A CHROMOSOME REPLICATION PATTERN DEDUCED FROM PERICARP PHENOTYPES RESULTING FROM MOVEMENTS OF THE TRANSPOSABLE ELEMENT, MODULATOR, IN MAIZE

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ABSTRACT

Modulator (*Mp*) was mapped after it transposed from the *P* locus on chromosome *1* by studying 105 light variegated/red twin sectors on medium variegated pericarp ears. Sixty-one percent of the receptor sites were detectably linked to *P*, and these showed an asymmetry of distribution adjacent to *P*. No transpositions were mapped in the 4 map units proximal to *P*, whereas 23 cases mapped to the same length distal to *P*. The remaining transpositions of *Mp* on chromosome *1*, both proximal and distal to *P*, were equally scattered. It has previously been shown that when Modulator transposes it replicates at the *P* locus and a second time at the receptor site. The pattern of transposition adjacent to *P* is consistent with a hypothesis that a replicon initiation site is situated proximal to *P*; that Modulator transposes at the time of replication; that it is not able to transpose into a replicated region but only into a replicating one. No difference in distribution of receptor sites was found when the Modulator was detected *vs.* not detected in the red co-twins by testing with a Dissociation element.

TRANSPOSABLE elements, discovered in maize by B. McClintock (1948, 1950, 1951) and now recognized in other organisms (Drosophila, yeast, man, bacteria, plasmids; see Cold Spring Harbor Symposium Quantitative Biology, Volume 45, 1981), appear to be genetically universal, as if they were a basic building block of chromosomes. The discoveries in eukaryotic organisms of transposable elements came initially from the analysis of highly mutable gene systems, whereas the discoveries in prokaryotic organisms largely resulted from the developments in DNA molecular techniques. These molecular approaches are only now being applied to the maize systems (BURR and BURR 1981, 1982; FEDOROFF, WESSLER and SHURE 1983). (For reviews see CALOS and MILLER 1980; GREEN 1980; FEDEROFF 1983.)

As with other systems, a striking feature of the transposable element, Modulator (*Mp*), in maize is that it moves from one site within the chromosome to other sites without any change in the linear relations of known chromosomal loci. MCCLINTOCK was the first to note that when such an element moved it

could subsequently be found at different sites on the same chromosome or on a nonhomologous chromosome. When the transposable element was at a new receptor location, she also showed that it was absent from the previous or donor location (McCLINTOCK 1948, 1950, 1951, 1956). These monumental studies in maize, although asking and ascertaining where an element can and did go, did not uncover any restrictions as to the receptiveness of chromosomal sites to the transposing element.

A nonrandom pattern of transposition within the genome was first noted in a quantitative study of 87 independent transpositions of the *Mp* element from the *P* locus on chromosome *1* in maize (VAN SCHAIK and BRINK 1959). Their conclusion was that *Mp* remained linked to *P* much more often than expected by chance. This finding, that sites close to the origin were favored as receptor locations during transposition, was substantiated by GREENBLATT and BRINK (1962) and A. CORTES (unpublished data).

Transposition of the *Mp* element back to a prior donor site can also occur (ORTON and BRINK 1966; BRINK and WILLIAMS 1973). In both of these studies it was clear that a transposable element could move in one direction and at a subsequent transposition move in the reverse direction.

Still another feature of transposition was elucidated by GREENBLATT and BRINK (1962). They showed that 62% of the transpositions of *Mp* replicated first at the *P* locus and again at the receptor site. Thirty-eight percent of the twinned spots showed no detectable *Mp* in the red co-twin. These findings suggested that two types of transpositions might actually be occurring: one type in which the transposable element replicated at the donor site (the *P* locus) and again at a recipient site and another type of transposition where Modulator replicated at the *P* locus but did not appear to replicate the second time at the receptor site (GREENBLATT and BRINK 1962, 1963).

The location of the receptor sites at which Modulator replicated a second time was determined by a three-point linkage test (GREENBLATT and BRINK 1962) to provide information on left-right direction with respect to the *P* locus. All prior mapping studies of the transposed Modulators (*tr-Mp*) from *P* were two-point tests and did not take into account the direction of the *tr-Mp* from *P*. These initial three-point linkage tests of six independently linked transpositions in which *Mp* replicated a second time indicated that the insertion was distal to *P*. In addition, the first attempt to locate *Mp*, when it appeared not to replicate at the receptor site, suggested a proximal location relative to the *P* locus (I. M. GREENBLATT, unpublished data). These results prompted an extensive analysis of Modulator transposition patterns to determine where it moves and when it appears to replicate and not replicate at receptor sites.

This report documents the locations of transposed Modulators (*tr-Mp*) after they left the *P* locus on chromosome *1*. The major salient findings show that there is no difference in the distribution of receptor sites of *tr-Mp* when the second replication occurs, or does not appear to occur, at a receptor site. A clear pattern was found, however, of an asymmetry in the distribution of linked sites on chromosome *1* for both types of receptor sites. The number of proximal locations of *tr-Mp* recovered was significantly less than the number of distal sites.

