In many organisms, allelic diversity generates phenotypic variations and contributes to many events, such as development, adaptation to changing environment, and genome evolution. Allelic diversity is generally defined by the difference in nucleotide sequences that code for a gene. However, a heritable epigenetic modification, in which the modification is attributable to the degree of methylation of a gene and not to the change in its sequence, sometimes occurs and can affect the level of gene expression by reducing its transcriptional level. Some examples of epigenetic phenomena mediated by allele-specific DNA methylation in plants found to date include genomic imprinting, nucleolar dominance, and paramutation. Unlike the case in mammals, epigenetic modifications of plant genes are thought to be mitotically and meiotically stable, but recent studies of allele-specific demethylation at the FWA and MEDEA loci and recessive allele-specific methylation of Brassica self-incompatibility alleles indicate that DNA methylation patterns in plants can vary temporally and spatially in each generation. In this review, we describe various epigenetic phenomena regulated by allele-specific DNA methylation and their possible underlying mechanisms.

Key words: RNA silencing; small RNAs; de novo DNA methylation; RNA-directed DNA methylation; dominance relationship

Epigenetic modification, a heritable modification of gene expression without a change in its DNA sequences, sometimes occur during cell propagation in plants and other eukaryotes. The main types of epigenetic modifications are DNA methylation and histone modification. DNA methylation at cytosine residues has been found to affect many important biological processes, including pathogen resistance, maintenance of genome integrity, developmental regulation, and regulation of gene expression.1-4) A majority of histone modifications includes histone methylation and acetylation/deacetylation. Methylation of lysine 9 histone H3 (H3K9) in particular appears to be a hallmark of silent chromatin. H3K9 methylation can act as a signal for DNA methylation, or can also be reinforced by DNA methylation.5) In this review, we focus on DNA methylation and its role in epigenetic phenomena of monoallelic gene silencing. Histone modifications will be mentioned only as necessary.

Differences in the pattern/degree of methylation of individual genes have been found to produce heritable altered states of gene expression and novel phenotypes.6) Genes with different degrees of methylation, rather than stable nucleotide mutations, are termed epialleles. Epialleles and their accompanying chromatin modifications can often affect phenotypic variations in natural plant populations. Recent studies of RNA silencing, which underlies epiallelic phenomena in plants, showed that these mechanisms are organized into a network of shared components with overlapping functions of small RNA-production pathways. Furthermore, monoallelic gene expression such as genomic imprinting, paramutation, and nucleolar dominance are observed in some situations according to parental origin, gene dosage compensation, or allele-inherent characteristics.7) Several studies have documented that monoallelic gene expression is also associated with increased DNA methylation or with RNA degradation caused by the binding of small RNAs on the recessive allele.8-10) Hence, the activity of these alleles is dependent on their epigenetic state, and many cases of monoallelic gene silencing can be defined as metastable epialleles. The phenotypic traits resulting from epiallelic phenomena are sustained within-generation (i.e., within an individual genome or developmental stage) or across-generation, both of which cases show combined spatial and temporal gene regulation during development.

In both plants and mammals, DNA methylation cor-
relates with monoallelic gene silencing, although the methylation dynamics during cellular proliferation are thought to be intrinsically different. In mammals, de novo methylation is restored to normal levels by the time of implantation, while epigenetic states of plant genes are often inherited over generations. However, recent contradictory reports such as allele-specific demethylation at the *FWA* and *MEDEA* loci in Arabidopsis and recessive allele-specific methylation of Brassica self-incompatibility alleles demonstrated that plants also use a mammalian-like control mechanism of methylation status during normal development. Thus the mechanisms for monoallelic gene silencing during normal development in plants remain controversial.

I. RNA Silencing Pathways in Plants

RNA silencing refers to a broad range of phenomena sharing the common feature that large, double-stranded RNAs or stem-loop precursors are processed to 21–26 nucleotide small RNAs, which then guide the cleavage of their cognate RNAs, block productive translation of these RNAs, or induce methylation of specific target DNAs. To date, a multiplicity of silencing pathways has been described (Fig. 1), and the core mechanism for silencing is similar in plants and animals. In plants, 21–26 nucleotide small RNAs (smRNAs) are generated from dsRNAs by an RNase III enzyme called Dicer or by Dicer-like (DCL) activities. These smRNAs, in association with the RNA-induced silencing complex (RISC), then guide either the cleavage of cognate RNA at a single site in the region of complementarities or the translational repression of target mRNAs by proteins of the Argonaute family. smRNAs are associated with RNA-directed DNA methylation (RdDM) and RNA-mediated heterochromatin formation.

