Paramutation and Development

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Abstract
Paramutation describes a heritable change of gene expression that is brought about through interactions between homologous chromosomes. Genetic analyses in plants and, more recently, in mouse indicate that genomic sequences related to transcriptional control and molecules related to small RNA biology are necessary for specific examples of paramutation. Some of the molecules identified in maize are also required for normal plant development. These observations indicate a functional relationship between the nuclear mechanisms responsible for paramutation and modes of developmental gene control.
INTRODUCTION

The induction and persistence of changes to gene regulation are fundamental events of both paramutation and developmental genetics (Brink 1960). Although the term paramutation has evolved to specifically represent meiotically heritable changes in gene regulation that are induced by homolog interactions (Chandler & Alleman 2008), Brink (1962) originally pointed to its conceptual parallels with mitotically heritable regulatory states that are coincidently induced at developmental transitions, such as the onset of the flowering phase in plants. The proposal that such contemporary views of paramutation requiring meiotic inheritance represent somehow aberrant examples of events taking place during normal development (Brink 1960, 1962) can begin to be evaluated in light of the recognition that common molecular components are required for both paramutation and development in Zea mays (Dorweiler et al. 2000, Parkinson et al. 2007). These molecular connections indicate that contemporary examples of paramutation may provide paradigms for understanding developmental gene control; the roles of transcription and RNA figure prominently in this regard.
parachromatin as Brink (1960) and Jorgensen (1994) have referred to it, that serve to define the heritable “genetic factor” persist. That examples of paramutation present only apparent exceptions to the basic tenets of genetics in no way diminishes their impact on the frameworks of quantitative and evolutionary genetics.

Paramutations can occur spontaneously among paramutable haplotypes without any obvious inductive event, or they can be brought about through interactions between paramutable and paramutagenic haplotypes. These events are most simply recognized and studied when the affected haplotype affects a visible trait such as plant color. This is one reason why specific haplotypes of the plant color1/booster1 (b1) and purple plant1 (Pl1) loci that encode transcriptional regulators of the anthocyanin biosynthetic pathways (Dooner et al. 1991) have been so useful for understanding the paramutation process. Maize geneticists have selected and maintained lines of strongly pigmented “reference” types since the first purple-colored corn plant came into culture in 1906 (Emerson 1921). Plants remain dark purple if they are homozygous for both highly expressed and paramutagenic states (Coe 1966, Hollick et al. 1995). The highly expressed and paramutagenic versions are termed B′ and P′, respectively (Figure 2). All the offspring derived from lightly pigmented (P'/P' or B′/B′) and darkly pigmented (Pl-Rh/Pl-Rh or B-I/B-I) parents are lightly pigmented and only transmit paramutagenic P' or B' versions to subsequent progeny (Figure 3b; Coe 1966, Hollick et al. 1995). This type of paramutation is often referred to as “induced,” a term that implies some type of required function from the paramutagenic haplotype. The term “facilitated” may be more appropriate because paramutable haplotypes have the innate ability to spontaneously change their heritable “genetic factor” persist. That exceptions to the basic tenets of genetics in no way diminishes their impact on the frameworks of quantitative and evolutionary genetics.

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As Coe (1968) pointed out, the time during development when induced paramutation occurs is conceptually constrained by the sectors having notably less pigment (Figure 3a) (Coe 1966, Hollick et al. 1995). Although Coe (1966) showed that gametes formed from somatic tissues with such reduced pigmentation transmitted B1-I haplotypes of similar pigmentation potential, he argued that the heritable changes that typify paramutation might actually be confined to the stages of meiosis (Coe 1961, 1966, 1968). Coe’s interpretation arose, in part, from his observations that most spontaneous paramutations at B1-I appeared to occur as separate events in germinal tissues. By crossing darkly colored females from lineages that typically gave a high frequency of weakly colored progeny by darkly colored males from lineages in which the high-expression reference state was stable, Coe (1961) found that the ear positions of particular kernels that gave rise to weakly colored progeny were inconsistent with any somatic clonal relationship (Figure 4).

Detailed pedigree analyses tracking linked genetic markers show that both the B1-I and Pl-Rh haplotypes can exist in either paramutable or paramutagenic states (Coe 1966, Hollick et al. 1995). The highly expressed and paramutable versions are referred to as B-I and Pl-Rh and, consistent with Brink’s nomenclature, the weakly expressed and paramutagenic versions are termed B′ and P′, respectively (Figure 2). All the offspring derived from lightly pigmented (P'/P' or B′/B′) and darkly pigmented (Pl-Rh/Pl-Rh or B-I/B-I) parents are lightly pigmented and only transmit paramutagenic P' or B' versions to subsequent progeny (Figure 3b; Coe 1966, Hollick et al. 1995). This type of paramutation is often referred to as “induced,” a term that implies some type of required function from the paramutagenic haplotype. The term “facilitated” may be more appropriate because paramutable haplotypes have the innate ability to spontaneously change their own (Styles & Brink 1969). Paramutagenicity might reflect the loss of, rather than the acquisition of, some activity, and thus the term “facilitated” accommodates both models.
Figure 2

Paramutable and paramutant plant phenotypes. Arrows indicate the color phenotypes displayed in male flowers (a-c) and leaves (d-f) of W23 inbred maize plants that are homozygous for the plant color1 booster1-Intense (B1-I) and purple plant1-Rhoades (Pl1-Rh) haplotypes of the indicated paramutable (a and d) or paramutant (b, c, e, f) states. Insets (a–c) highlight the specified anther types. The combined actions of the B and PL proteins direct plant color, whereas PL and the R protein encoded by the red color1 locus determine anther color (Dooner et al. 1991).

Meiotic transmission assay. Pl-Rh/Pl genotypes appear to become Pl'/Pl early because seedling tissues of such heterozygotes are already lightly pigmented (Figure 3h; Hollick et al. 1995). However, the pigmentation of clonal somatic sectors in which the chromosome arm carrying the Pl' version is lost at various points during development indicate that the mitotically heritable change of Pl-Rh to Pl' occurs progressively through development (J.B. Hollick, unpublished data). Similar analyses from Coe (1966) indicate that B-I remains largely unchanged throughout the somatic development of B-I/B' heterozygotes. Because the true measure of whether or not a paramutation has occurred requires the evaluation of a sexually transmitted
haplotype, it remains possible that apparent somatic occurrences (i.e., weak pigment phenotypes of PI-Rh/PP′ heterozygotes) reflect a predisposing form of repression in trans that is made permanent (i.e., PI-Rh′ changing to PP′) during meiosis or haploid gametophyte development (Coe 1966). Consistent with this idea, Gross & Hollick (2007) found that the strongly pigmenting pl1 haplotype from the W22 inbred line was suppressed in pl1-W22/PlP′ plants but that no heritable changes to the pl1-W22 haplotype were transmissible (Figure 3c).

