Controlling Elements and the Gene

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In a recent brief review (McClintock, 1956), a description was given of types of elements carried in the maize chromosomes that serve to control gene action and to induce, at the site of the gene, heritable modifications affecting this action. These elements were initially discovered because they do not remain at one position in the chromosome complement. They can appear at new locations and disappear from previously determined locations. The presence of one such element at or near the locus of a known gene may affect the action of this gene. In so doing, it need not alter the action potentials of the genic substances at the locus. Therefore, these elements were called controlling elements. It was also shown that controlling elements fall into groups, the members of each operating as an integrated system in the control of gene action.

In this report, some aspects of controlling elements will be considered that could not be discussed in the above-mentioned review. This will necessitate mention of some of the well known gene loci in maize, and symbols for them will be used in the discussion. So that a ready reference may be available, the pertinent information about each of these loci is given in the following list.

Chromosome 1: *P*, pericarp and cob color. A large number of alleles are known, including P^{vv}, which gives variegated pericarp and cob color.

Chromosome 3: A_1, anthocyanin pigment produced in kernel and plant; a_1, standard recessive allele, no anthocyanin in kernel or plant.

Sh_2, normal development of endosperm tissues; sh_2, recessive allele, shrunken endosperm. Very closely linked with A_1; less than a quarter of a crossover unit distal to it.

Chromosome 5: *Pr*, purple aleurone color; *pr*, recessive allele, red aleurone color. Located in long arm of chromosome 5.

A_2, anthocyanin pigment developed in kernel and plant; a_2, standard recessive allele, no anthocyanin developed in kernel or plant. Located in short arm of chromosome 5. Gives approximately 28 per cent recombination with *Pr*.

Chromosome 6: *Y*, yellow starch in endosperm; *y*, recessive allele, white starch.

Chromosome 9: *I*, dominant inhibitor of aleurone color in kernel.

C, allele of *I*, pigment produced in aleurone layer of kernel; *c*, recessive allele, no pigment in aleurone layer.

Sh_1, normal development of endosperm of kernel; sh_1, recessive allele, shrunken endosperm. Located approximately four crossover units proximal to *I*.

Bz, purple anthocyanin pigment in plant and aleurone layer of kernel; *bz*, standard recessive allele, bronze color in both plant and kernel. Located approximately two crossover units proximal to Sh_1.

Wx, amylose starch produced in pollen and endosperm, stains blue with solutions of I-KI; *wx*, recessive allele, starch is amylopectin, stains red-brown with I-KI solutions. Located approximately 15 crossover units proximal to *Bz*.

DISTINCTIONS BETWEEN CONTROLLING ELEMENTS AND GENE ELEMENTS

In maize, as in other organisms, a change at a particular locus in a chromosome is made evident by modification of a particular phenotype. Independently occurring alterations (mutations) at the same locus give rise to change in expression of this one particular phenotype, be it recognized by change in a particular enzymatic reaction or by means of some less well defined but identifiable modification of phenotypic expression. Because it is possible to predict which phenotypic character will be altered after modification at a particular known locus in a chromosome, it is inferred that some component is present there whose mode of action may be recognized within certain limits. These components appear to reside at fixed positions in the standard chromosome complement of maize, and they will be referred to in this discussion as the genes. Modification of action of a known gene component can result from insertion of a controlling element at or near the locus of the gene; and, in general, the types of change in phenotypic expression induced by its presence there are those that could be anticipated from previously acquired knowledge of mutant expressions that have resulted from modifications at this locus. Controlling elements, on the other hand, need not occupy fixed positions in the chromosome complement, and detection of the presence of one such element depends upon characteristics it exhibits that are independent of its position.

It is realized that our present knowledge of the gene does not allow formulation of a definition

of it based on structural organization, dimension, or primary type of activity. However, all the so-called gene loci with which we will be concerned are recognized because a change at the locus effects a change of some component that normally appears in the cytoplasmic region of the cell and therefore the alteration at the gene locus is reflected in this region. All the controlling elements so far identified, on the other hand, may have their area of activity confined within the nucleus itself, for they are known to serve as modifiers, suppressors, or inhibitors of gene action as well as mutators. They behave as if they were modulators of the genome. Each controlling element or system of interacting elements has its own mode of modulation, and this is expressed in an individualistic manner that is quite independent of the recognized type of action of the gene which it may be modulating. Our present knowledge would suggest that gene elements and controlling elements represent two different classes of primary components of the chromosome and that a close relationship exists between them.

The largest amount of evidence concerning the mode of behavior of controlling elements has been derived from study of the element called Activator (*Ac*), which by itself may control gene action at the locus where it resides, and also of another controlling element that responds to it. When this latter element is inserted at a known gene locus, changes in gene action may occur either immediately after its insertion or subsequent to it. Both the insertion of this element at the gene locus and the subsequent modifications in gene action it induces depend on the presence of the *Ac* element somewhere in the chromosome complement. In the absence of *Ac* no changes affecting gene action occur, and stability of gene expression will be exhibited as long as it is absent. Return of *Ac* to the nucleus through appropriate crosses will again effect activation of the second element of the system, and this will be expressed in a series of mutation-type changes at the locus of the gene where this element resides.

The second element of the system outlined above was originally given the designation Dissociation (*Ds*) because, in the presence of *Ac*, breaks appeared to be formed at the locus where it resided. It was later determined that the apparent "breaks" were produced because at this locus a dicentric chromatid and a corresponding acentric chromatid were formed. The acentric chromatid, composed of the segment of the chromosome from *Ds* to the end of the arm, was eliminated from the nucleus during a mitotic anaphase whenever such an event occurred. These dicentric-acentric chromatids were formed both in somatic and sporogenous cells, and the time during the development of a tissue when this took place was found to be a function of the dose of *Ac*: the higher the dose, the later the time of these occurrences. In the absence of *Ac*, however, no dicentric-acentric chromatids were produced nor was there any evidence that would suggest the presence of this *Ds* element at the locus. Return of *Ac* again initiated these dicentric-acentric chromatid formations. It is evident, therefore, that tests of the presence or absence of *Ac* in a plant may be made by crossing it with one that is homozygous for *Ds* but does not have *Ac*. These are the so-called *Ac* tester stocks, and descriptions of their usefulness for detecting the presence of *Ac* have been given elsewhere (McClintock, 1951, 1953; Barclay and Brink, 1954). If these *Ac* tester stocks carry recessive alleles of other known genetic markers, and if the plant carrying *Ac* is heterozygous for them, it is possible to determine the location of *Ac* and changes in location that may occur. By this means, various different positions of *Ac* have been detected. Evidence of its transposition from one known location to another has also been obtained.

In the presence of *Ac*, the *Ds* element undergoes transposition, and its insertion at various locations were detected. Sometimes *Ds* was inserted at or close to a known gene locus, and the effects of its presence on gene action were thereby discovered. In some cases both dicentric chromatid formations and changes in gene action were noted to occur at this gene locus, but only when *Ac* was also present in the complement; and the time of the occurrences in the development of a tissue reflected the dose of the *Ac* element that was present in the nucleus. In examining these cases, it was soon learned that the *Ds* element itself could undergo modifications that altered its mode of response to *Ac*. Some of them resulted in a reduced frequency of occurrence of dicentric chromatid formations, often correlated with an increased frequency of occurrence of change in gene action; and these latter were unaccompanied by gross change in chromosome morphology. Still other modifications of this *Ds* element resulted in almost complete elimination of dicentric chromatid formations, although mutations continued to occur at the locus of the gene where the *Ds* element resided. Thus, in such cases, the designation of "Dissociation" (*Ds*) for the element responsible for these mutations no longer appeared to be applicable. Nevertheless, this original designation has been retained because in the early studies it was possible to follow sequentially the changes in the *Ds* element that altered its type of response to *Ac*: from a high rate of dicentric chromatid formation to a low rate—and also the reverse, from a low rate to a high rate. Therefore, the designation "*Ds*" will be applied to any element at a known gene locus that responds to *Ac* in the following manner: in the absence of *Ac*, it undergoes no alterations that affect gene action; but in the presence of *Ac* such alterations occur, and the time of their occurrence during the development of a tissue, and cells of

the tissue in which they occur, are a function of *Ac*, particularly of its dose. The designation "*Ds*" for an element responding to *Ac* should not be construed to mean that this element will produce dicentric chromatids wherever it may be located. It may produce none. Nevertheless, its responses to *Ac*, as described above, are readily detected. It is also probable that there are different kinds of "*Ds*" elements, but all of them respond to *Ac* in this quite predictable manner.

It is clear that both the *Ac* and the *Ds* elements retain their characteristic modes of expression when located at various different positions in the chromosome complement, and that methods of detecting their presence in these different locations have been developed. The study of *Ac* and of the integrated *Ds-Ac* two-element system has been of considerable help in investigating other unrelated control systems. This applies particularly to the well known *Dt* (Dotted)-a_1 system originally discovered by Rhoades (1936, 1938, 1941, 1945). The standard recessive, a_1, is very stable in the absence of *Dt*. In its presence, however, mutations occur to the higher alleles of A_1 or to stable recessives that no longer mutate in the presence of *Dt*. The pattern of response to *Dt* is quite predictable. Dots of deep anthocyanin pigment appear in a nonpigmented background in the kernel, and streaks of anthocyanin appear in a nonpigmented background in the plant. The number of dots (mutations) that appear in the kernel is an expression of the dose of *Dt*: the higher the dose, the more frequent the mutations. The *Dt* element was located by Rhoades in the short arm of chromosome 9. Subsequently, Nuffer (1955) discovered the presence of *Dt* in two South American strains of maize. In one strain, it was located in chromosome 6 and in the other strain it was located in chromosome 7. The response of the standard a_1 allele to each of these newly detected *Dt* elements is the same as that expressed when the original *Dt* element, discovered by Rhoades, is present. Recently, Nuffer (personal communication) obtained evidence of transposition of the *Dt* element located in chromosome 7 to a new location in the chromosome complement. Thus, in this respect, also, *Dt* resembles *Ac*; both may undergo transposition without loss of identity.

