

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Paramutation: Epigenetic Instructions Passed Across Generations

Vicki Chandler^{*.1} and Mary Alleman[†]

^{*}BIO5 Institute and Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721 and

[†]Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282

PARAMUTATION is the fascinating ability of specific DNA sequences to communicate in *trans* to establish meiotically heritable expression states. Intriguingly, newly silenced sequences continue to issue instructions to naïve alleles in subsequent generations. The term “paramutation” was first coined in the 1950s by Alexander Brink to describe this puzzling phenomenon at the *r1* locus in maize (BRINK 1956); an interaction between specific alleles in heterozygotes led to heritable decreases in gene expression of one allele. Not only was the reduced expression state stable through meiosis, but also the low-expressing allele could induce silencing of another high-expressing allele in subsequent generations. The frequency of the change was 100% and the stability of the change was lower than typical mutations; hence the term “*paramutation*.” A few years later, Ed Coe, Jr., described another maize example in which interaction between alleles at the *b1* locus also led to heritable silencing (COE 1959) and Rudolf Hagemann described interactions at the *sulfurea* locus in tomato (HAGEMANN 1969). Since that time other examples of paramutation have been identified in maize and in other species (reviewed in CHANDLER and STAM 2004; STAM and MITTELSTEN SCHEID 2005; CHANDLER 2007), yet the two maize loci where paramutation was initially described, *r1* and *b1*, remain the most extensively characterized and best understood. The *r1* and *b1* loci encode closely related, functionally equivalent transcription factors that activate the anthocyanin pigment biosynthetic pathway (GOFF *et al.* 1990; LUDWIG *et al.* 1990). They are likely related to each other through a duplication resulting from an ancient allotetraploidization event during maize evolution (GAUT and DOEBLEY 1997). The two loci have multiple alleles with distinct expression patterns, which regulate the distribution of

anthocyanin pigments during development (STYLES *et al.* 1973; COE 1979). Recent work demonstrates a key role for RNA in mediating both *r1* and *b1* paramutation, as the *mop1* gene that encodes an RNA-dependent RNA polymerase (RDR; ALLEMAN *et al.* 2006) is absolutely required for paramutation at both loci (DORWEILER *et al.* 2000). Yet, there are striking differences in the properties of *r1* and *b1* paramutation, which hint at distinct mechanisms. In this article, the most striking differences between *r1* and *b1* paramutation are described and potential mechanisms are discussed relative to our current understanding of the role of RNA interference (RNAi) in mediating transcriptional silencing.

OVERVIEW OF *R1* AND *B1* PARAMUTATION

In spite of the close relationship, *r1* and *b1* have quite distinct gene organizations and there is no evidence of paramutant interactions between the loci (BRINK *et al.* 1960). Specific alleles at each locus participate in paramutation. At *r1*, all known alleles induce paramutation, respond to paramutation, or cause reversal of paramutation. The alleles that cause paramutation are generally structurally distinct from the alleles that become silenced (reviewed in CHANDLER *et al.* 2000; diagrammed in Figure 1). The *r1* haplotypes that participate in paramutation are expressed in the aleurone layer of the seed and the paramutant phenotype is strongest in this tissue (reviewed in BRINK *et al.* 1968; BRINK 1973). All *r1* “alleles” that undergo paramutation contain multiple copies of the coding region (Figure 1) and as such are referred to as haplotypes. In contrast, the *b1* alleles that participate in paramutation have a single coding region and identical DNA sequences and are thus epialleles (STAM *et al.* 2002a,b). These *b1* epialleles are expressed in the epidermal layer of the plant body, but not in the seed (Figure 2).

When the *R-r* allele, which confers dark purple seeds, is crossed to *R-stippled* (*R-st*), which confers purple

¹Corresponding author: BIO5 Institute, 1657 E. Helen St., University of Arizona, Tucson, AZ 85721. E-mail: chandler@ag.arizona.edu

