Progress in understanding of the genetic phenomenon paramutation

Sushil Kumar

Plants have evolved a variety of mechanisms to switch on and switch off genes in relation to their requirements for growth, development and adaptation to the environment. One of these processes is paramutation. This genetic phenomenon permits abrogation of certain traits for some to many generations without physical loss of the concerned genes. The calendar year 2006 is the golden jubilee year of the discovery of paramutation. There is an opportunity to discuss the progress made in recent years in the understanding of mechanism(s) underlying paramutation, especially in the light of the recent paper of Alleman *et al.*[1], which shows that an RNA-dependent RNA polymerase is required for paramutation in maize.

Diploid eukaryotic individuals receive a copy each of all genes from male and female parents. The two copies of a gene carried on separate homologous chromosomes may be in the form of identical or different alleles. Usually, the alleles derived from the two parents express independent of each other. When one allele silences the expression of the other allele in a heritable manner, the phenomenon has been called paramutation. First described by Brink[2] at the *r-1* locus in the maize (*Zea mays*) plant in 1956, now many examples of paramutation are known in animals and plants[3–10]. However, the best dissected case of paramutation pertains to *b-1* locus in maize.

The *B-1* gene of maize specifies a basic helix–loop–helix (bHLH) transcription factor[11], which together with the product of other genes is involved in the activation of anthocyanin biosynthesis pathway. Three alleles are known at the *b-1* locus of maize[7,9,12]. The homozygous *B-1*/*B-1* seedlings or mature plants are intensely pigmented. Contrastingly, plants homozygous for the null allele at *b-1* locus (*b-1*/*b-1*) lack pigmentation. The *B-1* allele is known to spontaneously give rise to *B′* allelic state, at a frequency of about 1 to 10%, in certain maize stocks[7]. The *B′* state once attained, is highly stable and penetrant. *B′*/*B-1* and *B′*/*B′* plants have little pigmentation. *B-1* is invariably changed to the *B′* state in *B′*/*B-1* plants (Figure 1). Thus in the paramutation system at the *b-1* locus, the *B-1* allele is paramutable, *B′* allele is paramutagenic and *b-1* allele is neither paramutable nor paramutagenic[12]. Recent studies address the question on how *B-1* turns to *B′*.

The *b-1* locus and adjoining regions have been sequenced in respect of *b-1*, *B-1* and *B′* allele[13]. A 6 kb region located 100 kb upstream of the transcription start site concerned with enhancer activity was also found to be necessary for paramutability and paramutagenicity. This region comprises seven tandem repeats of a 853 bp sequence, which are identical in *B′* and *B-1* alleles; only one copy of the 853 bp is present at the *b-1* allele. The occurrence of repeats indicated that the double-stranded RNA may be involved