The response of the standard a_1 allele to *Dt* is similar in many essential respects to that of *Ds* to *Ac* when the former is present at the locus of a known gene. It could be inferred, then, that a controlling element, responding to *Dt*, is also present at the standard a_1 locus and that it is this element which is responsible for the observed mutations and for their pattern of appearance in the plant and kernel tissues. Evidence of this is now available from studies aimed at analyzing the composition of the A_1 locus made by Laughnan (1952, 1955) and also by Nuffer (personal communication). When plants carrying an A_1 allele in one chromosome 3 and the standard a_1 allele in the homologue are crossed by plants homozygous for the latter recessive, some kernels exhibiting a mutant phenotype appear on the resulting ear. These express a lower level of intensity of pigmentation than that given by the A_1 allele. It has been shown that the majority of these mutants arise as a consequence of crossing over within a compound A_1 locus. When plants grown from these pale-colored kernels were crossed by plants carrying *Dt*, it was discovered that in about 5 to 10 per cent of them the mutant expression was unstable. Mutations to higher alleles of A_1 occurred, and the pattern of response (dots of deep pigmentation) was the same as that given by the standard a_1 allele. In these cases, however, the mutations were registered in a pale-colored background rather than in a colorless one. Plants that were homozygous for the A_1 allele also gave rise to pale mutants. It could be shown that many of these arose from crossing over within the compound A_1 locus. None of them, however, were unstable in the presence of *Dt*. From this it might be inferred that an element responding to *Dt* is not present at this A_1 locus. Because the pale mutants derived from crossing over in the heterozygote with a_1 do carry this element, it may be inferred that those crossovers that occur within a restricted region of the compound locus will introduce it. In the presence of *Dt*, it will respond in this new organization of the locus in the very same manner that it was responding before the crossover occurred.

On the self-pollinated ear of a plant that was A_1/a_1, *Dt*/*Dt* in constitution, a single kernel appeared that showed a striking change in pattern of mutation (Nuffer, 1951). A very large number of pigmented dots appeared in a colorless background, and the intensity of pigmentation in these dots varied from light to very dark. A plant was grown from this kernel, and an investigation of the nature of the change responsible for this altered mutation pattern was commenced. It proved to be a new allele of a_1 that responds to the presence of *Dt* by giving this strikingly different pattern of mutation: a high frequency of occurrence of mutation in plant and kernel in the presence of *Dt*, some of which gives rise to large sectors of mutant tissue and others to small areas. In the absence of *Dt*, however, no mutations occur and the mutant behaves as a stable recessive. By appropriate tests Nuffer (personal communication) was able to learn that the controlling element responsible for the dotted pattern of mutation and that responsible for this new pattern occupy different sites in the compound locus and that they can be separated by crossing over. It is now evident that the basic mechanism of control of gene action in the *Dt*-a_1 system is essentially the same as that in the *Ds-Ac* system. Interactions between the members of these two systems do not occur, however, for

Dt will not substitute for *Ac* in control of *Ds* (Nuffer, unpublished) nor will *Ac* substitute for *Dt* in control of gene action at a_1 (McClintock, 1953).

Recognition of other types of controlling elements is made possible by procedures that are essentially similar to those outlined above. This applies to the Suppressor-mutator system associated with control of gene action at a modified A_1 locus (a_1^{m-1}, McClintock, 1955, 1956), to a similar system operating to control gene action at A_2 (McClintock, unpublished), to several systems investigated by Dollinger (1955 and unpub.), and to still other systems now under investigation in several different laboratories. The presence of controlling elements in the maize chromosome complement is now well established. It is for the future to determine the extent to which such elements, and systems of interacting elements, operate in the over-all control of activity of the genome during development. The ever-increasing recognition of these elements and their modes of action suggest that they may perform a major function in this respect, and an understanding of the manner by which it is accomplished may be gained from examination of the modes of behavior of particular elements or systems of interrelated elements that are now known.

Detection of Transpositions of Controlling Elements

As stated earlier, the presence of elements, independent of the genes but controlling their action, was initially detected because they undergo transposition from one location to another within the chromosome complement, and the effects they produce when inserted at known gene loci are thereby made evident. Therefore, a description of some of the methods that have been used to detect such transpositions is of considerable importance for an appreciation of the nature of the behavior of controlling elements. A number of different methods have been used and they fall into two general categories, selective and nonselective. The nonselective method is more laborious than the selective method but its use may be required in the initial study of a controlling element. For example, in a cross of a plant having a single *Ac* element, whose location is unknown, to plants having no *Ac*, half the progeny can be expected to carry an *Ac* element as a result of meiotic segregations in the parent plant. If the individuals in this progeny are tested for the presence of *Ac* by the method described earlier, the expected ratio of presence and absence of *Ac* will usually be found. However, an occasional plant may be present in the progeny that has two *Ac* elements instead of the expected one. Again, if genetic markers have been introduced into the cross, and if the *Ac* element has shown no linkage with them in the parent plant, the progeny may include an occasional plant which has one *Ac*, as expected, but in which the *Ac* element now shows linkage with one of the markers (for an example, see Table 1, McClintock, 1951). If the progeny of this plant, in turn, is tested for the location of the *Ac* element, the majority of *Ac*-carrying individuals will show the same linkages of *Ac* with the given markers as exhibited by the parent plant. An individual may be found, however, in which this linkage is not expressed, and no linkage with the given markers will be exhibited in its progeny. In this manner, successive changes in location of the *Ac* element may be detected, but the method is quite unselective and it requires tests of a number of individuals in successive generations. Once the location of a controlling element has been determined, however, selective methods of detecting subsequent changes in its location may be applied, and these will be considered shortly.

A number of different selective methods of detecting transposition of controlling elements have been applied, and some of them take advantage of the particular mode of action of the controlling element under investigation. For example, the dose effects produced by the *Ac* element may be useful in this respect, for it is known that the higher the dose the later the time of occurrence of modifications affecting *Ac* itself or affecting *Ds* or *Ds*-type elements wherever the latter may be located. Changes in location of *Ac* usually occur rather late in the development of the somatic and sporogenous tissues, but occasionally they occur in a cell early in plant development and this can result in the appearance of sectors in which the *Ac* constitutions differ. If such a sector enters the ear, the altered *Ac* constitution in its cells is made apparent by the distinctive phenotypes of the kernels that develop within it after pollen from an *Ac* tester stock has been placed on the silks of the ear. They indicate either that *Ac* is absent in the cells that formed the sector or that it is increased in number. By selecting kernels from such a sector and by making test crosses with the plants derived from them, it is possible to verify the change in *Ac* constitution that has occurred in a somatic cell of the parent plant. Some sectors are twinned, in that the *Ac* element appears to be absent from one sector and increased in number in the twin; and verification of this is readily obtained from examination of the plants derived from the kernels in each component of the twinned sector.

The dose effects produced by *Ac* have also allowed detection of some of the changes in *Ac* location that occur late in the development of sporogenous cells and consequently are exhibited only in individual kernels on an ear. Their usefulness in this respect was described earlier (McClintock, 1951) in connection with tests devised to detect such changes in location of *Ac* that occur in plants carrying this element at allelic positions in a pair of homologous chromosomes. They also

allow detection of gametes carrying two *Ac* elements, produced by plants having only one. When such a plant is used in a test cross, the functioning of a gamete having two *Ac* elements gives rise to a kernel that exhibits a much delayed time of occurrence of the dicentric-acentric formations at *Ds* in chromosome 9 or of mutation at those gene loci where the *Ds-Ac* system is operating. Verification of the presence of more than one *Ac* element in these kernels may be obtained by testing the *Ac* constitution in the plants derived from them, provided, of course, that the change in *Ac* constitution occurred in a cell of the parent plant before gamete formation and thus provided for an endosperm and embryo that were alike in *Ac* constitution.

During studies of transposition of *Ds* and also of mutation occurring at those gene loci in which the *Ds-Ac* system of control of this operates, it has been noted that change in location of *Ac* often accompanies the event that affects the *Ds* element of the system. Such coincidences are so numerous that selection of individual kernels exhibiting modification of the *Ds* element is also useful as a method of selection for changes in location of *Ac*.

One of the most effective methods of selection for transposition of *Ac* utilizes those cases in which the *Ac* element resides at the locus of the gene whose action it is directly controlling: that is, the case of *Ac* (called Modulator and symbolized as *Mp*) at the *P* locus in chromosome 1, described by Brink and his collaborators (Brink and Nilan, 1952; Brink, 1954; Barclay and Brink, 1955; Fradkin and Brink, 1956), and that of *Ac* at the bronze locus in chromosome 9 (McClintock, 1956). Mutations at these loci are associated with events occurring to and instigated by the *Ac* element itself, and many of them are accompanied by removal of *Ac* from the affected gene locus and its insertion elsewhere. Therefore, if those kernels on an ear that exhibit germinal mutations are selected, and the plants grown from them are tested for *Ac*, it will be found that in a number of these plants *Ac* no longer resides at the mutant locus. However, *Ac* may still be present in the chromosome complement but located elsewhere. Tests of this type were made by Brink and his collaborators with regard to the *P* locus and by me with regard to the bronze locus. As an example of the type of result obtained from such tests, those conducted by me with the bronze locus will be given here.

The phenotypic expression produced when *Ac* is present at the bronze locus resembles that given by the standard recessive, *bz*. This latter recessive is completely stable in the presence of *Ac*, but mutations occur at the bronze locus where *Ac* resides. Some of these give the dominant *Bz* expression, and the majority of such mutants are thereafter stable in the presence of *Ac*. A larger fraction of the mutants express the recessive, *bz*, and this expression is thereafter stable in the presence of *Ac*. Some mutations, however, give rise to unstable dominants or to other types of change that will be considered later. Here we will consider only those changes that give rise to mutants that are stable in the presence of *Ac*. Kernels exhibiting mutant phenotypes were selected from ears produced by plants carrying only one *Ac*, and it was present at the unstable bronze locus in one chromosome 9. The homologous chromosome 9 in these plants carried the standard, stable recessive, *bz*. The short arm of each chromosome 9 carried other distinguishing genetic markers, to make it possible to ascertain that the observed mutations had occurred at the bronze locus where *Ac* resides. The ears from which the mutant-carrying kernels were selected were produced by these plants when they were crossed by plants having no *Ac* and homozygous for the recessive alleles of the selected genetic markers. On these ears, those kernels that were homozygous for the standard recessive, *bz*, were completely bronze; no *Bz* spots appeared in them. On the other hand, the majority of those kernels that received the chromosome carrying the bronze locus with *Ac* showed a number of deep purple (*Bz*) spots in a bronze (*bz*) background. A few kernels having this locus, however, showed an altered phenotypic expression and some of these, in turn, were either totally *Bz* or totally *bz*. Because of the constitution of these kernels with respect to the other genetic markers carried in chromosome 9, it was possible to refer the changed expression in each case to an event that had occurred, in a cell of the heterozygous parent plant, at the bronze locus where *Ac* resides. Some of these kernels were selected from the ears and plants were grown from them. These plants, in turn, were tested for presence or absence of *Ac*, for location of *Ac* if present, and for stability of the mutant expression. Among 16 *Bz* mutants selected, 14 proved to be stable. In six of the plants derived from these 14 *Bz* kernels, no *Ac* was present. In five plants, one *Ac* was present, but its position was altered. In four of these five plants, it was no longer linked with genetic markers carried in the short arm of chromosome 9, and in the fifth plant it was very closely linked with *Wx*. In the remaining three plants, *Ac* was present and its position was close to the locus of *Bz* but probably a short distance to the right of it. Thus, in at least 11 of these 14 cases of mutation to stable *Bz*, the association of mutation with removal of *Ac* from the bronze locus could be established with certainty, and in five of these cases the event could be related to transposition of *Ac* to a new location in the chromosome complement. It is probable that transposition of *Ac* to a new location was also associated with the mutation-inducing event in the six cases where *Ac* was absent in the plant derived from a *Bz* kernel. If this event occurred in a sporogenous cell before meiosis, segregation of the chromosome

carrying the *Ac* element in its new location at the following meiotic divisions could have resulted in the production of a gamete in which the *Bz* mutant was present but *Ac* was absent.

