Introduction to Epigenetic Gene Regulation

Epigenetic regulation is the process by which a gene’s activity is modulated through covalent modifications to the DNA, the histones around which it is wrapped, or the physical packaging of the chromatin in which it is embedded. Use of this term explicitly requires that the gene’s state be heritable, that is, it can be stably transmitted through one or more cycles of mitosis. Mechanisms of epigenetic regulation are likely to have originated as a defense against parasitic DNAs, such as transposons and viruses, but they also are used to control the expression of many genes crucial for development or environmental responses. Genetic studies have identified the molecular pathways for DNA and chromatin modification that underlie these processes, and genome profiling tools have revealed that they are pervasive and have a role in regulating most of the genome.

Eukaryotic DNA wraps around highly conserved histone octamers, a protein structure composed of two each of four histone proteins. The histone/DNA complex is called a nucleosome. Nucleosomes pack together in various ways along with additional proteins to make chromatin. Chromatin condenses dramatically during mitosis and becomes less densely packed during interphase. Classical cytogenetic studies identified regions of chromosomes called heterochromatin that remain relatively tightly condensed during interphase. Heterochromatin mostly comprises nongenic DNA including the centromeres and repeat-rich regions around the centromeres (pericentromeric regions), rDNA genes, repetitive elements, and transposons. Euchromatin consists of the gene-rich regions that are less densely packaged and accessible for transcription. DNA and histones in heterochromatin carry specific silencing modifications; collectively, these modifications (called epigenetic marks), along with the dense packaging, help act to suppress transcriptional activity in these regions. Epigenetic marks also regulate some genes in euchromatin, especially those involved in critical developmental transitions.

EPIGENETIC MARKS

DNA Methylation

DNA methylation is an important epigenetic mark in many organisms, including plants. DNA methyltransferases covalently attach methyl groups to cytosines, marking the DNA without interfering with base pairing. DNA methylation silences gene expression by interfering with binding of the protein complexes that carry out transcription (often collectively referred to as the transcriptional machinery). On the other hand, DNA methylation within transcribed regions can enhance transcription by unidentified mechanisms. Moreover, although it is not essential for splicing, DNA methylation may fine-tune alternative splicing in plants. In adjacent regions of splicing junctions, higher levels of DNA methylation in exons compared to introns seem to serve as markers to assist splicing.

Plants have three types of DNA methyltransferases with different target specificities and functions. METHYLTRANSFERASE1 (MET1) maintains methylation at symmetrical 5′-CG-3′ sites during DNA replication by copying the information from the template strand onto the newly synthesized strand. MET1 targets are mainly silenced transposons and heterochromatin, but MET1 also maintains marks on some imprinted genes including MEA, described below. CHROMOMETHYLASE3 (CMT3) methylates at 5′-CNG-3′ sequences and can initiate DNA methylation de novo at sites with certain histone modifications. DOMAINS REARRANGED METHYLTRANSFERASE1 (DRM1) and DRM2 are closely related proteins with redundant roles in methylating nonsymmetrical CHH sequences (where H can be an A, C, or T). These methylated sequences can lose information during each cycle of DNA replication because one template strand does not have a methylated cytosine.

DRM1 and DRM2 require targeting information that is often derived from the small interfering RNA (siRNA) pathway. The siRNA pathway directs gene silencing through complex mechanisms including RNA-directed DNA methylation (RdDM) (see Teaching Tools in Plant Biology 5: The Small RNA World). Essential components of RdDM include the plant-specific RNA Polymerases IV and V, long-noncoding RNAs (lncRNAs), RNA-dependent RNA polymerases (RDRs), dicer-like (DCL) RNA cleavage proteins, and ARGONAUTE (AGO) proteins that bind small RNAs and form RNA-induced silencing complexes.

CMT3, DRM1, and DRM2 are primarily required for regulating the expression of endogenous genes (as opposed to transposons), especially those that are critical developmental switches. A triple mutant of these three methylases is needed to show developmental abnormalities, indicating that CMT3, DRM1, and DRM2 have some functional redundancy.

The removal of DNA methylation is also important for proper gene regulation. Genetic studies in Arabidopsis identified a family of DNA glycosylase-type DNA demethylases that includes REPRESSOR OF SILENCING1 (ROSI), DEMETER (DME), DEMETER-LIKE2 (DML2), and DML3. These demethylases specifically excise methylcytosine, replacing it with unmethylated cytosine by a base-repair mechanism. Specific demethylation has been demonstrated or implicated during reproductive epigenetic resetting (described below), stomatal patterning, tomato fruit ripening, and responses to various stresses.