Recent mutant analyses (see below) also comport with the concept that the mechanism(s) responsible for somatic repression may be distinct from those required for ensuring the sexual transmission of paramutagenic states (Stonaker et al. 2009).

Based on the behaviors outlined above, open pollinated populations face extinction of the B-I and PI-Rh versions of the B1-I and Pl1-Rh haplotypes. Because the B′ state has never been observed to revert to a B-I reference state (Chandler & Alleman 2008), strong human selection is proposed to explain its persistence. How the B-I reference state came into culture originally remains enigmatic. The PP′ state can, however, revert to PI-Rh when carried in a hemizygous condition or when heterozygous with other pl1 haplotypes resident in various inbred lines (Figure 3d) (Gross & Hollick 2007, Hollick & Chandler 1998). Using pedigree analyses, Gross & Hollick (2007) found that certain pl1 haplotypes were either “neutral” in the sense that PP′ remained unchanged in PP′/pl1 heterozygotes (Figure 3c), or they were “amorphic” (similar to a deficiency) in allowing PI-Rh states to be transmitted from PI′/pl1 plants (Figure 3d). These results, together with earlier work at the red color 1 (r1) locus from Styles & Brink (1969), indicate that some high expression reference states persist by historical conditioning with specific haplotypes. The corollary can be inferred: Some form of homolog communication is important to maintaining certain paramutant states.

Despite the definitive types of inheritance that characterize paramutations as genetic changes (Brink 1956), paramutations are epigenetic in nature; they can be as reversible as imprinting marks, although such reversions are not necessarily tied to the sexual cycle. The term “epigenetic landscape” was applied by Waddington (1942) to help conceptualize the

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**Figure 3**

Inheritance, expression, and transmission behaviors of specific purple plant1 (pl1) haplotypes. Haplotype designations listed on the top and bottom lines represent their status in parental and progeny gametes, respectively. Color intensity (dark and light purple) represents phenotypic expression of the indicated haplotype. (a) Two examples of spontaneous paramutation are presented in which the timing of the change from PI-Rh to PP′ occurs either at meiosis or during somatic development. (b) Induction of paramutation in PI-Rh/PP′ heterozygotes takes place during somatic development, as indicated by the change from PI-Rh to PP′. (c) Trans-repression of the pl1-W22 haplotype occurs in pl1-W22/PlP′ heterozygotes, as indicated by the light purple text, yet pl1-W22 is transmitted unchanged. (d) Reversion of PP′ to PI-Rh states occurs when PP′ is heterozygous with a deficiency (−) or with other pl1 alleles that are referred to as “amorphic.”

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**Epigenetic:** pertaining to mitotically and/or meiotically heritable information that is not DNA in nature.
Figure 4

Spontaneous paramutation occurring on B1-I/B1-I cobs. Illustrations contrast (a) clonal and (b) non-clonal patterns of kernels arising on darkly-colored B1-I/B1-I plants that produce weakly-colored progeny (shaded kernels).

Canalization: the concept that persistent expression patterns of developmental regulators during ontogeny are buffered from environmental and genetic variations.

RDR2: a specific RNA-dependent RNA polymerase in plants responsible for accumulation of 24-nt small interfering RNAs.

behavior of developmental canalization. The apparent uniformity of organismal ontogeny in the face of environmental and genetic variation indicates that the expression of genes controlling development is, to some extent, buffered or canalized (Waddington 1942). The fidelity of replicating epigenetic information concerned with gene regulation through clonal lineages is expected to mirror the extent to which orderly development proceeds. In plants and animals, both chromatin modifications and DNA methylation have emerged as paradigms for such replicative epigenetic information (see Probst et al. 2009 for a review). Particularly transformative to this discussion, however, have been the more recent recognitions that transcription and RNA molecules are integral components of maintaining such epigenetic information systems (Moazed 2009, Zaratiegui et al. 2007). It is to this RNA component that the induction of paramutations in mouse together with mutant analyses in maize now point.

RNA AND PARAMUTATION

Reports from the Cuzin and Chandler groups in 2006 began to implicate a role for RNA in paramutation. In *Mus musculus*, Rassoulzadegan et al. (2006) obtained apparently paramutant haplotypes of the *kit* gene that conferred less hair pigment than normal by microinjecting various RNA preparations into fertilized eggs. Pedigree analysis showed that a particular *kit* knock-out transgene induced a paramutant state to the endogenous *kit* allele. Offspring lacking pigment at the ends of their tails persisted over successive generations in the absence of the inducing transgene in a manner reminiscent of paramutation. Remarkably, mice derived from eggs microinjected either with RNAs isolated from sperm or brain of mice carrying the *kit* transgene or with microRNAs (miRNAs) with potential targeting of the *kit* transcript also gave rise to offspring exhibiting reduced *kit* function (white-tipped tails). The results implied that maternal transmission of RNA molecules by themselves could deliver specific epigenetic information. Brennecke et al. (2008) recently drew similar conclusions regarding maternal transmission of small RNA molecules, the so-called piwi- or piRNAs (Klattenhoff & Theurkauf 2008), in *Drosophila melanogaster*.

In maize, Alleman et al. (2006) and Woodhouse et al. (2006b) identified an RNA-dependent RNA polymerase (RdRP) as essential for the paramutation process. This particular RdRP, which is related to RNA-DEPENDENT RNA POLYMERASE2 (RDR2) in *Arabidopsis thaliana*, provided a model for the persistence of double-stranded RNAs and their ribonuclease III-derived small RNAs. The maize RDR2 is required for both nonpolyadenylated sense and antisense RNAs representing certain long
terminal repeat (LTR) retrotransposons (Hale et al. 2009) as well as a predominant 24 nucleotide (nt) size class of RNAs known as small interfering RNAs or siRNAs (Nobuta et al. 2008). The siRNA signatures from both A. thaliana and maize floral tissues primarily correspond to repetitive features such as transposons (Kasschau et al. 2007, Lu et al. 2006, Nobuta et al. 2008). Thus much of the plant genome—approximately 85% of the maize genome being composed of repetitious elements (Schnable et al. 2009)—can be represented by double-stranded and si-type RNAs at certain phases of the life cycle via RDR2 action. Because the presumed RDR2 enzymology requires both an RNA template and an siRNA primer, direct repeats provide the ideal arrangement for facilitating a self-perpetuating source of RDR2-dependent siRNAs (Baulcombe 2006, Martienssen 2003).

