INDUCTION OF INSTABILITY AT SELECTED LOCI IN MAIZE

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Received April 14, 1953

IN previous reports (McCLINTOCK 1950, 1951), studies of the origin and expression of genic instability at a number of known loci in the maize chromosomes were summarized. It was concluded that changes in genic expression result from chromosome alterations at the locus of a gene and these are initiated by units other than those composing the gene itself. The mutations are considered, therefore, not as changes in the potentials of genic action, but rather chromosomal modifications at the locus effecting the kind and the degree of genic expression. The extragenic chromatin units have specificity in that differences among them may be recognized. Each exerts a specific type of control of the action of the gene with which it becomes associated. These units may be transposed from one location to another within the chromosome complement. When incorporated at a new location, each expresses its mode of control of the action of the associated gene, and in a manner similar to that which occurred at the former location. These conclusions have been supported by extensive examination of the action of one particular system that has modified genic action at a number of different loci. It is the so-called Dissociation-Activator (*Ds-Ac*) two-unit system.

Both *Ds* and *Ac* are single chromosomal units. Their locations in particular chromosomes may be determined by linkage relations to other established markers. Each, however, may move from one location to another within the chromosomal complement. Such transpositions of *Ds* or *Ac*, or both, may occur in a few sporogenous cells. Consequently, a few gametes may be formed with *Ds* or *Ac*, or both, located at new positions. Following such transposition, each remains at the new location until, in a subsequent cell or plant generation, transposition to another location again occurs. Either may be inserted at various locations within the complement. It has been determined that the process of transposition involves some initial change at either *Ds* or *Ac* that can result in chromosome breaks and fusions. It has also been learned that in order for transposition of *Ds* to occur, *Ac* must be present in the nucleus. Besides transposition, there are other known consequences of events initiated by *Ds* and again, these appear only when *Ac* is also present in the nucleus. They are dicentric chromatid formation, deficiency, duplication of segments, inversions, ring-chromosomes and reciprocal translocations between chromosomes. In all of these cases, the chromosome breaks are produced as the consequence of some initial change involving the *Ds* unit.

When *Ds* is transposed to the locus of a known gene, it may immediately or subsequently affect its action. This is expressed either by partial or complete inhibition, or by a previously unrecognized type of altered genic expression. As long as *Ds* remains in this position, genic action is subject to further change. It has been possible to determine that the subsequent changes are reflections of alterations occurring at the locus, and these are initiated by *Ds*. No *Ds*-initiated changes will occur, however, unless *Ac* is also present in the nucleus. If *Ac* is absent, no further modification of genic action occurs. The mutation present at the time of removal of *Ac* will be stable in expression in subsequent generations until *Ac* is again introduced into a zygote. In some cells of the plant arising from this zygote, alterations at the locus of the gene, initiated by *Ds*, again will occur. Some of these may result in further modifications of genic action—that is, new mutations. Both the initial mutation and subsequent mutations are expressions, therefore, of interaction between *Ds* and *Ac*. Thus, these two chromosomal units comprise a nuclear system capable of controlling genic expression; *Ds* initiates changes in genic expression and *Ac* controls when they will occur. Since *Ds* may enter various locations in the chromosome complement, *Ac*-controlled mutability can be expected to arise at a number of different loci of known genic action. In previous reports, the origin and subsequent behavior of *Ac*-controlled mutability at the *C*, *Bz* and *Wx* loci in the short arm of chromosome 9 were described. It is the purpose of this paper to indicate that, in conformity with expectation, *Ac*-controlled mutability can appear at other loci of known genic action. It will also be shown that this type of control of instability may be obtained at selected loci.

To test the prediction that genic instability under the control of *Ac* may be obtained at selected loci, two, considered to be particularly favorable for initial tests, were selected. These are the locus of A_1 in chromosome 3 long arm, and that of A_2 in chromosome 5 short arm. The genetically active components at these two loci affect anthocyanin pigment formation in both the plant tissues and in the aleurone layer of the kernel. The known recessive alleles are designated a_1 and a_2. When either is homozygous, no anthocyanin pigment is developed in the plant tissues or in the aleurone layer. Both recessives are stable in the presence of *Ac*; tests designed to show whether or not mutations of a_1 or a_2 would occur when *Ac* was present in the nucleus were negative. Consequently, it was possible to perform the following experiment. A number of plants were grown that had a *Ds* unit located in the long arm of one chromosome 5 and an *Ac* unit located in another chromosome of the complement. All plants were homozygous for A_1 and Sh_2 and for A_2 and Bm_1. (Sh_2, normal development of endosperm; sh_2, shrunken endosperm, located a quarter of a crossover unit distal to A_1. Bm_1, colorless secondary cell walls in plant tissues; bm_1, brown mid-rib, brown color in secondary cell walls; located 6 crossover units proximal to A_2 and in same arm of chromosome 5.) The silks of some of these plants received pollen from plants homozygous for a_1, sh_2 and A_2. The silks of other plants received pollen from plants homozygous for a_2, bm_1 and A_1. None of the plants used as pollen parents carried *Ac*. The ears resulting from

these crosses were examined to determine if any kernels exhibiting variegation for aleurone color were present.

If a sufficiently large number of ears were obtained from the described crosses, one or more variegated kernels should appear. This conclusion is based on the following reasoning. In plants carrying *Ds* and *Ac*, transposition of *Ds* may occur in a few sporogenous cells. Should one such transposition insert *Ds* at the locus of A_1, total or partial inhibition of A_1 action could result. The gametophyte and consequently the gametes derived from this sporogenous cell could carry an A_1 locus with a *Ds*-initiated altered capacity for action. Should *Ac* also be present, further *Ds*-initiated changes at the locus could occur in some cells during development of the kernel that arises from functioning of this gametophyte. In the progeny of such cells, these subsequent changes in A_1 action could be expressed if the pollen parent had contributed the recessive, a_1, which is stable in the presence of *Ac*. The aleurone layer of the mature kernel would exhibit a variegated pattern of anthocyanin pigmentation, either with respect to presence and absence of pigmentation, or to intensities of pigmentation, or both. This kernel could then be removed from the ear, a plant grown from it and tests conducted with this plant and its progeny to determine whether or not the variegation is an expression of instability at the A_1 locus and, if so, whether this instability is *Ac*-controlled. In a similar manner, alterations at the A_2 locus that arise in the *Ds* and *Ac* carrying plants could be detected if the pollen parent contributed the stable recessive, a_2. In order to establish that the unstable state in either case originates in the *Ds* and *Ac* carrying plant, it is necessary for one of the parents to introduce a second, closely linked genetic marker. In progeny tests, linkage of unstable A_1 with Sh_2 or of unstable A_2 with Bm_1 would indicate inception of instability in the *Ds* and *Ac* carrying parent plant.

