Published in final edited form as:

Trends Plant Sci. 2012 March ; 17(3): 149–154. doi:10.1016/j.tplants.2012.01.001.

Epigenetic and epigenomic variation in *Arabidopsis thaliana*

Robert J. Schmitz^{1,2} and Joseph R. Ecker^{1,2,3}

¹Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

²Genomic Analysis Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

³Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

Arabidopsis thaliana (*Arabidopsis*) is ideally suited for studies of natural phenotypic variation. This species has also provided an unparalleled experimental system to explore the mechanistic link between genetic and epigenetic variation, especially with regard to cytosine methylation. Using high-throughput sequencing methods, genotype to epigenotype to phenotype observations can now be extended to plant populations. We review the evidence for induced and spontaneous epigenetic variants that have been identified in *Arabidopsis* in the laboratory and discuss how these experimental observations could explain existing variation in the wild.

Natural genetic variation

Diversity within and between species is primarily driven by genetic variation and interactions with the environment. *Arabidopsis thaliana* has adapted to numerous environments, as it exists naturally and broadly throughout the northern hemisphere [1]. It is a selfing species that contains a largely isogenic genome, although residual heterozygosity does exist due to infrequent admixture [2]. The genome is compact and depleted of transposons and is relatively small (~150 Mb) compared to other plant species [3,4]. Recently, the *Arabidopsis* 1001 Genomes Project (see 1001genomes.org and signal.salk.edu/atg1001) was initiated with the goal of identifying the total genetic variation present within this species and to enable scientists around the globe to study their favorite quantitative trait of interest using association or linkage mapping techniques [1,5,6]. One challenge for adaptation of *Arabidopsis* to novel environments may be a limited set of mutations [7,8]. Therefore, it is essential to identify not only genetic variation but also natural epigenetic (see Glossary) variation, for which *Arabidopsis* is ideally suited [9,10]. In this regard, in addition to sequencing genomes [11–15], efforts are underway by the *Arabidopsis* 1001 Epigenomes Project (<http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=1122246>) to sequence transcriptomes and DNA methylomes, which will be useful for identifying natural epigenetic variants.

Epigenetic alleles (Epialleles)

The extent to which epigenetic variation contributes to phenotypes remains to be determined [16]. However, several examples of phenotypic variation that can arise through epigenetic variation are known, including the *peloric* (*Linaria vulgaris*) [17], *colorless non-ripening* (*cnr* – *Solanum lycopersicum*) [18] and *B'* (*Zea mays*) [19] epialleles. Studies in

Arabidopsis thaliana have provided the richest source of known epialleles [20–32]. Many of the *Arabidopsis* epialleles require the activities of the RNA-directed DNA methylation (RdDM) machinery [33] for their formation and stability. RdDM functions to silence repetitive and transposon sequences and in some cases genes by a complex set of machinery that initiates transcription, generates 24nt RNAs and methylates targeted DNA in all cytosine contexts (for review see [33]). In fact, a number of these epialleles were used in genetic screens or as markers [21,22,28,29] to uncover novel components and mechanisms of the RdDM pathway [34–48].

Epigenetics is the study of mitotically and/or meiotically heritable changes in phenotype that arise independent of genetic variation. This definition is especially difficult to reconcile in studies of natural epigenetic variation, as by definition the populations under study exhibit extensive genetic variation. Recognizing this conundrum and the complexity of epiallele formation, a classification system that grouped epialleles into one of three classes based on their dependence on genotype has been proposed [49]. The first class called obligate epialleles display a complete dependency on a genetic variant, whereas the second class called facilitated epialleles form from the presence of a genetic variant such as a nearby transposon insertion, but their maintenance is not necessarily dependent on this variant. The third class, pure epialleles, form independent of any genetic variation. The epialleles thus far identified in *Arabidopsis* are either obligate epialleles [21,24] or are facilitated epialleles, as their formation has been induced using mutations [23,27,28,50] in machinery required for maintenance of DNA methylation or they were recovered from mutagenesis experiments [22,29]. Mitotically pure epialleles exist [51], but until recently [52–54] the identification of naturally occurring meiotically pure epialleles has remained elusive.