![Fig. 1. RNA-Mediated Gene Silencing Pathways in Plants.](image)

Genomic sources of RNAs that are processed into smRNAs are listed above the dotted line. DCLx, dicer-like proteins; RdDM, RNA-directed DNA methylation; TGS, transcriptional gene silencing; PTGS, posttranscriptional gene silencing.
miRNAs originate from imperfect stem-loop precursors produced from non-coding RNA genes, which have their own promoters and are transcribed by RNA polymerase II. Primary transcripts of miRNA genes of approximately 1 kb are often spliced, capped, and polyadenylated. 29) Null mutations in the DICER-LIKE1 (DCL1) and ARGONAUTE 1 (AGO1) loci cause severe developmental defects because the encoded proteins are important for microRNA-mediated regulation of embryonic development. 30,31) In plants, miRNA biogenesis also requires HUA ENHANCER1 (HEN1) to protect miRNAs and siRNAs from degradation. 32,33) Both an exportin-5 homolog, HASTY (HST), and an R2D2 homolog, HYPTONASTIC LEAVES1 (HYL1), are also implicated in miRNA biogenesis. 34,35) In the case of siRNAs, they are processed from long double-stranded RNA (dsRNA) formed by convergent transcription, RNA-dependent RNA polymerization on sense RNAs, or transcription through inverted repeats. 36) siRNA-induced gene silencing pathways are variable and depend on the target loci. For instance, silencing at endogenous repeat loci is associated with histone H3K9 methylation and RdDM, which is correlated with the production of homologous siRNAs. This pathway therefore requires DICER-LIKE 3 (DCL3), ARGONAUTE 4 (AGO4), RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), and two forms of RNA POLYMERASE IV (POL IVa and POL IVb). 37-43) Mutations in the RNA-DEPENDENT RNA POLYMERASE 6 (RDR6), SUPPRESSOR OF GENE SILENCING 3/SILENCING DEFFECTIVE 2 (SGS3/SDE2), and SILENCING DEFFECTIVE 3 (SDE3) genes impair virus-induced gene silencing and also cause defects in the RNAi-like silencing that is induced by highly transcribed sense transgenes. 34-46)

II. RNA-Directed DNA Methylation and RNAi-Mediated Heterochromatin Formation

DNA methylation at cytosine residues is essential for the normal development of multicellular eukaryotes. 1) Targeted disruption of the DNA methyltransferase in mice results in lethality. 47,48) In Arabidopsis, both met1 (DNMT1-like gene, for maintenance of CG DNA methylation) and drm1 drm2 cmt3 triple mutants (DRM1 and DRM2, DNMT3-like genes, for de novo and maintenance of non-CG DNA methylation; CMT3, plant-specific DNA methyltransferase gene with chromodomain, for maintenance of CNG DNA methylation) also exhibit developmental abnormalities. 2,49-55)

Although DNA methylation is known to be a major modification of eukaryotic genomes that affects gene expression, 3,50) it is unclear how cytosine methylation is directed specifically to certain regions of the plant genome. The discovery of RNA-directed DNA methylation (RdDM) in plants provided the first indication that RNA can feed back on the genome to induce epigenetic modifications of the cognate DNA sequence. In this mechanism, both siRNA and miRNA are proposed to pair with homologous DNA sequences in the genome and to provide an attractive substrate for cytosine methyltransferases. 56) RdDM leads to de novo DNA methylation, which occurs at both symmetric and asymmetric sites in both DNA strands, 57) because both MET1 and DRM1/DRM2 can catalyze de novo methylation induced by RNA signals. Furthermore, DELETIVE IN RNA DIRECTED DNA METHYLATION 1 (DRD1), which encodes a plant-specific SW1/SNF2 ATPase for chromatin remodeling, is also required for the maintenance of siRNA-directed non-CG methylation. 58) RdDM is associated with at least three distinct RNA silencing systems in plants. 15) In the case of transgene RNA silencing and virus-induced gene silencing (VIGS), siRNAs can trigger methylation of the DNA of protein-coding genes in plants that is identical to their sequence. 59-61) Although it is still not known whether siRNAs have a role in effectively guiding RNA silencing functions, some experiments using Arabidopsis mutants suggest that DNA methylation is the essential process in PTGS. 45,62)