Histone Modifications

In nucleosomes, histone monomers have exposed N-terminal tails that are subject to covalent modification by histone-modifying
enzymes. Acetylation of the histones by histone acetyltransferases generally contributes to a more active chromatin state (i.e., higher levels of transcription); conversely, deacetylation is associated with a loss of gene expression. Histone methylation by histone methyltransferases can contribute to gene activation or silencing depending on the position of the methylation. The position and nature of histone modifications and the consequences of these modifications is known as the histone code. Histone marks are abbreviated as the histone name, the position of the mark, and the nature and number of the marks. For example, the epigenetic mark H3K27me3 is a trimethylation of Lys-27 (K) of Histone H3. H3K27me3 is a conserved silencing mark that is primarily found on developmentally regulated genes in euchromatin. Other well-conserved modifications include H3K9me, often associated with heterochromatin or silenced genes, and H3K4me, associated with actively transcribed genes.

There are many genes encoding histone modifying enzymes, and the specific functions of each are not fully characterized. H3K27me3 formation is strongly associated with the action of PRC2 (for Polycomb Repressive Complex), a multisubunit complex with histone methyltransferase activity. Beyond the formation of H3K27me3, plants and animals differ in how PRC2 silencing is mediated. In animals, H3K27me3 is bound by Polycomb (PC), a chromodomain protein that interferes with transcription by an unknown mechanism. Plants do not encode a Pc ortholog, but LIKE HETEROCHROMATIN1 (LHP1), also a chromodomain protein, seems to function similarly in that it binds H3K27me3 and is required to maintain silencing. In the epigenetically controlled vernalization response described below, maintaining the silenced state of FLC after vernalization requires the action of PRC2 and LHP1.

Histone modifications directly affect nucleosome structure. For example, histone acetylation weakens the attraction between DNA and histones, loosening the structure and facilitating access by RNA polymerase. Histone modifications also facilitate interactions between nucleosomes and other nonhistone proteins to promote or interfere with transcription.

Some histones have conserved variants that affect gene expression. For example, histone 2A has a variant, H2A.Z, which differs from H2A at the C-terminal region. Within the nucleosomes, the SWR1/SCAP complex can replace H2A with H2A.Z. This histone replacement is associated with high levels of transcription, perhaps by inhibiting DNA methylation. Reactivating expression of FLC during embryo genesis requires incorporation of H2A.Z into the nucleosomes near the FLC promoter. H2A.Z-containing nucleosomes also have been identified as important for the perception of ambient temperature changes and mediators of temperature-responsive transcriptional regulation and as controllers of plant immunity. There are three variants of histone H3. The establishment and maintenance of centromeres requires incorporation of the centromere-specific variant CENH3. H3.3 is associated with regions of elevated levels of gene expression, whereas H3.1 is associated with transcriptionally silent regions.

**Contributions of Chromatin Remodelers**

Several proteins have been identified that fall into the class of chromatin remodelers. Collectively, these proteins control the accessibility of chromatin DNA to various proteins such as transcription factors, histone modifying enzymes, and DNA methylases. Many chromatin remodeling proteins are members of the SWI/SNF family of ATP-dependent chromatin remodelers, which use the energy of ATP to change the interactions between DNA and histones. Some, including BRAHMA (BRM) and SPLAYED (SYD), have been identified genetically as contributing to developmental progressions or patterning and others as being involved in stress responses or hormone signaling.

Another class of chromatin remodeling enzymes was found through the characterization of mutants that show a derepression of DNA-methylated genes and transposons with no accompanying losses of DNA or histone methylation. AtMORC1 ATPases are required for heterochromatin silencing and the maintenance of chromatin architecture. Furthermore, the plant-specific protein MORPHEUS MOLECULE1 (MOM1) has a similar function, with atmorc1 mom1 double mutants showing an enhanced depression of silencing. The mechanisms by which these proteins act are still being explored.