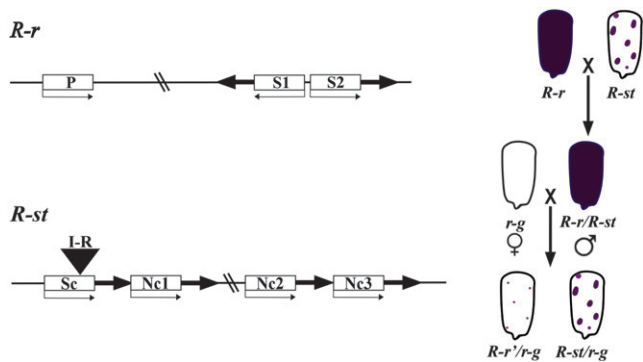


FIGURE 1.—Paramutation at *r1*. The structures of the paramutable (*R-r*) and paramutagenic (*R-st*) alleles are diagrammed as well as the crosses and phenotypes used to monitor paramutation. The open boxes represent the *r1* genes with the small arrows indicating the direction of transcription: P stands for plant and is a gene expressed in vegetative plant tissues; S1 and S2 are two genes expressed in the seed that are organized in an inverted repeat; Sc stands for self-color and is the most highly expressed gene in the *R-st* complex; Nc1–Nc3 stand for near colorless, three *r1* genes that are expressed at low levels in *R-st*. I-R indicates the transposable element inserted into Sc, which is responsible for the spotted, stippled phenotype. The thick arrows in *R-st* represent the large repeats spanning the *r1* genes. Only the repeats associated with the coding regions are shown. There are additional small, related sequences related to transposable elements that are not diagrammed, but are described in detail with primary references cited in CHANDLER *et al.* (2000). A comprehensive list of *r1* haplotypes with their tissue-specific expression patterns and paramutation properties can be found in NEUFFER *et al.* (1997).

spotted seeds (because of a transposon insertion that is not involved in paramutation), *R-r* is heritably changed such that it confers lightly pigmented seeds upon subsequent outcrosses (designated *R-r'*), while the *R-stippled* allele segregates unchanged (Figure 1). Intriguingly, when *R-r'* is crossed to *R-r*, *R-r* can be changed to *R-r'*; this is referred to as secondary paramutation to distinguish it from the primary paramutational interaction between *R-st* and *R-r*. At the *b1* locus, the low-expressing *B'* allele (which can also derive spontaneously from the high-expressing *B-I* allele) changes *B-I* into *B'* (new *B'* alleles are designated *B'**) with the silencing observed in the F₁ (Figure 2). The newly altered *B'** allele is indistinguishable from *B'* in its ability to paramutate naïve *B-I* alleles in subsequent generations. Thus, at *b1*, secondary paramutation is indistinguishable from primary paramutation. This contrasts with *r1* paramutation; the strength of *R-r'* secondary paramutation (the ability of *R-r'* to paramutate a naïve *R-r* allele) is fully penetrant only when *R-r'* has been heterozygous with *R-st* for multiple generations (BROWN and BRINK 1960).

Two assays are routinely used to monitor paramutation: (1) the ability of a paramutagenic allele to cause a heritable change in the expression of a paramutable allele (as measured at *r1* and *b1* by a reduction in

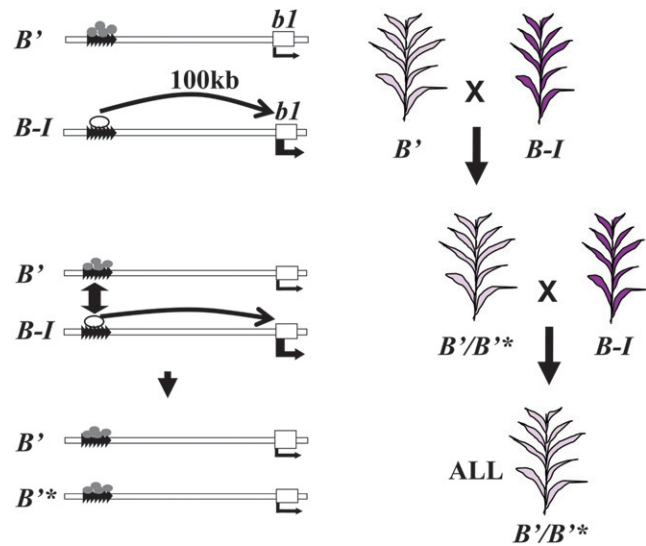


FIGURE 2.—Paramutation at *b1*. The paramutable (*B-I*) and paramutagenic (*B'*) epialleles have identical DNA sequences, but distinct chromatin structures represented by different proteins (circles) associated with the seven tandem repeats (arrowheads) that mediate paramutation and are located 100 kbp upstream of the coding region (open box). The arrows below the gene boxes indicate the direction of transcription and the thickness of the arrows indicate the relative transcription levels observed with the two epialleles. The crosses and phenotypes used to monitor paramutation are diagrammed with *B'**, indicating a *B'* allele that was *B-I* in the previous generation. *B'* and *B'** are indistinguishable in their ability to paramutate *B-I*.