The entire inequality of the proximal/distal difference, is accounted for by the finding that a region proximal to *P* and extending approximately four recombinational units from *P* is totally refractory to the reception of Modulator. The equivalent distal length of the chromosome contains the highest frequency of receptor sites. These genetic findings of the pattern of Modulator's movements (transpositions that have been shown to occur only during the period of chromosome replication (GREENBLATT and BRINK 1962; GREENBLATT 1968, 1974)) support the well-substantiated theory that chromosomes replicate in units from multiple initiation sites, replicons, along their length (JACOB and BRENNER 1963; TAYLOR 1963), and there is a time differential between the start and finish of these segments of replication. Since transposition at the time of replication has been previously demonstrated, the movements of Modulator, as described in this paper, are consistent with the replicon model of chromosome replication first deduced from pulse radioactive tracer analysis.

MATERIALS AND METHODS

Definition of terms

Red pericarp (P-RR): The dominant allele at the *P* locus in the central region of the short arm of chromosome *1* conditioning red pericarp and cob tissue. The *P-WW* and *P-WR* alleles condition colorless pericarp and colorless or colored cob, respectively.

Modulator (Mp): A transposable element discovered at the *P* locus. *Mp* can not as yet be distinguished by genetic tests with the Dissociation gene (*Ds*), from the transposable element, Activator (*Ac*), discovered by B. MCCLINTOCK (1948, 1950, 1951). Transposed modulator (*tr-Mp*) refers to an *Mp* element after it has left the *P* locus. Since in these studies *Mp* comes from the *P* locus, the donor site, *tr-Mp* is at a "receptor site" (different for each transposition). The central issue in this report is where these *tr-Mp* receptor sites are within the genome after *Mp* moves from the *P* locus. *tr-Mp* can subsequently move from a receptor site. Such movements are referred to as secondary transpositions and are eliminated from the data when identified. A full examination and justification of this consideration were given by GREENBLATT and BRINK (1962).

Medium variegated pericarp (P-RR-MP): Transposable element, Modulator (*Mp*), is conjoined with the *P* locus on chromosome *1*. The *P* locus allele present is *P-RR*, and its expression is suppressed by the presence of *Mp* to yield colorless pericarp. When *Mp* transposes during pericarp development, the *P-RR* allele is no longer inhibited and is expressed as a lineage of red pigment-producing cells on an otherwise colorless background. The frequency and timing of the red stripes found with a single *Mp* present at the *P* locus result in the medium variegated phenotype. Transpositions of *Mp* away from *P-RR* can produce sectors of red pigment that cover more than one kernel of pericarp tissue, the earlier the transposition the more kernels being covered by red pericarp. The embryo within the red-covered seeds shares the genotypes of the pericarp, barring segregational loss at meiosis (WOOD and BRINK 1956). Small late sectors of red cells are most frequent; the large early sectors are rare in the pericarp.

Light variegated pericarp (P-RR-Mp + tr-Mp): The same red stripes as found in medium variegated pericarp but with a 67% reduction in frequency of stripes are found in light variegated pericarp. This is due to the presence of a *tr-Mp* element interacting with the *Mp* still present at the *P* locus; they mutually suppress their rates of transposition.

Twin spots: These are red sectors contiguous with light variegated sectors; as physical twins they make up the majority of sector types on medium variegated pericarp (GREENBLATT 1974). The studies of GREENBLATT and BRINK (1962) and GREENBLATT (1968, 1974) have shown that these twin mutations arise from a single transposition of *Mp* away from the *P* locus during a mitotic chromosome replication. The *tr-Mp* element found in the light variegated co-twin resides at the same receptor site as the *tr-Mp* found in the red co-twin. Selection of twin spots with large kernel number allows for direct recovery, in the next generation, of plants with the genetic makeup of each of the two pericarp tissue types.

Ds *test*: Dissociation, *Ds*, (McCLINTOCK 1950; BARCLAY and BRINK 1954; GREENBLATT and BRINK 1962) in the "standard" position on chromosome *9* is proximal to the dominant aleurone pigment-conditioning gene, *C* (aleurone is 3n embryonic tissue and is the layer immediately within the pericarp wrapper of the kernel). When *Mp* is present with *Ds* in the same cells, *Ds* will produce chromosome breakage at its chromosome location resulting in the loss of the distal fragment of chromosome *9* carrying the *C* allele. Thus, *Ds* and *Mp* interaction results in a pattern of colored-colorless cell lineages in the aleurone and "tests" for the presence of a *Mp* otherwise not traceable at a receptor site.

When Modulator leaves the *P* locus it does so at the time of chromosome replication (GREENBLATT and BRINK 1962; GREENBLATT 1968, 1974, 1981). After *Mp* replicates at the *P* locus one of the two available copies transposes to a receptor site. At the receptor site it may replicate a second time (GREENBLATT and BRINK 1962; this report). When it does replicate the second time at the receptor site, a twin spot can result that will carry a *tr-Mp* element at the same chromosome location in both the red and light variegated cosectors of a twin spot (GREENBLATT and BRINK 1962). However, approximately one-fourth to one-third the twin sectors have no detectable *tr-Mp* in their red co-twin while, of course, still showing one *tr-Mp* present in the light variegated co-twin. An objective of the current experiments was to test where *tr-Mp* goes after leaving the *P* locus when it was not detectable in the red co-twin and where it goes when it was detectable in the red co-twin (*tr-Mp* was mapped from the light variegated co-twin).

