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## **DNA Methylation Readers in Plants**

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## 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.

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### **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.

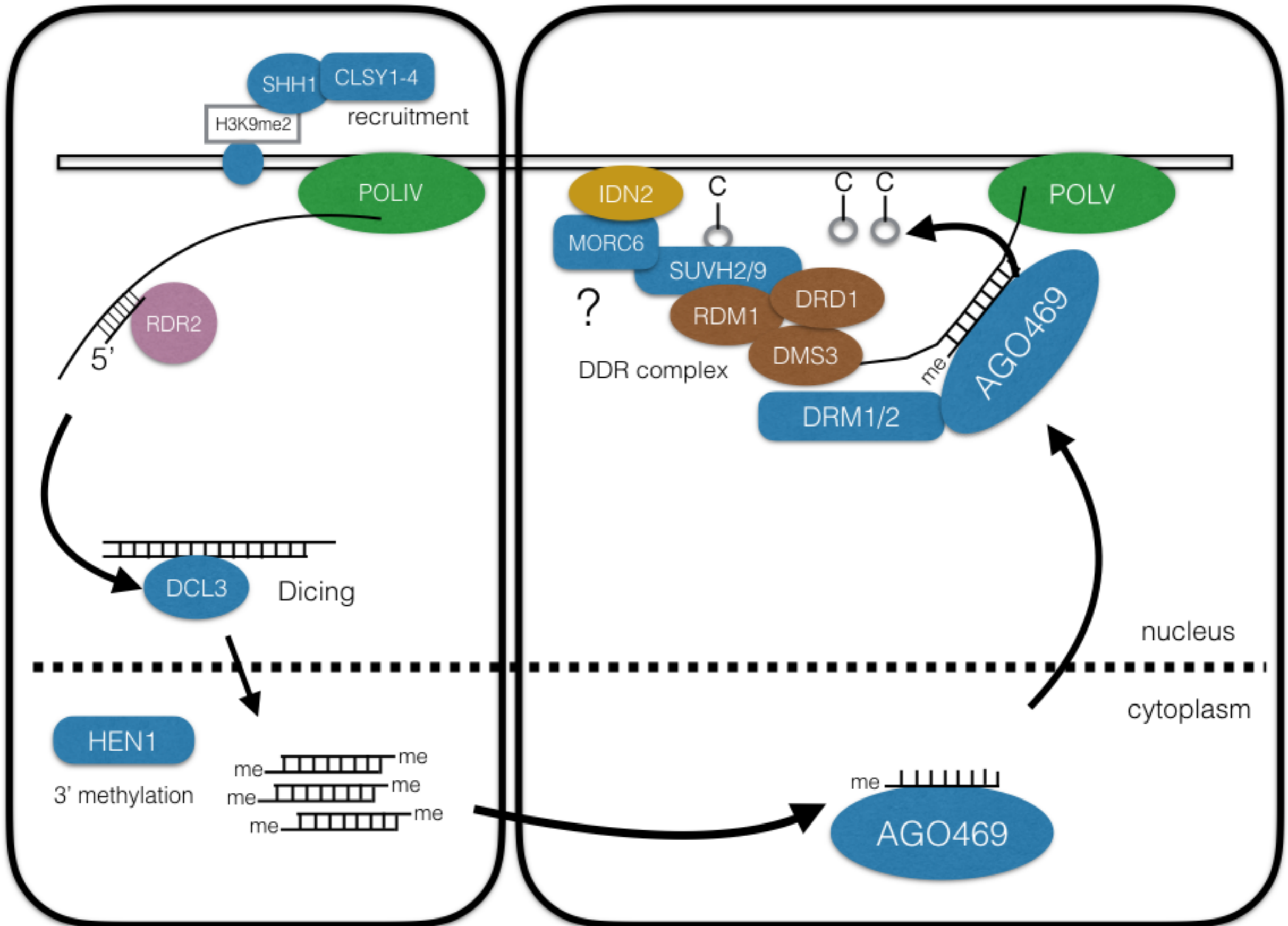


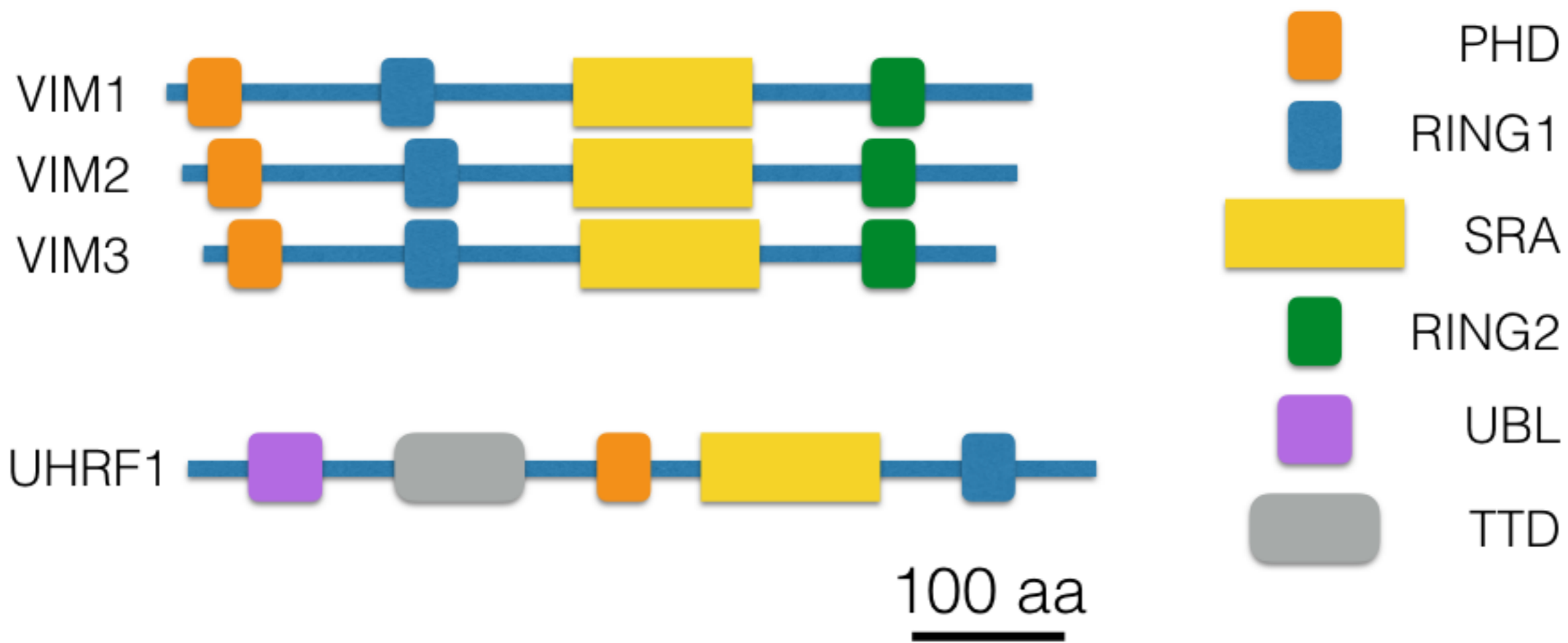
