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Phenotypic Diversity Mediated by the Maize Transposable Elements *Ac* and *Spm*

SUSAN R. WESSLER

Mutations caused by the insertion of members of the *Ac* or *Spm* family of transposable elements result in a great diversity of phenotypes. With the cloning of the mutant genes and the characterization of their products, the mechanisms underlying phenotypic diversity are being deciphered. These mechanisms include (i) imprecise excision of transposable elements, which can result in the addition of amino acids to proteins; (ii) DNA methylation, which has been correlated with the activity of the element; (iii) transposase-mediated deletions within elements, which can inactivate an element or lead to a new unstable phenotype; and (iv) removal of transcribed elements from RNA, which can facilitate gene expression despite the insertion of elements into exons. An understanding of the behavior of the maize elements has provided clues to the function of cryptic elements in all maize genomes.

MAIZE TRANSPOSABLE ELEMENTS ARE RESPONSIBLE FOR a remarkable variety of unstable mutant phenotypes. The phenotypes of several alleles, each containing the transposable element *Ds*, for example, can differ in (i) the frequency, timing, and quality of somatic mutations caused by excision of *Ds* from the gene during plant development and (ii) the residual level of gene expression observed in the absence of *Ds* excision. Plant geneticists had characterized these distinctive patterns of somatic mutation before recombinant DNA techniques were available. The accuracy of these early studies is a tribute to the geneticists and to their choice of experimental organism.

This review focuses on well-characterized examples of phenotypic diversity generated by transposable elements in maize. Remarks are largely confined to mutations caused by the *Ac* and *Spm* (or *En*) families of maize transposable elements. For a more detailed discussion of plant transposable elements, the reader is referred to several excellent reviews on the genetics and molecular biology of transposable elements in maize in particular (*1, 2*) and plants in general (*3, 4*).

The maize plant is an ideal genetic system for studying the phenotypic subtleties displayed by controlling element alleles. Sensitive genetic markers are available for observing the insertion and excision of the elements in virtually all organs. Notable among these are seven loci (*a*, *a2*, *c*, *c2*, *bz*, *bz2*, and *R*) involved in the biosynthesis and deposition of anthocyanin pigments in different organs of the maize plant. Mutation of any one of these loci eliminates or alters the wild-type purple color in some or all tissues. In addition to the anthocyanin loci, genes involved in the production of endosperm starch [that is, *shrunken* (*sh*) or *waxy* (*wx*)], when mutated, can alter the morphology of the kernel or the quality of the starch. These also are easily scored markers that have been important in the study of the genetics and molecular biology of maize.

Nomenclature

The maize transposable elements studied by McClintock were called controlling elements to emphasize their ability to regulate the

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expression of genes into which they are inserted (*5*). The first element recognized to be transposable was discovered as a site of chromosome breakage and was named *dissociation* (*Ds*) (*6, 7*). It was soon revealed that *Ds* could transpose or break chromosomes only in the presence of another genetic locus named *activator* (*Ac*), which could also promote its own transposition (*8*). Together *Ac* and *Ds* constitute a transposable element family that includes autonomous (*Ac*) and nonautonomous (*Ds*) elements.

The *Spm* family also has both autonomous and nonautonomous elements. The autonomous element was called *Suppressor mutator* (*Spm*) by McClintock (*9*) and *enhancer* (*En*) by Peterson (*10*). The nonautonomous elements studied by McClintock have recently been called defective *Spm* (*dSpm*) to reflect their relation to the autonomous element (*11*). Peterson called the nonautonomous elements *inhibitor* (*I*) (*10*). In this review, all nonautonomous elements of this transposable element family are called *dSpm*, autonomous elements characterized by Peterson are called *En*, and the autonomous elements discovered by McClintock are called *Spm*. Fully active *En* and *Spm* are nearly identical in their genetic (*12*) and molecular (*13, 14*) properties.

The Meaning of Phenotypic Diversity

Somatic mutations produce sectors. Plants exhibit clonal development, whereby areas of the plant are derived from single or small populations of meristematic cells (*15*). Thus, sectors of revertant tissue can result from the excision of transposable elements and the restoration of gene activity. The size of the sector is related to the number of mitotic divisions that have occurred subsequent to excision. The shape of the sector is determined by the cell lineage of the tissue. This type of development permits visual selection of unstable alleles with differences in timing, frequency, or intensity of these sectors (*16*).

