Epigenetics: Beyond Chromatin Modifications and Complex Genetic Regulation[1]

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Chromatin modifications and epigenetics may play important roles in many plant processes, including developmental regulation, responses to environmental stimuli, and local adaptation. Chromatin modifications describe biochemical changes to chromatin state, such as alterations in the specific type or placement of histones, modifications of DNA or histones, or changes in the specific proteins or RNAs that associate with a genomic region. The term epigenetic is often used to describe a variety of unexpected patterns of gene regulation or inheritance. Here, we specifically define epigenetics to include the key aspects of heritability (stable transmission of gene expression states through mitotic or meiotic cell divisions) and independence from DNA sequence changes. We argue against generically equating chromatin and epigenetics; although many examples of epigenetics involve chromatin changes, those chromatin changes are not always heritable or may be influenced by genetic changes. Careful use of the terms chromatin modifications and epigenetics can help separate the biochemical mechanisms of regulation from the inheritance patterns of altered chromatin states. Here, we also highlight examples in which chromatin modifications and epigenetics affect important plant processes.

In current usage, the term epigenetics (for review, see Haig, 2004) is used to describe several distinct concepts: some researchers use epigenetics to describe heritable differences not caused by DNA sequence changes, whereas others use epigenetics to describe any changes in chromatin modifications or simply describe unusual patterns of inheritance. This use of the same term to describe different concepts can limit our ability to communicate accurately. In this article, we use the term epigenetics to describe heritable patterns of phenotypic variation that are not solely attributable to differences in DNA sequence. The term heritable in this definition indicates stable transmission of information through mitosis or meiosis in the absence of the original inducing signal. Chromatin state often plays a critical role in gene regulation, but alterations in chromatin state may not necessarily lead to heritable changes. For example, the chromatin state change may depend on particular genetic sequences or the continual presence of an endogenous cue. Studies on the potential role of epigenetics in plant development or response to the environment generally involve genetically identical cells but can struggle to provide strong evidence of heritability in the absence of the primary signal. In contrast, studies investigating the role of epigenetics in natural phenotypic variation can struggle to provide evidence that changes in phenotype, which correlate with variation in chromatin modifications, are not the result of underlying genetic changes. Thus, providing clear evidence for a role of epigenetics has proven challenging in many studies.

The relatively liberal usage of the term epigenetics to describe different concepts can interfere with our ability to clearly describe novel research findings. In some cases, epigenetics has been used to describe any situation of inheritance that does not follow simple Mendelian expectations or any example of gene regulation involving chromatin changes. By reserving the term epigenetic to describe heritability without direct involvement of DNA sequence, we can distinguish this concept of inheritance from the biochemical mechanisms of gene regulation involving chromatin states. In an attempt to better delineate confirmed epigenetic phenomena, we next describe situations that would not be considered epigenetic by this definition.

One common usage of epigenetics is as a catchall term to describe any unexpected, non-Mendelian pattern of inheritance. In some cases, researchers studying unusual patterns of inheritance have found evidence for epigenetic phenomena. For example, the basis for the variable phenotype condition by some alleles of the *Agouti* locus in mouse (*Mus musculus*; Morgan et al., 1999), the unstable patterns of transposon activity (discussed in McClintock, 1984), and paramutation (Chandler and Stam, 2004) all involve non-Mendelian inheritance and epigenetics. However, caution should be taken, because there are many examples of unusual patterns of inheritance that can be attributed to genetic changes. For example, transposon insertions can cause

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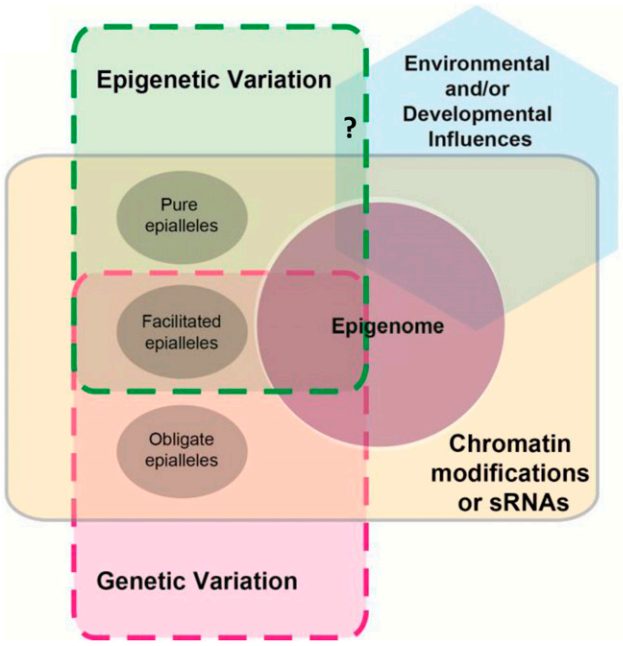
unstable phenotypes that behave in unexpected fashions. Studies on heritable changes of some flax (*Linum usitatissimum*) varieties in response to environmental stress also point to genetic rather than epigenetic changes (Johnson et al., 2011). Even examples of unusual inheritance in mutant plants defective in maintenance of chromatin modifications can reveal examples of genetic rather than epigenetic changes (Yi and Richards, 2008). Many non-Mendelian phenomena have genetic bases, such as quantitative traits, segregation distortion, and cytoplasmic inheritance. Although epigenetic phenomena can display unusual inheritance patterns, it is worth noting that genetic regulation can also lead to non-Mendelian patterns. The description of a phenomenon as epigenetic becomes particularly difficult in organisms that are not tractable to genetic studies. In species with complex polyploid genomes or crossing barriers, it is difficult to probe the actual mechanism of inheritance, and it can be easy to misapply the label epigenetic without showing the lack of primary sequence differences driving the phenomenon.

Another common usage of epigenetics is as a term to describe any gene regulation that involves chromatin modifications. The Latin prefix epi connotes over, and in one sense, chromatin modifications certainly provide potential information that may be over the genome sequence information. However, this definition of epigenetic does not have any requirement for transmission through mitosis or meiosis. Therefore, using epigenetic in this context may confound the description of chromatin state as opposed to inheritance patterns. In addition, using the term epigenetics to describe any chromatin changes would likely imply that all gene regulation is epigenetic, because chromatin modifications generally affect gene expression in eukaryotes. Many studies that find differences in chromatin modifications at a particular gene for two plants, tissues, or treatments will report epigenetic regulation of that gene. However, describing this as chromatin-based regulation of the gene would more precisely distinguish between chromatin changes and concepts of heritability. Chromatin modifications do function as an integral part of some epigenetic phenomena; however, some chromatin modifications are likely not heritable and therefore, might not be considered epigenetic (Fig. 1). For example, the phosphorylation of histone H3 Ser 10 (Nowak and Corces, 2004) is quite labile and changes during the cell cycle. There is little evidence that this particular modification provides heritable information that is transmitted through mitotic or meiotic cell divisions. Other chromatin modifications can exhibit stable inheritance, but this property varies substantially for different modifications. Next, we will discuss the potential avenues of heritability for a variety of chromatin modifications.