Seventy-one ears were obtained from the cross in which the pollen parent had contributed a_1. One kernel was found that clearly exhibited variegation for aleurone color. There were no colorless kernels on any of these ears. Among the 120 ears obtained when a_2 had been introduced by the pollen parent, three kernels exhibiting variegation for aleurone color appeared, each on a different ear. Again, there were no colorless kernels on any of these ears. Plants were grown from all four variegated kernels and tests initiated with each to determine the nature of the instability being expressed. The progeny, in turn, were further tested. From these studies, the following was determined. In the variegated kernel that had received a_1 from the pollen parent, and in the plant derived from it, instability of genic action at the locus of A_1 was being expressed. The alteration responsible for this instability occurred at the locus of A_1 in one of the chromosomes 3 of the *Ds-Ac* carrying female parent. Mutations at this modified A_1 locus, designated $a_1^{m\text{-}4}$, occur only when *Ac* is present. In the plants derived from the three variegated kernels that appeared when a_2 had been the pollen parent, the nature of the alteration at A_2 could be determined in only two of them. This was because in one of them, the chromosome 5 contributed by the *Ds-Ac* carrying female parent was not transmitted to the next

generation either through pollen or egg. Thus, no definite conclusions may be drawn concerning the nature of the instability, presumably at the A_2 locus, that was exhibited in the kernel from which this plant arose. In the two remaining plants, it could be shown that it was the A_2 locus in the chromosome 5 contributed by the female parent that had been modified. In one plant, the mutations occurring at this locus proved to be *Ac*-controlled. This mutable locus is designated $a_2^{m\text{-}4}$. In the second plant, the mutations were not *Ac*-controlled. This mutable locus is designated $a_2^{m\text{-}3}$.

Some of the methods used to determine whether or not mutation at a particular known locus is *Ac*-controlled will be outlined. For illustrative purposes, the A_1 locus has been chosen. Four independent inceptions of instability at A_1 have been detected in the Cold Spring Harbor cultures. Two of them, $a_1^{m\text{-}1}$ and $a_1^{m\text{-}2}$, are not *Ac*-controlled. Two of them, however, $a_1^{m\text{-}3}$ and $a_1^{m\text{-}4}$, are *Ac*-controlled. Both of the latter had their inception in plants homozygous for the normal A_1 locus and in which both *Ds* and *Ac* were present. The designation $a_1^{m\text{-}4}$ was given to the case whose origin was outlined above. In this report, the methods used to analyze the factors responsible for mutation at $a_1^{m\text{-}4}$ will be considered. These same methods, however, have been used to analyze all cases of *Ac*-controlled mutability. It is because of the very close linkage of Sh_2 to $a_1^{m\text{-}4}$, allowing the latter to be readily followed in progeny tests, that instability at the A_1 locus has been selected for illustrative purposes.

In considering *Ac*-controlled mutability, the following facts should be kept in mind. Mutations will occur only when *Ac* is present in the nucleus. In the absence of *Ac*, the modified genic action at the locus is stable. Instability, however, will return if *Ac* is again introduced into a nucleus having this modified locus. *Ac* controls when mutations will occur; the higher the dose of *Ac*, the later the time during the development of a tissue when mutations at the affected locus will occur. *Ac* is inherited as a single unit. Transpositions or alterations of *Ac*, however, may occur in a few sporogenous cells resulting in loss to one of two sister cells, change in location of *Ac* in the chromosome complement, or change in dose action (change in state). Some of the gametes produced by plants carrying *Ac* will be derived from cells in which such events occurred. Evidence for these statements will appear in the descriptions of the tests conducted with $a_1^{m\text{-}4}$.

The pattern of variegation in the original kernel having $a_1^{m\text{-}4}$ resembled that expected of mutable a_1. Within the aleurone layer, many small areas of color appeared in a colorless background. A plant was grown from this kernel in the greenhouse during the winter of 1951–52, and given the culture number 6110. This plant was: (1) self-pollinated, (2) crossed to two plants having no *Ac* factor that were $a_1\ Sh_2/a_1\ sh_2$, (3) crossed to a plant that was $A_1\ Sh_2/a_1\ sh_2$ and carrying a single *Ac* factor, and (4) crossed to a plant with a genetic constitution designed to test for the presence of *Ac* in plant 6110. The nature of the *Ac* tester stocks will be described shortly. The number of crosses conducted in the greenhouse was limited. The results of these crosses did indicate, however, that plant 6110 carried a newly arisen alteration at the A_1 locus; and

that this alteration was responsible for the expressed instability. The constitution of plant 6110 was a_1^{m-4} Sh_2/a_1 sh_2 and it carried one *Ac* factor. The evidence also suggested that the mutations occurring at a_1^{m-4} were *Ac*-controlled. From these greenhouse crosses, kernels with appropriate constitutions were selected and plants obtained from them in the summer of 1952 in order that more adequate tests of the nature of the instability could be conducted.

The self-pollinated ear of plant 6110 produced 228 kernels with the following appearances: 7 completely colored, Sh_2: 129 variegated (exhibiting areas of color in a colorless background), Sh_2: 49 colorless, Sh_2: 43 colorless, sh_2. In the crosses to plants that were a_1 Sh_2/a_1 sh_2 and in which no *Ac* factor was present, 888 kernels were produced. They could be segregated into the following classes: 13 completely colored, Sh_2: 225 variegated, Sh_2: 438 colorless, Sh_2: 212 colorless, sh_2. In the cross to the plant that was A_1 Sh_2/a_1 sh_2 and had 1 *Ac* factor, 273 kernels were produced: 145 completely colored, Sh_2: 1 completely colored, sh_2: 29 variegated, Sh_2: 17 colorless, Sh_2: 81 colorless, sh_2. The several distinctive patterns of colored spots exhibited by the variegated kernels derived from this latter cross suggested *Ac*-control of mutability at a_1^{m-4}. These resembled the patterns produced with single to triple doses of *Ac*. Kernels with these different doses of *Ac* were expected to be present on this ear because each parent carried one *Ac* factor. Other evidence also suggested that the mutations were *Ac*-controlled. This appears in the ratio of variegated to nonvariegated kernels resulting from the cross of plant 6110 to the plants that were a_1 Sh_2/a_1 sh_2 and had no *Ac*. If *Ac* were not linked to a_1^{m-4}, only one-half of the a_1^{m-4} Sh_2 carrying gametes produced by plant 6110 would also carry *Ac*. And, if *Ac* controls mutation at a_1^{m-4}, then in only one-half of the a_1^{m-4} Sh_2 carrying kernels could mutations occur. Since a_1^{m-4} and Sh_2 are very closely linked, the expected ratio of kernel types from the described cross should be: 1 variegated, Sh_2: 2 colorless, Sh_2: 1 colorless, sh_2. The observed ratios were: 225 : 438 : 212, which is a close approximation to this expectancy.