In the case of RNAi-mediated heterochromatin formation, arrays of centromere-associated repeats and of rDNA genes in plants are known to be heavily methylated, transcriptionally silent, and immobile. Loss of DNA methylation in met1 resulted in massive transcriptional reactivation in the centromeric and pericentromeric heterochromatic regions. 63-65) Most endogenous siRNAs in Arabidopsis target transposons and repeats, 63,66) and like transgene RNA silencing and VIGS, siRNAs can regulate processes that include centromere function and silencing of retrotransposon and endogenous RNA repeats. 67-69) However, unlike RdDM, which is largely confined to the region of RNA-DNA sequence homology, 70) RNAi-mediated heterochromatin formation can spread over several kilobases from the RNA-targeted nucleation site. 70,71) Differences between RdDM and RNAi-mediated heterochromatin assembly are due to the fact that dsRNA, generated from repeat sequences in heterochromatin, not only induces DNA methylation but also guide histone methyltransferase to the chromatin to modify histone H3K9, and then the methylated form of H3 is associated with the maintenance of heterochromatin. 17,72) In the case of silencing at endogenous repeat sequences, which are identical to promotor regions, RNA-directed de novo methylation causes transcriptional gene silencing (TGS) in conjunction with histone H3K9 methylation. 40) Double-stranded RNAs that contain sequences identical to promotor regions can induce methylation of unlinked homologous promoters and transcriptional gene silencing. 61,73-76) miRNAs can also trigger methylation of the DNA of protein-coding genes that share their sequences in plants, 77) but the implications of DNA methylation generated by miRNAs for PTGS have not been developed.
Although several miRNAs identified in Arabidopsis might target endogenous promoter sequences, the possible role of miRNAs in the regulation of gene expression at the transcriptional level remains controversial.

III. Plant Epialleles

Unlike in mammals, epigenetic modification of plant genes is usually mitotically and meiotically stable. Such heritable “epigenetic alleles” might be involved in diverse phenomena ranging from development to genome evolution and defense against transposons. To date, several stable epialleles have been found in plants (Table 1). The most widely analyzed epialleles are the SUPERMAN (SUP) and FWA genes. SUP encodes a transcriptional factor that defines inner floral whorl boundaries, and mutations in SUP alter floral pattern formation. Seven clark kent (clk) alleles were independently identified in sup mutants, and they were allelic to one another. DNA sequence analyses of SUP in clk plants revealed no nuclear changes, but they were found to be hypermethylated and transcriptionally silenced. The clk alleles were inherited relatively stably, so they were designated epialleles. The FWA epiallele was identified in the met1 and ddm1 (DECREASE IN DNA METHYLATION 1) mutants, which encode maintenance DNA methyltransferase and chromatin remodeling factor SW12/SNF2 respectively. Mutational analyses identified a stable dominant FWA allele that confers a late-flowering phenotype. This abnormal phenotype is ascribed to the stable demethylation of a SINE-related direct repeat around the FWA initiation site and the resulting ectopic expression of the gene. A similar phenomenon has also been observed in the pathogen-resistance (R) gene cluster, BALL (BAL). The ball mutants generated with the ddm1 background showed a dwarf phenotype, and this phenotype was ascribed to over expression of R genes. These data suggest that DNA methylation controls the overall level of R gene expression from a family of repeated genes. An example of influence on allelic methylation by combinations of endogenous sequences was found in the methylated duplicated genes in Arabidopsis, phosphoribosylanthranilate isomerase (PAI) genes. In some Arabidopsis ecotypes, the PAI2 gene was methylated and silenced at the transcriptional level because inverted duplication of similar sequences PAI1-PAI4 coexisted at different loci. In contrast, the PAI2 gene was unmethylated in other Arabidopsis ecotypes without PAI1-PAI4 repeats.