RDR2 is one upstream component of what has been described as an RNA-directed DNA methylation (RdDM) pathway (see Matzke et al. 2009 for a review). siRNAs produced from hairpin RNAs, sense/antisense RNA pairs, or RDR2 action can direct specific methyltransferase activities to complementary DNA sequences via potential base-pairing interactions with nascent RNA transcripts (Matzke et al. 2009, Wierzbicki et al. 2008). This pathway maintains de novo methylation patterns of cytosines found in the CHH (H representing any base but guanine) context, which are presumably independent of the mechanisms effecting maintenance methylation patterns of cytosines found in symmetrical CG and CHG arrangements. In A. thaliana, CHH methylation of transposons and noncoding intergenic sequences requires the actions of an RNA polymerase II (Pol II)-related helozenzyme complex known as RNA polymerase V (Pol V) together with a RAD54-type ATPase known as DRD1 (DEFECTIVE IN RNA-DIRECTED DNA METHYLATION1) (Herr et al. 2005; Kanno et al. 2004, 2005; Matzke et al. 2009; Onodera et al. 2005; Pontier et al. 2005). Wierzbicki et al. (2008) recently showed that Pol V and DRD1 together produce low levels of non-polyadenylated RNAs from noncoding regions that potentially serve as a tether for CHH-specific DNA methyltransferases. In a follow-up study, Wierzbicki et al. (2009) showed that recruitment of the ARGONAUTE4 (AGO4) protein, which serves as the siRNA chaperone (Qi et al. 2006) and presumed adaptor for the methyltransferase, is dependent on both functional Pol V and DRD1. Figure 5 provides a schematic view of the generic RdDM pathway currently believed to operate in both maize and A. thaliana.

RdDM in plants and the so-called heterochromatin maintenance mechanism in Schizosaccharomyces pombe both involve cotranscriptional processes involving nascent RNAs. Guided by base-pairing interactions with siRNAs bound to Argonaute1 (Ago1p), the RNA-induced transcriptional silencing (RITS) complex of S. pombe is recruited to nascent Pol II RNAs and causes Ago1p-based endonucleolytic cleavage (slicing) of complementary RNA duplexes (Buhler et al. 2006, Irvine et al. 2006).
These same siRNAs bound to Ago1p may also serve as primers for an RdRP [found in a RDR complex (RDRC)] to generate dsRNA for subsequent dicing by the ribonuclease III Dicer1 to produce more siRNA (Motamedi et al. 2008). Such a cotranscriptional gene silencing model was proposed to account for the apparent cis-restricted degradation of nascent transcripts from the reporter allele being assayed (Buhler et al. 2006). Thus in both S. pombe and A. thaliana, the transcription process itself appears integral to the mechanism(s) maintaining certain repressed epigenetic states.

miRNAs represent another class of small noncoding RNA that, through binding complementary sequences, ensure developmentally directed expression patterns of specific genes. Results from Bao et al. (2004) suggested that miRNAs also function to direct cytosine methylation at genomic targets. They found miRNA-dependent cytosine methylation at 3′ of the miR165/166 complementary site of the A. thaliana PHABULOSA (PHB) and PHAVOLUTA (PHV) genes, which each encode a class III homeodomain-leucine zipper (HD-ZIPIII) transcription factor. The miR165/166 complementarities in both PHB and PHV span introns, and dominant mutations that disrupt splicing of these introns (and therefore the miR165/166 contiguous complementarity) are associated with hypomethylation of the PHB and PHV coding regions. Hypomethylation associated with the dominant phb-1d mutation occurred in cis, which implicates that the miRNA interacts with the nascent RNA transcript to direct methylation to the PHB gene.

The identification of maize RDR2 from mutant screens (Dorweiler et al. 2000, Hollick & Chandler 2001) indicated that RdDM-type models account for the interhomolog communication required to induce paramutations. Paramutagenic haplotypes would produce siRNAs from RDR2-dependent dsRNAs, and these would act as effectors in recruiting DNA methyltransferases to homologous sequences on paramutable haplotypes. Assuming that such RdDM events induced the production of additional RDR2 templates, the general conceptual framework of paramutation occurring in maize was neatly accounted for. In support of this model, Stam et al. (2002a,b) had found that the tandem repeats of an 853 bp noncoding sequence found ∼100 kb 5′ were essential for paramutagenicity of the B1-I haplotype. Run-on transcription assays reported by Alleman et al. (2006) indicated that RNAs derived from these tandem repeats are synthesized in both sense and antisense orientations. It remains unreported, however, whether DNA-dependent transcription of these repeats is affected in the rdr2 mutants or whether any differential abundances of small RNA signatures representing these repeats reflect a paramutable B-I or paramutagenic B′ status.

Additional molecular components found to be required to maintain repressed paramutant states appeared to reinforce a role for RNA in maize paramutation. Mutant screens and positional cloning led Erhard et al. (2009), Stonaker et al. (2009), and Sidorenko et al. (2009) to identify both catalytic subunits of another Pol II-related holoenzyme similar to Pol IV in A. thaliana. Hale et al. (2007) similarly identified a DRD1-class ATPase that was named Required to Maintain Repression1 (RMR1). Similar to RDR2, RMR1 and the maize Pol IV subunits are responsible for the accumulation of 24 nt siRNAs representing repetitious features including both Type I and II transposons (Erhard et al. 2009, Hale et al. 2009, Stonaker et al. 2009). Whereas these findings appeared to support an RNA-induction model, Hale et al. (2007) used pedigree analysis to show that paramutation was still induced at the Pl1-Rh haplotype in the absence of RMR1 function. This result indicated that the siRNAs themselves are not an inductive substance responsible for establishing a meiotically heritable paramutant state. Similar genetic analyses, however, showed that the large subunit of Pol IV (RPD1) was essential for facilitating paramutation at Pl1-Rh (Hollick et al. 2005), and this led to models in which transcription rather than RNA per se might play a role (Erhard et al. 2009).
TRANSCRIPTION AND PARAMUTATION

Relative transcription rate measurements using radiolabeled ribonucleotide incorporation with isolated nuclei indicate that paramutation affects gene regulation at the transcriptional level (Hollick et al. 2000, Patterson et al. 1993, Sidorenko & Chandler 2008). Transcription rates are decreased but not eliminated, unlike some examples of transcriptional gene silencing. At B1-I, both regions within the bI gene and near the distant 5′ repeats also display a relatively nuclease-insensitive profile coincident with the reduced transcription rate of the paramutant B′ state (Chandler et al. 2000, Stam et al. 2002a). These results indicate that paramutation results in heritable alterations of chromatin structure that affect transcription. At Pl1-Rh, comparisons of pl1 RNA abundance using RNAse protections and corresponding transcription rates in different mutant backgrounds indicated that both transcriptional and posttranscriptional levels of regulation are affected. Hollick et al. (2005) found through mutational analyses that the large subunit of Pol IV was essential for maintaining transcriptional repression at Pl1-Rh, but Hale et al. (2007) showed that RMR1 only affects pl1 RNA stability. Paramutation occurring at the Pl1-Rh haplotype therefore results not only in transcriptional repression, but the type of RNA produced appears to be unaltered. This is conceptually similar to the cotranscriptional repression previously supported in S. pombe (Buhler et al. 2007, Moazed 2009) and A. thaliana RdDM in which transcription via Pol V is important to inducing and maintaining a repressed epigenetic state (Wierzbicki et al. 2008).