Before presenting the evidence that establishes *Ac*-control of mutation at a_1^{m-4}, the phenotypes produced by mutation will be described. The initial change at A_1 resulted in complete inhibition of anthocyanin pigment formation in the aleurone layer of the kernel and in the plant tissue. Subsequent mutations occur. Two main classes which result in pigment formation can be recognized. In one class, the mutations reestablish the original phenotypic expression of A_1: deep pigmentation in aleurone and plant. In the aleurone layer, the borders of such mutant areas are not precisely defined because of diffusion of pigment forming substances into the surrounding nonmutant cells. This produces "diffusion rims" about these mutant areas. Within the second class, the mutations give rise to a graded series, with respect to intensity of anthocyanin pigmentation; from light to relatively dark shades. The borders of areas having these mutations are sharp. Diffusion rims, characteristic of the first class of mutation either are not present or are very weakly expressed. The phenotypes exhibited by the kernels carrying germinal mutations also express these same

two main classes of mutation. In these cases, however, the color intensity in any one kernel is the same throughout the aleurone layer. The plant tissues likewise show a graded series of intensity of pigmentation among the mutant areas. Marked changes in state of a_1^{m-4} that result in altered relative frequencies of the various types of mutation, were rarely observed. This is in contrast to a_1^{m-1} and a_1^{m-3}, both of which have produced many modified states, each exhibiting a particular type or types of mutation and/or frequency of type.

In order to determine that *Ac* controls mutation at a_1^{m-4}, several types of tests must be applied and positive results obtained from each. These are: (1) establishment of the presence of *Ac* in plants showing mutations at a_1^{m-4}; (2) establishing that a_1^{m-4} is nonmutable in the absence of *Ac* but that return to mutability will occur when *Ac* is again introduced into the nucleus; (3) establishing in this case that it is the same *Ac* factor known to produce breaks at *Ds* and to control mutations at other loci of known genic action, which is responsible for controlling mutation.

The presence of *Ac* can be detected because it produces breaks at *Ds*, wherever it may be located, or mutations at some loci of known genic action (see table 6). In order to be able to test readily for the presence of *Ac* in a particular plant, it has been necessary to develop so-called *Ac* tester stocks. Many of them utilize genetic markers in the short arm of chromosome 9 affecting the phenotype of the endosperm of the kernel, and also *Ds* at its standard location in this arm. For illustrative purposes, the test procedure with one such stock may be described. This stock is homozygous for the chromosome 9 endosperm markers *I*, Sh_1, *Bz* and *Wx*, and for *Ds* at its standard location, proximal to *Wx*. (*I*, inhibitor of aleurone pigment formation; dominant to allele *C*, required for aleurone color development. Sh_1, normal development of endosperm; sh_1, shrunken endosperm. *Bz* in presence of *C* produces dark color in aleurone and plant; with the recessive, *bz*, color is modified to a bronze shade. *Wx* produces amylose starch in endosperm and pollen grain which stains blue with I-KI solutions; no amylose starch produced by recessive, *wx*, and starch stains red-brown with I-KI solutions. The order of these markers in the short arm of chromosome 9 is: *I* Sh_1 *Bz Wx Ds* centromere.) No *Ac* factor is present in the plants of this stock. Consequently, no breaks at *Ds* occur in these plants. If, for example, the *Ac* constitution of a plant homozygous for *C*, sh_1, *bz* and *wx* is to be determined, a cross to or by the *Ac* tester plant is made. If the plant being tested has no *Ac*, all the kernels on the resulting ear will be colorless, nonshrunken, and *Wx*. If one *Ac* is present, half of the gametes produced by the *C*, sh_1, *bz*, *wx* plant will carry *Ac*. The other half will have no *Ac*. The kernels on the resulting ear will exhibit this gametic ratio for *Ac*. Those that do not have *Ac* will be totally colorless, nonshrunken, and *Wx*. Those that have *Ac* will be variegated. In these kernels, sectors will be present that show the collective recessive phenotype: *C*, sh_1, *bz* and *wx*. These sectors are produced because *Ac* induces breaks at *Ds* in the *I* Sh_1 *Bz Wx Ds* carrying chromosome in some cells during the development of the endosperm. These breaks result in the formation of acentric fragments carrying all of the dominant

markers. The fragment, in each case, is lost to telophase nuclei in subsequent mitoses. Therefore, all cells arising from those in which the dominant markers have been removed will express the collective phenotype *C*, sh_1, *bz* and *wx*. If the plant being tested has two *Ac* factors, located at allelic positions in one homologous pair of chromosomes, all of the kernels resulting from this cross should be variegated. (Usually, however, a small percent of the kernels are nonvariegated either because no *Ac* is present or because of a high dosage action of *Ac* which so delays the time of *Ds* breaks that none occur during the development of the kernel. These conditions arise from transposition or change in state of *Ac* that can occur in a few sporogenous cells of *Ac* carrying plants.) If two *Ac* factors are present and these are nonlinked, a ratio approximating 1 nonvariegated to 3 variegated kernels will appear on the ears resulting from the test cross. If more than two *Ac* factors are present, not only the ratios obtained but also the timing of breaks at *Ds* may be used to detect the number of *Ac* factors that are present. In these cases, however, verification must be obtained by progeny tests.

The type of test outlined above may be used to detect positions of *Ac* when it is located in the short arm of chromosome 9. For example, plants having the constitution *Ac C* Sh_1 *Bz Wx/ac C* sh_1 *bz wx* or ones that are *C* Sh_1 *Bz Ac Wx /C* sh_1 *bz ac wx* have been crossed by *Ac* tester plants that are homozygous for *I*, Sh_1, *Bz*, *wx*, *Ds* and have no *Ac*. The phenotypes exhibited in the sectors of the variegated kernels on the resulting ears, and the frequency of kernels with sectors of a particular type, indicate in each of these cases, the location of *Ac* within the short arm of chromosome 9. Subsequent tests of the progeny arising from the various crossover classes of kernels in each of these two cases, affirm the particular location of *Ac*.

Tests showing that *Ac* controls mutation at $a_1^{m\text{-}4}$ may now be described. Kernels having particular phenotypes were selected from the self-pollinated ear of plant 6110, and from the ears derived from the two crosses described above. In plant 6110, the chromosome 3 factorial constitution was: $a_1^{m\text{-}4}$ Sh_2/a_1 sh_2; the chromosome 9 factorial constitution was: *C Wx/C wx*. The necessity for indicating this latter constitution will become apparent as the tests are described. One *Ac* was present in plant 6110 but it was not linked to the marker in chromosome 9 and appeared not to be linked to those in chromosome 3. Four classes of kernels were selected from the self-pollinated ear: (1) colorless, Sh_2, *Wx*, (2) colorless, Sh_2, *wx*, (3) variegated, Sh_2, *Wx*, and (4) variegated, Sh_2, *wx*. Since the locus of Sh_2 is very close to that of $a_1^{m\text{-}4}$, plants derived from the first two classes of kernels were expected to be either $a_1^{m\text{-}4}$ $Sh_2/a_1^{m\text{-}4}$ Sh_2 or $a_1^{m\text{-}4}$ Sh_2/a_1 sh_2 in constitution and to carry no *Ac* factor. Those derived from types (3) and (4) above were expected to have either of these two constitutions with respect to markers in chromosome 3; they should have, however, either one or two *Ac* factors, and if two, these should be located, in the majority of such plants, at allelic positions in one pair of homologues.