Since *tr-Mp* goes to so many different receptor sites, such studies necessitate recovering numerous independent transpositions. This was done by screening a large number of medium variegated ears for the presence of twin mutations in the pericarp tissue. Because meiosis intercedes between the transposition that gives rise to the twin mutation and transfer of the chromosome carrying the *tr-Mp* into the next generation, each twin spot must be large enough (arbitrarily ten kernels or more for each sector) so that segregational loss of the *tr-Mp*-carrying chromosome will not affect the subsequent testing results. Receptor sites near the *P* locus will tend to segregate with the donor *P* allele, but if the *tr-Mp* receptor site is far enough from *P* to recombine at random with it, 50% of the red pericarp plants reared from the red kernels will lack a *tr-Mp* because of meiotic recombination, not because it had not replicated a second time.

Each light variegated co-twin of all the identified twin spots serves as the material to test the linkage relationships of the receptor site of a *tr-Mp*. In the medium variegated stocks employed in these experiments the breakpoint of the reciprocal translocation, Tl-2b (1S.43, 2S.36) (LONGLEY 1958) was in coupling with the *P-RR-Mp* complex prior to transposition. Thus, after transposition a three-point backcross-type linkage test became available using Tl-2b, *P-RR-Mp* and *tr-Mp*. The phenotype of the heterozygous Tl-2b is semisterile seed set on the ear. The breakpoint Tl-2b is proximal to *P* (ANDERSON 1941; GREENBLATT 1981). *P-RR-Mp* is expressed as medium variegated pericarp. *Tr-Mp* is seen both as light variegated pericarp (interacting with *P-RR-Mp*) and as an activator in the *Ds* test. The *Ds* test allows for identification of a *tr-Mp* in all nonvariegated segregants in subsequent generations.

The initial mating to provide source pericarp materials with mutant twin sectors utilized a female parent that was homozygous for the *P-WR* (colorless pericarp and red cob) allele at the *P* locus, carried Texas male sterile cytoplasm and was backcrossed five times into the Wisconsin Inbred W23. Male sterile cytoplasm was used in order to ensure no pollen contamination from plants used as female parents. The pollen parent in these initial matings, backcrossed in excess of seven times to Iowa Inbred 4Co63, and self-mated once (thus, a mixture of heterozygous and homozygous variegated types in an expected ratio of 2:1) carried the *P-RR-Mp* complex in coupling with the reciprocal translocation Tl-2b. The pollen parent was also heterozygous for a normal chromosome *1* marked with a *P-WW* allele, had fertile cytoplasm and contained no restorer genes. The offspring of this mating were reared in an isolation plot and pollinated by an Inbred W23/Inbred 4Co63 hybrid that carried male fertile cytoplasm, no restorer genes, *P-WR/P-WW*, was homozygous for normal chromosome *1* and was known to be void of an active Modulator anywhere in its genome.

The testcross isolation plot was machine harvested; it yielded 6339 ears that expressed colored pericarp. Of these, 4033 were medium variegated and had semisterile seed set. That is, they carried a single *P-RR-Mp* complex in coupling with Tl-2b, and either *P-WW* or *P-WR* in coupling with normal chromosome *1*. These plants were pollen sterile due to their cytoplasm. It was from within this population of semisterile, medium variegated ears that 123 clearly defined red/light variegated twin

spots of the pericarp tissue were recovered. Kernels were selected from within each of the sectors of each of the twin spots and grown.

The plants derived from the kernels within the red sector were pollinated with the special tester stock, which carried *Ds* in the standard position on chromosome *9* (McCLINTOCK 1951) proximal to *C*. By the means outlined by GREENBLATT and BRINK (1962) this mating with *C-Ds* served to detect a Modulator element, if present, in all red pericarp derivatives independent of chromosome location. If any of the individuals reared from the kernels within the red sector showed a *tr-Mp* element present, the twin was scored positive (+) and was presumed to represent a case in which a second replication of *tr-Mp* occurred at the receptor site (GREENBLATT and BRINK 1962; GREENBLATT 1966). If, however, all derivatives from the red pericarp plants, both red and colorless, were found to yield a negative *Ds* test, the twin was scored as negative (−).

The plants reared from kernels selected from within the light variegated co-twins were backcrossed by plants genetically *P-WR/P-WR*, which carried normal chromosome *1* and were void of any active Modulators. By this means, individual ears could be selected that were phenotypically light variegated (*P-RR-Mp* + *tr-MP*) and had semisterile seed set (heterozygous for the reciprocal translocation, Tl-2b). They were also cytoplasmically male sterile.

The kernels from the selected light variegated, semisterile ears were in turn grown and pollinated with the same *C-Ds* pollen parent used to test *tr-Mp* in the red co-twins. Each plant reared was thus progeny tested for the presence of *tr-Mp* while concurrently providing the progeny to estimate recombination values between *P*, *tr-Mp* and the breakpoint of Tl-2b.

