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Molecular analysis of the *Ubiquitous* (*Uq*) transposable element system of *Zea mays*

A.G. Pisabarro, W.F. Martin*, P.A. Peterson[1], H. Saedler, and A. Gierl

Max Planck Institut für Züchtungsforschung, Abteilung Molekulare Pflanzengenetik, Carl-von-Linné Weg 10, W-5000 Köln 30, FRG
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA

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Summary. The *Uq* transposable element of maize is the most widely dispersed among different maize populations and genetic testerstrains. Despite intensive genetic characterization, little is known about its molecular structure. In order to obtain information relevant to this topic, we have cloned and sequenced three *ruq* receptors. Surprisingly, they are all *Ds1*-like receptor types of the *Ac-Ds* transposon family. Based on our molecular data, we present a model to explain the functional differences associated with the differential expression of the *Uq* and *Ac* transposon systems.

Key words: Maize – Transposable element – *Ac/Ds* – *Uq/ruq* – Methylation.

Introduction

Classical plant transposable elements (TEs) have short terminal inverted repeats (TIRs) and transpose via a non-replicative mechanism, i.e. the element leaves the donor site and integrates into a new chromosomal location (for review, see Döring and Starlinger 1986; Peterson 1988; Gierl et al. 1989). During integration, TEs generate small sequence duplications of the target site and are thus flanked by short DNA duplications of a size characteristic for a given TE family (Saedler and Nevers 1985). Two different kinds of elements can be distinguished in this class of transposons: autonomous elements, which are able to self-transpose, and receptor elements (usually deletion derivatives of the autonomous element), which are transposable only when an appropriate autonomous element is present and active in the genome. Each autonomous element codes for one or several proteins involved in its own transposition and in the trans-mobilization of receptor elements belonging to the same family (Frey et al. 1990; Kunze et al. 1987). These proteins recognize *cis*-determinants present at the termini of the elements (Gierl et al. 1988; Kunze and Starlinger 1989). Any element which, as a result of either genetic (mutations or internal deletions, Döring and Starlinger 1986; Merckelbach et al. 1986; Döring et al. 1989; Gierl et al. 1989) or epigenetic (methylation, Bennetzen et al. 1988; Schwartz and Dennis 1986) alterations, is unable to produce functional proteins, becomes a receptor element, provided that it conserves the TIRs and subterminal sequences required for its mobilization. It follows that, in the majority of cases, receptor elements are closely related in sequence to their autonomous counterparts. A well documented exception to this rule is the anomalous *Ac* receptor *Ds1* (Sutton et al. 1984), which shows no homology to the autonomous element *Ac* apart from the TIRs.

Among the different two-element transposon systems that have been described in maize (Peterson 1988), the *Ubiquitous* (Friedemann and Peterson 1980, 1982) family (*Uq*, the autonomous element and *ruq*, the receptor) deserves special attention because of its prevalence in numerous and diverse maize lines and populations (Lamkey et al. 1991; Cormack et al. 1988). Furthermore, in inbreds which have undergone a breeding program intended to stabilize the lines, *Uq* activity is not detected (Peterson and Friedemann 1983; Peterson and Salamini 1986). Receptor elements of this system (*ruq*) have been genetically identified at the *A1* (Friedemann and Peterson 1982; Pereira and Peterson 1985) and *C1* (Caldwell and Peterson 1989) loci of the anthocyanin synthesis pathway. In addition, several autonomous elements have been uncovered or activated (Pan and Peterson 1988, 1991 a, b), but in only one case (*Mn: : Uq*, Pan and Peterson 1989) has an autonomous element been found to cosegregate with an identifiable phenotype.

Despite the genetic characterization of the *Uq/ruq* element family, the sequence and structure of these elements have not been investigated. We initiated this study by cloning and sequencing *ruq* receptor elements in order

Offprint requests to: H. Saedler

* *Present address:* Institut für Genetik, Technische Universität Braunschweig, Konstantin-Uhde-Strasse 5, W-3300 Braunschweig, FRG

to use them as probes with which to clone the autonomous *Uq* element and to characterize it molecularly. Here we report the sequences of three *ruq* elements. They appear to belong to the well characterized *Ds1* group of elements. The relationship between the *Uq/ruq* and *Ac/Ds* systems is discussed.

Materials and methods

Plant material. The plant material used in this work is from P.A. Peterson's collection (Ames, Iowa) and has been described in detail elsewhere (Friedemann and Peterson 1982; Caldwell and Peterson 1989). The following mutant alleles were studied: *c-m80 4531, c-m81 6665, c-m81 6666,* and *c-m81 6667.* These are insertion mutations in the maize *C1* gene; the allele *a-ruq* is an *A1* allele in which the receptor *ruq* was initially discovered; the allele *a-m16* is described in Pereira and Peterson (1985). For the segregation analysis of *Uq* activity and DNA methylation, the plant material used corresponds to a *Uq3* segregating population (Pan and Peterson 1991 a).