IV. Genomic Imprinting in Plants

Genomic imprinting refers to an epigenetic modification of maternally and paternally inherited alleles that lead to different expression in a parent-of-origin-dependent manner. In both animals and plants, many events crucial to genomic imprinting occur during reproduction. However, unlike in mammals, where imprinting occurs in embryonic and extraembryonic lineages, imprinting in plants is mostly specific to the terminal endosperm tissue of the seed that is formed after fertilization of the central cell (a diploid sister cell of the egg) by a sperm cell. In Arabidopsis, several potential imprinted genes have been isolated from differential expression of maternally and paternally inherited alleles during seed development. The only exceptions are the FIS2 and FER-1 genes also have shown maternal mono-allelic expression patterns in the endosperm.

As described above, both the MEA and the FWA genes are expressed maternally in the endosperm, but it has been found that the MEA gene is biallelically expressed in the embryo and in other sporophytic tissues, while the FWA gene is silent in all vegetative

Table 1. Examples of Plant Epialleles

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>Biology of function</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>SUP, AG</td>
<td>Floral morphology</td>
<td>80, 82</td>
</tr>
<tr>
<td></td>
<td>FWA, FLC</td>
<td>Flowering time</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>BAL</td>
<td>Disease resistance</td>
<td>89</td>
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<tr>
<td></td>
<td>PAI2</td>
<td>Metabolic</td>
<td>90, 91</td>
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<tr>
<td></td>
<td>a-m2-7991AI</td>
<td>Pigmentation and transposition</td>
<td>168</td>
</tr>
<tr>
<td>Ipomoea tricolor</td>
<td>DFR-B</td>
<td>Pigmentation</td>
<td>169</td>
</tr>
<tr>
<td>Linaria vulgaris</td>
<td>Lyc</td>
<td>Floral symmetry</td>
<td>170</td>
</tr>
<tr>
<td>Lycopersicon esculentium</td>
<td>Sulf</td>
<td>Chlorophyll varigation</td>
<td>105</td>
</tr>
</tbody>
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and reproductive tissues except for the endosperm.\textsuperscript{12,81} What mediates maternal expression in the endosperm? Recently, the imprinting genes MEDEA and FWA were found to be regulated by \textit{DEMETER (DME)}\textsuperscript{100} in the endosperm. DME is a helix-hairpin-helix DNA glycosylase that activates the maternal MEA and FWA alleles before fertilization and ensures mono-allelic expression afterwards through some kind of lasting epigenetic modification.\textsuperscript{12,100} DNA glycosylases function in DNA repair to excise mismatched, altered, and damaged bases.\textsuperscript{101,102} Hence DME is associated with demethylation of the target locus. In fact, the maternal MEA and FWA alleles are hypomethylated in wild-type endosperm.\textsuperscript{12,13} In contrast, the maternal MEA gene is not hypomethylated in the \textit{dme} mutant, and no reporter signal was observed when the reporter gene was put under the control of the FWA promoter in the \textit{dme} mutant.\textsuperscript{12,13} These results suggest that DME was responsible for maternal-specific demethylation. In contrast, paternal-allele silencing in the endosperm is complicated. MET1 is the major maintenance DNA methyltransferase in \textit{Arabidopsis}, and it acts antagonistically towards DME, because \textit{met1} mutants show less extensive DNA methylation, but a hypomethylated paternal genome does not release paternal MEA silencing in the \textit{met1} mutant, whereas the paternal FWA allele is activated in the \textit{met1} mutant. Recently, it was found that Polycomb group proteins (including maternally expressed MEA) can maintain MEA paternal-allele silencing.\textsuperscript{13,58,59} These data imply that MEA protein is involved in regulating its own imprinted expression through two distinct mechanisms specific to the maternal and paternal MEA alleles.\textsuperscript{103} In contrast, maternal-specific expression of \textit{FWA}, established by a maternal-specific activation that is dependent on the DME and MET1, also regulates \textit{FWA} expression in the female gametophyte in a manner antagonistic to DME.\textsuperscript{12} In mammalian preimplantation embryos, the maternal genome is demethylated by a passive process along cleavage stages, whereas the paternal genome is demethylated by an active mechanism immediately after fertilization.\textsuperscript{104} Though it remains uncertain, the plant endosperm appears to be functionally analogous to mammalian extra embryonic membrane.

