REVIEW

The Suppressor-mutator element and the evolutionary riddle of transposons

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This review focuses on the epigenetic control of the maize *Suppressor-mutator* (*Spm*) transposon and the evolutionary origin of epigenetic mechanisms. Methylation of the *Spm* promoter prevents transcription and transposition, and the methylation of the adjacent GC-rich sequence renders the inactive state heritable. *Spm* encodes an epigenetic activator, TnpA, one of the two *Spm*-encoded transposition proteins. TnpA can reactivate an inactive, methylated *Spm* both transiently and heritably, and it is also a transcriptional repressor of the unmethylated *Spm* promoter. Features common to epigenetic mechanisms in general suggest that they originated as a means of decreasing the recombinogenicity of duplicated sequences.

The riddle of transposons

Transposable elements were discovered more than half a century ago. Initially they were seen as odd, perhaps even unique to maize, in which they were first identified by Barbara McClintock. Today we call them 'transposons' and we know that they are both prevalent and ubiquitous, often comprising as much as a third, and in some cases, more than half, of the genomes of higher plants and animals, including humans. However, their evolutionary role remains enigmatic: do they drive genome evolution or has evolution struggled to eliminate, control and contain transposons?

When transposons insert into genes or regulatory sequences, they commonly disrupt gene function (Fedoroff 1989a). But they can also reprogramme gene expression, and contemporary regulatory sequences occasionally contain relics of transposons and retrotransposons (Walker *et al*. 1995; Britten 1996, 1997). Transposons cause chromosome breaks that stimulate illegitimate recombination, rearrangements, and gene conversion. The chromosomes of most organisms are packed with dozens, hundreds or thousands of copies of transposable element sequences. The activation of just one family of transposons can simultaneously cause structural alterations at many chromosomal sites. Genetic changes are inevitable consequences of transposable element activity. Detailed genome analyses are tracing the evolutionary paths of the transpositions and rearrangements that have sculpted contemporary chromosomes. The rearrangements often begin and end at transposable element sequences (Walker *et al*. 1995; Saxena *et al*. 1996; Kim *et al*. 1998; Schwartz *et al*. 1998). Thus it is now no longer a matter of conjecture, but a fact that transposons are players in the evolutionary game.

Transposons outreplicate the genome, accumulating over time. Appreciating the implications of this property, a pair of speculative essays written almost two decades ago declared transposable elements to be the ultimate parasite: selfish DNA (Doolittle & Sapienza 1980; Orgel & Crick 1980). These essays sought to free us from the then prevalent notion that genome structure is optimized by phenotypic selection. But their most persistent influence, palpable even today, has been to circumscribe our view of transposable elements through their association with the terms 'parasitic' and 'selfish.' This is in part attributable to the power of Doolittle & Sapienza's simplifying assertion that DNA sequences that have an intracellular survival advantage need no other explanation of either their origin or their survival (Doolittle & Sapienza 1980). Not insignificantly, it is also because evidence has continued to accumulate that

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transposable elements can enter a species, and proliferate and spread in an evolutionary instant (Engels 1997).

The puzzle is therefore not how transposons survive in evolution, but how organisms survive their transposons and, perhaps, even accrue an evolutionary benefit. It is my view that transposons constitute a subset of the more general problem posed by genome duplications and the molecular machinery supporting homologous recombination. All organisms have the capacity to break and rejoin pairs of DNA molecules accurately, guided by stretches of sequence similarity or identity. Such homology-dependent recombination mechanisms underlie exchanges that generate new genetic combinations. However, homology-dependent recombination mechanisms both give rise to and eliminate duplications, and they permit recombination between homologous sequences at different chromosomal locations, generating rearrangements. Yet, eukaryotic genome evolution is dominated by the generation of duplications, both tandem and dispersed, by the combined processes of duplication and transposition.

Are there mechanisms that moderate the forces of transposition and homologous recombination? Indeed, there are. The impact of transposable element activity is minimized by both transcriptional and post-transcriptional mechanisms, inaccurate and incomplete replication, and preferential insertion in noncoding sequences (Howe & Berg 1989; Kidwell & Lisch 1997; Kim *et al.* 1998). In the present review, I will focus on the paradigmatic epigenetic regulatory mechanism of the maize *Suppressor-mutator* (*Spm*) transposon. While McClintock's discovery of transposable elements is well known, her pioneering observations on epigenetic regulation are less widely appreciated and I will summarize them here. Epigenetic studies have become increasingly popular recently, particularly as an awareness has grown that correct methylation is essential for development in mammals and higher plants (Jaenisch 1997; Richards 1997).