Twenty-four independent cases of mutation to a stable recessive were also examined. In nine of them, no *Ac* was present in the gamete that carried the stable recessive. In five cases, one *Ac* was present but it showed no linkage with markers in the short arm of chromosome 9. In nine cases, one *Ac* was present and showed linkage with these markers. In two of these nine cases, *Ac* was located close to *Wx*, and in one case it was located very close to *Sh*. In the remaining six of these nine cases, its exact location was not determined; it was linked with *Wx* and showed from 20 to 30 per cent recombination with it. In the remaining case of the 24 examined, two *Ac* elements were present, one located close to *bz*, and the other showing no linkage with markers in the short arm of chromosome 9. Thus, again, in this test, mutation could be associated with a change occurring to the *Ac* element at the bronze locus; for its removal from this locus was established with certainty in 17 of the 24 cases, and in eight of them the transposition of *Ac* to a new location could be determined.

It is obvious from the accounts given above that by selection of mutants one can also select for transpositions of *Ac*, and that this method is highly efficient. Somewhat similar results were obtained in tests of *Ds* in the case of c^{m-1} (*Ds* at the *C* locus). The majority of mutations to *C* were found to be associated with removal of *Ds* from the *C* locus, although its insertion at new locations could not be detected in most of the cases. Appropriate genetic markers that would have allowed detection of such insertion were not present in the tested plants.

Another useful selective method for detecting transpositions of controlling elements takes advantage of crossover techniques. For instance, a case was found in which *Ac* was inserted close to but to the left of *Wx*. Various tests were conducted to determine its location by means of crossover techniques. Recombinants appeared in about 10 per cent of the gametes of the plants tested; but this figure did not represent the true crossover value, as the example below will illustrate. In one test, the plants having *Ac* had the constitution *C Ac Wx* in one chromosome 9 and *C wx* and no *Ac* in the homologue; no other *Ac* element was present in these plants. When they were crossed with plants homozygous for *I*, *wx*, and *Ds* (located just to right of *wx*), and having no *Ac*, the majority of the *Wx* class of kernels on the resulting ear showed variegation for *C* areas in a colorless background. These kernels carried *Ac*. The *C* areas were produced by responses of *Ds* in the *I wx*-carrying chromosome to *Ac*, which resulted in dicentric-acentric formations at the locus of *Ds*. *I*, carried in the acentric fragment, was eliminated from daughter nuclei in the mitotic division which followed this event. The majority of the *wx* class of kernels, on the other hand, were totally colorless, since no dicentric-acentric chromatid formations occurred at *Ds* because no *Ac* was present in the nuclei of the endosperm. However, a few of the *Wx* kernels were nonvariegated (no *Ac*) and a few of the *wx* kernels showed *C* areas (*Ac* present). These last two classes of kernels represented the recombinants. When the plants grown from the variegated kernels in the *wx* class were tested for *Ac* location, its position, in about half of them, was no longer just to the left of *wx*, as might be expected from a crossover event. Instead it occupied a new location in the chromosome complement. In the other half of these plants, its location was that expected from crossing over. In other words, in this test, about half of the "recombinants" arose not from crossing over but rather from a transposition of *Ac*; and this probably occurred before the meiotic divisions and as a consequence allowed the *Ac* in the new location to be segregated at meiosis with a *wx*-carrying chromosome. Other examples illustrating this type of selective method will be considered in the following discussion of modes of detection of transpositions of the controlling element *Spm* (Suppressor-mutator) in the *Spm*-a_1^{m-1} system of gene control.

A series of studies has been made of transposition of the *Spm* element in the *Spm*-a_1^{m-1} system of control of gene action at the A_1 locus in chromosome 3, and it indicates that transposition of this element occurs with a high frequency. In this respect, it resembles the *Ds* and *Ac* elements, which also may undergo frequent transposition. The action of the *Spm*-a_1^{m-1} system was outlined in previous reports (McClintock, 1955, 1956), but its origin and mode of operation will be reviewed here. A modification at the standard A_1 locus in a sporogenous cell of a plant in a maize culture under investigation resulted in change in action of the genic materials at that locus. The change was discovered in a single kernel on an ear produced by the plant when it had been crossed by a plant homozygous for the standard recessive, a_1. Instead of being fully colored, as expected, this kernel was variegated for colored areas in a colorless background. The plant arising from this kernel also exhibited variegation for anthocyanin pigmentation. Subsequent tests indicated the mode of control of gene action at this modified A_1 locus. The basic mechanism involves two controlling elements: one resides at the modified A_1 locus (designated a_1^{m-1}) and directly controls gene action and the types of change in this action that may subsequently occur; and the other is an independently located element, designated Suppressor-mutator (*Spm*), which can modify this action in two distinctly different ways. When the *Spm* element is present, no anthocyanin pigment is formed. All action of the genic materials

at the a_1^{m-1} locus is inhibited by its presence until, in some cells, a mutation occurs at a_1^{m-1} that allows the genic materials to be active, the type of activity being a reflection of the type of modification produced by the controlling element at a_1^{m-1}. The expression of the gene induced by this modification is thereafter stable in the presence of *Spm* and a stable mutation is thereby effected. In the absence of *Spm*, on the other hand, the genic materials at the a_1^{m-1} locus are capable of some degree of activity and the kernels and plants are uniformly pigmented. This type of gene action at a_1^{m-1} is quite stable in the absence of *Spm*, and will be expressed without change in successive plant generations. When, however, *Spm* is again introduced into the endosperm and zygote nuclei having a_1^{m-1}, suppression of gene action is again made evident and the capacity of the *Spm* element to initiate stable mutation-type changes at a_1^{m-1} is also made evident. Unlike *Ac*, *Spm* does not show dosage effects, and the number of *Spm* elements present in a plant is indicated only by progeny tests. At least three and probably more independently located *Spm* elements were present in the initial plant having a_1^{m-1}, and because of this the majority of gametes produced by this plant, and by many of its progeny plants, carried *Spm*. Its detection was therefore obscured in the initial tests of a_1^{m-1}. Only after several generations of crosses to tester plants having no *Spm* was it possible to recognize this element, for individuals were then isolated that had only one or two *Spm* elements, and the part this element plays in the control of gene action and mutation at a_1^{m-1} was then clearly revealed. Clarification of the behavior of this system of control of gene action was thereafter readily accomplished.

When plants homozygous for a_1^{m-1} and having one *Spm* element are crossed by plants having no *Spm* but homozygous for the standard recessive, a_1, which is stable in its presence, the ratio of kernel types on the ears produced will usually approximate one uniformly colored (no *Spm*) to one variegated for deep colored spots in a colorless background (*Spm* present). If two independently located *Spm* elements are present, the ratio of kernel types approximates one uniformly colored (no *Spm*) to three variegated (*Spm* present); and if more *Spm* elements are present, the ratio of kernel types deviates in the expected manner. Again, tests of *Spm* constitutions may be made with plants that are homozygous for the standard a_1 allele (and therefore do not directly reveal the presence or absence of *Spm*) if these plants are crossed by ones homozygous for a_1^{m-1} but carrying no *Spm*. The ratios of uniformly colored to variegated kernels on the resulting ears reflect the *Spm* constitutions in the plants. Thus, plants homozygous for a_1^{m-1} and carrying no *Spm* can serve as tester parents in crosses made for the purpose of determining the presence or absence of *Spm* in individual plants and its numbers when present. If the plants being tested are heterozygous for other genetic markers, and if the tester stock carries the recessive alleles, linkage of an *Spm* element with one such marker may be detected after it has been inserted into the chromosome that carries this marker. By this means it was possible to detect linkages of *Spm* with genetic markers carried in chromosome 5, in chromosome 6. and in chromosome 9.

In plants that are a_1^{m-1}/a_1^{m-1} or a_1^{m-1}/a_1 in constitution and that have a single *Spm* element, large sectors may appear, some of which exhibit the pigmented phenotype that is characteristically produced in the absence of *Spm*. It can be shown that these sectors arise through loss of the *Spm* element from a cell early in development of the plant. The progeny of such a cell may contribute to the development of the ear, producing either all of it or only a part of it. When the ear is used in a cross with a plant that is homozygous for a_1^{m-1} but carries no *Spm*, in the former case all the kernels on the ear will be uniformly colored (no *Spm*), and in the latter case all the kernels within a well-defined sector will be uniformly colored (no *Spm*). If the plants derived from these uniformly colored kernels are again tested for *Spm* constitution, its absence in them may be verified. That they carry an a_1^{m-1} locus capable of responding to *Spm* may be shown by crossing them to plants that are homozygous for the standard a_1 allele and have one or more *Spm* elements. The typical variegated pattern of deeply pigmented areas in a nonpigmented background will appear in all kernels that have received a_1^{m-1} from one parent and *Spm* from the other.

In order to detect some of the changes in *Spm* constitution that may occur early in development of a plant, tests were conducted to determine its constitution in the cells that gave rise to an ear on the main stalk of the plant and also in those that produced an ear on one or more of its tillers (side branches). Tests of two ears per plant were obtained from 101 plants. In 95 of them, the number of *Spm* elements was the same in the cells that produced each ear (63 with one *Spm*; 26 with two *Spm*; 6 with three *Spm*). In six plants, the *Spm* constitution was not the same in the cells that gave rise to each ear (one case of one *Spm* in one ear and no *Spm* in the other; three cases of one *Spm* in one ear and two *Spm* in the second; two cases of one *Spm* in one ear, the second ear having a sector with no *Spm*). From twelve other plants, tests of three ears per plant were obtained; and correspondence in number of *Spm* elements was evident in each of the three ears of eleven of them (6 with one *Spm*; 4 with two *Spm*; 1 with three *Spm*). In one plant, the cells that gave rise to two ears carried one *Spm* element but the cells that gave rise to the third ear had two *Spm*.

