Epigenetic regulation of the maize *Spm* transposon

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Summary

Expression and transposition of the *Suppressor-mutator (Spm)* transposon of maize are controlled by interacting epigenetic and autoregulatory mechanisms. Methylation of critical element sequences prevents both transcription and transposition, heritably inactivating the element. The promoter, comprising the terminal 0.2 kb of the element, and a 0.35-kb, highly GC-rich, downstream sequence are the methylation target sequences. The element encodes two proteins necessary for transposition, TnpA and TnpD. There are multiple TnpA binding sites, both in the 5′ terminal promoter region and at the element's 3′ end. In addition to its role in transposition, TnpA is both a positive and a negative regulator of transcription. TnpA represses the element's promoter when it is not methylated. When the element is inactive and its promoter methylated, TnpA activates the methylated promoter and facilitates both its transient and heritable demethylation.

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A quandary

It has been almost half a century since Barbara McClintock discovered that certain bits of genetic material, which she called transposable elements and we now refer to as transposons, move from one chromosomal site to another. While transposons are both ancient and abundant, their role in organismal evolution remains largely enigmatic. What they can do is increasingly evident. Insertion of transposons into genes or regulatory sequences generally disrupts gene function, but it can also reprogram gene expression[1]. Transposons cause chromosome breaks that stimulate illegitimate recombination, rearrangements and gene conversion. Chromosomes of many organisms are laden with dozens or hundreds of copies of related transposable element sequences, referred to as an element family, and organisms often have many different element families. Mobilization of just one family of elements can cause structural alterations at many chromosomal sites simultaneously. Thus genetic changes, large and small, are inevitable sequelae of transposable element activity. By one means or another, transposable elements also outreplicate the genome, accumulating over time. The puzzle is not how transposable elements survive in evolution, but how organisms survive their transposable elements and, perhaps, even accrue evolutionary benefits.

The emerging answer is that there are many molecular devices for modulating transposition[1]. These include layers of *cis*- and *trans*-acting mechanisms that function at transcriptional, translational and post-translational levels. Here we will discuss a unique epigenetic regulatory system that governs expression of the maize *Suppressor-mutator* (*Spm*) transposon. *Spm* expression and transposition are regulated by a complex mechanism that has some of the characteristics of the familiar reversible regulatory mechanisms of prokaryotes and eukaryotes, but also has components resembling the more stable changes in gene expression associated with paramutation in plants and imprinting in animals[2,3]. While the underlying molecular mechanisms are at present best understood for the *Spm* element, it is likely that other maize transposons are regulated in a similar way[4].

The transcriptional activity of the *Spm* element is controlled negatively by an epigenetic mechanism. The element itself encodes an autoregulatory protein that pro-

motes the reactivation of an epigenetically silenced element. We will review the early genetic clues in McClintock's work pointing to the existence of both epigenetic and autoregulatory controls, then summarize our present understanding of the underlying molecular mechanisms. The *Spm* element's control system is currently the most thoroughly studied epigenetic regulatory circuit known. It is a paradigm uniquely accessible to both genetic and molecular analysis because it controls genes that are not essential for survival. But it can easily be anticipated from what is known about plant development and physiological adaptations to changing environmental conditions that similar mechanisms will surface in the integral regulatory circuitry of plants.

Genetic discovery of epigenetic control

Spm is the element that McClintock studied in the greatest genetic detail, perhaps because of the absorbing complexity of the interactions between the elements of this family. Her meticulous investigation of this genetic 'crosstalk' provided the subsequent generation of investigators with a compendium of clues about the *Spm* element's functions. McClintock clearly recognized the regulatory implications of her observations, although her interpretation that the transposons themselves were the regulatory elements has not withstood the test of time. At the present telling, it appears far more likely that her mutants revealed the inner workings of the element's own regulatory system. It is from this perspective that we have interpreted her genetic studies in succeeding paragraphs.

