A Single Genetic Unit Specifies Two Transposition Functions in the Maize Element *Activator*

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The self-mobile maize transposable element *Ac* (*Activator*) displays two *trans*-acting genetic functions: it induces transposition of the element *Ds* (*Dissociation*) but, as its dosage is increased, it also inhibits transposition. Previous work has shown that the 4563 base pair (bp)–long *Ac* element contains three open reading frames (ORF's) and that a deletion in ORF 1 in *wx-m9(Ds)*, a *Ds* derivative from *Ac* isolated at the *wx* (*waxy*) locus, results in loss of transposition. The *Ds* element in the *bronze* allele *bz-m2(DI)* is shown to have arisen from *Ac* by a 1312-bp deletion that is located almost entirely within ORF 2 and does not affect ORF 1. The *Ds* elements in *wx-m9(Ds)* and *bz-m2(DI)*, defective in ORF 1 and ORF 2, respectively, do not complement genetically to restore the transposition function of *Ac*; therefore, this function must be specified jointly by ORF's 1 and 2. Furthermore, since *bz-m2(DI)* does not contribute to *Ac*'s inhibitory dosage effect, both *Ac* properties result from the expression of the same genetic functional unit.

TRANSPOSABLE GENETIC ELEMENTS can move to different positions in the genome, sometimes causing mutations. They are of ubiquitous occurrence and may play a role in the generation of genetic diversity. The transposable elements of maize were the first ones to be discovered and remain the best characterized genetically (*1, 2*). The demonstration that they can serve as molecular gene tags (*3, 4*) and the prospect of using them as vectors in transformation have spurred a considerable effort to elucidate their molecular organization (*5*).

In the maize *Ac-Ds* transposable element system, *Ac* (*Activator*) is the self-mobile or autonomous element and *Ds* (*Dissociation*) the nonautonomous element (*1, 2*). Though both elements can transpose, only *Ac* can induce transposition of both itself and *Ds*; that is, only *Ac* has a *trans*-acting transposition function. Three *Ac* elements at two different loci have been isolated (*3, 6–8*). The two that have been sequenced completely are identical (*9–11*). The 4563-bp-long *Ac* element contains three open reading frames (ORF's) (*10*): ORF 1 (663 bp) and ORF 2 (1281 bp) read in the same direction and ORF 3 (453 bp) reads in the opposite direction (Fig. 1A). Mutants of *Ac* are particularly valuable in the analysis of the element's functional organization. McClintock (*12*) described instances of apparent mutations of *Ac* to *Ds* at two loci affecting seed characters, *bz* (*bronze*) and *wx* (*waxy*). Thus, the autonomously mutable alleles *wx-m9(Ac)* and *bz-m2(Ac)* gave rise, respectively, to the nonautonomously mutable alleles *wx-m9(Ds)* and *bz-m2(DI)*. An examination of the *wx-m9(Ds)* nucleotide sequence revealed that a deletion located entirely in ORF 1 (*10*) had converted *Ac* to *Ds* (*9*) (Fig. 1B). Other *Ds* elements, which cannot be proved to be derived from *Ac*, are also deficient for all or part of ORF 1, as well as for various parts of ORF 2. This observation lends support to the notion that ORF 1 encodes a *trans*-acting transposition function (*9, 11, 13*).

Southern blot analysis of *bz-m2(DI)* DNA indicated that the *Ds* derivative in this

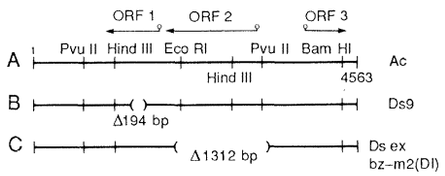

Fig. 1. Restriction maps of *Ac* and its *Ds* derivatives, showing the location of the three longest ORF's and of the deletions in the respective *Ds* elements. (A) Intact *Ac* element from *wx-m9(Ac)* and *bz-m2(Ac)*. (B) *Ds* element from *wx-m9(Ds)*. (C) *Ds* element from *bz-m2(DI)*.

allele had lost about 1.3 kilobases (kb) of DNA, including the Eco RI site and the Pvu II site 1.1 kb to the right (Fig. 1C). Since these sites are located in or very close to ORF 2, it was possible that the deletion in this *Ds* element affected ORF 2 but not ORF 1. If so, *bz-m2(DI)* would constitute appropriate material to investigate the involvement of ORF 2 in specifying the *trans*-acting transposition function of *Ac*.

To isolate the *Ds* element from *bz-m2(DI)*, a genomic library of Bgl II–digested DNA was constructed in EMBL 4 phage as described (*8*). Phages were screened with pAGS528, a 1.3-kb Kpn I–Pst I fragment internal to the transcribed region of the *bz* gene (*8*). Three clones were isolated and all yielded fragments that hybridized to *bz* and were the same size as expected from genomic Southern analysis of *bz-m2(DI)*. A 4.6-kb Kpn I–Pst I fragment was subcloned from one of these phages into pUC19. The fragment contained the entire 3.3-kb insert plus 1.3 kb of adjacent *bz* sequences. Overlapping fragments of the *Ds* insertion obtained by Bal 31 deletion (*14*) were subcloned into M13 phage (*15*) and sequenced by the dideoxy method (*16*).