Based on the observation that the many genomic retrotransposons present in mammals are extensively methylated, Bestor and colleagues recently proposed that epigenetic mechanisms evolved to control the activity of transposons (Yoder *et al.* 1997). This view has been adopted quickly (Henikoff & Matzke 1997; Selker 1997). My purpose here is to consider transposons in the wider context of tandem and dispersed duplications, which are a dominant but problematical theme in genome evolution. I will develop the thesis that epigenetic marking mechanisms underlie the retention of duplications and maintenance of the structure of repetitive eukaryotic genomes. I suggest that the ability of epigenetic mechanisms to interfere with expression of genes, including those encoded by transposons, and with transposition may be an important but secondary consequence of their success in sequestering genes from the recombination machinery.

Structure and expression of the *Spm* transposon

The 8.4-kb *Spm* element has a single transcription unit that occupies most of the its length (Fig. 1a; Pereira *et al.* 1986; Masson *et al.* 1987, 1989). The ends of the transposon contain sequences that are essential for both its transcription and transposition. The *Spm* termini are inverted repeats of the 13-bp sequence, CACTACAA GAAAA. These short-terminal inverted repeats (TIRs) delimit the DNA sequence that transposes. Between the TIRs and the transcription unit at both element ends is a region of several hundred base pairs containing multiple copies of a 12-bp sequence having 75% or more identity with the consensus sequence CCGAC ACTCTTA. These are binding sites for an element-encoded protein TnpA (Pereira *et al.* 1986; Masson *et al.* 1987; Gierl *et al.* 1988). The element's promoter lies within the first 0.2 kb at the element's 5′ end and contains TnpA binding sites (Raina *et al.* 1993). Adjacent to the promoter is a short (0.35 kb), unusually GC-rich (80% G + C) sequence containing 11 homologues of a 17-bp repeat (Masson *et al.* 1987).

The primary *Spm* transcript is alternatively spliced, yielding mRNA sequences with different open reading frames (ORFs), some with extensive overlaps. Four large transcripts have been identified and designated *tnpA-D* (Masson *et al.* 1989). Each transcript encodes either just one or one unique ORF, and the *tnpA* transcript is the shortest and most abundant. The TnpA and TnpD proteins are the only ones for which functions are known, and both are necessary for transposition (Masson & Fedoroff 1989; Frey *et al.* 1990). The structure of the *tnpA* and *tnpD* transcripts is shown in Fig. 1(b,c). In addition to its role in transposition, TnpA is both a positive and a negative regulator of the *Spm* promoter (Gierl *et al.* 1988; Schläppi *et al.* 1993, 1994). Although the role of TnpD in transposition is not yet known, TnpD binds to both free and DNA-bound TnpA, stabilizing the binding of TnpA to DNA (Raina *et al.* 1998).

McClintock's discovery of epigenetic regulation

McClintock observed that the *Spm* element could change to a number of different forms in which it was

