Paramutation's Properties and Puzzles

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Science **330**, 628 (2010);
DOI: 10.1126/science.1191044

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

PERSPECTIVE

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Paramutation refers to the process by which homologous DNA sequences communicate in trans to establish meiotically heritable expression states. Although mechanisms are unknown, current data are consistent with the hypothesis that the establishment and heritable transmission of specific chromatin states underlies paramutation. Transcribed, noncoding tandem repeats and proteins implicated in RNA-directed transcriptional silencing in plants and yeast are required for paramutation, yet the specific molecules mediating heritable silencing remain to be determined.

Alleles interact to establish heritable expression states in classic examples of paramutation, such as the well-studied *b1* locus in maize, which affects variation in coloration (Fig. 1). Paramutation-like interactions also can occur between transgenes or transgenes and endogenous genes and have been observed in multiple species [see (*1*–*3*) for recent reviews].

The nature of the interaction that leads to paramutation is unknown, but several lines of evidence suggest a role for RNA. The strongest evidence is that genetic screens for mutants unable to undergo paramutation identified several genes with homology to proteins that mediate RNA-directed transcriptional silencing (*3*). In *Arabidopsis*, transcriptional silencing of endogenous genes or transgenes can be mediated by 24-nucleotide (nt) siRNA (short interfering RNA) that target homologous sequences for silencing, which correlates with DNA cytosine methylation and histone modifications characteristic of heterochromatin (*4*). This pathway in *Arabidopsis* is referred to as RNA-directed DNA methylation (RdDM) or RNA-directed transcriptional silencing (*4*). In *Schizosaccharomyces pombe*, transcriptional silencing of mating-type loci and genes within centromeres is mediated by an RNA interference (RNAi)–heterochromatin pathway that shares several protein components with the RdDM pathway in *Arabidopsis* (*5*).

Additional data consistent with a role for RNA in paramutation is that the sequences mediating paramutation are transcribed noncoding tandem repeats (*1*, *3*). In both cases where the key sequences required for paramutation have been identified, they are either contiguous tandem repeats (Fig. 1) or direct repeats flanking unrelated sequences (*3*). These repeats contain enhancer sequences and are transcribed (*1*, *3*). At the *b1* locus, paramutation strength is correlated with the number of tandem repeats (*1*) because alleles with five to seven repeats exhibit strong paramutation, alleles with three repeats are intermediate, and alleles that do not participate in paramutation have only one copy. It is intriguing that repeats are also present within centromeres, transposons, and transgenes, which are also subject to RNA-directed transcriptional silencing (*4*, *5*), suggesting that underlying mechanisms regulating these elements may be shared with paramutation.

The *b1* tandem repeats are transcribed on both strands and generate 24-nt siRNAs (*3*, *6*). Given the *Arabidopsis* RdDM pathway (*4*), these *b1* repeat siRNAs are candidates for a direct role in paramutation, yet they are not sufficient to establish silencing, as evidenced by the fact that 24-nt siRNAs are produced from the single repeat unit in *b1* alleles that cannot induce silencing (*6*). The observation that a transgene, which produces *b1* repeat hairpin RNA and 24-nt siRNAs independently of *B′*, can change *B-I* into a *B′*-like allele (*6*) suggests that either double-stranded hairpin RNA or 24-nt siRNAs from the repeats are somehow mediating paramutation. One spec-

