Transposition of Reversed Ac Element Ends Generates Novel Chimeric Genes in Maize

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The maize Activator/Dissociation (Ac/Ds) elements are members of the hAT (hobo, Ac, and Tam3) superfamily of type II (DNA) transposons that transpose through a “cut-and-paste” mechanism. Previously, we reported that a pair of Ac ends in reversed orientation is capable of undergoing alternative transposition reactions that can generate large-scale chromosomal rearrangements, including deletions and inversions. We show here that rearrangements induced by reversed Ac ends transposition can join the coding and regulatory sequences of two linked paralogous genes to generate a series of chimeric genes, some of which are functional. To our knowledge, this is the first report demonstrating that alternative transposition reactions can recombine gene segments, leading to the creation of new genes.

Introduction

The maize Ac element is 4,565 base pairs (bp) in length and encodes an 807–amino acid transposase that catalyzes Ac/Ds transposition. The Ac/Ds element ends are delineated by complementary 11-bp terminal inverted repeat sequences, while the sub-terminal sequences are distinct from each other [1]. The individual Ac termini are designated as 5' or 3' according to their proximity to the beginning and end of the Ac transcript. Transposition requires one Ac 5' end and one Ac 3' end [2]. In standard transposition, the Ac 5’ and 3’ ends are part of a single transposon, and the outcome of transposition is the excision of the element from a donor site and insertion into a target site. However, transposition reactions can also involve the 5' and 3' ends of different Ac/Ds elements, which can be in either a direct or reversed orientation with respect to each other [3,4]. These alternative transposition events can generate deletions, duplications, inversions, and other sequence rearrangements. Because Ac/Ds preferentially transpose into genic regions, the rearrangements induced by alternative Ac/Ds transposition would be predicted to shuffle coding and regulatory sequences, and thereby generate new genes. We searched for such events in maize stocks containing a pair of reversed Ac ends in the p1 gene, which regulates kernel pericarp pigmentation. We obtained four chimeric alleles in which the promoter, exon 1 and exon 2 of the p2 gene (a paralog of p1) [5] is joined with exon 3 of the p1 gene. Because the p1 and p2 coding sequences are very similar, the new chimeric genes would encode proteins nearly identical to that encoded by the p1 gene. The p2 promoter is inactive in pericarp in the progenitor allele; however, these four new alleles show significant expression in kernel pericarp, and specify a novel orange pericarp phenotype. We propose that this new phenotype is largely caused by an altered expression pattern resulting from the chromosomal rearrangement. These results demonstrate that alternative transposition reactions can generate gene fusions and therefore may have been an important force in gene and genome evolution.

Results

Structures of Novel Chimeric Alleles

The maize p1 gene encodes a Myb-homologous transcriptional regulator required for synthesis of red pigments in kernel pericarp (Figure 1) and cob glumes [6]. The P1-rr11 allele (red pericarp, red cob) contains a truncated Ac element (fAc, fractured Ac) inserted in the second intron of p1, and a full-length Ac element inserted 13,175 bp upstream of the fAc element; the 5’ end of Ac and the 3’ end of fAc in P1-rr11 are oriented towards each other (Figure 1C). A paralog of p1, termed p2, is located approximately 60 kilobases (kb) upstream of the p1 gene in the chromosome containing the P1-rr11 allele [7] (Figure 1C). The p2 gene is not expressed in kernel pericarp and hence does not contribute to pericarp color [5,8]. Reversed Ac ends transposition in P1-rr11 would eliminate p1 gene function, and most mutants derived from P1-rr11 have colorless kernel pericarp and cob. However, we did isolate four alleles with orange pericarp and orange cob, and these were designated as P-oo32, P-oo1062, P-oo1067, and P-oo1068 (Figure 1A).

Genetic tests indicate that there is no Ac activity in the genome of the P-oo alleles. We characterized the structural rearrangements in the P-oo alleles by genomic DNA gel blot and PCR. Genomic DNA from plants carrying the P-oo32 allele was cut with HindIII and KpnI, and hybridized with

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Abbreviations: bp, base pair; kb, kilobase

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Transposable elements, or “jumping genes,” are DNA segments that can move to new sites in the genome. One type of transposable element from maize, called Ac/Ds, moves by a reaction known as “cut-and-paste.” In this mechanism, a transposase enzyme cleaves at both ends of a single Ac/Ds element, releasing the element from one site and inserting it at another location. However, if two Ac/Ds elements are situated near each other, the transposase may sometimes cut at the ends of two different elements. When these two Ac/Ds ends insert at a new location, a large rearrangement of the genome can occur; this process is termed alternative transposition. In this work, the authors studied alternative transposition events that affect the structure and expression of two genes that control maize kernel color. Alternative Ac transposition can cause fusions of the coding sequences of the two genes, generating a new functional chimeric gene that specifies a new maize kernel color. This mechanism of gene creation through alternative transposition is similar to the way that functional antibody genes are generated in the vertebrate immune system. These results show how the actions of transposable elements can reshuffle the genome to generate new functional genes.

