COMPONENTS OF ACTION OF THE REGULATORS *Spm* AND *Ac*

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Gene control systems other than those associated with the regulators *Spm* (Suppressor-mutator), *Ac* (Activator), and *Dt* (Dotted) are known to be present in maize. Just as proved true of the *Ac* and *Spm* systems, aspects of which still need to be elucidated, many types of test extending over a number of years will be

required to identify these additional systems, their components, and their modes of operation. Because time will not be available for such extended studies, it was decided to concentrate attention during the past year on some of the unresolved aspects of the *Ac* and *Spm* systems.

The Suppressor-mutator control system is so called because its regulator element, also designated *Spm*, has two components of action. The suppressor (or inhibitor) component, component-1, directly regulates the expression of a gene that has come under the control of the *Spm* system. Such control, it will be remembered, arises through insertion of the operator element of the system at the locus of a gene. When component-1 of *Spm* is in an active phase, the expression of the gene is suppressed; when it is inactive, gene action is expressed. One exception to this general rule concerns the modified gene locus a_1^{m-2}. This gene is active when component-1 is active, and suppressed when it is inactive. Component-2 of *Spm* is the mutator or transposition-inducing component. The response of the operator element to component-2 often gives rise to a mutant expression of the gene that releases it from control by the *Spm* system. Some responses, on the other hand, effect other modifications that are not associated with release but instead alter the subsequent types of response of the operator element to the components of *Spm*. These modifications have been called "changes in state" of the gene locus. With some altered states, the operator element loses its capacity to respond to component-2, although it retains its ability to respond to component-1. No further mutations occur, nor is the gene released from control by the system. Its action remains permanently under the control of component-1 of the *Spm* regulator element.

Both components of *Spm* may undergo change. Component-1 exhibits alternating cycles of activity and inactivity, whose regulation has been outlined in previous reports. Changes of component-2 resemble mutations in that they arise from single events, each of which alters the effectiveness of this component, both for inducing responses of the operator element that lead to changes in gene expression, and for inducing transpositions of *Spm*. Such alterations in action of component-2 have been detected only in those cells of the plant in which component-1 is active. Some alterations eliminate all activity of component-2 whereas others effect altered times and frequencies of occurrence of mutation-inducing responses of the operator element. Each mutant of component-2 may undergo still further mutation, the frequency of occurrence differing with different mutants.

The components of *Spm* and their characteristic modes of action were detected originally in observations of the activity of one gene that is under the control of the *Spm* system. Although it is possible to distinguish a change in action of either component through such observations, distinctions are greatly facilitated when two or more genes, each under the control of the system, are present in a plant or kernel. The operator elements at the different gene loci respond in like manner to modifications affecting either of the components. Through such combinations it has been possible to determine with a considerable degree of accuracy the types of modification undergone by each of the components of *Spm*.

Although direct evidence is lacking, the mechanism that releases a gene from the control of the *Spm* system is believed to be transposition of the operator element away from the gene locus. This assumption is based on studies of the *Ac* system, which have provided direct evidence of release of a gene from its control through transposition of the operator element away from the gene locus. Transpositions of the regulator element *Spm*, on the other hand, may be readily detected, and indirect evidence

had suggested that component-2 regulates them. Tests designed to obtain direct evidence were completed during the year, and are described below.

The original state of a_1^{m-2} has at the a_1^{m-2} locus an *Spm* element with a highly active component-2. This *Spm* is designated Spm^s. It induces many germinal mutations at the a_1^{m-2} locus, and the events responsible for them are usually associated with transposition of the *Spm* away from the gene locus. Often the event is accompanied by release of the gene from control by the *Spm* system. The germinal mutants that have been released from *Spm* control may be placed in two categories: those that resemble the wild-type gene in their action, and those that give rise to the diffuse-mottled phenotype, described in *Year Book 61* (pp. 448–460). In plants carrying the original state of a_1^{m-2}, component-2 of *Spm* may undergo a mutation that alters its capacity to induce mutations at a_1^{m-2} and to induce similar responses of the operator element at other gene loci. One of these mutations alters component-2 in such a manner that it induces change in gene action only very late in the development of a tissue, and in only a few cells. This mutant is designated Spm^w. In plants carrying Spm^w at the a_1^{m-2} locus, no germinal mutants are produced. Also, this *Spm* is not removed from the a_1^{m-2} locus in those cells of the plant that contribute to formation of the gametes. If component-2 back-mutates to a high level of activity, germinal mutants appear and *Spm* in many instances is transposed away from the gene locus. One Spm^w isolate, however, proved to be very stable; back-mutation of component-2 was rare. This isolate was selected for the tests to be described.