The potential importance of transcription to paramutation is further implied by studies in which enhancers and promoters appear to be functionally important for paramutagenicity. At the paramutable R-r:standard (R-r:std) haplotype, the two affected rI genes are arranged in inverted orientation centered on a small ∼400 bp promoter region (Figure 6). Internal deletions of this promoter region attenuate the ability of the haplotype to become paramutagenic, and larger deletions encompassing the 5′ untranslated regions abolish this ability completely (Kermicle 1996, Walker 1998). Ethylmethane sulfonate (EMS)-induced loss-of-function derivatives of Pl1-Rh affecting the PL1 protein can still become paramutagenic, but transposon- and irradiation-induced mutant derivatives failing to produce pl1 RNA cannot (Gross & Hollick 2007). Transgenic constructs containing just a single 1.2 kb transcriptional enhancer element of the pericarp1 Pl1-rr haplotype can become paramutagenic (Sidorenko & Chandler 2008, Sidorenko & Peterson 2001). Stam et al. (2002a,b) found recombinant derivatives of the B1-I haplotype in which the number of upstream tandem repeats affected both paramutagenicity and enhancer activity (Figure 6). Derivatives with a single upstream repeat have reduced bl gene expression and are nonparamutagenic, whereas those with three repeats confer moderate pigmentation and are weakly paramutagenic. These studies combined implicate a requirement for transcription of the affected coding regions themselves.

At both the R1-stippled (Rst) and R1-marbled (Rmb) haplotypes, it is the number of repeated r1 gene coding regions and large intervening regions that dictate the degree of paramutagenicity (Figure 6). Because these two r1 haplotypes are comprised of tandem direct duplications, derivatives with fewer repeats are obtained through recombination of misaligned homologs (Eggleston et al. 1995, Kermicle et al. 1995, Panavas et al. 1999). Both Kermicle et al. (1995) and Panavas et al. (1999) showed that such Rst and Rmb derivatives are quantitatively distinct in regards to r1 gene number and paramutagenicity. Kermicle et al. (1995) also used trisomics to change the number of r1 genes in the cell. The conclusion of these analyses was that no single r1 gene and intervening region were responsible for paramutagenicity; the ability to facilitate paramutation was quantitatively dependent on the total number of these large repeats regardless of their cis or trans arrangement. It remains unclear whether this correlation relates to the repeated nature
Figure 6

Structural features associated with paramutagenicity. Specific haplotypes and their deletion derivatives are illustrated (blue lines). Individual genic regions are indicated with gray arrows, and variable distances of intergenic sequences are represented with dotted blue lines (not to scale). The heptad repeat in B1-Intense is indicated with small purple arrows. Haplotype organizations and paramutagenicity assessments are presented for (a) R-stippled (Kermicle et al. 1995), (b) R-r:standard (Brink 1956, Brown 1966, Kermicle 1996, Walker et al. 1995), and (c) B1-Intense (Stam et al. 2002a).

per se or to the presumed differences in either r1 gene transcription or the resulting RNA.

The somewhat paradoxical requirement for transcription to maintain a repressed epigenetic state appears to be a recurring finding in the eukarya. In S. pombe, Volpe et al. (2002) first illustrated that repression of centromeric regions via RNA interference machinery required transcription of the centromeric repeats themselves. Kato et al. (2005) and Schramke et al. (2005) showed that repression was disrupted by mutations in the two catalytic subunits of Pol II, and Djupedal et al. (2005) made a similar discovery for the Pol II accessory subunit Rpb7. Just as
nascent Pol V transcripts in plants are required for the recruitment of specific DNA methyltransferases (Wierzbicki et al. 2009), nascent Pol II transcripts provide a scaffold for siRNA-targeted slicing via Argonaute proteins, RdRP-based siRNA amplifications, and recruitment of histone modifying factors (see Moazed 2009 for a review; Buhler et al. 2006). Thus the combination of nascent RNA transcripts, regardless of the polymerase, together with Argonaute-bound small RNAs have the potential to maintain repression through these cotranscriptional processes (Locke & Martienssen 2006).

ROLE OF RNA POLYMERASE IV

In maize, mutational analyses show that at least one Pol IV subunit is essential for both paramutation (Hollick et al. 2005) and normal plant development (Erhard et al. 2009, Parkinson et al. 2007). Phylogenomic analyses of both Luo & Hall (2007) and Erhard et al. (2009) indicate that all multicellular plants have Pol II-related DNA-dependent RNA polymerases (RNAPs) in addition to the ubiquitous eukaryotic RNAPs Pol I, Pol II and Pol III. The genetic analyses of Kanno et al. (2005) and Pontier et al. (2005) along with the biochemical analyses of Onodera et al. (2005) and mass spectrometry data of Ream et al. (2009) show that the alternate RNAPs in *A. thaliana* termed Pol IV and Pol V share subunits, but are distinguished by unique largest subunits. The biological roles of Pol IV and Pol V remain somewhat enigmatic in *A. thaliana* as the two complexes are largely dispensable for normal growth and development (see Pikaard et al. 2008 for a review). Deep sequencing of small RNA libraries from mixed-stage flowers indicates that Pol IV is required for the production of most siRNAs and that Pol V reproduces only a subset of those (Mosher et al. 2008). How the largest subunit of maize Pol IV affects both paramutation and transcriptional control (Cramer et al. 2008). The “G” domain that was recently shown to be required for α-amanitin sensitivity and nucleotide selectivity (Kaplan et al. 2008) is also highly divergent (Erhard et al. 2009). Although any RPD1-dependent RNA synthesis activity has so far remained undetectable (Erhard et al. 2009, Pikaard et al. 2008), Haag et al. (2009) used transgenic complementation tests to show that amino acid residues of the catalytic core are essential to siRNA generation. These results together suggest that Pol IV has very low synthetic capacity. Based on ultradepth sequencing of Pol IV-dependent siRNA, the inferred templates represent repetitive genomic features (Mosher et al. 2008, Zhang et al. 2007).

