FURTHER STUDIES OF THE SUPPRESSOR-MUTATOR SYSTEM OF CONTROL OF GENE ACTION IN MAIZE

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For many years, evidence of the presence in the chromosome complement of distinct genetic components that control the action of genes was available only from studies of maize, and these components were designated "controlling elements." Recently, however, controlling elements have been identified in bacteria, where it

is possible to explore at the chemical level some aspects of their mode of action. It is expected that continued examination of these elements in bacteria will lead to an appreciation of the mechanism of their operation, with a precision that would be difficult or impossible to duplicate in maize. Nevertheless, at the phenotypic level, the performances of the bacterial and maize control systems exhibit parallels that suggest basic similarities in gene control in these two widely separated organisms. The bacterial systems, as described by Jacob and Monod, are composed of two genetic elements, each distinct from the "structural" gene. One of them, termed the "operator," is adjacent to the structural gene (or sequence of structural genes), and directly controls its activation. The structural gene is considered to carry the code that is responsible for a particular sequence of amino acids and thus for the specificity of a protein. When the structural gene is activated, this protein is formed. The second element of the system, termed the "regulator," may be located either near the structural gene or elsewhere in the bacterial chromosome. The regulator is responsible for the production of a repressor substance, not a protein, that appears in the cytoplasm. The operator element responds in some yet unknown manner to a change in degree of effective action of the repressor substance by turning on or turning off the functioning of the structural gene in accordance with such change. Each operator-regulator system is specific, in that an operator will respond only to the particular product of the regulator of its system.

In maize, likewise, some of the control systems are composed, basically, of two elements. One, closely associated with the structural gene and directly controlling its action, can be likened to the operator element in bacteria. The other, which may be located near the first element or may be independently located in the chromosome complement, establishes the conditions to which the gene-associated element responds, a particular change in these conditions being reflected in a particular change in action of the gene. It thus is comparable to the regulator element in bacteria. In maize, as in bacteria, each operator-regulator system is quite specific: an operator element will respond only to the regulator element of its own system.

Discovery of the presence of controlling elements in maize was made possible by the fact that under certain conditions a controlling element may be transposed from one location to another in the chromosome complement. Transposition of the operator-like element to the locus of a gene will bring the action of that gene under the control of the system to which the element belongs.

During the year, studies of one of these systems were extended. The element that is comparable to the regulator in this system is designated Suppressor-mutator (*Spm*), and the system has been referred to in the past as the *Spm* system of control of gene action.

Knowledge of the function of the *Spm* system was initially obtained by studying gene action at the A_1 locus in chromosome 3 and the A_2 locus in chromosome 5 after the operator element of the system had been inserted at each of these loci. The modified loci were designated a_1^{m-1} and a_2^{m-1}. Three additional inceptions of control of gene action by this system have now been investigated. Two of them again involve the standard A_1 locus in chromosome 3, and are designated a_1^{m-2} and a_1^{m-5}. The symbols *m*–1, *m*–2, and *m*–5 refer to the order in time of inception of control of gene action of A_1 by the *Spm* system (*m*–3 and *m*–4 refer to inceptions of control of gene action at A_1 by the *Ds-Ac* control system). The third inception involves the locus of *Wx* in chromosome 9, and the modified locus is designated wx^{m-8}. (Both A_1 and A_2 are associated with anthocyanin pigment formation in plant and kernel. *Wx* is associated with production of amyl-

se in the pollen grain and the endosperm of the kernel.) The type of evidence that has allowed recognition of control of gene action at a_1^{m-2}, a_1^{m-5}, and wx^{m-8} by the Spm system will be reviewed in the following sections.

Control of a_1^{m-2} by the Spm System

Early in the study of a_2^{m-1}, an ear of a plant that was A_1/a_1 (standard recessive); A_2/a_2 in constitution was utilized in a cross with a plant that was homozygous for the standard recessive, a_1, and for the standard dominant, A_2. (The standard recessive, a_1, is completely stable in the presence of *Spm*. There is an operator component at the locus of standard a_1, but it belongs to the control system of which Dotted, *Dt*, is the regulator. The a_2 in the heterozygous parent plant was derived from mutation at a_2^{m-1}, which had occurred in a cell whose nucleus also carried active *Spm*.) The phenotypes of the kernels on the ear produced by this cross were those expected, with one exception. Instead of being either fully pigmented or totally colorless, the exceptional kernel exhibited spots of pigment in a nonpigmented background, suggesting that the A_1 locus had been modified in a cell of the ear-bearing parent plant, whose progeny cells gave rise to the kernel. The plant derived from this kernel also exhibited variegation for anthocyanin pigmentation, and tests conducted with it confirmed the presence of a modified A_1 locus, which was then designated a_1^{m-2}.