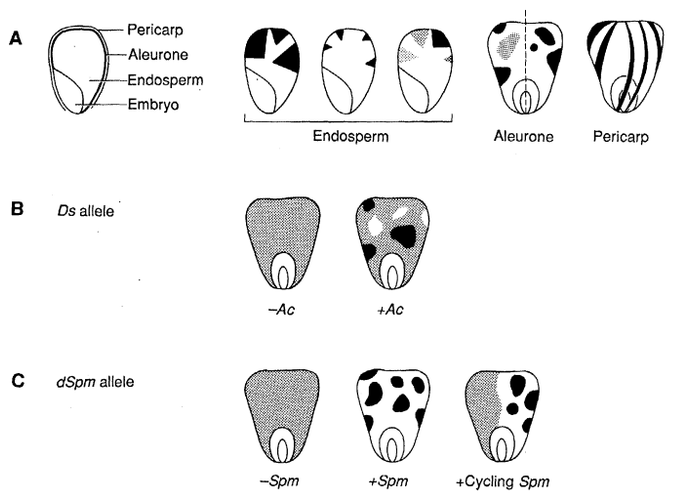

Fig. 1. Diagrammatic representation of phenotypes displayed by selected controlling element alleles. Each illustration represents a typical kernel of the designated allele and is not meant to suggest that the pattern of somatic mutation is invariant. (**A**) A longitudinal section of a kernel and examples of unstable phenotypes. Representing endosperm phenotypes are, from left to right, *Acwx-m7* (*29*), *wxB4* (+*Ac*) (*85*) and *wx-m1* (+*Ac*) (*8*). Kernels were stained with IKI to reveal the amylose sectors. Anthocyanin biosynthesis in the aleurone is illustrated by the *Ds* allele *c-m2* (+*Ac*) (*8*). Kernels were cut in the plane of the dotted line to produce the sections used to illustrate *waxy* expression in the endosperm. An unstable pericarp phenotype is illustrated by the *Ac* allele *P-vv* (*16*). (**B** and **C**) Examples of alleles with intermediate expression; the *Ds* allele *r-m3* (with and without *Ac*) (*86*), and the *dSpm* allele *a-m1(5996)* (with and without *Spm*) (*57*). The phenotype of this *dSpm* allele is also shown in the presence of an *Spm* element that cycles between active and inactive phases.

The maize kernel is ideal for analyzing the interactions of transposable elements and genes. Examples of the range of unstable mutant phenotypes expressed in three of the tissues that make up the kernel are shown in Fig. 1A. The endosperm is starchy tissue that nourishes the embryo during germination. The enzyme encoded by the *waxy* gene, uridine diphosphate (UDP)–glucosyl transferase, is responsible for the synthesis of amylose in the triploid endosperm. Insertions of transposable elements usually inactivate the gene, whereas excision of a transposable element can restore expression. Excisions of *Ds* from *waxy* that restore expression are monitored by staining sections of kernels with an iodine–potassium iodide solution, which indicates the presence of amylose. These excision events result in the formation of pie-shaped sectors; large sectors represent a single excision early in endosperm development, whereas small sectors signify later events.

The diversity of unstable *waxy* phenotypes is illustrated in Fig. 1A. For example, some unstable alleles of *waxy* give rise preferentially to large sectors, whereas others display only small sectors, suggesting a preference for late excision. Finally, other *waxy* alleles give rise to sectors with different levels of amylose, indicating that excisions can differ within the same kernel.

Most of what is known about *Ac* and *Spm* comes from the analysis of unstable phenotypes expressed in the single-cell-thick aleurone layer of the kernel (Fig. 1A). The aleurone layer is a site of anthocyanin pigment biosynthesis. Like the *waxy* alleles, unstable alleles of several genes affecting aleurone pigmentation give rise to characteristic patterns with respect to the size, frequency, and intensity of sectors. The analysis of anthocyanin expression is carried out in strains that have colorless pericarp.

The pericarp is the protective covering of the kernel that is genetically identical to the maternal parent because it is derived from the ovary wall. The *P* locus conditions red pigment in both the pericarp and cob. Variegated pericarp (*P-vv*) was the first unstable allele characterized in maize (*17*). Since the pericarp surrounds the kernel, somatic mutations resulting from the excision of *Ac* from *P-vv* produce red revertant stripes on a colorless background (Fig. 1A). Broad stripes are early excision events, whereas narrow stripes represent late events.

Insertion does not always abolish gene expression. Two additional features of allelic diversity are illustrated (Fig. 1, B and C). First, the insertion of maize controlling elements does not always abolish gene expression. Examples of *Ds* and *dSpm* alleles of genes responsible for aleurone pigmentation are shown. In the absence of either *Ac* or *Spm*, these elements cannot excise, and stable intermediate phenotypes are evident. Second, the introduction of *Ac* or *Spm* reveals a fundamental difference between these element families. *Ac*-mediated *Ds* excision can produce null sectors or revertant sectors; the background of intermediate expression is retained (Fig. 1B). However, *Spm* suppresses any residual gene expression seen in the absence of *Spm* and promotes *dSpm* excision, which can restore gene expression in sectors (Fig. 1C). In addition, there are alleles of *Spm* and *Ac* that can cycle between active and inactive phases. In the presence of a cycling *Spm* element, the *dSpm* allele reveals that both *sp* and *m* functions of *Spm* cycle on and off together (*18*) (Fig. 1C).