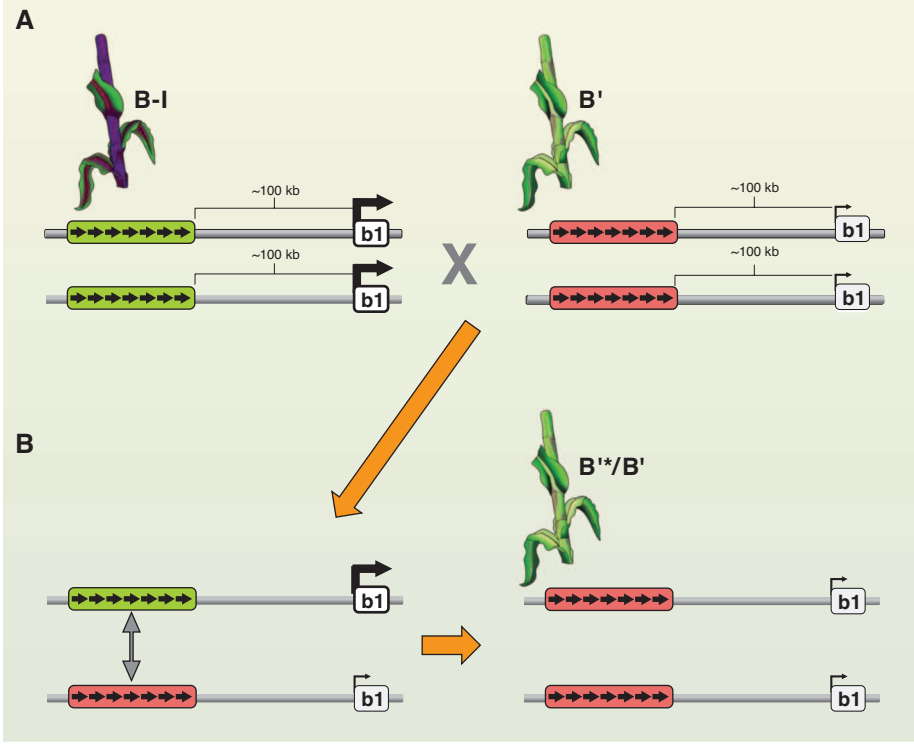

Fig. 1. Properties of *b1* paramutation. (**A**) *B-I* and *B′* phenotypes (dark and light colored plants, respectively) and diagrams of the *b1* locus and associated regulatory regions; because maize is diploid, the two diagrams for each plant represent the two alleles. The *b1* locus (white box labeled *b1*) encodes a transcription factor that activates the anthocyanin biosynthetic pathway, which produces purple coloration. When *b1* is highly transcribed (*B-I*, thick arrow above white box), a dark purple plant is observed. When transcription is low (*B′*, thin arrow above white box), a lightly pigmented plant is observed. *B-I* and *B′* have identical DNA sequences, including seven tandem copies of an 853–base pair (bp) repeat unit, located ~100 kb upstream of the *b1* coding region [indicated by seven black arrows within green (*B-I*) or red (*B′*) blocks]. The green and red blocks symbolize the distinct chromatin structures within the repeats (*7*) in *B-I* and *B′*, respectively. Extensive data demonstrate that the tandem repeats are required for *b1* paramutation and the high expression in *B-I* (*1*, *3*). The repeats have not been found elsewhere in the maize genome and are transcribed noncoding sequences that produce 24-nt siRNAs in both alleles (*3*, *6*). (**B**) The result of crossing *B-I* and *B′* is that *B-I* is always changed into *B′* by unknown mechanisms. The diagram portrays a two-step process (orange arrows), such that before establishment of paramutation both *B-I* and *B′* epigenetic states exist (left). Paramutation is established between early embryogenesis and the formation of 10 leaf primordia (*8*) through unknown mechanisms mediated by the repeats (symbolized by the double-headed gray arrow), resulting in *B-I* being changed into *B′* (right). The new *B′* allele (*B-I* in the previous generation) is denoted *B′**, is mitotically and meiotically stably silenced, and is as capable as *B′* at changing *B-I* into *B′* in subsequent generations (not diagrammed). [Modified from a drawing by M. Arteaga-Vazquez and J. E. Arteaga-Vazquez (*3*)]

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ulation is that repeat RNAs are the targeting signal, but whether the RNA signal (either siRNAs or larger RNAs) is received depends on the chromatin state of the alleles, which has been shown to differ between *B-I, B′*, and the single-copy repeat alleles that do not undergo paramutation (*7*). Another hypothesis that is not mutually exclusive is that differential production of paramutation-associated RNAs occurs in developing embryos (*6*), where paramutation is established (*8*) and where RNAs have not yet been examined. This latter hypothesis is exciting given that cis- and trans-acting small RNAs regulate epigenetic changes during gametogenesis, fertilization, and early zygotic development in multiple species (*9*).

Although the above evidence supports a role for RNA, other factors, such as protein-DNA interactions, could also be involved. For example, interactions between proteins that bind to the *b1* tandem repeats might mediate communication between alleles. Data consistent with that idea are that a transgene overexpressing a protein that binds to the *b1* tandem repeats and forms multimers, inducing a *B′*-like state in *B-I* (*10*). Another possible model, frequently discussed, is that the alleles communicate through DNA pairing (*1*, *2*). Although there is no experimental evidence demonstrating a role for DNA pairing, there is no evidence eliminating it either. It is of course possible that RNA, DNA, and protein interactions are all required for paramutation.

