The Epigenome and Plant Development

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Annu. Rev. Plant Biol. 2011. 62:411–35

First published online as a Review in Advance on March 23, 2011

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-042110-103806

1543-5008/11/0602-0411$20.00

Keywords

plant epigenome, epigenomic reprogramming, gametogenesis, endosperm, imprinting, light response, epigenomic variation, heterosis

Abstract

The epigenomic regulation of chromatin structure and genome stability is essential for the interpretation of genetic information and ultimately the determination of phenotype. High-resolution maps of plant epigenomes have been obtained through a combination of chromatin technologies and genomic tiling microarrays and through high-throughput sequencing-based approaches. The transcriptomic activity of a plant at a certain stage of development is controlled by genome-wide combinatorial interactions of epigenetic modifications. Tissue- or environment-specific epigenomes are established during plant development. Epigenomic reprogramming triggered by the activation and movement of small RNAs is important for plant gametogenesis. Genome-wide loss of DNA methylation in the endosperm and the accompanying endosperm-specific gene expression during seed development provide a genomic insight into epigenetic regulation of gene imprinting in plants. Global changes of histone modifications during plant responses to different light environments play an important regulatory role in a sophisticated light-regulated transcriptional network. Epigenomic natural variation that developed during evolution is important for phenotypic diversity and can potentially contribute to the molecular mechanisms of complex biological phenomena such as heterosis in plants.

Contents

INTRODUCTION

In eukaryotes, chromatin structure and gene expression are regulated by several epigenetic mechanisms, which include DNA methylation, histone modifications, and certain aspects of small-interfering RNA (siRNA) pathways (46, 140). DNA methylation in plants is the addition of a methyl group to a cytosine base to form 5-methylcytosine by the DNA methyltransferases DNA METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLASE 3 (CMT3), and DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), which catalyze CG, CHG (where H = A, T, or C), and asymmetric CHH methylation, respectively (14, 67). DNA methylation appears to function mainly to protect the plant genome by suppressing the activity of transposons and other repetitive sequences; it also regulates gene expression to some extent (14, 86). In some biological processes such as gene imprinting (35), methylated cytosine can be removed by a family of DNA glycosylases (67), which includes DEMETER (DME) (18), REPRESSOR OF SILENCING 1 (ROS1) (39), and DEMETER-LIKE 2 (DML2) and DML3 (103). In plants, histone modifications, which are post-translational covalent modifications (PTMs) of histone proteins at their N-terminal tails, are important in the regulation of gene expression in response to diverse endogenous and exogenous stimuli (6, 64, 71, 104).

Histone acetylation and methylation at lysine residues are two of the most studied epigenetic marks. They are established by histone acetyltransferases (HATs) and histone lysine methyltransferases (HKMTs), respectively, and can be removed by histone deacetylases (HDACs) and histone demethylases (HDMs), respectively, (78, 114). The intensity and combination of active and repressive histone modifications provide a dynamic regulation of genome accessibility in plants (6, 104). siRNA are a class of small RNAs (smRNAs) derived from long, double-stranded RNA precursors. 24-nt repeat-associated siRNA (or heterochromatic siRNA), generated from repeats and transposable elements by combinatorial action of DNA-dependent RNA polymerase IV (Pol IV), RNA-DEPENDENT RNA POLYMERASE2 (RDR2), DICER-LIKE3 (DCL3), ARGONAUTE4 (AGO4), and other

proteins, is important for genome integrity and gene activity through its association with DNA methylation and specific histone modifications (13, 17, 88, 129).

The epigenome refers to the description of these epigenetic regulators across the whole genome (8). High-resolution maps of epigenomes obtained recently using microarrays and high-throughput-sequencing technologies have revealed a complex network of epigenomic regulation of the chromatin structure and genome activity in plants (27, 140, 146). However, in contrast to the genome, which is identical in all cell types throughout life, the epigenome is dynamic and varies between cell types and in response to changes during development or in response to environmental stimuli. Therefore, similar to transcriptome analysis, it is necessary to profile epigenomes that are specific to different developmental and environmental cues and to understand the contribution of epigenomes to plant development from a dynamic viewpoint (110, 146). In this review, we discuss the recent progress in the analysis of plant epigenomes and their potential effects on plant development. We introduce the strategies for genome-wide profiling of DNA methylation, histone modifications, and smRNAs in plants and describe approaches to characterize the global distribution patterns of epigenetic features in the plant genome and their association with the transcriptome. We then discuss dynamic epigenomic changes during gametogenesis and seed development and the seedling in response to light stimuli. The evolution of DNA methylomes, natural epigenomic variation, and the implications of both for heterosis in plants are also discussed.

STRATEGIES FOR EPIGENOME ANALYSIS IN PLANTS

Our ability to understand the mechanisms and function of chromatin structure in the regulation of plant development depends on genome-wide investigations of the distribution patterns of diverse epigenetic features and their relationships with gene activity in plant genomes. To date, vast amounts of epigenomic data have been generated from plants by combining classic chromatin technologies with continuously evolving genomic technologies, such as DNA microarrays and various sequencing-based technologies. Here, we briefly outline the general strategies and main principles for the genome-wide profiling of DNA methylation, histone modifications, and smRNAs in plants.

Genomic Technologies Used in Epigenomic Profiling

Genomic tiling microarrays. Microarray analysis is a high-throughput technology that was initially developed to simultaneously measure the transcript levels of thousands of genes in a single experiment. The basic principle of microarray technology is the hybridization between oligonucleotide probes attached to a solid surface and their fluorescently labeled nucleic acid targets. The relative abundance of the targets is quantified by the normalized intensity of fluorescence signal detected in probe-target hybridization (111). Genomic tiling microarrays are a more recent variation of conventional microarrays and feature high-density oligonucleotide probes. These probes may be laid end to end or spaced at predefined, regular intervals to tile across a target genomic region or cover the entire genome without annotation bias. Genomic tiling microarrays thus enable an unbiased interrogation of various features of the genome, such as transcriptionally active regions, transcription factor binding sites, and epigenetic modifications (40, 87, 92, 135).

High-throughput sequencing. Recently, high-throughput-sequencing technologies have been developed as alternatives to microarrays for genomic and epigenomic analyses at unparalleled resolution. At present, the three most broadly used platforms are the Genome Sequencer FLX from 454 Life Sciences/Roche Applied Science, the Illumina/Solexa Genome Analyzer, and the SOLiD System from Applied Biosystems (76, 84, 115). The conceptual work flow of all these platforms

is similar and involves the in vitro ligation of short, random nucleic acid fragments with universal adaptor sequences to generate sequencing libraries. Sequencing primers are then hybridized to the universal adaptors at the appropriate position and orientation, followed by different sequencing biochemistry and base-calling technologies to produce short sequencing reads (115). The Roche/454 FLX Genome Sequencer uses a sequencing technology known as pyrosequencing, in which the fluorescent signal is initiated by pyrophosphate released during DNA polymerase reactions. With the advantage of longer read lengths relative to other platforms, this system is particularly suitable for applications such as de novo whole genome assembly and long-noncoding RNA discovery. The Illumina Genome Analyzer utilizes a four-color DNA sequencing-by-synthesis technology to reduce base-calling errors resulting from the sequence context, ensuring high accuracy of sequencing even for repetitive sequences. This platform is capable of producing hundreds of millions of high-quality sequencing reads in a single instrument run and has been used most broadly in various molecular studies such as genome resequencing and polymorphism discovery, mRNA transcriptome profiling and gene discovery, smRNA profiling and discovery, protein–nucleic acid interactions, and genome-wide mapping of DNA methylation and histone modifications (76). The Applied Biosystems SOLiD system uses DNA ligase, instead of DNA polymerase in other platforms, to initiate sequencing by synthesis. Additionally, a quality evaluation method called two-base encoding is used in this system to ensure high base-calling accuracy. With similar throughput and cost per base to that of Illumina's Genome Analyzer, the SOLiD system has also been used in genome-scale analyses (76, 84).