Somatic mutations can be inherited. Unlike the situation in animals, where a germ line is segregated early in development, plant germ cells differentiate from apical meristems that can produce both vegetative and reproductive organs. Thus, somatic mutations caused by the excision of transposable elements in the apical meristem can be passed on to gametes. Although endosperm does not form gametes, the patterns of somatic mutations seen in this tissue usually reflect the mutation frequency occurring in the rest of the plant.

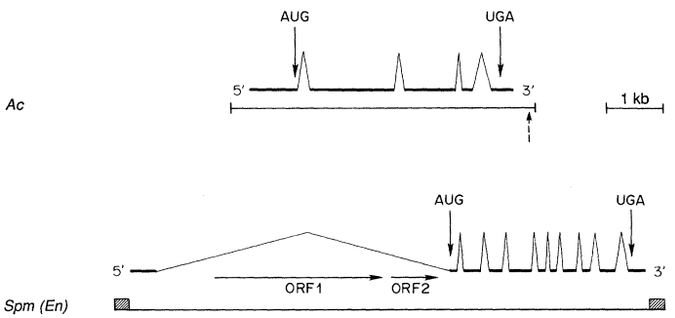

Fig. 2. Organization of the transcription units of the *Ac* (*26*) and *Spm*(*En*) (*13, 14*) elements. The 5′ and 3′ ends of the major transcripts are shown as are the positions of translation initiation and termination. The dashed arrow indicates the position of the splice donor sequences used to splice some *Ds* elements from exons (*65*). Heavy lines represent exons that are connected by fine lines representing introns. The shaded regions of *Spm* indicates the position of the subterminal repeats described in the text.

Consequently, unstable kernel phenotypes are often predictive of the frequency of new alleles that can be isolated from controlling element alleles and the phenotypes that will result. For example, unstable alleles that display large, early sectors in endosperm tissue usually give rise to a higher frequency of revertants or new mutants than unstable alleles with small, late sectors. Excisions that occur early in development, before the differentiation of gametes, are more likely to be inherited. Similarly, alleles that produce sectors with both intermediate and full expression will usually give rise to revertants that stably express either intermediate or full expression of the gene in question.

Structure of *Ac/Ds* and *Spm(En)/dSpm* Elements

The Ac *family of elements*. The six *Ac* elements cloned to date (*19–23*) are all 4.6 kb in length and have identical restriction maps. *Ac* elements from the *wx-m7* and *wx-m9* alleles have been sequenced and found to be identical (*24, 25*). *Ac* has a 10-bp inverted repeat in its 11-bp termini, which are flanked by an 8-bp direct repeat that is generated on insertion. The element encodes a 3.5-kb transcript that spans most of *Ac* and is present at low concentrations (one transcript in 20 cells) in all tissues of strains containing an active element (*26*) (Fig. 2). Previous genetic and molecular studies indicated that all of the functions of *Ac* are encoded by a single transcription unit. These functions are (i) the activation of *Ac* transposition and the transposition of one or many *Ds* elements in trans and (ii) contribution to an *Ac* dosage series; as the dosage of active *Ac* elements is increased, the time of transposition during plant development is delayed (*7, 27*).

Ds elements form a diverse group that can roughly be divided into three classes. The first class, deletion derivatives of *Ac*, was predicted by early genetic analysis of *Ac* alleles that gave rise, at low frequency, to *Ds* alleles (*28, 29*). These *Ds* derivatives led McClintock to conclude that *Ds* elements were defective *Ac* elements. A second class of elements, called *Ds2* elements, have internal sequences unrelated to the *Ac* element, flanked by less than 1 kb of sequences from the *Ac* termini. These composite structures are between 1.3 and 1.5 kb in size (*30*). The third class, *Ds1* elements, are short (about 400 bp) and are homologous only with the terminal 30 bp at the 3′ end and 11 bp at the 5′ end of *Ac*. Up to 75% of the rest of the element consists of A and T residues (*31*). The *Ac*-like elements and the *Ds1* elements are each repeated up to 30 times in all maize genomes examined and are present in the genomes of the maize relatives teosinte and *Tripsacum* (*31, 32*).

The Spm (En) *family of elements*. The *Spm(En)* element has a more complex structure than *Ac* (*13*) (Fig. 2). The *En* element shown in Fig. 2 was isolated from the *wx-844* allele; it is 4 bp shorter than the *Spm* element isolated from the *a-m2* allele and the rest differs by only 6 bp from the *Spm* element (*14*). All elements in this family have 13-bp inverted repeat termini and generate a 3-bp direct repeat upon insertion (*33*). The extended termini are highly structured ends resulting from a 13-bp sequence (different from the 13-bp termini) that is repeated 9 times in the left end and 15 times in the right (shaded area in Fig. 2). The major transcript isolated from strains harboring an active element spans most of the element and is called Gene 1 (*13*). Intron 1 contains two large open reading frames (ORFs), and minor transcripts homologous with this region have been detected (*13, 14*). Evidence (see below) suggests that Gene 1 encodes *sp* function and ORF1 may be part of the transposase.