Initial tests of a_1^{m-2}, conducted some years ago, showed that mutations occur at this locus and that they result in two distinct types of mutants, whose subsequent expressions are stable. Gene action, in both plant and kernel, expressed by one type (type 1) resembles that produced by the standard A_1. Mutants of the second type (type 2) occur far more frequently, and fall into a graded series with respect to ability to form anthocyanin pigment. A small percentage of them exhibit the null expression, but most of them produce pigment in the aleurone layer; the intensity of pigmentation, ranging from faint to rather deep, distinguishes the mutants from one another. All type-2 mutants that produce pigment, however, are characterized by differences in intensity among individual cells of the aleurone layer: the layer is speckled with cells in which the pigmentation is much more intense than in surrounding cells. The expression of type-1 and type-2 mutants also differs in the plant. With respect to distribution of anthocyanin pigment to various parts of the plant, and response of the pigment-producing system to sunlight, the expression of type-1 mutants resembles that associated with standard A_1. When a type-2 mutant that produces pigment is present, the pigment is restricted to certain parts of the plant, and the response of the pigment-producing system to sunlight is much retarded.

The early studies of a_1^{m-2} were conducted with only three successive generations of plants, and the system responsible for control of its behavior was not then identified. Some years later when the *Spm* system had been identified through analyses of a_1^{m-1} and a_2^{m-1} behavior, it was suspected that this system was also responsible for control of gene action at a_1^{m-2}. Consequently, examination of a_1^{m-2} was recommenced, and a number of exploratory tests were conducted with it during the past year. They revealed that gene action at a_1^{m-2} is under the control of the *Spm* system. The results also suggest that in this instance *Spm* is located close to the structural gene(s) of the A_1 locus, and that its transposition away from that locus is associated with the induction of the above-described mutants. It is possible here, as with bz^{m-2} (see Year Book 55), that the operator and regulator components both reside at or near the locus of the structural gene, and that mutation-inducing events are usually associated with removal of both elements from the vicinity of the locus. If so, removal of the regulator

element *Spm* without concomitant removal of the operator element should occur on rare occasions, and a search that may reveal this event is under way.

The conclusion that the *Spm* system controls gene action at a_1^{m-2} is derived from several types of observation. In the first place, *Spm* was present in each of 44 a_1^{m-2}-carrying plants that were tested for its presence. Its location was very close to a_1^{m-2} in plants that had only one *Spm* element; in plants having two such elements, one was located close to a_1^{m-2}. Some plants had three or more independently located *Spm* elements, but when so many were present the tests did not give conclusive evidence of their relative locations. The second pertinent observation concerns *Spm* constitution and location in plants carrying a stable mutant of a_1^{m-2}. In crosses of a_1^{m-2}-carrying plants to plants homozygous for standard a_1 and having no *Spm*, some kernels carrying a germinal mutation may be present on the ears in addition to those that have received unmodified a_1^{m-2}. Tests of the presence or absence of *Spm* in plants from both types of kernels revealed that *Spm* was present in each plant derived from a kernel carrying an unmodified a_1^{m-2}, and that when only one *Spm* was present it was located close to a_1^{m-2}. In contrast, some of the plants derived from the mutant class of kernels had no *Spm*, whereas in others, although *Spm* was present, it was never found to occupy a position adjacent to the locus of the mutant.

The third pertinent observation is concerned with *Spm* constitution in the parts of ears of a_1^{m-2}/a_1 plants that are derived from cells in which a mutation at a_1^{m-2}, producing a stable allele, has occurred early in plant development. If such an ear is tested for the presence or absence of *Spm* in individual kernels, it is possible to learn whether this element was present in the cells that produced the mutant sector, and, if so, its position in relation to the mutant locus. On some ears exhibiting such mutant sectors, no evidence of *Spm* was observed among the kernels within the sector, although it was present in kernels on other parts of the same ear. On other ears, *Spm* was present in the cells that produced the sector but was not closely linked with the mutant phenotype.