The constitutions with respect to sh_2 in the plants derived from the above

TABLE 1

Constitution of plants of culture 6424 (column 1) and types of crosses (columns 3 to 8) that have revealed these constitutions for each plant.

Constitution of plant	Plant no.	Crosses with plants of constitutions given in column headings					
		Self-pollinated	$a_1 sh_2/a_1 sh_2$ No *Ac*	$a_1 Sh_2/a_1 sh_2$ No *Ac*	$a_1 Sh_2/a_1 Sh_2$ No *Ac*	*Ac* tester plant	a_1/a_1 1 *Ac*
$a_1^{m-4} Sh_2/a_1^{m-4} Sh_2$; No *Ac*	1		+	+			+
(plants 1 to 5)	2						+
	3		+	+		+	+
	4		+	+			+
	5			+		+	+
$a_1^{m-4} Sh_2/a_1^{m-4} Sh_2$; 1 *Ac*	6		+			+	
(plants 6 to 8)	7		+			+	
	8		+				
$a_1^{m-4} Sh_2/a_1^{m-4} Sh_2$; 2 *Ac*	9	+	+	+		+	
$a_1^{m-4} Sh_2/a_1 sh_2$; No *Ac*	10			+			+
(plants 10 to 17)	11			+			+
	12		+	+		+	+
	13			+		+	+
	14			+		+	+
	15			+		+	+
	16		+		+	+	+
	17		+	+		+	+
$a_1^{m-4} Sh_2/a_1 sh_2$; 1 *Ac*	18		+				
(plants 18 to 25)	19	+	+	+		+	
	20	+	+	+	+	+	
	21		+	+		+	
	22		+	+		+	
	23		+			+	
	24		+	+	+	+	
	25		+				
$a_1^{m-4} Sh_2/a_1 sh_2$; 2 *Ac*	26		+	+		+	+
(plants 26 to 29)	27	+	+	+		+	
	28		+	+			
	29		+				

selected kernels were determined by crosses to plants that were either $a_1\ sh_2/a_1\ sh_2$ or $a_1\ Sh_2/a_1\ sh_2$. (Supply of plants of the former constitution was limited due to early death from disease of many of them. In some of the tests it was necessary, therefore, to substitute plants of the latter constitution.) *Ac* constitutions were determined for many of them by crosses with *Ac* tester stocks. Detection of the presence of $a_1^{m\text{-}4}$ in plants derived from the colorless kernels required the introduction of *Ac*. For this purpose, plants that were homozygous for a_1 and that carried one or more *Ac* factors were used in crosses with the plants derived from these kernels. To show that the factor controlling mutation at $a_1^{m\text{-}4}$ is the same as that which controls breaks at *Ds*,

TABLE 2

Ac constitution of gametes of plants of culture 6424 (table 1) determined by crosses to plants of Ac-tester stock. Constitution of tester stock: 1 Ds/1 Ds; A_1/A_1; no Ac.

Ac constitution of plant	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types on resulting ear	
			Colorless (no *Ac*)	Variegated: colored areas in colorless background (*Ac*)
Group I, No *Ac*	3	♂	324	0
	5	♂	128	0
	12	♂	260	0
	14	♂	123	0
	15	♂	11	0
	16	♂	216	0
Group II, 1 *Ac*	6	♂	251	248
	7	♂	252	288
	19	♂	377	397
	20	♂	135	142
	20	♂	206	188
	21	♂	151	135
	22	♂	154	128
	23	♂	42	46
	23	♂	145	134
	24	♂	182	191
Group III, 2 *Ac*	9	♂	47	410
	26	♂	15	457
	27	♂	23	434

some plants arising from the colorless, Sh_2, *wx* class of kernels were crossed by plants with the constitution: a_1/a_1; *Wx Ds/wx ds*; 1 *Ac*. The *Ac* factor was not linked to markers in chromosome 9.

The constitutions of 29 plants in the progeny derived from self-pollination of plant 6110 are entered in table 1 along with the tests conducted with each plant that served to indicate its constitution. These plants were grown in culture number 6424. Nine plants in culture 6424 were $a_1^{m\text{-}4}\ Sh_2/a_1^{m\text{-}4}\ Sh_2$. Five of them had no *Ac* (plants 1 to 5, table 1), three had one *Ac* (plants 6 to 8, table 1) and one had two *Ac* factors (plant 9, table 1). Twenty plants in culture 6424 were $a_1^{m\text{-}4}\ Sh_2/a_1\ sh_2$ and among them, eight had no *Ac* (plants 10 to 17), eight had one *Ac* (plants 18 to 25), and four had two *Ac* factors

TABLE 3

Types of kernels appearing on ears resulting from crosses of plants of culture 6424 (table 1) with tester plants having constitutions entered in column 1. Part I: Tester plants have no Ac. Part II: Tester plants have 1 or 2 Ac factors.

Part I

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
a_1sh_2/a_1sh_2 No *Ac*	1	♀C+♂	0	0	0	0	230	0	230
	3	♀C+♂	0	0	0	0	133	0	133
	3	♂♀	0	0	0	0	573	0	573
	4	♀	0	0	0	0	298	0	298
Totals, plants 1 to 4			0	0	0	0	1234	0	1234
	6	♀+♂	1	0	132	0	126	0	259
	6	♂♀+♂	7	0	507	0	554	0	1068
	7	♀+♂	1	0	132	0	151	0	284
	7	♂♀+♂	15	0	275	0	295	0	585
	8	♀+♂	3	0	168	0	188	0	359
Totals, plants 6 to 8			27	0	1214	0	1314	0	2555
	9	♂♀	26	0	584	0	95	0	705
	12	♀+C+♂	0	0	0	0	120	125	245
	16	♀+C+♂	0	0	0	0	131	120	251
	16	♂♀+♂	0	0	0	0	144	137	281
	17	♀+♂	0	0	0	0	65	55	120
Totals, plants 12 to 17			0	0	0	0	460	437	897
	18	♀+C+♂	0	0	75	0	73	134	282
	19	♀+C+♂	3	0	62	0	83	167	315
	19	♂♀+♂	12	0	199	0	216	375	802
	20	♀+♂	0	0	53	0	58	114	225
	20	♂♀+♂	12	0	234	0	267	444	957
	21	♀+C+C+C+♂	4	0	86	0	75	168	333
	22	♀+C+C+C+♂	1	0	99	0	79	154	333
	23	♀+C+C+C+♂	1	0	69	0	70	157	297
	23	♂♀+♂	20	0	179	1	185	333	718
	24	♂♀+♂	9	0	174	3	210	363	759
	25	♀+C+♂	1	0	54	0	72	123	250
Totals, plants 18 to 25			63	0	1284	4	1388	2532	5271
	26	♀+♂	1	0	166	1	0	152	320
	26	♂♀+♂	4	0	140	0	6	141	291
	27	♀+C+♂	0	0	134	0	35	178	347
	28	♀+C+♂	0	0	151	0	1	146	298
Totals, plants 26 to 28			5	0	591	1	42	617	1256
	29	♀	0	0	72	0	28	125	225