Molecular analyses. Genomic DNA was prepared and mutant alleles were initially analyzed by the Southern method, as described by Schwarz-Sommer et al. (1984) in order to map the insertion site of the transposon. They were then cloned by standard procedures using probes derived from the previously sequenced *A1* (Schwarz-Sommer et al. 1985) and *C1* (Paz-Ares et al. 1987) genes. In each case, genomic libraries of each mutant plant were prepared in either lambda EMBL4 or MN1149 phages and fragments containing the insertion elements were subcloned in pUC19 for sequencing using the dideoxynucleotide method (Sanger et al. 1977).

For the analysis of the alleles *c-m81 6665* and *c-m81 6667*, PCR amplification of the genomic region containing the elements was carried out as follows: Two single-stranded primers (corresponding to positions 1898 to 1923 in the coding strand, and positions 2211 to 2182 in the non-coding strand according to Paz-Ares et al. 1987) were synthesized and a standard PCR protocol was followed (Erlich 1989). Computer analysis and comparisons of the sequences were made using the WISGEN package (Devereux et al. 1989). For cluster analysis, the PHYLIP program package was used (Felsentein 1987).

To screen the plants for *Ac* sequences an internal *Ac* fragment (*Hin*dIII fragment extending from position 1171 to 2876, Müller-Neumann et al. 1984) was used as probe.

Results

Analysis of the ruq *receptor elements*

To date, no autonomous *Uq* element has been found integrated into a locus for which molecular probes are available. We set out to clone *ruq* receptor elements from molecularly characterized loci in order to isolate probes with which to clone the autonomous *Uq* element. Such receptors are known as unstable, *Uq*-controlled alleles of the *A1* and *C1* genes (Friedemann and Peterson 1982; Pereira and Peterson 1985; Caldwell and Peterson 1989).

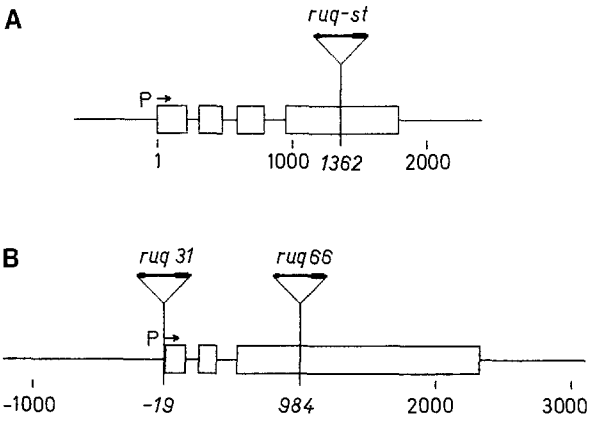

Fig. 1. **A** Map of the insertion of *ruq-st* in the *A1* locus. **B** Map of the insertion of *ruq31* and *ruq66* in the *C1* locus. The schemes represent the sequences published by Schwarz-Sommer et al. (1985) and by Paz-Ares et al. (1987) respectively. The exons are shown in *boxes,* insertion points are numbered according to the published sequences. In each *ruq* element the *circles* correspond to the CGG terminal motifs, indicated solely to show the transposon orientation. The *arrow* indicates the transcription direction from promoter P

Originally, the receptor element (*ruq-st*) integrated at the *A1* locus *a-ruq* was cloned and sequenced. Figure 1 A illustrates the integration site and the orientation of the element within the gene *A1* (Schwarz-Sommer et al. 1985). The *ruq-st* element is inserted in the fourth exon, as a 396 bp element that is 95% homologous in sequence to the *Ds1* element of *Zea mays* (Fig. 2; Sutton et al. 1984). In addition, it produces an 8 bp duplication at the integration site (target site duplication, TSD) upon insertion, as do other elements of the *Ac/Ds* transposon family. However, this *ruq* element has an alteration in one base pair (Figure 2, position 4) within the TIR that differentiates it from the other *Ds1* elements previously reported (Fig. 2). Further, the *ruq-st* element contains an internal deletion when compared with the *Ds1* elements found in *Wx-m1, Bz-wm* and *Adh-Fm* (Fig. 2, positions 354 to 364). This deletion is also found in the *ruq66* element (see below) and matches that reported for the *Ds101* element (Gerlach et al. 1987) and that of the *Ds1* element present in the *b-md-2* allele of the *B* locus (Clark et al. 1990).

The cloning and sequencing of the *Uq* receptor element present in the *a-m16* allele of *A1* produced the following results: a *Ds1*-like element, identical in its first 100 bp (Fig. 2, positions 1 to 100) to the *ruq-st* described above, was found to be inserted at the same position and in the same orientation into the *A1* locus as the *ruq-st* element. This allowed us to conclude that *a-m16* and *a-ruq* are reisolations of the same allele.