It was suspected that this Spm^w would undergo early transpositions if an *Spm* with a highly active component-2 was also present in the nucleus. To obtain direct evidence and to determine whether or not the components of Spm^w would be maintained unaltered after such a transposition, testcrosses were conducted with plants that commenced development with the selected Spm^w at the locus of a_1^{m-2} in chromosome 3 and an Spm^s located close to the *pr* marker in chromosome 5. The constitution of the plants was Spm^w a_1^{m-2} Sh_2/a_1 sh_2; Pr/pr Spm^s; wx/wx. The presence of Spm^s induced some germinal mutations at the a_1^{m-2} locus, most of them giving rise to the diffuse-mottled phenotype in the kernel. Kernels with this phenotype were detected on ears produced on the plants by a cross with plants that were homozygous for a_1, sh_2, and *pr* and had no active *Spm*. (In general the kernels on these ears were similar to those illustrated in *Year Book 63*, plate 1*B*, following page 601.)

Plants were grown from 30 of the kernels exhibiting a diffuse-mottled phenotype; 27 of the selected kernels had purple pigment and thus had received the *Pr* marker from the heterozygous parent, and three were red, having received the *pr* marker from this parent. Five of the plants derived from the *Pr* kernels contained Spm^w but no Spm^s, as shown by the response given to Spm^w by the gene wx^{m-8}, which had been introduced by the pollen parent in some of the crosses. All 30 plants were tested for the presence of *Spm*, its type, and its location with reference to the genetic markers carried in the plants.

In all, 78 fertile ears were produced by the 30 plants. The pollen parents in the crosses were of several types: homozygous for a_1^{m-1}, sh_2, *pr*, and wx^{m-8} and having no active *Spm*; homozygous for a_1, sh_2, *pr*, and wx^{m-8} and having no active *Spm*; or homozygous for a_1^{m-1}, sh_2, and *wx*, having no *Spm*, and either homozygous or heterozygous for the *Pr* marker. One particular state of a_1^{m-1} was utilized in these crosses because it responds in a very clear way to activity of the components of *Spm* and also allows the type of *Spm* in a plant to be registered in both the Sh_2 and the sh_2 class of kernels on the ears of the plant. The presence of wx^{m-8} in a kernel serves as an additional means of

scoring the *Spm* elements it may contain.

The tests revealed the following. Among the five plants derived from *Pr* kernels that were known to contain Spm^w, two had one Spm^w element, carried in chromosome 3 and linked with the diffuse-mottled locus but removed from it. Two others had one Spm^w, not linked with the diffuse-mottled locus. The fifth plant had two Spm^w elements, neither of which was linked with that locus. Of the remaining 22 plants derived from *Pr* kernels, 12 had no *Spm*, 4 had one Spm^w linked to the diffuse-mottled locus but removed from it, 3 had one Spm^w not linked with that locus, and 2 had one Spm^s closely linked with the *Pr* marker. One of these two plants had in addition an Spm^w, not linked with the diffuse-mottled locus. The remaining plant of the 22 had one Spm^s not linked with *Pr* or with the diffuse-mottled locus. Of the three plants derived from the *pr* kernels, one had one Spm^s, one had two Spm^s, and one had one Spm^s and also one Spm^w, not linked with the diffuse-mottled locus.

The evidence obtained from the tests indicates that an Spm^w element that is unable to induce its own transpositions early in development will undergo such transpositions if a potent component-2 is supplied by an *Spm* located elsewhere in the chromosome complement. It also indicates that the transposition event does not modify the components of the Spm^w; they remain unaltered after the event.

The Component of Spm *Responsible for Preset Patterns of Gene Expression*

To identify the component of *Spm* that is responsible for inducing the preset patterns of gene expression discussed in *Year Book 63* (pp. 594–599), plants carrying state 7977B of a_1^{m-2} and no active *Spm* were crossed with plants that had Spm^w at the locus of a_1^{m-2}, or Spm^w at the locus of a_1^{m-5}, or Spm^w linked with *Pr* in chromosome 5. Other crosses were made that introduced into some of the kernels an *Spm* with a highly active component-2. From the ears produced by these crosses, kernels were selected that had and that had not received the *Spm*. Plants were grown from both types of kernels, and testcrosses were conducted with their ears to determine whether or not they would bear kernels exhibiting types of anthocyanin distribution similar to those illustrated in *Year Book 63*, Plate 2*B*.