All *dSpm* elements analyzed to date are deletion derivatives of *Spm* (*11, 14, 33–36*). A determination of the sequences required for transposition was based on the analysis of *dSpm* elements with apparent alterations in excision frequencies (see below).

A Superfamily of Plant Transposable Elements?

The autonomous transposable elements *Tam1* (transposon *Antirrinhum majus*) and *Tam3* are remarkably similar to *Spm* and *Ac*, respectively (*37*). *Ac* and *Tam3* both generate 8-bp direct repeats upon insertion; the 7 bp at the ends of both elements are identical (*38*); and *Tam3* contains a single intronless transcription unit that, when compared to *Ac*, has colinear regions with 50% to 65% amino acid identity (*39*). Similarities between *Tam1* and *Spm* are even more striking. Both generate 3-bp direct repeats on insertion; 12 bp of the 13-bp inverted repeat termini are identical (*40*); the major transcription unit spans the length of both elements (*39*), even though *Tam1* is 14.9 kb long; and long ORFs within introns of the two elements have similar regions at the DNA (56%) and amino acid (50% to 70%) levels (*39*). Because of the similarity of these ORFs and the identity of the inverted termini and number of bases in the direct repeat, it has been suggested that ORF1 of *Spm* encodes all or part of the transposase (*m* function) (*39*).

A superfamily of plant transposable elements has been suggested with the finding that the *Tgm1* (transposon *Glycine max*) element isolated from a lectin gene of soybean has inverted repeat termini that are almost identical to those of *Spm* (11 of 13 bp) and generates a 3-bp direct repeat on insertion (*41*).

The finding of elements similar to *Ac* and *Spm* in distantly related dicotyledonous plants is surprising in light of recent estimates that the *Ac* and *Spm* families may be just two of possibly dozens or hundreds of transposable element families in maize (*42*). For some reason, the first elements characterized both genetically and molecularly in plants happen to be related. It is likely that these elements were studied in detail because they frequently cause unstable mutations, perhaps because they are the most active elements in maize and certain other plants.

Molecular Basis for Phenotypic Diversity

Insertion and excision. Elements of the *Ac* and *Spm* families can apparently insert into any maize gene, since unstable alleles containing *Ac* and *Spm* elements have been isolated for most loci (*43*). However, when *Ac* and *Ds* elements transpose, they usually transpose to regions on the chromosome near the site from which they excised (*44, 45*). Genetic analysis of the transposition of *Ac* from the variegated pericarp locus (*P-vv*) (Fig. 1A) indicated that two-thirds

of *Ac* transpositions occurred after *Ac* replication and were to an unreplicated site within four map units of the *P* locus (*45, 46*). Molecular characterization of these transposed *Ac* elements indicated that most insertion sites were hypomethylated (*47*). Since 80% of maize DNA is methylated (*48*), these results suggest that either *Ac* has a preference for hypomethylated targets or that the insertion of *Ac* leads to hypomethylation of adjacent DNA (*47*). *En* elements also have a tendency, though less pronounced, to transpose locally (*49*).

The new alleles derived from *Ac* alleles frequently contain the transposed element within four map units of the original site of insertion (*44, 45*). Revertants containing such tightly linked *Ac* elements are an excellent source of new *Ac* alleles resulting from transposition of the *Ac* element back into the gene from which it originally transposed. For example, several new *Ac* alleles of the *P* (*50*) and *R* (*51*) loci were isolated from revertants of the *Ac* alleles *P-vv* and *mR-nj*. One striking feature of these new *Ac* alleles of *P* and *R* was that each had a unique unstable phenotype with respect to the frequency, timing, and intensity of revertant sectors (*49, 51*). These alleles could have arisen from mutations within the *Ac* element or from differences in the sites of *Ac* insertion within the *P* or *R* genes. Since genetic tests indicated that the *Ac* elements harbored by these diverse alleles had not mutated, it was concluded that variations in the position of *Ac* within the gene resulted in distinct alleles (*51*).

The precise location of several independent insertions of members of the *Ac* and *Spm* families has been determined [reviewed in (*2*)]. Most mutations result from insertion into translated exons; however, targets in introns, untranslated exons, and 5′ flanking regions (within 200 bp of the start of transcription) have also been described. One reason that the position of insertion within a gene can strongly influence the unstable phenotype is that excision of controlling elements often changes the original target sequence (*24, 31, 32, 52, 53*). Although revertants of unstable alleles are phenotypically wild type, they usually contain, at the site of excision, all or part of the target sequence duplication generated on insertion. These additional nucleotides (called transposon footprints) often include transversions and inversions of the target sequence.

