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The evolving functions of DNA methylation

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DNA methylation is an ancient process found in all domains of life. Although the enzymes that mediate methylation have remained highly conserved, DNA methylation has been adapted for a variety of uses throughout evolution, including defense against transposable elements and control of gene expression. Defects in DNA methylation are linked to human diseases, including cancer. Methylation has been lost several times in the course of animal and fungal evolution, thus limiting the opportunity for study in common model organisms. In the past decade, plants have emerged as a premier model system for genetic dissection of DNA methylation. A recent combination of plant genetics with powerful genomic approaches has led to a number of exciting discoveries and promises many more.

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Introduction

One of the first reagents a student is likely to encounter in a lab class is a restriction endonuclease. The overwhelming majority of these are Type II enzymes with symmetric recognition sites. These enzymes are generally believed to protect bacteria from phages by destroying the genomes of invading viruses [1]. The host's genome is protected by a cognate DNA methyltransferase that modifies the same site recognized by the endonuclease [2]. Methylation prevents cleavage, thus ensuring host survival. Recent evidence indicates that some restriction–methylation systems might actually be selfish modules that confer little benefit to the host [3]. The modules ensure their survival because the endonuclease is more stable than the methyltransferase, so loss of the module results in cell death.

Type II restriction enzymes generally function as homodimers, so methylation of either strand is sufficient for protection from cleavage, a property that is particularly important during DNA replication [2]. Symmetry ensures that the sites generated by DNA replication are identically methylated and protected until full methylation is restored. DNA methylation is thus replicated semiconservatively, just like the underlying sequence. It is probable that the prevalence of palindromic recognition sites is driven in part by the need for protection during replication.

Whether restriction–methylation systems evolved as selfish modules or not, some have clearly been adapted by the host. Some bacterial methyltransferases have a preference for hemimethylated DNA [4], a property one would not expect in an invasive genetic parasite, but one very helpful in viral defense, because it ensures methylation of sites after replication but not of unmethylated viral DNA. Another example is the recognition of a newly synthesized DNA strand by MutH during mismatch repair [5]. MutH takes advantage of the Dam adenine methyltransferase that methylates the site GATC. Hemimethylated sites generated by replication allow MutH to identify newly synthesized DNA, thus ensuring that the original strand is used as the template for repair. The symmetry of the Dam recognition site is essential for this process. In fact the semiconservative replication of DNA methylation driven by preferential modification of hemimethylated symmetric sites is the single feature that unites virtually all the known functions of DNA methylation.

The star of eukaryotic DNA methylation, Dnmt1, inherited the key features of its bacterial ancestors [6]. Dnmt1 methylates a minimal palindromic site, the cytosine of dinucleotide CG (methylation of the fifth carbon of cytosine is the only type found in plants and animals), with a strong preference for hemimethylated DNA *in vivo* [7,8]. Dnmt1 is responsible for the maintenance of virtually all methylation in animals and most methylation in plants (where it is called MET1). Plants have an additional methyltransferase, CHROMOMETHYLASE3, which methylates CNG sites [9]. Dnmt1, in particular, appears to have the ability to faithfully propagate patterns of DNA methylation over many mitotic (and in plants meiotic) generations, an ability that has been put to a variety of uses [6]. A number of excellent recent reviews have covered the mechanisms of the establishment and maintenance of DNA methylation [10–14]. Here I will focus on the functions of DNA methylation within plant genomes.

Genomic immunity

A number of recent studies have mapped the distribution of DNA methylation in the entire genome of the model plant *Arabidopsis thaliana*, the latest using high-throughput sequencing of bisulfite-converted DNA to achieve single base pair resolution [15••,16••,17•,18,19]. The most striking feature of the resulting profile is virtually ubiquitous methylation of transposable elements of every variety, providing the strongest evidence yet that DNA methylation is used to control transposons. Although still controversial in animals [13], there is little doubt that plants and fungi use methylation in transposon defense. Some fungi actually do plants one better: *Neurospora crassa* couples DNA methylation with deamination to mutate cytosines to adenines in any duplicated sequence, thus destroying both copies of the repeat [20].