DNA methylation can be heritable through cell divisions, because the methyl group is covalently linked to DNA. DNA replication adds unmethylated cytosines, resulting in hemimethylation at previously methylated sites in all contexts (CG, CHG, and CHH, where H = A, C, or T). Methylation of the newly synthesized strand can occur through context-specific mechanisms (Law and Jacobsen, 2010). For CG dinucleotides, enzymes, such as Methyltransferase1 (MET1), recognize the hemimethylated DNA and direct the maintenance methyltransferase to methylate the symmetrical unmethylated cytosine (Bostick et al., 2007). CHG methylation can be transmitted to newly replicated DNA in a similar fashion by chromomethylase enzymes, such as Chromomethylase3 (CMT3), and evidence also shows that CHG methylation has a self-reinforcing loop with histone H3 lysine9 dimethylation (Lindroth et al., 2004; Johnson et al., 2007; Du et al., 2012). For CHH sites, inherited or newly generated small interfering RNAs (siRNAs) can direct DNA methylation by the Domains Rearranged Methyltransferase2 (DRM2) methyltransferase through the RNA-directed DNA methylation pathway (Aufsatz et al., 2002; Cao et al., 2003; Kanno et al., 2005; Onodera et al., 2005; Law and Jacobsen, 2010), or in some cases, newly accessible DNA resulting from chromatin remodeling undergoes CHH methylation by the CMT2 methyltransferase (Zemach et al., 2013).

Demethylation also influences DNA methylation patterns. Passive demethylation occurs through the lack of active maintenance of DNA methylation, and active DNA demethylation occurs through catalytic removal of 5-methylcytosine (Zhang and Zhu, 2012). Plants encode a family of glycosylases that can actively remove DNA methylation. These enzymes can act in a developmentally programmed fashion to induce locus-specific loss of DNA methylation (Choi et al., 2002; Ibarra et al., 2012) but also play a role in pruning DNA methylation patterns in other tissues (Zhu et al., 2007). Thus, although DNA methylation patterns can be stably inherited, especially in CG and CHG contexts, a variety of natural mechanisms result in gain or loss of DNA methylation and thus, potentially interfere with the heritability of DNA methylation.

Mechanisms for heritability of histone modifications or variants are less obvious and less frequently described in the literature (Margueron and Reinberg, 2010). In some cases, particular histone variants, such as centromeric histone H3, seem to be stably maintained at consistent genomic positions (Allshire and Karpen, 2008). However, many of the nucleosomes that are deposited after replication do not initially contain the same modifications as the parental chromatin (Xu and Zhu, 2010; Abmayr and Workman, 2012; Budhavarapu et al., 2013). Plants contain a variety of enzymes that can add or remove histone modifications (Chen and Tian, 2007; Pfluger and Wagner, 2007; Chen et al., 2011; Deal and Henikoff, 2011; Thorstensen et al., 2011). The targeting of these enzymes to the proper locations and the turnover rate for different modifications will influence the ability of that modification to be stably inherited. The histone modification with the most evidence for stable inheritance in plants is histone H3 lysine9 dimethylation, which is coupled with CHG DNA methylation and acts in a self-reinforcing loop that likely provides a mechanism for stable inheritance of this



Epigenetic variation: Heritable differences that are independent of changes in DNA sequence

Chromatin modifications: Differences in the presence or types of histones (variants) and modifications of DNA (methylation) or histones (methylation, acetylation, etc) and small RNAs that are often associated with epigenetic variation but can be influenced by genetic variation or development / environment

Epigenome: The genome-wide distribution of chromatin modifications or DNA methylation patterns that may include non-heritable changes or genetically influenced patterns

Epialleles: Meiotically heritable allelic differences in chromatin state
Pure epialleles: Epialleles that have differences in chromatin state that are independent of any genetic information
Facilitated epialleles: Epialleles for which a genetic difference (i.e. transposon insertion) leads to the potential to adopt alternate chromatin states
Obligate epialleles: Epialleles with altered chromatin state that is fully dependent upon genetic variants (either cis or trans-acting changes)

Figure 1. Definitions and overlap of concepts often associated with epigenetics. sRNA, Small RNA.

chromatin modification (Johnson et al., 2007). Much remains unknown about the potential mechanisms for heritability of histone modifications, but evidence indicates that differences in histone modifications between two cells will not necessarily be transmitted through mitosis or meiosis.

Another potential issue with equating chromatin modifications and epigenetics is that some chromatin modifications may result from genetic variation (Paszkowski and Grossniklaus, 2011; Pecinka et al., 2013). The identification of epialleles, alleles that vary in chromatin state, has increased with our ability to generate genome-wide maps of chromatin modifications, but understanding the mechanistic basis for epiallele formation remains challenging. Moreover, the epiallele designation can be misleading, because not all epialleles are epigenetic (Fig. 1). Obligate epialleles are completely dependent on genetic variation (Richards, 2006; Fig. 2) and persist, because the genome sequence encodes the instructions required to direct chromatin modifications. Therefore, the heritable information for this class of epialleles is genetic rather than epigenetic (Fig. 2).

The lines between genetic and epigenetic begin to blur when discussing the other two classes of epialleles, facilitated and pure, and any distinction between these epialleles may be very difficult to apply to natural situations. In both cases, the same genetic sequence can exhibit two potential chromatin states that show at least partial heritability. In some clear examples of facilitated epialleles, the genetic sequences at linked or unlinked genomic positions influence chromatin state. For example, in the *Agouti* spp. locus in mice, alleles that contain a transposon insertion upstream of the gene exhibit metastable inheritance of chromatin state (Morgan et al., 1999). Similarly, paramutation at the *B* locus of maize (*Zea mays*) requires certain sequences in the regulatory regions, but the presence of this genetic information does not always lead to one state or the other (Stam et al., 2002; Belele et al., 2013). In another scenario, a change in the DNA