Several insertions of *ruq* elements into the *C1* locus have been identified (Caldwell and Peterson 1989). To analyze the structure of these receptor elements, the different *Uq* responding alleles were cloned and studied.

```
          1                                                          80
ruq-st    TAGgGATGAAAGTGGTAATCCGAACTaTTAGaACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC
ruq31     TAGGGATGAAAacGGTAATCCGAACTGTTAGaACAAATTTAATATTTTAAAATAGATATaTATAAAATTTaATtTTGATC
ruq66     TAGGGATGAAAGTGGTAATCgGAACTGTTAGaACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC
Adh-fm    TAGGGATGAAAGTGGTAATCCGAACTGTTAGgAtAAATTTAATATTTTAAAATAGATATaTATAAAATTTGATaTTGATC
Wx-m1     TAGGGATGAAAGTGaTAATCtGAgCTGTTAGgACAAA.TTAATATTTTAAAATAGATATGTATAAAATTTGATagTGATC
Bz-wm     TAGGGATGAAAGTGGTAATCCGAACTGTTAGgACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTaATC
Ds101     .AGGGATGAAAGTGGTAATCCGAACTcTTAGaACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC
Ds103     TAGGGATGAAAGTGGTAATCCGAACTGTTAGaACAAATTTAATATTTTAAAATAGATATacATAAAATTTaATGTTGATC
Ds105     TAGGGATGAAAGTGGTAATCCGAACTGTTAGgACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC
Ds121     TAGGGATGAAAGTGGTAATCCGAACTGTTAGaACAAATTTAATATTaTAAAATAGATAcGTATAAAATTTGATGTTGATC
Ds123     TAGGGATGAAAGTGGTAATCCGAACTGTTAtgACAAATTTAATATTTTAAAATAaATATGTATAAAATTTGATGTTGATC
Ds130     TAGGGATGAAAGTGGTAATCCGAACTGTTAGgACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC
Ds132     TAGGGATGAAAGTGaTAATCCGAACTGTTAGgACAAATTTAATATTTTAAAATAGATATGTATAAAATTTGATGTTGATC

          81                                                         160
ruq-st    TTTTcTTATGTTATCAAGCACATTAGTACAAATATGAATAAAATA.TTAtATAAgTTGTTTTATGTATTATTTGCTCCCT
ruq31     TTTTcTTATGTTATCAAGCACATTAGTACAcATATGAATAAAATA.TTACAcAAATTaTTTTATGTATTATTTGCTCCCT
ruq66     TTTTcTTATGTTATCAAGCACATTAGTACAAATATGAATAAAATA.TTAtATAAgTTGTTTTATGTATTATTTGCTCCaT
Adh-fm    TTTTcTTATGTTATCAAGCACATTAGTAgAAATATGAAT.AAATA.TTACATAcATTGTTTTATGTATTATTTGCTCCCT
Wx-m1     TTgTgTTATtgTcTCAAGCA.tTTAtTACAAATAaGAATAAAATA.TTACATAAATTGTTTTATGTATTATTTGCTaCtT
Bz-wm     TTTTccTATGTTATCAAGCACATTAGTACAAATATGAATAAAATA.TTACATAAATTGTTTTATGTgTTATTTGgTCCCT
Ds101     TTTTcTTATGTTATCAAGCACATTAGTACAAATATaAATAAAATAcTTAtATAAgTTGTTTTtTGTATTATTTGCTCCCT
Ds103     TTTTaTTATGTTATCcAGCACATTAGTACAcATATGAATAAAATA.TTACAcAAATTaTTTTATGTATTATTTGCTCCCT
Ds105     gTTTcTTATGTTATCAAGCACATTAGTACAAATATGAATAAAATA.TTACAgAAATTGTTTTATGTATTATTTGgTCCCT
Ds121     TTTTgTTATGTTATCAAGCACATTAGTACAAATATGAATAAAATA.TTACATAAATTGTTTTATGTATTATTTGCTCCCT
Ds123     TTTTtTTATGTTATCAAaCACATTAGTACAAATATGAATAAAATA.TTACATAAATTGTTTTATGTATTATTTGgTCCCT
Ds130     TTTTcaTATcTTATCAAGCACATTAtcACAAATAaGAATAAAATA.TTgCATAAATTGTTTTATGTATTATTTGCTCCCT
Ds132     TTTTcaTATGTTATCAAGCACATTAtcACAAATAaGAATAAAATA.TTgCATAAATTGTTTTATGTATTATTTGCTCCCT

          161                                                        240
ruq-st    .ACAACAcAAAAaGTTGAAAAAA.TTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
ruq31     .ACAACAcgAAAcGTTG.AAAAAtTTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCTA.TTATTTaAGAAAA
ruq66     .ACAACAcAAAAaGTTGAAAAAA.TTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
Adh-fm    .ACAACAtAAAAgGTTGAAAAAA.TTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCTAtTTATTTGAGAAAA
Wx-m1     cACAgCAaAAAAaGTTGAAAAtAtTacCgacATTTgTTTtCGAATgCcataCcgAagTTtTATaTcaaTcTTTGAGAAAA
Bz-wm     .ACAACAcAAAtaGTTGAAAAAA.TTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCTA.TTATTTaAaAAAA
Ds101     .ACAACAcAAAAaGTTGAAAcAA.TTACCGAATTTaTTTtCGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
Ds103     .ACAACAcgAAAcGTTGAAAAAA.TTACCGAATTTaTTTCtGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
Ds105     .ACAACAcAAAtaGTTGAAAAAAcTTACCGAATTTgTTTCCGAATCCATACtGAAGTTTATATCTA.TTATTTGAGAAAA
Ds121     .ACAACAtAAAAaGTgGAAAAAA.TTACCGAATTTgTTTCCGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
Ds123     .ACAACAcAAAtaGTTGAAAAAA.TTACCGAATTTgTTTtCGAATCCATACCGAAGTTTATATCTA.TTATTTGAGAAAA
Ds130     .ACAACAaAAAAaGgTGAAAAAA..TACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCT.cTcATTTGAGAAAA
Ds132     acaAcaAaAAAAcGgTGAAAAAA.TTACCGAATTTaTTTCCGAATCCATACCGAAGTTTATATCT.cTcATTTGAGAAAA

          241                                                        320
ruq-st    TgTAGGATGAATTTGAGGTTTAcCTTTTATGAtTCTTaACAA.GcTGaATGTTAAAAACAAGAATACAAATTTGTATtGT
ruq31     TATAGaATGAATTTGAGGTTTAcCTTTcATGAATCTTTcACAA.GcTGgAcGTTAAAAAaAAGAATACAAATTTGTATtGT
ruq66     TgTAGGATGgATTTGAGGTTTAcCTTTTATGAATCTTaACAAgGcTGgATaTTAAAAACAAGAATACAAATTTGTATtGT