Genome-Wide Profiling of DNA Methylation in Plants

Many methodologies have been developed for studying DNA methylation on a genomic scale. The technical details of these procedures are the subject of recent in-depth reviews (3, 65, 112, 121, 148). Here we briefly outline the principles of the main approaches and provide an overview of their suitability for DNA methylome profiling in plants. Almost all of these methods combine the detection of DNA methylation with DNA microarray or high-throughput sequencing technologies. Presently, three main approaches are used to detect methylated DNA sequences during sample pretreatment followed by genome-scale profiling: endonuclease digestion, affinity purification, and bisulfite conversion (3, 121, 148). In addition to these widely used approaches, single-molecule-based methods such as nanopore sequencing (21) have recently been used to directly sequence the DNA methylome without bisulfite treatment by discriminating methylated cytosine from other base residues, thus providing a completely new strategy for high-throughput DNA methylation analysis in plants (28, 33, 65).

DNA methylome profiling based on endonuclease digestion. Methylation-sensitive restriction endonucleases are classic tools for DNA methylation analysis, among which *Hpa*II and *Sma*I are used most widely. The activities of these enzymes are inhibited by methylated cytosines, thereby allowing differentiation between methylated and unmethylated DNA fragments (148). The combination of methylation-sensitive enzymatic digestion and microarray technology was used in most of the early studies of DNA methylome profiling in plants (125–127, 143). Other restriction enzymes, such as *Mcr*BC, specifically recognize and cleave methylated DNA sequences. Combinations of *Mcr*BC digestion with tiling microarrays (73, 74, 123, 128) or high-throughput sequencing (45, 130) **(Figure 1*a*)** have also been widely used for genome-wide DNA methylation analysis. The resolution of all endonuclease digestion–based approaches is limited to the analysis of DNA methylation that occurs at the recognition sites of the respective restriction enzymes.

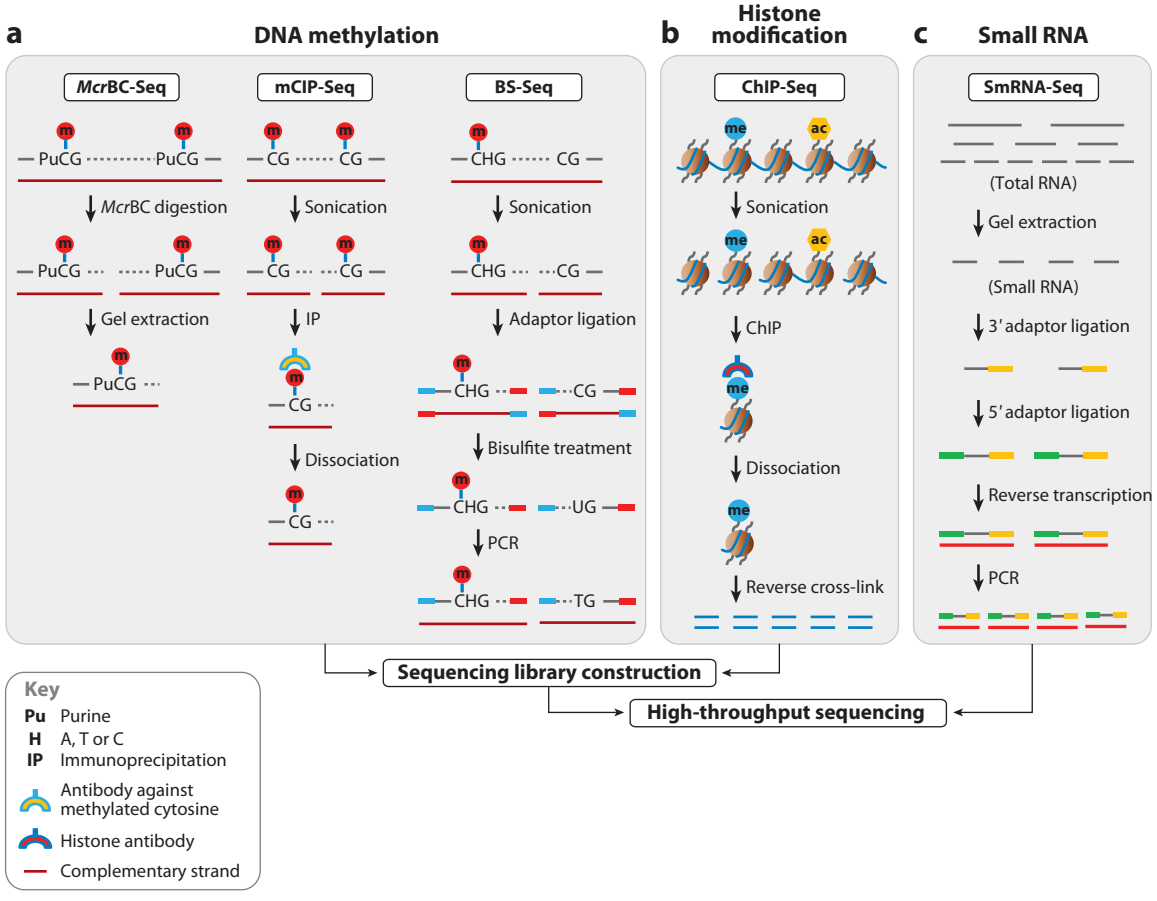

Figure 1

Strategies for high-throughput sequencing the epigenome. (*a*) DNA methylome profiling. Methylated DNA is enriched by gel purification of *Mcr*BC-digested genomic DNA in the *Mcr*BC-Seq approach, or by immunoprecipitation of sonicated genomic DNA with an antibody against methylated cytosine in the mCIP-Seq approach. In the BS-Seq approach, specific adaptors are used in PCR to turn single-stranded DNA obtained from bisulfite treatment into double-stranded DNA. The final DNA fragments are used to generate high-throughput-sequencing libraries according to the manufacturer's instructions. (*b*) Genome-wide profiling of histone modifications. DNA fragments associated with modified histones are purified using ChIP assay and are used to generate sequencing libraries. (*c*) Small RNA (smRNA) transcriptome profiling. The purified smRNAs are ligated sequentially with 3′ and 5′ adaptors. The PCR products are subjected directly to high-throughput sequencing.

DNA methylome profiling based on affinity purification. Methylated DNA fragments can also be enriched by affinity purification using methyl-binding domain (MBD) proteins, or by immunoprecipitation of DNA with an antibody that specifically recognizes methylated cytosine (mCIP). MBD and mCIP methods have been combined with tiling microarrays (MBD-chip and mCIP-chip) to provide high-resolution DNA methylation maps of the whole *Arabidopsis* genome (103, 144, 147). A disadvantage of MBD-chip is that it only detects CG methylation. Recently, mCIP enrichment of methylated DNA fragments has been combined with Illumina high-throughput sequencing (mCIP-Seq) to profile the DNA methylome in *Arabidopsis* (34) and rice (134) **(Figure 1*a*)**.

