DOE/ER/13922--1

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Grant # DE-FG02-88ER13922 FINAL REPORT

Contents

SUMMARY REMARKS

The objectives of the Workshop Series on the Genetics and Molecular Biology of Robertson's Mutator were to assess and consolidate interpretations of current Mutator research and to recognize and honor the outstanding contributions of Donald S. Robertson. To this end, a program of lectures, workshops, posters and opportunities for informal interaction was planned and carried out as indicated in the enclosed registration booklet. Within the context of the workshops, several topics were discussed. These discussions are summarized below.

Assays of Mu Activity

In this workshop the advantages and disadvantages of several methods of measuring Mu activity were discussed. It was considered important to be able to define and detect activity for several purposes, including transposon tagging (for gene cloning and induction of developmental mutations), and for the study of mechanisms of transposition. The following assays, and statistical methods for judging their reliability were discussed.

1) Germinal activity, as detected by the induction of new mutations in Robertson's Standard Test for Mutator Activity.

It was agreed that this assay is very reliable but it does have the following drawbacks. It requires a considerable amount of space and time; the effect of the Mutator-induced mutations is measured two generations after the event; new mutants can be detected only if they have a distinct and viable phenotype; a minimum number of copies of Mu1 elements is believed to be necessary for detection of new mutants (leading to an underestimation of germinal activity in active lines that happen to have few Mu1 elements in the genome); it is difficult to distinguish between low germinal activity and no germinal activity. A suggestion was made and discussed to modify the method by scoring defective kernel (dek) mutants instead of, or in addition to, seedling mutants.

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2) Somatic mutability, as detected by unstable phenotype, of a Mutator-induced allele in a reporter locus.

Several laboratories favor this method because it is simple, quick and relatively inexpensive. However, it was agreed to be potentially unreliable as an assay of insertion activity because it reflects the presence only of somatic reversions at a single locus. The presence of somatic mutability does not necessarily indicate that transposition or even excision of the Mu element from the locus has taken place. Moreover, lack of somatic mutability may reveal little about the activity of the plant of interest, because it can only be detected many cell generations after the transposition events to be measured took place.

3) Detection of non-parental Mu-hybridizing bands on genomic Southern blots.

This method is probably very reliable, but may be time consuming and expensive for detecting low levels of insertional activity.

4) Maintenance of high copy number of Mu1 elements.

This assay should probably be used only in conjunction with others, since it is possible to find active Mutator lines with few copies of Mu1 and, conversely, inactive lines with many Mu1 copies.

5) Lack of methylation of Mu1 elements.

Although there is a good correlation between inactivity of Mutator and methylation of the Mu1 elements, often methylated elements are found in lines that are judged by other methods to be active. Therefore, this method should be used in conjunction with others.

Search for an Autonomously Transposing, Trans-activating Mu Element

A considerable amount of time was spent in assessing whether any laboratory had clear evidence for a single segregating element that could trigger germinal and/or somatic Mutator activity. It was agreed that interpretation of classical genetic experiments, designed to detect the presence of such an element by scoring somatic mutability at a reporter locus, is confounded by an inactivation phenomenon that is associated with methylation of the Mu1 elements. This type of inactivation can produce progeny ratios similar to those expected from a segregating regulatory element and could account for the data reported by

several laboratories. One element, Cy, may exhibit the expected segregation patterns of a regulatory element without the concurrent complications of methylation-related inactivation, but the data were not available at this time. The agreement of many investigators to make all elements cloned in their laboratories available as hybridization probes, will potentially be of great help in finding an autonomously transposing and/or trans-activating element. As part of the discussion, it was emphasized that it is important to define the functional and structural features of the element to be identified.

Plant Developmental Biology

Although no workshop was specifically designated to cover the role of Mutator in developmental biology, the topic was discussed in a variety of contexts. From several laboratories a new awareness was being expressed concerning the importance of understanding the developmental biology of maize as a prerequisite for interpreting Mutator-induced phenomena. In addition, the relative non-specificity of Mutator insertion, as well as, the high mutagenicity of Mutator make Robertson's Mutator a potential tool for inducing and studying developmental mutants.