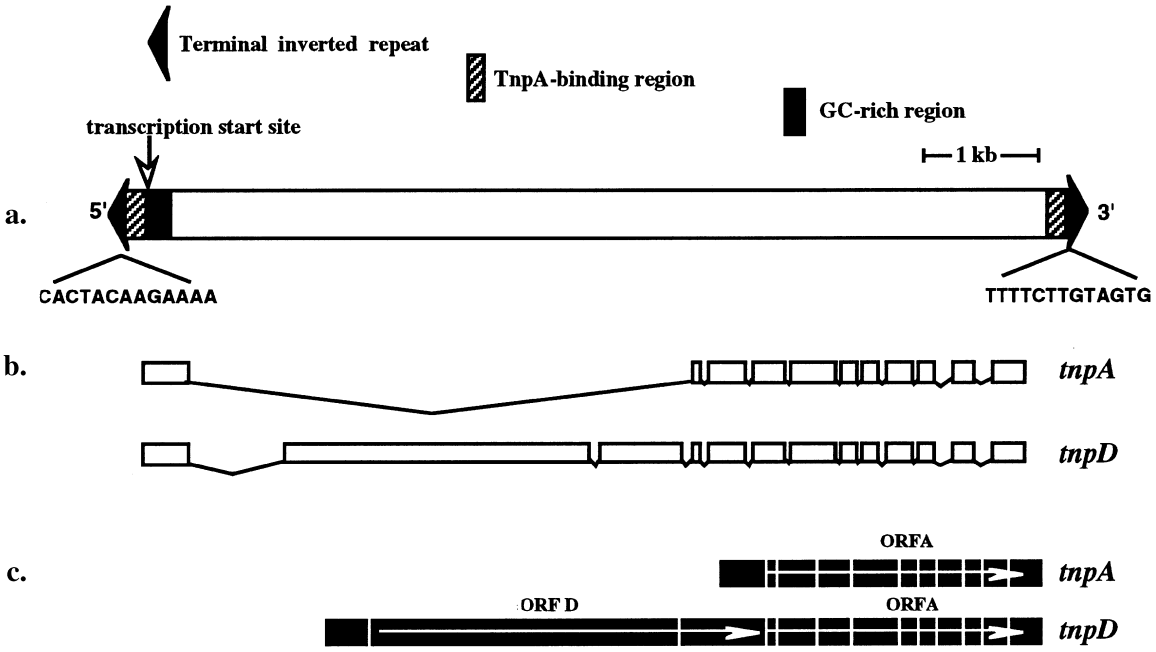

Figure 1 A diagrammatic representation of the *Spm* transposon. (a) Structure of *Spm*. The open box represents the 8.3-kb transposon. (b) The structure of the *tnpA* and *tnpD* transcripts; open boxes represent exons, and connecting lines represent introns. (c) The structure of *tnpA* and *tnpD* mRNAs. The transcripts are represented by filled boxes and the lines mark the location of introns. The arrows show the position and extent of the open reading frames (ORFs).

either silent, or it alternated between active and inactive phases during development (McClintock 1957, 1958, 1959, 1961, 1962, 1965). She described different developmental patterns of transposon activity and reported that a given developmental pattern is heritable, yet subject to further heritable change. She also found that elements could remain silent for multiple plant generations, returning to an active form at a very low frequency. She made the seminal observation that an inactive element could be activated by the presence of an active element, suggesting the existence of a transposon-encoded epigenetic activator (McClintock 1971). McClintock concluded that transposable elements are maintained in genomes in a deeply inactive, yet genetically competent state (McClintock 1946). This is now designated the 'cryptic' state (Fedoroff & Banks 1988; Fedoroff 1989b). In support of this inference, she showed that previously undetectable transposable elements could be converted to an active form under circumstances of extensive chromosome breakage (McClintock 1950a,b, 1951). These observations revealed the existence of a reversible (and therefore epigenetic) inactivation mechanism.

The molecular biology of epigenetic inactivation

Molecular studies showed that there are methylation differences between genetically silent and active *Spm* elements (Banks *et al.* 1988). Overall, both active and inactive elements are extensively methylated, but the sequences flanking the transposon at the insertion site are not (Banks *et al.* 1988). Active elements differ from inactive ones by the absence of methylation in a short region of about 0.6 kb surrounding the transcription start site. Active *Spm* transposons are unmethylated in the 0.2 kb promoter region and inactive transposons are methylated. The GC-rich region just downstream from the transcription start site is variably methylated, and the extent of C methylation in the GC-rich region is correlated with the heritability of the epigenetically inactive state. Extensively methylated *Spm* transposons are not transcribed, do not transpose autonomously and are mobilized at a very low frequency by an active element (Banks *et al.* 1988). This suggests that methylation inhibits both transcription and transposition. However, if an active *Spm* is genetically introduced into a plant with an inactive, methylated *Spm*, the latter is