Adh-fm    TATAGGATGAATTTGAGGTTTA.CTTTTATGAATCTTaACAA.GcTGgATGTTAAAAACAAGAATACAAATTTGTATtGT
Wx-m1     TATAtGATGAATTTGAGGaTTAaCTTTTATGAATCTTtACAA.GcTcaATGTTAtAAACAAGAATAtAAATTTGTATacT
Bz-wm     TATAGGATGAATTTaAGGTTTAtCTTTTATGAATCTTtACAA.acTGaATGTTAAAAACAAGAATACAAATTTGTATgaT
Ds101     TgTAGGATGAATTTGAGGTTTAtCTTTTATGAATCTTaACAA.GcTGtATGTTAAAAACAA.AATACAAATTTGTATtGT
Ds103     TATAGaATGAATaTGAGGTTTAcCTTTcATGAATCTTtACAA.GtTGgATGTTAAAAACAAtAATACAAATTTGTATtGT
Ds105     TATAGGATGAATTTaAGGTTTAtCTTTTATGAATCTTtACAA.GtTGgATGTTAAAAACAAGAATAtAAtTTTGTATtGT
Ds121     TATAGGATGAATTTGAGaTTTAcaTTTTATGAATgTTaACAA.GtTGgATGTTAAAAACAAGAATACAAATTTGTATtGT
Ds123     TATAGGATGAATTTaAGGTTTAtaTTTTATGAATCTTtACAA.GtTGgATaTTAAAAACAAGAATACAAATTTGTATtGT
Ds130     TATAGGATGAATTTGAGGTTTAcCTTTTATGAATCTTtACAA.GcTcaATGTTAAAAACAAGAATACAAATTTGTATaGT
Ds132     TATAGGATGAATTTGAGGTTTAcCTTTTATGAATCTTtACAA.GcTcaATGTTAAAAACAAGAATACAAATTTGTATaGT

          321                                                        400
ruq-st    AtATTCTATATCCTATTTATTCaCAATCAAAG.....AAAAAACTGATTACCGAATAAATACCGTTTCCGACCGT
ruq31     AgATTaTATATCtTATTTATTCGCAATCAAAGAAAgACgACtAAAAAACTGATTACCGAAT.AATACCGTTTCCGACCGT
ruq66     AtATTCTATATCCTATTTATTCGCAATCAAAG.....AAAAAACTGATTACCGAATAAATACCGTTTCCGACCGT
Adh-fm    AtATTCTATATCCTATTTATTCGCAATCAAAGAAAaACgACtAAAAAACTGATTACCGAAaAAATACCGTTTCCGACCGT
Wx-m1     gcATTaTATATCCTATTTATTCGtAATCAAAGAAAaACgACcAAAAAACTGATTACCGAATAAATACCGTTTCCGtCaGT
Bz-wm     AgATTCTATATtCTATTTATTCGCAATCAAAGAAAaACgACtAAAAAACTGATTACCGAATAAATACCGTTTCCGACCGT
Ds101     AtATTCTATATCCTATTTATTCGCAATCAAAG.....AAAAAACTGATTAtCGAATAAATACCGTTTCCGACCGT
Ds103     AgATTaTATATCCTATTTATTCGCAATCAAAGAAAaACgACtAAAAAACTGATTACCGAATAAATACCGTTTCCGACCGT
Ds105     AgATTCTATgcCCTATTTATTCGCAATCAAAGAAAaACgACtAAAAAA.TGATTACCGAATAAATAtCGTTTCCGACCGT
Ds121     AtATTCTATATCCTATTTATTCGCAATaAAAGAAAaACgACtAAAAAA.TGATTACCGAATAAATACCGTTTCCGACCGT
Ds123     AgATTCTATATCCTATTTATTCGCAATCAAAGAAAaACgACtAAAAAACTGATTACCGAATAAATACtGTTTCCGACCGT
Ds130     AgATTCTgTATCCTATTTATTCGCAATCAAtGAAAaACaACcAAAAAtCTGATTACCGAATAAATACCGTTTCCGACtGT
Ds132     AgATTCTATATCCTATTTATTCGCAATCAAtGAAAaACgACcAAAAAACTGATTACCGAATAAATACCGTTTCCGACCGT

          401   411
ruq-st    TTTCATCCCTA
ruq31     TTTCATCCCTA
ruq66     TTTCATCCCTA
Adh-fm    TTTCATCCCTA
Wx-m1     TTTCATCCCTA
Bz-wm     TTTCATCCCTg
Ds101     TTTCATCCCT.
Ds103     TTTCATCCCTA
Ds105     TTTCATCCCTA
Ds121     TTTCATCCCTA
Ds123     TTTCATCCCTA
Ds130     TTTCATCCCTA
Ds132     TTTCATCCCTA
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Fig. 2. Sequence alignment of the *Ds1* elements. Elements *ruq-st, ruq31*, and *ruq66* are described in this paper. In the alleles *Adh-fm, Wx-m1* and *Bz-wm*, only the sequence of the transposon is represented. That of *Adh-fm* is taken from Sutton et al. (1984) and the sequences of the rest of elements from Gerlach et al. (1987). The base change in *ruq-st* (position 4) and the 2 bp inversion in *ruq31* (position 12–13) are highlighted in *black;* the CGG motifs are in *shaded boxes. Upper case* shows base pairs conserved in at least 12 of the 13 sequences compared. All the sequences are isolated from maize, except for *Ds130* and *Ds132*, which are from *Tripsacum*

First, the *c-m80 4531* (*ruq31*) allele was cloned. The receptor element (*ruq31*) is inserted into the *C1* promoter region (Paz-Ares et al. 1987) just downstream of the TATA box (Fig. 1 B); it consists of a 405 bp element, which is 93% homologous to *Ds1* (Fig. 2); however, it features a 16 bp TIR the outermost 11 bp of which are identical to *Ds1*, and produces an 8 bp TSD.

Subsequently, the *ruq* element present in the allele *c-m81 6666* (*ruq66*) was also mapped, cloned and sequenced (Fig. 1 B). The insertion site is in the third exon of the *C1* locus. Its sequence is 397 bp long and is 95% homologous to the sequence of *Ds1* (Fig. 2). The element produces a 8 bp TSD and does not show any change in either the TIR or the subterminal regions, compared to the previously published sequence of *Ds1*. However, it does contain the 10 bp deletion already described for *rug-st*. Using specific primers, the region in which *ruq66* is inserted was PCR amplified from plants homozygous either for *c-m81 6665* or for *c-m81 6667* and hybridized with a probe specific for *Ds1*. In both cases, a band of the same size as the one reported for *ruq66* was detected (data not shown). Hence the alleles *c-m81 6665* and *c-m81 6667* are very likely identical to allele *c-m81 6666*. In fact, these three mutable kernels appeared on the same cob in a screen of more than five million kernels; however, their positions on the cob were not recorded when the cob was shelled (Caldwell and Peterson 1989). The insertion of *ruq66* into the *C1* locus must have occurred in the megaspore as can be deduced from the tagging protocol.