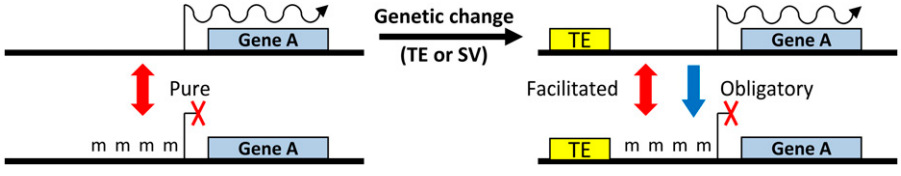


Figure 2. Classification of epialleles based on potential influence of genetic sequences. Pure epialleles shift between transmissible chromatin states (chromatin modification [m]) with no change in genetic sequence at linked or unlinked genomic positions. In other cases, a genetic change, such as a transposable element (TE) insertion or structural variant (SV), can produce directed chromatin changes, resulting in an obligatory allele. Alternatively, the genetic change can result in a poised allele that can exist in two potential chromatin states (facilitated).

sequence can trigger a stable change to the chromatin environment that persists in the absence of the original genetic variant as observed at the *Arabidopsis thaliana folate transporter1* (*AtFOLT1*) locus (Durand et al., 2012). Therefore, facilitated epialleles show a role for epigenetic influence and a role for genetic sequences that predispose an allele to epigenetic regulation (Fig. 2). Pure epialleles are completely independent from changes in the DNA sequence. Two apparent examples of pure epialleles, *Linaria cycloidea* (*Lcyc*; Cubas et al., 1999) and *Clark Kent* (*clk*; Jacobsen and Meyerowitz, 1997), exhibit stable differences in chromatin state without apparent genetic change. However, even these examples may have sequences that predispose these particular genes to being susceptible to altered chromatin state. These classifications attempt to provide a framework to consider the interaction between DNA sequences and epiallele formation, but it is clear in nature that the three classes exist in a continuum. These distinctions between epiallele types reveal the complexity in equating differing chromatin states at an allele with epigenetics.

Small RNAs are another example of a complex regulatory mechanism that can contribute to epigenetics but is not necessarily epigenetic itself (Bond and Baulcombe, 2014). Small RNAs, including siRNAs, microRNAs (miRNAs), and several other specific types, can influence transcription and RNA stability in plants. However, in most cases, the small RNA is produced from a genomic region and provides a sequence-specific genetic factor. Small RNAs can provide a mobile signal that affects distant genomic regions (Bender, 2004) or even distant cells (Melnyk et al., 2011). In some cases, the influence of the small RNA will only persist while the small RNA itself is present, but there are also mechanisms that can perpetuate the signal of small RNAs. Plant species have the potential to maintain small RNA signals, in some cases through RNA-dependent RNA polymerases; therefore, some small RNAs have the potential to create a self-perpetuating signal (Baulcombe, 2006). Some small RNAs can also trigger RNA-directed DNA methylation, thereby providing a signal that is later translated into a potentially heritable chromatin modification (Simon and Meyers, 2011). Although some small RNAs can exert heritable influences, many small RNAs exert only a transient influence on gene expression. For example, many miRNAs provide specific regulation that influences plant development or responses to the environment, but these responses would rarely fit the definition for epigenetics, because they are not heritable in the absence of the original inducing signal (Voinnet, 2009; Chen, 2012; Sunkar et al., 2012).

The term epigenomics complicates the distinction between chromatin modifications and epigenetics (Fig. 1). Originally, this term was used to describe studies that profile the genome-wide distribution of a particular chromatin modification, such as DNA methylation or histone modification (Callinan and Feinberg, 2006), that was often thought to contain epigenetic information. In recent years, epigenomics has come to encompass any study that profiles the genome-wide distribution of any chromatin-associated factors, such as histone modifications, histone variants, RNAs, and transcription factors (Bernstein et al., 2010), regardless of their heritability. Epigenomics studies provide comprehensive profiles of chromatin modifications, some of which may provide transmissible information. However, as discussed above, many of the chromatin modifications may be nonheritable or programmed by underlying DNA sequence. Therefore, it is important to distinguish between epigenetics and epigenomics, although the two words have similar connotations. Epigenomic studies improve our understanding of potential sources of epigenetic phenomena, and they can also reveal how genetic variation, development, or the environment influences the genome-wide distribution of chromatin modifications.

The above examples illustrate the complexity of gene regulation and some of the difficulties with invoking the term epigenetics. Before examining the potential contribution of epigenetics and chromatin modifications to plant processes, it is worthwhile to clearly distinguish the two types of heritable epigenetic information. Meiotic inheritance (or transgenerational) will often involve constitutive heterochromatin. Maintaining stable inheritance of information across generations requires this information to pass through many mitotic cell divisions. Given its stability, one would expect that this type of variation could play a role in natural variation, evolution, and polyploidy. By contrast, mitotic inheritance will often involve facultative heterochromatin that might vary among cells depending on their developmental history or environmental exposures. We will begin by considering the potential role for epigenetics and chromatin modifications in plant development and response to the environment. We will consider whether the line between mitotic and meiotic inheritance may sometimes be blurred. If some of the mitotically heritable changes in chromatin state that are influenced by development or the environment can be transmitted to offspring, this would create the potential for so-called soft inheritance, in which acquired characteristics could be transmitted to offspring (Richards, 2006). We will conclude by considering the potential role for epigenetics in natural variation and evolutionary processes.

EPIGENETICS AND PLANT DEVELOPMENT

Many open questions remain regarding the role of chromatin modifications and epigenetics in driving plant developmental processes. Development in multicellular organisms often involves differentiation of cells into specialized cell types that express different sets of genes. The silencing of certain genes in specific cell types could be considered to occur through facultative heterochromatin, with repressive chromatin in different locations based on cell type. The role of epigenetics in plant development is most likely limited to mitotic transmission of gene expression states. If the epigenetic memory of developmental decisions was

inherited through meiosis, it would likely interfere with development of the subsequent generation. Epigenetics may contribute to plant development by stably maintaining gene expression states that are initially directed by sequence-specific factors, such as transcription factors or small RNAs. The Polycomb group (PcG) of genes provides a classic example for a role of epigenetics in developmental control of gene expression (Simon, 1995; Francis and Kingston, 2001; Ringrose and Paro, 2004; Grimaud et al., 2006). A set of well-characterized regulatory mechanisms leads to differential expression of key regulators in *Drosophila melanogaster* larvae. Initially, these regulatory mechanisms depend on sequence-specific transcriptional activators and repressors, but these gene expression states become locked in by the PcG complex. This process has limited sequence specificity and can regulate different sets of genes in different cell types. Homologs of some PcG genes in plant species may play related roles in developmental regulation of gene expression (Bemer and Grossniklaus, 2012; Holec and Berger, 2012). The PcG genes contribute to molecular memory of gene expression choices that are made during cellular differentiation and provide mitotically heritable information, but we have limited evidence of their contributions to meiotic inheritance (Orlando, 2003; Breiling et al., 2007).