Decades ago McClintock observed that an *Spm* element could change or 'mutate' to one of a number of different forms in which it was either silent or showed a pattern of alternation between active and inactive phases during development[5-10]. Intrigued by the observation that any particular developmental pattern of element expression was heritable, McClintock devoted considerable effort to understanding the genetic basis of the patterning phenomenon. In the course of her studies, McClintock recognized different developmental patterns of element expression and understood that a given pattern is both heritable and yet somewhat labile, capable of changing to still different heritable expression patterns. Moreover, she observed that elements could remain silent for several plant generations, only occasionally returning to an active form.

Even in the results of some of McClintock's earliest studies leading to the discovery of transposition there is a hint that transposons are normally in genomes, but not active[11]. McClintock later successfully tested the hypothesis that previously undetectable transposable elements could be converted to an active form under circumstances of extensive chromosome breakage[12-14]. All of these early observations pointed to the existence of a mechanism by which the elements could be heritably maintained in a silent, yet genetically intact form – precisely the definition of an epigenetic mechanism. Because the differential expression patterns are heritable, but at the same time change to different types of heritable patterns at a relatively high frequency, these too appear to have an epigenetic basis. The multiplicity of developmental expression patterns that surfaced in McClintock's studies suggested either that there were many systems at work or that control was exerted by a single system of substantial versatility.

Genetic crosstalk between transposons

Although seemingly unrelated to element regulation at the time, McClintock's extensive studies of what she referred to as the 'states' of a locus provide insight into other facets of *Spm* regulation[10]. In retrospect, these studies constitute some of the earliest genetic data on interactions between a regulatory protein and its target DNA sequence. Like other transposable element families, the *Spm* family comprises fully functional autonomous elements and a host of moderately-to-severely disabled relatives. The non-autonomous family members, designated *defective Spm* (*dSpm*) elements, are dependent on the autonomous elements for mobility. McClintock found that *Spm* could both promote excision of a *dSpm* element from a mutant gene and change its phenotypic expression. Her studies focused on *dSpm* insertions into the maize *a* and *a2* loci, both of which encode enzymes in the biosynthetic pathway leading to the production of the plant's purple anthocyanin pigments. McClintock's *dSpm* insertion alleles fell into two different categories. In one type of allele, later designated *Spm-suppressible*, the mutant gene was expressed only in the absence of the autonomous *Spm* element. In the other type of allele, later designated *Spm-dependent*, the gene was generally expressed only in the presence of a fully functional *Spm*[10,15]. Among alleles of the second, *Spm-dependent* type, McClintock identified some that exhibited a special type of delayed gene expression after exposure to the *Spm* element, as described below.

While each allele exhibits its own unique response pattern to the *trans*-activating *Spm*, the overall message is simple and obvious (filtered through the clarifying lens of hindsight): *Spm* encodes one or more *trans*-acting gene products that interact with the element sequences intro-

duced into the locus by insertion of the *dSpm* element, altering gene expression and promoting element excision. According to this interpretation, the *dSpm* insertion alleles are analogous to contemporary promoter-reporter gene fusions, revealing the operation of autoregulatory functions. Some of the *Spm-dependent* alleles of the *a* locus exhibit an initially obfuscating, but ultimately enlightening, genetic property which McClintock called 'presetting'. The *a* gene of these alleles, expressed when *Spm* is present, can also be 'preset' to be expressed after the *trans*-activating *Spm* element is removed by transposition or genetic segregation[16-19]. To be 'preset', the mutant gene need only be exposed to an autonomous element early in plant development. The 'preset' gene is expressed in kernels receiving the gene, but not the *Spm* that 'presets' it. 'Presetting' has the unique genetic property that it is highly reproducible, requiring only the transient simultaneous presence of the mutant gene and an autonomous element, but that the 'preset' pattern is not heritable. The mutant gene relapses in subsequent generations into the inactive form typical of it when propagated in the absence of the *trans*-activating *Spm* element.