## ACKNOWLEDGEMENTS

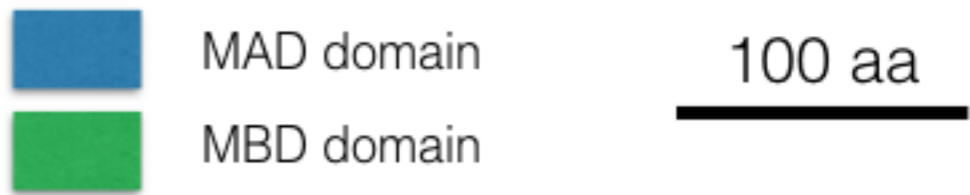
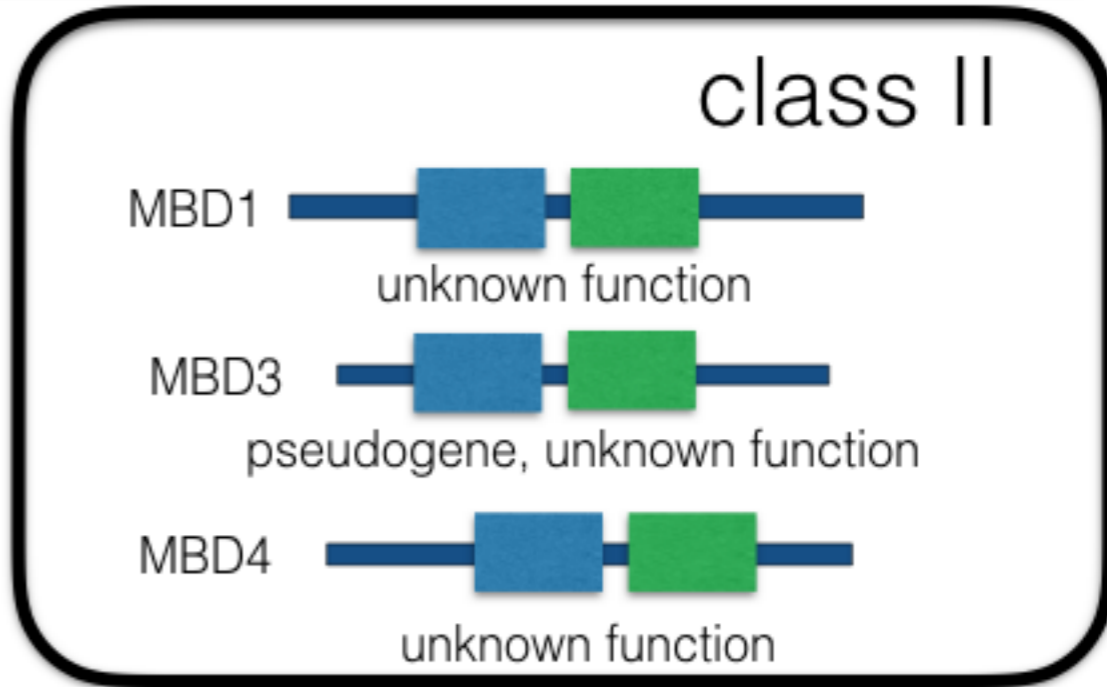
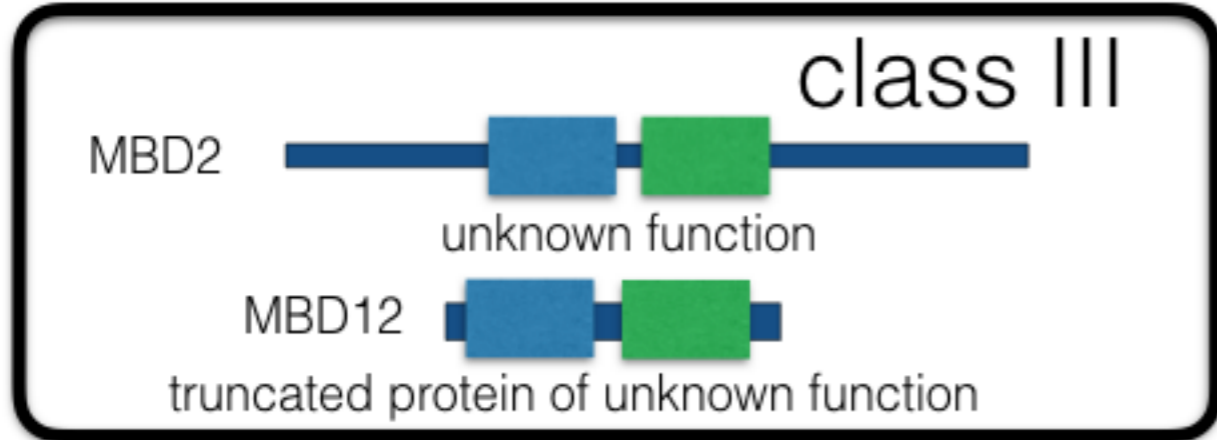
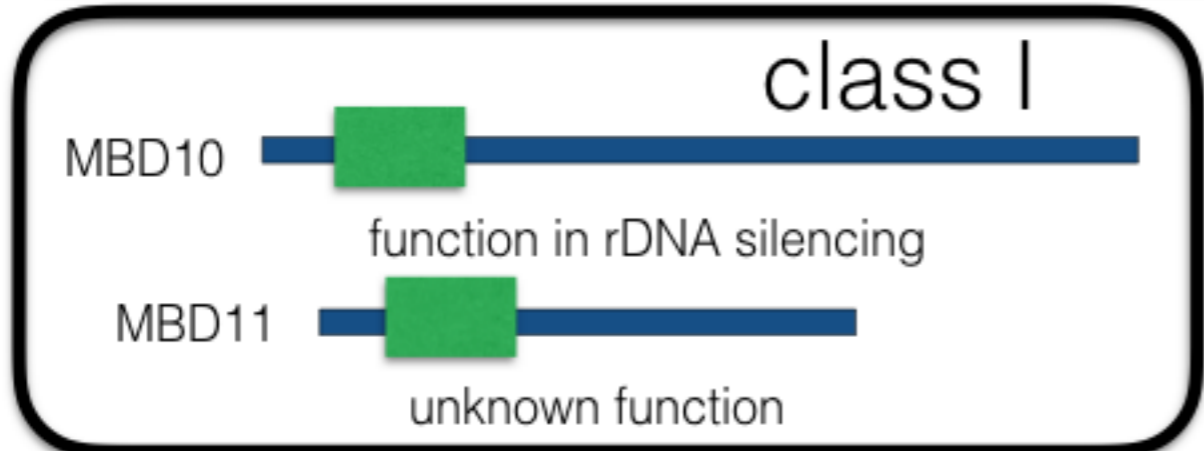