Sixty-one plants derived from kernels that had received the introduced *Spm* were tested. Since most of them produced two to four fertile ears, different types of tests could be conducted with many of them. At least one ear, and usually more than one, took part in a cross with a plant that was homozygous for a_1, sh_2, and wx^{m-8} and had no active *Spm*. On all the ears there were kernels that exhibited preset patterns of anthocyanin distribution and showed no indication of the presence of an active *Spm*. There was no evidence that would relate the type of preset pattern to the particular *Spm* present in the ear-bearing parent: Spm^s and Spm^w were equally effective in this regard. This observation suggested that component-1 of *Spm* is responsible for induction of preset patterns. Confirmation was provided by the kernel types on ears of four additional plants, which carried an *Spm* whose component-1 remained in an inactive phase throughout plant development but returned to an active phase in many cells during development of the kernels. No kernels with preset patterns appeared on any of the ears produced by these four plants.

Sixty-one plants derived from kernels that had not received *Spm* from the pollen parent were also examined. On 68 of the ears on these plants, produced either by self-pollination or by crosses with plants that were homozygous for a_1 and had no active *Spm*, kernels exhibiting preset patterns did not appear. The kernels were colorless or nearly so. In addition, 49 ears produced by 36 of the plants were utilized in a cross that introduced an active Spm^s. No kernels

with preset patterns appeared on these ears. The phenotype expressed by a_1^{m-2} (state 7977B) in those kernels that received *Spm* was typical: deep-pigmented spots in a lighter-pigmented background. Kernels that did not receive *Spm* from the pollen parent were colorless or nearly so.

Transmission of the Preset Pattern

In the study of preset patterns described in *Year Book 63*, pp. 594–599, it was found that the patterns usually did not reappear in the following generation. Most of the ears of plants derived from kernels exhibiting preset patterns showed no evidence of retention of the patterns: their kernels were colorless or nearly colorless. On a few ears, however, produced by plants carrying state 7995 of a_1^{m-2}, several kernels exhibited a pattern of anthocyanin distribution resembling that in the kernel from which the plant was derived. Five such exceptional kernels were present on one ear, four on another, and two on a third. This year, plants were grown from the exceptional kernels to determine whether or not the pattern would again reappear.

The constitution of the plants derived from these kernels was a_1^{m-2} (state 7995) $Sh_2/a_1\ sh_2$, wx/wx, and no active *Spm* was present in any of them. Nineteen of the fertile ears on these plants were utilized in a cross with a plant that was homozygous for a_1, sh_2, and wx or wx^{m-8} and had no active *Spm*. If more than two ears were produced by a plant, one of them was used in a cross with a plant that was homozygous for a_1 and sh_2 and had one or more *Spm* elements. This cross, conducted with one ear on each of five of the eleven plants, was made to test whether some modification had occurred at the a_1^{m-2} locus that would be revealed by the locus's response to an active *Spm* element. On all five ears the response to *Spm* was normal: deep-pigmented spots appeared in a lighter-pigmented background.

The 19 ears produced by the first-mentioned cross were examined for kernels with patterns of anthocyanin distribution and intensity resembling those in the parent and grandparent kernels. On the ears produced by plants derived from the parent ear that had four such kernels, no kernels of this type appeared. All were colorless or nearly so. On one or more of the ears produced by the remaining seven plants, some parent-type kernels did appear, in numbers ranging from one to five per ear. Their distribution on an ear was not random: most were located in the upper third, several at the base of the ear. When more than one was present on an ear, they were not clustered, exhibiting in that respect the same distribution as on the parent ears. There is no evidence that contamination contributed to their presence on the progeny ears, as all the remaining kernels were colorless or nearly so.

The exceptional kernels had pigment intensities resembling those of the kernels from which their respective plants arose. They did not show the wide range of intensities represented among kernels with preset patterns appearing on ears of plants that have an active *Spm*. It seems probable, therefore, that the condition responsible for a particular expression of a preset pattern was retained for two plant generations in the ancestor cells that produced the exceptional kernels, but was lost from those ancestor cells that produced the remaining kernels on the same ears. At present there is no adequate explanation for this phenomenon. Some of its aspects recall a type of gene expression that is produced by one of the operator elements of the *Ac* system. This operator functions at the loci of c_1^{m-2}, wx^{m-1}, and wx^{m-5}. It was noted that some responses of the operator to *Ac* did not immediately result in a stable expression of the gene; instead, the level of expression appeared to oscillate. This behavior was observed in the descendent cells of a cell in which such a response had occurred. The results were apparent in kernels that

had received one such "excited" gene locus from the pollen parent. Plants were grown from some kernels that exhibited this type of metastability at the wx^{m-5} locus. In kernels on the ears of the plants the level of action of the *Wx* gene was now uniformly expressed throughout the cells of the endosperm. The "oscillation" had ceased. Furthermore, no responses of the mutant locus to *Ac* occurred thereafter. The locus had acquired stability.