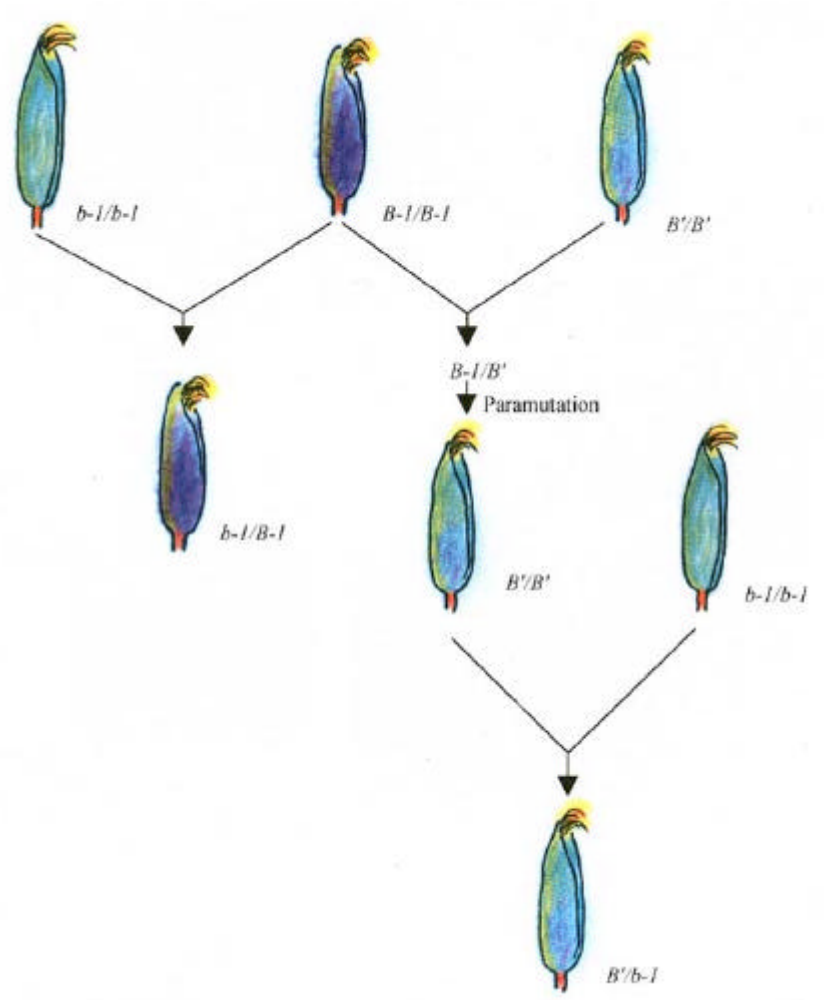
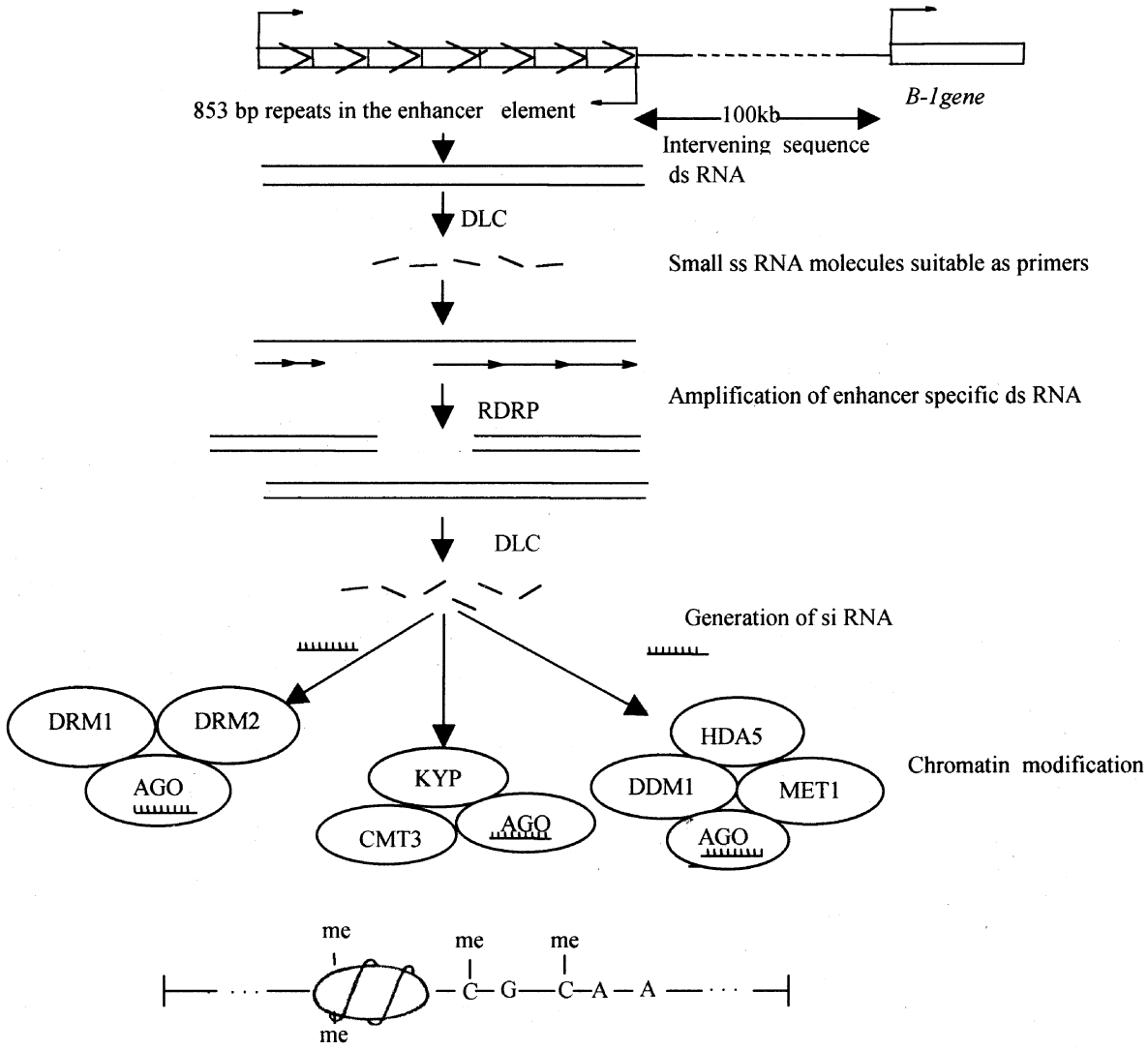