V. Paramutation

Paramutation is a widespread epigenetic phenomenon observed in plants, animals, and fungi.\textsuperscript{105,106} All examples of paramutation involve a \textit{trans}-interaction between alleles that results in a change in gene expression of one of the alleles.\textsuperscript{9,107} In general, when the two alleles are combined in a heterozygote, the target allele segregates out in a heritable transcriptionally silent state (Fig. 2). The alleles involved in paramutation are paramutagenic (able to induce a change in the epigenetic state of a paramutable allele) and paramutable (susceptible to a change in epigenic state induced by a paramutagenic allele). A paramutagenic allele changes the epigenetic state of a paramutable allele, which becomes a paramutated allele (the epigenetic state of a paramutable allele after trans-inactivation by a paramutagenic allele).\textsuperscript{9,107} Furthermore, once the paramutable allele has been changed by the paramutagenic allele, it sometimes displays a secondary paramutation. The epigenetic state of the paramutable allele can also spontaneously change into a paramutagenic state.\textsuperscript{9,107}

In several cases, a paramutagenic allele has copies of directly repeated sequences located far upstream of the transcribed region, and these repeated sequences are required for gene silencing.\textsuperscript{108,109} One example is the maize \textit{b1} gene, which encodes a basic helix-loop-helix transcriptional factor involved in the anthocyanin biosynthesis pathway in the epidermal cell layer of most vegetative parts of the plant. To date, many \textit{b1} alleles have been identified, and a few alleles undergo paramutation. In the case of the paramutable B-I and paramutagenic B' alleles, when light-colored B' plants are crossed with dark-colored B-I plants, trans-interactions between the B' and B-I alleles always lead to an irreversible change of B-I to the B' state, resulting in light-colored offspring.\textsuperscript{9} The \textit{b1} paramutation depends on several directly repeated copies of an 853-bp sequence located 100 kb upstream of the transcribed sequence.\textsuperscript{108} The number of repeated sequences depends on paramutagenicity. Neutral \textit{b1} alleles have only one copy of this sequence, and multiple repeats can induce paramutation and high \textit{b1} expression.\textsuperscript{106} Recent studies indicate that this silencing does not cause a change in the DNA sequence containing seven tandem repeats nor in DNA methylation within or around the transcribed region of B-I and B' alleles. However, DNA methylation at the repeated region and the chromatin structure around the transcribed region are different between B-I and B'.\textsuperscript{110,111} In the case of paramutation at the maize \textit{R} locus, which encodes myc-homologous helix-loop-helix proteins, the mechanism involves methylation of promoter sequences in both the trigger and the target DNA methylation, histone modifications, and chromatin-remodeling detected at the paramutagenic and paramutated alleles, confer silencing of transcription.
alleles. Furthermore, it is known that MOP1 (MEDIATOR OF PARAMUTATION 1) is required for the establishment and maintenance of paramutation; a mop1 mutant that blocks paramutation at the maize b1, r1, and Pl1 loci also shows loss of methylation from specific transposon sequences and results in reactivation of these sequences. These observations can be accounted for by the RdDM of regulatory sequences. In fact, recent study has shown that the mop1 gene encodes an RNA-dependent RNA polymerase 101 (RdRP101). RdRP101 is similar to RDR2 in rice and Arabidopsis, which is postulated to use siRNAs in a pathway that leads to the de novo DNA methylation of direct repeats. Therefore, paramutation might involve RNA-directed DNA methylation and chromatin modification. However, it is still an open question why repeated rather than single-copy enhancer alleles confer silencing of transcription through the locus. Detailed analysis of methylation patterning and smRNAs expressed from the upstream control region in each allele should help to clarify the paramutation machinery.