Tests were also conducted to determine *Spm* constitutions in progeny of plants in which one, two, or three *Spm* elements were known to be

present. Those conducted with 249 individuals in the progeny of plants having one *Spm* will illustrate the type of result obtained. The parent plants carrying *Spm* had been crossed by plants that were homozygous for a_1^{m-1} but had no *Spm*. A ratio on the resulting ears of one uniformly pale-colored kernel (no *Spm* present) to one that showed spots of deep color in a colorless background (*Spm* present) indicated the presence of one *Spm* element in the cells that gave rise to the ears. Variegated kernels were selected from these ears, and the plants grown from them were again crossed by plants homozygous for a_1^{m-1} but having no *Spm*. From this test it was learned that one *Spm* element was present in the cells that gave rise to the ear in 215 of these plants. In 20 plants two *Spm* elements were present, and in six plants three *Spm* elements were present. In the remaining eight plants, no *Spm* was present in any part of the plant. It appears from these tests that many of the modifications affecting *Spm* constitution occur in individual cells relatively late in development and may occur even in the gametophytic cells of the plants.

In order to examine more precisely these changes in *Spm* constitution, tests were made of the progeny produced by plants having one *Spm* element at a known location in the chromosome complement. *Spm* may be located at various positions, and several different positions in chromosome 5, chromosome 6, and chromosome 9 have been identified. Tests of the progeny of plants having *Spm* at these different locations were conducted. Several examples will illustrate the kinds of information such tests can give.

One plant that carried a single *Spm* element was *Y/y* in constitution. The test cross made with it indicated that this *Spm* element was linked with *y*. On the ear produced by the test cross, one of the two recombinant classes of kernels was *Y* and exhibited the variegated phenotype (*Spm* present). Plants were grown from some of the kernels in this recombinant class, and these, in turn, were tested for *Spm* constitution and location. The results obtained from one such plant are given in Table 1. This plant, a_1^{m-1} Sh_2/a_1sh_2, *Y/y* in constitution, was used as a female parent in a cross with a plant whose constitution was a_1^{m-1} sh_2/a_1sh_2, *y/y* and which had no *Spm* element. Linkage of *Spm* with *Y* was clearly expressed in those kernels carrying a_1^{m-1} in both the Sh_2 and the sh_2 classes. As expected, kernels homozygous for the standard a_1 allele were completely colorless, for this allele is stable in the presence of *Spm*. Plants were grown from 22 of the variegated kernels in the Sh_2 *Y* class, and these in turn were crossed by plants homozygous for a_1^{m-1}, Sh_2, and *y*, and having no *Spm*. This test cross was made in order to determine whether or not *Spm* would continue to show linkage with *Y* and, if so, the number of individuals that would show it. In 19 of these 22 plants, one *Spm* element was found to be present, and it was linked with *Y* in each of them. The percentages of recombinant classes of kernels were similar on the ears produced by all of these plants. Among a total of 7569 kernels produced by these ears, there were 3847 uniformly pale-colored kernels (no *Spm*), of which 683 were *Y* and 3164 *y*. Among the 3717 variegated kernels (*Spm* present) on these ears, 3033 were *Y* and 684 were *y*. In addition, there were five deeply colored kernels, which may be attributed to germinal mutation at a_1^{m-1}, for a few such mutations are to be expected. The recombinants were 18.0 per cent of the total, and this percentage compares well with that of the parent ear (see Table 1) which was 16.3. In two of the 22 tested plants, two *Spm* elements were present and one of them was linked with *Y* (39 pale colored, *Y*: 118 pale colored, *y*: 315 variegated, *Y*: 181 variegated, *y*). The remaining plant had one *Spm*, but it showed no linkage with *Y* (117 pale colored, *Y*: 112 pale colored, *y*: 101 variegated, *Y*: 100 variegated, *y*; and in addition 2 deeply colored kernels attributable to germinal mutation).

TABLE 1. TEST CROSS INDICATING LINKAGE OF *Spm* WITH *Y* IN CHROMOSOME 6

♀ $a_1^{m-1}Sh_2/a_1sh_2$; *Y Spm/y* × ♂ $a_1^{m-1}sh_2/a_1sh_2$; *y/y*; No *Spm*

	Phenotypes of Kernels						Totals
	Pale aleurone (No *Spm*)		Colorless aleurone with spots of deep color (*Spm*)		Colorless aleurone		
	Y	*y*	*Y*	*y*	*Y*	*y*	
Sh_2 class	20	113	111	30	0	0	274
sh_2 class	10	65	59	8	77	72	291
Totals	30	178	170	38	77	72	565

A much more extended series of tests of the type just outlined was conducted with progenies of plants carrying *Spm* in chromosome 6 but at another location in the chromosome, and these tests were extended into the fourth generation. A description would require more space than is justified here; it need only be stated that, in general, the results obtained were similar to those described above. In the interest of further clarification of the behavior of *Spm*, however, we should present an example of this kind of test made with plants carrying the *Spm* element in a different chromosome of the complement. In the following case, it was located in chromosome 5.

The silks of one ear of a plant that was a_1^{m-1} Sh_2/a_1sh_2, *Pr/pr* in constitution and carried *Spm*, received pollen from a plant that was homozygous for a_1, sh_2, and *pr* and had no *Spm* (cross 1). Another ear of this plant was used in a cross with a plant of similar constitution except that it was

homozygous for *Pr* (cross 2). The kernel types appearing on the ear produced by cross 1 indicated linkage of *Spm* with *Pr*. Plants were then grown from some of the variegated kernels in the *Sh*$_2$, *Pr* class on each of these ears, and these plants, in turn, were tested for *Spm* constitution. Examples of the results of tests of several of these plants are given in Table 2 (plant 6684D-1 and some of its progeny from cross 1, plant 6685F-2 and some of its progeny from cross 2, and also progeny of plant 6685G-2 from cross 2). Plant 6684D-1 proved to be a_1^{m-1} *Sh*$_2$/a_1*sh*$_2$, *Pr*/*pr* in constitution, and it had one *Spm* that was located in the chromosome 5 carrying *Pr*. Its linkage with *Pr* was evident in all the test crosses (rows 1 to 3 under A of Table 2). Plant 6685F-3 was also *Pr*/*pr* in constitution, but the *Spm* element was linked with *pr* (B, Table 2). The location of *Spm* in the *pr*-carrying chromosome may be attributed to a crossover in the parent plant carrying *Spm*, which introduced it into a *pr*-carrying chromosome. Most of the variegated plants in culture 6685 (derived from cross 2) were *Pr*/*Pr* in constitution. The *Spm* element in most of them could be expected to be located in one of the two *Pr*-carrying chromosomes. The results of tests of one plant of the culture, 6685G-2, which was a_1^{m-1} *Sh*$_2$/a_1*sh*$_2$, *Pr*/*Pr* in constitution, were as follows. This plant was crossed by one that was homozygous for a_1^{m-1}, *Sh*$_2$, and also for the recessive, *pr*, and had no *Spm*. The kernel types on the ear produced by this cross (132 pale-colored kernels: 137 kernels that had deep-colored spots in a colorless background) indicated that plant 6685G-2 had one *Spm*. All the kernels, however, were *Pr* in phenotype. Twenty plants were grown from the variegated kernels on this ear, and each was tested for *Spm* constitution and for the linkage of *Spm* with *Pr*. *Spm* was found to be present in 17 of the 20 plants but absent in three of them. Fifteen of these 17 plants carried a single *Spm* element; in 14 of them it was linked with *Pr* (rows 1 and 2 under C of Table 2), and in one it gave no evidence of linkage with *Pr* (row 3, C, Table 2). The remaining two of the 17 *Spm*-carrying plants had two *Spm* elements, one of which was linked with *Pr* (rows 4 and 5, C, Table 2).

The kernel types produced by the crosses shown in A of Table 2 indicated that *Spm* in plant 6684D-1 was located in the chromosome 5 that carried *Pr*. In order to determine whether or not this linkage would be expressed in the following generation, plants derived from the *Pr* class of variegated kernels produced by the crosses entered in lines 2 and 3 of A of Table 2 were examined for *Spm* constitution and location. Among the eleven tested plants in the progeny of the cross given in line 3, one did not have *Spm*. Each of the remaining ten plants had one *Spm*, and its linkage with *Pr* was clearly expressed in nine of them. The phenotypes of the kernels on the ears produced by these plants, after the given test cross, are entered in row 1 under D of Table 2. The *Spm* element in one plant did not show linkage with *Pr* (row 2, D, Table 2). In the second test, all ten of the examined plants derived from the variegated kernels in the *Pr* class on the ear produced by the cross given in line 2 of A, Table 2, carried *Spm*. In nine of them, one *Spm* was present, and in eight of these it was obviously linked with *Pr* (row 3, D, Table 2); in the ninth plant no linkage with *Pr* was observed (row 4, D, Table 2). In the remaining plant, three *Spm* elements were present, as the ratio of kernel types entered in row 5, D, Table 2 will indicate.

In plant 6685F-3, which was *Pr*/*pr* in constitution, *Spm* was found to be linked with *pr* (B, Table 2). Most probably, it was introduced into the *pr*-carrying chromosome as a consequence of crossing over. The variegated kernels in the *Pr* class on the ear produced by the cross given in B, Table 2, represent recombinants, and plants derived from ten of them were examined for *Spm* constitution and location. *Spm* was found to be present in nine of these ten plants, and absent in one of them. One *Spm* was present in eight of the nine plants, but in only four of them was it linked with *Pr*. The combined ratios of kernel types on the ears produced by these four plants are entered in row 1 of E, Table 2. In the remaining four plants having one *Spm*, no linkage with *Pr* was exhibted (rows 2 and 3, E, Table 2). In one plant, three or four *Spm* elements probably were present, as the ratio of kernel types on two tested ears of this plant indicates (row 4, E, Table 2). It was mentioned earlier, in connection with transposition of *Ac*, that selection of recombinants is an effective method of detecting transposition of this controlling element. The example given above illustrates the usefulness of this method for detecting transpositions of *Spm*. Another illustration of the effectiveness of this selection method will be outlined below.

A variegated plant that was a_1^{m-1}/a_1^{m-1}, *Wx*/*wx* in constitution was crossed by a plant homozygous for a_1^{m-1} and for *wx* but carrying no *Spm*. On the ear produced as the result of this cross, there were 365 kernels; 196 of them were uniformly pale colored (no *Spm*), and 169 had deep-colored spots in a colorless background (*Spm* present). It may be concluded that one *Spm* element was present in the plant that produced this ear. Among the pale-colored kernels, 156 were *Wx* and 40 were *wx*, whereas the ratio in the variegated class of kernels was 29 *Wx* to 140 *wx* (A, Table 3). Linkage of *Spm* with the *wx* allele carried in one chromosome 9 is obvious. Nine plants derived from the variegated kernels in the *Wx* class (the recombinant class) were examined for *Spm* and for its location. All of them carried *Spm*, and in seven of them one element was present. In none of the nine ears obtained from test crosses of these seven plants, however, was there any evidence of linkage of *Spm* with *Wx*. The com-

TABLE 2. TESTS OF *Spm* CONSTITUTION AND LOCATION IN THE PROGENY OF PLANTS CARRYING *Spm* IN CHROMOSOME 5

See text for origin of plants in A to E below, for the constitutions of plants entered in B to E, and for test crosses made with them.