pigment); and (2) the heritable alteration of the paramutable allele into a paramutagenic allele. At *b1*, these two phenotypes always occur simultaneously and completely. In contrast, at *r1*, the extent of paramutagenicity measured as the level of *R-r* pigmentation depends on the haplotype and the circumstances of the crosses (discussed in the next section and reviewed in CHANDLER *et al.* 2000). Specific nomenclature is used to describe the various steps in the paramutation process. Establishment describes the *trans*-interactions between alleles that produce the distinct expression states. In the case of both *r1* and *b1*, the new states show reduced expression (designated *R-r'* and *B'*), and the ability to maintain that silencing phenotype is referred to as “maintenance of silencing.” The extent to which the silencing and ability to cause paramutation is maintained in subsequent generations is referred to as heritability. The reason for distinguishing among establishment, maintenance, and heritability is that certain situations or genetic backgrounds can influence these processes differentially. The frequent association between paramutation and genes involved in pigmentation is likely to reflect ascertainment bias, because of the ease of scoring visible pigment phenotypes and the dispensable nature of anthocyanin for plant development.

STABILITY DIFFERENCES BETWEEN *rI* AND *bI* PARAMUTATION

The *rI* and *bI* systems differ in several properties, which hint at basic mechanistic differences. One dramatic example is the extreme stability of *B'* as compared with the instability of the *R-r'* silenced state. *B-I* is truly converted to a *B'* epiallele whereas *R-r'* states exist as a continuum, depending on the counter-allele and the genetic history. In wild-type genetic backgrounds, *B-I* is always changed to *B'** (COE 1966). *B'* and *B'** are indistinguishable, and both are extremely stable; they have never been observed to change back to *B-I* in wild-type genetic backgrounds and this is true whether they are maintained as homozygous or heterozygous with an allele that does not participate in paramutation (COE 1966). Spontaneous changes of *B-I* to *B'* occur at high frequencies, often 1–10% (COE 1966), and spontaneous *B'* alleles are as fully paramutagenic as *B'** alleles segregating from *B'/B-I* plants (Figure 2). Thus, the low-expression state associated with *B'* is invariably associated with strong paramutagenicity (COE 1966).

In contrast, *R-r'* is unstable with its pigment level and reversion frequency dependent on the strength of the *R-st* derivative to which it was crossed, the number of generations of heterozygosity with *R-st*, or when *R-r'* is homozygous. When *R-r'* is exposed to repeated generations of crosses with paramutagenic haplotypes, its seed pigmentation level continues to decrease to almost colorless and the haplotype becomes more paramutagenic (MIKULA 1961; McWHIRTER and BRINK 1962). Furthermore, expression of *R-r* is more reduced following passage through trisomics containing two doses of the paramutagenic allele relative to passage through disomic heterozygotes with one dose of the paramutagenic allele (KERMICLE *et al.* 1995). Paramutation can also be reversed gradually if *R-r'* is maintained as hemizygous or heterozygous with alleles that contain a single copy of the *rI* locus, such that seed color becomes even darker than nonparamutant *R-r* (STYLES and BRINK 1966, 1969). This set of experiments, together, suggests that there is communication between the various *rI* haplotypes and that this communication influences expression levels.

SEQUENCES REQUIRED FOR PARAMUTATION

One common feature of *rI* and *bI* paramutation is that tandem repeats are involved in paramutation at each locus, yet the actual sequences and properties of these repeats are quite distinct between the two loci. Within the *rI* paramutagenic haplotypes, the repeats are of unknown length but each is likely quite large, spanning the *rI*-coding regions (Figure 1; EGGLESTON *et al.* 1995). The regions associated with paramutagenicity have been mapped for two *rI* haplotypes using unequal crossing over between the multiple *rI* genes in paramutagenic and nonparamutagenic haplotypes to