A genetic hypothesis of *Spm* regulation

The odd capacity to be 'preset' for expression in the absence of *Spm* provides the key to *Spm* autoregulation. The first and most obvious implication of 'presetting' is that the mutant gene is not inactivated by the insertion itself, but by some type of heritable, yet reversible (hence epigenetic) modification of the insertion. Following this line of reasoning, *Spm* must encode a regulatory gene product that interacts with the inserted *dSpm* element to activate gene expression while it is present. But it must also 'erase' the epigenetic modification of the element's regulatory sequence at the locus, albeit transiently, to permit expression of the gene after the source of the regulatory gene product is gone. Recalling the observations reviewed earlier that *Spm* elements themselves are subject to epigenetic inactivation, this interpretation leads to the prediction that epigenetically inactive elements might respond to active elements, as do *Spm-dependent* alleles caused by insertion of *dSpm* elements. And indeed, McClintock's sophisticated genetic monitoring of the interactions between active and inactive elements provide the confirming evidence. An active element reactivates an inactive one, so long as both are together in the same plant[20].

But what about 'presetting'? McClintock reported that the active and inactive elements subsequently segregate from each other unchanged[20]. But a careful re-examination of the question revealed that the exposure of an active element exerts both a transient and a heritable effect on an inactive element[21]. Even a very inactive element, which we have termed a *cryptic Spm*, can eventually be fully reactivated under the influence of an active element. The cryptic form is extremely stable. The spontaneous reactivation frequency of a *cryptic Spm* approaches the spontaneous mutation frequency. The transition from the cryptic form to a heritably active form is slow. Even in the presence of an active element and with selection, the transition takes several plant generations[21]. But prior to full activation, the silent element exists in an intermediate form in which it is expressed only in the presence of a second, fully active element. Such an *Spm-dependent* element commonly exhibits 'presetting': a pattern of non-heritable expression following segregation of the *trans*-activating element[21]. The types of heritable developmental patterns of gene expression described by McClintock emerge somewhat later in the reactivation process.

Taken together, the foregoing genetic observations, most dating back to McClintock's extraordinary work, provided the genetic underpinnings of our hypothesis about the *Spm* element's regulatory circuitry[15]. We postulated the existence of a single multi-step negative mechanism responsible for the entire panoply of developmentally regulated *Spm* forms, as well as those that are inactive throughout development, but differ in the heritability of the inactive condition. And we further postulated that the element encodes one or more *trans*-acting regulatory gene products that can activate an epigenetically inactive element both transiently and heritably. Molecular genetic tests of the hypothesis became possible after the element was cloned and as its genetic components were first dissected and then reassembled in model transgenic systems.

Spm structure and expression

The autonomous elements of the *Spm* family are interchangeable genetically. Indeed, Peterson[22] reported that McClintock's *Spm* element was indistinguishable genetically from the *Enhancer* (*En*) element that he had identified some years earlier[23,24]. We now know that their DNA sequences are also almost identical[15,25]. Each is about 8.4 kb in length and superficially simple in organization (Fig. 1). Only a single transcription unit has been identified so far and it occupies most of the element's length. The few hundred base pairs outside of the transcription unit consist of sequences essential for both transcription and tranposition of the element. The very ends comprise inverted repeats of a short sequence, CACTACAAGAAAA. The presence of short terminal inverted repeats (or TIRs) is characteristic of one category of transposable element and the TIRs demarcate the segment of DNA that