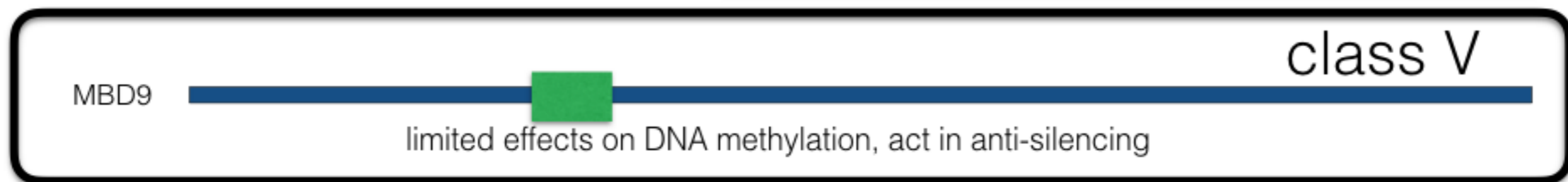
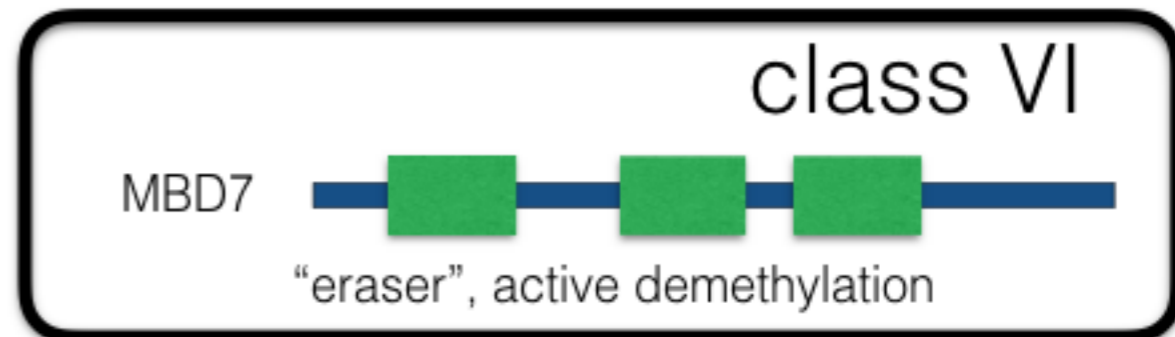
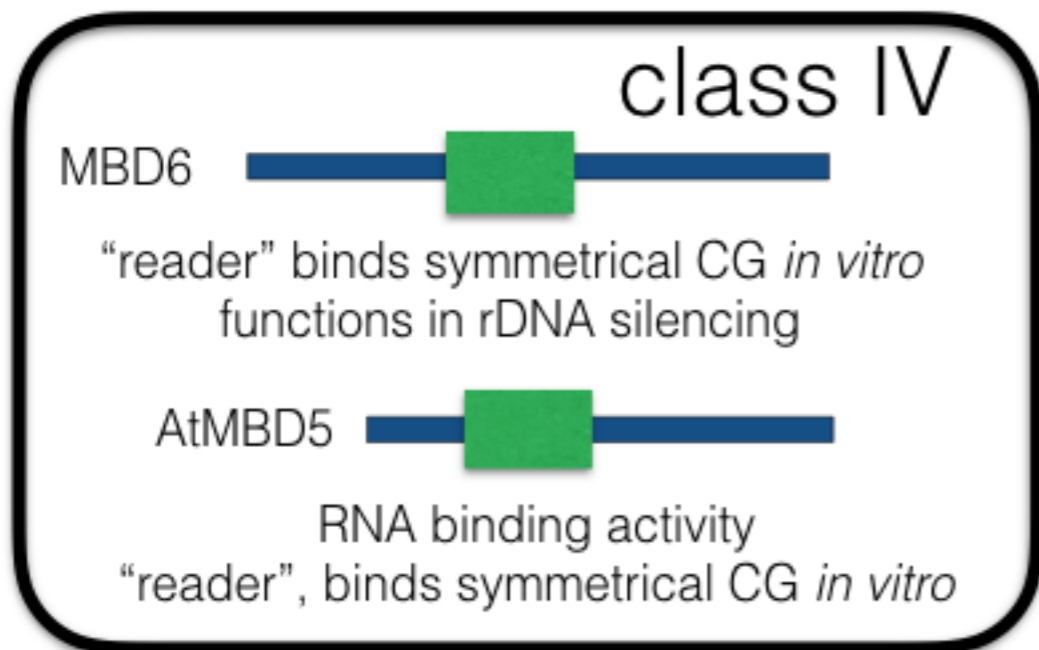
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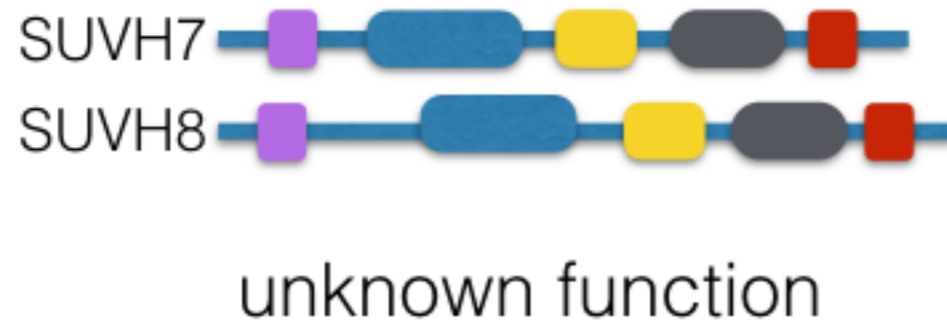
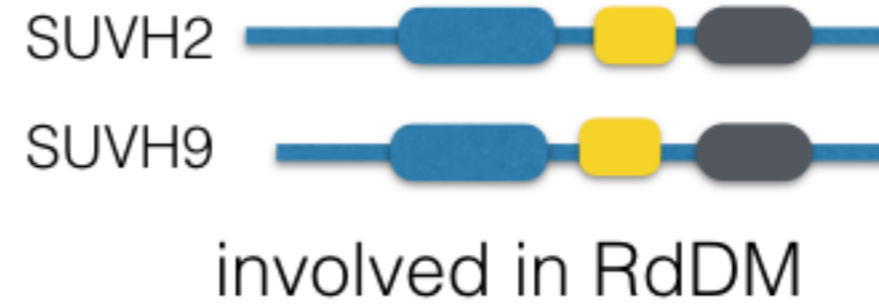
### Pol IV-dependent siRNA biogenesis

### Pol V-dependent *do novo* targeting









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# Graphical Abstract