generate a series of alleles with differing numbers of tandem repeats (KERMICLE *et al.* 1995; KERMICLE 1996; PANAVAS *et al.* 1999). Intriguingly, these results suggest that no specific region is required for *rI* paramutation, but that only repeat number matters: paramutagenicity is reduced as repeat number is reduced and paramutagenicity is increased as repeat number is increased. The region within *R-r* required to respond to paramutation has also been mapped and lies within the inverted repeat structure of the S component (BROWN and BRINK 1960; ROBBINS *et al.* 1991; KERMICLE 1996). Again, the *rI* genes themselves lie within the sequences associated with paramutation. The results are quite different at *bI* (Figure 2), in which specific sequences, an array of seven tandem repeats of 853 bp each, are required for paramutation; these are located >100 kb upstream of the single *bI*-coding region and share no sequence identity with the coding region (STAM *et al.* 2002a). Analyses of recombinant alleles differing only in the number of the upstream *bI* tandem repeats demonstrated that tandem repeats are required for paramutation and the high expression of *B-I* (STAM *et al.* 2002a). Alleles that do not participate in paramutation have a single copy of this sequence, which is unique in the maize genome, and thus is not shared with other genes that also undergo paramutation, such as *rI*. The observation that tandem repeats are associated with paramutation might suggest a mechanism of repeat expansion and contraction. However, as discussed below, the numbers of repeats do not change with paramutation.

POTENTIAL COMMUNICATION MECHANISMS

What might be the basis for the allele and haplotype communication? Two possibilities are the RNA-based model or the DNA pairing model, but these are not mutually exclusive (reviewed in CHANDLER and STAM 2004). Both Brink and Coe explored various mechanisms for the cause of *rI* and *bI* paramutation using the genetic approaches available to them at the time. Both were on the right track in understanding that paramutation must involve direct or indirect contact between paramutagenic and paramutable alleles. Brink understood that during paramutation *R-st* and *R-r* must communicate early in development in new heterozygotes. By attempting to block paramutation through the use of translocated chromosomes that would disrupt pairing, he tested whether chromosome pairing might mediate the communication. The experiment did not lead to conclusive results because the prior genetic history of the alleles used confounded the interpretation (J. KERMICLE, personal communication). Brink also tested for the existence of a “cytoplasmic particle,” loosely interpreted as a “*trans-acting*” or “communication molecule” that would be produced by *R-st* and transmit paramutation to *R-r*; but found no evidence for such (BRINK *et al.* 1964). On the

basis of studies at the *b1* locus, Coe favored a model in which the paramutagenic allelic transferred a physical entity, which he hypothesized could be DNA or RNA (COE 1968). We now know that there are no genetic changes among *b1* alleles, but there is good evidence that RNA signals are involved in allele interactions (see below).

REPEAT COUNTING MECHANISMS

At the *b1* locus, the full penetrance and stability of paramutation is conferred by the seven tandem repeats, as paramutation with *b1* alleles with three tandem repeats was less penetrant and less stable (STAM *et al.* 2002a). This result suggests that there is a mechanism for counting the numbers of repeats. Potential counting mechanisms could involve chromatin marks such as DNA and histone modifications, number of specific proteins bound, or RNA signals, whose level depends on the number of repeats.

BRINK *et al.* (1968) suggested that the *r1* haplotypes exist in a wide continuum of states and that the ability to move along the continuum is an inherent property of the haplotype itself with the extent and direction of movement along the continuum influenced by the nature of the other allele present. Brink proposed that the *r1* locus had two components: (1) the gene complex encoding the protein involved in anthocyanin synthesis and (2) a heterochromatic segment assumed to consist of varying numbers of a repeating unit called a metamere, which functioned to repress *r1*. He further proposed that the degree of repression was proportional to the number of metameres, which could change through misreplication during somatic mitosis (BRINK *et al.* 1968). This hypothesis sprang from Brink's being influenced by the description of position-effect variegation (PEV) in *Drosophila* (reviewed in LEWIS 1950). The influence likely resulted from the mosaic pattern of PEV in *Drosophila* eyes being strikingly similar to the mottled phenotype of paramutant *R-r'* kernels and the idea that both PEV and paramutation were caused by aberrations in gene expression systems.