Many of the key developmental regulators identified by forward genetics encode transcription factors (Ramachandran et al., 1994). However, there is strong evidence that chromatin modifications and complex gene regulatory mechanisms, such as small RNAs, play important roles in plant development. Forward genetics experiments have identified a number of genes that contribute to chromatin remodeling or chromatin modifications that can lead to developmental abnormalities (Goodrich and Tweedie, 2002; Reyes et al., 2002; Wagner, 2003; Reyes, 2006; Köhler and Hennig, 2010; Bemer and Grossniklaus, 2012; Holec and Berger, 2012). There is also very strong evidence for a role of miRNAs in controlling developmental transitions in many plant species, largely through their control of transcription factors (Carrington and Ambros, 2003; Jones-Rhoades et al., 2006; Chen, 2009). Direct analyses of chromatin modifications in different cell types have provided evidence that the profiles of some histone modifications change substantially during development (Roudier et al., 2009). For example, H3K27me3 contributes to regulation of important transcription factors in some cell types and shows clear tissue-specific patterns that are often associated with tissue-specific expression of the target genes (Lafos et al., 2011; Zheng and Chen, 2011; Makarevitch et al., 2013). Other marks, such as histone acetylation and H3K4me3, show tissue-specific patterns (Berr et al., 2010) but may be an effect, rather than a cause, of tissue-specific gene expression.

The role of DNA methylation in plant development has not been fully resolved. Plants that have lost functions for some components of the DNA methylation machinery can exhibit altered morphology and development (Finnegan et al., 1996; Ronemus et al., 1996; Richards, 1997). However, this may simply reflect ectopic expression of some genes that influence morphology and development as opposed to indicating a normal role for DNA methylation in regulation of development. For example, Arabidopsis plants compromised in certain key components of the DNA methylation machinery will express *SUPPRESSOR OF drm1 drm2 cmt3*(*SDC*). *SDC* is not normally expressed in vegetative tissues, but when ectopically expressed in certain DNA methylation mutants, it leads to altered development (Henderson and Jacobsen, 2008). Genome-wide analyses of DNA methylation do find examples of tissue-specific differences in vegetative tissues, but the frequency of these changes is relatively small compared to differences among ecotypes or the frequency of tissue-specific gene expression (Zemach et al., 2010; Zhang et al., 2011; Eichten et al., 2013a; Schmitz et al., 2013a). It remains possible that tissue-specific changes in DNA methylation play an important role in the regulation of certain aspects of development (Li et al., 2011; Zhong et al., 2013), but it seems that the bulk of DNA methylation in plants remains relatively stable during vegetative development.

In contrast to the generally stable patterns of DNA methylation in vegetative tissues, DNA methylation in reproductive and endosperm tissues shows dynamic changes (Lauria et al., 2004; Gehring et al., 2009; Hsieh et al., 2009; Zemach et al., 2010; Bauer and Fischer, 2011; Zhang et al., 2011; Gutierrez-Marcos and Dickinson, 2012). During male gametogenesis, the vegetative nucleus undergoes major alterations to DNA methylation patterns (Slotkin et al., 2009; Borges et al., 2012; Calarco et al., 2012; Ibarra et al., 2012; Jullien et al., 2012). Similarly, the central cell of the female gamete also experiences substantial changes in DNA methylation (Choi et al., 2002; Gehring et al., 2006; Hsieh et al., 2009; Ibarra et al., 2012). Neither of these cells will contribute genetic information to the vegetative tissues in the next generation. However, it has been hypothesized that the loss of epigenetic silencing in these cells allows the production of siRNAs that can reinforce silencing of transposons in adjacent reproductive cells that will contribute to the next generation (Slotkin et al., 2009; Baroux et al., 2011; Ibarra et al., 2012; Wollmann and Berger, 2012). This likely contributes to imprinted gene expression in endosperm tissue and the evidence that some targets of imprinting are key regulators of endosperm development (Li and Berger, 2012; Gehring, 2013).

Although Waddington originally used the term epigenetics in a developmental context (Waddington, 1942; Haig, 2004), here, we make the case that a definition of epigenetics requiring heritability makes it difficult to establish an epigenetic component for many of the chromatin modifications that occur during development. This is not to say that epigenetics does not play an important role in development but instead, highlights the complexities in truly assigning a role for epigenetics in the developmental regulation of gene expression. It is hard to show that the chromatin

differences among cells would be heritable in the absence of ongoing signals or positional cues. A key exception is imprinting, in which allele-specific expression is controlled by the parent of origin. The maternal and paternal alleles share the same DNA sequence and are present in the same nucleus, but they exhibit different expression levels (Gehring, 2013).

Plants and animals seem to have differences in how chromatin modifications and DNA methylation are reprogrammed before the next generation (Feng et al., 2010). In addition, they have differences in the ease of generating pluripotent cells. In many animal systems, generating pluripotent cells requires substantial treatments to remodel chromatin (Meissner, 2010; Adachi and Schöler, 2012). In contrast, in most plant systems, generating stable tissue culture lines often requires only treatment with combinations of plant hormones. However, tissue culture often causes chromatin changes (Kaeppler et al., 2000; Tanurdzic et al., 2008; He et al., 2012; Stroud et al., 2013). In summary, strong evidence shows that chromatin varies among different cell types and that some of these modifications play important roles in plant development, but whether specific examples are epigenetic (transmissible) during plant development remains less clear.

EPIGENETICS AND RESPONSE TO THE ENVIRONMENT

The term epigenetics is often invoked to describe plant responses to environmental cues (Finnegan, 2002; Boyko and Kovalchuk, 2011; Mirouze and Paszkowski, 2011; Paszkowski and Grossniklaus, 2011; Gutzat and Mittelsten Scheid, 2012; Pecinka and Mittelsten Scheid, 2012). The concept of environmental responses as epigenetic is often used to describe a range of findings from heritable phenotypic consequences to the observation of chromatin changes after environmental treatments. Stable phenotypic consequences after an environmental treatment may reflect physiological or morphological changes triggered by the initial environmental cues that are maintained without epigenetic contributions. However, the observation of chromatin changes does not necessarily imply epigenetics, because these changes may be transient effects that require the ongoing presence of the stimulus. For example, drought conditions may result in altered leaf wax deposition, and this morphological change could influence phenotypic responses to subsequent environmental conditions but would not reflect cellular inheritance of the memory of the stress. Conceptually, mitotically transmissible memory that programs responses to environmental cues may provide part of the mechanism that plants use to alter gene expression in response to the environment. There are some clear examples of epigenetic mitotic memory of environmental conditions, such as vernalization (see below), but there are many more examples in which dynamic changes in chromatin modifications provide a portion of the mechanism for transient gene expression changes in response to the environment (Fig. 3). We will begin by discussing the immediate and short-term effects of environmental stress. In the next section, we will touch on the more contentious issue of whether environmental exposures can lead to heritable changes in offspring reminiscent of Lamarckian inheritance (Daxinger and Whitelaw, 2010).