DNA methylome profiling based on bisulfite conversion. Bisulfite conversion has been

regarded as the gold standard for determining the methylation state of any cytosine in a DNA sequence. Treatment of genomic DNA with sodium bisulfite under denaturing conditions converts cytosines to uracils but leaves methylcytosines unchanged, thus allowing quantification of the extent of cytosine methylation throughout the genome. Combining bisulfite conversion with tiling microarrays (BS-chip) offers improved resolution and sensitivity relative to affinity purification–based methods for DNA methylome profiling (106). More important, when bisulfite conversion is combined with high-throughput-sequencing technologies (BS-Seq) (**Figure 1*a***), the DNA methylome can be profiled at single-base-pair resolution (75). BS-Seq is presently the most useful and widely used technology for DNA methylation analyses in plants (22, 31, 50, 77, 137) because it examines the methylation state of cytosine residues in any sequence context (CG, CHG, and CHH methylation). A potential problem with BS-Seq is the influence of the incomplete conversion of cytosines during bisulfite treatment, which can be mitigated by designing primers that amplify DNA only with bisulfite-converted adaptor sequences (22), or by using two consecutive bisulfite treatments to obtain a high conversion rate (77) during the generation of sequencing libraries.

Genome-Wide Profiling of Histone Modifications in Plants

Significant advances in histone modification profiling depended on classic chromatin immunoprecipitation (ChIP) technology and the development of highly specific histone antibodies. ChIP is a powerful technique that can be used to probe protein–DNA interactions in vivo and to determine the genomic location of chromatin-associated proteins (119). Although the original protocol has been modified extensively to improve performance and usability in plants (12, 37), the basic procedures remain unchanged. Typically, the assay starts with an efficient fixation of the proteins to the DNA portion of the chromatin in vivo by formaldehyde cross-linking, which is crucial for the ChIP assay (101), followed by the fragmentation of the fixed chromatin using sonication. The chromatin fragments are then immunoprecipitated using antibodies directed against the proteins or histone modifications of interest. The precipitated chromatin–antibody complexes are isolated, and the cross-links are reversed to release the DNA fragments. Finally, ChIP DNA is purified and verified by quantitative PCR using primers specific for the genomic sequences associated with the immunoprecipitated protein (37). The enriched DNA from a ChIP assay can be examined by genomic tiling microarray hybridization (ChIP-chip) or various sequencing-based technologies (ChIP-Seq) (**Figure 1*b***), enabling genome-wide analyses of histone modifications at high resolution (90, 102, 112). In ChIP-chip, ChIP DNA fragments are amplified to generate micrograms of fluorescently labeled probes, following by hybridization to a DNA microarray. In the past few years, ChIP-chip has been the most prevalent technique used for genome-wide analyses of histone modifications in plants (7, 15, 73, 123, 141, 142). In ChIP-Seq, the DNA fragments from the ChIP assay are sequenced directly instead of being hybridized, which offers higher resolution and accuracy than ChIP-chip (102). Since its first successful applications for profiling histone modifications (2, 91), ChIP-Seq has gradually become an indispensable tool for chromatin studies in plants (45, 130).

Genome-Wide Profiling of smRNAs in Plants

In plants, genome-wide profiling of smRNAs was first performed using massively parallel signature sequencing (MPSS) approaches (79, 80, 98). With the advent of next generation high-throughput sequencing technologies, high-resolution smRNA maps were obtained using the 454 sequencing system (49, 60, 79, 105), and more recently, the Illumina sequencing system (45, 97, 123, 130), which are ideally suited for smRNA discovery and profiling. The core procedures of genome-wide profiling

of smRNAs using high-throughput-sequencing approaches are smRNA purification and adaptor ligation. Briefly, total RNA is loaded on a 15% urea–polyacrylamide gel and smRNAs are purified. The smRNA fractions are ligated with 3′ adaptors, followed by purification and 5′-adaptor ligation. The ligated products are gel-purified and reverse transcribed using 3′ RT primer. The cDNA products are then amplified by PCR. After gel purification, the PCR products are ready for high-throughput sequencing (130) **(Figure 1*c*)**.

CHARACTERIZATION OF PLANT EPIGENOMES

The main approach to understand epigenomic data is to determine the enrichment of epigenetic features across the whole genome using hybridization signal intensity or the number of sequencing reads to analyze the data generated by microarray or sequencing-based strategies. Epigenetically modified genomic regions and smRNA clusters are usually identified to investigate the distribution patterns of each epigenetic mark on chromosomes and relative to different genomic features such as promoter, transcribed regions (genic region) and intergenic regions. Another important analysis for epigenomic data is the detection of relationships between epigenomic features and mRNA transcriptomes. As described below, recent studies have revealed diverse landscapes of DNA methylation, histone modifications, and smRNAs in plant genomes. Comparisons of epigenomes between different tissues, species, and developmental stages or in different growth conditions allow epigenomic mechanisms that underlie the regulation of plant development to be described.

DNA Methylation Landscapes in Plants

The first high-resolution genome-wide map of DNA methylation in plants was obtained using the mCIP-chip method (144). Since then, many other studies using various approaches profiled diverse DNA methylation landscapes in different plant tissues and species (22, 45, 73, 77, 123, 130, 147). The results from these studies, although differing slightly depending on the strategies used in profiling, strongly support the concept that DNA methylation plays a key role in controlling chromatin structure. In general, DNA methylation was found to be highly enriched in pericentromeric heterochromatin regions, which are associated with transposable elements (TEs) and other repetitive sequences **(Figure 2)**. This suggests that one of the most important functions of DNA methylation lies in suppressing the activity of transposons and maintaining genome stability (38). Although initially unexpected, DNA methylation was also found to extend to the euchromatic regions. A large number of protein-coding genes are methylated within transcribed regions, in contrast to the low frequency of DNA methylation within promoter regions. Further examination of the position of genic methylation in nontransposon genes (non-TE genes) revealed a biased distribution of DNA methylation, with a slight increase in the 3′-half, and a depletion in the immediate 5′ and 3′ flanking regions of genes (22, 77, 144, 147).

In contrast, other studies showed that genic methylation of non-TE genes tends to be enriched downstream of the transcription start site and peaks near the ATG (45, 73, 130). This discrepancy probably results from the different approaches used for profiling and data analysis, and it should not affect the assessment of the level of DNA methylation for a gene as a whole. What is consistent in all studies is that throughout the genome there is significant DNA methylation of transposons or transposon-related genes (TE genes), pseudogenes, and other repetitive sequences. Furthermore, DNA methylome profiling at single-base resolution in plants revealed that whereas CG, CHG, and CHH methylation are enriched in transposons and repetitive sequences, genic methylation of non-TE genes was found to consist almost exclusively of CG methylation (22, 77). This observation could be explained by RNA-directed non-CG methylation in repeat