Components of Action of Ac

Study of wx^{m-7}, first reported in *Year Book 63* (pp. 599–601), was extended in order to examine the activity cycles of *Ac* in greater detail. The wx^{m-7} modification arose by insertion of *Ac* at the *Wx* locus in chromosome 9. The initial effect was a marked reduction in activity of the gene. Transposition of *Ac* away from the locus restores a high level of gene action and releases the gene from control by the *Ac* system. *Ac* is known to regulate the time and frequency of occurrence of self-transposition and of responses of the operator element at other gene loci. Initially this *Ac* at the wx^{m-7} locus exhibited the type of dose expression that characterizes *Ac*: the higher the dose, the later the time of occurrence of such responses. It was observed that this *Ac* undergoes cycles of activity that alter the component responsible for the responses and for induction of transpositions. When that component is inactive, no such responses occur nor does the *Ac* contribute to dose expressions should an active *Ac* also be present elsewhere in the chromosome complement. It responds, however, to such an active *Ac* by undergoing transposition that releases the *Wx* gene from further control by the *Ac* system, and the time and frequency of occurrence of transposition reside in the active *Ac*. When the *Ac* at the *Wx* locus returns to an active phase, its capacity to induce responses of the operator element located elsewhere, to induce its own transpositions, and to contribute to dose expressions is restored. Thus, the activity cycles affect a component of *Ac* that is comparable to component-2 of *Spm*, but different in that it is also responsible for dose expressions, which are not exhibited by component-2 of *Spm*. No component of *Ac* comparable to component-1 of *Spm* has yet been identified.

A fascinating aspect of inactive *Ac* at wx^{m-7} relates to its control of the level of action of the *Wx* gene in the starch-bearing cells of the endosperm. Different levels of action are induced during kernel development. To examine this aspect it was necessary to utilize a technique that could reveal these levels in individual cells. The *Wx* gene is responsible for the production of amylose starch in the pollen grain, the embryo sac, and the starch-bearing cells of the endosperm of the kernel. The associated enzyme, which has been identified by O. E. Nelson, is affixed to the starch-forming granules within the cells. When the *Wx* gene is acting normally, approximately 25 per cent of the starch in the endosperm is amylose, the remainder being amylopectin. If the activity of the gene is reduced, the amount of amylose formed is also reduced. If the gene is totally inactive, all the starch is amylopectin. The two types of starch stain differentially with a solution of potassium iodide and iodine: the amylose stains blue and the amylopectin red-brown. The red-brown stain may be removed, either by hot water or by exposure of the cells to the rays of a lamp. To examine the level of *Wx* gene action in different cells of the endosperm of a kernel, a cut is made to expose a surface of endosperm cells, which are then stained with an I-KI solution. The intensity of blue coloring in the starch granules of different cells may be compared. It was learned many years ago that the intensity of this staining in the granules of a cell reveals the level of *Wx* gene action in that cell. It is believed, therefore, that the observed differences in intensity of blue coloring among the

endosperm cells of an individual kernel reflect differences in level of *Wx* activity in the cells. Such differences are illustrated in Plates 1 and 2.

To aid in interpreting the illustrations, a brief review should be made of endosperm development, some aspects of which were first revealed during the course of this study. The primary endosperm nucleus, produced by fusion of a sperm nucleus with two haploid nuclei contributed by the female gametophyte, divides in two, and then each nucleus divides again. Each of the resulting four nuclei gives rise by subsequent divisions to a column of nuclei, before cell walls are formed. The columns are arranged around a central, nonnucleated core. Cell-wall formation occurs later, but does not eliminate the central core, often visible in the mature kernel. The cells in the columns divide tangentially, and the outermost cells continue to divide, leaving behind cells in which endoreduplication of the chromosomes occurs. In the mature kernel the innermost cells of the endosperm are highly polyploid and very large, containing many starch granules. The degree of polyploidy becomes lower in cells farther removed from the middle of the kernel, and the cell size is correspondingly reduced. The chromosomes in the nuclei of cells toward the periphery do not undergo endoreduplication, and these cells are small. The aleurone layer is the outermost layer of cells of the endosperm; only in these cells is anthocyanin pigment produced. Although a change in control of action of a gene contributing to anthocyanin pigment formation may occur within a cell during endosperm development, it can be expressed only in cells of the aleurone layer that are descended from that cell.