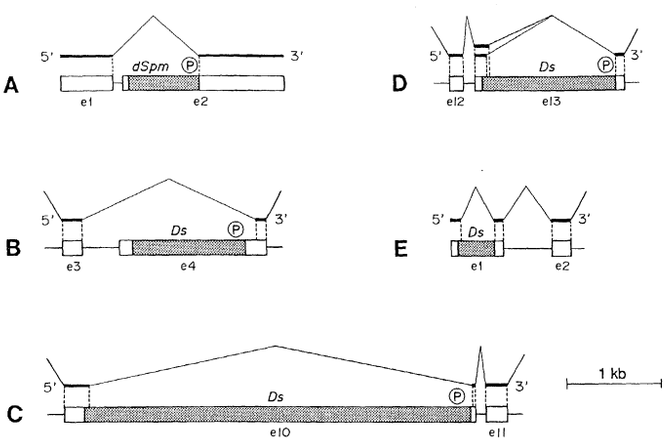

Fig. 3. The splicing of *dSpm* and *Ds* elements from RNA. The examples shown are (**A**) *bz-m13 CS9* (*63*); (**B**) *adh-2F11* (*64*); (**C**) *wx-m9(Ds)* (*65*); (**D**) *wxB4* (*65*), and (**E**) *adh-Fm335* (*32, 66*). For each, the unshaded boxes marked e1, e2, and so on represent the exons, and the fine lines connecting them are the introns in the wild-type gene. The shaded region indicates that a *dSpm* or *Ds* element has been inserted into that position of the exon. The pre-mRNA is drawn above these boxes. Heavy lines are above genomic sequences that are exons in the mutant gene; they are connected by diagonal lines that represent introns. A diagonal line rises from a donor site and descends to an acceptor site. The dashed lines indicate the position of donor and acceptor splice sites, either within the resident gene or the transposable element. P denotes the promoter end of the element and is included to orient the element with respect to the transcription unit of the resident gene. The designation of P is not meant to imply that these elements are transcribed. Except for (E), *Ds1* elements are not deletion derivatives of *Ac*. The terminus containing the donor splice sites in the *Ds1* elements is homologous for about 30 bp with the *Ac* terminus and is present in the same orientation.

When transposable elements excise from protein-coding regions, genetic selection of phenotypic revertants is biased toward those excisions that produce transposon footprints of 3 or 6 bp, thus restoring the correct reading frame. However, excision of maize controlling elements can lead to transposon footprints that (i) do not restore the correct reading frame (*53*) or (ii) restore the reading frame but result in the synthesis of an inactive protein (*54*). These excision events produce null sectors that are visible only if the original insertion does not abolish gene expression. An example of this phenotype is shown in Fig. 1B.

Excision of controlling elements provides a mechanism for generating proteins with altered enzymatic properties (*53*). Transposon footprints of 6 or 9 bp in revertants of the *wx-m1(Ds)* allele have been directly related to the reduced enzymatic activity of the *waxy* protein, UDP–glucosyl transferase (*55*) and provides a molecular basis for the somatic sectors of intermediate expression displayed by this allele (Fig. 1A). In addition, the reduced activity of a revertant of the *adh-2F11(Ds)* allele results from the addition of two amino acids, one of which is a cysteine, to a cluster of Cys codons that are believed to be involved in zinc binding (*56*).

Change in state. Unstable alleles can give rise to new alleles that yield a different unstable phenotype. This is referred to as a change in state (*7, 57*). Although a change in state has, in at least one case, been associated with intragenic transposition (*58*), most result from deletions within the transposable element. In the simplest case, *Ds* alleles have been derived from *Ac* alleles. The *wx-m9(Ds)* allele differs from its *Ac* progenitor by a deletion of 194 bp within exon 3 of the *Ac* transcription unit (*19, 24*). Similarly, the *bz-m2(Ds)* derivative of an *Ac* allele has a deletion of 1312 bp that removes all of exon 2 (*27*). These two *Ds* elements lack all *Ac* functions but can transpose if an active *Ac* element is elsewhere in the genome.

Deletions within the *Spm* element do not always result in the total loss of function. Derivatives of the *Spm*-containing *a-m2* (*14*) and *wx-844* (*59*) alleles have internal deletions that include ORF1 and ORF2 (Fig. 2) and produce about 10% normal-sized Gene 1 transcripts (*14, 59*). Since these *Spm-weak* (*Spm-w*) elements have normal *sp* function and are mutant for *m* function, it is likely that Gene 1 encodes *sp* function and that ORF1 or ORF2 (or both) encode all or part of the transposase (*59*). Homology between ORF1 and a portion of the *A. majus Tam1* element is consistent with this notion (*39*).

