Ac transposition is impaired by a small terminal deletion

Abstract In maize, the P1-vv allele specifies variegated pericarp and cob pigmentation, and contains an Ac transposable element inserted in the second intron of the P1-rr gene. Starting from P1-vv, we recovered a new allele, called P1-vv5145, which gives an extremely light variegated pericarp and cob phenotype. The P1-vv5145 allele contains an Ac element (Ac5145) at the same position and in the same orientation as in the progenitor P1-vv allele; however, the P1-vv5145 allele has a 2-bp deletion which removes the last nucleotide (A) from the 3′ end of the Ac element, and an adjacent flanking nucleotide (C) from the p1 intron. In crosses with a Ds tester stock, P1-vv5145 shows a normal ability to induce Ds transposition; however, Ac excision from P1-vv5145 is 3800-fold less frequent than from the progenitor P1-vv allele. Our results demonstrate that the alteration of the 3′ terminal base strongly impairs Ac transposition. The P1-vv5145 allele thus provides a relatively stable source of Ac transposable for controlling Ds transposition in genetic experiments. In addition, we describe two further alleles (P1-vv78, P1-vv9A46-3) that contain deletions of Ac and flanking p1 gene sequences. These latter deletions are larger and involve the 5′ end of the Ac element. A model is proposed to explain the formation of one-sided deletions as a consequence of Ac transposition during replication of the element.

Keywords Ac transposon · Transposition · P1-vv5145/p1 gene · Maize

Introduction

The concept of transposition was first proven when McClintock (1949) demonstrated that Dissociation (Ds), a locus of chromosome breakage, could move from one position to another in the maize genome in the presence of a second factor, Activator (Ac). McClintock identified Ac and Ds elements in the progeny of maize plants that had undergone cycles of chromosome breakage and fusion, and she concluded that the genomic stress of chromosome breakage events had induced or activated these elements from a quiescent state (McClintock 1984). However, even before McClintock’s experiments, Ac was present and highly active in certain maize stocks; in particular, Emerson (1917) published a detailed report of phenotypic variegation in maize kernel pericarp pigmentation associated with the P1-vv allele. Emerson described the P1-vv allele as an unstable factor that frequently mutated to a form giving full red color in pericarp and cob (P1-rr). It was later shown that the P1-vv allele carries an Ac insertion in the p1 gene (Barclay and Brink 1955; Chen et al. 1987). The p1 gene encodes a Myb-homologous regulator of genes encoding enzymes for flavonoid pigment biosynthesis (Grotewold et al. 1994), and hence the p1 gene has been an extremely useful reporter for the transpositional behavior of Ac, and the effects of Ac on gene expression (Greenblatt 1985). For example, analysis of Ac transposition from P1-vv provided the first evidence that Ac transposes during DNA replication (Greenblatt and Brink 1963), and inserts preferentially at linked sites (Greenblatt 1984). Starting from the original Ac insertion in Emerson’s P1-vv allele, a large number of cases of intragenic transposition of Ac to new sites within the p1 locus have been obtained (Atha et al. 1992; Moreno et al. 1992); the phenotypes of these mutants demonstrate that gene expression is affected by both the position and orientation of Ac (Peterson 1990). Moreover, Ac insertions in the p1 locus can induce homologous recombination between flanking repeat sequences (Atha and Peterson...
1991; Xiao et al. 2000), and can undergo so-called Non-Linear Transposition, thereby producing large interstitial deletions and duplications (Zhang and Peterson 1999). While research into the effects of Ac/Ds elements on gene expression and genome structure is still ongoing, the Ac/Ds system is being used extensively for gene tagging in maize (Walbot 2000) and other plant species, including Arabidopsis (Bancroft et al. 1992; Grevelding et al. 1992; Fedoroff and Smith 1993; Sunadaresan et al. 1995), petunia (Chuck et al. 1993), tomato (Meissner et al. 2000), and rice (Izawa et al. 1997; Chin et al. 1999). Transposition is the key property that underlies the biological effects of Ac/Ds elements and their application for transposon tagging; however, the mechanism of Ac/Ds transposition and the role of transposon sequences in the transposition process are still unclear.

