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Maize sex determination and abaxial leaf fates are canalized by a factor that maintains repressed epigenetic states

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Abstract

In maize (*Zea mays* ssp. *mays*), the meiotically heritable maintenance of specific transcriptionally repressed epigenetic states is facilitated by a putative RNA-dependent RNA polymerase encoded by *mediator of paramutation1* (*mop1*) and an unknown factor encoded by the *required to maintain repression6* (*rmr6*) locus. These so-called "paramutant" states occur at certain alleles of loci encoding regulators of anthocyanin pigment biosynthesis. Here we show *Rmr6* acts to canalize leaf and inflorescence development by prohibiting the ectopic action of key developmental regulators. Phenotypic and genetic analyses suggest that *Rmr6* ensures proper adaxial–abaxial polarity of the leaf sheath by limiting the expression domain of a putative adaxializing factor. Similar tests indicate that *Rmr6* maintains maize's monoecious pattern of sex determination by restricting the function of the pistil-protecting factor, *silkless1*, from the apical inflorescence. Phenotypic similarities with *mop1* mutant plants together with current models of heterochromatin maintenance and leaf polarity imply *Rmr6* functions to maintain epigenetic repression established by non-coding small RNA molecules.

Keywords: *Rmr6*; Epigenetics; Sex determination; *Sk1*; Leaf polarity; Paramutation; *Zea mays*

Introduction

The observation that largely uniform development occurs despite genotypic or environmental variations implies that the regulation of genes controlling developmental pathways is to some extent canalized or buffered (Waddington, 1942). Brink (1964) proposed a two-tiered system in which developmental patterns of gene expression are established by the action of developmental signals on specific DNA regulatory elements, while more generalized epigenetic chromosome-level alterations maintain these expression patterns independent of the original developmental stimuli. Consistent with Brink's hypothesis, patterns of activity and repression of *Drosophila* homeotic genes are initiated by a series of transcriptional regulators and subsequently maintained by specific chromatin structures (reviewed in Bantignies and Cavalli, 2006). In plants and many other animals, a similar relationship between specific chromatin structures and reversible DNA modifications is suggested by observations that the maintenance of cytosine methylation patterns is often important for ensuring proper development (reviewed in Attwood et al., 2002; Grant-Downton and Dickinson, 2006). It seems reasonable to presume that the degree to which a particular gene expression pattern is canalized, in a sense, reflects the fidelity of maintaining these chromosome-level epigenetic marks.

Recent studies suggest the establishment and maintenance of repressive chromatin structures in eukaryotes involves non-coding small RNA molecules (Bernstein and Allis, 2005). In *Arabidopsis*, heterochromatin appears to be maintained through the action of short interfering RNAs (siRNAs) produced by a process requiring a double-stranded RNase (DICER-LIKE3), RNA-DEPENDENT RNA POLYMERASE2 (RDR2) (Xie et al., 2004), and a form of RNA polymerase IV (Pontier et al., 2005). These siRNAs appear to target cytosine methylation to transposons and repetitive sequences through interaction with a small RNA binding protein, ARGONAUTE4 (Chan et al., 2006;

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Zilberman et al., 2004), and an alternate form of RNA Polymerase IV (Pontier et al., 2005).

MicroRNAs (miRNAs) represent another class of small non-coding RNA produced using similar cellular machinery, but these appear to act primarily at a post-transcriptional level to effect gene repression. Through binding complementary sequences, miRNAs can target RNA transcripts for degradation (Llave et al., 2002) or inhibit translation (Olsen and Ambros, 1999). By either of these two modes of action, miRNAs ensure developmentally directed expression patterns of specific genes. Recent results from Bao et al. (2004) suggest that miRNAs also function to direct cytosine methylation at genomic targets. They found miRNA-dependent cytosine methylation 3′ of the miR-165/166 complementary site of the *Arabidopsis PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) genes, which each encodes a class III homeodomain-leucine zipper (HD-ZIP III) transcription factor. The miR-165/166 complementarities in both *PHB* and *PHV* span introns, and dominant mutations that disrupt splicing of these introns (and therefore the miR-165/166 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 suggests that the miRNA interacts with the nascent RNA transcript to direct methylation to the *PHB* gene. These findings suggest a small RNA-based link between the gene-specific and chromosome-level tiers of control that Brink hypothesized are required for proper development.

Small-RNA-directed chromatin modifications are also implicated in paramutation, a meiotically heritable alteration in regulatory states influenced by certain allelic interactions (Alleman et al., 2006; Chandler et al., 2000). The maize (*Zea mays* ssp. mays) *mediator of paramutation1* (mop1; Dorweiler et al., 2000) locus, which encodes a protein similar to RDR2, is required for maintenance of epigenetic repression on alleles subject to paramutation (Alleman et al., 2006; Woodhouse et al., 2006). In *Arabidopsis*, *RDR2* is required for *de novo* methylation of tandemly repeated promoter sequences of a *FWA* transgene (Chan et al., 2004), for maintenance of cytosine methylation found in CpNpG and CpNpN contexts, and for histone 3 lysine 9 methylation (H3mK9) at *AtSN1* retroelement sequences (Xie et al., 2004). Because a putative maize RDR2 ortholog is required for maintaining repressed expression states of complex transgenes (McGinnis et al., 2006), RNAi-silenced transposons (Woodhouse et al., 2006), and paramutant alleles (Dorweiler et al., 2000), it has been suggested that paramutation involves the establishment of repressive chromatin structures (Alleman et al., 2006).