TABLE 3 (*continued*)

Part I (*continued*)

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
$a_1 Sh_2/a_1 sh_2$	1	♂	0	0	0	0	573	0	573
No *Ac*	3	♂	0	0	0	0	934	0	934
	4	♂	0	0	0	0	846	0	846
	5	♂	0	0	0	0	894	0	894
Totals, plants 1 to 5			0	0	0	0	3247	0	3247
	9	♂	22	0	392	0	49	0	463
	10	♂	0	0	0	0	364	106	470
	11	♂	0	0	0	0	731	236	967
	12	♂	0	0	0	0	705	210	915
	13	♂	0	0	0	0	715	234	949
	14	♂	0	0	0	0	644	200	844
	15	♂	0	0	0	0	431	127	558
	17	♂	0	0	0	0	192	86	278
Totals, plants 10 to 17			0	0	0	0	3782	1199	4981
	19	♂	4	0	182	0	385	198	769
	20	♂	8	0	108	0	185	88	389
	21	♂	12	0	205	0	438	224	879
	22	♂	2	0	206	0	525	249	982
	24	♂	6	0	130	1	237	134	508
Totals, plants 19 to 24			32	0	831	1	1770	893	3527
	26	♂	8	0	532	0	294	280	1114
	28	♂	3	0	457	0	243	194	897
Totals, plants 26 and 28			11	0	989	0	537	474	2011
$a_1 Sh_2/a_1 Sh_2$	16	♂	0	0	0	0	535	0	535
No *Ac*	20	♂	8	0	132	0	432	0	572
	24	♂	3	0	111	0	347	0	461
Totals, plants 20 and 24			11	0	243	0	779	0	1033
Part II									
$a_1 sh_2/a_1 sh_2$	4	♂	0	0	244	0	243	0	487
1 *Ac*	11	♂	0	0	78	0	75	145	298
	12	♂	0	0	76	0	60	123	259
	13	♂	0	0	125	0	111	268	504
	15	♂	0	0	34	0	35	60	129
	16	♂	0	0	68	0	62	135	265
	17	♂	0	0	54	0	61	149	264
Totals, plants 11 to 17			0	0	435	0	404	880	1719

TABLE 3 (*continued*)

Part II (*continued*)

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
a_1Sh_2/a_1sh_2 1 *Ac*	1	♂	0	0	251	0	210	0	461
	2	♀♂	0	0	161	0	158	0	319
	3	♂♂	0	0	387	0	384	0	771
	4	♂♂	0	0	157	0	157	0	314
	5	♀	0	0	119	0	115	0	234
Totals, plants 1 to 5			0	0	1075	0	1024	0	2099
	10	♀♀	0	0	117	0	203	113	433
	12	♀♀	0	0	98	0	184	83	365
	14	♀♀	0	0	103	0	202	100	405
	15	♂	0	0	122	0	207	122	451
Totals, plants 10 to 15			0	0	440	0	796	418	1654
a_1Sh_2/a_1Sh_2 1 *Ac*	10	♂	0	0	54	0	143	0	197
a_1Sh_2/a_1sh_2 2 *Ac*, non-allelic and non-linked	12	♂	0	0	248[1]	0	228	150	626

[1] 88 of these kernels had relatively few, late occurring mutations (4 *Ac*); 160 had many more mutations that occurred earlier in the development of the kernel (2 *Ac*).

(plants 26 to 29). The plants having no *Ac* factor were derived from the kernels that were colorless. Those carrying *Ac* were derived from the variegated kernels. There were no exceptions.

Direct tests for the presence of *Ac* were conducted with 19 of the 29 plants in culture 6424. The *Ac* tester stock used in tests of 17 of them was homozygous for A_1 and carried *I* and *Ds* (standard location) in each chromosome 9; no *Ac* was present. The types of kernels appearing on the ears when crosses were made to plants in the tester stock are entered in table 2. The nature of this test has been described above but may be summarized here. If all of the kernels are colorless, then the plant being tested has no *Ac*. If half of the kernels are colorless and the other half are variegated (colored areas in a colorless background), one *Ac* factor is present in the plant being tested. If nearly all of the kernels show colored areas in a colorless background, then the plant being tested carries two *Ac* factors. Two of the 19 plants directly tested for the presence of *Ac* (plants 13 and 17) carried *wx* in each chromosome 9. The silks of these two plants received pollen from plants homozygous for a_1 and carrying *C*, *Wx* and *Ds* at its standard location in one chromosome 9 and *C*, *wx* and *ds* in the homologue; no *Ac* was present in these plants. All of the kernels on the resulting ears were colorless; also, in those kernels that were *Wx*, no *wx* sectors were present, indicating the absence of *Ac* in these two plants.

The types of kernels produced on ears resulting from crosses of these 29 plants of culture 6424 to plants homozygous for a_1 are entered in table 3. The a_1 stocks were either homozygous for sh_2, heterozygous for sh_2, or homozygous Sh_2. Table 3 has two parts. In part I are entered the types and frequency of type of kernels appearing on ears derived from crosses of 28 of the 29 plants with plants homozygous for a_1 and having no *Ac* factor. In part II are entered the types and frequency of type of kernels appearing when plants derived from the colorless, Sh_2 classes of kernels in culture 6424 (plants 1 to 5 and 10 to 17) were crossed with plants homozygous for a_1 but carrying an *Ac* factor.

An examination of the data entered in part I of table 3 will reveal that all of the plants having one or two *Ac* factors, as determined by direct tests (Groups II and III, table 2), produced variegated kernels in crosses with plants having no *Ac* factor. Also, germinal mutations were evident. Those plants in culture 6424 with no *Ac* (Group I, table 2), on the other hand, produced only colorless kernels from the same type of cross. When, however,

TABLE 4

Relation of mutation at a_1^{m-4} in chromosome 3 to breaks at Ds in chromosome 9. Constitution of parents: ♀ a_1^{m-4}/a_1^{m-4}; wx/wx, no Ac × ♂ a_1/a_1; Wx Ds/wx ds; 1 Ac.

