Pattern of *Ac* Transposition in Maize

Drew Schwartz

Department of Biology, Indiana University, Bloomington, Indiana 47401

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ABSTRACT

Analysis of four nearby transpositions of *Ac* from the waxy locus indicate that the element can reinsert to either side. The cause of the asymmetry observed for *P-VV* transpositions is discussed and a model is presented to account for the high frequency of close reinsertions of *Ac*.

IN suitable genetic backgrounds the transposition of the maize activator (*Ac*) element can easily be followed. Transposition involves the excision of the element from its position in the chromosome and its reinsertion into another site. When the element is located in the locus of a gene such that gene function is repressed, excision can lead to recovery of function resulting in variegated somatic tissue. Using the unstable *P-VV* allele, which has an *Mp*(=*Ac*) element inserted in, and repressing the function of, the *P* gene controlling pericarp pigmentation, BRINK and his coworkers (VAN SCHAIK and BRINK 1959; GREENBLATT and BRINK 1962) were able to map the position of *Ac* reinsertion when it was transposed. *P-VV* has the advantage that somatic variegation of the maternal pericarp tissue can be detected on the ear and that kernels included in revertant somatic sectors are also germinally reverted. The mapping is facilitated by the unique dosage effect of *Ac* (MCCLINTOCK 1951). Distinct, easily distinguishable variegation phenotypes are produced by increasing doses of *Ac* per genome. One dose of *Ac* gives a medium variegated pericarp pattern while two doses produce a light variegated pattern (BRINK and NILAN 1952). Recombination distance between *P-VV* and the second *Ac* element can be directly measured by determining the frequency of change from the light variegated to medium variegated phenotype in progeny ears. A high tendency for reinsertion of *Ac* into a site close to its original position was observed. An analysis of twin spots, composed of a red revertant sector in which *Ac* was excised from the *P* locus, paired with a light variegated sector containing the parental *P-VV* allele and a second *Ac* element, indicated that *Ac* transposition occurs during chromosome replication (GREENBLATT and BRINK 1963; GREENBLATT 1968, 1974). In approximately two-thirds of the twin spots, the *Ac* element, excised from a *P* locus that had already replicated, was inserted into an as yet unreplicated chromosome site. In a more detailed study GREENBLATT (1984) reported that *Ac* reinsertion into closely linked sites is asymmetrical. Not a single reinsertion was found in the four map units proximal to the *P* locus, whereas there were 23 reinsertions in the four map units distal to *P*. This is a surprising and most interesting finding which could serve as a clue to the transposition process. Is polarity of *Ac* reinsertion a general phenomenon? Only a selected group of transpositions was analyzed in the *P-VV* study, *i.e.*, twin spots in which the *Ac* element excised from one chromatid was reinserted into the other chromatid or into an unreplicated chromosome segment. To answer this question, transpositions of *Ac* from the *wx-m9Ac* allele at the waxy locus were analyzed. The data from four closely linked reinsertions of *Ac* are presented as well as a model to account for the asymmetrical pattern of reinsertion reported by GREENBLATT.

MATERIALS AND METHODS

wx-m9Ac has an *Ac* element inserted into the 10th exon of the waxy gene (*Wx*) on chromosome *9* (MCCLINTOCK 1963; KLOSGEN *et al.* 1986). The presence of the inserted element causes a drastic but incomplete repression of the functioning of the gene. Autonomous excision of the element results in either restoration of full *Wx* gene function or complete inactivation.

The *I Wx Ds* genes are carried on the short arm of chromosome *9*. *I* is an allele of the *C* gene and inhibits anthocyanin pigmentation in the aleurone. It is located about 26 map units distal to *Wx*. The *Ds* element is of the state 1 class described by MCCLINTOCK (1951) which responds to *Ac* by undergoing dissociation of the chromosome with loss of distal markers. MCCLINTOCK (1948) reports that *Ds* is between 1 and 2 map units proximal to the *Wx* locus. I measured 1.9% recombination (36 of 1880) between the two loci.

wx-m6 is a 2.5-kb *Ds* insertional mutant of the *Wx* gene. The waxy phenotype is determined by filing the surface of the kernel and staining the starch with iodine potassium iodine.