Spontaneous epigenetic variation

Epialleles have the potential for reversibility and often exist in metastable states, as has been observed for both the *peloric* and *cnr* natural epialleles [17,18]. With the exception of transposon-induced events, this feature is unique to epialleles as genetic mutations are typically considered unidirectional over evolutionary time. The molecular spectrum and rate of spontaneous mutations are relatively well understood especially when compared to our understanding of spontaneous epigenetic variation. Genome resequencing of a collection of *Arabidopsis thaliana* mutation accumulation (MA) lines, which were created from a lone founder by single seed descent growth for 30 generations [55], revealed just under one mutation per line per generation [56]. Recently, base pair resolution DNA methylomes of this same population were determined, which uncovered a rate of base level spontaneous variation in DNA methylation (single methylation polymorphisms – SMPs) that was at least four orders of magnitude greater than genetic mutations [52,53]. Furthermore, these studies captured the formation of pure epialleles, some of which resulted in significant transcriptional variation (~10- to 1000-fold change) of the affected locus [52,53]. One study proposed that these pure epialleles may have arisen through incomplete reinforcement of methylation states that occurs postfertilization [52] (Figure 1), because the sites of epiallelic variation overlapped significantly with loci that are also affected in the maintenance methyltransferase *met1* and in the triple mutant of DNA demethylases *rdm1* (*REPRESSOR OF SILENCING1*; *DEMETER-LIKE2*; *DEMETER-LIKE3*) [57–59]. The involvement of epigenetic mechanisms that require the activities of small RNAs and/or DNA methylation machinery are widespread in plant reproductive development. In fact, both the maternally and the paternally inherited genomes in *Arabidopsis* require intricate mechanisms for ensuring their identity and for inheritance of epigenomic information from generation to generation [60,61]. One feature of these mechanisms is to correct errors (loss of DNA methylation) through the use of smRNA (small RNA) guides [62], but

inefficiencies in transferring this information across generations may result in the formation of a pure epiallele.

The variation in methylation observed in the MA lines was not uniformly distributed throughout the genome. This was especially the case for CG gene body methylation [9,63,64], which still has an unknown role and requires MET1 for its maintenance. In fact, there was a depletion of variation associated with transposon sequences, exons and the pericentromeres [52,53]. These genomic regions all are associated with nucleosome occupancy [65] and may reflect that methyltransferase enzymes such as MET1 maintain nucleosome-associated DNA methylation at a higher fidelity than nucleosome-free DNA methylation.

In general, the rates of spontaneous variation in DNA methylation were similar between all of the MA lines except for one (line 69). This hypervariability is unlikely due to the environment because the entire population was derived in a common greenhouse in synchrony, which would be expected to affect numerous lines if there were such pressures. Additionally, line 69 was identified as being hypervariable in two independent laboratories [52,53]. The basis for this hypervariability has been examined by resequencing the genome of line 69 [53]. Intriguingly, an additional nonsynonymous SNP (G–T, Ala–Ser) not previously reported in this line [56] was identified in *MEE57* [53], a homolog of the maintenance methyltransferase *MET1* [66]. It is conceivable that the variation in DNA methylation observed in line 69 may be due to this mutation in *MEE57*, which could potentially represent one route to the formation of genome-wide epigenetic variation.

Mutation-induced epigenetic variation

As described above, spontaneous genetic variation may provide a source of natural epigenetic variation. Proof-of-concept for this type of epiallele formation was originally demonstrated by using mutants in *DDM1* (*DECREASE IN DNA METHYLATION 1* [67]), which encodes a SWI–SNF2-like protein that is related to chromatin remodelers in mouse and humans [68]. *ddm1* mutants result in a genome-wide depletion of DNA methylation, especially in repeated regions of the genome [67]. Remarkably, remethylation of *ddm1* chromosomes does not occur immediately upon introduction to wild-type chromosomes, instead remethylation occurs gradually over generational time [67]. Genome-wide depletion of DNA methylation [57,63,69] and epiallele formation [25] can also be induced in *met1* mutants, which can result in a variety of developmental phenotypes [70,71]. It is noteworthy that *ddm1* mutants do not display major developmental abnormalities until successive generations removed from the originally mutated plant [30,72]. However, much of this mutation-induced epigenetic variation results in the stable transmission of morphological phenotypes across generations, even when outcrossed from the original mutant backgrounds [23,27,50,73,74]. Although the major molecular phenotype of *ddm1* or *met1* mutants is a depletion of DNA methylation, examples of genetic variation in the form of genomic rearrangements, copy number variants (CNVs) and frequent DNA transposition have also been observed and may account for a considerable amount of phenotypic variability [21,50,73,75–77].