The *Ds1* element in the three *ruq* alleles reported here is inserted in an orientation, with respect to the direction of host gene transcription, that prevents splicing of the element out of the mRNA, according to the model of Weil and Wessler (1990).

In summary, three different inserts of mutable alleles that respond to the autonomous *Uq* element have been studied. In each case, insertion of a *Ds1*-like element has been found. However, according to the results of genetic studies (Caldwell and Peterson 1991), *Uq* activity can be differentiated from *Ac* activity when challenged with reporter alleles such as *C Ds* or *C-I Ds* (McClintock 1951; Döring et al. 1989).

Sequence comparisons among Ds1 *elements*

Gerlach et al. (1987) have carried out a cluster analysis of the different *Ds1*-related sequences found in *Zea mays* and *Tripsacum*. In light of this, we wished to determine whether, the *Uq*-driven receptors are more closely related to each other than to the previously reported *Ds1* elements. For this purpose, the elements whose sequences are shown in Fig. 2 were subjected to cluster analysis using the DNAPENNY program of the PHYLIP package. To avoid the bias produced by the internal deletion in elements *Ds101, ruq-st* and *ruq66*, the sequences between positions 21 and 350 in Fig. 2 were used for the comparisons. The analysis showed a tree topology (Fig. 3) essentially identical to that of Gerlach et al. (1987); *ruq-st* and *ruq66* elements cluster together

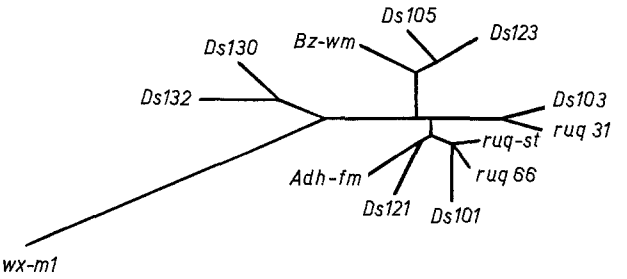

Fig. 3. Unrooted cluster of *Ds1*-related sequences. The Penny algorithm for DNA (version 3.0, Felsentein 1987) was used to find the most parsimonious tree. Only the internal sequences between positions 21 and 350 (Fig. 2) were used

and are close to *Ds101*, indicating the relatedness of these elements, which is in agreement with the fact that they also share the internal deletion; *ruq31* clustered with the element *Ds103* described by Gerlach et al. (1987) and, hence, is unrelated to the other two *ruq* elements.

The comparison of the sequences of *ruq* elements with other *Ds1* elements did not reveal any consistent characteristic that differentiates the receptors from the *Ds1* elements.

Autonomous element for Ds1 *receptors*

Since all the *ruq* elements found appear to be *Ds1* elements, and all respond to an autonomous element that is unable to mobilize other *Ac*-like *Ds* elements, the autonomous *Uq* element should somehow be specific for the *Ds1* receptors. To test whether sequences exist in the genome from which *Ds1* could be derived by deletion, we took advantage of the presence of *Fok*I restriction sites in both TIRs of the *Ds1* elements. In Southern experiments in which the DNA from plants segregating for *Uq* activity was digested with *Fok*I and probed with an internal *Ds1* sequence, it was found that all the hybridizing sequences corresponded to the size of 300–400 bp expected for *Ds1* elements (data not shown). Moreover, it was found that each plant contains more than a single type of *Ds1* element, as deduced from the restriction pattern. Therefore, the maize lines do not contain an autonomous element from which *Ds1* sequences could have been derived by deletion.

It is clear that genetically *Uq* recognizes only *Ds1* (Caldwell and Peterson 1991). This fact could be explained if *Uq* were a modified *Ac* element recognizing only *Ds1*. To test this hypothesis, DNA from plants with and without *Uq* activity (detected from kernel phenotypes by their ability to transactivate *ruq* elements; Pan and Peterson 1991 a, b) were analyzed by Southern hybridization to detect *Ac*-related sequences. In all cases, bands characteristic of the structurally intact *Ac* element were found (1.6 kb for *Hin*dIII and 3.1 kb for *Acc*I as predicted from Müller-Neumann et al. 1984) (data not shown). To determine if any of these *Ac* sequences represents an active element, digestions of DNA from plants segregating for *Uq* activity were carried out with the methylation-sensitive *Hpa*II enzyme. This enzyme

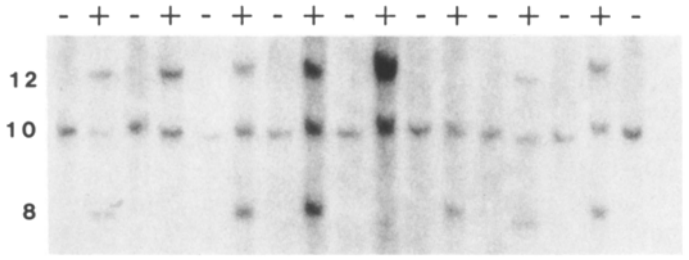