Homozygous *wx-m9 Ac* plants were crossed with *Wx* homozygotes and the *Wx/wx-9Ac* heterozygotes were used as female parents in backcrosses to *wx/wx* plants. The progeny ears were screened for *wx* mutant kernels resulting from germinal transposition of *Ac*, leaving the waxy gene nonfunctional. Since the *Ac* element is in an exon and the insertion of *Ac* is associated with an eight base duplication in the host DNA, precise excision of *Ac* would result in a frame shift mutation in the *Wx* gene. The waxy kernels were

1) *wx-m9/Wx* X *wx/wx*

select waxy kernels

2) (*Ac*?) *wx/wx* X *wx-m1/wx-m1*

select *wx-->Wx* variegated kernels

3) *Ac wx-m1/wx* X *I Wx Ds/I Wx Ds*

select *I Wx-->C wx* variegated kernels

4) *Ac C wx(m1)/I Wx Ds* X *C wx/C wx*

FIGURE 1.—Series of crosses to generate the material used to map the positions of the nearby transpositions of *Ac* from the waxy locus. The heterozygous kernels in cross #4 are either *C wx* or *C wx-m1*.

planted and crossed to *wx-m1/wx-m1* plants to test for the presence of an active *Ac*. Eleven mutants which showed *Ac* activity were further tested. Aleurone pigmented, *wx-m1→Wx* variegated kernels from each ear were grown and crossed to homozygous *I Wx Ds* (state 1) plants. Progeny kernels showing *IWx→Cwx* variegation (colored *wx* sectors in a colorless *Wx* background) were crossed to *C wx* tester plants which were homozygous for all the other genes required for aleurone pigmentation to determine the position of the *Ac* element relative to the *I*, *Wx*, and *Ds* sites. The series of crosses is shown in Figure 1. In six cases the transposed *Ac* was not linked to the waxy gene as equal numbers of *Wx* and *Wx→wx* kernels were found in the progeny of the final cross. In one case no crossovers were obtained between the *Ac* and the waxy locus. Data from the remaining four transposed *Ac* elements which were reinserted at positions close to the original site of *Ac* in the waxy locus were analyzed to determine if they had all transposed to only one side of the waxy locus, as was reported for *P-VV*.

RESULTS AND DISCUSSION

The *wx*-linked *Ac* element could be in one of three positions indicated by the arrows in Figure 2; distal to the *wx* locus, between *wx* and *Ds* or proximal to *Ds*. In the progeny of the cross in Figure 2, dissociation of the chromosome at the *Ds* position with the loss of the distal markers occurs only when there is a crossover between *Ac* and *Ds* placing both elements on the same chromosome. If *Ac* occupies a position between the *Wx* and *Ds* loci, a crossover between *Ac* and *Ds* would yield a *wx Ac Ds* combination and no *Wx→wx* variegated progeny kernels would be recovered. If *Ac* were distal to the *wx* locus, the majority of the *Wx→*

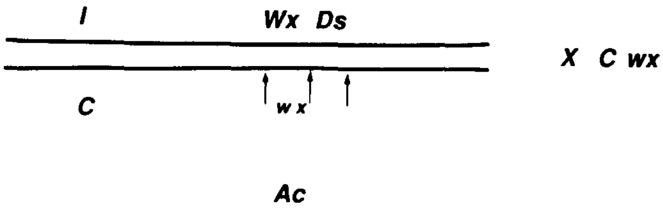

FIGURE 2.—Diagram of cross used to map transposed *Ac*'s. Possible positions of the *Ac* elements are indicated by the arrows.

wx variegated *Ac Wx Ds* crossover progeny kernels should receive the *C* allele from the *I/C* heterozygote parent and have colored aleurone. Only the double crossovers would carry the *I* inhibitor allele. The reverse condition would result if *Ac* is proximal to *Ds* with the majority of the *Wx→wx* variegated kernels carrying the *I* allele and showing no aleurone pigmentation.

Case 1 (*wx* mutant 7600-2): Nine variegated kernels were recovered in the *Wx* progeny population of 2312 kernels. Of these eight were *C Wx→wx* variegated and only one was *I Wx→C wx* variegated. Thus the *Ac* element is placed distal to the *Wx* locus at a distance of about 0.39 map unit. The *Wx→wx* variegation could also result from secondary transposition prior to meiosis moving the *Ac* element away from the proximal region of the short arm of chromosome *9* with subsequent cosegregation of the *Ac* and *Ds* chromosomes at meiosis. However, with the exception of crossovers between *I* and *Wx*, these would be *IWx→Cwx* in phenotype and only one kernel out of the nine was of this class.