Review of known Mu elements

Since the identification of the first Mu element (Mu1) a number of different Mu elements have been isolated. The feature that all these elements have in common is the approximately 200 bp terminal inverted repeats. The sequences internal to the inverted repeats are unique for each Mu element except for Mu1 and Mu2 (Mu1.7). Mu1 appears to be a deletion derivative of Mu2. Prior to this meeting no review of the number, sequence similarity, copy number, etc. was available. The number of different elements and their similarities were known by only a few investigators. This workshop brought together the individuals who have cloned and/or studied these elements and provided an opportunity to summarize their characteristics. This information is displayed in the following table. The compilation of this information was greatly appreciated by the participants and should provide a basis for a better understanding of the Mu system. It was estimated that 8 of these 9 elements represent approximately 60% of the elements which contain Mu1 homologous ends (data for the 9th element were not available). It was suggested that attempts to clone representatives of the remaining 40% should be continued and that one benefit of these studies may be the cloning of an element that can subsequently be shown to be an autonomously transposing element of the Mutator system.

In addition, it was established that 8 of the 9 elements were presently available to those interested in using them in transposon tagging experiments (see below).

Mu	Mu ends	Found in known gene	extrachromosomal forms detected	copy number in non-Mu lines
1	yes	yes	yes	0-4
2 (1.7)	yes	yes	NA	0-4
3	yes	yes	yes	1-7
4	yes	no	yes	2-4
5	yes	no	NA	20-30
6	yes	no	yes	4
7	yes	no	yes	4
8	yes	yes	NA	4
9 (rcy)	yes	yes	NA	NA

NA - data not available or unknown

Methylation of Mu elements

As is evidenced by the literature on Robertson's Mutator a great deal of investigation has been devoted to the involvement of DNA methylation in the activity of the Mu system. At the workshops the issue of methylation was addressed on numerous occasions. It was proposed by one group that the methylation of Mu elements could be accounted for by the action of a maintenance methylation system. While this theory was not formally challenged during the workshop proceedings it generated a great deal of informal discussions between the various groups represented at the workshop. It became clear that two models were considered to account for methylation related inactivation and reactivation. 1) The maintenance methylation model, mentioned above, which would predict that different Mu elements within the

same cell might have a different methylation status, and 2) a model that predicts that Mu-specific regulation of methylation takes place at the level of the cell suggesting that all Mu elements within one cell would have the same methylation status. Two very important implications of methylation of Mu elements were also discussed. 1) It was described that the 1:1 ratios of mutable to stable phenotype observed in some crosses, which on first observation might indicate the segregation of an autonomous element, could be explained entirely by a gradual, methylation-related inactivation process that results in stable plants. The consensus of the participants was that these phenomena would have to be considered when searching for the segregation of an autonomous element by classical genetic methods. 2) For one Mu-induced mutant a positive correlation was demonstrated between suppression of a mutant phenotype and methylation of the Mu elements in the suppressed mutant plants. These plants still contained the Mu element inserted in the mutant allele. The extent to which suppression takes place in the Mutator system was unclear, but it was agreed that the suppression of a mutant phenotype must be considered when interpreting transposon tagging experiments.