**EPIGENETIC REGULATION IN WHOLE-PLANT PROCESSES**

**Epigenetic Control of Transposons and Repetitive Elements**

A sizable fraction of most genomes (including human) is derived from transposons. Many of these transposon-derived sequences are not capable of transposing or being transcribed because of accumulated mutations, but others are only kept inert because of pervasive epigenetic silencing. The DNA of most transposons is extensively CG methylated; this is the most stable, heritable form of DNA methylation because of the relative simplicity of its maintenance by MET1 activity during DNA replication. DECREASE IN DNA METHYLATION1 (DDM1) is a chromatin-remodeling ATPase that is also required for full suppression of transposons activation. Loss-of-function ddm1 mutants have diverse, unstable abnormal phenotypes caused by the mutagenic action of the activated transposons, a phenotype that is even more dramatic in the ddm1 met1 double mutant. Silencing the full suite of transposons in a genome requires the concerted action of CMT3, DRM1, and DRM2 and RNA-mediated DNA methylation pathways.

DNA methylation is not sufficient for the establishment and maintenance of transposon silencing, as revealed by studies of the Évadé (EVD) family of transposons in Arabidopsis. Unlike many other transposons, EVDs are not activated in met1 mutant backgrounds. However, when the met1 mutation is combined with a mutation in a subunit shared by the plant-specific RNA Polymerases Pol IV and Pol V, the transposons are released from inhibition. A similar effect was observed in double mutants between met1 and kyp, the major histone H3 lysine 9 (H3K9) methyltransferase. Thus, these studies revealed that transposon silencing can involve both DNA and histone methylation, as well as the action of Pol IV and Pol V.

Further studies have identified several nonmutually exclusive pathways that initiate and maintain silencing of transposons. RNA transcripts from transposons, generated by RNA Pol II or IV, are converted to double-stranded RNAs by RNA-dependent RNA polymerases (RDRs) and subsequently cleaved to siRNAs.
by the action of DCL proteins. When bound to AGO proteins, siRNAs interact with transcripts produced by RNA Pol V to recruit DNA methylases (to methylate DNA) and histone methylases (to establish H3K9me histone marks) and so silence the transposons. This mechanism provides a robust and specific means for silencing transposons and repetitive elements, as well as genes, as described below.

**Epigenetic Control of Flowering Time**

One of the most thoroughly characterized epigenetic phenomena is the control of flowering time. Angiosperms grow vegetatively until endogenous and exogenous cues trigger the initiation of the reproductive phase of growth. Their reproductive success depends on the maternal plant having sufficient resources to support seed development and environmental conditions being mild enough to sustain the maternal plant during flowering and seed maturation. Many plants flower in the spring, ensuring that their seed matures before winter. These plants often grow vegetatively through the winter and flower in response to the changing daylengths of spring. To ensure that they flower in the spring, these plants must experience the cold of winter to become competent to flower, in a process called vernalization. The cold period can occur weeks or months of winter to become competent to flower, in a process called vernalization. To ensure that their seed matures before winter. These plants often grow vegetatively through the winter and flower in response to the changing daylengths of spring. To ensure that they flower in the spring, these plants must experience the cold of winter to become competent to flower, in a process called vernalization. The cold period can occur weeks or months before the plant flowers; the plant must remember vernalization over dozens or hundreds of cycles of DNA replication. The mediator of the vernalization response is the regulated inhibitor of flowering, *FLOWERING LOCUS C* (*FLC*).

*FLC* encodes a MADS box transcription factor that binds and represses the *FT* gene, the key activator of flowering. During vegetative growth, *FLC* is transcribed at a high level. Epigenetic marks maintain this transcriptional activation, including histone H4 acetylation, methylation of Lys-4 and -36 of histone 3, and incorporation of the histone variant H2A.Z into the nucleosome.

Prolonged cold treatment induces expression of the *VERNALIZATION INSENSITIVE3* gene, which is a component of the vernalization-specific *VRN2 PRC2* complex. This complex and others modify the histones at the *FLC* gene, removing the activating marks, such as H3K4 methylation and H4 acetylation, and adding silencing marks, such as H3K9me and H3K27me3. LHP1 associates with *FLC* during and after cold treatment to maintain its silenced state, allowing *FT* to be expressed and induce flowering.