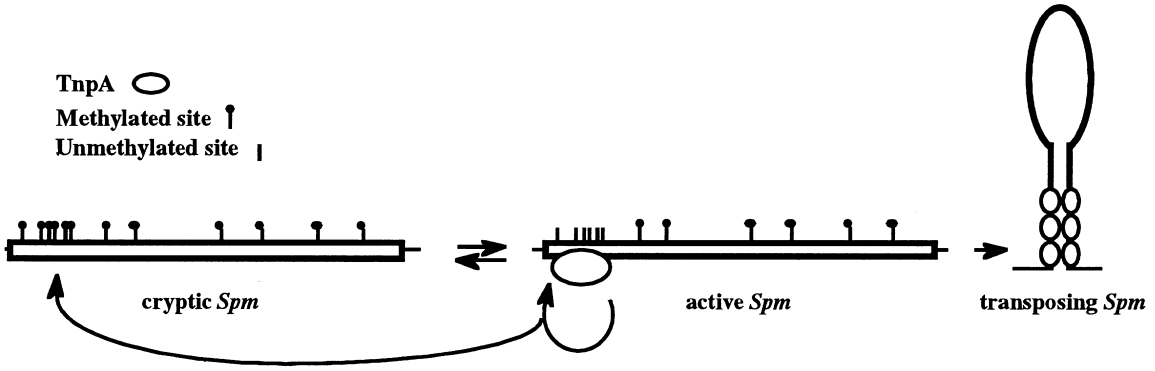

Figure 2 A diagrammatic representation of the *Spm* transposon's epigenetic regulatory scheme. The open boxes represent the element, and filled and open circles are used to represent methylated and unmethylated C residues within its sequence. TnpA is an element-encoded gene product that is required to bring transposon ends together in transposition and it affects transcription by triggering demethylation of the methylated promoter, on the one hand, and inhibiting transcription from the unmethylated promoter, on the other.

both transiently and heritably activated (Fedoroff 1989b). Interestingly, heritable reactivation occurs slowly, requiring several plant generations (Fedoroff 1989b). These observations suggested that *Spm* encodes an epigenetic activator (Fig. 2).

Epigenetic regulation of *Spm* in transgenic plants

Studies that provide a deeper understanding of inactivation and reactivation became possible when it was discovered that epigenetic regulation could be reconstructed in transgenic tobacco, which lacks the many background *Spm*-homologous sequences that interfere with molecular studies in maize (Schläppi *et al.* 1993). *Spm* is reversibly inactivated in tobacco, just as it is in maize, and the inactive transposon is methylated preferentially at the 5′ end (Schläppi *et al.* 1993). Of the 4 *Spm*-encoded proteins, only TnpA can reactivate the transposon, identifying it as the epigenetic activator (Fig. 2). Reactivation is accompanied by a decrease in 5′ terminal methylation of the transposon (Schläppi *et al.* 1993). To determine whether the *Spm* promoter is the target for inactivation, *Spm* promoter-firefly luciferase fusion constructs were created and tested (Schläppi *et al.* 1994). The promoter was readily inactivated and methylated in tobacco cells, but only if the GC-rich region was included within the promoter fragment (Schläppi *et al.* 1994). If the element's promoter lacked the GC-rich sequence, the luciferase gene continued to be expressed. Thus, if it contains the adjacent GC-rich region, the *Spm* promoter sequence is a sufficient target for inactivation and *de novo* methylation.

TnpA: both repressor and activator

Reporter gene assays were also used to assess the effect of TnpA on an unmethylated *Spm* promoter. Surprisingly, TnpA was found to inhibit the unmethylated *Spm* promoter, both in transient assays and in stably transformed tobacco (Schläppi *et al.* 1994). Using domain-swapping experiments, it was found that TnpA functions only as a repressor, although it can be converted to an efficient transcriptional activator by the addition of a transcription activation domain (Schläppi *et al.* 1996). DNA-bound TnpA readily forms intramolecular complexes by protein–protein interactions (Raina *et al.* 1998). Because there are multiple TnpA binding sites at both ends of the transposon, it is likely that TnpA promotes the formation of complexes containing element termini crosslinked by TnpA molecules (Raina *et al.* 1998). This may exclude the binding of transcription factors, explaining TnpA's repressor function. How TnpA activates the methylated promoter is not yet understood, but it may be analogous to its effect on transcription. Although TnpA binds less well to methylated than unmethylated transposon ends, binding may decrease the accessibility of the transposon's termini to methylase. Thus TnpA is a transposon-encoded transposition protein whose interaction with the transposon has both positive and negative regulatory consequences.