Transposon tagging

This workshop brought together a large number of scientists who are involved in isolating maize genes by transposon tagging. Included in this group were individuals who successfully cloned genes using Mutator as their only tag and others who have used Mutator, in addition to other transposable elements, to tag and clone genes. This workshop provided the opportunity to compare the basic strategies employed by the different groups. Four general strategies were discussed. 1) Identification and cloning of a Mu-hybridizing DNA fragment that segregates with the mutant phenotype of interest on Southern sibling segregation analysis. 2) Identification and cloning of a Mu-hybridizing fragment of DNA that is present in all plants with the Mu-induced mutant phenotype of interest. 3) Isolation of all Mu-hybridizing elements from a genomic library of a Mu-induced mutant of interest followed by the identification by differential RNA analysis of the one Mu hybridizing clone which is linked to the gene of interest. 4) Identification and cloning (by preparing a cDNA library) of Mu containing RNAs which segregate with the mutant phenotype of interest on northern hybridization analysis. Methods 1, 2 (full analysis to show conclusively that this clone is the gene of interest has not been completed) and 3 have been successfully employed with the Mutator system. Method 4 was presently being attempted in several labs. The feasibility of this technique is still being explored. The consensus was that methods 1 and 2 are the logical first steps in a tagging experiment and that method 3 was limited to mutants where

detailed knowledge about the expression of the gene (i.e., tissue specific or developmental) is known.

A good deal of discussion was devoted to the rate of mutation by the Mu1 element versus that of the other Mu elements (a total of 9 different elements have been cloned, see above). It was concluded that current data suggest that the Mu1 element has a higher capacity for mutagenizing genes than the other Mu elements. However, it was cautioned that, while some mutant alleles of particular genes appear to be caused exclusively by Mu1, not all mutations of genes have turned out to be induced by Mu1 (see table below). For example, of the two vp1 mutants found in a Robertson's Mutator stock, one was shown to be induced by Mu1 and the other by another "element" apparently unrelated to Mutator. In addition, of 4 mutations of the B gene induced in a Mutator stock none turned out to be caused by 8 of the 9 cloned Mu elements (the 9th element has not been tested). The participants concluded that it was prudent to try to work with mutants that appeared genetically and developmentally to be Mu1-induced.

GENE	NUMBER OF MUTANTS STUDIED	Mu1 INDUCED	OTHER Mu ELEMENT INDUCED
Adh	5	4	1
Wx	2	1	1
Bz1	17	17	0
Bz2	2	2	0
Vp1	2 (*)	1	
A1	1	1	0
B	4	0	

* mutation induced by "element" which shares no homology with any known Mu element.

In light of the possibility that some mutants are caused by Mu elements other than Mu1 it was established that 8 of the 9 known Mu elements were available to those would like to use them for the isolation of genes by transposon tagging. It was estimated that these 8 elements account for approximately 60% of all of the elements which contain Mu ends (see above) and that

the availability of the probes will greatly facilitate cloning genes where Mu1 is suspected to not be the element which has inserted into the gene.

In fairness to the critics of the Mutator system for tagging and isolating genes, we discussed the following question: "What is the basis of the belief of some non-Mu-taggers that Mutator is not a good system to try to clone genes". It was conceded that the large number of different Mu elements (homologous only in their terminal inverted repeats) and the high copy number of Mu elements does in some cases cause difficulty in the cloning of genes. However, it was the opinion of the participants that copy number can be readily reduced and that the availability of 8 of the 9 cloned Mu elements, and the on going efforts to clone the remaining Mu elements families, will diminish the severity of these drawbacks to the Mu system. There was overwhelming agreement that the strongest point in favor of the Mu system for tagging is the high frequency of mutations when compared to all other maize transposable element systems used for tagging. It was concluded that Mutator is a powerful system with which to induce mutations, particularly developmental mutants, and that the field size limitations of many investigators made it the best transposon system with which to induce mutations. It was also agreed, however, that if space was not limiting it would be prudent to try to tag both with the Mu system and another system such as En/Spm. The advantages of this approach are two-fold 1) the production of Mu induced mutations may precede that of the other system and expedite the isolation of the gene and 2) cloning the gene in two systems will give confirming data that the gene of interest has been cloned.

As might be expected the majority of the technical aspects of cloning and tagging were discussed individually during the poster sessions, meals and social events. It was clear that many of the participants benefited greatly from the opportunity to discuss these procedures with others who share common goals.