VI. Nucleolar Dominance

rRNAs are essential for protein synthesis. In eukaryotes, hundreds to thousands of copies rRNA genes organized as head-to-tail repeats are distributed evenly within the genome, which spans millions of base pairs. The level of rRNA gene transcription in eukaryotic cells is tightly regulated according to protein synthesis, which depends on cell activities. In addition, when eukaryotic cells have aberrant copies of rRNA genes than they need, which is the case with polyploidy and allotetraploidy, only a subset of the rRNA genes can be active. For example, inbred lines of maize can have 10-fold differences in rRNA gene content with no apparent difference in growth.

Nucleolar dominance is a phenomenon observed in cells of interspecific hybrids in which NORs (nucleolar organizer regions) derived from one parental species are dominant over the other. In a typical case, only rRNA genes inherited from one parent are transcribed in interspecific hybrids. These reversible and epigenetic events have been described in many plant hybrids, such as *Brassica* and *Arabidopsis*, as well as in animal hybrids. A form of nucleolar dominance is also observed in human-mouse somatic cell hybrids.

Nucleolar dominance is phenotypically and characteristically different from other epigenetic events such as gametic imprinting in that it is independent of maternal and paternal effects and occurs not at random, which is not the case for X chromosome inactivation in the somatic cells of female mammals. In synthetic hybrids, the same parental set of rRNA genes is always silenced, but silencing requires an enforcement process for at least two generations to be fully established.

As for the mechanisms of nucleolar dominance, some plausible evidences that DNA methylation and histone modification play important roles in gene silencing of rRNA genes have been uncovered. For example, in *Brassica napus*, the allotetraploid hybrid of *B. rapa* and *B. oleracea*, rRNA transcripts from *B. oleracea* is suppressed at the level of transcription, but DNA methylation inhibitor- and histone deacetylase (HDAC) inhibitor-treated *B. napus* seedlings resulted in transcriptional derepression of the silent *B. oleracea* rRNA genes. The same results have been demonstrated in allotetraloid hybrids in the genera *Arabidopsis*. Furthermore, mapping of the *Arabidopsis* rRNA gene promoter using bisulfite-mediated DNA sequencing indicated that the methylation pattern was to be categorized into two: most promoters were extensively methylated in all cytosine contexts, but a less abundant class of promoters was methylated under overall but hypomethylated in the core promoter region. In addition, chromatin immunoprecipitation (ChIP) by antibodies specific for histone H3 dimethylated on lysine 9 (H3\textsuperscript{dimethyl}K9) and histone H3 trimethylated on lysine 4 (H3\textsuperscript{trimethyl}K4) revealed that a switch in *A. thaliana* rRNA genes from association with H3\textsuperscript{dimethyl}K9 to association with H3\textsuperscript{trimethyl}K4 is accompanied by a loss of promoter DNA methylation. In the case of *A. suecica*, the natural allotetraploid hybrid of *A. thaliana* and *A. arenosa*, analyses of silenced and active rRNA genes confirmed and extended these results. These results suggest that the dominant class of genes is still in excess of the number of rRNA genes needed, and that these excess genes are dosage-controlled, as in non-hybrids, by a mechanism involving cytosine hypermethylation. In addition, an RNAi-mediated knockdown screen of HDACs (HDT1 or HDA6) and HDACs-inhibitor treatment resulted in transcriptional derepression of the *A. thaliana* genes concomitant with a transition from heterochromatin to euchromatin. This result implies that HDACs are associated with epigenetic switch mechanisms that silence rRNA genes through concerted histone and DNA modification.

To date, it is still unclear why discrimination between parental sets of rRNA genes to completely inactivate one set occurs in the process of generating hybrids, and why not dosage-control both parental set of rRNA genes. In mammals, methylcytosine-binding domain protein (MBD)-like domains are critical in targeting rRNA gene silencing. MBDs are known to figure in multi-protein complexes that include one or more HDACs. Further identification of the components in MBDs involved in nucleolar dominance and detailed analysis of target regions of DNA methylation and histone modification at the silenced and active loci involved in rRNA gene silencing should help to clarify the silencing machinery of nucleolar dominance.