Many published reports have documented changes in chromatin or siRNA abundance in response to environmental conditions (Gutzat and Mittelsten Scheid, 2012; Khraiwesh et al., 2012). Histone modification patterns can vary after environmental exposure and often are associated with altered expression at some loci (Kwon et al., 2009; Kim et al., 2010; van Dijk et al., 2010; Luo et al., 2012; Zong et al., 2013). In some cases, environmental treatments, such as heat, can affect heterochromatin (Lang-Mladek et al., 2010; Pecinka et al., 2010; Tittel-Elmer et al., 2010). Environmental stress can also affect the abundance of miRNAs or siRNAs in plants (Ruiz-Ferrer and Voinnet, 2009; Khraiwesh et al., 2012). Transposons can also respond to changes in environmental conditions (Grandbastien et al., 2005; Pecinka et al., 2010; Ito et al., 2011) and may influence the responsiveness of nearby genes to environmental cues (Feschotte, 2008; Naito et al., 2009; Ito et al., 2011; Bucher et al., 2012; Wheeler, 2013; Cavrak et al., 2014). Together, these reports provide clear evidence for an important role of chromatin and siRNAs in plant responses to the environment. Although chromatin changes or small RNAs have the potential to contribute to heritable changes, they can also lead to transient changes in gene expression. Few examples have shown actual mitotic or meiotic memory of environmental treatments.

Vernalization provides an example of mitotic epigenetic memory during the lifetime of a plant. Certain plant varieties germinate in the fall, overwinter as a vegetative rosette, and subsequently, flower early in the spring season when the days lengthen. Vernalization is the exposure to long-term cold that occurs during this overwintering period and renders plants competent to flower early in the spring (Amasino and Michaels, 2010). Plants with this lifecycle can take advantage of an ecological niche that enables successful reproduction in the early spring, when many other plant varieties have just begun to germinate. A phenotypic analysis of the impact of temperature and day length on flowering in henbane (*Hyoscyamus niger*) elegantly showed the epigenetic basis for vernalization (Lang and Melchers, 1947). Although the specific mechanisms of vernalization vary between species, clear evidence shows an epigenetic basis of vernalization in Arabidopsis (Schmitz and Amasino, 2007). Before the extended period of cold, the floral repressor *FLOWERING LOCUS C* (*FLC*) is expressed and suppresses the transition from vegetative growth to flowering. During extended periods of cold, *FLC* is silenced, in part, by changes to chromatin, including histone modifications (Bastow et al., 2004; Sung and Amasino, 2004; Song et al., 2012). This altered chromatin and expression state is then stably transmitted mitotically and renders plants

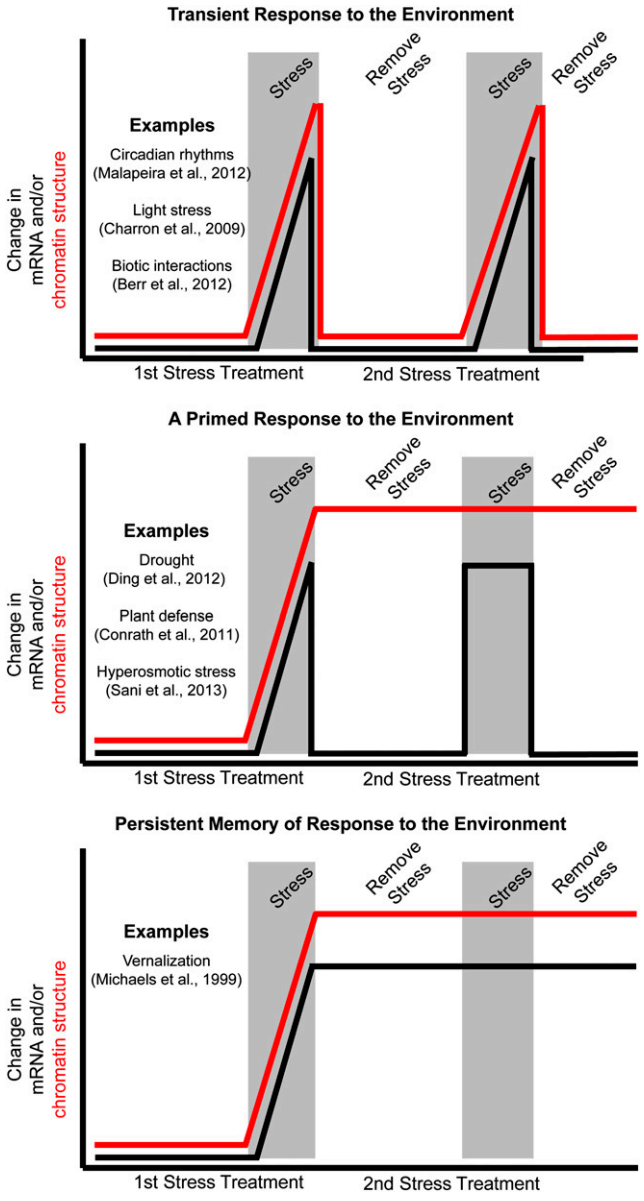


Figure 3. Differential expression and chromatin alterations in responses to the environment. Top, Genes in this class respond transiently and have no memory to the environment by undergoing changes to both the local chromatin state and mRNA levels. Middle, Genes in this class respond to a change in the environment by altering chromatin states and changing mRNA levels. This alteration in chromatin states can persist, which enables a more rapid mRNA response after a subsequent exposure to that environment. Bottom, A memory of the environment is enabled by persistent changes in both gene expression and local chromatin states after exposure to the environment.

competent to flower, even in the absence of the original stimulus (cold temperature). Vernalization is not meiotically heritable, because it resets every generation. Failure to reset the requirement for vernalization would be detrimental, because it would lead plants to flower rapidly before the onset of winter, reducing overall reproductive success.