Epigenetic inactivation of transposons

The methylation-associated inhibition of transposon activity is extensively documented in plants (Fedoroff

1996; Martienssen 1996). The general properties of methylation-associated transposon inactivation in plants are, that it is highly heritable and generally affects a single element, although there are examples of concerted silencing of all of the members of a transposon family throughout the genome (Martienssen 1996). A majority of the methylated sequences in the human genome are retrotransposons (Yoder *et al.* 1997). Based on this observation, it has recently been proposed that methylation evolved to control the potentially deleterious effects of transposition by suppressing their transcription (Yoder *et al.* 1997). Although there is, as yet, no direct evidence that methylation is responsible for the inactivity of retrotransposons, there is ample evidence that methylation silences gene expression (Hershkovitz *et al.* 1990; Nyce 1991; Holliday & Ho 1995). However, epigenetic inactivation need not be associated with methylation. Nor does it require that a sequence be transcriptionally active. Transposon silencing has been observed in *Drosophila,* which exhibits little or no DNA methylation (Preston & Engels 1989; Jensen *et al.* 1995). The common thread connecting inactivating sequences is neither the sequence, whether coding or noncoding, nor a particular mechanism, but rather the detection of homology. The capacity to detect and mark duplications appears to be the essential common feature of epigenetic mechanisms and may well hold the key to understanding their evolutionary origin.

An evolutionary paradox

Duplications are a by-product of the properties of the DNA replication and recombination machinery. Short stretches of homology suffice to give rise to duplications by slippage during replication, homology-dependent unequal crossing-over, and double-strand breakage/repair (Gorbunova & Levy 1997; Liang *et al.* 1998). However, duplications are problematical. Once a duplication exists, the mechanisms that generated it also permit unequal crossing-over between identical repeats (Anderson & Roth 1977; Perelson & Bell 1977; Koch 1979). In micro-organisms, the frequency of unequal crossing-over between tandem repeats is higher than the duplication frequency (Romero & Palacios 1997). Unequal crossing-over between repeated sequences produces organisms with higher numbers of repeats, and organisms with only one remaining copy of the duplicated sequence (Perelson & Bell 1977).

This gives rise to a fundamental paradox: chromosomes with a single remaining copy of a repeat are stable, while larger and larger numbers of copies of the duplications are segregated to a smaller and smaller fraction of the population by unequal crossing-over. Absent mechanisms to stabilize duplications or permit their asymmetric generation, organisms with many copies of a given sequence can never come to dominate a population without phenotypic selection. Prokaryotes bear out this theoretical expectation, transiently generating and readily losing large tandem duplications in response to selection (Koch 1971; Anderson & Roth 1977; Romero & Palacios 1997). The DNA segments most commonly amplified are, importantly, often bounded by transposons, which provide the dispersed homologies necessary to originate duplication (Romero & Palacios 1997).

Stability of duplicated sequences

Whatever the theoretical difficulties, duplications arise and are maintained in all known genetic systems. As much as one-third of the human genome and half or more of the genomes of higher plants consist of repetitive sequences, a majority of which are transposons and retrotransposons (Flavell *et al.* 1974; Smit *et al.* 1995; SanMiguel *et al.* 1996; Smit & Riggs 1996; Smit 1996; Bennetzen & Freeling 1997). Perfect tandem repeats are highly unstable in prokaryotes (Bi & Liu 1996b,a; Feschenko & Lovett 1998). And ectopic recombination between homologous sequences occurs at a high frequency in prokaryotes and yeast (Lichten *et al.* 1987; Kupiec & Petes 1988; Goldman & Lichten 1996). An extrapolation of the recombination frequencies in these organisms to the highly redundant maize and human genomes leads to the prediction of multiple rearrangements per cell at meiosis. However, recombination frequency decreases with increasing genome size (Thuriaux 1977), and while there is evidence in both plants and animals for ectopic homologous recombination in higher eukaryotes, the reported frequencies are surprisingly low, of the order of $1–3\times10^{-6}$ (Gorbunova & Levy 1997; Schiebel *et al.* 1997; Shalev & Levy 1997; Ciotta *et al.* 1998; Cooper *et al.* 1998). At the same time, there is both sequence conservation between repeats, and the higher order conservation of genome layout, suggesting the existence of mechanisms for protecting duplications and stabilizing the structure of chromosomes in the face of extensive homologies (Bennetzen & Freeling 1997; Schwartz *et al.* 1998).

Duplications, methylation and recombination

Differences in the basic recombination machinery account for some of the differences in homologous

and ectopic recombination frequencies between prokaryotes and lower eukaryotes, on the one hand, and higher eukaryotes with large genomes, on the other (Petes *et al.* 1989; Gorbunova & Levy 1997; Liang *et al.* 1998). However, eukaryotes have additional mechanisms for the recognition, retention and regulation of redundancy. These include the repeat induced point mutation (RIP) in *Neurospora crassa and* methylation induced premeiotically (MIP) in the fungus *Ascobolus immersus* (reviewed in Selker 1997). Both RIP and MIP modify both copies of a pair of repeats. In MIP, duplicated sequences are just methylated, while in RIP, both copies of the duplicated sequence are both C-methylated and mutated at a variable number of the GC base pairs.