VII. Dominance Relationships between Self-Incompatibility Alleles in *Brassica*

Many species of hermaphrodite plants have evolved
mechanisms to prevent self-fertilization. Self-incompatibility (SI) is one physiological means of avoiding self-fertilization through recognition of self-pollen in or on the female pistil. SI of the cruciferous plant genus *Brassica* is known to be controlled sporophytically by the multiallelic *S*-locus. Studies have revealed that the *S*-locus of *Brassica* encodes male and female *S*-determinant molecules, *S*-locus protein 11 (SP11, also known as *S*-locus cystein rich protein) and *S*-locus receptor kinase (SRK) respectively, and that their *S*-haplotype-specific interaction triggers an SI response in the stigma epidermis (Fig. 3). Extensive analyses of dominance relationships between the two *S*-haplotypes in *Brassica* have demonstrated that a certain dominance hierarchy in determining the SI phenotypes of pollen can occur with corresponding changes in the expression of *SP11*, depending on their allelic partner (Fig. 4). Similar phenomena have been demonstrated in self-incompatible *Arabis lyrata*, implying that dominance among *SP11* alleles is determined extensively at the RNA level in the family Brassicaceae.

Repression of recessive *SP11* transcripts in *S*-heterozygotes is a typical example of monoallelic gene silencing, but this was different from well-documented phenomenon such as genomic imprinting, because it was not affected by the parental origin of the genes.

Dominance among *SP11* alleles was also different from nucleolar dominance because the silencing of recessive *SP11* does not require an enforcement process.

Consequently, the *S*-haplotype of pollen is determined by the expression level of each *SP11* allele, which can be reversibly suppressed by particular allelic combinations.

Fig. 3. Self-Incompatibility Model in *Brassica*.

The *S*-locus consists of at least two genes, SRK and *SP11*. The SRK receptor kinase is the female determinant, and it spans the plasma membrane of the stigma papilla cell. SP11 is predominantly expressed in anther tapetum and accumulates in the pollen coat during pollen maturation. Upon pollination, SP11 penetrates the papilla cell wall and binds SRK in an *S*-haplotype-specific manner. This binding induces the autophosphorylation of SRK, triggering a signaling cascade that results in the rejection of self-pollination.
similar to the 5' promoter region of the SP11 genes. It is possible that monoallelic methylation depends on such short repetitive sequences in the SP11 intergenic region conferring the structural genomic differences observed between dominant and recessive S-haplotypes in cruciferous SI (Fig. 4d). These data suggest that plants too use a mammalian-like control mechanism for methylation status, although it is important to know how general it is.

VIII. Concluding Remarks

In the past several years, the remarkable progress in plant epigenetics has established a basis for the molecular mechanisms of gene silencing. Studies of various RNA silencing phenomena indicate that these mechanisms are organized into a network of shared components with overlapping functions among the small RNA-production pathways. The underlying epigenetic mechanisms of monoallelic gene expression, such as genome imprinting, paramutation, X-chromosome inactivation, and nucleolar dominance remain unclear, but as described in this review, smRNA-mediated silencing mechanisms might be involved in all of these systems.

In plants, sequence-specific DNA methylation is a general phenomenon observed during the process of RNA silencing via dsRNAs. In fact, mutations in a predicted RdRP blocked both RNA silencing and DNA methylation. However, different silencing mechanisms lead to different modes of action to suppress gene expression. In the case of TGS, promoter hypermethylation around cis-elements, followed by chromatin remodeling, can affect transcriptional repression by directly or indirectly interfering with the transcription machinery. In this case, DNA methylation does not result in degradation of target RNAs, and target RNA levels decrease with increasing DNA methylation because transcription of the target RNA decreases.

It is obvious that DNA methylation and chromatin remodeling are associated with gene silencing, but the
causal relationships and concrete roles of methylation in the RNA-degradation pathway remain poorly understood. In the TGS pathway, relatively uniform, intermediate DNA methylation leads to partial silencing and recruits histone modification enzyme HDA6. In the case of generating interstitial heterochromatin in Arabidopsis, high levels H3K9 methylation co-localized with DNA methylation at transposon sequences in the chromosome 4 knob. However, a controversial argument that RdDM recruits histone modification is suggested by the pattern of DNA methylation. RdDM does not usually infiltrate substantially into adjacent sequences, and the minimum DNA-target size for RdDM is about 30 bp, which is not possible with histone modifications. In paternal-allele silencing of the MEA gene, where Polycomb group proteins play a crucial role in gene silencing, DNA methylation is a consequence of chromatin modification. Consequently, DNA methylation might assist or recruit chromatin-remodeling factors through RNA silencing pathways to reinforce its repressive effect on gene activity even though its precise role in RNA degradation and correlation with histone modification remains to be clarified.