Another potential example of epigenetic contributions to environmental response is the priming or training response to environmental conditions. Priming refers to changes in phenotype or gene expression after repeated exposures to an environmental stress. A phenotypic response or gene expression change will be trained by initial exposures to stress and will exhibit more pronounced or rapid responses to subsequent treatments of the same environmental condition (Fig. 3). There are examples of priming in response to abiotic (Sung et al., 2003; Ding et al., 2012; Sani et al., 2013) and biotic (Conrath, 2011) environmental cues. It is important to note that the observations of priming are often rooted in phenotypic observations of how responses to subsequent treatments differ from initial treatments. In some cases, the priming response has been associated with alterations in chromatin modifications (Jaskiewicz et al., 2011; Sani et al., 2013) or DNA methylation (Dowen et al., 2012; Yu et al., 2013). However, it is difficult to fully document the role of epigenetics in this phenomenon (Sani et al., 2013). The initial treatment of an environmental stress often results in numerous physiological and morphological changes. These differences may provide a different response to subsequent treatments in the absence of a true epigenetic memory.

EPIGENETICS AND TRANSMISSION OF ENVIRONMENTAL EFFECTS TO OFFSPRING

The section above focused on how plants respond to environmental cues with chromatin changes, including mitotically transmissible changes. In this section, we will evaluate the evidence that environmental exposures of the parents may transmit altered expression states to offspring. It is possible that environmental stresses during the parental generation could promote changes, leading to offspring being more prepared to deal with a similar environment. This may be especially true in plant systems with limited seed dispersal, leading to very similar environmental conditions for both parent and offspring. There are some hints that this might occur (Boyko and Kovalchuk, 2011; Bilichak et al., 2012; Luna et al., 2012; Rasmann et al., 2012; Slaughter et al., 2012), but it is still unclear if this soft inheritance occurs (Pecinka and Mittelsten Scheid, 2012) and how large an impact on phenotype it may have.

There are clear examples of maternal effects in which the environment of the maternal parent can influence seed or seedling traits of the offspring (Galloway, 2005). In many cases, these are most pronounced if the environmental stresses occur during seed development and maturation. However, the observation of maternal effects does not necessarily translate to epigenetics per se. In an extreme case, in which the maternal parent is subjected to severe environmental stress, the seeds will often be smaller and have reduced viability, likely because of direct physiological changes during exposure rather than inheritance of expression states altered by stress. The separation of seed viability or physiology

from true inherited epigenetic changes is complex. Imprinting provides a clear example of epigenetic parental effects that influence the relative expression of the maternal and paternal alleles in offspring endosperm tissue. However, there is little evidence that imprinting is sensitive to environmental conditions.

What are the hints of epigenetic memory of environmental stresses that could be transmitted to offspring? Reports indicate that somatic homologous recombination rates in offspring can be affected by parental environmental exposures (Molinier et al., 2006; Yao and Kovalchuk, 2011; Puchta and Hohn, 2012), but this may not be a highly reproducible response (Pecinka et al., 2009; Ülker et al., 2012). Several recent articles provide evidence for heritable phenotypic changes or chromatin changes during development or in the offspring of plants exposed to biotic stress (Boyko and Kovalchuk, 2011; Dowen et al., 2012; Luna et al., 2012; Rasmann et al., 2012; Slaughter et al., 2012). Groups have also reported heritable changes in DNA methylation or other chromatin modifications in the progeny of plants subjected to abiotic stresses (Verhoeven et al., 2010; Bilichak et al., 2012; Verhoeven and van Gurp, 2012; Colaneri and Jones, 2013). There are also reports that tissue culture, a very severe environmental condition, results in heritable changes in DNA methylation levels (Stroud et al., 2013). Despite these reports, there are still concerns about whether they represent bona fide examples of epigenetics that are induced by the environment (Pecinka and Mittelsten Scheid, 2012).

Parental environmental exposures certainly can affect the phenotype of offspring, but this might have little effect on phenotype. For most agronomic species, there is limited evidence that performing selection using environmental variation as opposed to genetic variation can lead to improved performance. It is clear that using particular environments for selecting ideal genetic combinations has resulted in more locally adapted varieties. However, there is limited evidence that breeders have been successful in performing local adaptation of a variety using repeated growth of materials in a particular environment without allowing for genetic diversity or recombination of diverse alleles. That being said, this fascinating topic deserves additional attention and will likely reveal nuances in how plants adjust to environmental conditions.

A POTENTIAL ROLE FOR EPIGENETICS IN EVOLUTION AND PHENOTYPIC VARIATION WITHIN SPECIES

The above sections focused on the potential for epigenetics and chromatin modifications to contribute to changes in gene expression associated with development or environmental responses. Epigenetics could also contribute to natural variation within species and potentially, local adaptation. Epigenetics could theoretically impact natural variation as a faster acting and less permanent method of gene regulation compared with genetic variation. The potential reversibility of epiallelic states opens the possibility of temporary adaptation or exploration of cryptic genomic information (Richards, 2006, 2011; Weigel and Colot, 2012). In a similar vein, the potential instability of epiallelic states and the requirement for continued active silencing may suggest that longer term evolutionary changes in plants would often use genetics as opposed to epigenetics. This may leave a role for epigenetics in natural variation within a species but suggests that stable differences between species likely involve primarily genetic changes.

There are many examples of natural variation for DNA methylation or other chromatin modifications. The main difficulty in assigning natural variation for chromatin modifications as epigenetic is the problem of separating chromatin changes from genetic changes that also occur between individuals of the same species. There are some well-characterized examples of epigenetic natural variation that may contribute to phenotypic variation within plant species (Fig. 4). In recent years, there has been substantial progress in characterizing natural variation for DNA methylation or other chromatin modifications. However, it has become clear that this variation can be driven by both genetic and epigenetic mechanisms. The development of epigenetic recombinant inbred lines (epiRILs), populations that segregate for variation in DNA methylation patterns with limited genetic variation, has provided a tool for showing the effects of epigenetics on natural variation (Johannes et al., 2009; Reinders et al., 2009).

There are examples in which natural phenotypic variation within a species seems to be regulated by epigenetics (Fig. 4). *Linaria vulgaris*, a snapdragon plant named after Carl Linnaeus, shows natural variation in flower symmetry (Cubas et al., 1999). The *peloric* variant displays radial symmetry and has increased levels of DNA methylation in the promoter and coding region of *Lcyc*. The underlying sequence of the *Lcyc* alleles and surrounding regions is identical, suggesting an epigenetic basis for this phenotype. The symmetry phenotype is heritable through generations, and occasional reversion to wild-type symmetry can occur on branches because of the loss of promoter methylation (Cubas et al., 1999). Similar examples at the *colorless nonripening* locus in tomato (*Solanum lycopersicum*; Manning et al., 2006), the *Clark Kent* alleles of the *SUPERMAN* locus (Jacobsen and Meyerowitz, 1997), and the *qui-quinine starch* locus (Silveira et al., 2013) in Arabidopsis display aberrant DNA methylation patterns with no apparent dependence on changes in genetic sequence. There is also evidence for natural variation for DNA methylation levels at ribosomal DNA (Riddle and Richards, 2002) and some repetitive elements (Rangwala et al., 2006; Rangwala and Richards, 2007) in Arabidopsis; however, it is not clear whether these differences contribute to morphological changes.