The repressive action of Polycomb group proteins is antagonized by the action of histone methyltransferases known as trithorax group proteins (trxG), which activate gene expression through trimethylation of histone H3. In plants, the trxG histone methyltransferase *SET DOMAIN GROUP26* (SDG26) activates expression of the flowering activator *SUPRESSOR OF OVERXPRESSION OF CONSTANS1* (*SOC1*) by inducing H3K4me3 and H3K36me3 modifications in nucleosomes positioned at its promoter. Chromatin remodeling proteins such as BAF60 (a SWI/SNF subunit) also affect *FLC* expression by altering the three-dimensional structure of chromatin at the *FLC* gene and affecting its expression level.

The expression of long non-coding RNAs (lncRNAs) also contributes to the control of expression levels of *FLC*. In animal systems, lncRNAs have been shown to act as scaffolds for the recruitment of chromatin modelling complexes. An lncRNA called *COLDAIR* is encoded by an *FLC* intron and it physically interacts with the PRC2 complex to repress *FLC* gene expression during vernalization. *COLDAIR* coordinates H3K27me3 enrichment at the chromatin around the *FLC* locus, leading to repression of this genomic region. Alternative splicing of an antisense *FLC* RNA also affects *FLC*’s chromatin state and expression level.

**Plant Developmental Programs**

Epigenetic regulation and reprogramming of genes occurs throughout plant development. Epigenetically regulated genes include genes encoding transcription factors, microRNA genes, and genes involved in auxin synthesis and response. Regulatory mechanisms include the incorporation of histone variants, changes in DNA methylation patterns, and PRC2-mediated H3K27me3 deposition. Many tissue-specific genes and regulatory genes that are specifically repressed in undifferentiated or proliferating tissues are regulated by the action of polycomb group proteins and trxG proteins. As examples, hundreds of genes synchronously gain or lose H3K27me3 marks during the transition from callus tissue to leaf, shoot apical meristem to leaf, root apical meristem to differentiating root cells. Thus, the developmental progression through the plant life cycle, which involves numerous developmental transitions, is extensively regulated through large-scale epigenetic reprogramming of gene activity, in addition to other, well-described regulatory mechanisms, such as the production and activation of transcription factors and the actions of small RNAs.

In the 18th century, Linnaeus puzzled over a mysterious, spontaneously arising floral variant that he named Peloria (meaning monster) in toadflax (*Linaria vulgaris*). Many years later, the origin of this variant was found to be an epigenetically regulated allele of the floral homeotic gene *CYCLOIDEA*. More recently, a mystery involving abnormal fruit formation in the economically important oil palm (*Elaeis guineensis*) was solved. These plants are propagated vegetatively, which has been observed to lead to epigenetic alterations and somaclonal variation. In some plants, the fruit spontaneously develop an abnormality known as mantling, which reduces oil production. The source of this abnormality was traced to the hypomethylation of a transposon (known as *Karma*) present in a floral patterning gene, which leads to alternative mRNA splicing, a nonfunctional protein, and abnormal fruit development.

**Epigenetic Response to Stress**

There is now strong evidence indicating that many abiotic and biotic stress responses involve some epigenetic changes. Numerous studies have demonstrated genome-wide epigenetic changes in response to stress or during recovery from stress, and other studies have revealed altered stress responses in mutants deficient in epigenetic machinery. As examples, low humidity causes an increase in methylation and decrease in expression level of master genes required for stomatal development, leading to a reduction in stomatal numbers, which
is an adaptive response to low humidity. Similarly, drought stress has been shown to enrich activating histone marks such as H3K4Me3, on dehydration-responsive genes. In some cases, the epigenetic and gene expression changes are transient. In others, the chromatin marks persist, “priming” the plant to respond more rapidly to subsequent stress.

Large-scale, genome-wide studies show that the stress effects on the epigenome can be far-reaching. For example, widespread changes in DNA methylation leading to changes in gene expression were found in response to biotic stress. Many of the differentially methylated genes are known to be involved in defense responses, but transposons are also sites of differential methylation.

Interestingly, there is accumulating evidence that stress-induced transposon activation may have specific functional roles in plant stress responses, through several different mechanisms. Transposon activation can increase the expression of stress-responsive genes located nearby. Activation of an *Athila* transposon leads to the production of small RNAs that in turn regulate the expression of a key gene involved in stress tolerance. Heat-induced activation of an *ONSEN* transposon could, in some mutant backgrounds, lead to an increase in transposition in the next generation.