Crosses		Phenotypes of Kernels						
		Pale Sh_2 (No *Spm*)		Colorless with spots of deep color; Sh_2 (*Spm* present)		Colorless sh_2	Germinal mutant; Sh_2	Totals
♀	♂	*Pr*	*pr*	*Pr*	*pr*			
A. Types of kernels on ears produced by test crosses of plant 6684D-1 which was $a_1^{m-1}Sh_2/a_1sh_2$, *Pr Spm/pr* in constitution								
6684D-1	a_1sh_2/a_1sh_2 ; *pr/pr*; No *Spm*	50	90	91	48	321	2	602
a_1sh_2/a_1sh_2 ; *pr/pr*; No *Spm*	6684D-1	120	223	214	114	622	5	1298
$a_1^{m-1}Sh_2/a_1^{m-1}Sh_2$; *pr/pr*; No *Spm*	6684D-1	80	188	114	46	—	1	429

Spm Constitution of Plants	No. of Plants	Phenotypes of Kernels					
		Pale (No *Spm*)		Colorless with spots of deep color (*Spm* present)		Germinal mutant	Totals
		Pr	*pr*	*Pr*	*pr*		
B.							
1 *Spm*	1	275	91	67	214	4	651
C.							
1 *Spm*	6	434	1646	1512	415	9	4016
1 *Spm*	8	1798 (*Pr* and *pr*)*		1429	341	2	3570
1 *Spm*	1	281 (*Pr* and *pr*)*		114	132	0	527
2 *Spm*	1	49	105	209	148	1	512
2 *Spm*	1	128 (*Pr* and *pr*)*		132	87	1	348
D.							
1 *Spm*	9	429	1174	1133	366	3	3105
1 *Spm*	1	67	73	71	77	0	288
1 *Spm*	8	1089 (*Pr* and *pr*)*		616	251	1	1957
1 *Spm*	1	234 (*Pr* and *pr*)*		98	99	1	432
3 *Spm*	1	31	36	167	176	0	410
E.							
1 *Spm*	4	149	550	510	113	1	1323
1 *Spm*	3	321	290	248	254	1	1114
1 *Spm*	1	185	171	134	152	1	643
3 or 4 *Spm*	1	15	14	235	246	2	512

* In some crosses, difficulty was encountered in discriminating between *Pr* and *pr* kernels because of segregation of another factor that modifies pigment color in *pr/pr* kernels but only, however, in the pale class.

bined ratio of kernel types on these ears is given in row 1 under B of Table 3. One plant had two *Spm* elements, but linkage with *Wx* was not indicated in either of the two tested ears of this plant (line 2, B, Table 3). The ratio of kernel types on two ears produced by the remaining plant indicated the presence in it of three *Spm* elements, as shown in row 3, B, Table 3. When such a high number of *Spm* elements is present in a plant, evidence of linkage of one of them with a given marker is obscured. Thus it cannot be stated whether or not in this plant one of the elements was linked with *Wx*. It is clear, however, that there is no evidence among these recombinants of linkage of *Spm* with *Wx*. It may be concluded that the *Spm* element in the parent plant was located very close to *wx* and that the recombinants appearing on the ear of this plant arose mainly from a premeiotic transposition of the *Spm* element to a new location, which allowed it to be segregated at meiosis with the chromosome carrying the *Wx* allele.

In Table 2, the results of tests of 47 variegated plants are entered. The variegated plant was used as female parent in test crosses of 46 of them. In eight of these 46 plants, two ears per plant were used for such tests, and agreement with regard to *Spm* number and location was shown by the two ears in seven of the eight plants. In one plant, the kernel types on the ear produced by the main stalk indicated the presence of two *Spm* elements, one of which was linked with *Pr*, and these are entered in line 5 under C of Table 2. Tests of the tiller ear of this plant indicated the presence of only one *Spm* in the cells that gave rise to it, and this *Spm* was linked with *Pr* (9 pale colored, *Pr*: 84 pale colored, *pr*: 69 variegated, *Pr*: 11 variegated, *pr*). In two additional plants, three ears were used for the test cross, and agreement with regard to *Spm* number and location was shown in all three ears produced by each plant. The nine plants that gave the kernel types entered in B of Table 3 were all used as female parents in the test cross, and in four of them two ears per plant were so tested. Agreement with regard to *Spm* number was shown by both ears produced by each of the four plants.

Before we leave the subject of methods of detection of transposition of controlling elements, one other fact should be emphasized. If, in any one test, the controlling element is found to be linked to a known marker, and if other known markers are present in the same chromosome, linkage with them will also be exhibited, and in the expected manner. If genetic markers on other chromosomes are also present, and are followed in the tests, no linkage with them will be exhibited. In other words, the basic mode of inheritance is like that of other known genetic markers, because the controlling element resides at a particular locus until some event causes it to be transposed elsewhere, where its position may again be

TABLE 3

Spm Constitution of Plants	No. of Plants	Phenotypes of Kernels: Pale (No *Spm*) *Wx*	Pale (No *Spm*) *wx*	Colorless with spots of deep color (*Spm* present) *Wx*	Colorless with spots of deep color (*Spm* present) *wx*	Germinal mutants	Totals
A. Test cross indicating linkage of *Spm* with *wx* in chromosome 9 ♀ a_1^{m-1}/a_1^{m-1}; *Wx/wx Spm* × ♂ a_1^{m-1}/a_1^{m-1}; *wx/wx*; No *Spm*							
	1	156	40	29	140	0	365
B. Tests of *Spm* constitution in 9 plants derived from variegated kernels in the *Wx* class of A, above. All plants were used as females in crosses with plants that were a_1^{m-1}/a_1^{m-1}, *wx/wx*, and that had no *Spm*							
1 *Spm*	7	860	953	801	824	1	3439
2 *Spm*	1	52	66	197	208	0	523
3 *Spm*	1	46	36	402	377	0	861

identified. The time of occurrence of these changes in location, during development of a tissue, and the frequency of their occurrence, depend on several factors: dose (the *Ac* element and the control it exerts on transpositions of *Ds*), environmental conditions such as temperature and nutrition (Eyster, 1926; Rhoades, 1941; van Schaik, 1954), the genetic background (Brink, personal communication), and the location of the element in the chromosome complement (see account of nontransposing controlling elements in McClintock, 1956).

THE STRUCTURE OF CONTROLLING ELEMENTS

Controlling elements and gene elements are alike in one important aspect, and this is related to the replication of their structural organization during chromosome reduplication. With respect to both types of elements, the newly constituted chromosome is a replica of the parent chromosome, provided no event occurs to alter the structure of the controlling elements or that of the locus where it may rseide; and in most division cycles such events do not occur. The most effective demonstration of maintenance of organization of a controlling element through successive mitotic cycles is provided by that element of a two-element system which undergoes modification only when the second element of the system is also present, such as *Ds* in the *Ds-Ac* system, or the element at the A_1 locus in the a_1-*Dt* system and the a_1^{m-1}-*Spm* system. In the absence of *Ac*, *Dt*, or *Spm*, in each of these systems the organization at the locus where the complementary controlling element resides must be replicated without altera-

TABLE 4. EXAMPLES OF TYPES OF ALLELES OF A_1 PRODUCED BY THE OPERATION OF THE *Ds-Ac* SYSTEM, THE a_1-*Dt* SYSTEM, AND THE a_1^{m-1}-*Spm* SYSTEM OF CONTROL OF GENE ACTION AT THE A_1 LOCUS IN CHROMOSOME 3

A. The *Ds-Ac* System Example: a_1^{m-3}

Phenotype produced in absence of *Ac*	Phenotype produced when 1 *Ac* is present
Allele 1 Colorless kernel. No anthocyanin pigment in plant. No mutations.	Allele 1 Dots of the full A_1-type expression in restricted regions of kernel. Few streaks of A_1 pigment in plant. Few germinal mutations to higher alleles of A_1 . Some chromosome "breaks" at a_1^{m-3} locus.
Allele 2 Uniformly light pale-colored kernel and lightly pigmented plant. No mutations.	Allele 2 Numerous spots of various sizes showing deep pigmentation in pale-colored background in kernel. Sectors of deep pigmentation in pale-colored background in plant. Many germinal mutations to higher alleles of A_1 . Few if any "breaks" at a_1^{m-3} locus.
Allele 3 Uniformly pigmented kernel and plant. Intensity of pigment much darker than that given by allele 2. No mutations.	Allele 3 Similar to allele 2 above except that mutant spots in kernels and sectors in plants appear in background coloration of medium intensity.

B. The a_1-*Dt* System

Phenotype produced in absence of *Dt*	Phenotype produced when 1 *Dt* is present
Allele 1 (standard a_1) Colorless kernel. No anthocyanin in plant. No mutations.	Allele 1 Few dots of deep A_1-type in pigmentation in colorless background in kernel. Fine streaks of A_1-type pigmentation in plant. Few germinal mutations to higher alleles of A_1 and to stable recessives. Most germinal mutants are stable in presence of *Dt*.
Allele 2 Colorless kernel. No anthocyanin in plant. No mutations.	Allele 2 Large number of pigmented spots or dots in colorless background in kernel. These show various grades of intensity of pigmentation from light to deep. Plant shows many sectors of mutant tissue in nonpigmented background. Many germinal mutations to alleles giving various grades of intensity of pigmentation, the majority of which are stable in the presence of *Dt*; a few, however, are unstable.