The best characterized changes in state are in derivatives of *dSpm* alleles that give rise to new phenotypes with respect to the patterns of somatic mutation caused by excision of the element. The *bz-m13* and *a-m1* alleles both result from insertion of an identical 2.2-kb *dSpm* element into exons of genes responsible for aleurone pigmentation (*11, 35, 60*). In the presence of an active *Spm* element, these alleles produce large pigmented sectors and give rise to new alleles at a high frequency. DNA sequencing reveals that changes in state that display smaller sectors, suggestive of reversion later in development, have sustained deletions within the 2.2-kb *dSpm* element. For the changes in state of *a-m1 5719A1*, *a-m1 1112*, and *bz-m13 CS9*, one deletion end point extends into the subterminal repeat region (Fig. 2), potentially disrupting the extended base pairing of the termini. It has been hypothesized that deletions involving the subterminal repeats may interfere with transposase binding and result in the observed low frequency of excision (*35, 36, 61*).

Deletions within elements of the *Spm* family often involve the subterminal repeats (*14, 36*). This finding, coupled with genetic

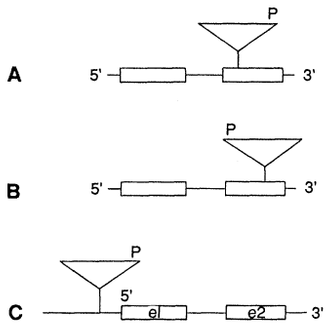

Fig. 4. The orientation of transposable elements with respect to the direction of transcription of the resident gene. Specific examples are described in the text. P stands for promoter and indicates which end of *Ds* or *dSpm* contains the 5′ end of the transcription unit. Boxes represent exons. (**A** and **B**) Insertion into exons. (**C**) Insertion upstream from the transcription start site.

evidence that the frequency of producing new states from any unstable allele correlates with the excision frequency of the element (*57*), led to the suggestion that deletion formation may be mediated by the *Spm* transposase (*14, 36*). A similar analysis of revertants of the *Drosophila melanogaster Rp11215* allele harboring a *P* element showed that altered phenotypes were correlated with deletions within the *P* element (*62*). These intra-element deletions were also believed to be catalyzed by the transposase.

Ds and dSpm elements as introns. A few *Ds* and *dSpm* alleles with insertions in exons are expressed and encode wild type–sized mRNAs. The discovery that these elements can be removed from RNA provides an explanation for these observations. The processing of one *dSpm* and four *Ds* elements from RNA has been analyzed in strains where these elements cannot transpose; that is, they lack *Spm* and *Ac*, respectively, in the genome. In each case, RNA polymerase apparently initiates at the promoter of the resident gene and reads through the entire element, creating pre-mRNAs that are larger than wild-type mRNA by the size of the element. Various mechanisms by which the elements are spliced from these pre-mRNAs are summarized in Fig. 3. When the 902-bp *dSpm* element of the *bz-m13 CS9* allele is present, the normal 3′ acceptor is not recognized; instead, a cryptic acceptor in the 13-bp inverted termini of *dSpm* is used (Fig. 3A) (*63*). Despite the loss of 33 bp from the exon, the reading frame is maintained and a wild-type *bz* phenotype is observed. A similar mechanism removes the 1.3-kb *Ds* element from the *adh-2F11* allele; however, a cryptic acceptor is used in exon 4 and not in the terminus of the element (*64*) (Fig. 3B). The removal of 133 bp from exon 4 is probably responsible for the null phenotype.

The removal of *Ds* elements from *wx-m9*, *wxB4* (*65*), and *adh-Fm335* (*32, 66*) transcripts has characteristic features. In all three transcripts a new intron is created by the recognition of donor sites in a region adjacent to the *Ds* inverted repeat terminus (Fig. 2, dashed arrow) and acceptor sites within the resident gene just downstream from the site of insertion. At least three donor consensus sequences are found in the 20 bp adjacent to one *Ds* terminus (Fig. 2, dashed arrow). For *wxB4*, at least two of these donors are used in vivo (*65*) (Fig. 3D). Although one of these transcripts is in frame, this allele has a null phenotype in the absence of *Ac*, presumably because of the additional nucleotides of the *Ds* terminus that persist in the mRNA. The *wx-m9* allele is significantly expressed; yet the one sequenced cDNA does not represent a transcript with the correct reading frame. The use, in vivo, of one of the other splice donor sites present in the *Ds* terminus may result in a functional gene product. It may be significant that the position of these donor sites is such that when coupled with a single acceptor, three different reading frames are generated (*67*).

Only about 40 bp of the *Ds1* element of the *adh-Fm335* allele are identical with *Ac*. This includes the 11-bp inverted termini and the splice donor sequences adjacent to one terminus. Now that *Ds* elements can be mutagenized and assayed for transposition in tobacco (*68*), it will be interesting to see whether these donor sites have been conserved because they are required for transposition, or whether conservation is related to a selective advantage conferred by an ability to be spliced from RNA.