### Epigenetic Control of Imprinted Genes

Like animals, angiosperms reprogram epigenetic marks during reproduction. The mechanisms by which the reprogramming occurs evolved separately in mammals and angiosperms, but both may function similarly to silence transposons in the new generation. Interestingly, mammals and angiosperms also selectively silence some genes in a parent-of-origin manner, in a phenomenon called imprinting. The nonequivalency of the maternal and paternal genomes was demonstrated by introducing nuclei from an egg and sperm, or two eggs or two sperm into an enucleated cell. Only the cells that contained both maternal and paternal genomes gave rise to viable embryos. Subsequent studies have shown that this effect is due to selective epigenetic silencing at certain imprinted alleles in each genome.

In angiosperms, the familiar green plant is the diploid sporophyte. Meiosis produces haploid spores, which undergo one or more cycles of mitotic division to produce a multicellular haploid male or female gametophyte. The female gametophyte has seven cells with eight nuclei. The binucleate central cell is the progenitor of the endosperm; another cell of the female gametophyte is the egg cell. The male gametophyte ultimately produces one cell encompassing one vegetative nucleus and two sperm nuclei. One sperm nucleus fertilizes the central cell to produce a triploid endosperm, while the other fertilizes the egg to produce the zygote.

*MEDEA* (*MEA*) encodes a subunit of the PRC2 complex. *MEA* is an imprinted gene; reciprocal crosses (in which the genotypes of the parents are reversed) generate progeny with different phenotypes. Progeny that receive a loss-of-function *mea* allele from their mother die, whereas those that receive it from their father have a wild-type phenotype. This effect is due to selective epigenetic silencing. In vegetative tissues and pollen, *MEA* is silenced. In the central cell of the female gametophyte, the silencing marks (including DNA and histone methylation) are removed, allowing *MEA* expression. After fertilization, the PRC2 complex, including MEA, maintains silencing of the paternal allele, ensuring that only the maternal allele is expressed. By applying RNA sequencing towards hybrids of inbred lines, it has been possible to identify more than 100 genes with parent-of-origin effects in Arabidopsis, rice, and maize. A small number of genes are imprinted in all three species (which diverged more than 100 million years ago), suggesting that imprinting of these genes confers a selective advantage.

### Gene Silencing in Trans

Like *MEA*, the imprinted gene *FWA* is silenced during vegetative growth and released from silencing in the female gametophyte. Abnormal *FWA* alleles were identified that are not silenced in vegetative tissues. These expressed alleles are identical in DNA sequence to wild-type *FWA* but differ in their epigenetic marks; they are epialleles. The *FWA* epialleles lack DNA methylation at a repeated element near the transcription initiation site; this hypomethylation leads to an expressed rather than silenced gene in vegetative tissues. Surprisingly, when a copy of the *FWA* gene is introduced into wild-type plants via *Agrobacterium tumefaciens*–mediated transformation, the transgene becomes methylated and epigenetically silenced in vivo, indicating that the silenced endogenous *FWA* transmits information to the newly introduced gene. In the *drm2* mutant background, the endogenous *FWA* gene remains silenced, but the introduced transgene is not silenced, indicating that DRM2 is necessary for de novo silencing of the introduced transgene.

Subsequent studies have shown that siRNAs are required for de novo DNA methylation, revealing that the expressed *FWA* allele silences the introduced allele through an siRNA mechanism. Interestingly, both *FWA* alleles are associated with siRNAs, but only the methylated, silenced allele is capable of silencing in trans, indicating that although necessary, siRNA production is not sufficient for *FWA* DNA methylation.

A similar RNA-based mechanism seems to be responsible for an unusual genetic event called paramutation. In paramutation, one allele of a gene can alter the epigenetic state of another in trans. The classic example of this is the maize (*Zea mays*) *b1* locus, which encodes a transcription factor involved in pigment synthesis. The wild-type allele, *B-I*, is transcribed at high levels, and plants homozygous for *B-I* are highly pigmented. The paramutagenic epiallele *B′* is expressed at a lower level due to epigenetic silencing; plants homozygous for *B′* alleles are not strongly pigmented. In an F1 hybrid, the *B′* allele paramutates (silences) the *B-I* allele, converting it to *B′*; the lightly pigmented F1 plant becomes homozygous for *B′* through paramutation. Paramutagenic silencing requires an RNA-dependent RNA polymerase and is thought to involve the same type of RNA-dependent DNA methylation pathway as *FWA* silencing.