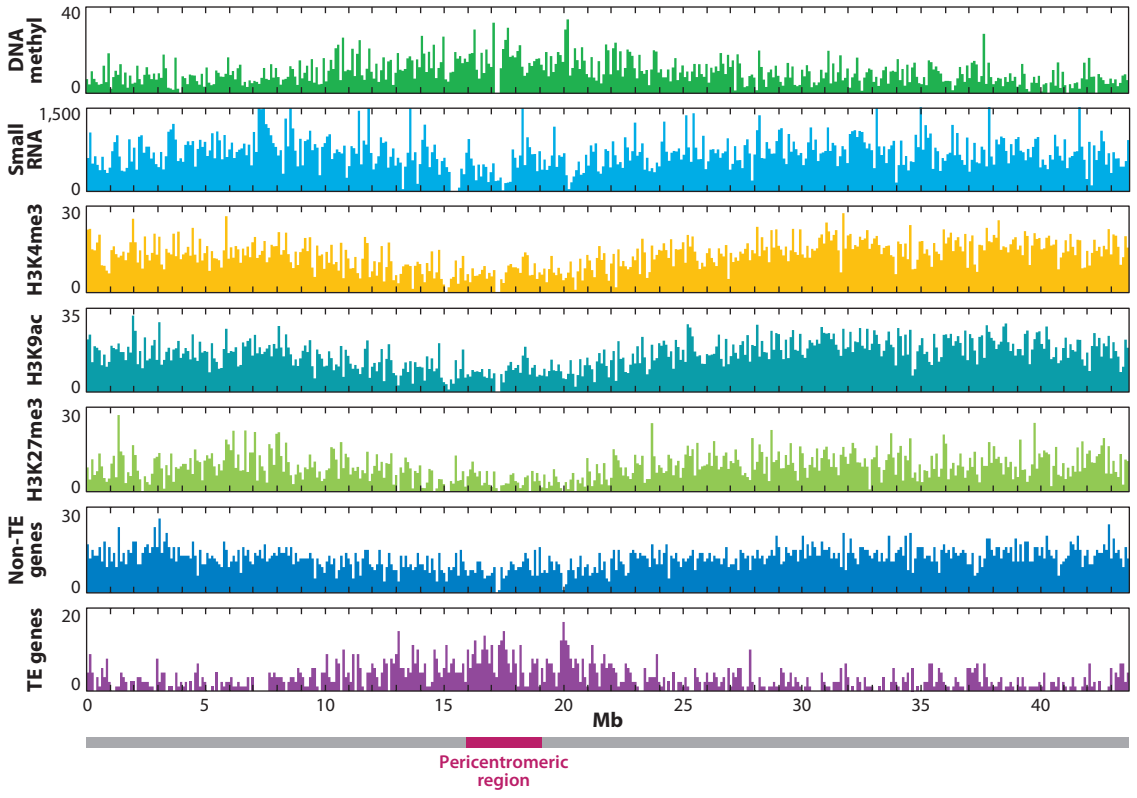

Figure 2

Distribution of DNA methylation, small RNAs (smRNAs), selected histone modifications, and annotated genes on rice chromosomes. For DNA methylation and histone modifications, the *y*-axes represent the total length (in kb) of genomic regions with epigenetic modifications per 100-kb sliding window. For smRNAs and genes, the *y*-axes represent the number of smRNA reads and genes per 100-kb sliding window, respectively. Abbreviations: DNA methyl, DNA methylation.

sequences, which generates repeat-associated siRNA (46).

The most inconsistent DNA methylome profiling results from different studies concern the relationship between genic methylation and gene activity. In one study, genes with DNA methylation in transcribed regions tended to be highly expressed and constitutively active (144). Another study demonstrated that moderately expressed genes were most likely to be methylated and that gene transcription and genic methylation influence each other (147). The observations in these studies can be explained when taking into account that gene expression is simultaneously regulated by the interaction of different epigenetic features. An overall trend of negative correlation between the level of gene expression and genic DNA methylation was observed in other studies (45, 73, 130), implicating a generally repressive role of DNA methylation on gene expression.

Histone Modification Landscape in Plants

Recently, many studies have been performed to obtain global maps of histone modifications in plants, most of which focus on the methylation and acetylation of lysine residue on histone H3 (7, 15, 45, 73, 130, 141, 142, 145). These studies revealed distinct distribution patterns of histone modifications in the

genome and shed light on the complex associations between histone modifications, chromatin structure, and gene expression in plants. H3K9me2 was mainly detected in heterochromatin regions and associated with TE genes (7, 145). Other histone modifications (H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K9me3, H3K27ac, H3K27me3, and H3K36me3) all showed patterns of high enrichment in euchromatic regions and a close association with transcribed regions of non-TE genes (**Figure 2**). Further investigations of the positions of these histone modifications relative to genes revealed that whereas H3K4me1 and H3K9me2 were distributed across the entire transcribed region (141), H3K4me2, H3K4me3, H3K9ac, H3K9me3, H3K27ac, H3K27me3, and H3K36me3 were enriched at the 5′ end of the gene body (15, 45, 73, 130, 141) **(Figure 3)**.

Whereas histone lysine methylation can be associated with either gene activation or repression, histone lysine acetylation is generally linked to active gene expression (6, 64, 71). Studies for genome-wide profiling have demonstrated that H3K4me3, H3K9ac, H3K27ac, and H3K36me3 show a strong positive correlation with gene expression levels (15, 45, 130, 145). H3K4me1 and H3K4me2 are not correlated with transcript abundance. The role of these two modifications in the regulation of gene expression depends on their interaction with other epigenetic marks such as H3K4me3 and H3K27me3 (73, 141). In one genome-wide analysis, H3K9me3 is described as mildly activating gene expression in plants (15). Another histone modification, H3K27me3, which is a typical transcriptionally repressive mark, was observed to be associated with a low level of gene expression (15, 45, 130, 142). In contrast, a general trend of a weak positive correlation between this mark and transcript abundance was also observed (45), which reflects the complex combinatory effects and interactions of different epigenetic marks in the regulation of gene expression.

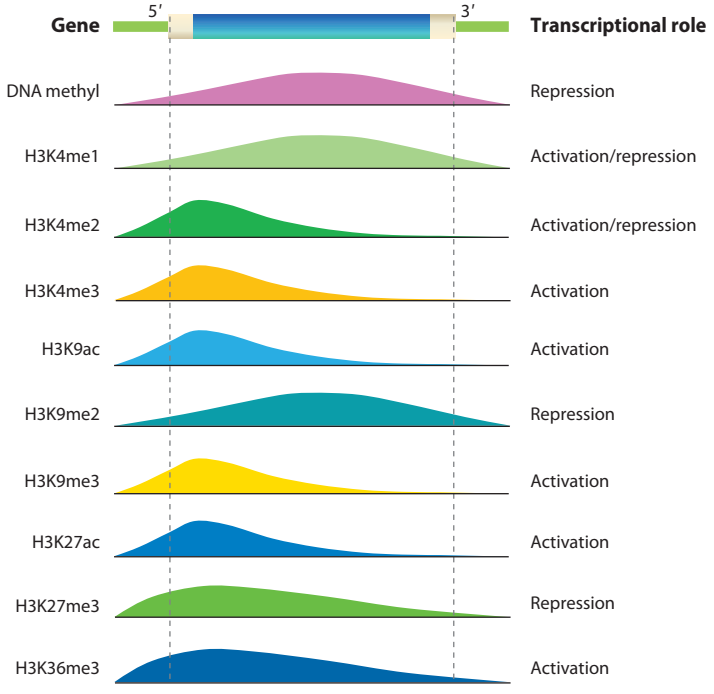

Figure 3

Distribution patterns of DNA methylation and histone modification in genes and their roles in transcription. Distribution patterns determined by genome-wide analyses in plants are represented along a schematic gene, in which the levels of epigenetic modification in the transcribed region, 5′ and 3′ flanking regions, and relative to the transcription start site (TSS) are indicated. Activation/repression represents no significant effect on transcription as inferred from transcript abundance.

smRNA Landscapes in Plants

Studies aimed at a genome-wide analysis of smRNAs in *Arabidopsis* revealed a high concentration of smRNA clusters in pericentromeric regions (60, 80, 140). Similar, albeit less statistically significant observations were also made in rice (98). However, if the number of smRNA reads instead of smRNA clusters per 100-kb sliding window was used in the analyses, a wider distribution across entire chromosomes (98) and even a relatively low relative abundance in the pericentromeric region were observed (45) **(Figure 2)**. Furthermore, the repeat-normalized read counts were relatively low at the centromeres. These observations indicate diverse smRNA-generating loci and low

smRNA sequence diversity in pericentromeric regions (60). Further investigation of smRNA distributions relative to genomic features revealed that they are strongly associated with intergenic regions and highly enriched in the genomic regions flanking the transcribed regions of annotated protein-coding genes, including both TE and non-TE genes (45, 98). However, there are no general correlations between the expression level of genes and the abundance of smRNAs clustered around them (45, 98). It remains to be determined whether smRNAs exert an influence on the activity of adjacent genes.