Banquet in honor of Donald S. Robertson

On Saturday night, June 11, 1988, a banquet was held to honor Donald S. Robertson. All participants showed a warm appreciation for Dr. Robertson's contributions and generosity. Tribute was paid to him by Dr. Daniel Zaffarano, Vice-President for research and Dean of the Graduate School at Iowa State University, and by maize geneticists Virginia Walbot from Stanford University, Michael Freeling from the University of California at Berkeley, William Sheridan from North Dakota State University, and M. G. Neuffer from the University of Missouri.

ROBERTSON'S MUTATOR WORKSHOP
06/11/88 to 06/12/88

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ROBERTSON'S MUTATOR
1978 1988

In the study of living beings, history displays a pendulum movement, swinging to and fro between the continuous and the discontinuous, between structure and function, between the identity of phenomena and the diversity of beings. From these oscillations, the architecture of the living gradually emerges, revealed in ever deeper layers. In the living world as elsewhere, the question is always to 'explain the complicated and visible by the simple and invisible', according to Jean Perrin's expression. However, with living beings as with inanimate objects, there are wheels within wheels. There is not one single organization of the living, but a series of organizations fitted into one another like nests of boxes or Russian dolls. Within each, another is hidden. Beyond each structure accessible to investigation, another structure of a higher order is revealed, integrating the first and giving it its properties. The second can only be reached by upsetting the first, by decomposing the organism and recomposing it according to other laws. Each level of organization thus brought to light leads to a new way of considering the formation of living beings.

from the Logic of Life
Francois Jacob

It is our sincere hope that together we can explore the architecture of Robertson's Mutator, defining the properties of the layers already described, and peering ahead into the next level of organization.

Brent Buckner Jette Foss

MUTATOR WORKSHOP

JUNE 11-12, 1988

Scheman Continuing Education Building
Iowa State University
Ames, Iowa 50011

PROGRAM

Friday, June 10

Afternoon: Tour of Don Robertson's experimental fields.

Evening: 7:30-8:30 Registration ----------First Floor Desk

7:30-9:30 Social----------First Floor Lobby

Saturday, June 11

7:30: Registration--first floor desk/continental breakfast-------Lobby

8:00 Welcome and Introduction----------Benton Auditorium

8:15 Review of Robertson's Mutator - Don Robertson---Benton Auditorium

8:45 Lecture: Regulation of Mu Excision Activity - Virginia Walbot
Benton Auditorium

9:15 Lecture: Loose Ends of Mutator Research - Loverine Taylor
Benton Auditorium

9:45 Coffee Break----------First Floor Lobby

10:15 Workshop: Assays of Mu Excision Activity - Virginia Walbot
Rooms 250-252

12:15 Lunch----------Room 179

1:15 Lecture: Robert Martienssen----------Benton Auditorium

1:30 Poster Session----------Lounge 182

2:30 Lecture: Run-on Transcription Assays - Judith Strommer
Benton Auditorium
3:00 Lecture: Mu3 - Karen Oishi----------Benton Auditorium

3:30 Break----------First Floor Lobby

4:00 Workshop: Genetic Effects of Mu - Michael Freeling---Rms 250-252

7:00 Champagne Social----------First Floor Lobby

8:00 Banquet----------Room 179

Sunday June 12

7:30 Continental Breakfast----------First Floor Lobby

8:30 Lecture: Insertional Specificity of Mu - Jeffrey Bennetzen
Benton Auditorium
9:00 Lecture: Extrachromosomal Forms of Mu - Venkatesan Sundaresan
Benton Auditorium
9:30 Coffee Break----------First Floor Lobby

10:00 Workshop: Mechanisms and Regulation of Mu transposition -
Vicki Chandler------Rooms 250-252
12:00 Lunch----------Scheman Courtyard

2:00 Workshop: Transposon Tagging with Mu - Jeffrey Bennetzen-
Rooms 250-252
4:00 Summary of Meeting - Michael Freeling----------Rooms 250-252

4:30 Speakers Available for general questions--------First Floor Lobby

5:00 Dinner----------Scheman Courtyard

Submitted Questions

1. How does Mutator cause deletions and other rearrangements?

2. What does the high incidence of somatic stability (60%?) among mutants newly induced by Mutator imply about mechanisms of mutagenesis?