Brink clearly sought a hypothesis that incorporated the essence of heterochromatin and repeated sequences without the requirement for large centric blocks that were known to be part of the PEV phenomenon. He attempted to avoid these inconsistencies with an alteration of terminology, for example, "ortho" and "parachromatin," in which parachromatin is the part of the genome responsible for chromatin condensation and gene silencing (BRINK *et al.* 1960). Parachromatin is assumed to be a product of a special class of chromosomal elements that might be called determinants of communication. It is elaborated by these determinants in response to the cellular environment (BRINK 1960). This description sounds provocatively similar to RISC-complex-mediated gene silencing (ZARATIEGUI *et al.* 2007).

The large tandem repeats associated with paramutagenic alleles are consistent with the metamere hypothesis, but molecular studies have demonstrated that the number of repeats does not change during plant development (M. ALLEMAN, unpublished data); thus differences in *r1* expression are likely to be mediated by epigenetic mechanisms (such as changes in DNA methylation and histone modifications). Potentially, the larger number of tandem repeats in paramutagenic alleles facilitates the communication with the inverted repeat at the sensitive locus—either directly through pairing or through some type of RNA-signaling molecule. At *r1*, a larger number of repeats could result in an increase in the amount of communication signal sent by paramutagenic haplotypes or an increased frequency of direct pairing. The inverted repeat nature of the paramutable haplotypes may make them particularly receptive to this signal.

TRANSACTING FACTORS REQUIRED FOR PARAMUTATION

Characterization of *trans*-acting factors required for paramutation has begun to provide mechanistic clues. Recent results strongly suggest that the communication signal is likely to be RNA molecules produced and interpreted by genes involved in the RNAi transcriptional silencing pathway. Several *trans*-acting factors that affect paramutation have been identified (DORWEILER *et al.* 2000; HOLLICK and CHANDLER 2001; HOLLICK *et al.* 2005). Two of these genes have been cloned: *mop1*, which encodes an RDR (ALLEMAN *et al.* 2006), and *rmr1*, which encodes a putative chromatin-remodeling protein (HALE *et al.* 2007). Both of these proteins are closely related to proteins involved in RNAi-mediated transcriptional gene silencing in *Arabidopsis thaliana*, suggesting that the RNAi pathway mediating heritable chromatin structures is required for paramutation. The tandem repeats mediating *b1* paramutation are transcribed on both strands (ALLEMAN *et al.* 2006) and MOP1 is required for an increased quantity of 24-nucleotide short interfering RNAs (siRNAs) coming from these repeats (M. ARTEAGA-VAZQUEZ and V. CHANDLER, unpublished data). In addition to affecting paramutation at multiple loci (DORWEILER *et al.* 2000), the MOP1 protein also influences the epigenetic regulation of *Mutator* transposons (LISCH *et al.* 2002; SLOTKIN *et al.* 2005; WOODHOUSE *et al.* 2006a,b) and certain transcriptionally silenced transgenes (MCGINNIS *et al.* 2006), processes associated with RNAi mechanisms in multiple species.

Both establishment of paramutation and maintenance of the transcriptional silencing at *B'* absolutely depend on *mop1* (DORWEILER *et al.* 2000), suggesting a role for RNA in both processes. However, *mop1* has a more subtle role in the heritability of the reduced expression state. When *mop1* mutations are outcrossed to wild type to reintroduce the wild-type MOP1 pro-

tein, most of the progeny have *B'* expression levels (DORWEILER *et al.* 2000). This result suggests that the MOP1 protein can efficiently resilence the “active” epiallele transmitted from the *mop1* mutant plant. Another possibility is that the “active” allele remembers that it was *B'* in the previous generation in spite of its high expression promoted by the absence of MOP1 and is therefore expressed at a low level in the progeny. The latter hypothesis would require that some type of heritable mark remain at the *B'* allele even when it is expressing at a *B-I* level and would require that mark to be efficiently transmitted through meiosis. Recently we noted, when growing thousands of progeny in the absence of the MOP1 protein, that at a low frequency the *B'* epiallele is changed to a *B-I* state that is heritable and immune to resilencing in the presence of the MOP1 protein. This contrasts with wild-type backgrounds in which *B'* has never been observed to change to *B-I*. Similarly, in the absence of the MOP1 protein, a transcriptionally silent transgene can be reactivated such that it stays active for multiple generations even in the presence of the MOP1 protein introduced by outcrosses (MCGINNIS *et al.* 2006). This result is consistent with the establishment of chromatin states that are relatively immune to silencing.