### Resetting the Epigenome…or Not!

After vernalization, the transcriptional repression of *FLC* is maintained through many mitotic cycles before and during flowering.
Like many epigenetically controlled animal genes, the epigenetic state of FLC is reset during reproduction. It is actively silenced during gamete formation and then is reactivated during fertilization or early embryogenesis, a process that requires the contribution of the SWR1 complex and incorporation of the activating H2A.Z variant. This resetting process ensures that plants in the next generation must again be vernalized prior to flowering.

Recent surveys of genome-wide epigenetic marks, using deep sequencing, chromatin immunoprecipitation (ChIP), and microarray approaches (see below), suggest that extensive epigenetic reprogramming occurs during angiosperm reproduction. Compared with vegetative tissues, heterochromatin marks and DNA methylation are highly reduced not only at the site of imprinted genes, but throughout the endosperm genome. Also, there is an extensive proliferation of RNA Polymerase IV–derived siRNAs in the developing endosperm. In the pollen vegetative nucleus, but not the sperm nuclei, transposons are activated and hypomethylated. Notably, the vegetative nucleus and the endosperm genomes are not incorporated into the embryo; these demethylation programs do not occur in the egg cell or sperm nuclei that are the progenitors of the zygote. In combination, these results are consistent with a model in which a release of silencing in the pollen vegetative nucleus and endosperm ensures transposon repression in the zygote through the siRNA pathway. Because siRNAs can move short distances between cells, perhaps siRNAs induced by transposon activation in the vegetative nucleus and endosperm are mobilized into the germ tissues to initiate and maintain epigenetic silencing controls.

Natural epigenetic variation is common in plants, and the epigenome is not necessarily reset during gametogenesis. Indeed, transgenerational epigenetic inheritance of qualitative (single-gene) traits has been demonstrated, for example, in nonripening tomato and the peloric floral morphology of toadflax. The epigenetic heritability of complex (multigene) traits was difficult to determine without comparing methylation patterns in the genomes of isogenic epimutants. Therefore, it remained controversial for a long time whether transgenerational epigenetic inheritance could actually be used for breeding purposes. Recently, however, methylome profiling revealed that certain methylated alleles are stable across generations and can be used as authentic epigenetic quantitative trait loci (QTL<sup>epi</sup>) that are amenable for artificial selection. Interestingly, the phenomenon of hybrid vigor, in which the F1 hybrid of two genetically distinct parents can show enhanced growth relative to each parent, may be contributed to by epigenetic effects including altered DNA methylation and siRNA patterns, although the magnitude and generality of such effects remains unclear.

METHODS

Methylation-Sensitive Nucleases

Many restriction enzymes are sensitive to DNA methylation. Digestion of genomic DNA with methylation-sensitive restriction enzymes can be used to compare against DNA digested by their methylation-insensitive isoschizomers. Endonucleases that digest specifically methylated DNA (McrBC) can also be used to detect methylated genomic regions. Following digestion, the products are further processed for characterization of specific regions (DNA gel blots and PCR) or genome-wide analysis (microarray hybridization and DNA sequencing).

Affinity Purification of Methylated DNA

Antibodies that recognize methylated cytosine are available and can be used to isolate methylated DNA by affinity purification or immunoprecipitation. These methods are useful to produce genomic maps showing methylcytosine-rich regions, but do not establish the exact methylation sites.

Analysis of DNA Methylation by Bisulfite Sequencing

When DNA is treated with bisulfite, cytosines are deaminated and converted to uracil, whereas methylated cytosines are protected from deamination. To determine the extent of methylation in a region, the sequence of a bisulfite-treated sample must be compared with an untreated sample. The cytosines that are conserved in the treated and untreated samples are those that were methylated; unmethylated cytosines are read as thymines in the bisulfite-treated sample and cytosines in the untreated control sample.

Analysis of Histone Modification

ChIP is a technique that allows specific protein-DNA interactions to be examined. ChIP was initially developed as a method to identify the interactions between transcription factors and their DNA binding sites. However, the availability of antibodies that selectively bind modified histones makes this method useful for epigenomic studies as well. Chromatin is cross-linked by formaldehyde treatment and then sheared to ~500-bp fragments. Antibodies are used to purify the fraction of chromatin that includes the modified histone of interest. The DNA-protein cross-linking is reversed and the DNA purified for analysis by PCR, sequencing (ChIP-Seq), or microarray hybridization (ChIP-chip).

