Mol Gen Genet (1988) 211:364–366

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Short communications

An RFLP adjacent to the maize *waxy* gene has the structure of a transposable element

Marion L. Spell, George Baran*, and Susan R. Wessler
Botany Department, University of Georgia, Athens, GA 30602, USA

Summary. Two maize inbred lines harbor non-mutant *waxy* (*Wx*) genes that display restriction fragment length polymorphism (RFLP) upstream from the start of *Wx* transcription. Sequencing of this region in the two strains revealed a DNA insertion with the structural features of a transposable element. The insertion is 316 bp in length, has 15 bp imperfect inverted repeats and is flanked by a 5 bp direct repeat generated upon insertion. Sequences homologous to this insertion are present in multiple copies in maize and its relatives teosinte and *Tripsacum* but not in the more distantly related dicot tobacco. Finally, this element is not homologous with any previously described maize DNA insertion.

Key words: Transposable element – RFLP – Maize – *Waxy* gene

The *waxy* gene of maize is an excellent experimental system for understanding the factors contributing to genomic diversity in higher plants. The gene is responsible for the synthesis of amylose in endosperm and pollen tissues. Mutant alleles are plentiful because they are viable and easily distinguished phenotypically. In addition to the unstable alleles caused by the insertion of transposable elements, there are many phenotypically stable alleles of spontaneous origin. Recently we have demonstrated that many of these stable mutations are also caused by the insertion of large DNA elements (Wessler and Varagona 1985). This analysis has also revealed that the non-mutant *Wx* genes carried by many closely related inbred lines display extensive restriction fragment length polymorphism (RFLP) in 5′ flanking regions. In this study we have characterized one of these RFLPs and shown it to be a transposable element-like DNA insertion.

Southern blot analysis of the *Wx* gene harbored by the two inbred lines HY and W23 revealed several RFLPs believed to be caused by insertions or deletions within and around the *Wx* transcription unit. To understand the precise nature of these changes we cloned a 10 kb *Eco*RI fragment harboring the *Wx* gene in the W23 allele and compared it with the HY clone isolated previously (Shure et al. 1983). There are three differences between the restriction maps of these clones (Fig. 1A); we focused on the apparent insertion in the *Sst*I-*Eco*RV fragment of W23 (Fig. 1A, compare CAS1 and CAS2). To determine whether the 0.3 kb difference in size between CAS1 and CAS2 resulted from an HY deletion or a W23 insertion, this region in both strains was subcloned and sequenced (Figs. 1B and 2). We found that the RFLP results from an additional 316 bp in CAS2 (Fig. 2B) located 42 bp from the *Sst*I site. (Fig. 1B). This region is 1.6 kb upstream from the start of *Wx* transcription (Klosgen et al. 1986). For the reasons described below, we believe that the additional 316 bp in W23 arose by the insertion of a transposable-like element.

Virtually all of the maize transposable elements analyzed to date can be distinguished by three distinctive structural features: (1) inverted repeat termini, (2) a direct repeat of host target sequences flanking these termini and (3) multiple copies of homologous sequence in all maize genomes and the genomes of maize relatives (reviewed in Nevers et al. 1985). The insert in W23 satisfies all of these criteria.

Comparison of the sequences at the site of insertion reveals that 5 bp of HY [CTAAG] have been duplicated and reside adjacent to the termini of the inserted element (Fig. 2A). The presence of the direct repeat suggests that a transposase mediated the insertional event. The direct repeat flanks 15 bp imperfect inverted repeat termini; 3 of the 15 bp are mismatched (Fig. 2A). The integrity of the termini of plant elements has been correlated with the ability of the element to transpose (Schwarz-Sommer et al. 1985). The W23 allele has been crossed into several maize backgrounds and the insert has not undergone transposition (Wessler and Varagona 1985; Schwarz-Sommer et al. 1984). Thus the imperfect nature of the inverted termini may indicate that the insert can no longer transpose. Alternatively, the element may transpose at a very low frequency or in an untested genetic background.