C. The a_1^{m-1}-*Spm* System

Phenotype produced in absence of *Spm*	Phenotype produced in presence of *Spm*
Allele 1 Colorless kernel. No anthocyanin pigment in plant. No mutations.	Allele 1 Many areas of different sizes showing various low levels of intensity of pigmentation in colorless background in kernel and in nonpigmented background in plant. Many germinal mutations to give alleles producing these low levels of pigment intensity. Mutants so obtained are stable in presence of *Spm*.
Allele 2 Very pale color in kernel. Intense anthocyanin pigmentation in plant. No mutations.	Allele 2 Small dots of full A_1-type pigmentation in colorless background in kernel. Fine streaks of A1-type pigmentation in nonpigmented background in plant. Very few germinal mutations. These are stable in presence of *Spm*.
Allele 3 Intense pigmentation in both kernel and plant. No mutations.	Allele 3 Many medium-sized dots showing full A_1-type pigmentation in colorless background in kernel, and many fine streaks of A_1-type pigmentation in nonpigmented background in plant. Few germinal mutations to higher alleles of A_1 . These are stable in presence of *Spm*.
Allele 4 Pale color in kernel. Intense pigmentation in plant. No mutations.	Allele 4 Many spots and large areas of full A_1-type pigmentation in nonpigmented background in kernel, and numerous sectors and streaks of A_1-type pigmentation in nonpigmented background in plant. Many germinal mutations to higher alleles of A_1 that are stable in presence of *Spm*.

tion in each mitosis in the germ line, and because of this it is maintained through successive plant generations. Only when, by appropriate crosses, the second element of the system is introduced, will alterations occur, and then only in some cells of the plant. Illustrations of the type of evidence that has revealed this are given below.

When *Ds* was first discovered, many dicentric-acentric chromatid-forming events were observed at the locus of *Ds* when *Ac* was also present in the nuclei of a plant. The frequency of their occurrence was high and they could be observed readily in both the plant and the endosperm tissues. Gametes having no *Ac* were produced by such plants, and in the next generation those plants having *Ds* but no *Ac* showed no dicentric-acentric chromatid formations at the locus of *Ds*, nor any other type of change that would reveal its presence. When, however, *Ac* was reintroduced in a subsequent generation by an appropriate cross, the presence of *Ds* was revealed, because dicentric-acentric chromatids were now formed at the previously determined position of *Ds*, and the frequency of their occurrence was the same, with the same dose of *Ac*, as that observed before *Ac* had been removed. Besides dicentric-acentric chromatid formation at the locus of *Ds*, other types of change in the presence of *Ac* were also noted, and some of them effected transposition of *Ds* to a new location. Occasionally, another type of modification occurred at the locus of *Ds*, but again only when *Ac* was also present, and this was recognized by a decided change in the relative frequency of occurrence of the above-mentioned types of response of *Ds* to *Ac*. The location of *Ds*, however, was not altered by the event that was responsible for this. If, by meiotic segregation, *Ac* was removed from the chromosome complement that carried such an altered *Ds*, the particular modification responsible for it was maintained without further change through successive plant generations. This was made evident because return of *Ac* in a later generation elicited from it the very same pattern of response that it had given before *Ac* was removed. Through selections based upon such clear-cut changes in the particular types of response of *Ds* to *Ac*, it was possible to isolate several different alleles of *Ds*, and to maintain them unaltered in successive generations in plants that did not have *Ac*. The behavior of each of them, when *Ac* was returned to the nucleus, could be predicted in advance if its behavior before the removal of *Ac* was known. Such predictions are possible only if the particular organization at the locus of *Ds* in each such case is replicated in each successive mitotic cycle in the germ line. It is difficult in the face of such evidence to avoid the conclusion that these alleles of *Ds* reflect organizational and thus structural differences either of the *Ds* element itself or of other components at the locus where it resides, even though the type and dimension of such differences are not yet known.

In all examined cases where a two-element system of control of gene action is known to operate, changes have been noted in the type of response of the element at the affected gene locus, and isolates showing different types of change have been made. The different isolates so far tested have behaved in inheritance as alleles of one another; and the type and pattern of response shown by each, when the second element of the system is present, is predictable. Table 4 was constructed in order to illustrate some of the kinds of differences these isolates may exhibit. Each of the alleles listed in this table demonstrates the presence of a particular type of organization at the gene locus where the controlling element resides. This organization is reproduced in each successive mitosis, and this is maintained through successive plant generations, provided the complementary element of the system concerned is absent.

It might be considered that a controlling element represents some kind of extrachromosomal substance that can attach itself or impress its influence in some manner at various positions in the chromosome complement and so affect the action of the genic substances at these positions. The modes of operation of controlling elements do not suggest this, however. Rather, they suggest that controlling elements are integral components of the chromosomes themselves, and that they have specific activities and modes of accomplishing them, much as the genes are presumed to have. Any proposed view of the structure and organization of controlling elements must consider not only the origin and maintenance of distinct alleles, as described above, and the modes of interaction exhibited by two-element systems, but also the nature of the change responsible for alteration in gene action that appears after either insertion or removal of a controlling element at a known gene locus. Insertion need not effect mere inhibition of gene action, as the analysis of the *Spm-*a_1^{m-1} system illustrates. Also, removal of the identifiable controlling element is not usually accompanied by exact restoration of the type of action shown by the genic substances before the controlling element appeared there, although sometimes it may give rise to a similar type of action. In many cases there is a clearly expressed difference, ranging from slight to marked. Thus, the presence of a controlling element at a gene locus need not effect mere inhibition of gene action, and its removal from the locus need not effect mere release of such inhibition. Other types of modification occur. Some form of organizational or structural change in chromosome materials must occur, both when a controlling element is present at a locus and also as a consequence of its removal. The available evidence suggests that the disappearance of an identifiable controlling element from a known location in a chromosome is associated with its appearance at a new location. The mechanism of transposition, then, is significant in any considera-

tion of the structures concerned, and our knowledge of this mechanism, inadequate as it is, should be evaluated.

The Mechanism of Transposition

Transposition of *Ds* from its first known position in the short arm of chromosome 9 to another position within that arm was detected very early in the study of the *Ds-Ac* system, and attempts were made to obtain information about the manner by which transposition is accomplished. Besides dicentric-active chromatid formation at the locus of *Ds*, other types of chromosomal aberration were noted in these early studies; they were made evident by the appearance of translocations, inversions, duplications, ring chromosomes, and deficiencies, but only when *Ac* was also present in the nucleus. In all such cases it was found that one of the two positions in the chromosome or chromosomes involved in the origin of the rearrangement was always at the previously identified locus of *Ds*. It was obvious, therefore, that the *Ds* element was primarily responsible for them. It was then decided to determine whether or not transposition of *Ds* accompanied such events, and for this purpose those cases that produced a duplication of a segment of the short arm of chromosome 9 were examined. They were chosen because the genetic markers located in this arm would allow the most precise analyses to be made. Three such cases were examined in detail, and from all three it was learned that transposition of *Ds* had accompanied the event that accomplished the duplication. A description of the origin and constitution of the chromosome having the duplication, in two of these three cases, was given in an earlier publication (McClintock, 1951) and need not be repeated here. It could be determined from a study of the three cases, however, that the *Ds* element that was inserted into the new location came from only one of two sister chromatids 9. It was also learned that the insertion of *Ds* at the new location was accompanied by its removal from its previous location. In each of the three examined cases, the involvement of sister chromatids in the origin of the duplication, the orientation of the duplicated segment, and the location of the two *Ds* elements suggested that contact of the locus of *Ds* with another locus in this chromosome preceded the event that produced the duplication and the transposition of *Ds*; and that the transposition could be associated with the mechanism of the subsequent chromosome reduplication itself. It was also learned from these cases that insertions of *Ds* into new locations could take place without effecting gross chromosomal rearrangements (see positions of *Ds* in diagrams given in McClintock, 1951).

The allele of *Ds* that gives rise to the types of chromosomal aberrations mentioned above is one that produces many dicentric-acentric chromatid formations at the locus of *Ds* itself, but only, of course, when *Ac* is also present in the nucleus. It might be considered that such configurations could arise either from lack of reduplication of the components at the locus where *Ds* resides, or from "stickiness" of these materials. The cases mentioned above indicate, however, that the *Ds* element is reduplicated when chromosomal aberrations occur, for the two *Ds* elements produced by the reduplication process can be accounted for even though one of them occupies a new location. This suggests that the dicentric-acentric chromatid formations might arise not from lack of reduplication at the locus but rather as a consequence of the reduplication mechanism itself. Some component of this particular allele of *Ds* may be so ordered that it will allow a reverse bonding between linearly arranged components during the reduplication process; this, in turn, would lead to the dicentric-acentric chromatid formations that are so clearly expressed in some cells of the somatic and sporogenous tissues of plants having the allele.

It should be emphasized, in this discussion of transposition, that the *Ds* element undergoes alterations which modify its type of action. These were mentioned earlier. Some of them give rise to an allele of *Ds* that no longer produces dicentric chromatids (or only a few of them) or other types of chromosomal rearrangement. Transpositions continue to occur, however. The controlling elements *Ac* and *Spm* also undergo frequent transpositions and these are not usually accompanied by chromosomal translocations. Thus it appears that transposition is not the consequence of "stickiness" at the locus where these elements reside; for, if it were, many cases of chromosomal aberration should have been detected in association with their transposition. The absence or infrequence of such cases is conspicuous.

One can conclude, then, that transpositions of controlling elements either arise from some yet-unknown mechanism or occur during the chromosome reduplication process itself and are a consequence of it. At present, the latter interpretation is favored, for it will account for many of the observations of change in number and location of controlling elements. If, in a plant having one such element at a known location, the element is transposed from one of two sister chromatids of one chromosome to a new location in one of two sister chromatids of another chromosome, segregation at the following mitotic anaphase could result in several different types of change in constitution of the element in the sister nuclei. Either one nucleus would have this element at the previous known location and the sister nucleus would have it at a new location, or one nucleus would have no element and the sister nucleus would have two, one at the previous known location and one at a new location. As a consequence of such segregations, cells could be formed having no such element, or one that was unchanged in its location,

or one at a new location, or two elements, one at the previously known location and one at a new location. In the gametes produced by plants having a transposable element, just such types of change in location and number of elements have been found; examples were given earlier. Changes in constitution of *Ac* occurring in somatic cells give rise to sectors that exhibit these changes, and in some of them it is clear that the dose of *Ac* has been increased. It is possible to observe that subsequent changes in *Ac* constitution may also occur, for subsectors showing them may appear within the larger sectors. If sequential transpositions of a controlling ele ment occur in the cells of the germ line in the manner outlined above, gametes carrying more than two elements may be produced by plants whose zygote nuclei had but one; and examples of this have been found (see examples under D and E of Table 2).