Why are repeats required for paramutation? Tandem repeats create a characteristic sequence at their junctions relative to single-copy sequences; the *b1* tandem repeat junctions have distinct chromatin structures, which have been hypothesized to affect silencing (*7*), potentially through specific proteins or RNAs that associate with these sequences. It has also been suggested that RNAs synthesized from repeats, but not a single-copy sequence, trigger silencing (*6*). A model proposed to explain how centromeric tandem repeats maintain heterochromatin silencing (*11*) offers an hypothesis for how tandem repeats could generate a distinct pool of RNAs relative to nonrepeats. That model suggests a mechanism by which multiple cycles of amplification of RNAs from tandem repeats [as outlined in (*9*)] results in distinct populations of RNA that span the full repeat sequence, as compared to RNA amplification from dispersed copies or single-copy sequences that have reduced sequence complexity (*11*).

Once paramutation is established (*8*), it is maintained through mitotic and meiotic cell divisions. Although the nature of the heritable molecule(s) is unknown, it is unlikely to be *b1* tandem repeat siRNAs, as mitotic silencing is maintained when a mutation dramatically reduces these siRNAs in juvenile and adult tissues (*3*). Analyses of cytosine methylation and histone modifications in *B-I* and *B′* revealed more cytosine methylation within the *b1* tandem repeats in *B′* relative to *B-I* (*7*), whereas histones associated with the *b1* repeats in both alleles did not carry modifications characteristic of silent chromatin. Future studies on the paramutation properties of mutants impaired in DNA methylation and various histone modifications should shed light on the potential role for these marks in paramutation. The observations that RdDM in *Arabidopsis* is associated with cytosine methylation and heterochromatin histone modifications (*4*), yet paramutation does not occur between RdDM silenced alleles (see below), leads to the speculation that paramutation involves additional mechanisms, such as RNA or proteins that remain associated with the *b1* repeats during mitosis and meiosis.

It is puzzling that RNAi-mediated heterochromatin in *S. pombe* and RdDM-silenced genes in *Arabidopsis* do not undergo paramutation (*4*, *5*). For example, specific alleles of *b1* and *FWA* in *Arabidopsis* are both silent when cytosine residues of the respective tandem repeats are methylated and active when hypomethylated. In both systems, the tandem repeats required for silencing are transcribed and produce small RNAs regardless of whether the alleles are active or silent. The methylated, silenced *FWA* allele can initiate trans methylation of an unmethylated transgene, yet, unlike the maize paramutation system, the unmethylated allele segregates normally and is active and unchanged (*12*). It is unclear whether the "natural" active *FWA* allele is protected from silencing, or the transgene is hypersensitive to silencing, or both (*12*). Additionally, the mechanism that makes *B-I* in maize highly sensitized to silencing is also unknown, although several hypotheses have been proposed (*13*).

The relationship with other RNA silencing pathways suggests that paramutation, despite being rare, may underlie fundamental mechanisms for gene regulation (*2*). Speculations on potential roles and consequences include that paramutation provides an adaptive mechanism through the transfer of favorable expression states to progeny, that paramutation could be a mechanism for establishing functional homozygosity in polyploids, and that it might function in inbreeding depression and hybrid vigor or inheritance associated with complex human diseases (*13*).

Independent of paramutation's function or frequency, our understanding of its mechanisms should shed light on potentially novel mechanisms for transmitting epigenetic information across generations.

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10.1126/science.1191044

PERSPECTIVE

Epigenetics in the Extreme: Prions and the Inheritance of Environmentally Acquired Traits

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Prions are an unusual form of epigenetics: Their stable inheritance and complex phenotypes come about through protein folding rather than nucleic acid–associated changes. With intimate ties to protein homeostasis and a remarkable sensitivity to stress, prions are a robust mechanism that links environmental extremes with the acquisition and inheritance of new traits.

In its modern usage, "epigenetics" encompasses all mechanisms for the inheritance of biological traits that do not involve alterations of the coding sequence of DNA (*1*). Considered elsewhere in this issue are well-known epigenetic mechanisms that control access to DNA by modifying nucleotides or associated histones, or involve the transmission of information through RNA. Here, we discuss an extreme case of epigenetic inheritance with a mechanism that is not based on heritable changes in nucleic acid. Instead, it is based on robust self-propagating changes in the folding of certain proteins known as prions.

Prions operate outside the canonical steps of molecular biology's central dogma. As protein-