The above-described observations conformed with the assumption that *Spm* is responsible for control of gene action at a_1^{m-2}; but the decisive evidence was obtained from observations of the response of a_1^{m-2} to change in phase of activity of *Spm*. When *Spm* is in its inactive phase, no gene action at a_1^{m-2} is expressed in either plant or kernel, and consequently anthocyanin pigment is absent. When *Spm* is in its active phase, however, mutations occur that permit production of anthocyanin. Moreover, if a plant or kernel begins development with *Spm* in an inactive phase, the time of occurrence of such mutation at a_1^{m-2} is a function of the time during development when *Spm* changes to an active phase: the later the time of change, the later the occurrence of mutation.

A Third Inception of Control of Gene Action at the A_1 Locus by the Spm System

In the course of investigation of the *Ds-Ac* system of control of gene action, it was necessary to maintain a stock culture carrying a particular combination of gene markers and also *Ac*. This stock was homozygous for the standard dominant alleles of all but one (i.e., *Bz*) of the gene loci associated with anthocyanin pigment formation. To maintain this stock, sib crosses were made each year. In 1957, an ear produced by one of these sib crosses revealed the presence among some of its kernels of somatically occurring change in action of a gene concerned with anthocyanin pigment formation. The following year, testcrosses were made with a few plants grown from these kernels, to determine whether the locus involved was one that had previously been identified. It proved to be A_1 in chromosome 3; and

the modified locus was designated a_1^{m-5}. Other crosses with these plants indicated that the *Ac* system was not in control of gene action at a_1^{m-5}. *Spm*, however, was found to be present in each a_1^{m-5}-carrying plant that exhibited somatically occurring changes in gene action; and the ratio of kernel types on ears produced by testcrosses with these plants suggested that gene action at a_1^{m-5} might be under the control of the *Spm* system. Tests conducted during the past year have shown this to be so.

The originally isolated state of a_1^{m-5}, in the presence of an independently located, active *Spm*, is characterized by the production of somatic mutations to stable alleles of A_1, often occurring early in plant or kernel development. Most of them fall into two distinct groups: those that express high levels of A_1 gene action, and those that express either low levels or the null level. Mutants of these two main types occur with nearly equal frequencies, and both are stable in the presence of active *Spm*. The original state of a_1^{m-5} also gives rise to new states in the presence of active *Spm*, either class I or class II.

The original state of a_1^{m-5} produces so many early-occurring mutations in the presence of active *Spm* that it was not as useful as some of the derived states for the purpose of determining whether or not the *Spm* system is responsible for control of gene action at a_1^{m-5}. The derived class I states that give rise to mutations only late in development of plant and kernel served this purpose well, however, and four such states were selected for tests. It was learned that when *Spm* is absent (or present in an inactive phase) each of these states is characterized by a low level of A_1 gene action, in both plant and kernel, an expression that continues as long as *Spm* is absent or inactive; but in the presence of active *Spm* all gene action is suppressed, until in some cells, late in the development of plant or kernel, a mutation-inducing event at a_1^{m-5} allows some level of A_1 gene action to be expressed.

A class II state of a_1^{m-5} was also examined. In the absence of active *Spm*, this state produces a low level of gene action in both plant and kernel, whereas in the presence of active *Spm* all gene action is suppressed. With this state, however, in contrast to the class I states, no mutation-inducing events or alterations of state were observed. It behaves as a typical class II state.

One unusual state of a_1^{m-5} was identified and further examined. In the absence of active *Spm*, this state expresses a low level of A_1 gene action in both plant and kernel. When an active *Spm* is present, all gene action is suppressed but alterations of a_1^{m-5} do occur, many of them resulting in the appearance of new states. The new states are distinguished from one another by the types of mutation they give in the presence of active *Spm*, by the relative frequency of occurrence of each type (if more than one type is produced), by the time during development when the mutations occur, and by the frequency of occurrence of mutation at any one time during development. Thus an array of new states has been derived from this one state.