If transposition occurs during the chromosome reduplication process, then the means by which it is accomplished is of considerable importance. It is conceivable that it is brought about by some mechanism similar to that proposed to account for transduction in bacteria (Demerec, Blomstrand, and Demerec, 1955; Demerec and Demerec, 1956) —a form of exchange or "crossing over" between the component contributed by the phage and that present in the bacterium, which requires a reduplication of both components. If such an event accounts for transposition, then reciprocal substitutions of components at the loci concerned should be produced as a consequence. There is evidence to suggest that such substitutions may occur. In the study of *Ac* at the bronze locus, described earlier, three cases of change in mode of control of mutation at this locus were noted. Each was associated with removal of *Ac* from the bronze locus, and in two of the three cases it could be established with certainty that *Ac* had been transposed to a new location. In two of the three cases, a recessive, bronze, phenotype appeared as a consequence of the removal of *Ac*; but instead of being stable in its presence, as in most such cases, this phenotype was stable only in its absence. In its presence, mutations to the higher alleles of *Bz* occurred, and the type of response was the same as that exhibited in other cases in which the *Ds-Ac* system of control of gene action operates. The mode of control in the third case was similar. In this case, however, removal of *Ac* was associated with partial expression of the genic substance at the bronze locus, for some *Bz*-type pigment appeared both in the plants and in the kernels having it. This expression was quite stable in the absence of *Ac*, but in its presence mutations occurred to give alleles that are associated with the appearance of higher or lower levels of intensity of the pigment. The origin of these three cases could be explained if a *Ds*-type element (one responding to *Ac*) had been substituted for *Ac* during the transposition process.

There is other evidence that should be mentioned in considering the possibility that substitution may accompany transposition, and this is related to the types of mutation that are produced when a known controlling element is removed from a locus. As stated earlier, the mutations so produced need not be alike; in some cases, wide differences in their mode of expression can be observed. It is possible that some of the differences they exhibit are the consequence of substitution of one type of controlling element for another. Only through further investigation, however, woult it be possible to verify this; and except in the three above-mentioned cases evidence is not yet available, although in several other cases there is evidence suggestive of it.

Positions in the Chromosome Complement at Which Controlling Elements May Be Inserted

It is known that controlling elements may be inserted at various locations within the chromosome complement. In order to learn whether these positions are randomly distributed or selectively located, a large number of independent transpositions of a particular element from a known location to new locations needs to be determined. Even though many sequential transpositions of the elements *Ds*, *Ac*, and *Spm* have been detected, the evidence obtained from any one of these elements is insufficient to allow definite conclusions to be drawn. The evidence does suggest a degree of nonrandomness, which, however, may merely reflect degrees of viability following upon insertions at particular positions rather than selectivity of positions at which the elements may be inserted. Such inviabilities were discovered in studies aimed at detecting the positions within the short arm of chromosome 9 into which *Ds* may enter. This arm carries genetic markers that allow easy detection in kernels of insertion of *Ds* between any two of them. Plants having *Ds* at a known location in this arm produce some gametes having *Ds* at new locations, some of which are also in the same arm. When plants carrying *Ds* and also dominant alleles of some of the known genetic markers in this arm were crossed to plants that were homozygous for the recessive alleles, kernels that arose from functioning of a pollen grain in which *Ds* occupied a new position in the arm were readily detected. The endosperm and embryo of a number of such kernels were quite normal in appearance, but other kernels were abnormal in various ways. Some of them were smaller than normal, others were germless or had defective embryos, and still others exhibited distorted growth in the endosperm tissues. Most of these kernels did not germinate. Some of the normal-appearing kernels also did not germinate. In other words, dominant lethality was exhibited among a number of kernels

having *Ds* at new positions within the short arm of chromosome 9. The new position of *Ds* could be verified only in plants derived from the kernels that did germinate. The positions occupied by *Ds* in these plants seemed not to be randomly distributed but to be clustered about certain locations within the arm. It is suspected that they represented only some of the positions into which *Ds* may enter, and that the insertion of *Ds* at other positions results in dominant lethality. This inference is supported by the results of examinations of cases that exhibited semidominant lethality of the following type. In endosperms with two normal chromosomes 9 not carrying *Ds* and a chromosome 9 with *Ds* at the new location, growth was so distorted that many kernels failed to mature. Only a few reached maturity but all of them were obviously aberrant in morphology. Plants could be obtained from some of the latter, however. Four independent cases of this type were examined, and in all four the semilethal effect was associated with a modification induced by *Ds* in a chromosome component located to the right of *Bz*. In these plants, the chromosome 9 carrying *Ds* was quite normal in morphology. The semidominant lethality was not due, primarily, to inhibition of gene action, for it is known that endosperms that are deficient for all of the short arm of one chromosome 9 develop normally. Some change in gene action other than localized inhibition was induced in these cases.

Because dominant lethality may be expressed when controlling elements are inserted at some positions in the chromosome complement, as described above, difficulties are encountered in attempts to determine whether a particular element can enter any site in the chromosome complement or is restricted to certain sites. At present, no definite statement can be made regarding this. It is known, however, that controlling elements may be inserted at a number of different positions. Those that seem to be preferred, on the basis of present knowledge, may represent only a selected number of possible sites at which the presence of the element does not induce inviability at some stage in development.

Influence of Controlling Elements in Modifying Gene Action

It is now known that controlling elements may modify gene action in a number of different ways. They may influence the time of gene action in the development of a tissue, and also determine the cells in which it will occur. Again, they may influence the type of action, with regard to either degree (quantitative aspects) or kind (qualitative) aspects). They may also act as inhibitors, suppressors, and modifiers, as well as inducers of types of change at a gene locus that resemble those often referred to in the past as point or gene mutation.

When a particular controlling element is inserted at a gene locus, its presence there may be detected by the changes in phenotypic expression that appear. Tests may then be conducted to determine the position in a chromosome at which the responsible changes are occurring, and thus the locus involved may be identified. Subsequent tests may be made to examine the mode of operation of the particular controlling system concerned with these changes and to determine its components. From such procedures it was learned that one system can operate at a number of different gene loci, and that the action at one gene locus may be controlled by different systems; the evidence has been presented in previous publications (McClintock, 1953, 1955, 1956).

When a controlling element is inserted at the locus of a known gene, a recognizable change in phenotypic expression may be observed as an immediate consequence, or no immediate change may result. In the latter case, the presence of a controlling element at the locus is detected subsequently, for it will initiate recognizable changes in gene action. The origins of a_1^{m-3} and a_1^{m-4} will illustrate this fact. Both arose from insertion of a *Ds*-type element (one responding to *Ac*) at the standard A_1 locus in chromosome 3, and the *Ds-Ac* two-element system controls gene action in both cases. In the case of a_1^{m-4}, inhibition of gene action probably occurred as an immediate consequence of insertion of a *Ds* element at the standard A_1 locus, as its presence there was made evident a few cell generations after that event occurred. In the case of a_1^{m-3}, on the other hand, insertion of the *Ds* element at the A_1 locus did not produce an immediate change in gene action. Its presence was revealed later, however, by altered expressions of the gene substance at the locus, which were recognized in some of the progeny of one particular plant of a culture. At least a full plant generation intervened between insertion of the *Ds* element at A_1 and recognition of its presence there. Had *Ac* been removed from the nucleus shortly after this insertion, the presence of the *Ds* element at the locus would not have been detected, for in the absence of *Ac* the phenotypic expression would have remained unaltered. Only by some fortuitous cross that again introduced *Ac* into a plant carrying this original state of a_1^{m-3} would the presence of *Ds* have been revealed, for only then would frequent change in gene action occur. Thus, the presence of a controlling element at a particular locus is revealed by the types of change that occur under given conditions, and if these conditions do not prevail its presence at the locus may not be recognized.

That the presence of a controlling element at a locus need not effect inhibition of gene action is also shown by studies of those cases in which *Ac* resides at the bronze locus in chromosome 9 and at the *P* locus in chromosome 1. The phenotype produced when these cases were initially recognized was that of the recessive, or null, expression. In both cases, mutations occurred, and the part

that *Ac* plays in these was reviewed earlier. It was found that removal of *Ac* from the locus concerned was associated in some cases with a change in gene action from one that gives the null expression to one that gives a high degree of activity, and also that the mutant so formed was subsequently stable in the presence of *Ac* when the latter was located elsewhere. Some of the mutants were not stable, however, and subsequent mutations occurred. In the case of *Ac* at the bronze locus, two of the 16 *Bz* mutants examined were of this type. From studies of both of them it was learned that the event that gave rise to the *Bz* expression did not result in removal of *Ac* from the immediate vicinity of the *Bz* locus and that *Ac* was responsible for the subsequent mutations that occurred. Some of these later mutations resulted in stability of the *Bz* expression, and in the three examined cases it was learned that *Ac* had been removed from the locus of *Bz*. Other changes were detected by the reappearance of the unstable recessive, and in the several examined cases it was learned that *Ac* was still present at the bronze locus. Still other types of mutant expression were noted, but these need not be discussed here. It is desired only to emphasize that the presence of a controlling element at a gene locus need not effect inhibition of action but may instead condition a mode of control of gene action in subsequent cell and plant generations, which will follow in a predictable manner. The ability to predict depends, of course, upon the extent of knowledge of the controlling system in operation in any one case.

The presence of a particular controlling element at a known gene locus can influence gene expression in different ways, which may range from complete suppression to various degrees of action. Moreover, the types of action may differ not only quantitatively but also qualitatively. There is evidence to suggest that in some cases these various types of mutation are reflections of modifications affecting different components of a compound locus, and that each component of the locus is concerned in its own way with development of one particular phenotype. Extensive evidence based on crossover studies of the compound nature of the A_1 locus in chromosome 3 has been presented by Laughnan (1949, 1952, 1955a, b) and evidence regarding the *R* locus in chromosome 10 has been obtained by Stadler and colleagues (Stadler and Nuffer, 1953; Stadler and Emmerling, 1954, 1956). Instability of expression of A_1 has appeared rather frequently, and cases of this have been examined by several maize geneticists (Rhoades, 1936, 1938, 1941, 1945; McClintock, 1951, 1953, 1956; Nuffer, 1955, 1956; Laughnan, 1956; Peterson, 1956; Richardson, 1956). On the basis of our present knowledge of the origin and behavior of such "unstable loci" it is inferred, when not determined with certainty, that a controlling element resides at the A_1 locus in each such case. The modifications in gene action it induces there can affect the action of one or another of the known components of this locus, or it may affect all of them simultaneously. Other gene loci, such as that of *C* in chromosome 9, also appear to be compound. Evidence of this was obtained by study of several distinguishable types of mutation that occur in the case of c^{m-2}. (For origin of this case and the controlling system involved, see McClintock, 1951.) These are associated with the production of at least two different diffusible substances, both of which are required for pigment formation. It was also noted that the dose expression given by the *C* allele commonly used in genetic studies is related to the limited production of one of these substances by this allele: the more *C* alleles present, the greater the amount of this substance formed, and, consequently, the denser the pigmentation. It is conceivable, then, that some of the qualitative differences in expression of mutants of a given locus reflect alterations in action of different components of a compound locus, and that a controlling element as the consequence of one event, may affect the action of only one component or of more than one; or the modification induced by any one event may affect the action of all of them.