The sequenced segment of the *Ds* element in *bz-m2(DI)* matches the published *Ac* sequence except for a 1312-bp deletion from nucleotide 1993 to nucleotide 3304 (Fig. 2). This deletion covers most of ORF 2: it extends from 74 bp upstream of the putative TATA box to 107 bp upstream of the putative termination codon (*10*). In agreement with previous studies (*11, 13*), we find no evidence for the involvement of direct repeats in the origination of the deletion. The only secondary structure of interest that we can detect is a 16-bp palindromic sequence, 3 bp to the left of the right deletion end point, containing one mismatch. ORF 1 was sequenced in its entirety to test whether single base pair changes in ORF 1 could have resulted in the loss of the *trans*-acting transposition function in *bz-m2(DI)*, and no changes were found. Thus, both intact ORF's 1 and 2 are required for *Ac*'s *trans*-acting transposition function.

We also tested for genetic complementation between two *Ds* elements altered in ORF 1 and ORF 2, respectively. Homozygous *bz-m2(DI) wx* plants were pollinated with *bz-R wx-m9(Ds)* (*17*) and the resulting heterozygous seed were examined for revertant purple (Bz) sectors in the aleurone and blue (Wx) sectors in the I/KI-stained endosperm starch (*18*). All the kernels from the resulting cross were uniformly bronze and had red staining (wx) starch, indicating that the intact ORF 1 and ORF 2 in the two *Ds* elements do not complement (Fig. 3A). Control crosses between *bz-m2(DI) wx* and *bz-R wx-m9(Ac)* gave spotted kernels, as expected (Fig. 3B). This is genetic evidence that the two long ORF's of the transposable element *Ac* encode the same *trans*-acting transposition function. Possibly they are joined together by RNA splicing to produce a single protein (transposase). In an analogous two-element system in *Drosophila*, mutations in any of the four ORF's of the *P* transposable element were each sufficient to eliminate the *trans*-acting transposition function of the element and failed to complement with each other when tested in pairwise combinations (*19*). Recently, it has been shown that these four ORF's are

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Fig. 2 (top). Nucleotide sequences of the left (top) and right (bottom) junctions of the 1312-bp deletion in the *Ds* element of *bz-m2(DI)* and of the adjacent sequences in the progenitor *Ac* element in *bz-m2(Ac)*. A 16-bp palindromic sequence (underlined) occurs 3 bp to the left of the right deletion endpoint. Fig. 3 (bottom). Kernel phenotypes produced by the following genotypes: (A) *bz-m2(DI) wx/ bz-m2(DI) wx/ bz-R wx-m9(Ds)*; (B) *bz-m2(DI)/bz-m2(DI)/bz-R wx-m9(Ac)*; (C) *bz-m2(Ac)/ bz-m2(Ac)/ bz-R wx-m9(Ac)*.

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                                        1992
                                         |
bz-m2(DI)    ACATGTACAACAATTGAGACA
bz-m2        ACATGTACAACAATTGAGACAAACATACCTGCGAGGATCA...

bz-m2(DI)                                    TTCTGAATCCGACTAGAAGA
bz-m2     ...CCACGCGCAC|GTGCACGTACATTCTGAATCCGACTAGAAGA
                                         |
                                        3305
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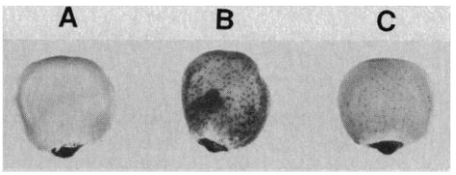

spliced in the *Drosophila* germline to produce a single transposase protein (*20, 21*).

Since the short ORF 3 does not appear to qualify as a potential protein coding gene (*10*), it is likely that the postulated transposase plays a dual role, mediating *Ac*'s *trans*-acting transposition function and the well-documented inverse dosage effect of *Ac* upon somatic mutations (*1, 2*). Evidence for this derives from a comparison between the following triploid aleurone phenotypes. Kernels of the genotype *bz-m2(Ac)/bz-m2(Ac)/bz-R wx-m9(Ac)* show a variegation pattern typical of three doses of *Ac*; that is, very small Bz sectors occur, indicative of somatic mutations occurring late in the development of the aleurone (Fig. 3C). In contrast, kernels of the genotype *bz-m2(DI)/ bz-m2(DI)/bz-R wx-m9(Ac)*, with two copies of *Ds* and one copy of *Ac*, produce a variegation pattern typical of one dose of *Ac*, a mixture of large and small sectors, indicative of mutations occurring both early and late in development (Fig. 3B). Thus, the 1.3-kb deletion in ORF 2 of the *Ds* element in *bz-m2(DI)* affects the element's capacity both to induce transposition in *trans* and to contribute to *Ac* dosage. These observations implicate ORF 2 in both transposition properties of *Ac* but do not exclude the possibility that ORF 3 might play a role in transposition.

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