Synopsis

Transposable elements are DNA segments that can move to new sites in the genome. One type of transposable element from maize, called Ac/Ds, moves by a reaction known as “cut-and-paste.” In this mechanism, a transposase enzyme cleaves at both ends of a single Ac/Ds element, releasing the element from one site and inserting it at another location. However, if two Ac/Ds elements are situated near each other, the transposase may sometimes cut at the ends of two different elements. When these two Ac/Ds ends insert at a new location, a large rearrangement of the genome can occur; this process is termed alternative transposition. In this work, the authors studied alternative transposition events that affect the structure and expression of two genes that control maize kernel color. Alternative Ac transposition can cause fusions of the coding sequences of the two genes, generating a new functional chimeric gene that specifies a new maize kernel color. This mechanism of gene creation through alternative transposition is similar to the way that functional antibody genes are generated in the vertebrate immune system. These results show how the actions of transposable elements can reshuffle the genome to generate new functional genes.
605 bp as expected for p1 expression in kernel pericarp. The p1-ww1112 allele was used as a negative control; it has a deletion of the p1 coding sequence, but retains the sequences upstream of p1, including the p2 gene [10]. As expected, no products were amplified from this allele. The P-oo alleles generated RT-PCR products of 522 bp, which is consistent with expression of the fusion genes that include a 5' UTR derived from the p2 gene (Figure 3). Sequencing of the RT-PCR products confirmed that the P-oo transcripts contained exon 1 and exon 2 of p2, and exon 3 of p1, as predicted by the gene structures. The chimeric P-oo genes would encode a protein identical to that encoded by the p1 gene except for a change in the fourth amino acid residue [5,12].

No expression of the unrearranged p2 gene was detected in either p1-ww1112 or P1-rr11. This is consistent with previous reports, and supports the conclusion that the native p2 gene is not expressed in kernel pericarp [5]. It is somewhat surprising that p-ww2 and the P-oo genes, each of which contain the p2 promoter, generate transcripts in kernel pericarp. It has previously been shown that sequences nearly identical to genomic fragment 15 of the p1 gene form part of an enhancer located approximately 5 kb upstream of the p1 transcription start site [13]. In p-ww2 and the P-oo alleles, the p1 fragment 15 is located at new positions ranging from 6.2 kb to 14.4 kb 3' of the p2 transcription start site. At these new sites, the fragment 15 sequence may enhance expression of the fusion genes in pericarp. This idea is consistent with the observation that the intensity of pericarp pigment specified by the indicated alleles.

(A) The kernel pericarp pigmentation phenotypes specified by the indicated alleles.
(B) Genomic Southern blot. Genomic DNA from plants homozygous for the indicated alleles was cut with KpnI and HindIII, and hybridized with probes 15 or 8B from the p1 gene. Lanes marked P-oo32 contain approximately twice as much DNA as lanes marked P1-rr11; this DNA overloading enables the detection of the 7.6-kb band in the KpnI 8B blot, but also results in the intense 6.5-kb band in the HindIII 15 blot.
(C) Restriction map. The solid and gray boxes are exons 1, 2, and 3 (left to right) of p1 and p2, respectively. Red triangles indicate Ac or fAc insertions, and the open and solid arrowheads indicate the 3' and 5' ends, respectively, of Ac/fAc. Sequences hybridizing with Southern blot probes are indicated by the solid bars above (probe 8B) and below (probe 15) the map. The short horizontal arrows indicate the orientations and approximate position of PCR primers. Primers are identified by numbers below the arrows. The sequence of the junction of each fusion allele is shown here; the black letters indicate p2 sequence, while the red letters indicate fAc sequence. K, KpnI; H, HindIII. Lines below the map indicate the restriction fragments produced by digestion with KpnI or HindIII and hybridizing with the indicated probe; asterisks indicate HindIII restriction sites located within Ac or fAc sequences. DOI: 10.1371/journal.pgen.0020164.g001
Transposon-Induced Exon Shuffling

Figure 3. RT-PCR Analysis of P-oo Transcripts

RNA was extracted from kernel pericarp (20 DAP), reverse transcribed, and PCR-amplified using primers complementary to both p1 and p2 transcripts. The progenitor allele (P1-rr11) shows amplification of a 605-bp band from p1. The p-ww2 and P-oo alleles show amplification of a 522-bp band characteristic of the 5' region of the p2 gene. The p1-ww1112 allele has a deletion of p1; the native p2 gene is intact in this allele, but is not expressed in kernel pericarp.