In plants, as in most eukaryotes, transposable elements cluster around centromeres, forming cytologically distinguishable blocks of condensed chromatin known as chromocenters. Plenty of transposons, however, are found in the euchromatic arms even in the small genome of *Arabidopsis*. So what happens if a transposon integrates too near a gene, or even within an intron, or if duplication causes genic sequence to be 'mistaken' for a transposon by the methylation machinery? Generally, this means trouble, because even if the transposon is methylated and silenced, a nearby gene can still be adversely affected by being silenced along with the element. Occasionally, though, a fortuitous insertion or duplication followed by methylation can confer an advantage. One example of this is the *Arabidopsis FLC* gene, which controls flowering time [21]. *Arabidopsis* accessions with a hypoactive *FLC* gene flower early, a potential advantage under certain climactic conditions. Two *Arabidopsis* strains harbor independent insertions of different transposable elements within the first intron of *FLC* [22–24]. The methylation of these elements renders *FLC* insensitive to upregulation by the activator FRIGIDA, thus conferring early flowering. Transposons and repeats are thus capable of bringing gene expression under the control of DNA methylation. An intricate example of this is parent-specific gene expression, discussed below.

Genomic imprinting

A number of genes in mammals and flowering plants have a peculiar property: they are only expressed from the genome of one of the parents [25,26]. This genomic imprinting negates one of the major benefits of diploidy for these genes by preventing a defective allele from being covered by the other copy. The most popular theory for why this takes place postulates that in organisms whose embryos derive nourishment directly from the mother and where the offspring of a female can be sired by multiple males, the interests of the parents are not congruent [27]. The male benefits when his offspring extract the maximum resources possible at the expense of current and future siblings, whereas the female prefers to distribute resources equally. Thus genes favoring the former outcome tend to be expressed from the male's genome and genes favoring the latter from the female's.

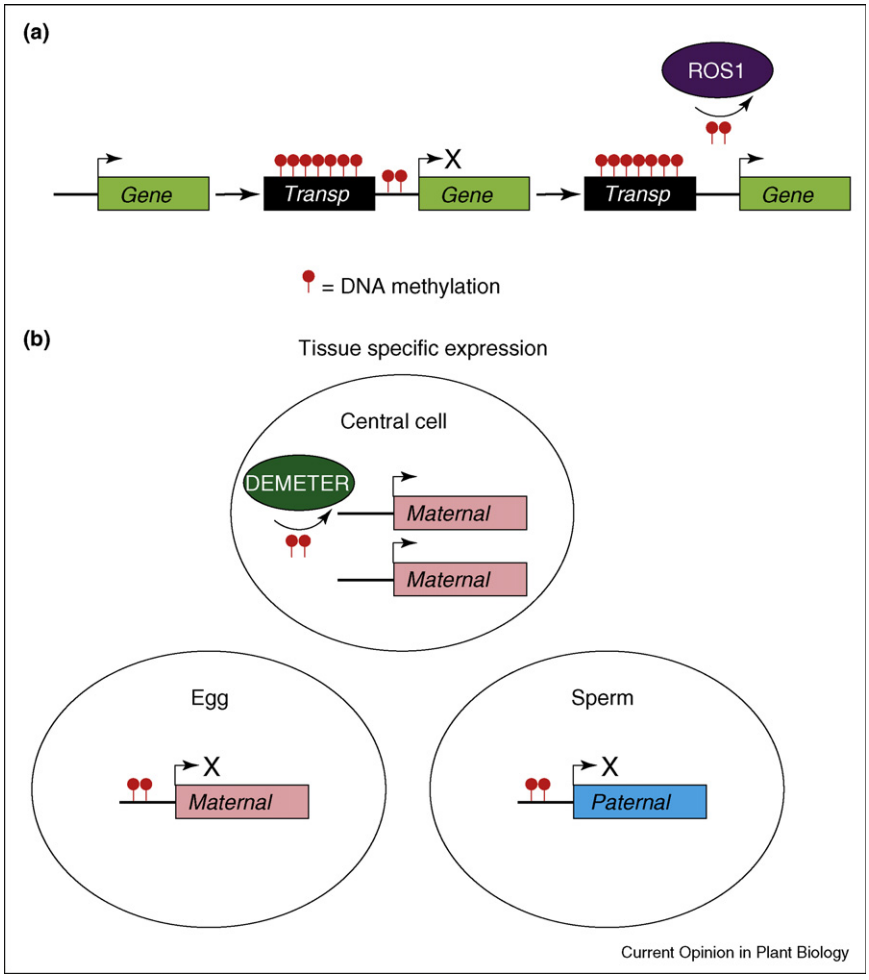
Imprinting of a number of mammalian and plant genes is controlled by DNA methylation. In mammals, methylation of imprinted genes is established in the gametes, with female-expressed genes generally methylated in the male gametes and vice versa [26]. In *Arabidopsis*, all known imprinted genes that are controlled by DNA methylation (*FWA*, *MEDEA*, and *FIS2*) are expressed from the female genome in the endosperm, a tissue that nourishes the developing embryo [25]. Methylation of these genes is the default state: *FWA* and *FIS2* are methylated at the promoter, while *MEDEA* is methylated 3′ of the gene [28–30]. A DNA demethylase, *DEMETER*, is expressed in the central cell, the precursor of the endosperm, before fertilization, removing methylation from and activating the imprinted genes (Figure 1) [28,31]. Because the endosperm is consumed by the embryo, there is no need to re-establish methylation of these genes.

