Plant Transposable Elements. A Hard Act to Follow

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The discovery and genetic characterization of plant transposable elements (TEs) led to a revolution in our understanding of the composition and dynamic potential of the genetic material in virtually all organisms. Most of these breakthroughs occurred between 30 and 50 years ago. It was during this time that TEs were discovered in maize (*Zea mays*) and several aspects of their genetic behavior were characterized. Through the study of spotted kernels and sectored flowers, McClintock and her contemporaries discovered: (a) the existence of multiple TE families with autonomous and nonautonomous members that are normal residents of the genome, (b) that elements can move within and between chromosomes where they can alter gene expression or serve as sites of chromosome breakage or rearrangement, (c) that excision is often imprecise and reinsertion is often to a linked locus, and (d) that elements can exist in the genome in a quiescent state that is subject to reactivation by biotic and abiotic means collectively termed "genomic stress." This era of discovery and its relevance to modern biology is reviewed by Fedoroff (9). In addition, the story of how the genomic stress hypothesis came to fruition is summarized in McClintock's Nobel lecture (18). This view of the genome as responsive and dynamic, that is, something more than a collection of genes, heralded the start of the current genomics era.

The purpose of these historical notes is to review conceptual breakthroughs that have occurred over the past quarter century. I would venture to guess that for most of us involved in the study of plant TEs during this time, the historical legacy has been a hard act to follow. However, I will argue that recent studies, especially those in the last 5 years, have raised the bar on what constitutes the dynamic genome and have placed plants once again at the forefront of transposon studies.

PHASE I: THE DNA ELEMENTS— CHARACTERIZING OUR GENETIC LEGACY

Although the historical legacy may have been a hard act to follow, the large collections of TE-induced alleles generated during that era provided most of the raw materials used by the first generation of plant molecular biologists. What I have arbitrarily called

phase I in the molecular analysis of plant TEs focused on two areas: (a) the isolation of genes and their TE-induced alleles and the subsequent characterization of TE families, and (b) understanding the mechanisms underlying the diversity of unstable phenotypes. Many of the first plant genes cloned in the early 1980s were chosen because they had TE-induced alleles. These include the *sh1*, *adh1*, and *wx* genes from maize and the *nivea* gene from *Antirhinnum majus*. From their mutant alleles came the first characterized *Ds*, *Ac*, and *Tam* elements (3, 11, 21). The isolation of members of the other prominent plant TE families including *Mutator*, *Spm/En*, and other *Tam* elements occurred in numerous labs around the world including those of the Burrs, Freeling, Peterson/Saedler, Starlinger, Walbot, and Carpenter/Coen (for review, see 17)

This first wave of plant elements was distinguished by their high frequency of somatic and germinal instability. All were DNA or class 2 elements, a group that is characterized by short inverted terminal repeats and transposition via a DNA intermediate. That is, the element usually excises from one site and reinserts elsewhere. Analyses of complex and diverse mutant phenotypes induced by insertion and excision of class 2 elements into plant genes revealed a myriad of ways that these elements can modify gene regulation. These included: (a) the discovery that transposon footprints are usually left behind when elements exicise (21), (b) that *Ds* and *dSpm* elements function as introns (15, 23), and (c) that promoter insertions can alter tissue-specific patterns of expression (8).

In retrospect, the most important discoveries that came out of this period were the analyses of what McClintock called changes in phase, or the reversible switch in element activity. The correlation between element activity and methylation state was first documented for the *Mutator* element (6) and foreshadowed similar correlations for other TEs and normal plant genes.

With these elements in one hand and a large collection of previously isolated TE-induced alleles in the other, transposon tagging strategies were developed (for review, see 10) that permitted the rapid isolation of many structural and regulatory genes in both maize and *Antirrhinum majus.* The importance of transposon tagging protocols for gene isolation was recognized by those working with plants that lack

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well-characterized endogenous systems. This recognition led to the introduction of the maize elements into a variety of plant species, beginning with tobacco (*Nicotiona tubacum*; 1), and has resulted in the isolation of many genes, the first being a floral color gene from petunia (*Petunia hybrida*; 7) and the most prominent being the *N* resistance gene from tobacco (24). It is unfortunate that the success of forward genetic approaches like transposon tagging is severely limited by the large size of most plant genomes and the requirement for a visible mutant phenotype. To circumvent these problems, site-selected transposon tagging protocols (also called reverse genetics) using the *Mutator* element were developed (2).