All flowering plants appear to have further derivatives of RPD1 and RPD2 known as RPE1 and RPE2 (Luo & Hall 2007) that correspond to Pol V. In *A. thaliana*, the one functional RPD2 (known as NRPD2a or NRPD2/E2) is shared between Pol IV and Pol V whereas in maize, the *required to maintain repression?/mediator of paramutation2 (rmr7/mop2)* locus encodes one (RPD2a) of three, presumably functional, RPD2-like genes (Sidorenko et al. 2009, Stonaker et al. 2009). Recent mass spectroscopy analyses of purified Pol IV (*A. thaliana* callus) and Pol V (cauliflower heads and *A. thaliana* callus) complexes show that additional divergence, and presumed specialization, has occurred among other Pol II subunits (Huang et al. 2008, Ream et al. 2009). Relative to the Pol II subunit profile from the same callus source, Pol IV and Pol V share 7 of 12 subunits with Pol II, but Pol IV and Pol V share a unique RPD4/E4 subunit, Pol IV has its own RPD7, and Pol V has specific RPE7 and RPE5. Three other RPB5-related genes
in the *A. thaliana* genome noted by Ream et al. (2009) indicate further complexity. Whether or not this diversity of RPD2-like subunits in maize and RPB5-related subunits in *A. thaliana* predicts the existence of distinct functional holoenzymes remains to be seen. However, recent comparisons between maize plants deficient for either RPD1 or RPD2a hint that this may be the case (Stonaker et al. 2009).

RPD1 and RPD2a appear to overlap only for certain functions. Mutational analyses show that both maize RPD1 and RPD2a are required to maintain the somatic repression of paramutant *Pl* (Hollick et al. 2005, Stonaker et al. 2009) and to maintain nearly 85% of the 24 nt siRNAs in early seedlings (Stonaker et al. 2009). This last finding implicates both RPD1 and RPD2a in comprising the primary Pol IV core required for initiation of siRNA production, although it is unknown if the same populations of siRNAs are affected in the two different mutants. Plants homozygous for *Pl* and deficient for either RPD1 or RPD2a are darkly pigmented, but revertant *Pl-Rb* states are typically only transmitted from RPD1 mutants (Hollick et al. 2005, Stonaker et al. 2009). Furthermore, RPD1 mutants display several developmental defects (Erhard et al. 2009, Parkinson et al. 2007) whereas RPD2a mutants are morphologically normal (Stonaker et al. 2009). Contrasting phenotypes are also manifest at the molecular level. Using mutant analyses, Hale et al. (2009) found that RPD1 represses the expression of polyadenylated RNAs from certain LTR retrotransposons, but Stonaker et al. (2009) found no such changes in RPD2a mutants. These differences between RPD1 and RPD2a imply either that RPD1 has functions independent of an RNAP holoenzyme or that it can form functionally distinct holoenzyme complexes with alternate RPD2-like partners.

The means by which RPD1, but not RPD2a, maintains a meiotically heritable feature responsible for *Pl* transmission and maintains proper developmental canalization is, at present, unknown. The siRNAs derived from Pol IV/RMR1/RDR2 action do not appear to be required for either, as mutants that lack RPD2a-derived siRNAs do not facilitate reversions of *Pl* to *Pl-Rb* (Stonaker et al. 2009), and mutants that lack RMR1-dependent siRNAs still manage to facilitate paramutations of *Pl-Rb* to *Pl* in *Pl-Rb/Pl* heterozygotes (Hale et al. 2007). Both *rmr1* and *rmr7/rpd2a* mutants have no recognizable problems with development (Hale et al. 2009, Stonaker et al. 2009). By extension, cytosine methylation patterns maintained by the siRNA-dependent RdDM pathway would also not be responsible for either paramutation or development. Hale et al. (2007) showed that patterns of CG or CHG cytosine methylation within *doppia*-type DNA transposon sequences are affected by RPD1 action, although no differences in such patterns have been found to be coincident with paramutable and paramuntant states of *Pl-Rb* and *B1-I* (Hollick et al. 2000, Stam et al. 2002a). Both Walker (1998) and Walker & Panavas (2001) did, however, find that paramutagenic *r1* haplotypes were typically hypermethylated across their *r*-gene coding regions relative to paramutable and neutral haplotypes. Other possible RPD1-imposed chromatin structures have not been evaluated. The meiotic inheritance of epigenetic regulatory states independent of DNA methylation can certainly occur in *S. pombe* (Nakayama et al. 2000), *D. melanogaster* (Cavalli & Paro 1998), and *Caenorhabditis elegans* (Alcazar et al. 2008, Vastenhouw et al. 2006).

**TRANSPOSONS AND PARAMUTATION**

McClintock (1951) was the first to ascribe cytological heterochromatin a regulatory function in the control of transposons and to surmise more generally that asymmetric distributions of submicroscopic heterochromatin to replicated daughter chromatids might, through its action on transposons, underlay the process of cellular differentiation during development. Although the conceptual paradigm of heterochromatin as a repressive form of
chromatin structure was aided by additional studies of position effect variegation in *D. melanogaster* and X-chromosome inactivation in mammals, the cytological definition appears to have become antiquated. The term heterochromatin is now broadly applied to describe a variety of chromatin structures in which gene function is repressed. The 24 nt class of small RNAs in plants has been referred to as the “heterochromatic” or “chromatin-associated” siRNA, although Hale et al. (2009) recently showed that the appearance of cytological heterochromatin in maize is not dependent on these molecules. In large genome cereals such as maize, transposons are ubiquitous both between and within genic regions (Hale et al. 2009, Schnable et al. 2009), thus making it very likely that control of transposons via siRNA pathways plays significant roles in gene regulation. As an example, Liu et al. (2004) showed that mutations in AGO4 affect RNA expression from the FLOWERING LOCUS C allele of *A. thaliana* ssp. *Landsberg erecta* that contains an intronic *Mutator*-like DNA transposon.