Case 2 (*ax* mutant 7603-2): Three variegated kernels were found among the 2131 *Wx* progeny. This is a recombination frequency of 0.14%. Two were *C Wx→wx* variegated and one was *I Wx→C wx* variegated. Additional data are required for positioning of the *Ac* element because of the low number of recombinants recovered. The *Ac* element can either be 0.14 map unit distal to *wx* or 0.14 map unit proximal to the *Ds* element. Both locations would allow recovery of *Wx→wx* variegated progeny. A decision between these alternatives required determination of the amount of recombination between the *Ac* and *wx* loci. *Ds* is about two map units from *Wx*. If *Ac* is located proximal to *Ds* it should show about two percent recombination with *Wx*. When plants heterozygous for *Ac wx* and the *wx-m6* allele were backcrossed to *wx/wx* tester plants only two recombinant *wx-m6→Wx* variegated kernels were recovered in a population of 4914 waxy kernels. Since only half of the recombinants between the two loci can be detected in this cross, those which couple *Ac* with *wx-m6*, the number of recombinants should be doubled giving a recombinant frequency of 0.08 (4 of 4914). Thus the transposed *Ac* must be distal to the waxy locus at a distance estimated to be 0.1 of a map unit (7 of 7045) when both sets of data are combined.

Case 3 (*wx* mutant 7594-2): No *Wx*→*wx* variegated kernels were recovered in a population of 2201 *Wx* kernels in the *I Wx Ds* cross indicated in Figure 1. However, 19 *wx-m6*→*Wx* variegated kernels were obtained in a population of 3473 waxy kernels from the *Ac wx*/*wx-m6* backcross putting *Ac* 1.1 map units from the *wx* locus when the data are corrected by doubling the number of recombinants, 38 of 3473. *Ac* must be proximal to the *wx* locus, between it and the *Ds* element. Secondary transposition can be ruled out in this case since equal frequencies of variegated progeny kernels would be expected in the crosses to *I Wx Ds* and *wx-m6* plants.

Case 4 (*wx* mutant 7598-1): No *Wx*→*wx* variegated kernels were recovered in a population of 3732 *Wx* progeny kernels in the Figure 1 type cross involving *I Wx Ds*. One *wx-m6*→*wx* variegated kernel was recovered in a population of 1453 waxy kernels in the *Ac wx*/*wx-m6* backcross. Doubling the number of recombinants places *Ac* 0.14 of a map unit proximal to the *wx* locus (2 of 1453), between *wx* and *Ds*.

Thus, in two of the cases cited, *Ac* transposes distally from the waxy locus to a position approximately 0.1 and 0.39 map unit away. In the other two it moved proximally, at a distance of about 0.14 and 1.1 map unit. The relative positions are critical, not the actual distances. Southern blots of DNA digested with *Sal*I restriction enzyme and probed with the Sal#3 fragment of the waxy gene (WESSLER and VARAGONA 1985) confirmed that in each of the four cases of the *Ac* element had moved away from its original position in the waxy gene. These results differ from those reported for *P-VV* where within a distance of four map units from the point of origin 23 transpositions of *Ac* were clustered distally and no transpositions were proximal, but different chromosomal regions were involved in the two studies.

To account for this difference, it is proposed that the asymmetry observed in the clustered nearby transpositions of *Ac* from *P-VV* is not real but results from restricting the analysis to those transpositions which give rise to a twin spot of red and light variegated pericarp sectors.

The high frequency of twin spotting and the fact that at least half of the twins carry the transposed *Ac* in both the red and light variegated sectors indicates that transposition of *Ac* occurs during chromosome replication (GREENBLATT and BRINK 1963). GREENBLATT (1984) presented a model in which he proposed that the *P* locus is located about two map units from an origin of replication in chromosome *1*. In this model, *Ac* is excised as a single stranded DNA segment which is reinserted into the unreplicated DNA upstream from the replication fork. To account for the asymmetry and the absence of transpositions downstream into the already replicated chromatids, GREENBLATT suggests that *Ac* can only transpose into unreplicated regions of the chromosome. The occurrence of frequent *tr-Mp*-negative type twin spots in which the transposed element is present only in the light variegated sector of the twin and is absent in the red sector, is contrary evidence which seems to indicate that *Ac* can transpose into already replicated chromatids (GREENBLATT and BRINK 1962). In an attempt to circumvent this difficulty, GREENBLATT modified his explanation of these *tr-Mp*-negative twin spots and suggested that in these cases the *Ac* element is actually present in both sectors of the twin but is silent and undetected phenotypically in the red sector. However, a recent study by CHEN, GREENBLATT and DELLAPORTA (1987) argues against this explanation, since the *Ac* element cannot be detected in the red sectors at the molecular level in SOUTHERN (1975) blots.