Wherever possible, repeats of the backcross mating with light variegated parents were employed in order to produce as many progeny as possible for linkage counts. This caution of repeat sibships has been shown to aid in the identification of secondary transpositions of *tr-Mp* (GREENBLATT and BRINK 1962). Although each of the two Modulator elements in a light variegated plant, one still conjoined at the *P* locus and the other *tr-Mp* at a receptor site, tend to stabilize each other and reduce the potential number of secondary transpositions, they occur nevertheless. Detection of such secondary transpositions is limited to those cases in which the multiple sibships derived from a single transposition result in heterogenous linkage data by statistical test (KRUSKAL and WALLIS 1952). In such a case, if there is a single differing sibship in a population of three or more sibships, it is omitted from the calculation of receptor site location and the statistical test is run again to ensure that those estimates of receptor site location are homogeneous. In two instances, twins 1 and 10, one progeny in each produced a highly significant test of heterogeneity when pooled with the other repeat progenies. When these single progenies were omitted from the calculation, the two remaining samples for twin 1 and the three remaining samples of twin 10 gave values that were not significantly different for a within-twin comparison. Because linkage data from multiple samples were too heterogeneous to be pooled, twins 28 and 107 were removed from all consideration of estimating the primary position that Modulator took upon leaving the *P* locus. When only a single progeny was available for linkage analysis no attempt was made to uncover secondary transposition. As an overall caution no progenies were pooled for the final estimates of *tr-Mp* location if the exclusion of one of them would result in an altered decision as to the proximal-distal localization of the *tr-Mp* receptor site.

RESULTS

One hundred twenty-three red/light variegated twin sectors from backcross-type matings were analyzed for recombination of *tr-Mp* with the chromosome *1* markers *P*, *T* (the breakpoint of the translocation Tl-2b). Of these, 105 provided linkage data with the chromosome *1* markers from the light variegated co-twin, and the presence of *tr-Mp* in the genome of their co-twinned red sector was successfully tested for by the *Ds* test. These results are presented in Tables 1 through 4 and show the linkage relationship between the *P* locus and the *tr-Mp*.

Table 1 lists 39 light variegated sectors, each from an independently occurring twin mutation. Each show *tr-Mp* to be linked to the *P* locus and located distal to *P* on chromosome *1*.

TABLE 1

Listing of twin spots in which tr-Mp *was found to be distal to the* P *locus*

Twin no.	*Ds* positive or *Ds* negative	No. of families scored	No. of individuals	% recombination		
				P and *tr-Mp*	*tr-Mp* and *T*	*T* and *P*
1	+	2	281	2.14	8.19	6.05
2	+	3	610	0.49	5.90	5.74
7	+	4	523	2.68	6.69	4.40
10	+	3	393	3.81	5.84	2.03
11	−	5	608	2.31	3.14	2.15
12	+	1	138	21.74	23.91	2.17
17	−	4	488	1.84	7.38	5.94
19	−	1	180	1.67	3.89	3.33
20	+	1	91	5.49	6.59	5.49
22	+	2	257	2.71	6.23	4.28
23	+	1	122	5.74	8.20	4.10
25	+	3	390	1.54	2.82	2.31
26	+	3	407	7.86	9.34	3.44
27	+	2	404	23.76	24.50	2.72
29	+	3	330	3.03	4.55	2.73
32	+	2	296	28.72	30.07	2.70
37	−	1	225	18.22	19.56	3.11
42	+	3	457	3.06	7.44	4.81
51	+	3	433	12.01	14.55	4.39
52	+	3	441	2.72	4.31	2.49
55	+	3	363	1.10	3.86	2.75
57	−	1	141	29.79	31.91	4.97
60	+	4	497	2.82	3.62	2.41
61	−	3	411	2.92	4.38	2.43
69	+	3	408	5.88	9.07	5.64
70	+	3	335	5.07	7.76	3.88
76	−	3	390	6.92	9.74	2.82
77	−	1	103	0.97	2.91	1.94
83	+	3	374	3.74	5.35	3.21
84	−	2	303	7.59	9.24	2.97
85	+	3	444	6.76	11.26	5.41
88	+	3	349	1.43	2.29	0.86
91	+	3	408	0.98	4.41	3.92
92	+	1	100	2.00	3.00	1.00
96	+	2	182	10.99	11.54	0.55
103	+	2	256	10.55	13.67	3.13
108	+	2	282	2.84	3.90	1.06
113	−	3	419	3.10	4.06	2.86
114	+	2	273	3.66	4.03	2.56

Ds test data from red sector; recombination data from co-twinned light variegated sector progeny.

Table 2 lists 18 light variegated sectors, again of independent origin, in which *tr-Mp* has been found linked proximal to *P* on chromosome *1*.

Table 3 contains the 41 cases of light variegated sectors that showed *P* and *tr-Mp* recombining at random, *i.e.*, greater than 43% recombination. Their location remains unknown except that they are not near the *P* locus.