As discussed throughout this review, smRNAs are essential for the regulation of transcripts, and have been implicated in controlling various epigenetic phenomena observed in plants and other organisms. Whole-genome approaches to the identification of smRNAs in model organisms have yielded unexpectedly high levels of smRNAs, non-coding RNAs, and antisense RNAs throughout the genome. Genome-wide distribution of smRNAs suggests that there is ample opportunity to target promoter sequences including causal units of transcription. Endogenous smRNAs that regulate the expression of complementary or homologous sequences of the genome at either the transcriptional or the translational level can potentially originate from hairpin precursors encoded in intergenic regions or by hybridization of overlapping sense-antisense transcripts. In fact, in Schizosaccharomyces pombe, heterochromatic siRNAs were generated from outer centromeric repeats. In Arabidopsis, as in S. pombe, a certain portion of centromeric satellite repeats, composed of thousands of methylated tandem copies, are transcribed. Similarly, in plants, several direct repeats are associated with monoallelic gene silencing, such as the disease resistance gene cluster and imprinting- and paramutation-related genes. De novo silencing of the FWA epiallele was induced by a transgene which covered direct repeats around the transcription start site. A direct repeat was identified as the critical element for control of B paramutation and an RdRP deficient mutant (mop1) blocked B paramutation. Hence, repeat associated smRNAs, which are derived from various types of transcripts, might contribute to monoallelic silencing in plants. The expression of many miRNAs in plants is also known to be regulated temporally and spatially. Therefore, it is possible that, like common gene regulation mechanisms, various types of smRNAs generated from non-coding RNAs are induced in tissue- and temporal-specific manners or under stressed conditions, and consequently smRNAs-mediated gene silencing might occur in time and space. For example, some natural cis-antisense transcript-generated siRNAs can be detected only under high salt conditions. Many miRNAs have been found to be essential for controlling development in plants. It is possible that some genes related to development are regulated monoallelically by smRNAs. More precise functional characterization of repeat associated smRNAs should provide a deeper understanding of epigenetic phenomena in plants.

Whether DNA methylation affects transcriptional repression directly by interfering with the transcription machinery or indirectly by association with RNA degradation in conjunction with histone modification, identification of the degree and genome-wide distribution of DNA methylation should provide important clues to the epigenetic mechanisms associated with development in plants and other organisms. Recently, comprehensive maps of DNA methylation of the genome of A. thaliana have been reported using whole-genome tiling arrays. To understand the relationships between DNA methylation and transcription, genome-wide expression analyses of both strands of the Arabidopsis genome using the same tiling array were performed. In addition, to elucidate the involvement of DNA methylation in transcriptional regulation, DNA methylase-deficient mutants (met1, cmt3, drm1, and drm2) too have been used in tiling array analyses. ChIP-on-chip experiments using methylcytosine-specific antibodies that recognize DNA methylation in all sequence contexts revealed that about 20% of the Arabidopsis genome was methylated. Methyltion maps of the Arabidopsis genome showed that heavily methylated regions include not only centromeres, pericentromeres, transposable elements, and other repetitive sequences, but also euchromatic regions. Recent advances in massively parallel sequencing technology should accelerate comprehensive analyses of transcription and smRNAs of the genome. Detailed comparative analyses of each profile of DNA methylation and transcription and smRNAs obtained from different tissues and organ samples at various stages of Arabidopsis should provide deep insight into the role of DNA methylation in plant development. Hopefully, similar approaches to the identification of the relationships between transcripts and DNA methylation using different accessions of Arabidopsis will identify typical monoallelic expression genes in plant development, which will contribute to a deeper understanding of epigenetic phenomena in plants mediated by allele-specific DNA methylation.
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