Barbara McClintock proposed the concept of gene regulation by transposon insertion within or near genes [78]. It is now commonly accepted that variation in gene expression can be regulated by the position of nearby transposons [79–82]. For example, transposon insertions in certain accessions of *Arabidopsis thaliana* can result in a rapid cycling flowering behavior [24]. Most fascinating is the recent identification of the ONSSEN class of transposons that are heat responsive [83]. The transcriptional activation of ONSSEN can persist for at least three days after the heat stress has been removed and can result in transposition in the germline of

RNAi mutants [83]. Moreover, genome reinsertion of *ONSEN* in the RNAi mutants in the nonstressed next generation confers heat responsiveness to nearby genes [83]. Even though plant species have developed a wide range of mechanisms to ensure faithful transposon silencing, transpositions can occur over generational time either in the wild or in cell culture as well as in mutant backgrounds [62,76,83,84] and can provide a substrate for epigenetic variation to nearby genes (Figure 2).

Epigenetic recombinant inbred lines (epiRILs)

The relatively stable transmission of epigenetic variation observed in *ddm1* and *met1* mutants provided the basis for creation of epigenetic recombinant inbred lines or epiRILs [23,27]. RIL populations are commonly constructed in plants and are used for mapping causal variants for traits that are variable between two naturally occurring parental lineages. The epiRIL populations provide a unique opportunity to reduce genetic variability and increase epigenetic variability. Two independent epiRIL populations were created using either *ddm1* [23] or *met1* [27] mutants that were crossed to wild-type Col-0 plants to generate F1 hybrids. Segregating populations of plants were screened for the presence of wild-type alleles of either *DDM1* or *MET1* and propagated by single seed descent for multiple generations to increase homozygosity. Each epiRIL within the population essentially contains a mosaic epigenome derived from either wild type and *ddm1* or wild type and *met1*. Using microarray-based epigenomic approaches, it was determined that although some of the epialleles remained stable throughout construction of the epiRIL population, many slowly reverted back to the wild-type status over generational time [23,27]. This type of reversion is not uncommon and probably results from *de novo* methylation through smRNA guides [62]. In addition to epigenetic variation, there was clear evidence of genetic variation in the form of transpositions in the epiRILs [23,27,76].

Both epiRIL populations contained extensive phenotypic variation that was stable throughout each population for numerous traits such as time to flowering, plant height and fruit size [23,27]. Phenotypes present within the *met1* epiRIL population resembled variation observed in natural *Arabidopsis* accessions [27]. Moreover, significant heritability estimates and continuous distribution of phenotypes were observed in the *ddm1* epiRILs, indicating that multiple loci contribute to the observed phenotypic variation and these loci can potentially be identified through linkage mapping approaches [85]. Recently, the *ddm1* epiRIL population was subjected to extensive phenotyping alongside natural *Arabidopsis* accessions in a common garden to test the contribution of epigenetic variation to phenotypic diversity [86]. The findings demonstrate that phenotypic variation in the epiRIL population displays heritability estimates similar to natural (wild) accessions grown in parallel [86]. Although epiRILs represent a set of artificially induced epialleles, these results provide compelling evidence that if spontaneous mutations do occur in components of the methylation machinery then an explosion of largely heritable epigenetic variation may quickly arise, providing a substrate for natural selection to act upon. As described above, a spontaneous mutation occurring in line 69 of the MA population was found in a component of methylation machinery (*MEE57*) [53], which could account for the increased hypervariability of methylation polymorphisms in these plants [52,53].

Natural epigenetic variation

Although natural epigenetic variation exists in *Arabidopsis* [9,10], understanding the basis for this variation presents a complex challenge due to the inherent genetic variation also present in these same accessions [11–15]. The experimental systems mentioned above provide alternative routes by which epigenetic variants can arise and spread within populations. There is, however, evidence for other events occurring in the wild

[21,24,87,88]. A survey of *Arabidopsis* accessions for variation in methylation of the 178-bp centromeric satellite repeat identified hypomethylation of this region in the Bor-4 accession [88]. The causal variant for the hypomethylation was identified through linkage mapping as it segregated as a single recessive locus and encodes a methylcytosine binding protein named *VIMI* (*VARIANT IN METHYLATION 1*) [88]. Because the methylation status of the centromeric repeat was entirely dependent on the presence of the *VIMI* variant, methylation variation of centromeric repeats in Bor-4 represents an example of ‘obligate’ epigenetic variation [49]. This case nicely illustrates the ability of genetic variants to affect epigenetic variation *in trans* similar to what is observed in the epiRIL population. Another example of natural epigenetic variation that uncovers the complexity of epiallele formation exists at the *PAI* (*PHOSPHORIBOSYLANTHRANILATE ISOMERASE*) loci in certain *Arabidopsis* accessions [21,89]. In the Col-0 genome the *PAI* loci are present as a three-member gene family with high nucleotide sequence identity. In some accessions, the *PAI1* locus exists as a duplicated inverted repeat as well as in other rearranged forms depending on the accession [89]. This inverted repeat leads to silencing by the RdDM pathway both *in cis* at *PAI1* as well as *in trans* at *PAI2* and *PAI3* [45,90]. The methylated *PAI* loci provides an additional example of naturally occurring ‘obligate’ epialleles as the silencing of these loci dissipates when either *PAI2* or *PAI3* are segregated away by outcrossing from the inverted repeat at *PAI1* [21].