Fig. 4. Analysis of an *Uq* segregating population for the presence of *Ac*-related sequences. DNA from plants segregating for *Uq* (+ and − indicate presence or absence of *Uq*) was digested wtih *Hpa*II and hybridized to an internal (HindIII fragment) *Ac* probe

should yield fragments ranging in size from 3 to 4 kb, depending upon whether or not the promoter region of the *Ac* element is methylated (Schwartz and Dennis 1986). In our population, no *HPa*II bands smaller than 8 kb to were seen to hybridize to an internal HindIII *Ac* fragment probe, indicating that all *Ac*-related sequences in the population were heavily methylated. Three higher molecular weight fragments are observed (8 kb, 10 kb and 12 kb, Fig. 4). The 10 kb band is seen irrespective of the *Uq* activity, while either the 8 kb or the 12 kb or both bands are detected only in segregants showing *Uq* activity. This seems to indicate that a particular state of a methylated chromosomal region containing *Ac* homologous sequences is cosegrating with *Uq* activity.

Discussion

Structure of the receptor element ruq

The *ruq* receptor elements described in this paper appear to be closely related to the previously reported *Ac* receptor elements *Ds1* (Sutton et al. 1984) (Figs. 2 and 3) based on the following features: 1) the sequence differences found when *ruq* elements are compared with authentic *Ds1* elements are not statistically significant; 2) the different *ruq* elements are not significantly clustered in a tree analysis of *Ds1* elements, suggesting that they do not have a distinct evolutionary origin, 3) their TIRs are very similar (except for some changes that will be discussed below (Figs. 2 and 5); and 4) they produce an 8 bp TSD, as do other members of the *Ac*/*Ds* family (Döring and Starlinger 1986).

Element	Sequence	Length	Organism
ruq-st	TAgaCaTCaaA.	11	Maize
ruq31	TAggCaTCaaAcggtt	16	Maize
Ac/Ds	TAggCaTCaaA.	11	Zea/Tripsacum
Ipsr	TAggCgTCgcAa	12	Pea
105::Tam3	TAaaCaTCgcAa	12	Antirrhinum
Tam3	TAaaCaTCtcAa	12	Antirrhinum
TpcI	TAggC.TCtaAa	12	Parsley

Fig. 5. Comparison of the terminal inverted repeats (TIR) of several members of the *Ac*-like type of transposons from different organisms. The consensus sequences are highlighted. The outermost nucleotide of the sequence (T) is not shown as consensus because of the variability of this position in *Ac*, in the *Ds1* found in *Bz-wm*, and in *Ds101* (see text). The sequences of *Ipsr* (Bhattacharyya et al. 1990), *105::tam3*, *Tam3* (Sommer et al. 1985, Martin et al. 1989); and *TpcI* (Herrmann et al. 1988) TIRs are as published

The identity of *ruq* and *Ds1* raises the question of the relationship between the *Uq*/*ruq* and *Ac*/*Ds* systems. It has been conclusively shown genetically as well as in transgenic assays that *Ac* is able to mobilize *Ds1*, recognizing it as an efficient substrate for transposition (Lassner et al. 1989). This confirms, from a molecular point of view, that *Ds1* is a true *Ac* receptor. Earlier genetic data indicated that the element *ruq-st* did not respond to the presence of *Ac* in the genome (Friedemann and Peterson 1982; Pereira and Peterson 1985), while it responds more efficiently to *Uq* than do the other *ruq* elements (Caldwell and Peterson 1989). It is now clear that all the *ruq* elements do in fact respond to *Ac* (Caldwell and Peterson 1991) while the autonomous *Uq* elements are only able to mobilize *Ds1* receptor elements (both *ruq* elements and *Ds1* present in alleles like *wx-m1*). This receptor specificity of the autonomous *Uq* element supports the view that *Uq* differs from *Ac*, but shares at least the *Ds1* class of receptor elements.

While negative dosage effect is taken as a dogma for the behavior of *Ac* in *Zea mays* (McClintock 1948), recent experiments by M. Heinlein and P. Starlinger (Maydica, in press) clearly show that the issue is more complicated, and that positive dosage relations can also be observed for *Ac* in maize, which is more compatible with the behavior of *Ac* in transgenic plants (Belzile et al. 1989). *Uq* clearly shows a positive dosage effect, the higher the dose the earlier and more frequent the excision (Friedemann and Peterson 1981); hence dosage seems not to be a distinguishing feature between *Ac* and *Uq*. Moreover, preliminary data show that there is a positive cooperative effect between *Ac* and *Uq* when both are present in the same plant (P.A. Peterson, unpublished results).