TABLE 2

Listing of twin spots in which tr-Mp *was found to be proximal to the* P *locus*

Twin no.	*Ds* positive or *Ds* negative	No. of families scored	No. of individuals	% recombination		
				P and *tr-Mp*	*tr-Mp* and *T*	*T* and *P*
3	+	2	214	6.07	2.80	5.14
13	+	3	355	8.45	4.51	7.32
15	−	5	731	25.17	24.76	3.15
31	+	2	334	5.09	3.29	2.99
35	−	2	291	27.15	23.71	4.81
36	+	4	484	17.56	16.94	3.10
44	+	2	276	25.00	21.38	3.62
58	+	3	287	6.27	3.83	6.62
62	−	2	256	8.98	5.86	3.91
67	−	3	536	7.09	4.66	3.54
71	+	2	218	4.59	3.21	2.29
90	+	2	175	19.43	18.29	3.43
93	+	2	388	8.76	5.93	3.35
98	+	2	198	8.59	7.58	5.05
100	+	1	144	9.72	6.25	3.47
104	+	3	404	3.96	1.73	3.22
116	+	3	438	5.02	2.28	3.20
117	−	1	112	38.39	36.61	1.79

Ds test data from red sector; recombination data from co-twinned light variegated sector progeny.

In addition, seven light variegated sectors showed *P* and *tr-Mp* tightly linked, but the distal/proximal relationship could not be determined by the available data; these are listed in Table 4.

There were 18 twin mutations that did not yield the necessary data for them to be listed in Tables 1 through 4. These twins are presented in Table 5 in order to identify the basis for their exclusion. The 18 twin sectors listed in Table 5 represent 15% of the original samples of twin spots chosen on the basis on pericarp phenotypes and, in fact, measure the efficiency of the selection and recombination techniques.

A prime concern in these experiments is a comparison of the location of *tr-Mp* after it has left the *P* locus in the light variegated cosector, when it is detectable *vs.* nondetectable in the red co-twin sector as measured by the *Ds* test. Although the *Ds* test findings are also listed with the individual light variegated groupings from each twin in Tables 1 through 4, a summary of the positive *vs.* negative *Ds* test findings are presented in Table 6.

The rightmost column in Table 6 lists the percentage of cases in which *tr-Mp* yielded a positive *vs.* negative *Ds* test result. The percent of the *Ds*-positive types is quite uniform irrespective of *tr-Mp* location. There is an average of 73% positive tests with *Ds* for the entire 105 cases. A close screening of the data of both proximal and distal linked *tr-Mp* sites does not disclose any regions of receptor site locations in which the *Ds*-positive test result is occurring exclusively or more frequently than elsewhere (see Figure 1). In other words, the pattern of sites found for the *tr-Mp* in light variegated sectors co-twinned with red sectors

TABLE 3

Listing of twin spots in which tr-Mp *was found to be recombining at random with the* P *locus*

Twin no.	*Ds* positive or *Ds* negative	No. of families scored	No. of individuals	% recombination		
				P and *tr-Mp*	*tr-Mp* and *T*	*T* and *P*
4	+	3	309	47.90	48.54	2.59
8	+	1	116	43.97	43.10	4.31
14	+	2	254	50.00	50.39	2.76
18	−	2	315	49.21	48.89	1.59
21	+	1	68	51.47	51.47	0.00
30	+	2	234	41.45	41.03	3.85
33	+	2	200	49.00	49.50	1.50
34	−	3	389	51.67	52.19	4.63
38	−	2	201	42.79	45.77	5.97
40	+	1	56	48.21	44.64	3.57
41	+	2	262	38.93	38.55	2.67
43	+	2	226	46.90	47.35	3.10
45	−	2	203	46.31	47.78	2.46
53	+	1	20	60.00	60.00	0.00
56	+	2	212	48.11	48.11	2.83
59	+	2	275	44.73	45.82	3.27
61A	−	2	328	49.70	49.70	1.83
63	−	2	356	50.84	50.00	4.21
64	+	2	101	45.54	46.53	4.95
65	+	1	115	55.65	53.91	6.96
66	+	2	305	45.25	46.56	2.62
68	−	1	76	44.74	46.05	3.95
72	+	2	300	47.33	46.67	2.67
73	+	2	281	49.11	50.89	2.45
75	+	2	246	47.15	47.97	4.07
79	+	2	273	49.08	51.28	2.20
80	+	1	118	43.22	44.92	5.08
81	+	1	127	48.03	47.24	0.79
82	−	3	545	43.85	44.95	2.94
86	−	2	201	45.27	44.78	4.48
87	+	2	351	46.44	46.72	2.56
95	+	1	127	42.52	41.73	2.36
97	−	1	132	46.97	50.00	6.06
99	+	3	289	50.17	49.13	4.50
105	+	2	313	46.01	47.60	1.60
106	+	1	254	40.94	40.55	1.18
109	+	2	207	48.31	48.79	2.42
115	+	3	429	44.76	42.89	3.26
118	+	1	71	46.34	57.75	7.04
119	+	1	78	46.15	44.87	3.85
122	+	3	301	40.86	40.86	3.99

Ds test data from red sector; recombination data from co-twinned light variegated sector progeny.

of the *Ds*-negative and *Ds*-positive type appear to be randomly placed throughout the linkage map of chromosome *1*. Thus, location of the receptor site, on a scale here measured, does not appear to be involved in determining which of the two types of twin red sectors will arise.