Epigenomic approaches for studying natural epigenetic variation

The type of epiallelic variation present at the *PAI* gene family in some accessions of *Arabidopsis* is prevalent within this species. Using a comparative epigenomic approach, extensive methylation variation was identified between the Col-0 and *Ler* accessions; some of which overlapped with copy number variants identified within these same accessions [9]. Two major groups of methylation variants were identified in this study; one group contained hypervariable genic methylation patterns although a second group included transposons, which were invariably methylated. This second group was strongly associated with smRNAs typical of the RdDM pathway, whereas the genic methylation showed no such relationship [9].

An array-based epigenomic approach [10] was used to examine the genome-wide patterns and heritability of methylation polymorphisms between two different *Arabidopsis* accessions. This study revealed that inheritance of methylation was largely additive, as intermediate methylation levels were observed in F1 hybrids. They also determined that methylation variation immediately upstream of genes was strongly correlated with transcriptional variation, whereas variation of gene body methylation had no significant impact on gene expression levels [10].

There are currently a relatively small number of natural epialleles known in *Arabidopsis*, but with the use of epigenomic approaches this situation is likely to rapidly change [91]. Early attempts at profiling genome-wide patterns of histone modifications and DNA methylation resulted in low-resolution maps (~50–1000 bp) and were unable to provide information regarding specific (base resolution) DNA methylation contexts [9,10,64,92,93,63,94]. However, it is now possible to survey complete genomes for DNA methylation at base pair resolution by combining the gold standard for methylation detection sodium bisulfite conversion [95–101], with high-throughput DNA sequencing [57,69]. The cytosine DNA methylome maps produced using these new methodologies make it possible to distinguish between the commonly observed gene body CG methylation and the rarely observed gene body methylation that occurs in all three sequence contexts (CG, CHG, CHH), which is typically targeted by the RdDM pathway. As described above in the spontaneous epigenetic variation section, the ability to survey base resolution methylomes will rapidly accelerate the

identification of natural epigenetic variants in any plant species that contains a reference genome.

Concluding remarks and future outlook

There exists natural epigenetic variation within *Arabidopsis*, but it is becoming increasingly clear that many forms of *cis* and *trans* genetic variation can contribute to the formation of epialleles. In the near future, it will be possible to use association mapping techniques that combine both nucleotide (CNV or SNP) and methylation variation [differentially methylated region (DMR) or SMP] present within this species to map causal variants for much of this epigenetic variation. It is also clear that perturbations to the machinery required for RNAi and/or DNA methylation probably result in a release of transcriptional silencing, promoting both genetic variation as well as the formation of novel epialleles. Methods now exist for detecting base resolution DNA methylomes, shifting the need for improvement to input sample preparation. Essentially all published studies of plant epigenomes have utilized complex tissues, comprising numerous cell types, for DNA methylome profiling. Recent developments now enable enriching samples for specific cell types [102–104]. Future improvement may even allow interrogation of the epigenomes of single cells, which would provide unprecedented resolution for detection of epigenomic variation. These higher resolution views will be essential to unravel the intricate relationship between genotype, epigenotype and phenotype, allowing this knowledge to be applied to a wide range of species, including plants and people.

Acknowledgments

We would like to thank Milos Tanurdzic and anonymous reviewers for their helpful suggestions to improve the clarity and thoroughness of this review. R.J.S. was supported by an National Institutes of Health National Research Service Award (NIH NRSA) postdoctoral fellowship (F32-HG004830). This work was supported by the National Science Foundation (MCB-0929402 and MCB1122246), the Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation (J.R.E.). J.R.E. is a Howard Hughes Medical Institute–Gordon and Betty Moore Foundation (HHMI–GBMF) Investigator.