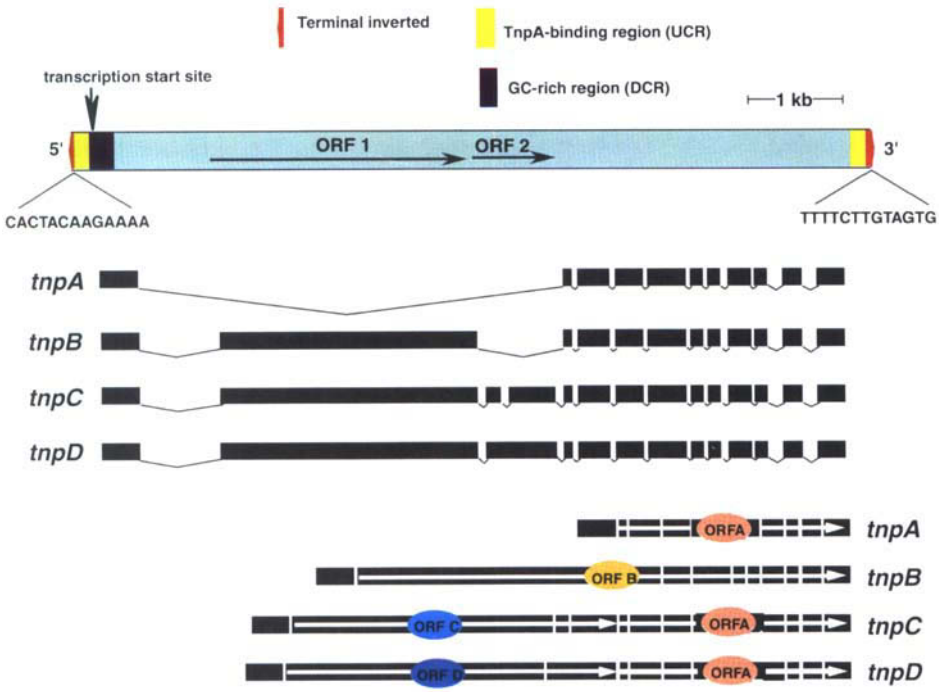

Fig. 1. A diagrammatic representation of the *Spm* element's structure, transcripts and ORFs. Black boxes represent exons. Arrows show the extent of each ORF. ORF C and ORF D contain sequences derived from ORF 1 and ORF2, located in the first intron on the *tnpA* transcript

transposes. Between the TIRs of the *Spm* and its transcription unit at both element ends, there are several hundred base pairs that we have designated the 'subterminal repetitive regions'. Each contains multiple copies of a repeated sequence (and homologs of it) that binds to TnpA, one of the element-encoded proteins[26]. The element's promoter is contained within the first 0.2 kb at the element's left end and is coextensive with the subterminal repetitive region[27].

Spm's seemingly simple sequence organization belies considerable genetic complexity. The element's primary transcript is alternatively spliced, yielding mRNA sequences with different open reading frames (ORFs), some with extensive overlaps. We have identified at least four transcripts generated by different patterns of splicing from the *Spm* element's single primary transcript. These have been designated *tnpA*, *tnpB*, *tnpC* and *tnpD*, in order of increasing size[28]. The *tnpA* transcript is the shortest and was the first one identified[25]. It is by far the element's most abundant transcript, being 50-100 times more abundant than any of the other transcripts[28]. The spliced transcripts and their ORFs are shown at the bottom of Fig. 1. Two of the transcripts contain a single ORF, while the others contain two ORFs. Each transcript has one ORF that is unique to it and it is this coding sequence that has been given the transcript's name. Thus the potential proteins encoded by these transcripts are called TnpA, TnpB, TnpC and TnpD.

Using a tobacco test system, the TnpA and TnpD proteins were shown to be both necessary and sufficient to mobilize a transposition-defective *Spm* (*dSpm*) element[29-31]. To date, the other proteins (TnpB and TnpC) have been found neither to help nor to hinder transposition (M. Schläppi and N. Fedoroff, unpublished). Because TnpA does not bind to the element's TIRs, but to its subterminal repeats, it has been postulated that TnpD is the element-encoded protein responsible for correct cleavage of the transposon ends[31]. The results of recent experiments reveal that TnpD does not bind *Spm* directly, but rather interacts with TnpA bound to DNA containing TnpA binding sites (R. Raina, M. Schläppi and N. Fedoroff, unpublished). It has also been postulated that TnpA serves to bring the ends of the element together for transposition[26,31], a role for which there is some direct evidence (R. Raina and N. Fedoroff, unpublished). Much yet remains to be learned about the manner in which TnpA and TnpD interact with each other and with the *Spm* element to promote transposition.

The molecular basis of epigenetic inactivation

We began studies on the molecular differences between genetically silent and active *Spm* elements some years ago. The sequence just downstream from the transcription start site of the element is very rich in GC base pairs (Fig. 1) and contains many sequences in which the C residues can be modified by methylation[15,25]. We analysed DNA from plants with active and inactive elements with restriction enzymes whose ability to cut DNA is inhibited by methylation of C residues in their cleavage sites. We found

that both active and inactive elements were extensively methylated, although the adjacent sequences at the insertion site studied were not[32]. However, active elements differ from inactive ones by the absence of methylation in a restricted region of about 0.5 kb surrounding the transcription start site. Active elements are unmethylated in the 0.2 kb promoter region (initially designated the upstream control region or UCR[32]). Methylation is variable in the GC-rich region just downstream from the transcription start site (designated the DCR[32]). The more stably and heritably inactive the *Spm* element, the greater the extent of C methylation in the DCR (Fig. 1). Moreover, developmental patterns of element expression, as well as the conversion of a *cryptic Spm* to an active one, are paralleled by changes in methylation of the UCR and DCR, implying either that methylation is causal to element inactivation or that it is a direct reflection of it[32,33].