PHASE 2: TEs OF HIGH COPY NUMBER

Although the characterization of class 2 elements dominated the first 10 years of the molecular era, it soon became clear that the low copy numbers of these elements precluded their having a significant impact on genome size, structure, or evolution. One exception to this generalization may be miniature inverted repeat TEs (MITEs) which appear to be high copy number class 2 elements that, in some cases, are preferentially associated with grass genes (4).

TE studies in the 1990s have been dominated by long terminal repeat (LTR) retrotransposons, which are members of the class 1 or retro-element group. LTR retrotransposons are flanked by long terminal repeats and usually encode all of the proteins required for their transposition. For all class 1 elements, it is the element-encoded transcript, and not the element itself, that forms the transposition intermediate. It is for this reason that they can attain much higher copy number than class 2 elements. Transcription of most of the active plant elements characterized to date is largely quiescent during normal development but can be induced by biotic and/or abiotic stresses including cell culture, wounding, and pathogen attack (12). Because the element-encoded transcript is also the transposition intermediate, LTR retrotransposons may have the ability to rapidly alter genome structure in response to environmental cues (see below).

Given their large size (from 4–10 kb on average) and potential to amplify on a massive scale, it is not surprising that LTR retrotransposons comprise the largest fraction of TE-derived genomic DNA in almost all plant genomes examined to date (for review, see 16). An important series of recent experiments, led by the Bennetzen lab (20), has demonstrated that differential amplification of LTR retrotransposons largely accounts for the C-value paradox among the agronomically important members of the grass clade. The C-value paradox is the observed lack of correlation between increases in DNA content and an organism's complexity. It has been documented for both animal and plant species, but to date only appears to be "solved" for the members of the grass tribe.

The focus on high copy number elements in plants necessitated the development of new protocols to assay TE movement on a whole-genome basis. Unlike the low copy number class 2 elements discussed above, MITEs and retrotransposons rarely transpose and are not associated with mutant genes. Thus their activity could not be visualized in the traditional manner of examining spotted kernels (Fig. 1A). Instead, a modification of the gel-based amplified fragment length polymophism technique called transposon display was developed to simultaneously monitor the movement of hundreds of elements (Fig. 1B; 22). Transposon display of the stable and highly polymorphic MITE families of maize has led to their use as a new class of molecular marker that is preferentially associated with genic regions (5).

THE FUTURE OF PLANT TRANSPOSONS: POISED FOR NEW BREAKTHROUGHS

Given that a large fraction of the DNA sequence output from plant genome projects will be derived from TEs, there will be no shortage of new elements to be discovered, categorized, and exploited as potentially valuable molecular tools. However, three recent papers exemplify for me the areas where major breakthroughs are most likely to arise. The first, by Hirochiki and coworkers, reports the amplification of the tobacco retrotransposon *Tto1* in Arabidopsis plants that are methylation deficient (*ddm1*; 13). In the near future we should know how epigenetic
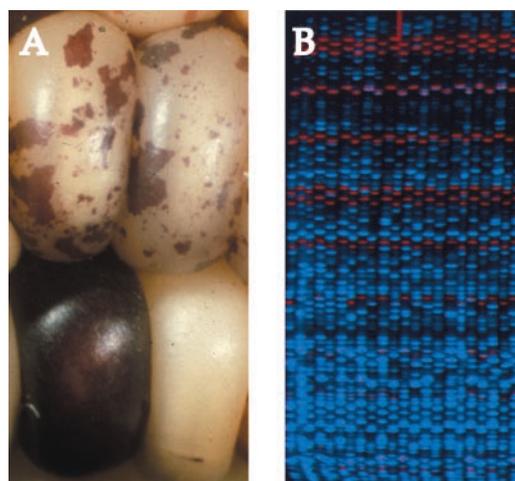
Figure 1. Assaying TE activity the old way (A) and the new way (B). A, Maize kernels displaying patterns characteristic of TE excision from a gene in the anthocyanin biosynthetic pathway. B, Computer-generated image of a transposon display where blue fluorescent bands are PCR products that are anchored in a restriction site and in a member of the MITE family *Hb2* (courtesy of Zenaida Magbanua [University of Georgia, Athens]). Red bands are M_r markers. Samples were resolved on an ABI377 sequencer where the loading was staggered.

mechanisms regulate TEs, whether this control is influenced by environmental cues, how TE organization influences global chromatin structure (and in turn gene expression), and whether epigenetic regulation evolved to regulate TEs.