Although the underlying mechanisms vary, most or all eukaryotes have the capacity to recognize and modify either the structure or the nuclear form of the duplicated DNA. This capacity was often first recognized genetically, as in paramutation in plants and the position-effect variegation in *Drosophila* (Brink 1958; Coe 1968; Tartof *et al.* 1989; Wallrath & Elgin 1995; Hollick *et al.* 1997). Many higher eukaryotes, including plants and animals, methylate the duplicated DNA (Flavell 1994; Ronchi *et al.* 1995; Cerutti *et al.* 1997; Perticone *et al.* 1997; Garrick *et al.* 1998). However, even organisms such as yeast and *Drosophila*, which do not appear to methylate DNA either at all or very much, have mechanisms which recognize and alter the accessibility or expression of duplicated DNA (Urieli-Shoval *et al.* 1982; Preston & Engels 1989; Sabl & Henikoff 1996; Dorer & Henikoff 1997; Pirrotta 1997; Sherman & Pillus 1997).

Extra copies of genes introduced by the transformation of plants can result in either the sporadic or consistent inactivation of both the introduced and endogenous copies (Assaad *et al.* 1993; Meyer 1996; Ye & Signer 1996). While the phenomenon was often first detected because it caused transcriptional inactivation, it appears likely that the underlying mechanism is based on the detection of homologies. This, in turn, implies that there exist mechanisms that scan even very large genomes for homologies. A copy number-dependent inactivation mechanism similar to that in plants is now known to exist in animals (Garrick *et al.* 1998). Silenced genes are often, but not always, methylated and silencing may or may not be heritable. If it is heritable, silencing can be reversed (Paszkowski 1994; Wolffe 1998). Although it was hypothesized some time ago that duplication-targeted DNA methylation might serve to mark and differentiate duplicated sequences, the first direct evidence that DNA methylation inhibits recombination appeared only recently (Kricker *et al.* 1992; Maloisel & Rossignol 1998). Additional strong indirect evidence comes from a report that mutations, a majority of which are attributable to deletions and other types of illegitimate recombination events, are more common by an order of magnitude in ES cells lacking the methyltransferase gene than in cells that are either hemi- or homozygous for it (Chen *et al.* 1998)

Conclusions

In conclusion, I propose that the evolutionary origins of epigenetic mechanisms lie in the necessity to sequester duplicated sequences from the homologous recombination machinery. This is likely to be true for dispersed transposons as well as tandem duplications, since homologous recombination between ectopic sequences generally results in chromosome rearrangements, which are often lethal. It may be that the evolutionary invention of an efficient marking and sequestering mechanism for duplicated sequences was actually a prerequisite for their rapid accumulation in eukaryotic evolution. The undeniable fact is that genome organization and chromosome structure are relatively stable, despite great differences in the proportion of genomic DNA comprising highly recombinogenic tandem and dispersed repeats, among them the transposons and retrotransposons that are so abundant in many higher eukaryotic genomes. Yet the ability to prevent recombination between homologous duplicated sequences is also demonstrably imperfect. Analyses of large genomic sequences have begun to reveal traces of historic recombination and transposition events (Walker *et al.* 1995; Saxena *et al.* 1996; Kim *et al.* 1998; Schwartz *et al.* 1998).

Just as insertions have occasionally provided genes with new developmental gene regulation profiles, so it appears that epigenetic mechanisms are now used for much more than homology recognition. McClintock first described the ability of transposons to be expressed in regular, heritable developmental patterns (McClintock 1965). Today it is increasingly evident that epigenetic mechanisms are central to the developmental regulation of certain genes in both plants and animals. Chromatin complexes formed by the Polycomb Group of genes in *Drosophila*, the stable inactivation of imprinted genes in mammals, and the methylation of vernalization and flowering genes in plants, are all examples of contemporary epigenetic developmental regulatory mechanisms (Finnegan *et al.* 1996; Jaenisch 1997; Pirrotta 1997; Richards 1997; Finnegan *et al.* 1998). The underlying molecular mechanisms are just now being elucidated. The *Spm* transposon's TnpA may constitute the first

member of a more general class of epigenetic regulatory proteins.

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