Although plant and mammalian imprinting mechanisms are in many ways analogous, the processes evolved independently. This brings up an important question: how did these pathways evolve? Recent studies in *Arabidopsis* offer an important clue. *DEMETER* is part of a family of four *Arabidopsis* genes, three of which (*ROS1*, *DML2*, and *DML3*) are widely expressed and appear to play no role in imprinting [32•]. All three have demethylase activity [32•,33]. Mutation of all three genes, or of just *ROS1*, leads to hypermethylation of a number of loci (nearly 200 in the case of the triple mutant) [32•,34,35•]. There is strong preference for hypermethylation just 5′ and 3′ of genes [32•]. Methylation of these areas is strongly disfavored in wild type [18,19,36], which makes good sense, at least for upstream sequences, because promoter methylation is generally incompatible with transcriptional initiation in plants and animals [6]. The demethylase genes thus appear to protect the genome from inappropriate methylation, preventing endogenous genes from being 'mistaken' for transposons. *FWA*, *FIS2*, and *MEDEA* are methylated in the same regions that are normally targeted by the demethylases, and the methylated sequences of *FWA* and *MEDEA* are repeated [28,29,37,38]. It is thus easy to imagine how imprinting might have evolved from a more ancient genome protection system by restricting the expression of a demethylase during seed development to the central cell (Figure 1).

Beyond the promoter

Perhaps the biggest surprise revealed by genomic studies of *Arabidopsis* DNA methylation was the prevalence of DNA methylation in the bodies of genes [15••,16••,17•,18,19]. Furthermore, there was a link to transcription, because methylation of genes transcribed at

Figure 1



Model for the evolution of gene imprinting via DNA methylation in plants. (**a**) A transposition event near a gene leads to DNA methylation and silencing of both the transposon and the gene. Mistaken DNA methylation of the endogenous gene is reversed by DNA demethylases (ROS1), reactivating the gene. (**b**) Duplication of a demethylase gene and subsequent restriction of expression of one copy (DEMETER) to the central cell leads to tissue-specific demethylation and activation of genes.