Glossary

Accession	a natural (wild) isolate from a specific geographic location
CNV	copy number variant
DMR	differentially methylated region
Epiallele	a heritable variant of an allele that is not reflected by a change in its DNA sequence, but does have an effect on gene expression
Epigenetics	the study of mitotically and/or meiotically heritable changes in phenotype that arise independent of genetic variation
Epigenomics	the genome-wide study of modifications to DNA and associated proteins such as a variety of modified and variant histones, nucleosome positioning, transcription factors as well as RNAs
epiRIL population	recombinant inbred lines that have wild-type genotypes, but contain mosaic epigenomes based on the parental lines used to establish the population
MethylC-Seq	a genome-wide technique to survey base resolution maps of DNA methylation using high-throughput sequencing

RNA-directed DNA methylation (RdDM)	a mechanism that is involved in silencing through a feedback loop consisting of small RNAs guiding DNA methylation and histone modifications
Small RNAs (smRNAs)	21–24 nucleotides (nt) RNA species that can guide machinery to silence loci post-transcriptionally (21 nt smRNAs) and/or transcriptionally (24 nt smRNAs)
SMP	single methylation polymorphism

References

1. Nordborg M, Weigel D. Next-generation genetics in plants. *Nature*. 2008; 456:720–723. [PubMed: 19079047]
2. Tang C, et al. The evolution of selfing in *Arabidopsis thaliana*. *Science*. 2007; 317:1070–1072. [PubMed: 17656687]
3. Schnable PS, et al. The B73 maize genome: complexity, diversity, and dynamics. *Science*. 2009; 326:1112–1115. [PubMed: 19965430]
4. Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*. 2000; 408:796–815. [PubMed: 11130711]
5. Atwell S, et al. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*. 2010; 465:627–631. [PubMed: 20336072]
6. Weigel D, Mott R. The 1001 genomes project for *Arabidopsis thaliana*. *Genome Biol*. 2009; 10:107. [PubMed: 19519932]
7. Hancock AM, et al. Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*. 2011; 334:83–86. [PubMed: 21980108]
8. Fournier-Level A, et al. A map of local adaptation in *Arabidopsis thaliana*. *Science*. 2011; 334:86–89. [PubMed: 21980109]
9. Vaughn MW, et al. Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol*. 2007; 5:e174. [PubMed: 17579518]
10. Zhang X, et al. Global analysis of genetic, epigenetic and transcriptional polymorphisms in *Arabidopsis thaliana* using whole genome tiling arrays. *PLoS Genet*. 2008; 4:e1000032. [PubMed: 18369451]
11. Cao J, et al. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat Genet*. 2011; 43:956–963. [PubMed: 21874002]
12. Gan X, et al. Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*. *Nature*. 2011; 477:419–423. [PubMed: 21874022]
13. Ossowski S, et al. Sequencing of natural strains of *Arabidopsis thaliana* with short reads. *Genome Res*. 2008; 12:2024–2033. [PubMed: 18818371]
14. Schneeberger K, et al. Reference-guided assembly of four diverse *Arabidopsis thaliana* genomes. *Proc Natl Acad Sci USA*. 2011; 108:10249–10254. [PubMed: 21646520]
15. Santuari L, et al. Substantial deletion overlap among divergent *Arabidopsis* genomes revealed by intersection of short reads and tiling arrays. *Genome Biol*. 2010; 11:R4. [PubMed: 20067627]
16. Richards EJ. Natural epigenetic variation in plant species: a view from the field. *Curr Opin Plant Biol*. 2011; 14:204–209. [PubMed: 21478048]
17. Cubas P, et al. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*. 1999; 401:157–161. [PubMed: 10490023]
18. Manning K, et al. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet*. 2006; 38:948–952. [PubMed: 16832354]
19. Stam M, et al. Differential chromatin structure within a tandem array 100 kb upstream of the maize b1 locus is associated with paramutation. *Genes Dev*. 2002; 16:1906–1918. [PubMed: 12154122]