Paramutation also provides an example of epiallelic variation within populations (Chandler, 2007; Hollick, 2012). Paramutation describes a directed allelic

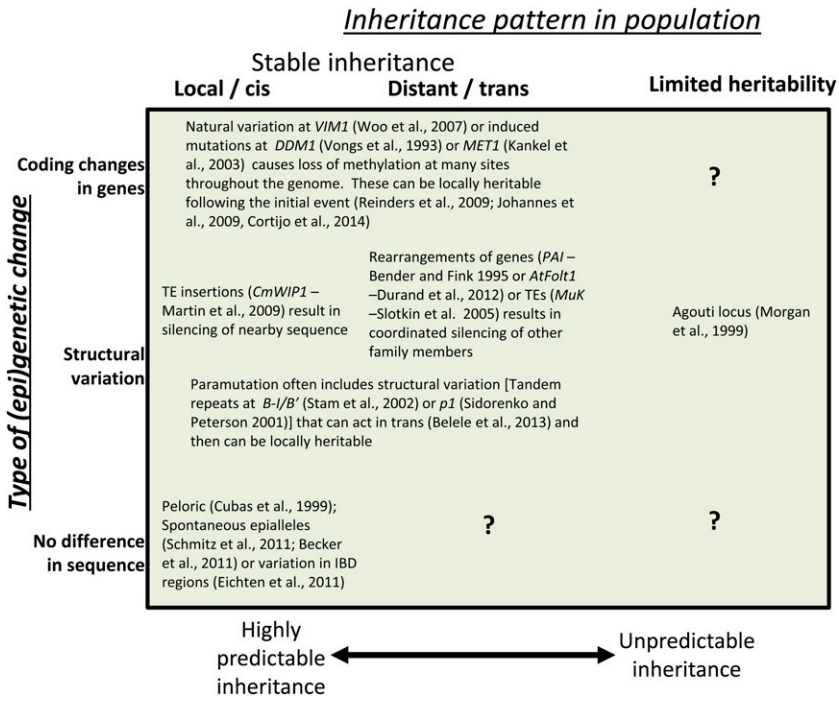


Figure 4. Classification of examples of variable DNA methylation levels in populations. The inheritance patterns for the DNA methylation patterns can include examples of local (cis) inheritance, examples of distant (trans) inheritance, or highly unstable examples that have limited or no heritability. These examples also can be classified based on whether a genetic change is involved and if so, what type of genetic change occurs. PAI, *Phosphoribosylanthranilate isomerase*; TE, transposable element; *VIM1*, *Variant in methylation1*; *CmWIP1*, *Cucumis melo WPP-domain interacting protein1*; *MuK*, *Mu killer*; IBD, identity by descent.

interaction that can occur in heterozygous individuals and results in differences in expression levels without affecting primary sequence (Chandler, 2007; Hollick, 2012). Paramutation can clearly result in epigenetic variation, but only certain alleles at a particular locus are subject to regulation by paramutation (Chandler and Stam, 2004), which may reflect the need for certain genetic elements to create an environment that is permissive for a role of epigenetics for the regulation of facilitated epialleles. Similarly, there are many examples of potential chromatin-based regulation that may be linked to genetic changes, such as transposon insertions (Kinoshita et al., 2007; Saze and Kakutani, 2007; Martin et al., 2009; Robbins et al., 2009).

Technological advances have provided the opportunity to study genome-wide changes in DNA methylation (Schmitz and Ecker, 2012), histone modifications (Dong et al., 2012; Makarevitch et al., 2013), or small RNAs (Zhai et al., 2008; Shivaprasad et al., 2012) in different individuals of the same species. Genome-wide studies of DNA methylation have provided evidence for natural variation in DNA methylation patterns (Vaughn et al., 2007; Eichten et al., 2011, 2013b; Regulski et al., 2013; Schmitz et al., 2013a, 2013b). Each of these studies identified numerous examples of differentially methylated regions between different individuals of the same species. However, identification of a differentially methylated region does not simply imply that this variation is epigenetic in nature, because genetic variation can lead to variation in DNA methylation, which is seen from an obligate epiallele within the *phosphoribosylanthranilate isomerase* (*PAI*) gene family in Arabidopsis (Bender and Fink, 1995). Some accessions of Arabidopsis have an inverted repeat of the *PAI1* locus that leads to the production of small RNAs and silencing of the entire gene family by small RNAs that direct DNA methylation (Melquist and Bender, 2004). Variation for transposable elements may contribute to DNA methylation of nearby low-copy sequences (Hollister and Gaut, 2009; Eichten et al., 2012). The effects of genetic changes on DNA methylation state can be confounded by examples of DNA methylation variants that may have arisen as a result of a genetic variant but are no longer dependent on that genetic variant for their maintenance over generational time. This is exemplified by the *AtFOLT* copy number variants of Arabidopsis (Durand et al., 2012). *AtFOLT1* exists as a single copy locus in some Arabidopsis accessions, but a complex rearrangement and duplication of this locus, *AtFOLT2*, exists in other accessions. The nature of this duplication leads to silencing by small RNAs and DNA methylation of the *AtFOLT1* locus. Therefore, segregating populations from two parents that differ in the *AtFOLT* copy numbers results in rare individuals that contain only the silenced *AtFOLT1* locus. Even more fascinating is that this silenced locus can be maintained over at least six generations in the absence of the inducing trigger (the complex rearrangement and duplication). This finding suggests that the DNA methylation patterns present in one individual in a population may reflect both the exposures to other alleles that occurred in past generations.