When W23 and HY genomic DNA are digested with *Eco*RI (an enzyme that does not cut within the DNA insertion), we find a single fragment homologous to the HY probe (Fig. 3A) but at least 50 fragments homologous with all or part of the 316 bp insert (Fig. 3B). Southern blot analysis of genomic DNA from teosinte, a close maize relative and *Tripsacum dactyloides*, a more distant relative, indi-

* *Present address:* Molecular Genetics Inc., 10320 Bren Rd. East, Minnetonka, MN 55343, USA

Offprint requests to: S. Wessler

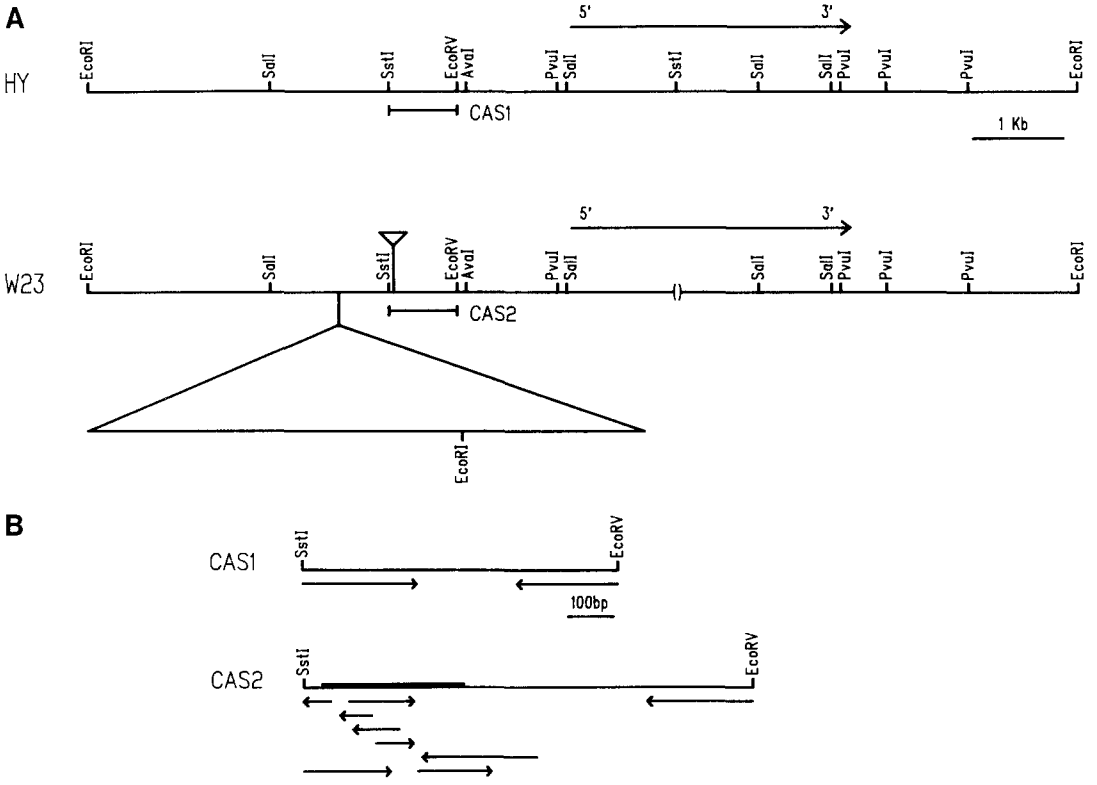

Fig. 1. A A comparison of the *Wx* gene and flanking DNA harbored by the maize inbred lines HY and W23. Insertions and deletions in the W23 allele are denoted by *triangles* and *parentheses* respectively. The W23 allele was isolated from a library (a gift from S. Dellaporta) that was generated by inserting a complete *Eco*RI digest of maize genomic DNA into the λ vector EMBL 4. In this strain the entire *Wx* gene is contained on a 10 kb *Eco*RI fragment that extends from the *Eco*RI site in the large DNA insert to the *Eco*RI site downstream from the 3′ end of the gene. The 5′ to 3′ *arrow* above W23 represents the direction and approximate limits of the *Wx* transcription unit (Klosgen et al. 1986). **B** DNA sequencing strategy. The *Sst*I-*Eco*RV fragments designated CAS1 and CAS2 were subcloned into the phage vectors M13mp18 and 19 (Yanisch-Perron et al. 1985) and sequenced (Sanger et al. 1977). Random subclones were produced using the shotgun cloning procedure of Messing (1983). The *arrows* indicate the regions sequenced. The *heavy line* in CAS2 indicates the location of the insert