Figure 1. Paramutation at the *b-1* locus in maize, *Zea mays.* The *b-1*/*b-1* homozygous plants do not carry anthocyanin pigmentation, because *b-1* is a null allele of the wild type gene *B-1* that is responsible for activation of the anthocyanin biosynthesis pathway. Contrastingly, *B-1/B-1* plants are darkly pigmented with anthocyanin. The *B′*/*B′* plants carry paramutated/paramutagenic allele of *b-1* locus in homozygous condition and because *B-1* is paramutated or silenced to the *B′* state, the plants are lightly pigmented with little anthocyanin. While the F_1 progeny from the cross *b-1*/*b-1* × *B-1*/*B-1* is intensely pigmented, progenies from the crosses *B-1*/*B-1* × *B′*/*B′* and *B′*/*B′* × *b-1*/*b-1* are lightly pigmented. The *B-1* allele in the heterozygous *B-1*/*B′* plants gets paramutated to the *B-1′* or *B′* state. Therefore, all the progeny plants from *B-1′*/*B′* or *B′*/ *B′* × *b-1*/*b-1* crosses are highly pigmented.



Heterochromatic silencing of the enhancer element and thereby the paramutation of B-1 gene to its B' state.

Figure 2. Model for paramutation at the *b-1* locus. The enhancer element of the *B-1* gene located 100 kb upstream has seven copies of a 853 bp sequence. The transcription product(s) of this region is/are double-stranded RNA (dsRNA) molecules. The DICER (DCL3)-mediated cleavage of this product generates small single-stranded RNA (ssRNA) molecules. More dsRNA of the enhancer sequence is synthesized by the action of RNA-dependent RNA polymerase (RDRP). Thus more of small ssRNA or small interfering RNA (siRNA) becomes available. The locus-specific siRNA or RNAi then guides silencing of the enhancer element via its heterochromatization. Chromatin modification may occur variously. First, by the action of a complex of *DOMAIN REARRANGED METHYLTRANSFERASEs* (*DRM1* and *DRM2*) that carries out *de novo* cytosine methylation and *ARGONAUTE* (*AGO4*) protein and siRNA. Second, by the complex included with *CHROMOMETHYLTRANSFERESE 3* (*CMT3*) that methylates cytosine in non-CG-contexts and *KRYPTONITE* (*KYP*) histone methyltransferase involved in methylation of lysine-9 position of H3 histone (H3mK9). The third siRNA-guided heterochromatizing complex has in it the cytosine methyltransferase for CG-methylation (*MET1*), histone deacetylase (*HDA6*) and *DECREASE IN DNA METHYLATION* (*DDM1*), which is essential for both cytosine and H3mK9 methylation. Heterochromatization or gene silencing is probably achieved by histone methylation or histone and cytosine DNA methylations of the target chromatin. This model of *B-1* silencing is based on the current understanding of the heterochromatic gene silencing process[14,15]. Alleman *et al.*[1] have shown that *B-1* to *B′* silencing does not occur in *RDRP* or *MOP-1* mutants in *Zea mays.* Why paramutation occurs in a fraction of progeny of some seemingly wild-type strains, but not in others remains an enigma.

in paramutational silencing of the *b-1* gene and related functions. Indeed, nuclear run-on assays revealed that the tandem repeats required for paramutation at the *b-1* locus are transcribed from both strands[1].