To achieve a deeper understanding of *Spm* inactivation and reactivation, we needed to be able to reproduce the associated genetic phenomena in a transgenic system devoid of the many background *Spm* elements present in maize. In time, we found that the element was subject to reversible inactivation in tobacco plants, just as it was in maize, and that inactivation was accompanied by preferential methylation of the element's 5′ end, again mirroring observations made in maize[34]. We then established that the inactive element could be reactivated by introducing a TnpA cDNA, whereas introduction of any of the element's three other coding sequences had no effect[34]. This clearly identified TnpA as the element's positive autoregulatory protein. In analysing the progeny of TnpA-containing plants, we found that the reactivated *Spm* element commonly segregated in its original inactive form, just as McClintock had observed in maize[20,34]. But we also found that in some cases the *Spm* element had been 'preset' for continued expression following its exposure to TnpA protein, and this was reflected in reduced methylation of its 5′ end.

To determine whether the element's 5′ terminal segment contained sufficient information for methylation and silencing of the adjacent transcription unit, we fused either the promoter alone, or both the promoter and the DCR, to a firefly luciferase gene and investigated the activity of the gene, both in transient assays and in stably transformed cells[35]. The element's promoter was rapidly inactivated and methylated when the fusions were stably transformed into plant cells, but only if the DCR was present[35]. If the element's promoter lacked the DCR, the luciferase gene continued to be expressed. In earlier studies, we had reported that while the DCR sequence has no effect on the adjacent *Spm* promoter, the presence of the DCR made the promoter insensitive to an upstream enhancer[27]. We also reported that the DCR could effectively silence a heterologous promoter[27]. Thus the DCR sequence has emerged as a central component of the *Spm* element's regulatory mechanism. Not only does the DCR insulate the element from external promoters and enhancers, but it appears to be required for inactivation and methylation of the element's promoter.

TnpA is both a repressor and an activator

Early genetic data reviewed above imply that *Spm* encodes both positive and negative regulatory gene products[10]. Using luciferase reporter gene assays, we investigated the effect of TnpA on both an active, unmethylated *Spm* promoter and an inactive, methylated *Spm* promoter. We found that TnpA represses the active, unmethylated *Spm* promoter[35]. Only the DNA-binding and dimerization domains of the protein are required for repressor activity, suggesting that TnpA represses transcription by competing with transcription factors for binding sites on the *Spm* promoter[35,36]. In sharp contrast, TnpA activates the *Spm* promoter if it is inactive and methylated. Moreover, promoter reactivation is invariably accompanied by a decrease in promoter methylation[35].

To investigate this apparent paradox, we asked whether the different abilities of TnpA to inhibit and activate might be determined by differences in the way the protein interacts with methylated and unmethylated DNAs. We reasoned that if TnpA is inherently a transcriptional activator, then it should activate a reporter gene with a different DNA-binding site when fused to the corresponding DNA-binding domain, while if it is a repressor, the resulting hybrid protein should still repress transcription. We found that TnpA functions as a repressor when fused to a different DNA-binding domain and becomes a transcriptional activator of its own promoter only when a heterologous activation domain is added to the DNA-binding and dimerization domains of TnpA (M. Schläppi, R. Raina and N. Fedoroff, unpublished). We concluded that TnpA is not a conventional transcriptional activator. We had earlier observed that TnpA-mediated activation of the methylated promoter is always associated with reduced methylation of both the promoter region and the DCR[34,35] and that activation requires the protein's C-terminal sequence, as well as the DNA-binding and dimerization domains[35]. Both of these observations suggest the possibility that TnpA interacts with other proteins to effect promoter activation and demethylation of the UCR and DCR sequences[35]. Thus we think that TnpA probably activates the methylated promoter by a different mechanism from the one it uses to repress the unmethylated promoter.