A

HY "TARGET" SITE GATTGG CTAAG ACTATC

W23 GATTGGCTAAG GGTcTaTTTGgTTGA.. 286bp ..TCAAaCAAAcAcACC CTAAGACTAT

B

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10          20          30          40          50          60
GGTCTATTTG GTTGAGCTGT GCGTGTGAAA AAAGTTTGCT ATGGACTGTG AGCTGTGAAA

70          80          90          100         110         120
AAATCTGCTG TAGGCTGTAA GCTGTTAAAA AGCTAAAAAC CGTTTGGTTG AAACCACTAA

130         140         150         160         170         180
AAGTCGTTAA AAATTCCTTC GATATATGTT TCACAGTTCC ATCCGAAAAA CCACTAAAAG

190         200         210         220         230         240
CAGGTCTAGA GGTGCTTTCA GATTTGCACT ACGAGAAAGT CGGCTTTTAG AAAAAGTTGC

250         260         270         280         290         300
TTCCTAGATC CAGCCCTTTG GTTGGCTTTT GGCTTTTAGG GGTGCAAAAC AAAGCCAAAA

310
GTCAAACAAA CACACC
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Fig. 2A, B. Sequence of the DNA insertion in the W23 allele. **A** Sequence comparison of the region of insertion in HY and W23. The *boxed* region represents the 5 bp host sequence that is duplicated upon insertion. The *arrows* underline the imperfect inverted repeat termini of the insert. *Lower case letters* indicate base pair mismatches within the repeat. **B** DNA sequence of the 316 bp insert

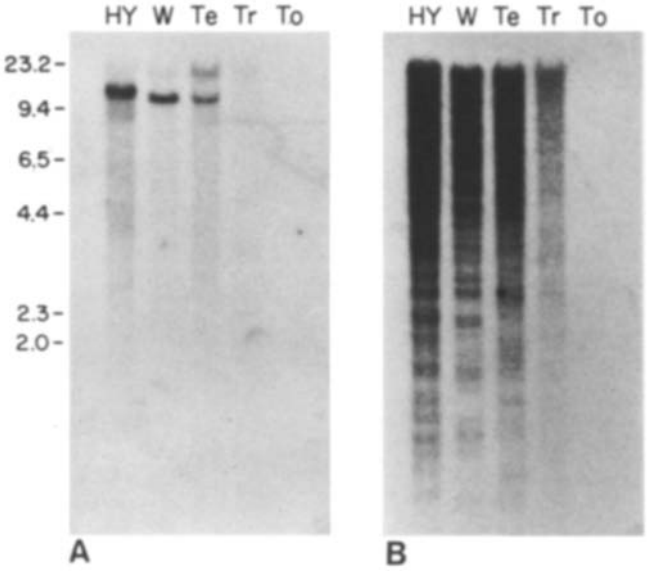

Fig. 3A, B. Southern blot analysis of genomic DNA probed with CAS1 and CAS2. **A** *Eco*RI-digested genomic DNA (2 μg) was electrophoresed through 0.7% agarose, transferred and probed with nick translated CAS1 (10^8 cpm/μg, Rigby et al. 1977) by methods described previously (Wessler et al. 1986). Filters were washed using high stringency conditions (67° C, 0.1 × SSC, 0.5% SDS for 1 h) and autoradiography was for 60 h with an intensifying screen. The probe was eluted at 42° C using the Gene Screen manufacturers protocol. W, W23; Te, teosinte; Tr, *Tripsacum*; To, tobacco. **B** The filter described in **A** was reprobed with CAS2 (Fig. 1). All conditions described in **A** were repeated for **B**. Molecular weight markers are in kb

cates that sequences homologous with the insert are also present in multiple copies (Fig. 3B). Homologous sequences are not detected in the more distantly related dicot, tobacco (Fig. 3B).