To examine the effects produced by *Ac* in its active and inactive phases, it is necessary to know when it is in one or the other. This is made possible by the presence of a gene that is associated with anthocyanin pigment formation and is under the control of the *Ac* system. The modified A_1 locus a_1^{m-3} was chosen for the purpose. The state of a_1^{m-3} selected for the experiments produces a lightly pigmented aleurone layer when *Ac* is absent or inactive. When *Ac* is active, the responses it induces in the operator element at the a_1^{m-3} locus give rise to altered A_1 gene expressions, often restoring full or nearly full activity to the gene. The time of occurrence of these responses is controlled by the *Ac* element that is present in the kernel. An illustration is given in Plate 1*A*. The responses to the active *Ac* in this kernel were registered in like manner by a_1^{m-3} and wx^{m-7}; they occurred late in development of the kernel.

Kernels that commence development with a_1^{m-3} and an inactive *Ac* at the locus of wx^{m-7} will show no deeply pigmented areas in the aleurone layer at maturity if the *Ac* remains inactive throughout endosperm development. If *Ac* returns to the active phase in an individual cell during development, it will induce responses of the operator element leading to change in gene action at a_1^{m-3} in some of the progeny of that cell. The event will be evidenced by an area in the aleurone layer that exhibits pigmented spots. *Ac*'s return to activity will also induce many reversions of the *Wx* gene to full expression. The size of an area having pigmented spots indicates the time during development when the change of phase occurred.

Progeny were obtained from a number of plants that developed from kernels having a_1^{m-3} and an inactive *Ac*, for examination of the cycles of activity that *Ac* would subsequently undergo. Only that aspect of the study relating to control of expression of the *Wx* gene will be reported here. In a number of progeny kernels, the action of a_1^{m-3} gave no indication of a change of phase of *Ac* from inactivity to activity. The level of *Wx* gene expression in the starch-bearing cells of these kernels was not uniform, as shown in Plate 1(*C*, *D*, and *E*). Although most such kernels exhibit a basic pattern of *Wx* gene action, produced by

a low level of this action in the cells of the upper mid-region and at the base of the endosperm, many changes in level may occur during endosperm development. It was noted that the initial level of *Wx* gene expression imposed by the inactive *Ac* at the time of fertilization, regardless of whether it was contributed by the male or by the female gametophyte, had a marked effect on the levels of *Wx* gene expression appearing in the kernel. Some kernels commenced development with a low level of *Wx* gene action (Plate 1*C*), others with a much higher level (Plate 1*D* and *E*). If such kernels had started development with one active *Ac*, located elsewhere than at wx^{m-7}, both a_1^{m-3} and wx^{m-7} would have responded to it. Early and late changes in A_1 gene expression and changes to full *Wx* gene expression would have occurred, in the manner exhibited by the kernel in Plate 1*F*. In that kernel the pattern of *Wx* gene expression produced by the presence of active *Ac* is superimposed on the pattern produced by inactive *Ac*.

The kernel shown in Plate 1*B* and all three kernels shown in Plate 2 illustrate the responses of a_1^{m-3} and wx^{m-7} to a change in phase of *Ac* from inactive to active during endosperm development. The legends give the constitutions of the kernels and describe the effects produced by the changes in phase. It can be noted particularly in Plate 1*B* and Plate 2*B* and *C* that the progeny cells of a cell in which *Ac* underwent activation are readily distinguished because they express either a low or a high level of *Wx* gene action. In cells where *Ac* remained in an inactive phase throughout endosperm development, on the other hand, a wide range of levels may be expressed, resembling the range exhibited by kernels in which *Ac* remains inactive throughout development.

In conclusion, it may again be stated that the resemblance between the regulators *Ac* and *Spm* resides in a component of each that initiates responses of the respective operator element and of the regulator itself which effect transpositions. If the component is inactive, such responses do not occur. It is this component of *Ac* that is comparable to component-2 of *Spm*. In *Ac*, but not in *Spm*, the component is also responsible for dose effects. The operator element of the *Ac* system has not yet given evidence of differential control of action of the gene in response to activity cycles of *Ac*, in the manner exhibited by the operator element of the *Spm* system. Thus, *Ac* has no recognizable component that corresponds to component-1 of *Spm*.

It is possible that the different levels of *Wx* expression produced by inactive *Ac* at wx^{m-7}, the "preset" patterns within the *Spm* system, and the "oscillations" in gene expression produced by responses to active *Ac* of one *Ac* operator may all reflect a common type of event occurring at the locus of a gene to initiate temporary metastability of its action.

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