Regulatory hypothesis revisited

On the basis of genetic data, we postulated the existence

of a single multi-step negative mechanism responsible for the entire range of inactive *Spm* forms, as well as those that exhibit differential patterns of element expression in development[15]. The results of subsequent molecular studies suggest that negative epigenetic regulation is attributable to methylation of the *Spm* element's promoter and GC-rich DCR sequence at multiple sites. We have learned that promoter methylation does not require element-encoded gene products and can occur in tobacco cells, as it does in maize. Rapid methylation requires only the promoter and the adjacent DCR sequence. We have established a close correlation between methylation and the heritability of the inactive form of the element, as well as a similar correlation between the genetic activity of the element in development and promoter methylation[32,33]. The existence of more than 100 methylatable C residues on each strand within the first 0.6 kb from the element's 5′ end suggests that differences in both the heritability of the inactive state, as well as the diverse developmental patterns of element expression, may find their molecular explanation in the extent or pattern of methylation.

We also postulated that the element encodes one or more *trans*-acting regulatory gene products that can activate an epigenetically inactive element both transiently and heritably[15]. TnpA has been identified as the *trans*-acting regulator and it does, indeed, function both to activate and promote demethylation of a methylated, epigenetically inactivated *Spm* promoter. In formulating our initial regulatory hypothesis, we assumed that *Spm* inhibited expression of certain *dSpm* alleles by the same mechanism that it activated gene expression[15]. Molecular experiments have established that instead, TnpA is *both* a repressor and an activator of *Spm* expression, depending on whether or not the promoter is methylated.

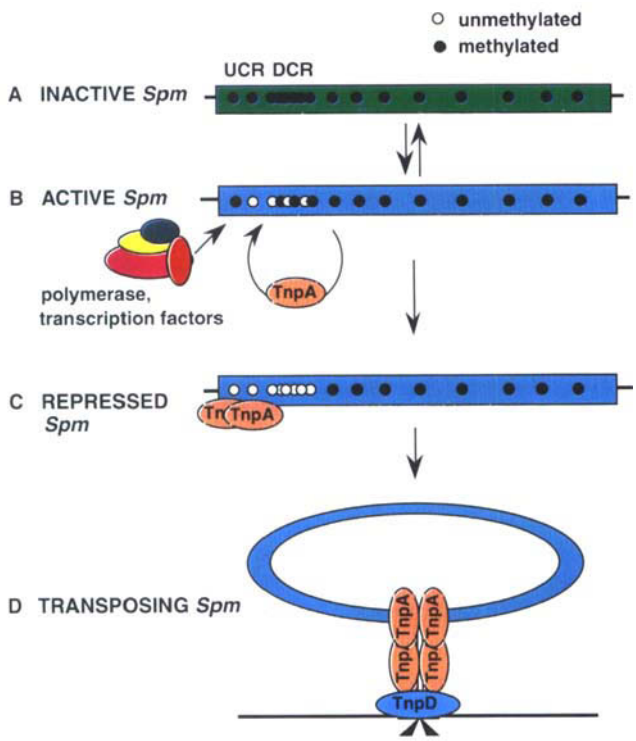

Fig. 2. Transposition and regulation of *Spm*: a model. Filled and open circles represent methylated and unmethylated C residues in the element's sequence. Arrowheads indicate the sites at which the transposon is cleaved during transposition.