It is now known that the presence of a controlling element at a known locus can effect change in gene action not only of the genic components located close to it, but also of other genic components located some distance to either side of it. A number of examples of this kind affecting gene action in a particular segment within the short arm of chromosome 9, extending over a region 5 or more crossover units in length have been examined; and these have been reviewed in previous publications (McClintock, 1953, 1954, 1955, 1956). Recently, Richardson (1956) found a case of "spreading effect" that appeared to be induced by a controlling element located at A_1 in chromosome 3. The nature of the changes responsible for such spread of mutation along the chromosome is of considerable importance for an understanding of the manner by which controlling elements can induce their effects, and those involving the segment of chromosome 9 that includes the loci of *I*, *Sh*, and *Bz* are particularly useful for this purpose. The "spreading effect" in these cases is known to be induced by the presence of a *Ds* element that is located just to the left of *Sh*; and it is also known that the mutation-inducing process is not accompanied by change in location of *Ds*. One might consider that the "spreading effect" is merely the expression of a deficiency for the loci involved, even though most such cases give viable homozygotes, or, barring this, that the organization of the chromosome segment concerned or the structure or organization of components within it is altered in some particular manner by the *Ds* element. Therefore, tests of some of these cases are being conducted in order to determine whether or not crossing over occurs within the

affected segment. It has been learned, in several of these cases, that either crossing over does not occur or its frequency of occurrence is very low. In others, however, crossing over takes place within the affected segment and the frequency of occurrence differs among the several examined cases. Plants derived from reciprocal crossovers are now under investigation in order to determine if mutant expressions of genic components within the affected segment will appear in their progeny, and if so, the types each may give. That separation by crossing over can occur between the individual genic components whose modification is responsible for the compound mutant expression has already been shown by Richardson in the case of the "spreading effect," mentioned above.

Examination of the mode of operation of two-element controlling systems has revealed the breadth of influence of such systems in modifying gene action. The study has concerned not only the number of different genes that such systems control, but also the manner of this control, which is of greater significance. It has been possible to examine the mode of operation of the *Ds-Ac* system at seven different gene loci (for references, see McClintock, 1953, 1956), and to learn that the system operates in essentially the same way in each case. The mode of control of gene action resides in the system itself. It is the *Ds*-type element at the locus of the gene that is directly responsible for control of gene action and for the changes that occur in gene action; and it is the *Ac* element of the system that is responsible for initiating these modifications where the *Ds* element resides, and also for the time of their occurrence. In some of the cases examined, the change in gene action is usually associated with removal of *Ds* from the gene locus, and stability of mutant expression in the presence of *Ac* is thereby effected (c^{m-1}, bz^{m-1} are examples). In other cases, the *Ds* element is not usually removed when a change in gene action is initiated, and, in the presence of *Ac*, subsequent changes may occur (c^{m-2}, wx^{m-1}, a_1^{m-3} are examples). Any one of the mutants so produced, however, will be stable in the absence of *Ac*. Thus, by removing *Ac* from the nucleus, it is possible to isolate a number of alleles that are distinguishable from each other by different modes of gene expression (see Table 4 for examples). Extensive examination of the operation of this *Ds-Ac* system as well as other systems has thus provided a large body of knowledge, from which it is possible to conclude that controlling systems are composed of distinct and well-defined entities in the nucleus, that these are independent of the gene elements as defined earlier, that they need not reside at fixed sites in the chromosome complement, that they retain their identities when transposed from one location to another, and that they operate in much the same general manner wherever they may be located.

The mode of operation of the *Spm*-a_1^{m-1} two-element system, which was considered in some detail earlier in this discussion, is impressive because one aspect of control of gene action expressed by the *Spm* element of the system is suggestive of the mode of operation of suppressors, inhibitors, and modifiers that have been identified in other organisms. In its presence, all action at the a_1^{m-1} locus is suppressed, but in its absence all but one of the alleles of a_1^{m-1} are active to some degree, and some of them produce kernels and plants that exhibit intense pigmentation (see Table 4). The *Spm* element undergoes many transpositions without suffering loss of identity, for its mode of control of gene action at a_1^{m-1} has been found to be the same when variously located. In contrast to *Ac* in the *Ds-Ac* system, increments of *Spm* do not effect modification in time of mutation at a_1^{m-1}. The same phenotypes appear when either one or more *Spm* elements are present in the nuclei of a plant.

Another two-element system has been examined in which the mode of control of the independently located element resembles, in certain respects, those of both *Spm* and *Ac*. The element in this system that is comparable to *Ac*, *Spm*, or *Dt*, exhibits a suppressor-mutator type of control of gene action and mutation at a modified A_2 locus, designated a_2^{m-1}, and in a manner that is similar to that of the *Spm* element of the a_1^{m-1} system. For example, in its absence, gene action at the a_2^{m-1} locus in one of the isolates (one of the alleles of a_2^{m-1}) resembles that given by the standard A_2, for the plants and kernels are intensely and uniformly pigmented and no mutations occur. In its presence, however, all gene activity at a_2^{m-1} is suppressed until the occurrence there of a mutation-inducing event that allows pigment to be produced; and the time of occurrence of such events during the development of a tissue depends on the dose of this suppressor-mutator-type element that is present in the nucleus: the higher the dose, the later the time of occurrence. In this respect, it very much resembles *Ac*.

Differences in phenotype produced by increments of *Ac* or of the *Spm*-type element just described are sharply defined. However, the effects of additions of elements that exhibit this type of dosage expression need not be so contrasting. It is known from other evidence that these effects may be expressed in a somewhat different manner, and an example will be given here. It involves two elements, one of which resides at the A_2 locus in chromosome 5; the other element is independently located. A low dose of the independently located element of this system effects early-occurring mutations at the locus of A_2, from a nonactive allele to one that gives an apparently standard A_2-type expression. Added increments of this element effect step-wise delays in time of occurrence of these mutations, until mere specks of pigment appear in a nonpigmented background in the kernel and only very fine streaks of an-

thocyanin pigment appear in a nonpigmented background in the plant. Increments of the element above that which gives this speckled pattern result in a striking change in phenotypic expression. Now, pigment is produced in both plant and kernel. Although the intensity of color is low, the pigment is uniformly distributed and no mutations occur. This change in gene action is not the consequence of mutation at the modified A_2 locus, but rather the expression of a high dose of the independently located element of the system. This may be shown by crossing the pale-colored plants to standard a_2 tester stocks in which the element is absent. Doses of it are thereby reduced in some of the progeny, and this reduction is evidenced by the reappearance in kernels and plants of the variegated phenotype: a nonpigmented background in which pigmented spots or areas appear. It has also been determined that this element undergoes transposition. This may sometimes be exhibited in sectors in the plant or kernel, which express the change in number of the element that occurred in the ancestor cell giving rise to the sector.

It was stated earlier that the presence of a controlling element at a particular gene locus may not be recognized unless favorable conditions for revealing it are also present; and examples were given. The origin of a number of mutants might well be traced to effects produced by controlling elements, but this might be as difficult as it initially appeared to be for the standard a_1 mutant in maize. This mutant responds to *Dt* by producing higher alleles of A_1, but in the absence of *Dt* the recessive mutant expression is most stable. The mutant was used in genetic studies long before *Dt* was discovered or its control over the element at the a_1 locus recognized. Had *Dt* not been discovered, the a_1 mutant would still be considered an example of stable gene change, just as many of the other known mutants are now considered. In order to identify readily the presence of controlling elements and to be able to examine their different modes of behavior, only those cases were selected, originally, that gave clear-cut evidence of their presence by modification of gene action in both somatic and sporogenous cells. These are the so-called "mutable genes" or "mutable loci." It is quite evident, however, that the standard a_1 mutant is as good a member of the "mutable gene" class as any other that has been described in this or previous reports. How many other known mutants, whose behavior appears to be stable in the existing experimental cultures, also belong to this class? Can conditions be altered so as to expose the presence of a controlling element? Efforts in this direction have not yet been made, except with the standard a_1 mutant itself (McClintock, 1951a, b). The method used gave positive results in that case; and this suggests that positive results might also be obtained if similar experiments were conducted with other known mutants which appear to be stable in the genetic stocks now being used.

Controlling elements appear to reflect the presence in the nucleus of highly integrated systems operating to control gene action. The modes of operation of the known two-element systems bring into sharp relief one level of this integration. Other levels are now under investigation, and these are related to the effects produced by modifiers of particular systems that have appeared within the cultures under examination or been introduced by strain crosses. For example, in the *Spm*-carrying cultures, a certain type of modifier arises with rather constant frequencies. In its action it resembles, in certain respects, the *Spm* element itself, and it may be derived from this element in some manner. It differs from the original *Spm*, however, in quite recognizable ways. In the absence of *Spm*, this modifier effects suppression of gene action at a_1^{m-1}, but only in the aleurone layer of the kernel. The kernels may be totally colorless or they may show one or several small dots of deep color. The plants, on the other hand, show the pigmented phenotype that is exhibited in the absence of *Spm*. In the presence of *Spm*, the modifier behaves as a recessive, and in test crosses of plants carrying both of them typical segregation ratios for both elements are exhibited. The modifier probably undergoes transposition just as *Spm* does, for it has been located at several different positions in the chromosome complement. It is a controlling element and also it is a component of this system. Still other types of modifiers of this system have appeared, each expressing a characteristic type of modification of the system, or, in other words, a characteristic type of integrative action within the system as a whole. Recognition of the presence and operation of a so-called two-element system, then, represents only recognition of the lowest integrative level of those elements in the chromosome complement that are directly concerned with modification of the genome as a whole.

Mendelizing units associated with phenotypic expressions that are similar in many essential respects to those produced by known controlling elements in maize, such as modifiers, suppressors, and some types of inhibitors, have been described in a number of organisms. Are many of their effects also attributable to the activities of controlling elements; and what kinds of criteria may be used to discriminate between the proposed two classes of genetic elements, that is, gene elements and controlling elements? Transposability, which made possible the recognition of controlling elements in the chromosome complement of maize, may not serve in all cases as a reliable criterion for discrimination between the two classes of elements, because the frequency of its occurrence may be so low, under certain conditions, that detection may be difficult (McClintock, 1956). Nevertheless, as far as my knowledge goes, little

if any effort has been made to detect transposition of mutators, modifiers, suppressors, or some types of inhibitors in other organisms, and the degree to which it may occur is not yet known. It would be surprising, indeed, if controlling elements were not found in other organisms, for their prevalence in maize is now well established.

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DISCUSSION

CATCHESIDE: I would like to suggest that Dr. McClintock has in the transposition of controlling elements the explanation of the phenomenon which has been referred to by several speakers as negative interference. It would follow that evidence for controlling elements in organisms other than maize should be sought in those cases which show apparent negative interference.