MOP1 is also absolutely required to establish *r1* paramutation (DORWEILER *et al.* 2000) as *R-r* is not changed to *R-r'* by *R-st* in homozygous *mop1* mutant plants. Intriguingly, the MOP1 protein is not required to maintain the reduced expression associated with *R-r'* (J. KERMICLE, personal communication). There are several potential explanations. First, an RNA mechanism may not be involved in maintaining the silencing of *R-r*. Second, at *r1*, the silencing might be stabilized through the more extensive DNA methylation that occurs. A third possibility could be that the *R-r'* allele does not require the RDR to maintain an RNA signal; potentially, the inverted repeat structure seen at *R-r* (Figure 1) could generate hairpin RNA in sufficient quantities. This latter hypothesis is similar to what is seen with the *MuK* locus of maize, which generates a double-stranded RNA hairpin homologous to the fully functional autonomous Mutator element, *MuDR*, silencing it (SLOTKIN *et al.* 2005); this silencing does not require MOP1 (WOODHOUSE *et al.* 2006a,b). Further experiments to test the role of other RNAi pathway mutants should reveal whether an RNAi pathway is involved in maintaining *R-r'* silencing. Another complication in interpreting the *r1* result is that the phenotype is observed only in paternally transmitted alleles, which are subject to genomic imprinting (ALLEMAN and DOCTOR 2000). DNA methylation within the inverted repeats in *R-r* does correlate with its silencing (WALKER 1998) and this methylation may contribute to the maintenance of silencing in the absence of the MOP1 pathway. Another possibility is that the silencing mediated by imprinting is epistatic to the silencing mediated by MOP1 and thus MOP1's absence is inconsequential.

OPEN QUESTIONS

Given the dramatic differences between *r1* and *b1* paramutation, it is striking that both involve repeated sequences of some type and that an RNA *trans*-effect links the two processes. Transgene silencing and heterochromatin silencing in multiple species also involve repeated sequences, and RNA-directed chromatin changes are mediated by components of the RNAi pathway (HENDERSON and JACOBSEN 2007; ZARATIEGUI *et al.* 2007). While similar proteins are involved in maize paramutation and RNA-directed DNA methylation in *A. thaliana*, it is interesting that paramutation has yet to be described in *A. thaliana*. The *FWA* locus in *A. thaliana* is the system most similar to *b1* paramutation in that methylated tandem repeats producing siRNAs from a silenced endogenous locus can communicate with an incoming transgene to silence it (HENDERSON and JACOBSEN 2007). Yet a silenced endogenous *FWA* locus does not silence an active endogenous allele. Why is the active *FWA* locus immune to silencing while the *FWA* transgene is silenced?

Many additional intriguing questions remain. What process enables the extremely efficient and highly heritable *trans*-communication associated with paramutation in maize? What is the nature of the RNA that triggers paramutation? Why are tandem repeats required? What are the heritable molecules or marks? Why does paramutation exist and is it really rare? Thoughts on some of these questions are discussed in CHANDLER (2007). Current approaches directed toward identifying additional key genes required for paramutation and understanding the relationships between tandem repeat RNA and chromatin structure offer hope for eventually understanding this intriguing process.

Paramutation studies in the Chandler laboratory were supported by grants to V.C. from the National Science Foundation (NSF) and the National Institutes of Health. M.A. received a sabbatical supplement from the NSF. We thank Jerry Kermicle for helpful discussions.

LITERATURE CITED

- ALLEMAN, M., and J. DOCTOR, 2000 Genomic imprinting in plants: observations and evolutionary implications. *Plant Mol. Biol.* **43**: 147–161.
- ALLEMAN, M., L. SIDORENKO, K. MCGINNIS, V. SESHADRI, J. E. DORWEILER *et al.*, 2006 An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature* **442**: 295–298.
- BRINK, R. A., 1956 A genetic change associated with the *R* locus in maize which is directed and potentially reversible. *Genetics* **41**: 872–889.
- BRINK, R. A., 1960 Paramutation and chromosome organization. *Q. Rev. Biol.* **35**: 120–137.
- BRINK, R. A., 1973 Paramutation. *Annu. Rev. Genet.* **7**: 129–152.
- BRINK, R. A., D. F. BROWN, J. KERMICLE and W. H. WEYERS, 1960 Locus dependence of the paramutant *R*-phenotype in maize. *Genetics* **45**: 1297–1312.
- BRINK, R. A., J. L. KERMICLE and D. F. BROWN, 1964 Tests for a gene-dependent cytoplasmic particle associated with *R* paramutation in maize. *Proc. Natl. Acad. Sci. USA* **51**: 1067–1074.
- BRINK, R. A., E. D. STYLES and J. D. AXTELL, 1968 Paramutation: directed genetic change. Paramutation occurs in somatic cells and heritably alters the functional state of a locus. *Science* **159**: 161–170.