Plants deficient for *mop1* display stochastic occurrences of delayed flowering, decreased stature and tassel feminization (Dorweiler et al., 2000), implying a mechanistic connection between paramutation and developmental gene control. Developmental phenotypes associated with mutations in *required to maintain repression6* (*rmr6*), another *trans*-acting factor involved in maintaining paramutation-induced repression, strengthens this connection. The particular defects seen in *rmr6* mutant plants suggest a relationship between the epigenetic marks maintained on alleles subject to paramutation and those used to canalize the developmental programs responsible for domesticated maize leaf and inflorescence architecture. Using a genetic approach, we find *Rmr6* maintains tissue-specific repression of developmental regulators controlling flowering time, internode elongation, developmental phase transition, leaf adaxial/abaxial (dorsal/ventral) polarity, and sex determination. The phenotype of double mutants with the adaxializing *Rolled1-O* (*Rld1-O*) mutation leads to the hypothesis that *Rmr6* ensures proximal leaf adaxial–abaxial polarity by maintaining regional expression domains of an adaxializing factor. Epistasis data show that *Rmr6* restricts the *silkless1* (*sk1*)-encoded pistil protection activity from the terminal inflorescence of the primary axis (apical inflorescence or tassel). These results imply that the expression patterns of key developmental regulators are maintained, at least in part, by the same cellular machinery that restricts transcription of specific alleles of the *purple plant1* (*pl1*), *booster1* (*b1*), and *red color1* (*r1*) loci subject to paramutation (Hollick et al., 2005).

Materials and methods

Nomenclature

Nomenclature designations follow species-specific guidelines (Lawrence et al., 2007; http://www.arabidopsis.org/portals/nomenclature/namerule.jsp). Maize chromosomes, loci, alleles, and allelic states are designated by italic type. Recessive alleles and loci are designated in lower case, while the first letter of dominant alleles, including non-mutant alleles, is capitalized. In the presentation of double mutant analysis between *rmr6* and *sk1* mutants, non-mutant alleles are followed by a "+" and mutant alleles (including both *rmr6-1* and *rmr6-2* alleles) are followed by a "−". Chromosome translocation breakpoints are designated with a "*T*". Regulatory states of the *Pl1-Rhoades* allele are written as *Pl-Rh* for the fully expressed state, and *Pl′* for the repressed paramutant state (Hollick et al., 2005). Diploid genotypes are written with the pistillate (maternal)-derived allele first, followed by the staminate (paternal)-derived allele. For *Arabidopsis*, wild-type alleles are written in upper case italics while mutant alleles are written in lower case italics. Gene products for both species are written in upper case unitalicized type.

Germplasm and genetic crosses

Hand pollinations were used for all genetic crosses and detailed pedigree information is available upon request. For morphometric measurements, lines were constructed as detailed in Hollick et al. (2005) and the mean values were compared using 2-sample *z* tests. For comparisons of flowering time and plant height, 4 and 7 individual progenies segregating 1:1 for +/*rmr6-1* and *rmr6-1*/*rmr6-1* sibling plants were used, respectively. Internode length and leaf wax distribution were measured in *Pl′*/*Pl′* lines homozygous for the *T6-9 (043-1)* (*T*) interchange chromosome (Longley, 1961) that were segregating 1:1 for either +/*rmr6-1* and *rmr6-1*/*rmr6-1* or +/*rmr6-2* and *rmr6-2*/*rmr6-2* siblings. Stocks containing recessive *tassel seed* mutations (*ts1*, *ts2*, *ts4*, *ts*-PI200203*, *ts*-PI251881*, *ts*-PI267209*, *ts*-N2490*) and *sk1* were obtained from the Maize Genetics Cooperation Stock Center (USDA-ARS, University of Illinois, Urbana, IL). The *Rld1-O* allele was provided by Dr. M. Timmermans (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

For *sk1* epistasis analysis, F2 families segregating for mutations in both *rmr6* and *sk1* were derived from +/*rmr6-1*; *T Pl′*/*T Pl′* and *pl1*/*pl1*; *sk1*/*sk1* parents. To ensure proper identification of *rmr6* mutant homozygotes using anther pigment phenotypes, *Pl′*/*Pl′* individuals were enriched among F2 kernels. With the exception of rare recombination events, kernels with waxy endosperm are homozygous for both the *T6-9* interchange and *Pl′* as both *wx1* and *pl1* loci are linked (2.3 and 1.4 cM, respectively) to the interchange breakpoint (Hollick et al., 2005). To increase the likelihood of obtaining *rmr6-1*/*rmr6-1*; *sk1*/*sk1*