Sequence comparisons indicate that neither the termini nor the central region of this element has significant homology with any known maize element analyzed to date (Sutton et al. 1984; Muller-Neumann et al. 1984; Shepherd et al. 1984; Pohlman et al. 1984; Periera et al. 1985; Barker et al. 1984). Thus, these sequences identify a new class of middle repetitive, potentially transposable DNA in maize and its relatives.

It has been suggested that up to half of the maize genome either is or was mobile (Freeling 1984). If there is even a fraction of this much mobile DNA present, evidence for DNA insertions should be widespread. The *Wx* RFLP described here is the third reported example of transposable element involvement in RFLP formation in maize (Shepherd et al. 1984; Sachs et al. 1986). This, coupled with the involvement of DNA insertions in both unstable and stable mutant alleles, underscores the important contribution of transposable elements in the evolution of the maize genome.

Acknowledgements. We thank Beth Johnston, Steve Ludwig, Russell Malmberg and Alan Jaworski for critical reading of the manuscript. This work was supported by NIH grant GM 32528 (S.R.W) and an NIH postdoctoral fellowship (G.B.).

References

Barker RF, Thompson DV, Talbot DR, Swanson J, Bennetzen JL (1984) Nucleotide sequence of the maize transposable element *Mu1*. Nucleic Acids Res 12:5955–5967

Freeling M (1984) Plant transposable elements and insertion sequences. Annu Rev Plant Physiol 35:277–298

Klosgen RB, Gierl A, Schwarz-Sommer Z, Saedler H (1986) Molecular analysis of the *waxy* locus of Zea mays. Mol Gen Genet 203:237–244

Messing J (1983) The M13 vectors for cloning. Methods Enzymol 101:20–78

Muller-Neumann M, Yoder JI, Starlinger P (1984) The DNA sequence of the transposable element *Ac* of *Zea mays*. Mol Gen Genet 198:19–24

Nevers P, Shepherd N, Saedler H (1985) Plant transposable elements. Adv Bot Res 12:102–203

Pereira A, Schwarz-Sommer Z, Gierl A, Bertram I, Peterson P, Saedler H (1985) Genetic and molecular analysis of the *Enhancer* (*En*) transposable element system of *Zea mays*. EMBO J 4:17–23

Pohlman R, Fedoroff NV, Messing J (1984) The nucleotide sequence of the maize controlling element *Activator*. Cell 37:635–643

Rigby PW, Dieckmann JM, Rhodes C, Berg P (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Sachs MM, Dennis ES, Gerlach WL, Peacock WJ (1986) Two alleles of maize *alcohol dehydrogenase I* have 3′ structural and poly(A) addition polymorphisms. Genetics 113:449–467

Sanger F, Nicklen S, Coulsen AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 56:5463–5467

Schwarz-Sommer Z, Gierl A, Klosgen RB, Wienand U, Peterson P, Saedler H (1984) The *Spm* (*En*) transposable element controls the excision of a 2-kb DNA insert at the *wx-m8* allele of *Zea mays*. EMBO J 3:1021–1028

Schwarz-Sommer Z, Gierl A, Berndtgen R, Saedler H (1985) Sequence comparison of states of *a1-m1* suggests a model of *Spm* action. EMBO J 4:2439–2443

Shepherd NS, Schwarz-Sommer Z, Blumberg vel Spalve J, Gupta M, Wienand U, Saedler H (1984) Similarity of the *Cin1* repetitive family of *Zea mays* to eukaryotic transposable elements. Nature 307:185–187

Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the *waxy* locus in maize. Cell 35:225–233

Sutton WD, Gerlach WL, Schwartz D, Peacock WJ (1984) Molecular analysis of *Ds* controlling element mutations at the *Adh1* locus of maize. Science 223:1265–1268

Wessler SR, Varagona M (1985) Molecular basis of mutations at the *waxy* locus of maize: Correlation with the fine structure genetic map. Proc Natl Acad Sci USA 82:4177–4181

Wessler SR, Baran G, Varagona M, Dellaporta S (1986) Excision of *Ds* produces *waxy* proteins with a range of enzymatic activities. EMBO J 5:2427–2432

Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC 19 vectors. Gene 33:103–109

Communicated by R.B. Goldberg

Received August 9, 1987