Orientation of the element. All known spliced *dSpm* and *Ds* elements have the same orientation with respect to the direction of transcription of the resident gene (Fig. 4A). In this orientation, and in the absence of *Ac* or *Spm* elements in the genome, RNA polymerase II can efficiently read through the elements shown in Fig. 3. In contrast, the *wx-m8* allele has a 2.2-kb *dSpm* element in exon 10 of the *waxy* gene in the opposite orientation (*33*) (Fig. 4B). In the absence of an active *Spm* element, transcription initiated at the *waxy* promoter terminates prematurely within the element and results in a truncated polyadenylated transcript (*69*). In the presence of an active *Spm* element, both the leaky expression displayed by many *dSpm* alleles and the truncated transcripts seen for the *wx-m8* allele are suppressed (*69*). These data are explained by proposing that *sp* function results from the binding of a protein (the S protein) to the ends of *dSpm* elements, thus preventing formation of either the readthrough transcripts seen in one orientation or the prematurely terminated transcripts seen when the element is in the other orientation (*59, 63*). This notion is supported by the finding that the Gene 1 protein (S protein), which is believed to encode *sp* function, binds selectively to the *Spm* termini (*59*).

If the *sp* function interferes with transcriptional readthrough, then unstable alleles with insertions in nontranscribed regions should not be suppressible. This is in fact the finding for the *Spm* allele *a-m2* and a few of its *dSpm* derivatives, which are mutants arising from an insertion 100 bp from the 5′ end of the *a* gene transcript (*60*) (Fig. 4C). Unlike most other *Spm* alleles, expression of the *a* gene of *a-m2* is dependent on the presence of *sp* function rather than being suppressed by it (*70*). It has been suggested that the proximity of the *Spm* and the *a* gene promoters has put *a* gene expression under *Spm* control (*14, 60*).

Inactivation and Activation of *Ac* and *Spm* Elements

Inactivation. Deletions within *Ac* and *Spm* elements lead to their permanent inactivation. Reversible inactivations, referred to as changes in phase, also occur (*29, 71*). Active elements can become inactive and remain so for several cell divisions (cycling elements, Fig. 1C) or for several generations (inactive elements). In addition, there are alleles of *Spm* elements that are only active in certain parts of the plant (*72*). Inactive *Ac* elements (*Ac-i*) behave like *Ds*; that is, they can transpose only in the presence of an active *Ac* element; they cannot transactivate other *Ds* elements in the genome; and they do not contribute to an *Ac* dosage series (*70*). Inactive *Spm* elements (*Spm-i*) behave like nonautonomous *dSpm* elements except they can be transiently reactivated in the presence of an *Spm-s* (standard, fully active element) or *Spm-w* element (*14, 71, 73*). Finally, both *sp* and *m* functions of *Spm* become active or inactive simultaneously (*72*) (Fig. 1C). *Ac-i* and *Spm-i* elements behave as if they do not make functional gene products, although, *Spm-i* elements can do so in response to the product of an active element elsewhere in the genome.

The fact that inactive elements can be transiently or permanently reactivated suggests that the loss of activity does not involve rearrangements or deletions within the element. Consistent with this notion is the finding that inactive derivatives of two *Ac* alleles, *wx-m9* and *wx-m7*, are structurally identical to their active progenitor alleles (*74, 75*). However, unlike their progenitors, both elements exhibit DNA modification involving cytosine methylation of

Ac sequences but not flanking *waxy* DNA. Reactivation of the inactive *wx-m9* allele is correlated with demethylation of 900 bp at the 5′ end of *Ac* (*75*). This region includes the promoter, the transcription start site, and the untranslated leader exon (Fig. 2).

The correlation of *Ac* activity with a hypomethylated state may provide an explanation for the finding that *Ac* usually transposes just after replication (*46*), when it is presumably hemimethylated and thus possibly more active. Similarly, the rate of transposition of the bacterial element Tn*10* increases when it is hemimethylated (*76*).

Activation. Even though most strains of maize do not have active *Ac* or *Spm* elements, they contain multiple copies of sequences homologous to these elements (*19, 34*). This finding, together with demonstrations that various stresses to the genome [such as x-rays (*77*), ultraviolet rays (*78*), the breakage-fusion-bridge cycle (*79*), and growth in culture (*80*)] can activate several maize transposable elements, including *Ac* and *Spm*, leads to the tentative conclusion that agents causing chromosome damage may alter the normal methylation pattern and activate cryptic elements (*81*).