20. Foerster AM, et al. Genetic rearrangements can modify chromatin features at epialleles. *PLoS Genet.* 2011; 7:e1002331. [PubMed: 22028669]
21. Bender J, Fink GR. Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of *Arabidopsis*. *Cell.* 1995; 83:725–734. [PubMed: 8521489]
22. Jacobsen SE, Meyerowitz EM. Hypermethylated SUPERMAN epigenetic alleles in *Arabidopsis*. *Science.* 1997; 277:1100–1103. [PubMed: 9262479]
23. Johannes F, et al. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* 2009; 5:e1000530. [PubMed: 19557164]
24. Liu J, et al. siRNAs targeting an intronic transposon in the regulation of natural flowering behavior in *Arabidopsis*. *Genes Dev.* 2004; 18:2873–2878. [PubMed: 15545622]
25. Mathieu O, et al. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell.* 2007; 130:851–862. [PubMed: 17803908]
26. Mittelsten, Scheid O., et al. Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. *Nat Genet.* 2003; 34:450–454. [PubMed: 12847525]
27. Reinders J, et al. Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* 2009; 23:939–950. [PubMed: 19390088]
28. Saze H, Kakutani T. Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1. *EMBO J.* 2007; 15:3641–3652. [PubMed: 17627280]
29. Soppe WJ, et al. The late flowering phenotype of *fwa* mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. *Mol Cell.* 2000; 6:791–802. [PubMed: 11090618]
30. Kakutani T, et al. Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc Natl Acad Sci USA.* 1996; 93:12406–12411. [PubMed: 8901594]
31. Rangwala SH, et al. Meiotically stable natural epialleles of *Sadhu*, a novel *Arabidopsis retroposon*. *PLoS Genet.* 2006; 2:e36. [PubMed: 16552445]
32. Riddle NC, Richards EJ. Genetic variation in epigenetic inheritance of ribosomal RNA gene methylation in *Arabidopsis*. *Plant J.* 2005; 41:524–532. [PubMed: 15686517]
33. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet.* 2010; 11:204–220. [PubMed: 20142834]
34. Greenberg MV, et al. Identification of genes required for de novo DNA methylation in *Arabidopsis*. *Epigenetics.* 2011; 6:344–354. [PubMed: 21150311]
35. Johnson LM, et al. SRA-domain proteins required for DRM2-mediated de novo DNA methylation. *PLoS Genet.* 2008; 4:e1000280. [PubMed: 19043555]
36. Woo HR, et al. Three SRA-domain methylcytosine-binding proteins cooperate to maintain global CpG methylation and epigenetic silencing in *Arabidopsis*. *PLoS Genet.* 2008; 4:e1000156. [PubMed: 18704160]
37. Chan SW, et al. RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in *Arabidopsis*. *PLoS Genet.* 2006; 2:e83. [PubMed: 16741558]
38. Chan SW, et al. Two-step recruitment of RNA-directed DNA methylation to tandem repeats. *PLoS Biol.* 2006; 4:e363. [PubMed: 17105345]
39. Chan SW, et al. RNA silencing genes control de novo DNA methylation. *Science.* 2004; 303:1336. [PubMed: 14988555]
40. Zilberman D, et al. *ARGONAUTE4* control of locus-specific siRNA accumulation and DNA and histone methylation. *Science.* 2003; 299:716–719. [PubMed: 12522258]
41. Cao X, Jacobsen SE. Role of the *Arabidopsis* DRM methyltransferases in de novo DNA methylation and gene silencing. *Curr Biol.* 2002; 12:1138–1144. [PubMed: 12121623]
42. Jackson JP, et al. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature.* 2002; 416:556–560. [PubMed: 11898023]
43. Lindroth AM, et al. Requirement of *CHROMOMETHYLASE3* for maintenance of CpXpG methylation. *Science.* 2001; 292:2077–2080. [PubMed: 11349138]
44. Ebbs ML, Bender J. Locus-specific control of DNA methylation by the *Arabidopsis* SUVH5 histone methyltransferase. *Plant Cell.* 2006; 18:1166–1176. [PubMed: 16582009]