- BROWN, D., and R. BRINK, 1960 Paramutagenic action of paramutant *R-r* and *R-g* alleles in maize. *Genetics* **45**: 1313–1316.
- CHANDLER, V. L., 2007 Paramutation: from maize to mice. *Cell* **128**: 641–645.
- CHANDLER, V. L., and M. STAM, 2004 Chromatin conversations: mechanisms and implications of paramutation. *Nat. Rev. Genet.* **5**: 532–544.
- CHANDLER, V. L., W. B. EGGLESTON and J. E. DORWEILER, 2000 Paramutation in maize. *Plant Mol. Biol.* **43**: 121–145.
- COE, E. H., JR., 1959 A regular and continuing conversion-type phenomenon at the *B* locus in maize. *Proc. Natl. Acad. Sci. USA* **45**: 828–832.
- COE, E. H., JR., 1966 The properties, origin and mechanism of conversion-type inheritance at the *B* locus in maize. *Genetics* **53**: 1035–1063.
- COE, E. H., JR., 1968 Heritable repression due to paramutation in maize. *Science* **162**: 925.
- COE, E. H., JR., 1979 Specification of the anthocyanin biosynthetic function by *b* and *r* in maize. *Maydica* **24**: 49–58.
- DORWEILER, J. E., C. C. CAREY, K. M. KUBO, J. B. HOLLICK, J. L. KERMICLE *et al.*, 2000 *Mediator of paramutation1* is required for establishment and maintenance of paramutation at multiple maize loci. *Plant Cell* **12**: 2101–2118.
- EGGLESTON, W. B., M. ALLEMAN and J. L. KERMICLE, 1995 Molecular organization and germinal instability of *R-stippled* maize. *Genetics* **141**: 347–360.
- GAUT, B. S., and J. F. DOEBLEY, 1997 DNA sequence evidence for the segmental allotetraploid origin of maize. *Proc. Natl. Acad. Sci. USA* **94**: 6809–6814.
- GOFF, S. A., T. M. KLEIN, B. A. ROTH, M. E. FROMM, K. C. CONE *et al.*, 1990 Transactivation of anthocyanin biosynthetic genes following transfer of *B* regulatory genes into maize tissues. *EMBO J.* **9**: 2517–2522.
- HAGEMANN, R., 1969 Somatic conversion (paramutation) at the *sulfurea* locus of *lycopersicon esculentum* Mill. III. Studies with trisomics. *Can. J. Genet. Cytol.* **11**: 346–358.
- HALE, C. J., J. L. STONAKER, S. M. GROSS and J. B. HOLLICK, 2007 A novel Snf2 protein maintains trans-generational regulatory states established by paramutation in maize. *PLoS Biol.* **5**: 2156–2165.
- HENDERSON, I. R., and S. E. JACOBSEN, 2007 Epigenetic inheritance in plants. *Nature* **447**: 418–424.
- HOLLICK, J. B., and V. L. CHANDLER, 2001 Genetic factors required to maintain repression of a paramutagenic maize *pl1* allele. *Genetics* **157**: 369–378.
- HOLLICK, J. B., J. L. KERMICLE and S. E. PARKINSON, 2005 *Rmr6* maintains meiotic inheritance of paramutant states in *Zea mays*. *Genetics* **171**: 725–740.
- KERMICLE, J. L., 1996 Epigenetic silencing and activation of a maize *r* gene, pp. 267–287 in *Epigenetic Mechanisms of Gene Expression*, edited by V. E. A. RUSSO, R. A. MARTIENSSSEN and A. D. RIGGS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- KERMICLE, J. L., W. B. EGGLESTON and M. ALLEMAN, 1995 Organization of paramutagenicity in *R-stippled* maize. *Genetics* **141**: 361–372.
- LEWIS, E. B., 1950 The phenomenon of position effect. *Adv. Genet.* **3**: 73–115.
- LISCH, D., C. C. CAREY, J. E. DORWEILER and V. L. CHANDLER, 2002 A mutation that prevents paramutation in maize also represses *Mutator* transposon methylation and silencing. *Proc. Natl. Acad. Sci. USA* **99**: 6130–6135.