Two papers have raised the bar on our concept of the dynamic genome and have positioned the grass clade as a focal point for future studies. In a follow-up to their study of intergenic retrotransposons in maize, SanMiguel et al. (19) provide evidence that a burst in retrotransposon activity doubled the size of the maize genome within the past 3 million years. This result demonstrated for the first time that TEs could rapidly restructure a genome. In the second paper Kalendar et al. (14) present a dramatic example of TE-mediated genomic restructuring within populations of the wild barley *Hordeum spontaneum* growing in distinct regions of a canyon in Israel. In this case, genome restructuring takes the form of genome size variation due to retrotransposon amplification (the *BARE-1* element) and intra-element deletion. Correlation between *BARE-1* copy number, genome size, and local environmental conditions suggest for the first time a testable molecular mechanism linking habitat with TE induction in natural populations.

Taken together these two studies suggest that the grass clade is in a dynamic period of genomic restructuring and, for this reason, may be the system of choice for understanding the extent of TEs involvement in both macroevolutionary and microevolutionary processes. Given the rapid pace of recent discoveries, it may be reasonable to expect that in the not-too-distant future this line of research will provide mechanisms to explain how evolution works at the molecular level.

LITERATURE CITED

1. **Baker B, Schell J, Lorz H, Fedoroff N** (1986) Proc Natl Acad Sci USA **83:** 4844–4848
2. **Bensen RJ, Johal GS, Crane VC, Tossberg JT, Schnable PS, Meeley RB, Briggs SP** (1995) Plant Cell **7:** 75–84
3. **Bonas U, Sommer H, Saedler H** (1984) EMBO J **5:** 1015–1019
4. **Bureau TE, Wessler SR** (1992) Plant Cell **4:** 1283–1294
5. **Casa AM, Brouwer C, Nagel A, Wang L, Zhang Q, Kresovich S, Wessler SR** (2000) Proc Natl Acad Sci USA **97:** 10083–10089
6. **Chandler VL, Walbot V** (1986) Proc Natl Acad Sci USA **83:** 1767–1771
7. **Chuck G, Robbins T, Nijjar C, Ralston E, Courtney-Gutterson N, Dooner HK** (1993) Plant Cell **5:** 371–378
8. **Coen ES, Carpenter R, Martin C** (1986) Cell **47:** 285–296
9. **Fedoroff NV** (1983) *In* Mobile Genetic Elements. Academic Press, New York, pp 1–63
10. **Fedoroff NV, Furtek DB, Nelson OE** (1984) Proc Natl Acad Sci USA **81:** 3825–3829
11. **Fedoroff NV, Wessler SR, Shure M** (1983) Cell **35:** 235–242
12. **Grandbastien M-A** (1998) Trends Plant Sci **3:** 181–189
13. **Hirochika H, Okamoto H, Kakutani T** (2000) Plant Cell **12:** 357–369
14. **Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH** (2000) Proc Natl Acad Sci USA **97:** 6603–6607
15. **Kim HY, Schiefelbein JW, Raboy V, Furtek D, Nelson OE** (1987) Proc Natl Acad Sci USA **84:** 5863–5867
16. **Kumar A, Bennetzen JL** (1999) Annu Rev Genet **33:** 479–532
17. **Kunze R, Saedler H, Lönnig W-E** (1997) Adv Bot Res **27:** 331–470
18. **McClintock B** (1984) Science **226:** 792–801
19. **SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL** (1998) Nat Genet **20:** 43–45
20. **SanMiguel P, Tikhonov A, Jin Y-K, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen, JL** (1996) Science **274:** 765–768
21. **Sutton WD, Gerlach WL, Schwartz D, Peacock WJ** (1984) Science **223:** 1265–1268
22. **Van den Broeck D, Maes T, Sauer M, Zethof J, De Keukeleire P, D'Hauw M, Van Montagu M, Gerats T** (1998) Plant J **13:** 121–129
23. **Wessler SR, Baran G, Varagona M** (1987) Science **237:** 916–918
24. **Whitham S, Dineshkumar SP, Choi D, Hehl R, Corr C, Baker B** (1994) Cell **78:** 1101–1115