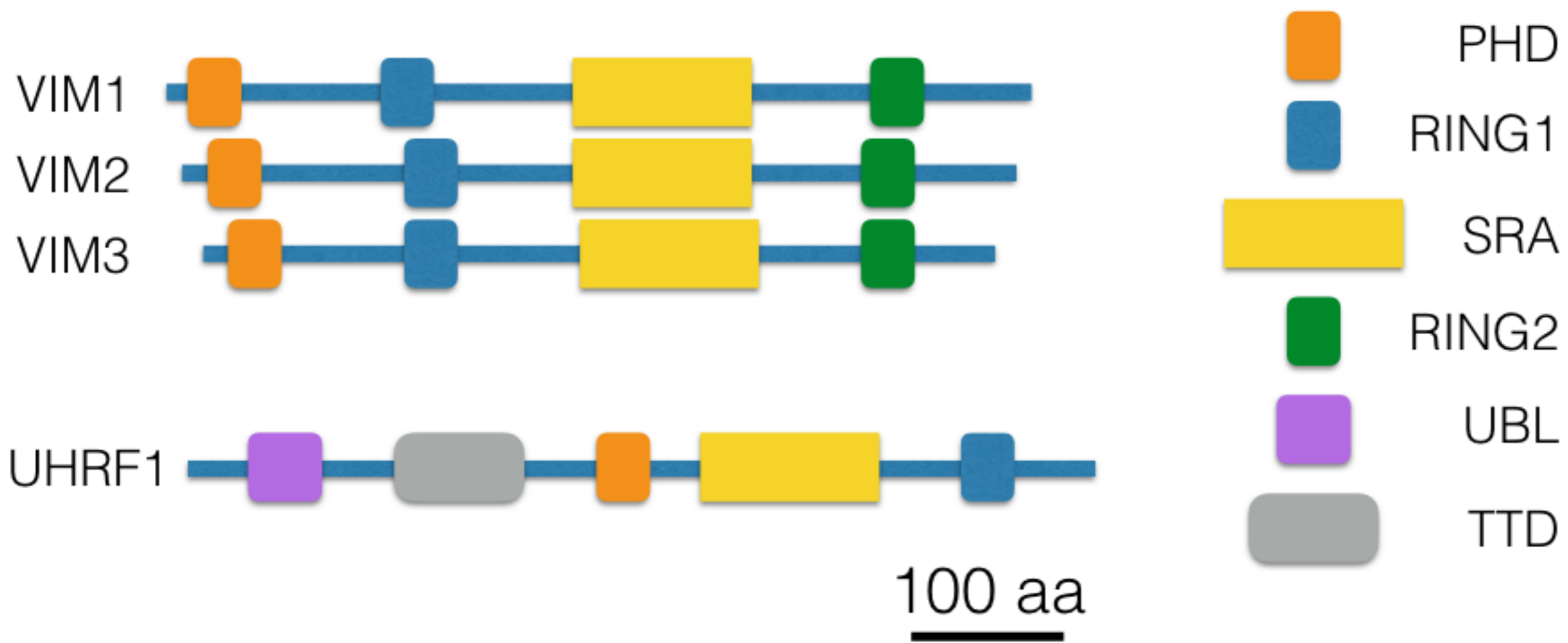
## ACKNOWLEDGEMENTS

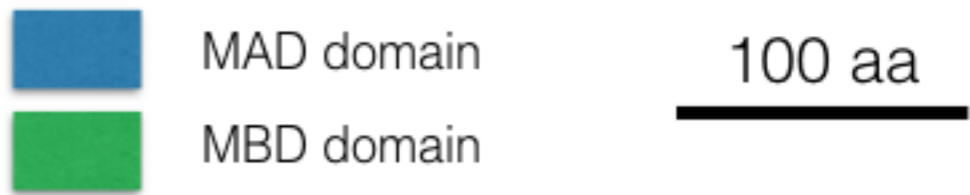
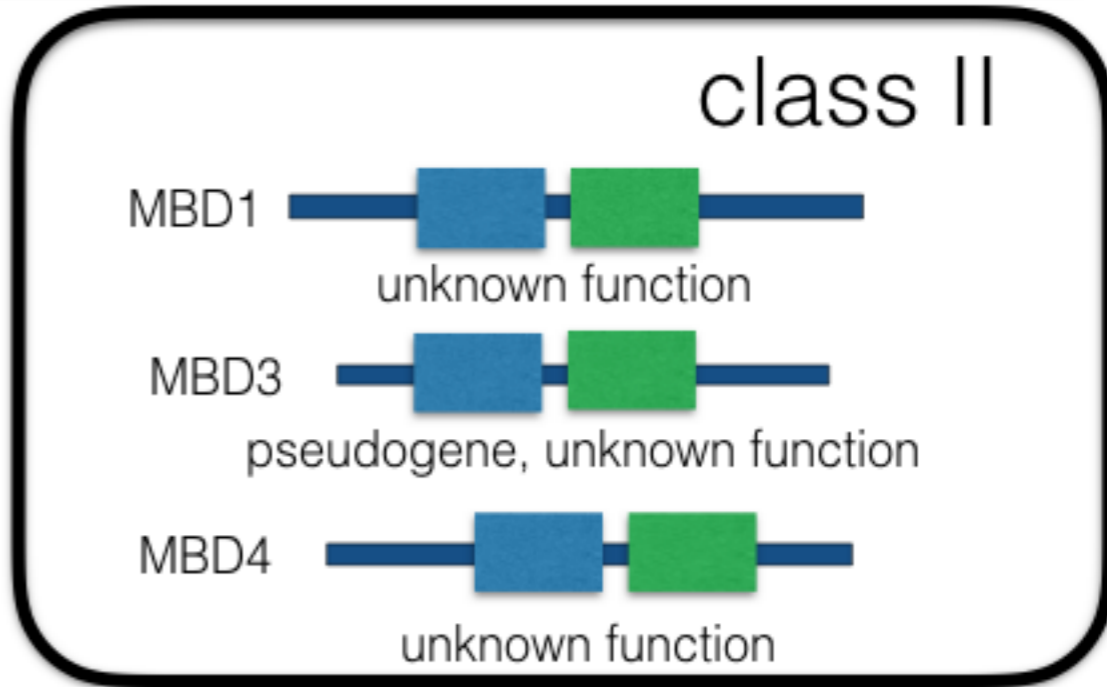
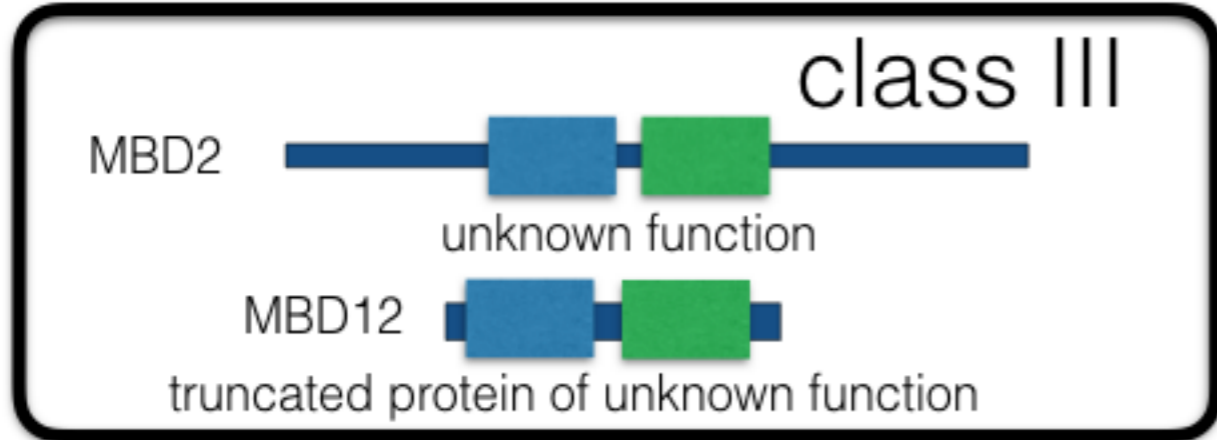
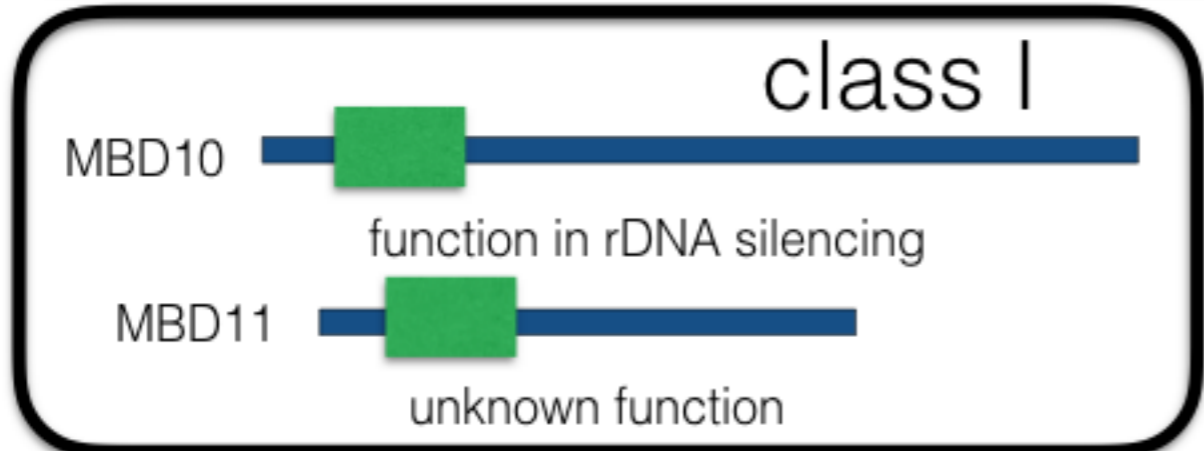