3. What is the evidence for an autonomous regulator of Mutator activity? How many laboratories claim to have evidence for such a regulator?

4. Is a difference expected between regulation of somatic instability and regulation of germinal activity by an autonomous element? What observations are pertinent to this question?

5. Could sequences of any known Mu elements be part of an autonomous element? Why?

6. Is Mu1 related/unrelated to any autonomous Mu element?

7. Is a minimum number of copies of Mu elements required for Mutator activity?

8. Why does Mu1 seem to be the most prevalent/mutagenic element found in Mutator-induced mutants?

9. What is the largest known element that **moves** with Mu ends?

10. What, if any, is the relationship between different Mu elements?

11. What, if any, is the relationship between Mutator and repetitive DNA? Have any Mutator elements been found in repetitive DNA?

12. What do we know about the origins of the Mutator system?

13. What are the potential uses of the Mutator system in cell culture (e.g. mutagenesis, tagging)?

14. How can the unique features of cell culture be used to advantage to study the Mutator system?

15. What experiments should be done to prove whether or not a Mutator-tagged gene has been cloned?

16. Do excision products (i.e. the sequence surrounding the place from which a Mutator element has excised) have any characteristic features?

17. What models are offered to explain the existence of very light (i.e. less than one copy per haploid genome) Mu1-hybridizing bands on Southern blots of genomic DNA cut with "outside cutters" in active Mutator lines?

18. Is there consensus that a limited range of stages in development exists when insertion of a Mu1 element can take place? What are the limits? What factors influence the limits?

19. Is there consensus that excision can take place only in a limited range of developmental stages? What are the limits? How can they be changed?

20. What, if any, is the relationship between excision and insertion?

21. Is transposase necessary for excision?

22. What evidence exists for Mu-end transposition?

23. Are Mu elements clustered on the chromosome ("bonanza" chromosomes).

24. Can some stable, newly-induced mutations be rendered mutable while others cannot?

25. What evidence exists for cytoplasmic mutations induced by Mutator?

26. How are extrachromosomal forms of Mu related to cytoplasmic or nuclear events?

27. Where are the extrachromosomal forms?

28. What, if any, is the evidence for a co-integrate type of transposition intermediate for Mutator?

29. Is Mu-loss from inbreeding correlated with high Mu copy number?

30. Of the three related phenomena, inbreeding, loss of germinal or somatic activity and methylation of the Mu1 element, which, if any are believed to be causally related to the others?

31. What evidence exists for insertional specificity?

32. What are the similarities and differences between Mutator and other transposable elements systems?

33. Can dominant mutations be induced by Mutator and, if so, what are the mechanisms?

34. Is the pattern of progress of methylation during the development of the plant reproducible? What is the nature of it?

35. What is the consensus/model regarding the necessary conditions required for somatic mutability to occur at a given locus? (e.g. 1) presence of a regulator element; 2) presence of an element with intact Mu ends at the locus; 3) either the regulator or the reporter or both need to be unmethylated?? everywhere?? at the ends only?? middle only??

36. What do models for explaining germinal Mutator activity require? (e.g. 1) presence of a regulator element; 2) enough elements with intact Mu ends to generate a detectable frequency of new mutants; 3) absence of methylation of Mu elements? all?? regulator only??

37. What is the evidence for and nature of any position effects of Mu insertion?

38. What, if anything, can be inferred from similarities and differences in the direct-repeat flanking sequences that have been cloned along with Mu elements?

39. What are the most efficient techniques for using Mutator in gene tagging experiments?

40. What is the significance of "early" somatic mutable patterns recently found in several seemingly Mu-induced mutants?

41. What do we know about germinal reversion of Mutator-induced mutants?

42. What are the most appropriate or best assays for Mutator activity (e.g. new seedling mutants, plant striping, new defective kernel mutants, somatic mutability, etc.)?