TABLE 4

Listing of twin spots in which tr-Mp *was linked to the* P *locus but proximal-distal relationships remain unknown*

Twin no.	*Ds* positive or *Ds* negative	No. of families scored	No. of individuals	% recombination		
				P and *tr-Mp*	*tr-Mp* and *T*	*T* and *P*
5	+	2	309	1.94	4.53	4.53
6	+	1	60	3.34	3.34	0.00
9	+	2	310	2.26	1.94	2.26
16	+	2	239	0.00	2.09	2.09
50	−	3	308	0.00	2.92	2.92
112	−	1	201	4.96	4.96	1.99
121	−	1	46	6.52	6.52	0.00

Ds test data from red sector; recombination data from co-twinned light variegated sector progeny.

TABLE 5

Disposition of those twin sectors which do not appear in Tables 1 through 4

Basis of exclusion	Twin no. involved
Offspring from the light variegated sector were not semisterile and thus lacked the Tl-2b marker	24, 39, 46, 47, 48, 54, 74, 78, 89, 94, 101, 102, 110, 111, 120
Linkage data from the multiple samples were too heterogeneous to be pooled	28, 107
The reciprocal translocation was not the original Tl-2b marker but some unknown semisterile	49

TABLE 6

Distribution of transposed Modulator when it is detectable (Ds *positive*) *or not detectable* (Ds *negative*) *from co-twin red sectors relative to* tr-Mp *linkage with the* P *locus in their co-twinned light variegated sectors*

Location of *tr-Mp*	No. of twin spots			
	Ds positive	*Ds* negative	Total	% positive
Distal to P	29	10	39	74
Proximal to P	13	5	18	72
At random with P	31	10	41	76
Linked—unknown proximal or distal	4	3	7	57
Totals	77	28	105	73

Figure 1, a histogram representation of the data in Tables 1 and 2, depicts the mapped positions of *tr-Mp* insertion, into chromosome *1*. The single most striking feature of the data presented for the linked sites of *tr-Mp* with *P* is a region immediately proximal to the *P* locus and approximately four recombinational units in length which was totally void of a *tr-Mp* receptor site. Yet, the equivalent

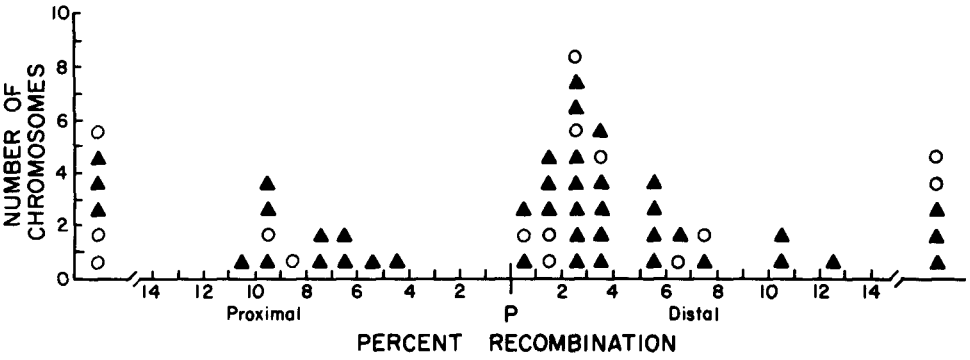

FIGURE 1.—The distribution of *tr-Mp* along chromosome *1* after transposition from *P* locus in light variegated co-twins. (The sites beyond 14% recombination of *P* are grouped together.) ○, *Ds*-negative test of red co-twin; ▲, *Ds*-positive test of red co-twin.

distance distal to *P* had 23 cases of *tr-Mp* insertion; these represent 59% of the distal cases and 40% of all linked sites. *tr-Mp* inserted into the region of the chromosome proximal to *P* but beyond this refractory interval in 18 cases. This was equivalent to the 16 cases of insertion in the corresponding length of the distal portion of the chromosome. Thus, the major difference in *tr-Mp* transposition to distal sites compared with proximal sites can be totally accounted for by the 4 map units distal to *P* compared with the corresponding proximal region. These differences of *tr-Mp* insertion with respect to *P* have occurred irrespective of whether *tr-Mp* was found by *Ds* testing to be present in the red co-twin or not.

The breakpoint of the translocation, Tl-2b, the third chromosomal member in this three-point backcross linkage analysis, was found to reside within the region proximal to *P* refractory to *tr-Mp* insertion. By utilizing the *tr-Mp* to *T* linkage data listed in Tables 1 and 2, it can be seen that *tr-Mp* does not insert immediately adjacent to *T*. The closest *tr-Mp* proximal insertion shows 1.73% recombination with *T*, whereas the nearest distal case shows 2.29% recombination with *T*. Thus, the breakpoint of Tl-2b lies within the length of chromosome not receiving *tr-Mp*.

Of the original population of 4033 medium variegated ears used to select large-sized twin spots, 3911 ears had none of the required size, whereas 121 had one twin spot of the required size. A single ear, no. 61, had two different and apparently independently occurring twin spots listed as no. 61 (Table 1) and no. 61A (Table 3). Twin 61 showed *tr-Mp* to be linked distal to *P* (2.92% recombination with *P*), whereas in twin 61A, *tr-Mp* recombined at random with *P*. In both twins the red sector was void of a *tr-Mp* and are listed as *Ds*-negative types.