Although much of the natural variation in DNA methylation in plant populations may reflect contributions of genetic variation, evidence indicates that at least a portion of this variation reflects pure or facilitated epialleles. Identification of pure or facilitated epialleles is

challenging, because any dependence on genotype needs to be ruled out as a direct causal factor. This is especially difficult in plant populations that contain abundant genetic variation. Therefore, the strongest evidence for pure epialleles has arisen in controlled plant populations with known pedigrees and limited genetic variation. Recent studies in Arabidopsis and maize that combined epigenomic profiling methods with populations of plants that were derived from multiple generations of successive growth revealed evidence for these pure epialleles (Becker et al., 2011; Eichten et al., 2011; Schmitz et al., 2011, 2013b). In these experiments, the variation in genotype for whole genomes or specific regions of the genome was reduced to spontaneous mutations. The frequency at which these epialleles naturally appear is greater than the known spontaneous genetic mutation rate, indicating their independence from genotype. Moreover, one of the identified epialleles from Arabidopsis reverted to the wild-type methylation state after one additional generation of growth (Becker et al., 2011). These pure epialleles may arise within inbred populations and contribute to spontaneous variation (Havecker et al., 2012).

Epialleles have the potential to exhibit unexpected patterns of inheritance, such as paramutation or high levels of instability (Cubas et al., 1999; Becker et al., 2011; Schmitz et al., 2011). Several recent studies have attempted to document the patterns of inheritance for differential methylation using association mapping with natural populations (Eichten et al., 2013b; Schmitz et al., 2013a) or biparental populations (Eichten et al., 2013b, Regulski et al., 2013; Schmitz et al., 2013b). Although a minority of the loci exhibits patterns that would suggest paramutation-like patterns or unstable inheritance (Greaves et al., 2014), the majority of DNA methylation variation seems to be under local (cis) control and inherited in a relatively faithful manner (Schmitz et al., 2013a). In some cases, the inheritance of DNA methylation patterns is locally controlled, even in the absence of DNA sequence variation (Eichten et al., 2011), providing evidence for stable inheritance of pure epialleles.

The creation of epiRILs provides another tool for studying the potential contribution of epigenetics to natural variation (Johannes et al., 2009; Reinders et al., 2009). Populations of Arabidopsis were generated that contained perturbed epigenomes, but genetic variation was largely held constant. These populations segregate for genomic regions that have been stripped of DNA methylation by passage through a mutant background (*decrease in DNA methylation1* [*ddm1*] or *met1*). The resulting lines segregate for altered DNA methylation state as well as some novel sequence changes caused by the reactivation of some transposon families in these mutant backgrounds (Miura et al., 2001; Singer et al., 2001; Kato et al., 2004). The analysis of the epiRILs reveals evidence for phenotypic variation within these populations (Johannes et al., 2009; Reinders et al., 2009; Roux et al., 2011; Latzel et al., 2012, 2013; Zhang et al., 2013). This result suggests that the loss of DNA methylation releases cryptic information in the Arabidopsis genome, and the segregation for these changes can impact morphology. To support this hypothesis, differentially methylated regions (DMRs) in the epiRILs were recently identified, and a subset of DMRs was used as markers to identify quantitative trait loci associated with phenotypic variation. The results indicated that the altered DNA methylation levels in the epiRILs lead to phenotypic changes (Cortijo et al., 2014). The phenotypic variation produced by the epiRILs resembles that found in natural strains of Arabidopsis (Roux et al., 2011), and therefore, much of the variation captured in epiRILs may also be segregating in natural plant populations that have not had intentional perturbation of epiallelic states. In fact, many of the DMRs identified in the epiRILs overlap with DMRs present in natural strains (Cortijo et al., 2014). Although not all variation observed in epiRILs is fully epigenetic, they are powerful in that they reveal the potential for epigenetic variation to affect phenotypic variation in plant genomes.

Understanding the role of epialleles in evolution is still in its infancy (Finnegan, 2002; Rapp and Wendel, 2005). Given that there are examples of pure epialleles that affect phenotype, it should be possible for natural selection to act on epigenetic variation and drive changes in epiallele frequency within populations. However, unresolved questions remain. First, given the overall longer periods of time that are relevant for evolutionary change among species, it is likely that potentially unstable inheritance of epialleles could be supplanted by genetic variation. If it is advantageous to silence expression of a gene throughout development, it is likely that, over time, mutations will arise that will abolish function of that gene. Second, a major question about the potential role of epigenetics in evolutionary processes is whether the environment influences the rate and nature of epiallelic variation. The evidence for directed, stable, meiotically heritable epialleles induced by environmental conditions is somewhat rare and may actually be unexpected. If epigenetic information can be influenced by directional environmental conditions, it might be expected that it could fluctuate back if the environment changes again. These types of changes would be unlikely to provide long-term stability that would contribute to differences between species but instead, might contribute to shorter term evolutionary processes, such as local adaptation. However, the potential for environmental stress to increase the rate of epigenetic variation may provide an alternative role for epigenetics in evolution. If environmental stress results in frequent alterations in chromatin modifications that are then heritable, it could provide a source of increased morphological variation that could be subject to natural selection. Depending on the stability of these changes, they would have the potential to contribute to the origin of novel epialleles that could be driven to fixation.

Epigenetics may also contribute to evolutionary processes that occur in the formation of polyploids or the accommodation of transposons. There is evidence

that the combination of genomes in polyploids can be accompanied by changes in chromatin modifications (Chen, 2007; Doyle et al., 2008; Ng et al., 2012; Hegarty et al., 2013; Madlung and Wendel, 2013). These novel chromatin changes may provide the variation needed to identify individuals that can balance the contributions of the different genomes and may be necessary for the successful stabilization of polyploids. There is abundant evidence that many epigenetic processes affect transposons. Many epigenetic mechanisms silence transposons and prevent their proliferation (Slotkin and Martienssen, 2007; Lisch, 2009; Bucher et al., 2012). Evolutionary pressures have likely driven a balance between silencing of transposons and allowing for expression of nearby genes (Hollister and Gaut, 2009; Tenaillon et al., 2010; Levin and Moran, 2011; Lisch and Bennetzen, 2011).

CONCLUSION

In this article, we considered the role for chromatin modification and epigenetics in contributing to various plant processes. The distinction between chromatin modifications and epigenetics is useful, because it allows the term epigenetics to connote stable transmission or inheritance of information. Although by our more restricted definition, chromatin-based regulation of gene expression is not necessarily epigenetic, we do not wish to diminish the importance of this fascinating type of gene regulation. A careful use of these terms can help delineate the mechanisms for gene regulation versus the type of inheritance for gene regulation. Epigenetics has become a widely used term, and the word is in some danger of losing any real meaning if it is applied to too many distinct types of concepts. Although there are clear examples for a role for epigenetics in plant evolution, development, response to the environment, and parental effects, there are also many other interesting mechanisms that contribute to these processes. We have attempted to highlight the potential role for both epigenetics and chromatin-based processes in contributing to each of these areas of plant research and hope that we have conveyed our excitement about the future of these research areas.

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