43. What is a good definition for the Mutator system? What characteristics are present in Mutator plants that are not present in non-Mutator plants?

44. How can we promote better agreement on terminology?

45. Are Mu clones unstable?

46. Is the rearrangement of Mu clones random or reproducible?

47. Are the rearrangements E. coli strain specific? Vector specific?

48. Should primary screening of a lambda library be done in a strain of E. coli that will not allow recombination of direct or indirect repeats (i.e., CES 200, CES 201, etc.)? Secondary screening, etc.?

49. Is the best way to ^{32}P label the DNA flanking Mu in a clone by using oligomers to the ends of Mu and Klenow synthesis?

50. Can Tag I polymerase amplification be used for cloning a Mu containing sequence of interest?

51. Are all of the known Mu sequences available for use as hybridization probes for the isolation of genes?

52. What is the best way to "prove" you have cloned your gene of interest?

53. What are the pros and cons of RFLP mapping to demonstrate you have cloned your gene of interest?

54. What is the basis of the belief of some non-Mu-taggers that Mutator is not a good system to tag and isolate genes?

55. Are there any methods that will help to eliminate the possibility of attempting to clone a DNA that segregates with the phenotype of interest but is merely linked?

GENETICS AND MOLECULAR BIOLOGY

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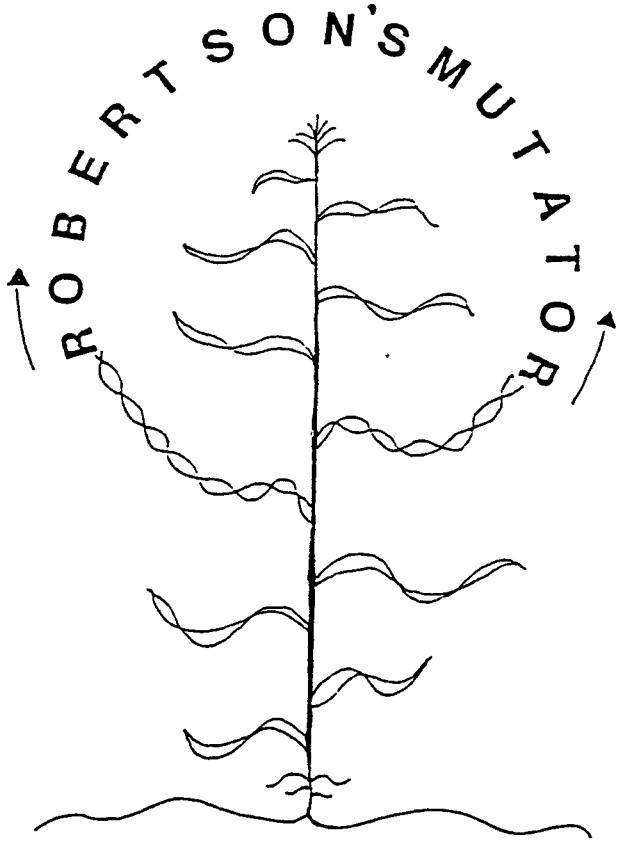

A WORKSHOP SERIES

June 11-12 1988

ABSTRACTS

Analysis of Additional *Mu* Elements

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Most *Mu*-induced mutations have been found to be due to the insertion of the 1.4 kb transposable element *Mu1*. However, two other transposable elements have been found to be capable of transposing in *Mutator* lines (Chen *et al.*, Genetics *116*, 469-477 [1987]; Wessler *et al.*, in Proc. Intl. Symp. on Plant Transposable Elements [1988]). These elements have *Mu* termini, but share no internal sequence with *Mu1*. Furthermore, additional elements that contain *Mu*-termini surrounding unique internal sequences have been found in both *Mutator* and non-*Mutator* lines (Chandler *et al.*, in Proc. Intl. Symp. on Plant Transposable Elements [1988]).