Of the 105 estimates of *tr-Mp* locations, 61% (64 cases) were found to be linked to the *P* locus, its original site on chromosome *1* (Tables 1, 2 and 4 pooled). Thus, there is a nonrandomness of insertion following transposition both on chromosome *1* and between chromosome *1* and other potential locations throughout the genome. In other words, the mechanism of transposition of Modulator when it is at the *P* locus favors chromosome *1* sites over all other chromosomes combined and, in addition, favors sites immediately distal to the *P* locus over all other locations. As discussed in the next section, these results are

believed due to the pattern of chromosome replication, since Modulator moves only when the chromosome is replicating and, consequently, the pattern of transposition reflects the pattern of chromosome replication.

With respect to the total of 105 cases of *tr-Mp* site locations, only two cases listed in Table 4 as twins 16 and 50 showed *tr-Mp* so close to the *P* locus that no recombinant individuals were observed in populations of 239 and 308 individuals, respectively. In spite of these two exceptions, slightly more than 98% of the cases showed sites of *tr-Mp* insertion that do recombine with the site of origin, the *P* locus.

DISCUSSION

The outstanding feature of the data obtained by mapping *tr-Mp* after it leaves the *P* locus is the pattern of receptor sites that was found immediately adjacent to the *P* locus. On the proximal side, a region of approximately 4 map units was totally void of *tr-Mp* insertions. In contrast, on the distal side, just the opposite was occurring. Here, in the same length of chromosome, the highest frequency of insertions, 23 cases, took place. The remainder of the chromosome both distally and proximally does not seem to vary significantly: 16 cases on the distal side and 18 on the proximal side (Figure 1). A hypothesis that attempts to explain this asymmetry in transposition pattern can be constructed from the evidence that Modulator leaves the *P* locus at the time of mitotic chromosome replication (GREENBLATT and BRINK 1962, 1963; GREENBLATT, 1966, 1968, 1974, 1981). The assumptions are that *tr-Mp* enters a segment of a chromosome that is only actively replicating. The region immediately proximal to *P* (that is void of any *tr-Mp* insertions) has completed replication at the time the *P* locus and its conjoined *Mp* are replicated. The region immediately distal to *P* begins its replication when the *P* locus and Modulator are completing their replication. The just replicated Modulator at *P* can now move to a replicating section, be inserted and be replicated a second time (Figure 2). If this is true, the pattern of Modulator transpositions becomes a device that allows visualization of the pattern of chromosome replication.

The pattern of chromosome replication discerned by *tr-Mp* insertion appears to correspond with the pattern deduced by pulse labeling of replicating chromosomes. That is, there are sites of replication initiation along the chromosome and replication proceeds from these sites. Consequently, there are, at any one time, discrete lengths that are replicated and adjacent regions that are yet to be replicated. This is the replicon model of chromosome replication (JACOB and BRENNER 1963; TAYLOR 1963).

Superimposition of the replicon model on the pattern of *tr-Mp* transposition sites suggests the presence of a replicon initiation site or sites proximal to *P*. Based on this model, replication may be presumed to start proximal to *P* and proceed toward *P*. *P* and *Mp* are then replicated, and it is the replicated *Mp* that transposes. It is inserted only into a region that is then being replicated: the region distal to *P* or equivalent. The ability to utilize *tr-Mp* to resolve the limits of other replicons distant from the *P* locus is poor at best. All that can be concluded is that the chance of an insertion either proximally or distally when

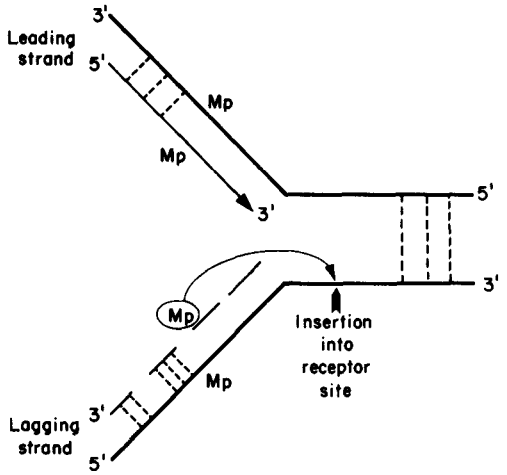

FIGURE 2.—Adaptation of the KORNBERG (1979) model of a DNA replication fork showing Modulator transposing. The arrow does not connote active movement. See text.

viewed beyond the adjacent replicon appears equal. This is what would be expected if the number of replicons is the same along equal lengths of chromosome distal and proximal to *P*. These results fit well with the known location of the *P* locus: the midregion of the short arm of chromosome *1*. The equal numbers of *tr-Mp*, proximally and distally, beyond the immediate replicon suggests that the probability of *Mp* moving left or right is equal.

The estimated length of the proposed replicon appears to be very long. In the present study it is based on recombination values between the breakpoint of the reciprocal translocation, Tl-2b, the *P* locus and a *tr-Mp* element. GREENBLATT (1981) has shown that the recombination values of the breakpoint of this reciprocal translocation and the *P* locus are subject to modifications by the presence or absence of *Mp* adjacent to this region. Modulator was found to nearly double the recombination values. Thus, the measured values of recombination in these studies may be too high. The presence of an *Mp* element is not expected to alter distal/proximal relationship judgments only the estimate of the actual distances.