Aside from deep-sequencing data of Nobuta et al. (2008) indicating that maize RDR2 amplifies small RNAs representing repetitious features, targeted analyses of both Type I LTR retrotransposons (*Prem2/ji* and *CRM4*) and Type II DNA transposons (*Mutator* and *doppia*) indicate that they are similarly targeted by RPD1 and RMR1 action. Woodhouse et al. (2006a) showed that *Mutator* elements that were heritably silenced following exposure to a hairpin RNA source are “reactivated” by passage through multiple generations in the absence of RDR2 function, and Hale et al. (2009) found that cytosine hypermethylation of the *Mutator* terminal inverted repeats was dependent on both RMR1 and RPD1 action. Terminal fragments of *doppia* transposons are found in the promoter regions of both *Pl1-Rb* and *R-rstd*. The *Pl1-Rb doppia* region is persistently hypermethylated in all cytosine contexts in both *Pl-Rb* and *Pr* states (Hollick et al. 2000) by the combined actions of RPD1, RMR1, and RDR2 (Hale et al. 2009). In the absence of RPD1, methylation of the *doppia* cytosines representing all sequence contexts is significantly reduced, transcription rates of *Pl1-Rhoades* are increased approximately threefold, and heritable reactivation of *Pr* to *Pl-Rb* can occur. In the absence of RMR1, methylation of only CHH residues is lost and transcription rates remain unchanged, but heritable reversions of *Pr* to *Pl-Rb* also occur. Because RPD1 confers no detectable RNA synthesis, models involving either titration of shared Pol II subunits or competition for promoter RNA polymerase assembly sites have been proposed to accommodate these data (Erhard et al. 2009, Hale et al. 2007). Recent findings regarding *CRM4* regulation serve to illustrate these competitions.

The maize genome harbors thousands of *CRM4* LTR retrotransposons, and it is impossible to know if all of them are similarly regulated. Their sense and antisense sequences are enriched in both small RNA populations (Hale et al. 2009, Nobuta et al. 2008) and in RMR1/RDR2-dependent non-polyadenylated large RNA fractions (Hale et al. 2009). This is consistent with the view that *CRM4* elements are initially transcribed by some RNA polymerase and then processed into double-stranded RNA via RMR1/RDR2 action. When RPD1 is absent, sense-specific polyadenylated *CRM4* transcripts appear that presumably are the products of normal Pol II function (Hale et al. 2009). *Figure 7* presents a polymerase competition model proposed by Hale et al. (2009) to account for these data. As a general feature of genomes with repetitive elements, such Pol II competitions could be essential to focus an otherwise limiting amount of Pol II action to primarily genic regions. Based on the significant differences in transposon content between maize and *A. thaliana*, this type of model could reconcile the apparent dysregulation of certain developmental factors in maize RPD1 mutants (Hale et al. 2009, Parkinson et al. 2007) with the apparent normal development of similar *A. thaliana* mutants.

Although the current evidence is circumstantial with regard to paramutation, a role for...
transposons in mediating meiotic inheritance and trans-homolog interactions is not without precedence (see Slotkin & Martienssen 2007 for a review). In *M. musculus*, cytosine methylation profiles of the LAP retroelement found that ≈100 kb 5′ of the agouti gene in the *A"* haplotype are correlated with agouti-dependent coat coloration, and these LAP methylation patterns can be inherited (Morgan et al. 1999). In *Z. mays*, presetting of specific Spm/En transposition patterns via prior exposure in the previous generation to other Spm/En elements (McClintock 1964) could be described as an example of paramutation (Martienssen 1996). Several of the maize DNA transposons cycle between active and inactive states in parallel with changes to the cytosine methylation patterns at their terminal inverted repeats (Banks et al. 1988, Chandler & Walbot 1986).

It now appears that small RNAs, especially those representing transposons, may serve a more fundamental role in maintaining the inheritance of repressed states than previously thought. Recent results in *D. melanogaster* show that transposon-derived piRNAs in the haploid egg and associated nurse cells are correlated with subsequent silencing of related transposons of paternal origin (Brennecke et al. 2008). These data explain the parent-of-origin nature of hybrid-dysgenesis; novel transposons such as *P* or *I* only cause a dysgenic syndrome when transmitted from the male (Bregliano et al. 1980). Perhaps similarly, the RNAs microinjected into one-celled mouse embryos can sometimes phenocopy specific mutations (Rassoulzadegan et al. 2006, Wagner et al. 2008). Ciliated protozoans use parental macronuclei (mac)-sifted small RNAs derived from postmeiotic micronuclei (mic) to target precise resections of mic-specific sequences as new macs mature (Meyer & Chalker 2007).

Slotkin et al. (2009) recently documented a perhaps similar behavior in *A. thaliana* pollen grains in which *Athila* transposon sequences from the vegetative nucleus were expressed and 21 nt siRNA products were found in both the vegetative nucleus and in purified sperm cells that do not express *Athila* RNA transcripts. These studies present a remarkable view of inheritance in which small RNAs themselves may act as sexually transmissible vectors of epigenetic information.

Figure 7
Model for long terminal repeat (LTR) retrotransposon regulation via RNA polymerase IV (Pol IV) competition. Adapted from Hale et al. (2009), the schematic provides a model accounting for the loss of small interfering RNAs (siRNAs) in *rpd1*, *rpd2a*, *rmr1*, and *rdr2* mutants; the loss of nonpolyadenylated transcripts in *rpd1*, *rmr1*, and *rdr2* mutants; and the production of polyadenylated RNAs from CRM4 LTR retrotransposons only in *rpd1* mutants. These results indicate that Pol IV competes with Pol II for access to retrotransposon promoters in a manner that is independent of the RMRI- and RDR2-dependent small RNAs. Subunits (yellow) are hypothesized to be shared between Pol II and Pol IV RNA polymerases as they are in *A. thaliana* (Ream et al. 2009).
MAIZE DEVELOPMENT IN PARAMUTATION MUTANTS

Although abnormal development has been documented for both maize rdr2 and rpd1 mutants (Dorweiler et al. 2000, Parkinson et al. 2007), the majority of these phenotypes only arise after one or more parental genomes has been passed through at least one generation in such mutant backgrounds. Inbreeding of rpd1 null mutants for three or four generations invariably leads to either embryonic lethality or to progeny plants that are unable to self-fertilize (Parkinson et al. 2007, J.B. Hollick, unpublished data). Mutant F1 progeny derived from sibling matings of heterozygous effective phenotypes reappear in subsequent F2 outcrossing to normal inbreds. When such matings, but all such defects are corrected upon defects increases following continued sibling defects of both RPD1 and RDR2 will therefore be documented for both maize rdr2 and rpd1 mutants (Dorweiler et al. 2000, Parkinson et al. 2007), the majority of these phenotypes only arise after one or more parental genomes has been passed through at least one generation in such mutant backgrounds. Inbreeding of rpd1 null mutants for three or four generations invariably leads to either embryonic lethality or to progeny plants that are unable to self-fertilize (Parkinson et al. 2007, J.B. Hollick, unpublished data). Mutant F1 progeny derived from sibling matings of heterozygous females and homozygous rpd1 mutant males begin to display both general and specific defects including reduced plant height, delayed flowering, elaboration of female flowers in the tassel, failure to abort secondary pistils in the ear, adaxialized leaf sectors, and lateral outgrowths of various tissue types (Figure 8; Erhard et al. 2009, Parkinson et al. 2007). In certain lineages, the severity of these defects increases following continued sibling matings, but all such defects are corrected upon outcrossing to normal inbreds. When such defective phenotypes reappear in subsequent F2 progenies, they exclusively cosegregate with the rpd1 defect (Parkinson et al. 2007). This observation indicates that meiotically stable epialleles are created that manifest their dysregulation only in the absence of rpd1 function.