Molecular basis of the Ds1 *response to* Ac

The mechanism of *Ac*-driven *Ds1* mobilization is not yet clear. To address this question, we should consider the different structures present in, or formed by, an integrated *Ds1* element: the target site duplication (TSD), the subterminal *Ds1* regions, and the *Ds1* terminal inverted repeats (TIR).

Upon insertion, *Ds1* elements produce 8 bp TSD as do all elements of the *Ac* family. However, the presence of this duplication is not required for the excision of an *Ac* element (Dennis et al. 1988; Dooner et al. 1988). Furthermore, it has been reported that the insertion of the *Ds101* element reported by Gerlach et al. (1987) produces only a 6 bp TSD, although it is not known whether this element can excise from this site. On the other hand, the mechanism of *Ac*-driven transposition of *Ds1* may differ from the mechanism of the *Ac*-driven mobilization of standard *Ds* elements, which are deletion derivatives

of *Ac*. Coupland et al. (1988) report that *Ac* subterminal regions are required for transposition of *Ds* elements in transgenic tobacco, and Lassner et al. (1989) report similar results in tomato. These subterminal regions are not present in the *Ds1* elements, as their internal sequence is not homologous to *Ac*. If the ends of the element are important, then obviously *Ds1* and normal *Ds* elements should respond differently.

The ends are made up of the 11 bp TIR plus subterminal regions needed for transposase binding (Kunze and Starlinger 1989). Kunze and Starlinger (1989) have reported the hexamer AAACGG as the putative binding sequence for the *Ac*-encoded transposase. This sequence is present once at one end of *Ds1* elements (Fig. 2, position 386), and the central part of this motif (CGG) is present eight times in highly conserved positions in all the *Ds1* elements compared here (Fig. 2). It is worth noting that *ruq31* differs from all other *Ds1* and *Ac*/*Ds* elements in the sequence immediately adjacent to the 11 bp TIR. Due to the inversion of 2 bp (Fig. 2, positions 12 and 13), *ruq31* contains a 16 bp long TIR. This alteration creates a new, perfect *Ac* transposase binding site. However, whether this is relevant for transposition of *ruq31* is questionable, since *ruq31* behaves as a normal *Ds1* receptor.

It is well known that the outermost base pair of the TIR is not required as a perfect repeat: the ends of *Ac* elements show a mismatch in this position (Müller-Neumann et al. 1984; Pohlmann et al. 1984a, b). The TIRs of the *Ds1* element found in *Bz-wm* also differ in this position (see Fig. 2), and *Ds101* has only a 10 bp TIR (Gerlach et al. 1987). On the other hand, elimination of the 4 terminal nucleotides at one end of *Ac* prevents transposition of this element in transgenic tobacco (Hehl and Baker 1989). These data are complemented by our finding that the single base pair change found at position 4 in *ruq-st* does not preclude transposition of the element.

The mechanism of Uq *activity – a hypothesis*

The fact that a larger version of *Ds1* is not present in *Uq* active lines (see Results) excludes the possibility that *Ds1* (*ruq*) is a deletion derivative of *Uq*. Concerning the nature of the autonomous *Uq* element, two alternatives can be considered. Either *Uq* is a particular version of *Ac*, which recognizes only *Ds1* as receptor, or *Uq* is a completely new transposon element system that shares with *Ac* the ability to transactivate *Ds1*-like elements. The first possibility is discussed in the following.

In order to explain the specificity of *Uq* in the mobilization of the *Ds1* elements one must consider the models for the molecular basis of transposition described for other elements. In the case of the *En*/*Spm* transposon (Pereira et al. 1986), Frey et al. (1990) propose as a mechanism for transposition, the coordinated action of two different proteins: one holding the ends of the transposon together by binding to the highly structured subterminal regions of *En* (protein TnpA) and a second protein with endonucleolytic activity, which removes the transposon from the target gene (TnpD protein). These two proteins are the only element-encoded factors required for *En*/*Spm* transposition in transgenic tobacco (Frey et al. 1990; Masson et al. 1991). The situation in the *Ac* family is somewhat different because only one protein has been shown to be encoded by the *Ac* element. The ORFa protein (Kunze and Starlinger 1989) has been found to bind selectively to subterminal regions of *Ac* and not to the TIRs that are the likely sites for endonucleolytic action. Thus, the *Ac*-encoded protein could be functionally equivalent to the protein TnpA of *En*. However, the possibility that this protein also functions as endonuclease, fulfilling the function of TnpD, cannot be ruled out because ORFa is the only *Ac*-encoded protein required for transmobilization of receptors in transgenic plants (Hehl and Baker 1989).