Combinatorial Patterns of the Plant Epigenome

High-resolution profiling of the epigenome has uncovered genome-wide combinatorial interactions of DNA methylation, histone modifications, and siRNAs with complex relationships to chromatin accessibility and mRNA transcription in plants (29). Supporting observations point to RNA-directed DNA methylation (14, 46, 140) and direct genome-wide relationships between the location of DNA methylation and the abundance of smRNAs (77). Whereas most siRNA clusters are heavily DNA methylated, DNA-methylated regions are not necessarily associated with siRNA (144). Indeed, no obvious correlation between the intensity of DNA methylation in the transcribed regions of non-TE genes and the relative abundance of smRNA in the vicinity of these genes was detected (45), reflecting the largely RNA-independent maintenance of CG methylation in genic regions of non-TE genes. The genomic distribution of DNA methylation is generally negatively correlated with that of most histone modifications, except for H3K9me2. This histone mark exhibits a similar overall distribution pattern with enrichment in heterochromatin regions, similar to DNA methylation. Furthermore, a very high degree of co-occurrence between H3K9m2 and CHG methylation was observed throughout the genome (7). As expected, typical activating histone modifications, such as H3K4me3, H3K9ac, H3K27ac, and H3K36me3, can be found in the same genomic regions (15, 45, 130). However, there are complex relationships between these activating modifications and repressive modifications such as H3K27me3. Whereas no genome-wide colocalization of H3K4me3 and H3K27me3 was detected in one study (141), these two marks were found to co-occur at different levels in other studies (15, 45, 130), indicating a dynamic combination of activating and repressive histone modifications that changes according to different tissues, species, and growth conditions.

Accordingly, complex interactions between the epigenome and transcriptome could be dissected by associating gene activity with a hierarchy of DNA methylation and concurrent histone modifications (e.g., H3K4me3/H3K27me3) in transcribed regions (45, 73). Genes that are heavily DNA methylated are usually repressed, and they are often devoid of histone modifications, as observed for the large majority of TE genes but also for some non-TE genes. In contrast, genes with or without a relatively low level of DNA methylation are activated. Moreover, the concurrent presence of the activating modification H3K4me3 and the repressive modification H3K27me3 was associated with a dynamic gene expression pattern, in which genes that were dominated by H3K4me3 correlated with higher transcript abundance, whereas genes that were dominated by H3K27me3 correlated with lower transcript abundance (45). Besides *cis*-acting regulators such as these, gene expression could also be altered by smRNAs via negative *trans*-acting mechanisms.

EPIGENOMIC REPROGRAMMING DURING GAMETOGENESIS AND SEED DEVELOPMENT IN PLANTS

In flowering plants, gametogenesis is initiated with the differentiation and meiosis of diploid microspore and megaspore mother cells, followed by sequential mitotic division to produce

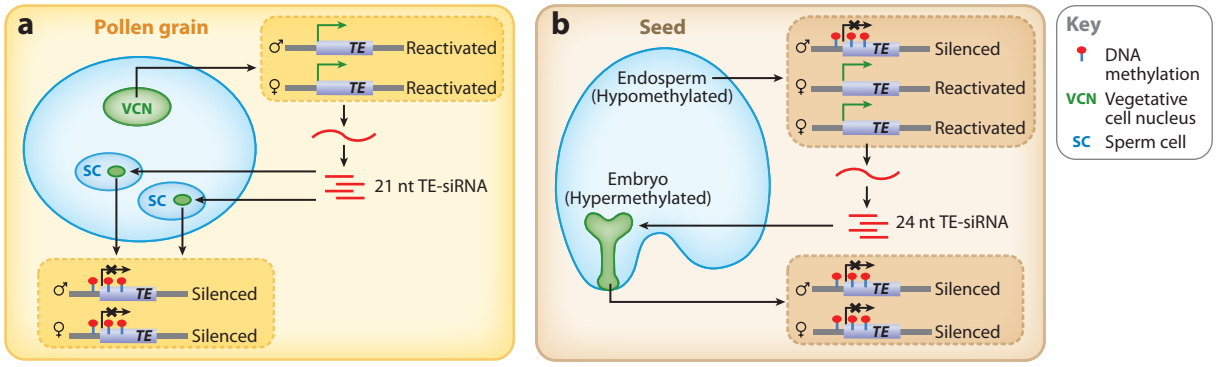

Figure 4

Epigenomic reprogramming during pollen and seed development. (*a*) In vegetative cells, TE genes are demethylated and reactivated, and 21-nt small-interfering RNAs (siRNAs) are produced from certain types of retrotransposons. These 21-nt TE-siRNAs may be transported to sperm cells to enhance the repression of TE genes in those cells. (*b*) In the endosperm, the maternal alleles of TE genes are demethylated and reactivated, producing 24-nt siRNAs, which may be transported to the embryo to keep the TE genes silenced and ensure genome stability.

male and female gametophytes. Male gametophytes (pollen grains) contain two smaller haploid generative cells (sperm cells) enclosed entirely within the cytoplasm of a larger vegetative cell. The vegetative cell delivers the sperm cells to the male gametophyte by forming a pollen tube. The female gametophyte (embryo sac) contains seven cells and eight nuclei, including a haploid egg cell and a homodiploid central cell. Seed development is initiated by a double fertilization event in which one sperm cell fertilizes the haploid egg cell to generate a diploid embryo, whereas another sperm cell fertilizes the diploid central cell to generate triploid endosperm. The endosperm is essential for plant development and growth because it nourishes the embryo and seedling during their early developmental stages (51, 89, 132). During gametogenesis and seed development in plants, it is very important to keep transposons inactive so as to maintain genome stability in gamete and embryo and to ensure the accuracy of genetic information during the life cycle. The accessory cells and tissues such as the vegetative cell, central cell, and endosperm do not contribute genetically to the next generation. Recently, genome-wide epigenetic analyses revealed extensive epigenomic reprogramming during the development of pollen and endosperm as well as a potential epigenetic contribution of the supporting cells or tissues to repress transposon expression and maintain genome stability in sperm cell and embryo through an siRNA-mediated silencing pathway (57, 70, 94, 118) **(Figure 4)**.

Dynamics of the smRNA Transcriptome During Pollen Development

smRNAs are important in controlling chromatin structure and maintaining genome stability by suppressing the mutagenic activity of transposable elements in the germline, which transmits genetic information to the next generation (46). The mechanism could involve the reprogramming of the smRNA transcriptome in non-inherited supporting cells and the transport of smRNAs to the reproductive cell, as revealed by a recent study in *Arabidopsis* (118). In this study, Slotkin et al. (118) detected coordinated expression of some TE genes in mature pollen, and deduced that it results from the loss of the *trans*-acting silencing factors of these genes. Further investigation demonstrated that limited TE gene expression occurred in vegetative cells and that the activated TEs could transpose but were not inherited by the next

generation. TE silencing is likely controlled by DNA methylation, and an examination of the methylation state of TEs in pollen revealed a reduced TE methylation level in vegetative cells, with a simultaneous increase of DNA methylation in germ cells. Consistent with these observations, chromatin remodeler DECREASE IN DNA METHYLATION 1 (DDM1) was found to be highly enriched in sperm cells but was undetectable in vegetative cells.