Parent plants		Kernel types						Totals
		Variegated aleurone		Colorless aleurone		Variegated aleurone	Colorless aleurone	
♀	♂	*Wx*	*wx* areas in *Wx* background	*Wx*	*wx* areas in *Wx* background	*wx*	*wx*	
6424-2	6427B-9	5	61	77	0	102	103	348
6424-3	6427B-8	7	89	101	0	86	105	388
6424-4	6427B-7	6	50	60	0	63	55	234
Totals		18	200	238	0	251	263	970

these same plants (3 and 5; 12 to 17) were crossed with plants homozygous for a_1 but carrying an *Ac* factor, variegated kernels appeared (part II, table 3). In both part I and part II of this table it may be seen that the variegated kernels were present in the expected classes, and in the expected frequency within each class, on the assumption that *Ac* is the factor responsible for the occurrence of mutation at a_1^{m-4}.

Results of the test, recorded in table 4, leave no doubt concerning *Ac*-control of mutation at a_1^{m-4}. It is the same *Ac* factor that is responsible for producing breaks at *Ds*, wherever it may be located, and for controlling mutability at some other loci of known genic action (see table 6). This test was conducted with plants 2, 3 and 4, each of which was homozygous for a_1^{m-4} and Sh_2 in chromosome 3 and for *wx* in chromosome 9. Pollen from plants homozygous for a_1, and carrying *C*, *Wx* and *Ds* (standard location) in one chromosome 9 and *C*, *wx* and *ds* in the homologue and also a single *Ac* factor, not linked with the markers in chromosome 9, was placed on the silks of these three plants. If *Ac* is the factor controlling mutation at a_1^{m-4}, the types of kernels on the resulting ear, and the frequency of each type, may be predicted. In the *wx*

class, two types of kernels should appear. Half of them should be colorless (no *Ac*) and half should show variegation for color in the aleurone layer due to mutations at $a_1^{m\text{-}4}$ (*Ac* present). In the *Wx* class of kernels, three types should appear. Half of the *Wx* kernels should be colorless, and no *wx* sectors should appear within these kernels (no *Ac*). The other half of the *Wx* kernels should be variegated for aleurone color (*Ac* present) and the majority of them should also have sectors showing the *wx* phenotype. This is because *Ds* is located close to *Wx*. Relatively little crossing over occurs between the loci of these two factors. Thus, the majority of the *Wx* class of kernels will have *Ds* in the *Wx*-carrying chromosome; in only a small percentage of them will *Ds* be absent. When *Ac* is present, breaks occur at *Ds* in some of the cells during the development of the kernel. These result in elimination of *Wx*. As a consequence, sectors having the *wx* phenotype will appear in the mature kernels.

Among a total of 970 kernels entered in table 4, 501 were completely colorless and 469 were variegated for aleurone color. Within the colorless class, 263 were *wx* and 238 were *Wx*. In these *Wx* kernels, no sectors showing the *wx* phenotype appeared. Within the variegated class of kernels, 251 were completely *wx* and 218 were *Wx*. Among these *Wx* kernels, on the other hand, sectors exhibiting the *wx* phenotype were present in 200 of them. In only 18 of them were *wx* sectors absent. It is obvious, therefore, that the factor controlling breaks at *Ds*—that is, *Ac*—is also responsible for mutation at $a_1^{m\text{-}4}$. Without *Ac*, neither breaks at *Ds* nor mutations at $a_1^{m\text{-}4}$ will occur. When *Ac* is present, both will occur. With this information in mind, it is possible to interpret readily the data entered in tables 2 and 3 that have not received detailed comment. It is only necessary to indicate the essential correlations that these data reveal.

In direct tests of *Ac* constitutions, table 2, eight plants proved to have one *Ac* factor (plants 6 and 7, and 19 to 24). Plants 6 and 7 were homozygous for Sh_2. Since Sh_2 is very closely linked to $a_1^{m\text{-}4}$, these plants could be expected to be homozygous for $a_1^{m\text{-}4}$. When they are used in crosses to plants homozygous for a_1 and carrying no *Ac*, a ratio of 1 variegated to 1 nonvariegated kernel should appear on the resulting ear. This ratio was obtained, as shown in part I of table 3. Plants 19 to 24 were Sh_2/sh_2. Therefore, they are expected to be: $a_1^{m\text{-}4}\ Sh_2/a_1\ sh_2$. When crossed to plants homozygous for a_1 and having no *Ac*, a ratio of 1 variegated to 3 nonvariegated kernels should appear on the resulting ear. If the tester plant is also homozygous for sh_2, the variegated kernels would be present almost exclusively in the Sh_2 class, and the ratio of kernel type in this class would be 1 variegated to 1 nonvariegated. The ratios obtained from tests of these six plants (part I, table 3) fit this expectancy.

Direct tests of *Ac* constitutions showed that plants 9, 26 and 27 had two *Ac* factors (table 2). Plant 9 was homozygous for Sh_2 and therefore could be expected to be homozygous for $a_1^{m\text{-}4}$. The ratio of variegated to nonvariegated kernels on the ears resulting from crosses to plants homozygous for a_1 and having no *Ac* factor, should closely reflect the gametic ratio of presence and absence of *Ac*. The ratios obtained (part I, table 3), show that this expectation

is fulfilled. (Because some of the gametes produced by these plants will not carry *Ac*, a small percent of the kernels should be colorless, nonvariegated. Also, a few of them may have *Ac* but carry an altered state of a_1^{m-4} that no longer gives visible mutations or gives so few of them that, in individual kernels, none may have occurred. In order to distinguish between these two possibilities, it is necessary to conduct progeny tests with the plants arising from the nonvariegated kernels.) Plants 26 and 27 were Sh_2/sh_2 and therefore could be expected to be heterozygous for a_1^{m-4}. Approximately 1 variegated to 1 nonvariegated kernel should appear in the backcross test to plants homozygous for a_1 but having no *Ac*. If the tester plant is also homozygous for sh_2, the variegated kernels should be confined, with few exceptions, to the Sh_2 class. As the data in table 3 show, this expectation is fulfilled.

Once the fact is established that *Ac* controls mutation at a_1^{m-4} and that this mutable locus responds in the expected manner to increased doses of *Ac*, it is no longer necessary to apply to each plant all of the tests that have been outlined above. Constitutions may be determined by ratios of kernel types in backcross tests. Plants carrying altered states of a_1^{m-4} or changes of *Ac* affecting its state, location, or number, may be identified by such tests. In these latter cases, however, progeny tests often must be conducted to establish with certainty a particular conclusion drawn from the backcross test.

Mutation or change in state of an *Ac*-controlled mutable locus rarely occurs early in the development of a plant, although a few such occurrences have been noted in all examined cases. Most of the changes at the locus take place in the later developmental stages of the sporophytic tissues. It is such late occurring mutations that are responsible for the completely colored kernels recorded in part I of table 3. Such kernels did not appear in the tests recorded in part II of this table. In this part of the table, the a_1^{m-4} carrying plants did not have *Ac*. Thus, no germinal mutations were expected and none were found. The types of germinal mutation occurring in plants having *Ac* resemble in kind those appearing in the variegated kernels. Among them are darkly pigmented kernels and kernels showing various lighter shades of pigmentation.