Concluding Remarks

It has been proposed for maize and several other organisms that transposable elements may serve to generate diversity in life threatening situations (*82*). The available data on the *Ac* and *Spm* family of elements are consistent with the following scenario. Drought conditions, extremes in temperature, viral infection, or other genomic shocks that threaten the survival of the organism may lead to the demethylation of cryptic *Ac* or *Spm* elements. Under such conditions, many maize elements may be activated; in addition to *Ac* and *Spm* elements, the activity of the maize transposable element *Mutator* also correlates with its methylation state (*83*). Once activated, the impact of autonomous elements is amplified since they can transactivate the numerous nonautonomous elements in the genome to transpose. It is primarily the excision of the nonautonomous elements that creates genetic diversity by adding nucleotides to coding or regulatory regions, thus altering proteins or tissue specificity of expression. Following excision, elements are likely to transpose into active genes since they prefer unmethylated target sites. This enhances their effectiveness as mutagens. Since *Ac* and *Spm* are active in all tissues, and somatic mutations can be inherited, the potential for genetic diversity in the progeny is greatly enhanced. Mutations will accumulate until the *Ac* and *Spm* elements are inactivated by methylation or transposase-mediated intra-element deletions. Once *Ac* and *Spm* elements are inactivated all elements in the family are immobilized; the deleterious effects of insertion into certain active genes may be mitigated by the splicing of elements from exons and the production of some functional gene product.

Although the properties of *Ac* and *Spm* suggest such a scenario, these properties are also consistent with the alternative view that transposable elements are selfish DNA (*84*). These arguments do not have to be mutually exclusive; characteristics of the elements that benefit the organism, such as the ability to function as introns, may also enhance their success.

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Research Articles

MyoD1: A Nuclear Phosphoprotein Requiring a Myc Homology Region to Convert Fibroblasts to Myoblasts

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Expression of a complementary DNA (cDNA) encoding the mouse MyoD1 protein in a variety of fibroblast and adipoblast cell lines converts them to myogenic cells. Polyclonal antisera to fusion proteins containing the MyoD1 sequence show that MyoD1 is a phosphoprotein present in the nuclei of proliferating myoblasts and differentiated myotubes but not expressed in 10T1/2 fibroblasts or other nonmuscle cell types. Functional domains of the MyoD1 protein were analyzed by site-directed deletional mutagenesis of the MyoD1 cDNA. Deletion of a highly basic region (residues 102 to 135) interferes with both nuclear localization and induction of myogenesis. Deletion of a short region (residues 143 to 162) that is similar to a conserved region in the c-Myc family of proteins eliminates the ability of the MyoD1 protein to initiate myogenesis but does not alter nuclear localization. Deletions of regions spanning the remainder of MyoD1 did not affect nuclear localization and did not inhibit myogenesis. Furthermore, expression of only 68 amino acids of MyoD1, containing the basic and the Myc similarity domains, is sufficient to activate myogenesis in stably transfected 10T1/2 cells. Genetic analysis maps the MyoD1 gene to mouse chromosome 7 and human chromosome 11.

THE IDENTIFICATION OF THE MYOD1 COMPLEMENTARY DNA (cDNA) (*1*) depended on the initial observation that a brief treatment of C3H10T1/2 fibroblasts (10T1/2 cells) with 5-azacytidine yields, at high frequency, colonies capable of forming muscle (up to 50 percent) (*2*), presumably because 5-azacytidine incorporation into DNA results in the demethylation and subsequent expression of specific loci involved in myogenesis. It was later shown (*3*) that the frequency of myogenic conversion after treatment with 5-azacytidine is consistent with the activation of a small number of genes and possibly a single gene. Furthermore, genomic DNA transfections (*4, 5*) have shown that myoblast DNA, but not DNA from 10T1/2 cells, has the capacity to convert 10T1/2 cells to muscle at a frequency consistent with the transfer of a single genetic locus, an indication that 5-azacytidine does, in fact, lead to an altered structure (that is presumably related to demethylation) of 10T1/2 DNA.

We used subtracted cDNA hybridization to isolate the MyoD1 cDNA (*1*). MyoD1 is not expressed in 10T1/2 cells but is expressed in myoblast lines derived from 10T1/2 cells after treatment with 5-azacytidine, as well as in other myoblast lines and primary muscle both in vivo and in vitro (*1*). The MyoD1 cDNA, when expressed under the control of a viral long terminal repeat (LTR) and transfected into several different fibroblast or adipoblast cell lines, converts these cells to myoblasts (*1*). It is important to distinguish between the azacytidine gene (that is, the gene activated by 5-azacytidine treatment of 10T1/2 cells), the genomically transferred myogenic gene, and MyoD1 (the myogenic regulatory gene ultimately identified by subtracted cDNA screening). Whether these are all the same or different genes remains to be determined. In this regard, it was recently shown (*6*) that transfection of a genomic cosmid DNA sequence distinct from MyoD1 can result in the activation of myogenesis in 10T1/2 cells.

The sequence of the MyoD1 cDNA contains a major open reading frame of 318 amino acid residues. The sequence has a region of 22 amino acid residues with a marked similarity to a region that is

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