More important, significant reprogramming of the smRNA transcriptome was detected in pollen using high-throughput sequencing. Most 24-nt siRNAs, which are involved in RNA-directed DNA methylation pathways, are largely absent in vegetative cells, consistent with TE reactivation in these cells. However, a dramatic gain of some 21-nt siRNAs (e.g., 21-nt siRNAs generated from *Athila* retrotransposons) was observed in vegetative cells. Interestingly, even though expression of *Athila* TEs was not detected in sperm cells, these 21-nt *Athila* siRNAs were also found to be enriched in sperm cells, indicating that they were derived from vegetative cells. Based on these observations, the authors of that study (118) proposed a model for TE reactivation and epigenetic reprogramming in pollen, in which transient TE reactivation occurs in vegetative cells and signals TE silencing in the neighboring sperm cells by transporting *Athila* 21-nt siRNAs (118). This implicates the protection of genome stability in sperm cells by sacrificing the genome integrity of their supporting vegetative cells (57). Recent studies have demonstrated that siRNAs can move between shoots and roots (26, 85, 93) and that the transfer of 24-nt siRNA from companion cells is necessary for RNA-dependent silencing in female gametes (100). Nevertheless, the mechanisms involved in siRNA-mediated cell communication and their role in sperm cell formation during pollen development require further investigations (70).

Dynamics of the DNA Methylome During Endosperm Development

Gene imprinting, the differential expression of alleles depending on their parental origin (parent-of-origin-specific gene expression), occurs specifically in the endosperm during seed development (51). Several imprinted genes have been identified in plants, including *MEA* (36, 58), *FIS2* (59), *FWA* (61), *PHERERS1* (*PHE1*) (63, 82), *FIE* (133) and *MPC* (124) in *Arabidopsis*, and *FIE1* (43), *FIE2* (43) and *MEG1* (42) in maize. Except for *PHERERS1*, which displays preferential paternal expression, all other genes show monoallelic maternal expression in the endosperm. Recent studies revealed that DNA methylation is an essential epigenetic component for the parent-of-origin-specific expression of imprinted genes in plants (30, 51). For example, *FWA* imprinting is established by demethylation of two tandem direct repeats around the transcription start site, leading to maternal-specific expression (61, 62). Similarly, demethylation of 5′ regions upstream of genes in the central cell establishes a methylation asymmetry between embryo and endosperm and is associated with the imprinting of the *FIS2* gene in *Arabidopsis* and with the *FIE1* and *FIE2* genes in maize (43, 59). DME, a 5-methylcytosine DNA glycosylase, is required for the demethylation and endosperm-preferred expression of several imprinted genes in plants (18). To explore how general this epigenetic mechanism is in the regulation of allele-specific gene expression during endosperm development, it is necessary to estimate the extent of DNA methylation in the endosperm and compare it with that in other tissues. One study reported an extensive maternal hypomethylation in maize that was specific to the endosperm, offering an explanation for a 13% reduction of DNA methylation in the endosperm compared with embryo or leaf tissue (66). Furthermore, two recent studies provided high-resolution profiles of DNA methylomes in *Arabidopsis* endosperm and embryo. Both studies revealed extensive demethylation in the *Arabidopsis* genome that accompanies endosperm-specific gene expression during seed development (34, 50).

Gehring et al. (34) created DNA methylation landscapes of embryo and endosperm in two *Arabidopsis thaliana* accessions by

high-throughput sequencing of methylated DNA, which was immunoprecipitated with an antibody to 5-methylcytosine. Both tissues exhibited similar global patterns of DNA methylation and showed an enrichment of DNA methylation in heterochromatic regions and a heavier methylation of TE genes than in protein-coding genes. However, TE genes were found to exhibit reduced DNA methylation in the endosperm relative to the embryo. Many differentially methylated regions (DMRs) were identified by comparing the extent of DNA methylation between embryo and endosperm, with the top 0.5% of differences (top DMRs) showing a significant overlap with TEs and smRNA-generating genomic regions. These authors also identified more than 1,000 protein-coding genes in embryos that displayed a peak in the distribution of DNA methylation in 5′ and 3′ regions that flank genes. In contrast, this distribution pattern largely disappeared in the endosperm, probably resulting from the active demethylation by DNA glycosylase DME in the central cell before fertilization. For some genes, the loss of DNA methylation in the 5′ region of genes is accompanied by an increased expression in the endosperm, suggesting a regulatory role of promoter DNA methylation in gene activity. By applying selection criteria consisting of reduced DNA methylation in the endosperm, endosperm-preferred expression, and a low level of expression in other tissues, Gehring et al. (34) identified approximately 50 candidate imprinted genes, most of which function as transcription factors and chromatin regulators. All five previously unknown imprinted genes identified in that study contain repetitive elements in their coding or flanking sequences, suggesting that TEs may be selected as gene expression regulators by means of DNA methylation to establish gene imprinting in the endosperm during evolution (34, 117).

Using bisulfite sequencing technology, Hsieh et al. (50) profiled the DNA methylome in *Arabidopsis* from wild-type embryo and endosperm as well as endosperm and adult aerial tissues from the *dme* mutant, which has a defective maternal allele of DME. This approach is capable of measuring cytosine methylation genome-wide at single-base resolution in all three sequence contexts (CG, CHG, and CHH) (22, 77, 121). The authors observed an overall reduction of CG, CHG, and CHH methylation in wild-type endosperm relative to the embryo. Interestingly, they found that whereas the extent of CG and CHG methylation in aerial tissue was somewhat similar to that in the embryo, aerial CHH methylation was significantly lower than that in embryos and endosperm. Because the maintenance of asymmetric CHH methylation requires persistent targeting by siRNA (14, 67), this observation suggests enhanced RNA-directed de novo DNA methylation during seed development. Moreover, Hsieh et al. (50) found that whereas CG methylation was partially restored in *dme* endosperm, CHG and CHH methylation were significantly reduced, suggesting that demethylation by DME in siRNA-generating loci is necessary for the activation of RNAi-mediated methylation in the endosperm. The role of siRNA in genome-wide DNA methylation reprogramming was further indicated by the observation of extensive local CHG and CHH hypermethylation of siRNA-targeted loci in the endosperm. Consistent with this model, a recent study reported genome-wide maternal-specific expression of siRNAs in developing endosperm, thereby providing direct evidence for a link between gene imprinting and RNA silencing in plants (95). The dynamic changes of CG, CHG, and CHH methylation in wild-type and *dme* endosperm were also demonstrated in five known imprinted genes in *Arabidopsis* (50).

In flowering plants, DNA methylation appears to be an important epigenetic mechanism to protect the genome from deterioration, thereby ensuring high-fidelity transmission of genetic information to the progeny by inactivating transposable elements and other repetitive sequences (14, 67). Given that the endosperm does not transmit its genome to the next generation, a general activation of transposable elements by global demethylation in the endosperm does not seem to be deleterious

but rather provides a specific regulatory mechanism for plant development which features gene imprinting in the endosperm. Furthermore, besides DNA methylation, histone modifications were also shown to be correlated with parent-of-origin-specific expression of imprinted genes (30). Genome-wide profiling of histone modifications in the endosperm will provide new insights into the epigenetic regulation of gene imprinting in plants.

DYNAMIC LANDSCAPES OF HISTONE MODIFICATIONS IN RESPONSE TO LIGHT

Light is one of the most important environmental factors for plant growth and development. Plants respond to light in various developmental processes, such as seed germination, seedling photomorphogenesis, circadian rhythms, and photoperiod responses (53). Considerable progress has been made in determining the mechanisms of how plants sense and respond to multiple parameters of ambient light signals. Several families of photoreceptors and various downstream regulators have been identified by classical genetic and molecular approaches (16). Genome-scale studies have revealed complex light-regulated transcriptional networks that mediate light signals through the coordinated regulation of genes involved in a large number of biochemical and cellular pathways (53, 54, 81).