An alternative method, DamID, involves expressing a histone binding protein as a fusion with Dam, a sequence-specific DNA adenine methylase from Escherichia coli. The Dam methylase is specifically recruited to the binding site of its fusion partner, where it methylates nearby sites. Subsequent cleavage of the DNA with a methylation-sensitive enzyme allows methylated sites to be preferentially amplified and purified by PCR. This method has been used to identify the chromatin-wide distribution of the plant protein LHP1.

Next-Generation DNA Sequencing Methods

The convergence of robust methods for identifying epigenetic marks with the development of next-generation sequencing methods has facilitated a tremendous amplification in our study and understanding of epigenetic controls of the genome. For many years, the advances in DNA sequencing technologies were focused on generating longer stretches of sequence information from individual molecules to facilitate the assembly of genomic sequences. Recently, the usefulness of obtaining short sequence reads from many molecules in parallel has become evident; these methods are referred to as high-throughput or deep sequencing. For example, massively parallel sequencing of mRNA (reverse transcribed to cDNA) is an important method for examining transcript abundance within and between tissues. Used in combination with ChIP, deep sequencing allows the relative abundance of histone modifications to be assessed genome wide.
wide. Deep sequencing is also an important tool in examining small RNA accumulations within and between tissues.

There are several different types of next-generation sequencing that share the features of low-cost, massive parallelism and speed. For example, Roche/454 sequencing is a synthesis-based pyrosequencing technology, which uses bioluminescence to detect the release of pyrophosphate by incorporation of a nucleotide into extending sequence. Like conventional dyeideoxy sequencing, this method uses DNA polymerase and a primed template. The addition of a deoxynucleotide triphosphate to the elongating primer is quantified by the emission of light; when a deoxynucleotide triphosphate is attached, pyrophosphate is released. The pyrophosphate is then used as a substrate in the production of ATP, which is used by the enzyme luciferase to produce light. Light emission across is collected in real time by a CCD camera. Using this method, more than a million templates can be sequenced at a time.

Other next-generation technologies include cyclic-reversible termination (Illumina/Solexa), which uses technology similar to conventional dyeideoxy sequencing, but with the added feature that the terminators can be reverted to allow additional rounds of template extension, eliminating the need for electrophoretic separation of products. In addition to sequencing by synthesis, other methods, such as support oligonucleotide ligation detection (SOLID), depend on sequencing by primer ligation. Ion Torrent semiconductor sequencing (Thermo Fisher Scientific, formerly Life Technologies) is based on detection on a primer ligation. Ion Torrent semiconductor sequencing (Thermo Fisher Scientific, formerly Life Technologies) is based on detection on a chip of H+ released during DNA polymerization. Recent sequencing advances, such as the synthesis-based Single Molecule Real Time sequencing (SMRT) from Pacific Biosciences, incorporate real-time sequencing by single DNA polymerases on single-molecule DNA templates. Addition of a single nucleotide is detected by a fluorescent tag that is released after incorporation and diffuses away. By acting on single DNA molecules, massively parallel real-time methods substantially improve the length of DNA reads and eliminate the need for template amplification.

Future Prospects

Our understanding of epigenetic programming of plant genomes is growing exponentially. Facilitated by genome-wide surveys in wild-type and mutant plants, new pathways integrating DNA methylation, histone modification, and siRNA production are being identified. As we learn more about the mechanisms by which plant genes are epigenetically regulated, we are also learning more about the diverse processes regulated by epigenetic controls, including the role of epigenetic programming in developmental pattern formation. It is also becoming clear that epigenetic processes are involved in the responses of plants to pathogens and environmental stresses. Natural variants of Arabidopsis thaliana that have evolved in diverse environments reveal the role of epigenetic controls in recent environmental adaptations and the roles of epialleles in natural selection. Epigenomic studies of plants other than Arabidopsis are revealing the processes that occur during interspecific hybridization and polyploidization and in plants that carry a large number of active transposons, such as maize. From cell differentiation to evolution and adaptation, there’s not a lot to be silent about in epigenetics.

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RECOMMENDED READING

(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

Reviews


Seminal Articles


Osorba, T., Questa, J.I., Sun, Q., and Dean, C. (2014). Antisense COOLAIR mediates the coordinated switching of chromatin states at


March 2016 9


Methods