Our lab is interested in finding out what types of elements are capable of transposing in Mutator lines. To address this, experiments were undertaken to trap elements moving in our *Mutator* stocks into genes for which probes were available.

Last summer, three *Bz1Sh1 Mutator* stocks were crossed to testers for the *bronze1* (*bz1*) and *shrunken1* (*sh1*) genes. Of the three *Mutator* stocks used, one had a typical number of *Mu-1* hybridizing sequences and two had been screened for low *Mu-1* copy number. The progeny were scored for stable and unstable *bz1* and *sh1* mutants and 39 *bz1* and 25 *sh1* putative mutants were isolated. This past winter, 13 *bz1* and 6 *sh1* putative mutants were crossed to *bz1sh1* tester to determine if the mutations were transmitted to the progeny. Eight of the 13 *bz1* mutants transmitted a mutable *bz1* phenotype and five of the *sh1* putative mutants transmitted a *sh1* mutant phenotype. The remaining mutants either transmitted a wildtype phenotype or failed to produce progeny.

Of the 13 mutants that transmitted a mutant phenotype all 13 contain insertions ranging from 500 bp to around 5 kb in size. Currently, these insertions are being analyzed using Southern blots and genomic cloning.

"An Independently Segregating Factor Which Increases the Reversion Rate of bPerumu5"

Garth Patterson and
Vicki Chandler

Our group is interested in the expression of the B gene, which is a regulatory gene of the anthocyanin pathway in Zea Mays. Vicki Chandler and Virginia Walbot have used stocks which have Mutator activity to generate four unstable mutations of the B-Peru allele. One of the mutants, bPmu5, has a very low germinal reversion rate and small somatic revertant sectors, which is a phenotype typical of mu-induced mutations. We have discovered that the reversion rate of bPmu5 changes dramatically in some crosses. In these crosses, some progeny have a drastically increased somatic and germinal reversion rate, while other progeny retain the low reversion rate of the bPmu5 parent. The segregation ratios ot these two types of individuals has led us to the hypothesis that the increase in reversion is due to a single dominant gene which is unlinked to the B locus. We call this gene Modulator.

Seedling Screen for a Site-specific Mutation in a Mutator Line

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Little is known about timing mechanisms that delay assembly of the major light harvesting complexes during chloroplast morphogenesis. We are interested in understanding how mutation at V24 elicits precocious assembly of these chlorophyll a/b light harvesting complexes. One strategy is to clone the locus using transposon-tagging. I report the use of a seedling screen to obtain new mutations at v24, using a Mutator line as the female parent. The line employed contains Mu1 and mutable b of undefined origin, but possibly induced by a Mu1 relative. Test crosses were largely made to heterozygous v24-424 material. Homozygous v24-424 plants have poor survival frequency in the field, produce small ears and flower 2-4 weeks later than non-virescent siblings. On screening approximately 80,000 seedlings, in the sand bench, three strongly virescent individuals were observed that were derived from three separate ears. Therefore, the mutation frequency at V24 was at least 7.5×10^{-5}. Two of the virescent individuals survived to maturity, and one was outcrossed as a male to a rapid-cycling maize line (.75 Gaspe, .25 Mo17). Neither of the two survivors set seed, presumably due to the weak nature of the plants. The use of a small, rapid-cycling line permitted maintenance of material at all maturation stages within limited greenhouse space. We also report independently isolated virescent mutations with a similar phenotype to v24, and induced in Mutator lines by M. Freeling.