When large size populations of *B′/B-1* were examined, some plants were found to be darkly anthocyanin-pigmented. The pigmented plants were homozygous for mutation in a separate locus called *mop-1* (mediator of paramutation 1)[12]. Thus,

whereas *B′/B-1*, *Mop-1/Mop-1* or *B′/B-1*, *Mop-1*/*mop-1* plants are lightly pigmented, the *B′/B-1*, *mop-1*/*mop-1* plants are densely pigmented. That is, *mop-1* mutation modifies the phenotype of *B′/B-1* plants to that of *B-1*/*B-1* plants, by preventing the establishment of the *B′* state in the *B-1* allele in the *B′/B-1*, *mop-1*/*mop-1* plants. The initial *mop-1-1* mutant allele was due to mutator-caused spontaneous mutation. Subsequently, an ethylmethane sulphonate-induced *mop-1-2* allele has been recovered[1]. A syntenic comparison between the maize and rice maps with respect to the *mop-1* region and sequencing of *mop-1* established that the *mop-1* gene specified an RNA-dependent RNA polymerase gene (RDRP), equivalent to RDRP known to be associated with the production of short interfering RNA (si RNA) that targets chromatin in plants[1].

Although the presence of siRNA has not yet been demonstrated in *B′/B-1*, *Mop-1*/*Mop-1* or *B′/B-1*, *Mop-1*/*mop-1* plants, data gathered by Alleman *et al.*[1] favour trans-acting RNAi-directed establishment of epigenetic marks, in the form of DNA and/or histone modification, on *B′* for its continued heritable silencing over generations (Figure 2). This model of establishment of paramutation will find additional support, if mutations in the gene for the argonaute protein(s), dicer enzyme(s), and other protein components of the RNA-induced transcriptional silencing (RITS)[14,15] apparatus, besides RNA-dependent RNA polymerase, are also found to lose paramutagenic activity of the *B′* allele. An intriguing fact that remains to be understood is why the *B-1* allele spontaneously turns into the *B′* allele in certain maize lines with high frequency[7]. What is the genetic cause of instability in these lines? Are the events in such lines associated with the hyperactivity of the RITS complex based on certain internal or external biotic and abiotic signals?

The fact that the mutated *mop-1* gene obviates occurrence of paramutation, not only at the *b-1* locus but also at the other loci such as *r-1* and *pl-1*, means that the mechanism of paramutation at different loci may be common in maize, i.e. via synthesis of double-stranded RNA from repeat sequences which somehow silences the corresponding *b-1*, *r-1* and *pl-1* 1oci in trans[1,12]. Recently, evidence has been adduced for si-, mi- or some other kind of small regulatory-RNAs to be responsible for the occurrence of a kind of paramutation in mouse[10]. Thus RNA has been identified as the medium of crosstalk between alleles of genes in diploid nuclei, for affecting expression of one by the other, both in plant and animal systems. Undoubtedly, understanding of paramutation will parallel developments in the field of genetics of epigenetics and will uncover new principles about the dynamics of gene regulation in eukaryotic organisms.

1. Alleman, M. *et al.*, *Nature*, 2006, **442**, 295–298.
2. Brink, R. A., *Genetics*, 1956, **41**, 872–890.
3. Cavalli, G. and Paro, R., *Cell*, 1998, **93**, 505–518.
4. Henikoff, S. and Comai, L., *Cell*, 1998, **93**, 329–332.
5. Garrick, D., Fiering, S., Martin, D. I. and Whitelaw, E., *Nature Genet.*, 1998, **141**, 347–360.
6. Rassoulzadegan, M. *et al.*, *Nature*, 2006, **441**, 469–474.
7. Coe Jr., E. H., *Genetics*, 1966, **53**, 1035–1063.
8. Brink, R. A., *Annu. Rev. Genet.*, 1973, **7**, 129–152.
9. Chandler, V. L., Eggleston, W. B. and Dorweiler, J. E., *Plant Mol. Biol.*, 2000, **43**, 121–145.
10. Vaucheret, H. and Fagard, M., *Trends Genet.*, 2001, **17**, 29–35.
11. Goff, S. A. *et al.*, *Genes Dev.*, 1992, **6**, 864–875.
12. Dorweiler, J. E., Carey, C. C., Kubo, K. M., Hollick, J. B., Kermicle, J. L. and Chandler, V. L., *Plant Cell*, 2000, **12**, 2101–2118.
13. Stam, M., Belele, C., Dorweiler, J. E. and Chamdler, V. L., *Genes Dev.*, 2002, **16**, 1906–1918.
14. Baulcombe, D., *Nature*, 2004, **431**, 356–363.
15. Reyes, J. C., *Curr. Opin. Plant Biol.*, 2006, **9**, 21–27.

Sushil Kumar is in the National Centre for Plant Genome Research, Post Box 10531, Aruna Asaf Ali Marg, New Delhi 110 067, India
e-mail: sushil2000_01@yahoo.co.in