The results recorded in table 3 suggest that very little crossing over occurs between a_1^{m-4} and Sh_2. Only 6 variegated kernels appeared in the sh_2 class, and 4 of these 6 appeared when pollen from one plant was used, plant 24. Three of them appeared in crosses that were made with a single collection of pollen from this plant. This suggests that mutation rather than crossing over may be responsible for some of the variegated kernels in the sh_2 class.

Similar tests, although less extensive than those outlined above for the progeny derived from self-pollination of plant 6110, were conducted with plants arising from selected kernels resulting from crosses of this plant to plants that were $a_1\ Sh_2/a_1\ sh_2$, and had no *Ac*, and to a plant that was $A_1\ Sh_2/a_1\ sh_2$ and carried one *Ac*. From this latter cross, variegated kernels considered to have received an *Ac* factor from each parent were selected. These exhibited only a few specks of color scattered over the aleurone layer. Tests of 6 plants arising from them showed the presence of two *Ac* factors, but these were not linked

to each other and not linked to the marked factors in chromosome 3. Direct tests for *Ac* were conducted with several of these plants. A gametic ratio of 3 with *Ac* to 1 with no Ac was obtained. The ratio of variegated to nonvariegated kernels obtained when these plants were crossed to ones homozygous for a_1 and having no *Ac* gave the expected ratios: 3 variegated to 1 nonvariegated kernel among those that received a_1^{m-4} (table 5).

The same types of tests that were used in the analysis of a_1^{m-4} were also conducted with plants carrying a_2^{m-3} and a_2^{m-4}. It was soon evident that mutations occurring at a_2^{m-3} are not *Ac*-controlled. At a_2^{m-4}, however, mutation is

TABLE 5

Types and frequency of type of kernels on ears resulting from cross of ♀ a_1/a_1; *no Ac* × ♂ a_1^{m-4} Sh_2/a_1 sh_2; *2 non-linked Ac factors.*

Constitution of ♀ parent with reference to Sh_2 and sh_2	♂ Parent	Kernel types						Totals
		Colored		Variegated		Colorless		
		Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	
sh_2/sh_2	6426A-1	1	0	48	0	13	65	127
	" -5	6	0	214	0	88	318	626
	" B-2	1	0	97	1	22	107	228
Totals		8	0	359	1	123	490	981
Sh_2/sh_2	6426A-1	8	0	112	0	142	87	349
	" -2	29	0	443	0	479	315	1266
	" B-1	9	0	145	0	173	107	434
	" -2	8	0	213	0	252	155	628
	" -3	29	0	380	0	412	234	1055
Totals		83	0	1293	0	1458	898	3732
Sh_2/Sh_2	6426A-1	12	0	153	0	302	0	467
	" -2	26	0	267	0	576	0	869
Totals		38	0	420	0	878	0	1336

Ac-controlled; and a_2^{m-4} responds to *Ac* in a manner comparable to that observed for all other known *Ac*-controlled mutable loci (see table 6). A detailed account of this analysis will not be included here.

DISCUSSION

From examination of instability of genic action at a number of known loci in maize, it is concluded that mutations need not express changes in genes, but may be the result of changes affecting the control of genic action. It can be shown that this control is effected by nongenic agents carried in the chromosomes. Different agents are present, and may be distinguished by specificities they exhibit in their mode of control of the action of genes. It has been learned that the same agent may operate at different loci of known genic action and that different agents may operate at any one locus. These agents, therefore, reflect the presence of " extragenic " systems carried in the chromosomes, which control genic expression. One of them is the *Ds-Ac* two-unit system,

TABLE 6

Known loci (column 1) at which mutability, under the control of Ac, has arisen (column 2) and mutability at the same loci controlled by other systems (column 3). (The figures following the symbols represent the sequence of appearance in the Cold Spring Harbor cultures.)

Symbol of normal, dominant factor at locus	Instability controlled by *Ac*	Instability controlled by system other than *Ac*
C	c^{m-1}	c^{m-3}
	c^{m-2}	
	c^{m-4}	
Sh_1	$sh^{m\,1}$	
Bz	bz^{m-1}	bz^{m-3}
	bz^{m-2}	
	bz^{m-4}	
Wx	wx^{m-1}	wx^{m-2}
	wx^{m-5}	wx^{m-3}
	wx^{m-6}	wx^{m-4}
A_1	$a_1{}^{m-3}$	$a_1{}^{m-1}$
	$a_1{}^{m-4}$	$a_1{}^{m-2}$
A_2	$a_2{}^{m-4}$	$a_2{}^{m-1}$
		$a_2{}^{m-2}$
		$a_2{}^{m-3}$

[1]For origins of sh^m from *Sh*, see MCCLINTOCK 1952.

whose action at the locus of A_1 has been considered in this report. Detailed summaries of its operation are given elsewhere (McCLINTOCK 1951). Therefore, considerations of the behavior of this system need not be repeated here.

Instability of genic action, under the control of the *Ds-Ac* system, has been examined at six known loci (table 6). At four of them (*C*, *Bz*, A_1 and A_2) genic action is associated with development of anthocyanin pigmentation. At one of them, *Wx*, it is associated with the chemical constitution of starch in endosperm and pollen grain. In the sixth case, Sh_1, it is associated with a morphological structure of the endosperm. At five of these six loci, instability under the control of other systems has appeared in the Cold Spring Harbor cultures, as indicated in the table. There appears to be no relation between the primary type of action of the gene and the type of system that can serve to control it.

Inception of instability at a locus of known genic action is interpreted as the result of the insertion of a specific controlling unit at or adjacent to the locus. Such insertion is an expression of the phenomenon of transposition, which is characteristic of controlling units. Transposition in the case of *Ds* or *Ac* may be detected readily, and its mechanism has been analyzed (McCLINTOCK 1951). It is initiated by chromosome breaks and fusions that occur at the sites of the controlling units; and it results in their removal and insertion at other positions in the chromosome complement.

In this two-unit system, it is the *Ds* component that is responsible, when inserted at the locus of a gene, for modification of the action of the gene; whereas the function of *Ac* is to control the occurrence of changes at *Ds*. Some of the initiating events at *Ds* result in detectable chromosome alterations. Others produce changes that are not microscopically visible and these probably are responsible for most of the observed cases of altered genic expression. Removal of *Ds* from the locus of the gene is one of them. Others may result in modifications of the chromatin material at the locus, either structurally, quantitatively, or by insertion of chromatin from elsewhere. In this case, a particular type of change in genic expression might reflect a particular type or types of change in chromatin components at the locus. Evidence in support of this assumption has been obtained (McCLINTOCK unpublished).