Control of Gene Action at the Locus of Wx by the Spm System

During examination of the relative frequencies of production of different types of stable mutants of A_2 given by the original state of a_2^{m-1} in the presence of active *Spm*, an ear of a plant having the constitution a_2^{m-1} bt/a_2 Bt; Wx/wx was used in a cross with a plant that was homozygous for a_2, *bt*, and *wx* and had no *Spm*. (a_2 = standard recessive, stable in the presence of active *Spm*; *bt* = brittle endosperm, located approximately 6 crossover units from the locus of A_2 or its alleles.) The kernel types on the resulting ears were those expected, with one exception. The exceptional kernel was colorless and *Bt*, but was neither totally *Wx* nor totally *wx*. The endosperm was a mosaic of sectors exhibiting different levels

of *Wx* gene action, from the null level (*wx*) in some sectors to the full *Wx* level in others, the pattern resembling that produced by the particular state of a_2^{m-1} in the ear-bearing parent plant in the presence of active *Spm*. It was suspected that the operator element of the *Spm* system had been incorporated at the *Wx* locus in chromosome 9 in a cell of this plant, and that the descendants of this cell, which gave rise to the exceptional kernel, expressed control of gene action at the *Wx* locus by the *Spm* system. A plant was grown from the exceptional kernel (plant number 7774) and subjected to tests that would determine whether or not the hypothesis was correct. The results gave clear evidence of control by the *Spm* system of gene action at wx^{m-8}, as the newly modified *Wx* locus was designated.

Plant 7774 proved to be a_2 *Bt*/a_2 *bt*, wx^{m-8}/wx in constitution, and it had two independently located active *Spm* elements in most of its tested parts. The plant produced three tillers; and testcrosses were made with pollen from the main stalk and from each of the tillers. In addition, it produced four fertile ears, and a testcross was made with each. The types of testcross that established *Spm* control of gene action at wx^{m-8} were as follows:

1. Reciprocal crosses with plants having no *Spm* that were homozygous for the standard, stable recessive *wx* and carried a class I state of a_2^{m-1}. Three distinctly different class I states of a_2^{m-1} were utilized in this test.
2. Crosses to plants homozygous for *wx* and carrying a class I state of a_2^{m-1} and also Spm^w (a weak-acting *Spm;* see Year Book 56).
3. Crosses to plants homozygous for *wx* and carrying a class II state of a_2^{m-1}. Some of the plants had no *Spm;* others carried *Spm* in an inactive phase of long duration.

In the kernels on the ears produced by these crosses, a_2^{m-1} served as an indicator of the presence or absence of *Spm*, and of its type of action if present. In each kernel that had received both a_2^{m-1} and wx^{m-8}, their behavior could be compared. Correspondence in response of a_2^{m-1} and wx^{m-8} in these kernels was so complete that there could be no doubt of control of wx^{m-8} by the *Spm* system. They responded in like manner to presence or absence of active *Spm*, to Spm^w, and to an inactive *Spm* that underwent change to the active phase in a few cells late in kernel development.

The behavior of this original isolate of wx^{m-8} may be summarized as follows: In the absence of active *Spm*, it exhibits a very low level of gene action, an expression that remains stable as long as *Spm* is absent or is present in an inactive phase. With a fully active *Spm*, all gene action is suppressed until a mutation-inducing event at wx^{m-8} results in a stable mutant. Some such mutants give rise to the apparent full *Wx* phenotype; others produce an intermediate level; and still others give a very low level or the null expression. Such mutations may occur in a cell either early or late in plant or kernel development. Plant 7774 exhibited some early-occurring mutations. One of its ears, for example, was derived from a cell in which a mutation had restored full *Wx* gene expression; and one of its tassels had a large sector carrying a stable mutant that gave the null (*wx*) expression.

Control of Reversals in Spm Activity Phase

An important aspect of the *Spm* control system is the cyclically occurring change in phase of activity of *Spm*—from active to inactive and back to active. It was early noted that the duration of a phase is controlled in some manner. It may be short, lasting only a few cell generations, or it may extend through many cell generations or even plant generations. The early studies also suggested that control of the duration of a phase resides, primarily, in the event that initiates the reversal of phase. To obtain more evidence, additional experiments were performed

this year, all of them conducted with plants descended from a single plant having one *Spm* closely linked with the *Wx* gene in chromosome 9. The behavior of this particular *Spm* element has been observed through six generations of plants, and the initial studies of reversal of phase of activity of *Spm* were conducted with it.

The studies this year had two objectives: (1) to compare the times and frequencies of occurrence of phase reversal of *Spm* in sister plants, all carrying an *Spm* that had undergone reversal of phase in a cell of the mother plant; and (2) to determine the stability of phases of *Spm* activity when two elements were present in a plant, occupying allelic positions but in alternate phases of activity at the beginning of plant development. In all tests, a class II state of a_2^{m-1} was employed to detect the times and frequency of occurrence of changes in *Spm* activity phase, as described in previous Year Books.