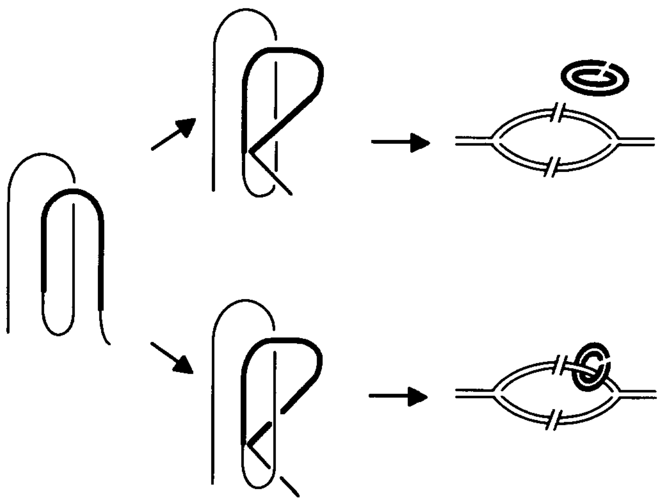

FIGURE 3.—Model of *Ac* trapping to account for the high frequency of nearby transpositions. The *Ac* element is indicated by a heavy line. *Ac* transposition occurs after the element is replicated. Association of the *Ac* termini will produce a ring-like structure following excision. The ring can either be free or trapped by the replicating chromosome, as indicated. See text.

The frequent close reinsertions of *Ac*, with about half of the transpositions from *P-VV* to sites within 12 map units, 46 of 105 (GREENBLATT 1984), suggests that the excised DNA segment can remain associated with the chromosome and be reinserted as it slides along its length. Excision of *Ac* as a double stranded DNA segment and its frequent association with the chromosome from which it was excised can readily account for the pattern of transposition observed with *P-VV*. When the excised element slides in the direction of the replicating fork it can be reinserted either into the unreplicated chromosome at the fork, giving rise to the *tr-Mp*-positive type twin, or into the already replicated *P-VV* containing chromatid just behind the fork, giving rise to the *tr-Mp*-negative type twin spot. When the excised *Ac* element remains associated with the chromatid from which it was excised and moves in the opposite direction, away from the replicating fork, reinsertion will not produce a twin spot until the element reaches the fork replicating in the opposite direction on the other side of the origin of replication and result in the observed asymmetry. Reinsertion of the element into the chromatid from which it was excised will give rise to a single red sector. The length of the silent sector in which reinsertions do not produce twin spots will depend on the distance between the *P-VV* locus and the origin of replication.

The frequent association of the excised *Ac* element with the chromosome, resulting in nearby reinsertions, is a predicted consequence of the excision process. No chromosome dissociation, detected by loss of distal markers, occurs when *Ac* is excised from the chromosome. This indicates simultaneous cleavage of both junctions between the ends of the *Ac* element and the chromosome, with 100% rejoining of the cleaved chromosome ends. It suggests that the two ends of the *Ac* element are held in close proximity at the time of excision, probably by binding to the transposase. The coupling of the ends to form a closed rink-like structure would very likely persist until reinsertion occurs when the two inverted repeat termini of the element are ligated to the cleaved ends of the chromosome at the point of reinsertion. Since the chromosome is a highly coiled structure it can readily be interlocked with the transposable element when the two *Ac* termini are brought together, as shown in Figure 3. In such cases the chromosome would entrap the ring produced by the excision of the element. Its movement would be limited to the length of the chromosomal region between the two replicating forks and the reinsertion would be in a site close to the original position.

In conclusion, this communication presents a model to account for the high frequency of nearby reinsertions of *Ac* following excision and suggests that the asymmetry reported for *Ac* reinsertions from the *P-VV* allele results from the limitation of the analysis to transpositions which give rise to twin spots of red and light variegated pericarp.

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