The *Ac* component also undergoes transposition, and its insertion at new positions has been noted; but no cases of direct *Ac* control of genic change have been observed. *Ac* controls the occurrence of changes at *Ds*, wherever *Ds* may be located. Because methods of detecting the presence of *Ac* are both simple and sure, it is possible to determine, in any case of mutability, whether or not it is controlled by the *Ds-Ac* system. Also, because *Ds* may be transposed to various positions in the chromosome complement, inception of *Ac*-controlled mutability at different known loci can be anticipated. It is only necessary to provide adequate means for their detection, such as those outlined in this report.

Examination of many different cases of instability of genic expression has shown that the basic mechanism producing it is alike in all cases. The mutation results from a change occurring at the locus of a gene during a mitotic cycle. The locus in each sister chromatid may be altered, but the modification need not be alike in both. Consequently, genic expression may differ in the two resulting cells and in their progeny. The factor controlling the time of occurrence of changes at the locus of a gene in future cell and plant generations—that is, the *Ac* factor in the case described here—may likewise be altered during mitotic cycles. As a consequence, sister chromatids may differ with respect to this controlling unit. Segregation of sister chromatids at anaphase will give rise to two cells that may differ with regard to this component. In the progeny of these two cells, this difference will be expressed at the locus of the gene whose mutation it controls. A twin sector or a twin spot may appear in the mature tissue. The two components of the sector or spot may show marked differences in mutation time and/or frequency. (A twin sector may be recognized if the mutation affects a phenotypic change that can be expressed in any cell of the tissue, such as mutation from *wx* to *Wx* in the endosperm. A twin spot will appear if the mutation can be expressed only in a restricted part of the tissue, such as anthocyanin pigmentation in the endosperm tissue which is confined to the outermost layer, the aleurone layer. In this latter case, however, the presence of twin spots reflects the presence of twin sectors.) The two sectors of a twin may express differences in various ways: by absence of mutation in one and presence in the other, by changes in mutation time in the sector

showing mutation, or by changes in mutation time in each sector. If, during a single mitosis, a change occurs at the locus of the gene and also at the locus of the controlling unit, the result may be twin sectors that exhibit differences in type of mutation as well as in time of occurrence; that is, they may differ in presence or absence of mutation, in mutational type in each sector, and in time of mutation. In the *Ds-Ac* system, it is known that such coincidences occur with high frequency.

Recently, BRINK and NILAN (1952) and BRINK (1953) have presented evidence, from a study of variegated pericarp in maize, that supports the generalizations stated here and elsewhere (MCCLINTOCK 1950, 1951) concerning the existence and behavior of controlling units. Recognizing the significance of twin spots, they analyzed the progeny of several of them. The examples selected were characterized by mutation to dominant *P* in one spot and change in pattern of variegation in the twin. The time and frequency of occurrence of mutation was much altered in the latter. Examination of progenies derived from spots of the latter type revealed the presence of a mendelizing unit, which BRINK and NILAN call Modulator because its presence is responsible for the altered pattern of mutation. This unit, in the progeny of some spots, was inherited independently of *P*. In the progeny of others, it showed linkage to *P*. A striking similarity to *Ac* is evident in this case. With respect to *Ac* it is already known that plants having one *Ac* factor may produce sectors in which two *Ac* factors are present. In some of them, the two *Ac* units occupy different positions in the chromosome complement: one at the former location and one at a new location, or both at new locations. They may also show independent inheritance in some cases and linked inheritance in others.

Evidence obtained by PETERSON (1953) in a study of mutability at a locus in maize concerned with chlorophyll development (pale green to green) suggests the presence in this case also of a controlling unit, which likewise appears to undergo transposition.

In a discussion of the possible significance of the evidence obtained from studies of genic instability (MCCLINTOCK 1951), it was suggested that controlling units are present in all nuclei, and that they serve to regulate genic action during the development of an organism. Their presence, in the cases of observed genic instability, has been revealed by their displacement. Such displacement, involving units of any one system, might arise in any strain of maize. Detection of a displaced unit would depend upon available methods. In the case of *Ac,* tester stocks have been developed that can reveal its presence. *Dt* (Dotted), a controlling unit somewhat similar to *Ac* in its mode of action, can be detected through the behavior of the recessive a_1; when *Dt* is present, a_1 mutates to higher alleles of A_1. NUFFER (1953) examined a number of strains of maize from various geographic regions in order to determine if *Dt* was present in any of them. It was found in one strain from Brazil and in another strain from Peru. The original *Dt,* discovered by RHOADES, appeared in a strain originating in Mexico; and its location was determined as in or adjacent to the terminal knob of the short arm of chromosome 9. The locus of

Dt in the strain coming from Brazil is not in the chromosome 9 short arm; linkage studies suggest that it is located in chromosome 6. The locus of *Dt* in the Peruvian strain has not yet been determined. All three of these *Dt* factors behave alike in their mode of producing mutation at a_1. Since transposition, often without change in action, characterizes controlling units, it is not surprising that *Dt* units are present in unrelated strains of maize, and that their locations in the chromosome complement differ. As further methods are devised to detect controlling units, it is anticipated that more of them will be discovered.

SUMMARY

Previous studies of the origin and mode of expression of genic instability at a number of known loci in maize led to the following conclusions. Extragenic units, carried in the chromosomes, are responsible for altering genic expression. When one such unit is incorporated at the locus of a gene, it may affect genic action. The altered action is detected as a mutation. Subsequent changes at the locus, initiated by the extragenic unit, again can result in change in genic action; consequently, a new mutation may be recognized. The extragenic units undergo transposition from one location to another in the chromosome complement. It is this mechanism that is responsible for the origin of instability at the locus of a known gene; insertion of an extragenic unit adjacent to it initiates the instability. The extragenic units represent systems in the nucleus that are responsible for controlling the action of genes. They have specificity in that the mode of control of genic action in any one case is a reflection of the particular system in operation at the locus of the gene.

One extragenic system controlling genic expression is composed of two interacting units. It is the so-called Dissociation-Activator (*Ds-Ac*) system. Both *Ds* and *Ac* undergo transposition. The *Ds* component, when inserted at the locus of a gene, is responsible for modification of genic expression. Subsequent changes at the locus, initiated by *Ds*, result in further modification of genic expression. The *Ac* component in this two-unit system controls when the changes at *Ds* will occur. From the conclusions stated above, it was anticipated that the *Ds-Ac* system could operate at any locus of known genic action. This is because the *Ds* unit may be transposed to various locations in the chromosome complement. To obtain this type of instability at any one locus of known genic action, it is only necessary to provide adequate means for its detection. The methods used to obtain and detect this type of instability at the A_1 locus in chromosome 3 and at the A_2 locus in chromosome 5 are described. A detailed analysis of one such case is presented in this report.

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