1. Kernels were selected from an ear of each of five different plants, either *Wx +/wx Spm* or *Wx Spm/wx +* in constitution, in which a part of the ear had developed from a cell in which a reversal in phase of *Spm* activity had occurred. Plants were grown from kernels within the sector exhibiting the altered phase of activity, and also from kernels that had appeared on the remaining part of each ear, where no such alteration had occurred. Comparisons were made with regard to initial phase of activity of *Spm*, as well as times and frequency of occurrence of reversals of phase. These observations revealed marked similarities in pattern of phase reversal among the plants derived from kernels within a sector, contrasting with the pattern in plants derived from kernels on other parts of the same ear.

2. Plants were constructed that carried one inactive *Spm* element, which for four generations had exhibited a long duration of the inactive phase, and in addition an active *Spm* that was known to undergo phase reversal late in plant and kernel development. (Both elements, as was stated earlier, were derived from a single ancestor plant having one *Spm* closely linked to *Wx* in chromosome 9.) In the tested plants, the two elements occupied allelic positions in chromosome 9, the inactive *Spm* closely linked to *Wx* and the active *Spm* closely linked to *wx*. To test whether or not, under these conditions, control of phase reversal would continue to reside in each *Spm* element itself, the ears produced by each plant were crossed by plants that were homozygous for *wx* and had no *Spm*, and also by plants that were homozygous for *wx* but carried *Spm* in its active phase. The second cross reveals the presence of inactive *Spm*, as described in Year Book 57. The *Wx* and *wx* classes of kernels on the resulting ears were compared with respect to *Spm* activity phase. It was apparent that control of the duration of activity phase had not been modified in either *Spm* element by their association at allelic positions in the nuclei of the ear-bearing plants: the characteristic duration of phase of each *Spm* was exhibited in the progeny. This evidence confirmed the previously drawn conclusion that control of reversal of *Spm* activity phase resides in the *Spm* element itself.

Nonrandom Selection of Genes Coming under the Control of the Spm System

Five independent inceptions of control of gene action by the *Spm* system have been examined so far: three at the locus of A_1 in chromosome 3, one at the locus of A_2 in chromosome 5, and one at the locus of *Wx* in chromosome 9. A_1 and A_2 are associated with anthocyanin pigment formation in plant and kernel, and *Wx* is concerned with development of amylose starch in pollen and endosperm. There is no reason to believe that control of gene action by this system is confined only to genes associated with anthocyanin production or with the structure of starch. The ease of detection of any change in action of these genes is believed to be responsible for the ready determination of the con-

trol system responsible for the change. It should be recalled that early in the study of controlling elements many independent inceptions of control of action of genes associated with chlorophyll development were recognized. Study of some of these was initiated, but it was soon decided to discontinue their examination in order to concentrate on systems that affect genes associated with anthocyanin development in the kernel or in both plant and kernel. It was realized that much more could be learned about the action of a controlling element on a gene if the phenotypic expression of the gene was exhibited in both plant and kernels or even in kernels alone. The kernels are especially valuable in this regard, because the phenotype of the endosperm registers the genetic constitution in the succeeding generation. Very large numbers of kernels may be obtained in progeny tests of a single individual, and with little technical difficulty. To obtain similar numbers of progeny plants would introduce technical difficulties of some magnitude, which might reduce the efficiency of the studies. Before the studies of control systems associated with chlorophyll genes were discontinued, however, the system associated with one such gene had been examined in some detail. The locus concerned was named "mutable luteus" (lu^m). As was mentioned in Year Book 57, the system responsible for control of lu^m resembles in detail the *Spm* system. Moreover, a_2^{m-1} originated in a plant carrying lu^m. It is very probable that lu^m represents a case of control by the *Spm* system of a gene associated with chlorophyll production, although direct proof has not been obtained.

In analyses of the mode of operation of a gene-control system, the phenotypic expression of the gene controlled is of paramount importance, particularly during initial examinations of the system. Once a control system has been identified, through its action on appropriate genes, and methods have been devised that will readily detect its action at other gene loci, it becomes practicable to examine its control of genes whose phenotypic expressions are less well adapted for such studies. In the studies reported so far, genes that are most appropriate for initial examination of control systems have consistently been chosen.