45. Ebbs ML, et al. H3 lysine 9 methylation is maintained on a transcribed inverted repeat by combined action of SUVH6 and SUVH4 methyltransferases. *Mol Cell Biol.* 2005; 25:10507–10515. [PubMed: 16287862]
46. Mull L, et al. A histone methylation-dependent DNA methylation pathway is uniquely impaired by deficiency in *Arabidopsis* S-adenosylhomocysteine hydrolase. *Genetics.* 2006; 174:1161–1171. [PubMed: 16951055]
47. Bartee L, et al. *Arabidopsis cmt3* chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. *Genes Dev.* 2001; 15:1753–1758. [PubMed: 11459824]
48. Ausin I, et al. IDN1 and IDN2 are required for *de novo* DNA methylation in *Arabidopsis thaliana*. *Nat Struct Mol Biol.* 2009; 16:1325–1327. [PubMed: 19915591]
49. Richards EJ. Inherited epigenetic variation – revisiting soft inheritance. *Nat Rev Genet.* 2006; 7:395–401. [PubMed: 16534512]
50. Tsukahara S, et al. Bursts of retrotransposition reproduced in *Arabidopsis*. *Nature.* 2009; 461:423–426. [PubMed: 19734880]
51. Michaels SD, Amasino RM. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell.* 1999; 11:949–956. [PubMed: 10330478]
52. Schmitz RJ, et al. Transgenerational epigenetic instability is a source of novel methylation variants. *Science.* 2011; 334:369–373. [PubMed: 21921155]
53. Becker C, et al. Spontaneous epigenetic variation in the *Arabidopsis thaliana methylome*. *Nature.* 2011; 480:245–249. [PubMed: 22057020]
54. Eichten SR, et al. Heritable epigenetic variation among maize inbreds. *PLoS Genet.* 2011; 7:e1002372. [PubMed: 22125494]
55. Shaw RG, et al. Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. *Genetics.* 2000; 155:369–378. [PubMed: 10790410]
56. Ossowski S, et al. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science.* 2010; 327:92–94. [PubMed: 20044577]
57. Lister R, et al. Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell.* 2008; 133:523–536. [PubMed: 18423832]
58. Penterman J, et al. Genetic interactions between DNA demethylation and methylation in *Arabidopsis thaliana*. *Plant Physiol.* 2007; 145:1549–1557. [PubMed: 17951456]
59. Saze H, et al. Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nat Genet.* 2003; 34:65–69. [PubMed: 12669067]
60. Mosher RA, et al. Uniparental expression of PolIV-dependent siRNAs in developing endosperm of *Arabidopsis*. *Nature.* 2009; 460:283–286. [PubMed: 19494814]
61. Slotkin RK, et al. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell.* 2009; 136:461–472. [PubMed: 19203581]
62. Teixeira FK, et al. A role for RNAi in the selective correction of DNA methylation defects. *Science.* 2009; 323:1600–1604. [PubMed: 19179494]
63. Zhang X, et al. Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell.* 2006; 126:1189–1201. [PubMed: 16949657]
64. Zilberman D, et al. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat Genet.* 2006; 39:61–69. [PubMed: 17128275]
65. Chodavarapu RK, et al. Relationship between nucleosome positioning and DNA methylation. *Nature.* 2010; 466:388–392. [PubMed: 20512117]
66. Pagnussat GC, et al. Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. *Development.* 2005; 132:603–614. [PubMed: 15634699]
67. Vongs A, et al. *Arabidopsis thaliana* DNA methylation mutants. *Science.* 1993; 260:1926–1928. [PubMed: 8316832]
68. Jeddeloh JA, et al. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. *Nat Genet.* 1999; 22:94–97. [PubMed: 10319870]

69. Cokus SJ, et al. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature*. 2008; 452:215–219. [PubMed: 18278030]
70. Ronemus MJ, et al. Demethylation-induced developmental pleiotropy in *Arabidopsis*. *Science*. 1996; 273:654–657. [PubMed: 8662558]
71. Finnegan EJ, et al. Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc Natl Acad Sci USA*. 1996; 93:8449–8454. [PubMed: 8710891]
72. Jacobsen SE, et al. Ectopic hypermethylation of flower-specific genes in *Arabidopsis*. *Curr Biol*. 2000; 10:179–186. [PubMed: 10704409]
73. Miura A, et al. Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. *Nature*. 2001; 411:212–214. [PubMed: 11346800]
74. Kakutani T, et al. Meiotically and mitotically stable inheritance of DNA hypomethylation induced by ddm1 mutation of *Arabidopsis thaliana*. *Genetics*. 1999; 151:831–838. [PubMed: 9927473]
75. Yi H, Richards EJ. Gene duplication and hypermutation of the pathogen *Resistance gene* SNC1 in the *Arabidopsis bal* variant. *Genetics*. 2009; 183:1227–1234. [PubMed: 19797048]
76. Mirouze M, et al. Selective epigenetic control of retrotransposition in *Arabidopsis*. *Nature*. 2009; 461:427–430. [PubMed: 19734882]
77. Singer T, et al. Robertson's *Mutator* transposons in *A. thaliana* are regulated by the chromatin-remodeling gene *Decrease in DNA Methylation* (DDM1). *Genes Dev*. 2001; 15:591–602. [PubMed: 11238379]
78. McClintock B. The origin and behavior of mutable loci in maize. *Proc Natl Acad Sci USA*. 1950; 36:344–355. [PubMed: 15430309]
79. Ahmed I, et al. Genome-wide evidence for local DNA methylation spreading from small RNA-targeted sequences in *Arabidopsis*. *Nucleic Acids Res*. 2011; 39:6919–6931. [PubMed: 21586580]
80. Lippman Z, et al. Role of transposable elements in heterochromatin and epigenetic control. *Nature*. 2004; 430:471–476. [PubMed: 15269773]
81. Hollister JD, Gaut BS. Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res*. 2009; 19:1419–1428. [PubMed: 19478138]
82. Hollister JD, et al. Transposable elements and small RNAs contribute to gene expression divergence between *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Proc Natl Acad Sci USA*. 2011; 108:2322–2327. [PubMed: 21252301]
83. Ito H, et al. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature*. 2011; 472:115–119. [PubMed: 21399627]
84. Tanurdzic M, et al. Epigenomic consequences of immortalized plant cell suspension culture. *PLoS Biol*. 2008; 6:2880–2895. [PubMed: 19071958]
85. Johannes F, et al. Epigenome dynamics: a quantitative genetics perspective. *Nat Rev Genet*. 2008; 9:883–890. [PubMed: 18927581]
86. Roux F, et al. Genome-wide epigenetic perturbation jumpstarts patterns of heritable variation found in nature. *Genetics*. 2011; 188:1015–1017. [PubMed: 21596900]
87. Riddle NC, Richards EJ. The control of natural variation in cytosine methylation in *Arabidopsis*. *Genetics*. 2002; 162:355–363. [PubMed: 12242246]
88. Woo HR, et al. VIM1, amethylcytosine-binding protein required for centromeric heterochromatinization. *Genes Dev*. 2007; 21:267–277. [PubMed: 17242155]
89. Melquist S, et al. *Arabidopsis PAI* gene arrangements, cytosine methylation and expression. *Genetics*. 1999; 153:401–413. [PubMed: 10471722]
90. Melquist S, Bender J. Transcription from an upstream promoter controls methylation signaling from an inverted repeat of endogenous genes in *Arabidopsis*. *Genes Dev*. 2003; 17:2036–2047. [PubMed: 12893775]
91. Schmitz RJ, Zhang X. High-throughput approaches for plant epigenomic studies. *Curr Opin Plant Biol*. 2011; 14:130–136. [PubMed: 21470901]
92. Zhang X, et al. Genome-wide analysis of mono-, di- and trimethylation of histone H3 lysine 4 in *Arabidopsis thaliana*. *Genome Biol*. 2009; 10:R62. [PubMed: 19508735]