The model we propose for *Uq* activity is based on the following assumption: the smaller the receptor element, the easier it is to bring the ends together in order to facilitate excision, a prerequisite for transposition. Therefore, the number of ORFa molecules required to transpose a *Ds1*-like element might be smaller than the number required to transpose a larger *Ac* or *Ds* (*Ac* deletion derivative). In such a scenario, weak *Ac* activity should only be detectable when *Ds1* elements are used as receptors, whereas stronger *Ac* activities are required for mobilization of *Ds* receptors. Such weak *Ac* activity could be the result of a very low level of expression of the *Ac* transposase due either to mutations in the promoter region of the element or, more probably, to a heavily methylated state of the promoter that severely reduces but does not abolish transcription of ORFa.

As a consequence, the *Ds1* transposition rate would depend, on the following two factors, among others: 1) the quality of the transposon ends for binding of ORFa protein, and 2) the actual transposon length, which should allow both ends to come together with a high probability. Changes in either of these factors should drastically affect *Ds1* transposition.

There is some evidence to support this model: 1) Lassner et al. (1989) have presented data suggesting that normal *Ds* elements transpose less frequently than *Ds1* elements in transgenic tobacco plants carrying the same trans-acting *Ac* element. 2) Data from different groups reviewed by Haring (1991) show that the strength of the promoter controlling ORFa expression can affect the rate of *Ac* transposition in tobacco and tomato. 3) *Ds1* elements have ORFa binding sites that are concentrated at one of the ends of the element (see Fig. 2, shaded boxes). 4) The right end of *Ds1* has been shown by Kunze and Starlinger (1989) to bind ORFa protein although with a low affinity. Such a low affinity is not surprising if we consider it as a mechanism to regulate the transposition rate of *Ds1* (few ORFa proteins together with a high affinity binding site would make *Ds1* transpose at a very high frequency). 5) The unusual structure of the promoter region of *Ac*, which lacks TATA or CAAT boxes and resembles promoters of some mammalian housekeeping genes somewhat (Kunze et al. 1987), points to a subtle control of ORFa protein expression in which a very low basal transcription level can be expected.

This model allows us to make some testable predictions concerning *Uq* activity: 1) *Ac* transcripts should be detectable in plants showing *Uq* activity but lacking *Ac* activity provided that a sufficiently sensitive technique is used for detection. 2) *Uq* activity should be seen with *Ds1* as a reporter allele and not with normal *Ds;* however, *Uq* could evolve into *Ac* activity if selected with standard *Ds*. 3) Likewise, *Ac* could lapse into an *Uq*-like form which would be seen only with *Ds1* receptors.

The prevalence of *Uq* among maize lines and testers may actually reflect the prevalence of *Ac* since *Uq* is a special form of *Ac*: a version showing low levels of activity. In any case, the evolutionary implications are linked to constitutive transposition for *Ds1* elements, and possibly to the maintenance of this receptive element within *Zea mays* even though no master element for *Ds1* exists in maize from which it could have been derived via deletion. This raises again the question of the origin of *Ds1* elements which has yet to be clarified.

The hypothesis discussed in this paper should allow the cloning of *Uq* elements inserted at known positions. On this basis the cloning of the *Mn::Uq* element described by Pan and Peterson (1989) is underway in our laboratory.

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The new nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the following accession numbers: *ruq-st* X59774, *ruq31* X59775 and *ruq66* X59776.