Although we are far from a full understanding of the mechanisms that regulate *Spm* transcription and transposition, it is becoming increasingly evident that they are intimately interrelated (Fig. 2). There are binding sites for TnpA at both element ends, and there is preliminary evidence that TnpA serves to bring the element's termini together during transposition[30,31]. Deletion of either the DCR or TnpA binding sites from either element end diminishes transposition frequency[24]. The element's promoter sequence is coextensive with the 5′-terminal TnpA-binding region, implying that transcription-factor binding sites either overlap or coincide with TnpA binding sites (Fig. 2B). Deletion of the DCR reduces TnpA's ability to repress the promoter (M. Schläppi, R. Raina and N. Fedoroff, unpublished). These observations are consistent with the simple hypothesis that transcription from the *Spm* promoter leads to the accumulation of TnpA, with progressive occupation of TnpA binding sites eventually inhibiting further transcription from the promoter and resulting in formation of a transposition complex containing both element ends held together by DNA-bound TnpA dimers (Fig. 2D). Consistent with this hypothesis, the negative effect on transposition of both DCR and TnpA binding site deletions might be attributable to changes in either TnpA binding affinity or, perhaps, in the stability of the transposition complex.

Methylation of the *Spm* promoter and DCR (Fig. 2A) inhibits element transcription and decreases the ability of the element to transpose, even when supplied with TnpA and TnpD *in trans*[32]. An intriguing and poorly understood aspect of *Spm*'s epigenetic regulatory mechanism is the role of the DCR in promoting element methylation and inactivation. The DCR facilitates methylation in the promoter region upstream from it and, conversely, TnpA facilitates demethylation of both the promoter and the downstream DCR sequence. These observations suggest that the DCR is an important element in the epigenetic inactivation and reactivation of the *Spm* element. Its influence may be mediated by its high GC content, its effect on DNA conformation or its ability to interact with proteins. While we know that TnpA can reactivate an inactive, methylated *Spm* promoter and facilitate its demethylation, we know little about the underlying molecular mechanism. The obser-

vations that TnpA lacks a conventional transcriptional activation domain and promotes demethylation of sequences at considerable distances from its binding sites rule out the simplest type of explanation, in which demethylation and activation are a consequence of competition between a transcription factor and a methylase for binding to the same or overlapping sites. Because of its novelty and the possibility that similar mechanisms are used in plant development and differentiation, the mechanism by which TnpA activates the methylated *Spm* promoter continues to be a major focus of our present studies.

References

1 Berg, D. and Howe, M. (ed.) (1989). *Mobile DNA*. American Society for Microbiology, Washington.

2 Brink, R. A. (1973). Paramutation. *Ann. Rev. Genet.* **7**, 129-152.

3 Lyon, M. F. (1993). Epigenetic inheritance in mammals. *Trends Genet.* **9**, 123-127.

4 Fedoroff, N. V. and Chandler, V. (1994). Inactivation of maize transposable elements. In *Homologous Recombination in Plants* (ed. J. Paszkowski), pp. 349-385. Kluwer Academic Publishers, Dordrecht, The Netherlands.

5 McClintock, B. (1957). Genetic and cytological studies of maize. *Carnegie Inst. Wash. Yrbk* **56**, 393-401.

6 McClintock, B. (1958). The suppressor-mutator system of control of gene action in maize. *Carnegie Inst. Wash. Yrbk* **57**, 415-452.

7 McClintock, B. (1959). Genetic and cytological studies of maize. *Carnegie Inst. Wash. Yrbk* **58**, 415-429.

8 McClintock, B. (1961). Further studies of the suppressor-mutator system of control of gene action in maize. *Carnegie Inst. Wash. Yrbk* **60**, 469-476.

9 McClintock, B. (1962) . Topographical relations between elements of control systems in maize. *Carnegie Inst. Wash. Yrbk* **61**, 448-461.

10 McClintock, B. (1965). The control of gene action in maize. *Brookhaven Symp. Biol.* **18**, 162-184.

11 McClintock, B. (1946). Maize genetics. *Carnegie Inst. Wash. Yrbk* **45**, 176-186.

12 McClintock, B. (1950). The origin and behavior of mutable loci in maize. *Proc. Natl Acad. Sci. USA* **36**, 344-355.

13 McClintock, B. (1950). Mutable loci in maize. *Carnegie Inst. Wash. Yrbk* **49**, 157-167.

14 McClintock, B. (1951). Mutable loci in maize. *Carnegie Inst. Wash. Yrbk* **50**, 174-181.

15 Masson, P., Surosky, R., Kingsbury, J. and Fedoroff, N.V. (1987). Genetic and molecular analysis of the *Spm-dependent a-m2* alleles of the maize *a* locus. *Genetics* **177**, 117-137.