93. Zhang X, et al. Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. PLoS Biol. 2007; 5:e129. [PubMed: 17439305]
94. Bernatavichute YV, et al. Genome-wide association of histone H3 lysine nine methylation with CHG DNA methylation in *Arabidopsis thaliana*. PLoS ONE. 2008; 3:e3156. [PubMed: 18776934]
95. Clark SJ, et al. High sensitivity mapping of methylated cytosines. Nucleic Acids Res. 1994; 22:2990–2997. [PubMed: 8065911]
96. Frommer M, et al. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc Natl Acad Sci USA. 1992; 89:1827–1831. [PubMed: 1542678]
97. Hayatsu H, et al. Reaction of sodium bisulfite with uracil, cytosine, and their derivatives. Biochemistry. 1970; 9:2858–2865. [PubMed: 5459538]
98. Shapiro R, et al. Deamination of cytosine derivatives by bisulfite. Mechanism of the reaction. J Am Chem Soc. 1974; 96:906–912. [PubMed: 4814744]
99. Shapiro R, et al. Nucleic acid reactivity and conformation. II. Reaction of cytosine and uracil with sodium bisulfite. J Biol Chem. 1973; 248:4060–4064. [PubMed: 4736082]
100. Hayatsu H. Bisulfite modification of nucleic acids and their constituents. Prog Nucleic Acid Res Mol Biol. 1976; 16:75–124. [PubMed: 2948]
101. Wang RY, et al. Comparison of bisulfite modification of 5-methyldeoxycytidine and deoxycytidine residues. Nucleic Acids Res. 1980; 8:4777–4790. [PubMed: 7443525]
102. Deal RB, Henikoff S. A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Dev Cell. 2010; 18:1030–1040. [PubMed: 20627084]
103. Deal RB, Henikoff S. The INTACT method for cell type-specific gene expression and chromatin profiling in *Arabidopsis thaliana*. Nat Protoc. 2011; 6:56–68. [PubMed: 21212783]
104. Birnbaum K, et al. A gene expression map of the *Arabidopsis* root. Science. 2003; 302:1956–1960. [PubMed: 14671301]

Figure 1.

Incorrect maintenance of methylation states over generational time at certain loci may lead to the formation of pure (nongenetic) epialleles. It has been proposed that the activities of MET1 and RDD are required to maintain methylation states in the germ line during postfertilization [52]. A failure to maintain the appropriate methylation state by plants wild type for either MET1 [(a) – loss of DNA methylation] or RDD [(b) – gain of methylation] or additional enzymes (X) may result in the formation of pure epialleles, unlinked to any *cis* or *trans* genetic variation. Gold lines, CG methylation; blue lines, CHG methylation; pink lines, CHH methylation.