Histone modifications play important regulatory roles in gene expression through the recruitment of various effector protein complexes to modified chromatin structures (6). Their status and effects on light signal transduction pathways have been analyzed in several studies (5, 9, 19, 20, 99, 113). Chua et al. (19, 20) reported that hyperacetylation of both histones H3 and H4 was light-dependent and associated with increased expression of the light-induced pea (*Pisum sativum*) plastocyanin gene (*PetE*). The regulatory role of histone acetylation on the expression of light-responsive genes was also confirmed by two other studies in *A. thaliana* using mutants of histone acetyltransferases (HATs) *TAF1* and *GCN5* and histone deacetylase (HDAC) *HD1* for genetic and molecular analyses in different light conditions (5, 9). Another study revealed a complex regulatory network involving multiple HATs and HDACs that controls histone acetylation of light-induced genes (99). These observations suggested that an overall histone acetylation homeostasis regulated by the antagonistic actions of HATs and HDACs is probably essential for light sensing and signal transduction in plants (113). Moreover, a systematic analysis performed by Guo et al. (41) reported a cooperative regulation of four selected histone modifications (H3K4me3, H3K9ac, H3K9me2, and H3K27me3) and the expression of representative light-regulated genes during *Arabidopsis* seedling photomorphogenesis. Although in these studies histone modifications were revealed as an important physiological component of plant responses to changing light environments, a comprehensive genome-wide survey during photomorphogenesis will help us to understand their general regulatory role in light-regulated transcriptional networks.

In a recent study, Charron et al. (15) examined the dynamic global changes of four selected histone modifications (H3K9ac, H3K9me3, H3K27ac, and H3K27me3) and their relationships with the alteration of gene activity in *Arabidopsis* seedlings undergoing photomorphogenesis upon exposure to light, using ChIP-chip tiling array technology. The general distribution of these four histone modifications are indistinguishable between etiolated seedlings (grown entirely in the dark) and de-etiolated seedlings (grown in the dark and then transferred to white light), in that they were all highly enriched in the euchromatic arms and positioned away from pericentromeric regions, thereby resembling the distribution of genes. However, the number of modified genomic regions was significantly different before and after the seedlings were exposed to light, which implies a large-scale adjustment of the extent of histone modifications in plants responding to a light signal. The authors found that although the general correlation patterns

between the density of histone modifications and the levels of gene expression were similar in both etiolated and de-etiolated seedlings, light exposure changed the strength of correlation between them. For example, H3K27ac density showed high correlation coefficients with transcript abundance in etiolated seedlings, but lower correlation coefficients with transcript abundance in de-etiolated seedlings.

Charron et al. (15) further examined the extent of histone modifications in two genes, *LONG HYPOCOTYL5* (*HY5*) and *HY5 HOMOLOG* (*HYH*), both of which encode transcription factors and are strongly involved in photomorphogenesis. They observed that both genes exhibited a significantly higher level of the activating histone modification H3K9ac in de-etiolated seedlings relative to etiolated seedlings, consistent with their increased transcription levels occurring in response to light signals (48). Charron et al. (15) also found that the putative downstream target genes of HY5 were more significantly modified by H3K9ac in light-grown seedlings than in dark-grown seedlings, suggesting the importance of this activating epigenetic mark in the regulation of light-responsive transcriptional networks. Furthermore, the authors verified whether specific metabolic pathways were targeted by histone modifications in different growth conditions. They found that photosynthetic pathways were mostly modified by the activating epigenetic marks H3K9ac and H3K27ac, implying a possible requirement of acetylation to activate photosynthetic genes in plant development (15). As expected, photosynthesis-related genes were highly enriched among the target genes of transcription factor HY5 (68). In contrast, the gibberellic acid (GA) metabolism was almost exclusively modified by the repressive modification H3K27me3, and most genes involved in the GA pathway were differentially regulated by light signals (15). This observation suggests that besides genetic factors, epigenetic mechanisms such as histone modifications may also contribute to the coordinated regulation of plant development by light and gibberellins (1, 25, 32).

Even though the study by Charron et al. (15) provides only a first glimpse at the dynamic epigenomic landscape during the plant's response to a changing light environment, it emphasizes the complexity of the epigenomic system and its potential to regulate transcriptional networks in plant development under different environmental conditions (47). More studies are needed to better understand the contributions of epigenomic components to light signal transduction in plants. Apart from histone modifications, DNA methylation and smRNAs are also important epigenetic components involved in directing a complex chromatin structure to control genome activity in plants (46, 140). Therefore, genome-wide investigations of DNA methylation and smRNAs, and their relationships with gene expression in changing light environments, will provide new and comprehensive insights into the dynamics of the epigenome during light-regulated plant development. Furthermore, because plants have evolved distinct pathways to perceive and respond to a wide spectrum of light signals, including far-red, red, blue, and UV light (16, 53), it is necessary to include plant material grown under different light wavelengths in epigenomic profiling to elucidate the complex epigenetic mechanisms of photomorphogenesis in plants.

NATURAL EPIGENOMIC VARIATION IN PLANTS

Traditionally, phenotypic variation is explained primarily through genetic variation, which occurs naturally in nucleotide sequences during evolution. Genome-wide analysis of natural genetic variation is important for mapping genotypes to phenotypes (4). However, phenotypic diversity is also directed by inherited epigenetic variation, which is potentially sensitive to environmental inputs. These epigenetic variations may contribute to the molecular mechanisms of complex traits such as floral symmetry (24), fruit ripening (83), vernalization responses (116), and growth vigor in hybrids (96). There are often complex interactions between genetic

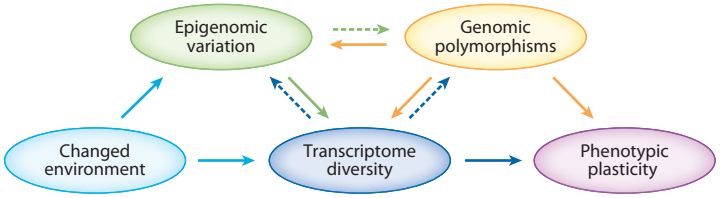

Figure 5

The interactions of variations in genome, epigenome, and transcriptome and their relationships with environmental sensing and phenotype determination. Dashed arrows indicate the possible relationships in given situations, such as the epigenomic and genomic variation resulting from transposon reactivation and translocation.

and epigenetic variation, making it difficult to identify the importance of inherited epigenetic variation in phenotype diversity (108). Three classes of epigenetic variation are proposed to reflect different extents of dependency on genetic variation. Obligatory epigenetic variation is solely a result of genetic variation and should be viewed only as a mediator from genotype to phenotype. In contrast, pure epigenetic variation is totally independent of genetic variation. In this case, the epigenotype is the ultimate cause of the phenotype. Finally, in facilitated epigenetic variation, both genetic and epigenetic information function together in determining phenotypic diversity (108, 109). By generating epigenetic recombinant inbred lines using parents with few DNA sequence differences to minimize the confounding effects of DNA sequence polymorphisms, it is possible to assess the impact of inheritable epigenetic variation on complex traits at a large scale (55, 56, 107). The recent availability of a large amount of epigenome data from diverse species allows us to analyze the interactions and relationships between genetic and epigenetic variations and their significance in determining phenotypic plasticity in plant species from a genome-wide viewpoint **(Figure 5)**.