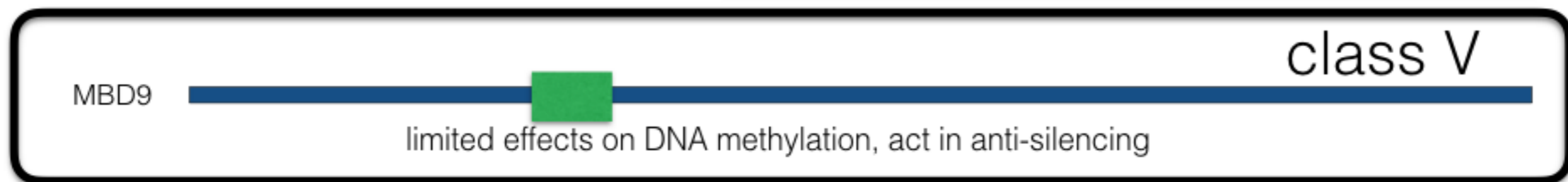
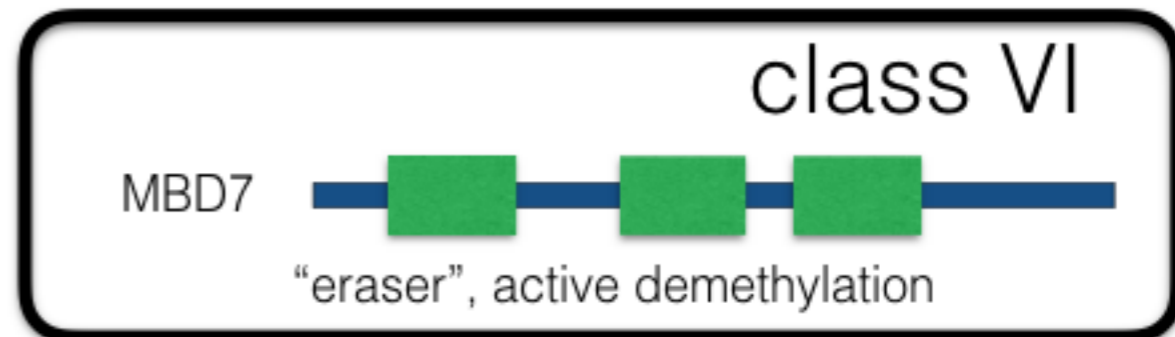
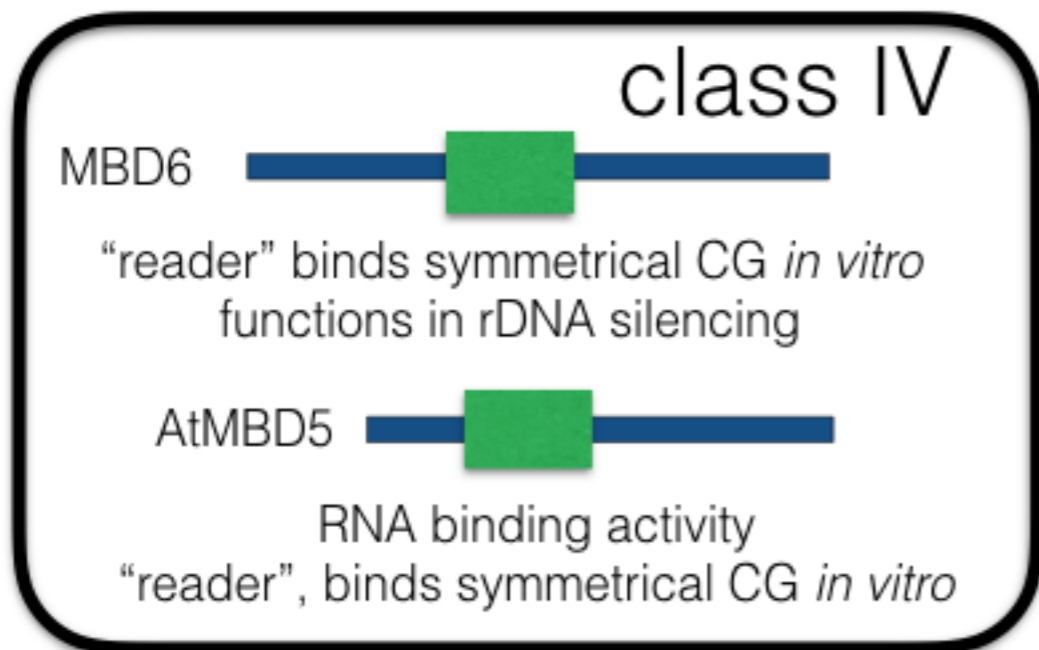
The work in our laboratory is funded through ANR grants REMETH (ANR-15-CE12-0012), and CHROMOBREED (ANR-18-CE92-0041). We thank members of the lab in input in the preparation of this review, and apologize to numerous authors whose work could not be cited due to space limitations.

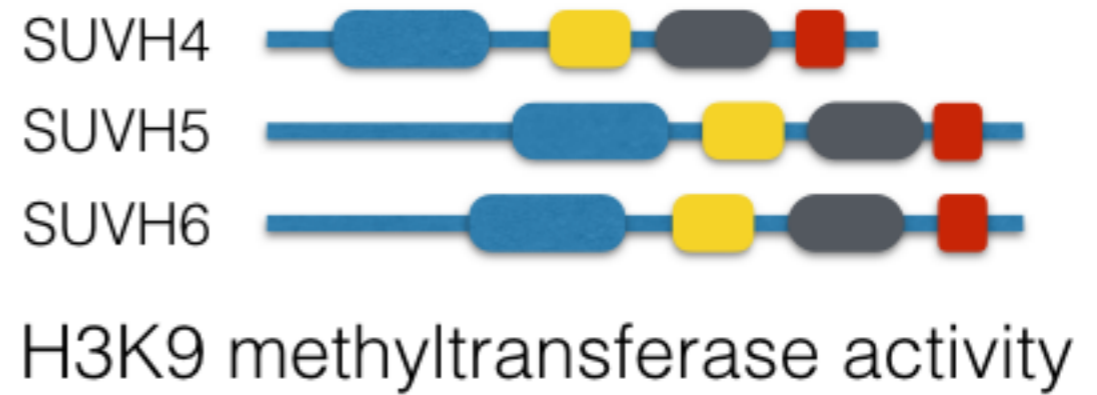
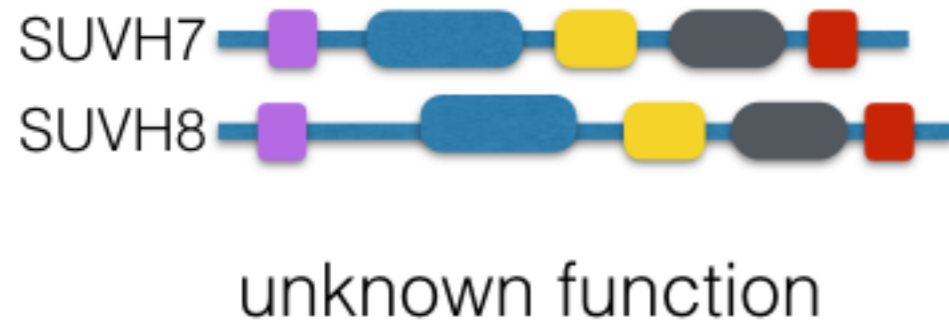
Pol IV-dependent siRNA biogenesis

Pol V-dependent *do novo* targeting









100 aa

# Graphical Abstract