16 McClintock, B. (1963). Further studies of gene-control systems in maize. *Carnegie Inst. Wash. Yrbk* **62**, 486-493.

17 McClintock, B. (1964). Aspects of gene regulation in maize. *Carnegie Inst. Wash. Yrbk* **63**, 592-602.

18 McClintock, B. (1965). Components of action of the regulators *Spm* and *Ac*. *Carnegie Inst. Wash. Yrbk* **64**, 527-534.

19 McClintock, B. (1967). Regulation of pattern of gene expression by controlling elements in maize. *Carnegie Inst. Wash. Yrbk* **65**, 568-578.

20 McClintock, B. (1971). The contribution of one component of a control system to versatility of gene expression. *Carnegie Inst. Wash. Yrbk* **70**, 5-17.

21 Fedoroff, N.V. (1989). The heritable activation of cryptic *Suppressor-mutator* elements by an active element. *Genetics* **121**, 591-608.

22 Peterson, P.A. (1965). A relationship between the *Spm* and *En* control systems in maize. *Am. Nat.* **99**, 391-398.

23 Fedoroff, N.V. (1983). Controlling elements in maize. In *Mobile Genetic Elements* (ed. J. Shapiro), pp. 1-63. Academic Press, New York.

24 Fedoroff, N. (1989). Maize transposable elements. In *Mobile DNA* (ed. M. Howe and D. Berg), pp. 375-411. American Society for Microbiology, Washington.

25 Pereira, A., Cuypers, H., Gierl, A., Schwarz-Sommer, Z. and Saedler, H. (1986). Molecular analysis of the *En/Spm* transposable element system of *Zea mays*. *EMBO J.* **5**, 835-841.

26 Gierl, A., Lütticke, S. and Saedler, H. (1988). *TnpA* product encoded by the transposable element En-1 of *Zea mays* is a DNA binding protein. *EMBO J.* **7**, 4045-4053.

27 Raina, R., Cook, D. and Fedoroff, N. (1993). The maize *Spm* transposable element has an enhancer-insensitive promoter. *Proc. Natl Acad. Sci. USA* **90**, 6355-6359.

28 Masson, P., Rutherford, G., Banks, J.A. and Fedoroff, N. (1989). Essential large transcripts of the maize *Spm* transposable element are generated by alternative splicing. *Cell* **58**, 755-765.

29 Masson, P. and Fedoroff, N. (1989). Mobility of the maize *Suppressor-mutator* element in transgenic tobacco cells. *Proc. Natl Acad. Sci. USA* **86**, 2219-2223.

30 Frey M., Reinecke J., Grant S., Saedler H. and Gierl, A. (1990). Excision of the *En/Spm* transposable element of *Zea mays* requires two element-encoded proteins. *EMBO J.* **9**, 4037-4044.

31 Masson, P., Strem, M. and Fedoroff, N. (1991). The *tnpA* and *tnpD* gene products of the *Spm* element are required for transposition in tobacco. *The Plant Cell* **3**, 73-85.

32 Banks J.A., Masson P. and Fedoroff, N. (1988) Molecular mechanisms in the developmental regulation of the maize *Suppressor-mutator* transposable element. *Genes Dev.* **2**, 1364-1380.

33 Banks, J.A. and Fedoroff, N. (1989). Patterns of developmental and heritable change in methylation of the *Suppressor-mutator* transposable element. *Devl Genet.* **10**, 425-437.

34 Schläppi, M., Smith, D. and Fedoroff, N. (1993). TnpA *trans*-activates methylated maize *Suppressor-mutator* transposable elements in transgenic tobacco. *Genetics* **133**, 1009-1021.

35 Schläppi, M., Raina, R. and Fedoroff, N. (1994). Epigenetic regulation of the maize *Spm* transposable element: novel activation of a methylated promoter by TnpA. *Cell* **77**, 427-437.

36 Trentmann, S.M., Saedler, H. and Gierl, A. (1993). The transposable element *En/Spm*-encoded TNPA protein contains a DNA binding and a dimerization domain. *Mol. Gen. Genet.* **238**, 201-208.

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