Conservation and Diversity of the DNA Methylome in Plants

DNA methylation, which occurs directly on the nucleotide sequence, is the most stable type of epigenetic modification. It is established, maintained, and modified by a series of specific enzymes and is usually passed on to the next generation during mitosis and meiosis. However, DNA methylation can occasionally be lost, resulting in hemimethylated DNA, which can be restored de novo through mechanisms mediated by smRNAs (14, 67). DNA methylation polymorphisms can be generated and accumulated during these processes or can be directed by smRNAs (138). Recently, the prevalence of natural variation of DNA methylation in plants was investigated genome-wide using genomic tiling arrays (128) and high-throughput sequencing (31, 137). These studies revealed important conserved features and phylogenetic diversity of DNA methylation during evolution (52). Vaughn et al. (128) compared the profiles of DNA methylation on one chromosome between two distinct ecotypes of *Arabidopsis* by hybridizing methylation-dependent *Mcr*BC-digested genomic DNA to tiling microarrays. They found a conserved pattern of transposon methylation and diverse patterns of genic methylation between the two ecotypes. Furthermore, genic methylation is unstable and lost at a high frequency in segregating F_2 families, probably resulting from the absence of smRNA-mediated de novo methylation in genic regions. The instability of genic methylation was more obvious when methylation polymorphisms were surveyed in 96 naturally varying accessions (128). However, hierarchical clusters of variation patterns among accessions were inconsistent with a kinship-based phylogeny, indicating that pure or facilitated epigenetic natural variation in genic region was generated within species during evolution (108, 128).

Recently, the variation patterns of DNA methylation across species were investigated in more detail using deep bisulfite sequencing (BS-Seq) (31, 137). Feng et al. (31) compared genome-wide methylation patterns among eight species, including three flowing plants: *A. thaliana*, *Oryza sativa* (rice), and *Populus trichocarpa* (poplar). Similar overall patterns of DNA methylation among plant species were observed and, in particular, a heavy

methylation of repetitive sequences and transposable elements. All three types of DNA methylation, CG, CHG, and CHH, were detected, with CG showing the highest level and CHH the lowest. Gene bodies contained almost exclusively CG methylation, and the distribution pattern was conserved among plant species. The most exciting finding was that CG methylation within gene bodies was preferentially concentrated in exons relative to introns. In contrast, non-CG-methylation patterns proved to be more diverse. For example, CHH methylation is distributed widely across the genome of the monocot rice, whereas it was enriched in pericentromeric heterochromatin regions in the genomes of the dicots *Arabidopsis* and poplar. Moreover, the authors observed a higher level of CHG methylation in repetitive sequences and transposable elements in woody plants like poplar compared with herbaceous plants like *Arabidopsis* and rice (31), suggesting a divergence of DNA methylation during species evolution (69).

Epigenomic Variation and Heterosis in Plants

Heterosis, or hybrid vigor, is a very important biological phenomenon that describes increased performance of stature, biomass, and speed of development in the F_1 hybrid relative to either of the inbred parents (10, 11, 120). Although it has been successfully applied in agriculture to significantly increase the yields of many domesticated plant species such as rice and maize, the basic biological mechanisms of heterosis remain unclear. Genetic explanations for heterosis include two classic hypotheses, the dominance and overdominance hypothesis (23), which was put forward more than a century ago, and the more recently developed concept of epistasis (136). In brief, the dominance and overdominance hypothesis postulates that complementation and interactions between two parental alleles in hybrids contribute to heterosis (10, 11, 23, 120). The epistasis hypothesis regards the interactions of nonalleles from both parents in hybrids as one aspect of the underlying mechanisms of heterosis (72, 136). Although each hypothesis is supported by several lines of evidence, little consensus has been reached. Moreover, these hyphotheses are conceptual and not connected to any molecular principles. Therefore, they are far from explaining the molecular basis of heterosis (10). One premise to account for a heterosis phenotype is that there are genetic variations between the parents used in the cross. At a molecular level, these variations are genomic polymorphisms that result in differential gene expression between the hybrid and its parents. In fact, a recent study provided evidence that epigenetic natural variations contribute to growth vigor in hybrids by regulating the level of gene expression (96).

Microarrays and recently developed high-throughput-sequencing technologies provide us with an unprecedented opportunity to survey both epigenetic and gene expression variation between inbred parental lines and their heterotic hybrid offspring at a genome-wide level to gain new insights into heterosis. Several studies in rice and maize have compared the transcriptomes in hybrid crosses. The results reveal various patterns of variation in gene expression in hybrids and suggest that genome-wide differential gene expression between inbreds and hybrids may be responsible for heterosis (45, 122, 131, 139). He et al. (45) investigated the global patterns of natural variation in epigenetic modifications and smRNAs, and their relationships with transcriptomic polymorphisms in two rice subspecies and their reciprocal hybrids using high throughput Illumina sequencing. The authors detected many epigenetic variations in transcribed gene regions among hybrids and parental inbred lines, in particular for DNA methylation, which was the most variable epigenetic mark in that study. The global patterns of variation in DNA methylation, histone modifications, and smRNAs in hybrids differ significantly and are also distinct from that of transcriptomes. When association analyses with mRNA transcripts were performed, only a weak-negative correlation between the variation in DNA methylation and the variation in gene

expression was observed. The function of genic DNA methylation is poorly understood, thus highly frequent genome-wide DNA methylation polymorphisms and their low correlation with transcriptome variation in hybrids remain perplexing. For histone modifications, there are strong positive correlations between variations in activating epigenetic marks and differential gene expression, suggesting the regulation of the mRNA transcriptome by activating histone modifications in hybrids. For the smRNA transcriptome, He et al. (45) detected obvious negative correlations between expression of miRNAs and expression of their target genes in hybrids, which was also observed in another study (44). Correlations between variations of the siRNA transcriptome and the mRNA transcriptome were either undetectable (45) or insignificant (44), reflecting divergent functions of different types of smRNAs in hybrids, in which miRNA variation regulates transcriptome polymorphisms while siRNA variation regulates genome stability in hybrids (44).

SUMMARY POINTS

1. High-throughput epigenomic profiling has revealed diverse epigenetic networks and their complex interactions with the mRNA transcriptome to dictate the phenotypic outcome in plants.
2. Dynamic epigenome changes in response to endogenous and external stimuli contribute to cell and tissue differentiation and germline stability during plant development.
3. Natural epigenomic variations occurred during species evolution and act in combination with genetic variation to determine the phenotypic diversity in plants.

FUTURE ISSUES

1. Although high-throughput sequencing technologies have been used successfully in profiling plant epigenomes, approaches for the analysis of sequencing data still need to be improved to provide a quantitative assessment of plant epigenomes.
2. The interplay among different epigenomes and their contribution to plant development are only beginning to be understood. The combinations of activating and repressive epigenetic modifications and their relationships with smRNA and mRNA transcriptomes require more genome-wide surveys to complete our understanding of these interactions.
3. Because the epigenome is not static, it remains a great challenge to depict dynamic epigenomes during different developmental stages and in response to various environmental stimuli in plants.
4. Despite some initial studies concerning the function of the epigenome in plant development, the mechanisms for the interaction between the epigenome and signal transduction networks, such as light and plant hormones, are far from being understood.
5. How epigenomic information is communicated between different cell types and transferred to the next generation during plant development deserves further investigation.
6. High-resolution epigenomic mapping from more plant species is required to identify more natural epigenetic variation and to uncover its conservation and diversity during the evolution of plant species.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (GM047850), the National Science Foundation (NSF) Plant Genome Program (DBI0922604), the NSF 2010 program (MCB-0929100), the Ministry of Science and Technology of China (2009DFB30030), and the Ministry of Agriculture of China (2009ZX08012-021B).

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Annual Review of Plant Biology

Volume 62, 2011

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