Attempts to translate length of the short arm of chromosome *1* in maize deduced by recombination estimates and the amount of DNA in the total genome will result in overestimates of the amount of DNA per map unit. The most distant marker on the short arm of chromosome *1*, Striate, is given the linkage designation, zero, because there is no known marker distal to it. The physical distance between Striate and the end of the chromosome is unknown and may be very large. Therefore, any calculation of the amount of DNA in the proposed replicon in which *P* is found will yield values that are biased upward to an unknown extent. Distortion of linkage values is also expected by having Tl-2b in heterozygous condition (BURNHAM 1962).

The picture of transposition pattern uncovered by VAN SCHAIK and BRINK (1959) appears different from that uncovered by means of twin spot analysis

(GREENBLATT and BRINK 1962; this report). The current study found only two cases of transposition that failed to show any recombination between *P* and *tr-Mp*, whereas in the VAN SCHAIK and BRINK (1959) report this class represented the largest fraction of the linked sites. GREENBLATT and BRINK (1962) analyzed additional twin-spots and did not uncover any cases of *tr-Mp* not recombining with *P*. This difference in results of mapping the insertion of *tr-Mp* close to the *P* locus is an important issue. It raises the question of whether the means of uncovering the transpositions could influence the recovery of receptor site locations. VAN SCHAIK and BRINK (1959) used light variegated ears and light variegated sectors (within a medium variegated pericarp ear) as their source of a transposed Modulator population, whereas GREENBLATT and BRINK (1962) and the current report used only transpositions from twin spot mutations. The basis for this major difference in *tr-Mp* receptor site locations is not resolvable at this time. In addition, VAN SCHAIK and BRINK (1959) utilized a two-point backcross linkage method that prevented their detection of the proximal/distal receptor site difference adjacent to *P* reported here.

I would like to suggest that the actual movement of *Mp* occurs by means of the chromosome coiling and twisting back on itself, and the actual transport of *tr-Mp* through nuclear space doesn't actually take place. Transposition occurs when *Mp* is replicated at *P* and is not yet ligated into the replicating DNA strand. While in the "unattached condition" *Mp* comes into proximity of a later replicating state of a replicon (due to chromosome coiling) and is *"taken in"* by the replication of that section of the double helix (Figure 2).

A unique feature of Modulator transposition is its second replication at the receptor site. The first published report of this double replicative behavior (GREENBLATT and BRINK 1962) suggested that the difference in *tr-Mp* behavior at the receptor site [*Ds* positive (+) *vs. Ds* negative (−)] was due to the condition of replication where Modulator arrived. They suggested that *tr-Mp* replicated at the donor site and again, a second time, at the receptor site if the receptor site was replicating. Such events would then yield a *Ds*-positive test from the co-twin red sector. The *tr-Mp* in each of the two sectors of a given twin spot would then be at the same chromosomal location. The data of GREENBLATT and BRINK (1962) fit this expectation. To explain those twin spots, which gave a negative *Ds* test from the red co-twin, it was suggested that *tr-Mp* had moved to chromosomal receptor sites that had completed replication at the time *tr-Mp* arrived (GREENBLATT and BRINK 1963). The current results suggest that *tr-Mp* does not insert within already replicated segments and the original hypothesis to explain *Ds*-negative tested red sectors of twin spots needs to be modified. A recent finding by YAGIL *et al.* (1980) offers a new perspective to this problem of Modulator behavior in *Ds* tests. This research group discovered that a transposon in *E. coli* can become "quiet" in a number of cases. By quiet, they mean the standard phenotypes used to detect the element do not detect its presence, but subsequent analysis revealed the presence of the element. They have evidence that it is quiet due to a reversal in the direction of linear incorporation. With such an insight from DNA base sequence analysis in *E. coli*, it is very possible that the *tr-Mp*-negative cases may be reversed insertions, in which case a *tr-Mp*

may be present but quiet to the *Ds* detection system employed for their disclosure. Such a hypothesis for *tr-Mp*-negative types is testable and is being pursued.

It appears that, in both the prokaryotic and eukaryotic chromosome systems, the transposition event is governed most likely by the chromosome replication pattern. The current work suggests that the location of the *tr-Mp* element and the number of replications, one at the donor site and one at the receptor site, appear to be governed by the native pattern of chromosome replication. Transposition and replication are intimately involved. In the case of the *Mu* lytic cycle in *E. coli*, transposition appears to occur within the context of chromosome replication, but in this situation, due to the high number of copies, there are approximately 100 replicated *Mu* elements available for insertion throughout a similar number of chromosomes (CHACONAS, HARSHAY and BUKHARI 1981).

Models of transposable element movement, as viewed with the prokaryotic perspective, currently abound (see for example: FAELLEN, TOUSSAINT and DE LA FONTAYNE 1975; GRINDLEY and SHERRATT 1978; SHAPIRO 1979; CHACONAS, HARSHEY and BUKHARI 1981; HARSHEY and BUKHARI 1981). What their relationship is to a eukaryotic chromosome is not as yet clear, but the current work, using solely genetic analysis, points to a crucial role of chromosome replication in the transposition process.

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