- LUDWIG, S. R., B. BOWEN, L. BEACH and S. R. WESSLER, 1990 A regulatory gene as a novel visible marker for maize transformation. *Science* **247**: 449–450.
- MCGINNIS, K. M., C. SPRINGER, Y. LIN, C. C. CAREY and V. CHANDLER, 2006 Transcriptionally silenced transgenes in maize are activated by three mutations defective in paramutation. *Genetics* **173**: 1637–1647.
- MCWHIRTER, K., and R. BRINK, 1962 Continuous variation in level of paramutation at the *R* locus in maize. *Genetics* **47**: 1053–1074.
- MIKULA, B. C., 1961 Progressive conversion of *R*-locus expression in maize. *Proc. Natl. Acad. Sci. USA* **47**: 566–571.
- NEUFFER, M. G., E. H. COE and S. R. WESSLER, 1997 *Mutants of Maize*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- PANAVAS, T., J. WEIR and E. L. WALKER, 1999 The structure and paramutagenicity of the *R-marbled* haplotype of *Zea mays*. *Genetics* **153**: 979–991.
- ROBBINS, T. P., E. L. WALKER, J. L. KERMICLE, M. ALLEMAN and S. L. DELLAPORTA, 1991 Meiotic instability of the *R-r* complex arising from displaced intragenic exchange and intrachromosomal rearrangement. *Genetics* **129**: 271–283.
- SLOTKIN, R. K., M. FREELING and D. LISCH, 2005 Heritable transposon silencing initiated by a naturally occurring transposon inverted duplication. *Nat. Genet.* **37**: 641–644.
- STAM, M., and O. MITTELSTEN SCHEID, 2005 Paramutation: an encounter leaving a lasting impression. *Trends Plant Sci.* **10**: 283–290.
- STAM, M., C. BELELE, J. E. DORWEILER and V. L. CHANDLER, 2002a Differential chromatin structure within a tandem array 100 kb upstream of the maize *b1* locus is associated with paramutation. *Genes Dev.* **16**: 1906–1918.
- STAM, M., C. BELELE, W. RAMAKRISHNA, J. E. DORWEILER, J. L. BENNETZEN *et al.*, 2002b The regulatory regions required for *B'* paramutation and expression are located far upstream of the maize *b1* transcribed sequences. *Genetics* **162**: 917–930.
- STYLES, E. D., and R. A. BRINK, 1966 The metastable nature of paramutable *R* alleles in maize. I. Heritable enhancement in level of standard *R* action. *Genetics* **54**: 433–439.
- STYLES, E. D., and R. A. BRINK, 1969 The metastable nature of paramutable *r* alleles in maize. IV. Parallel enhancement of *R* action in heterozygotes with *r* and in hemizygotes. *Genetics* **61**: 801–811.
- STYLES, E. D., O. CESKA and K. T. SEAH, 1973 Developmental differences in action of *r* and *b* alleles in maize. *Can. J. Genet. Cytol.* **15**: 59–72.
- WALKER, E. L., 1998 Paramutation of the *r1* locus of maize is associated with increased cytosine methylation. *Genetics* **148**: 1973–1981.
- WOODHOUSE, M. R., M. FREELING and D. LISCH, 2006a Initiation, establishment, and maintenance of heritable MuDR transposon silencing in maize are mediated by distinct factors. *PLoS Biol.* **4**: e339.
- WOODHOUSE, M. R., M. FREELING and D. LISCH, 2006b The *mop1* (*mediator of paramutation1*) mutant progressively reactivates one of the two genes encoded by the MuDR transposon in maize. *Genetics* **172**: 579–592.
- ZARATIEGUI, M., D. V. IRVINE and R. A. MARTIENSSSEN, 2007 Non-coding RNAs and gene silencing. *Cell* **128**: 763–776.