The Ac element is 4.6 kb in length and encodes, so far as is known, a single transposase that catalyzes transposition of both Ac and Ds elements (the non-autonomous counterpart of Ac) (Kunze and Starlinger 1989). Based upon their structures, Ds elements can be grouped into four classes. (1) The simple Ds elements, like Ds9 (Pohlman et al. 1984), contain deletions of the internal sequences of Ac elements. (2) Composite Ds elements, like Ds2 (Merrickbach et al. 1986) and wxB4::Ds (Varagona and Wessler 1990), have rearranged Ac sequences or unrelated sequences in the internal region. (3) Ds1 elements are short (approximately 400 bp long) and have very limited regions of homology to Ac at their 5′ and 3′ ends (Sutton et al. 1984; Gerlach et al. 1987). (4) Double Ds elements contain one internally deleted Ds element inserted into another identical Ds element, in opposite orientation (Döring et al. 1984). Despite their structural diversity, Ac and Ds elements share similar 11-bp terminal inverted repeat sequences (TIRs). These TIRs are critical for transposition: Ac transposition is abolished by replacement of four terminal bases in the 3′ TIR (Hehl and Baker 1989), or five terminal bases in the 5′ TIR (Healy et al. 1993). In addition to the TIRs, transposition requires an additional ~250-bp of the subterminal sequence at each end (Coupeland et al. 1988, 1989; Varagona and Wessler 1990). These sub-terminal sequences contain multiple copies of a hexamer sequence (AAACGG) that binds Ac transposase in vitro (Kunze and Starlinger 1989). Mutation of individual copies of the AAACGG motifs reduces excision frequency (Chatterjee and Starlinger 1995). The 400-bp Ds1 element has a single AAACGG motif close to the 5′ TIR; mutation of this motif completely abolishes Ds1 excision (Bravo-Angel et al. 1995). The Ac transposase contains a bipartite DNA binding domain that recognizes both the subterminal sequences and the terminal inverted repeats. Transposase binding to the subterminal sequences exhibits strong cooperativity (Becker and Kunze 1996, 1997; Ros and Kunze 2001).

Here, we describe a novel Ac element (Ac5145) which has a normal ability to trans-activate Ds elements, but which itself transposes rarely. The Ac5145 element is contained within P1-vv5145, a derivative of the standard P1-vv allele; while P1-vv specifies variegated pericarp and cob pigmentation (Emerson 1917), P1-vv5145 causes extremely light variegation of pigmentation. P1-vv5145 contains an Ac element (Ac5145) at the same position and orientation as in P1-vv, but the terminal “A” at the 3′ end of the element and the adjacent “C” from the flanking pi gene sequence are deleted. These results indicate that, even though the terminal nucleotide at the 5′ end can differ between transposition-competent Ac and Ds elements, the terminal nucleotide at the 3′ end of Ac is critical for transposition. We also describe two additional pi alleles in which much larger deletions occurred at the 5′ end of the Ac element. To explain the origin of these deletion derivatives of Ac, we propose a new model for Ac transposition in which the ends of Ac are cut in sequential reactions, the order of which is determined by the direction of replication of Ac.

**Materials and methods**

Genetic nomenclature, and isolation of the P1-vv5145 allele

The maize pi gene regulates the pigmentation of kernel pericarp, cob, tassel glume margins, husk and silk. Alleles of the pi gene are commonly identified by a two-letter suffix corresponding to their expression in kernel pericarp and cob: P1-rr gives red pericarp and red cob; P1-wr gives white (colorless) pericarp and red cob; and P1-ww gives white (colorless) pericarp and white (colorless) cob. The P1-vv allele described by Emerson (1917) conditions variegated kernel pericarp and cob pigmentation. The P1-vv allele contains the transposable element Ac inserted in the large second intron of the P1-re gene (Athma et al. 1992). The P1-vv5145 allele originated from a sector of kernels with nearly colorless pericarp on an ear of genotype P1-ry/P1-wr. Kernels from within the colorless sector were grown and self pollinated to recover the P1-vv5145 allele in homozygous condition. To test Ac trans-activation functions, the P1-vv5145 and progenitor P1-ry alleles were crossed with the r-sc/m3(Ds) tester, which carries a Ds element in the R1-sc locus. In the absence of Ac, Ds blocks r1 function, resulting in kernels with colorless aleurone; in the presence of Ac, Ds excises from R1-sc, giving rise to sectors of purple aleurone (Kermicle 1980).

Determination of transposition frequency

Red sectors on P1-vv kernel pericarps are due to excision of Ac and consequent reversion to P1-rr. The frequencies of Ac excision in P1-vv and P1-vv5145 were calculated as the number of visible red sectors divided by the number of kernels examined. For P1-vv, red sectors were counted on 10 randomly selected kernels of genotype P1-vv/P1-wr. For P1-vv5145, red sectors were counted on 10 randomly selected ears of genotype P1-vv5145/P1-wr; ear kernel number was estimated as the product of the number of kernel rows times the mean number of kernels per row.

PCR and DNA gel-blot hybridization

For P1-vv and P1-ovov1114, Ac excision footprints were isolated by PCR of genomic DNA samples from 2-week-old seedlings as described by Scott et al. (1996). Because of the low excision frequency in P1-vv5145, footprints from this allele were isolated by PCR of genomic DNA from germinal revertants obtained from the progeny of exceptional kernels with red pericarp. Genomic DNA was isolated as described (Dellaporta et al. 1983) and amplified by PCR for 35 cycles as follows: denaturation at 94°C for 30 s, annealing at...