Lineages in which the genome has been previously conditioned by the absence of RDR2 function also have elaborate female flowers in the tassel and reduced plant heights, although the occurrence of such defects is sporadic and variable (Dorweiler et al. 2000). Breeding the rpd1-I mutant allele into different inbred lines also produces inbred-specific defects including disease lesion mimicry in the B73 inbred (K.F. Erhard and J.B. Hollick, unpublished data). Defects of both RPD1 and RDR2 will therefore need to be evaluated in the same inbred following a similar breeding design before it can be determined whether or not the same developmental pathways are similarly affected.

Many of the ontogeny defects seen in rpd1 mutants appear to phenocopy known miRNA regulatory mutants. Of particular note is the way in which plant height is reduced in rpd1 mutants. Parkinson et al. (2007) showed that this defect was related to reduced internode elongation during the adult phase of growth. Maize plants utilize a balance of miRNA-regulated genes to properly position the juvenile-to-adult developmental transition. One particular miR172 represses expression of the glossy15 transcription factor that promotes juvenile characters (Lauter et al. 2005), and a miR156 promotes juvenile growth by repressing teosinte glume architecture1 (Chuck et al. 2007a). By evaluating the timing at which juvenile leaf characters are replaced by adult traits, Parkinson et al. (2007) also found that this juvenile-to-adult transition period is delayed. Thus, rpd1 mutant plants have difficulty both transitioning to and executing the adult phase of growth.

Leaf polarity defects in rpd1 mutants (Figure 8b) are nearly identical to the phenotype of a dominant rolled leaf1 allele (Rld1-O) in which the miR165 and miR166 binding sites are disrupted (Juarez et al. 2004). The rld1 gene encodes an HD-ZIPIII factor that promotes adaxial leaf fates. In situ hybridizations from Juarez et al. (2004) indicate that abaxial positioning of miR166 in the leaf primordia is required to repress rld1 expression from that
domain. Lateral outgrowths similar to those seen in *rpd1* mutants (Figure 8c) are also seen in another misregulated HD-ZIPIII from dominant gain-of-function *PHB* mutants in *A. thaliana* (McConnell & Barton 1998).

Production of female flowers in the tassel (Figure 8a) is also a hallmark of misregulation via another *miR172* that targets the *indeterminate spikelet1* transcription factor (Chuck et al. 2007b), although this defect is distinct from that seen in *rpd1* mutants. Epistasis analysis from Parkinson et al. (2007) indicates that the female flowers seen in *rpd1* mutants are due to misexpression of the *silkless1* locus that normally functions to protect female pistils from abortion in the developing ear (Calderon-Urrea & DellaPorta 1999). The silks formed in *rpd1* mutant tassels are extensions of unabort ed pistils found in abnormal bisexual flowers. The nature of the *silkless1* gene remains unknown, but it has been postulated to be a miRNA precursor gene (Malcomber & Kellogg 2006). Thus many, if not all, of the primary developmental defects seen in *rpd1* and *rdr2* mutants are similar to misexpression phenotypes of transcription factors normally controlled by miRNAs. In accord with these parallels, Hultquist & Dorweiler (2008) recently showed that feminized tassels of *rdr2* mutants have elevated levels of a SBP-box-type transcription factor that is normally repressed by a particular *mir156*.

### MAKING SENSE OF REPRESSION VIA SMALL RNAs

The potential for both *cis-* and *trans-* regulation via small RNA molecules is staggering (see Hollick 2008 for a review). Hairpin RNAs can generate miRNAs that initiate the phasing of *trans*-acting siRNA that subsequently interfere with the expression of one or more genes (Ghildiyal & Zamore 2009). Both biotic and abiotic stresses can induce naturally occurring, convergently transcribed gene pairs known as *cis* -NATs (natural *cis*-antisense transcripts) (Borsani et al. 2005; Katiyar-Agarwal et al. 2006, 2007), and Pol IV can maintain strand-specific small RNAs from the overlapping transcripts that target biologically relevant genes for repression. Potential sense and antisense overlaps from transcripts produced in *trans* (*trans*-NATs) could also be responsible for other regulatory small RNAs. Regulatory cross-talk between various small RNA pathways (Dunoyer et al. 2007) implicates possible regulatory effects based on metabolic fluxes of specific small RNA species. Small RNAs can be trafficked both intercellularly (Dunoyer et al. 2007, 2010; Molnar et al. 2010; Nogueira et al. 2007) and systemically in plants (Molnar et al. 2010, Yoo et al. 2004), and small RNAs obtained from the environment can effect responses in certain animals (Whangbo & Hunter 2008). These observations hint at a significant level of regulatory complexity that we are only beginning to recognize. The larger regulatory roles of noncoding RNAs in maintaining imprinting marks (see Koerner et al. 2009 for a review), organizing chromosome architecture in the nucleus (Lei & Corces 2006), and mediating homolog interactions (Grimaud et al. 2006) remain largely unexplored.

As Brink (1962) suggested, the types of orderly and regularly occurring epigenetic changes associated with developmental events such as juvenile-to-adult transitions now appear to be mechanistically related to those responsible for paramutations. Some events take place during so-called phenocritical periods: times of weak canalization in which normal development can be perturbed by environmental stresses. For example, Waddington (1953) showed that heat shock pulses administered to *D. melanogaster* embryos at particular times could phenocopy *crossveinless*-type mutations affecting wing development. Cavalli and Paro (1998) later showed that specific stages of embryogenesis coincided with the establishment of mitotically heritable repression or activation via the so-called Polycomb and Trithorax response elements (PRE/TREs). Transgenes with an attendant *Fab-7* PRE that were transiently activated during these times not only remained active during development, but these active transgenes often persisted in those states through female transmission. Mikula (1995) has
shown that paramutations occurring at \(r1\) are similarly sensitive to environmental influences during early tassel development. The extent of heritable repression occurring in heterozygotes of paramutable and paramutagenic \(r1\) haplotypes is significantly greater when sibling seedlings are grown at 32°C under constant light compared with 22°C and constant light for only the first 15 days followed by light/dark cycles for days 16–21. This stage-specific environmental sensitivity of paramutation draws a parallel with the phenocritical points of organismal development.