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Discussion

Our results document four cases of exon shuffling induced by members of the hAT superfamily of DNA transposons. hAT elements are widespread in plants, animals, and fungi. The somatic rearrangement of vertebrate immunoglobulin genes through V(D)J recombination is catalyzed by proteins (Rag1/Rag2) that are functionally related to hAT family transposases [14,15]. Indeed, the formation of the P-oo alleles described here through transposase-induced intra-chromosomal deletion is analogous to the mechanism of vertebrate antibody gene rearrangement [16,17]. In contrast to the situation in vertebrates in which the immunoglobulin rearrangements are limited to somatic cells, the genome rearrangements detected in maize can be inherited because of the late recruitment of gametophytic cells during plant development [18].

Recent sequence analysis of the rice and maize genomes have shown that the Mutator and Helitron transposon families are involved in large-scale duplication and shuffling of coding sequences [19–21]. Although it is not yet known whether the resulting chimeric genes are functional, their sheer abundance suggests that these transposon-induced rearrangements could be an additional large potential source of chimeric genes.

Previous reports of exon shuffling in cultured human cells have been associated with illegitimate recombination, or retrotransposition of long interspersed nuclear elements [22,23]. Exon shuffling via retrotransposition can occur only when retroelements are inserted in or near exon sequences. In rice, the Tos17 retrotransposon inserts preferentially into low-copy-number sequences [24]. In contrast, the vast majority of retroelement sequences in the maize genome are located predominantly in intergenic regions [25] and hence would not be expected to contribute to exon shuffling, whereas the tendency of Ac to insert preferentially into genic regions [26] greatly enhances its potential role in mediating exon shuffling reactions. Some cases of exon shuffling may confer a positive selective advantage that could promote fixation of variant chromosomal structures, such as inversions or reciprocal translocations, in sympatric populations [27,28].

Chromosomal rearrangements have been reported for other, non-hAT, transposon systems. In the fungus Fusarium, transposition involving termini of different Tc1-mariner elements can generate deletions and inversions that also may shuffle coding and regulatory sequences [29]. In Drosophila, transposition of Foldback elements and an associated white gene can result in activation of white gene expression, although little is known about the mechanism of Foldback transposition [30]. Also in Drosophila, transposition involving the termini of different P elements can induce
various chromosomal rearrangements including deletions and inversions [31,32]. It seems likely that alternative transposition reactions of the type we report here are not unique to the hAT transposon superfamily, but may be a common feature of “cut-and-paste” eukaryotic transposomes. Some transposable elements, such as Ac/Ds and Sleeping Beauty, tend to transpose to linked sites [33,34], leading to transposon clusters in which the termini of the linked transposons could be in either direct or reversed orientation. Alternative transposition reactions may then act upon these clustered transposon termini to generate large-scale chromosomal rearrangements. In support of this idea, a recent report has demonstrated that transgenic mice containing clusters of transposition reactions may then act upon these clustered transposon termini to generate large-scale chromosomal rearrangements [35]. Given the abundance of tandemly duplicated segments in plant and animal genomes, our results suggest that the alternative transposition events could represent an important evolutionary mechanism for the generation of new genes.

Materials and Methods

Genetic stocks. Alleles of the maize p1 gene are identified by a twoletter suffix that indicates their expression pattern in pericarp and cob, e.g., P1-rr (red pericarp and red cob); and P1-sew (white pericarp and white cob). The P-oo (orange pericarp and orange cob) alleles described here were derived from P1-w99D9A [4].

Genomic DNA extractions and Southern blot hybridization. Total genomic DNA was prepared from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol [36]. Agarose gel electrophoresis and Southern hybridizations were performed as described [37], except hybridization buffers contained 250 mM NaHPO4 (pH 7.2), 7% SDS, and wash buffers contained 20 mM NaHPO4 (pH 7.2), 1% SDS.

PCR amplifications. PCR amplifications were performed as described [38] using the following oligonucleotide primers: CGCGGACCAGCTGCTACCGG, CCAAGGGAGGAAGAG CATCCTCATCAAG, GCACGTGTGCTGATCGTCAG, and GCAGCTTCATGTCCATGGGC. HotMaster Taq polymerase from Eppendorf (Hamburg, Germany) was used in the PCR reaction. Reactions were heated at 94 °C for 3 min, and then cycled 35 times at 94 °C for 20 s, 63 °C for 30 s, and 65 °C for 1 min per 1 kb length of expected PCR product, then 65 °C for 8 min. In most of the PCR reactions 2 M betaine and 4%–8% DMSO were added. The band amplified was purified from an agarose gel and sequenced directly.

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