very low and very high levels is strongly disfavored [18,19]. Animal genes have long been known to be methylated, but *Arabidopsis* genes have been widely believed to be methylation free with few exceptions. The finding that roughly a third of *Arabidopsis* genes were methylated begged the question of what all this methylation might be doing. Loss of gene body methylation caused by the mutation of *MET1* resulted in only a minor change in expression [19], so it is improbable that this methylation is required for normal transcription. Notably, however, a recent study has shown that CG methylation (the type found in *Arabidopsis* genes) of an exon of the *Arabidopsis* phytochrome A gene causes strong transcriptional repression, demonstrating that intragenic methylation can influence transcription. [39]. We have proposed that methylation of gene bodies might repress inappropriate transcriptional initiation [19,36], by analogy with a yeast pathway that prevents aberrant initiation from gene bodies by ensuring proper chromatin assembly following passage of RNA polymerase [40]. This theory remains to be tested, and it is possible that body methylation in plants does little of anything, but is instead tolerated in a subset of genes. One thing is clear: although methylation of genes can be highly variable between different strains of *Arabidopsis* [17•], it is not random.

An important clue came from a recent study that examined DNA methylation in the *met1* mutant [16••]. Mutation of *MET1*, which results in essentially complete loss of CG methylation [15••,16••], has long been known to cause hypermethylation of CNG sites [41]. Such methylation has been generally assumed to be highly stochastic, and yet appears to ameliorate the phenotype of *met1* plants [42•]. By combining bisulfite treatment, which converts unmethylated cytosine to uracil, with high-throughput sequencing [43,44], Lister *et al.* [16••] showed

that CNG hypermethylation in *met1* preferentially occurs in gene bodies and that 78% of hypermethylated genes had CG methylation in wild type. This overlap is far too high to be explained by the effects of transcription alone, because most genes are unmethylated regardless of how strongly they are transcribed. Thus, genes that are normally methylated are either specifically targeted for methylation or other genes in the genome are protected. Given how widespread genic methylation is, deciphering what it might be doing and how it is regulated will be a major priority for future research.

Arabidopsis as a model for DNA methylation analysis

Current evidence suggests that the involvement of DNA methylation in plant development beyond imprinting is likely to be limited [10,11]. *Arabidopsis* plants with a knockout of *de novo* methylation activity (required for the establishment of methylation at unmethylated sequences) are morphologically normal [45], as are the demethylase triple mutants [32•], indicating that changes in DNA methylation are not required for normal development under laboratory conditions. In animals, however, methylation is much more dynamic, with differential methylation of a number of crucial developmental regulators detected in different tissues [46–49]. In particular, genes required for the maintenance of stem cell fate tend to be methylated in differentiated cells. It thus should not be surprising that DNA methylation is linked to a number of human diseases, including cancer [50] — methylation changes are an early and universal hallmark of all known tumors [51].

Superficially, the relatively minor role that DNA methylation plays in plant development might suggest that plants are a poor model for understanding human diseases linked to methylation. However, the machinery of DNA methylation is highly conserved between plants and animals — the major differences have to do with targets [6]. In fact, the relative insulation of development in plants is extremely useful [52], because *Arabidopsis* plants survive simultaneous mutations in multiple DNA methyltransferases [53], whereas any such single mutation is lethal in mice [6]. What makes plants even more valuable is that DNA methylation has been lost several times in the course of animal and fungal evolution, so the common model organisms that have proven so powerful in deciphering other processes — *Drosophila*, *C. elegans*, *S. cerevisiae*, and *S. pombe* — are of no use, except as surrogates for methyltransferase transgenes [6,54,55]. *Arabidopsis* also has the advantage of a genome 20 times smaller than that of human or mouse, thus greatly simplifying genomic studies. DNA methylation in *Arabidopsis* has been mapped at single base pair resolution throughout the entire genome [15••,16••], an accomplishment not matched by other systems. Plants are thus uniquely positioned to help answer outstanding questions about the biology and functions of DNA methylation.

Conclusions

Through evolution DNA methylation has been put to many uses: genome defense against transposable elements, gene regulation, and perhaps repression of cryptic transcription within genes. This versatility derives from the unique ability of methyltransferases to faithfully propagate methylation patterns through rounds of cell division. Until recently we had little information about the methylation profiles of eukaryotic genomes, but this is rapidly changing. Powerful genomic technologies are allowing new insights into this ancient and long-studied process. Plants are well positioned to be at the forefront of future methylation research that is likely to directly impact human health.

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