Waddington’s (1959) genetic assimilation hypothesis provided a model to resolve the presumed improbability of accumulating the precise combination of adaptive traits without passing through a state of decreased fitness. First, individuals that optimally respond to environmental stimuli, regardless of the magnitude of that stimulus, are selected. Because the response is canalized in these individuals, they can accumulate genetic polymorphisms that mimic the canalized response independent of the stimulus. Such events could arise without compromising fitness. Heat-shock protein 90 (Hsp90) provides a protein-based mechanism supporting Waddington’s hypothesis (Flatt 2005). Loss of Hsp90 function leads to sporadic morphological variations that are presumably caused by unstable and dysfunctional protein conformations reflecting amino acid polymorphisms arising in otherwise highly inbred populations of \(D.\ melanogaster\) and \(A.\ thaliana\) (Queitsch et al. 2002, Rutherford & Lindquist 1998). Because of its phenotypic buffering capacity, Hsp90 relieves selective pressure that would otherwise prevent accumulation of these deleterious yet potentially adaptive morphological variations. Abnormalities found in \(rpd1\) mutants indicate that RPD1 also carries out a phenotypic buffering role. In this case, RPD1 may buffer against regulatory variations involving transposons and other repetitive genomic features.

Because RPD1 and RDR2 maintain both the transcription states of paramutant alleles and the fidelity of proper developmental programs, it is likely that canalization of some genes controlling maize development require RPD1-dependent small RNAs. The fidelity of some miRNA-initiated repression may require RPD1 function if such miRNAs can engage the RdDM pathway. More generally, RPD1 could provide developmental canalization by stabilizing transient or persistent siRNA-directed expression patterns. Alterations in timing or spatial action of siRNAs, or the RNAPs responsible for their biogenesis, could provide important sources of phenotypic variation on which selection can operate. In accord with Waddington’s (1959) concept of genetic assimilation, such variations could precede the accumulation of genetic changes that faithfully produce these potentially adaptive traits. Thus, part of the paramutation mechanism may prove to have both ontogenetic and phylogenetic importance.

**SUMMARY POINTS**

1. The paramutation process results in meiotically heritable alterations in gene regulation that are not associated with changes to the DNA sequence.

2. Because the assay for paramutation requires meiosis, the mechanism responsible cannot be assumed to be mitotic in nature. Evidence from maize is cited to support the concept that trans-homolog repression is distinct from the repression required for meiotic inheritance of paramutant states.

3. A role for RNA-directed mechanisms in the paramutation process is implicated by RNA microinjection experiments in \(M.\ musculus\) and mutational analyses in maize. Mutations of upstream RdDM components including both catalytic subunits of Pol IV, a RAD54-type ATPase (RMR1), and an RdRP (RDR2) have been identified in forward genetic screens for components needed to maintain the somatic repression of paramutant states at the \(Pl1-Rh\) and \(B1-I\) haplotypes.
4. The paramutation process occurring at \textit{Pl1-Rh} does not require the siRNA component derived from RMR1 function, thus calling into question models that infer interhomolog communication via small RNA molecules.

5. Genetic analyses indicate that \textit{cis}-acting components related to transcription of the assayed gene are essential for paramutagenicity.

6. Developmental defects seen in \textit{rpd1} mutants implicate a failure to canalize the action of developmental regulators related to phase changes, leaf polarity, sexual identity, and lateral meristem repression. These phenotypes are similar to miRNA-regulated defects.

7. Conditioning the maize genome through multiple generations in the absence of RPD1 and RDR2 function lead to fundamental dysregulation of developmental homeostasis.

8. The parallel between phenocritical times during development and the environmental sensitivity of paramutation occurring at \textit{r1} is emphasized and molecular frameworks in which to consider Waddington’s concept of genetic assimilation are discussed.

**FUTURE ISSUES**

1. The nature of the meiotically-heritable information acting to repress paramutant states remains unknown. More examples of functionally important sequences and RPD1/RMR1-dependent chromatin modifications at those features are needed to address this issue.

2. Are there diagnostic structural features that can predict alleles subject to paramutation? How frequent are such alleles? More examples are needed to entrain or enable such searches.

3. Is homologue contact required either to facilitate or to maintain paramutant states? This remains a difficult question as such interactions could be transient and/or confined to a particular cell type.

4. To what extent does paramutation affect phenotypic variation? This question may now be approachable by comparing the variation manifest in \textit{rmr}-type mutant and non-mutant populations.

5. It remains unclear why only the mutations affecting RPD1 and RDR2 function also interfere with normal plant development. Most, if not all, of the small RNA biogenesis components are required to maintain the somatic repression of paramutant states. Comparisons of small RNA profiles may yield clues to this question.

6. Do aberrant polymerase competitions between genic regions and repetitious elements account for the apparent dysregulation of developmental regulators? Genome-wide profiles of Pol II occupancy and/or action in both mutant and non-mutant materials should begin to address this issue.

7. How is the paramutation mechanism integrated with the perception of environmental stimuli? Although our understanding of the regulatory roles of environmentally-induced small RNAs is nascent, it now seems likely that they have the capacity for epigenomic reprogramming. How such environmental perceptions are passed on to future generations remains to be determined.
DISCLOSURE STATEMENT
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LITERATURE CITED
Brink RA. 1956. A genetic change associated with the R locus in maize which is directed and potentially reversible. *Genetics* 41:872–79
IN ADVANCE

Genetic and molecular analyses support a model in which Pol IV competes with Pol II for genomic templates.


Hultquist JF, Dorweiler JE. 2008. Feminized tassels of maize *map1* and *ts1* mutants exhibit altered levels of *miR156* and specific SBP-box genes. *Planta* 229(1):99–113


Genetic analyses showing that paramutagenicity is proportional to *r1*-gene copy number and that no single *r1*-gene is required.
An incredibly prescient description of the nuclear systems controlling development motivated by McClintock's observations of transposon behavior.


An incredibly prescient description of the nuclear systems controlling development motivated by McClintock's observations of transposon behavior.

A definitive study showing that meiotically heritable changes can be induced by environmental conditions during early development.

Developmental phenotypes resembling defective miRNA regulation implicate a mechanistic relationship between paramutation and development.

RNA microinjections indicate that heritable changes in gene function may be RNA-directed.


Woodhouse MR, Freeing M, Lisch D. 2006b. The mnp1 (mediator of paramutation) mutant progressively reactivates one of the two genes encoded by the MuDR transposon in maize. Genetics 172:579–92