ABSTRACT
Run-on Transcription Assays
Judy Strommer
Mu Workshop, June 1988

One direction of Mu-related research has been to examine effects of Mutator element insertion at specific loci in order to learn how genic sequences and structures affect the level of gene expression. The direct measurement of transcription in isolated nuclei has proved important for such work, permitting a distinction between transcriptional and post-transcriptional determinants of mRNA levels. There are reasonable concerns about the use of such run-on transcription assays, due both to the inherent difficulty in defining good controls and to the variability in different investigators' methods. Because the approach is so promising and so susceptible to misinterpretation, I would like to inititiate a discussion of the method and its use by (1) describing the run-on transcriptional system as we envision it to be operating and (2) presenting experimental evidence in support of this view.

MODIFICATION STATUS OF *Mu* ELEMENTS

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This poster is divided into two sections in which related aspects of the modification of *Mu* elements are addressed.

I. DNA Modification at *bz2-mu1* The mutable allele *bz2-mu1* was recently cloned (McLaughlin & Walbot, 1987), and a detailed restriction map extending about 2 kb on either side of the element insertion is available. In addition, the sequence of the *Mu1.4* insert and about 1 kb of 5' and 3' flanking sequence has been determined (See Poster by Luehrsen & Walbot at this meeting). We have used this information and subclones of the *Bronze-2* gene sequence to study the modification status of the *Mu1.4* element at *bz2-mu1* in Active (spotted kernels) and Inactive (no somatic mutability) lines by digesting genomic DNA with many C-methylation sensitive enzymes. By hybridizing the same blots with gene-specific probes we have studied the modification of sites within *Bronze-2* and *Adh1*.

Our basic findings are [1] The modification of the *Mu1.4* element at *bz2-mu1* parallels the behavior of the family as a whole; in the Inactive line we find modification throughout the element sequence, [2] In the Inactive line there are 6 restriction sites in the coding region of *Bronze-2* proximal to the *Mu* insert that show decreased digestion; digestion at one site within 100bp of the distal insertion site is also depressed. Sites more than 1kb proximal or distal to the *Mu* insert appear to be unaffected. [3] In the Active line we found three sites within the proximal coding region that are hypomodified relative to the *Bronze-2* progenitor allele. *The implications of the correlation between the modification state of the* Mu *element and the nearby gene sequences are discussed.*

II. Buoyant Density Measurements on *Mu* Elements in Active and Inactive Lines Our strategy is to use the observation that DNA sequences containing methylated bases have a decreased buoyant density to ask whether *Mu* elements are hypomethylated in Active lines and/or hyper-methylated in Inactive lines. We digest genomic DNA with *Taq*I, an enzyme insensitive to C-methylation, to generate the same fragment lengths containing the centers of *Mu1.4* and *Mu1.7* elements from both Active and Inactive Mutator lines. After centrifugation to equilibrium in CsCl, the buoyant density of fractions taken from each gradient is determined by refractometry. Aliquots of each fraction are displayed on a slot blot, and the position of *Mu* elements is determined by hybridization to pA/B5 (an internal segment of *Mu1*). In an Inactive line we find that the *Mu* elements hybridizing to this probe are modified to the extent expected for bulk maize DNA of this base composition and CpG + CpNpG content. In contrast, *Mu* elements in an Active line are hypomodified.

PLANS TO CLONE THE *A2* LOCUS USING EXISTING MUTABLE ALLELES

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Why clone another gene?

A2 is the only required structural gene of the anthocyanin pathway for which no molecular evidence is available. Because *Bz1*, *Bz2* and *A2* are coordinately regulated but are not "choice points" in the anthocyanin:flavonoid pathway, these genes may share the "minimum" information required for regulation. Of interest to research on Mutator is elucidation of the type, position and orientation of the putative *Mu* elements in each of the *a2-mu* alleles available.

How to do this?

In 1982 we isolated 3 *A2* mutables using the Mutator line that yielded the *Bronze-2* and *Bronze-1* alleles we have characterized as containing *Mu* inserts. All three of the *a2* mutables show the "standard" late sectors in the aleurone. For 6 years these mutables have been propagated by outcrossing, but no molecular analysis was conducted until 2 months ago. To clone *A2*, we will employ the same strategy used by McLaughlin & Walbot to obtain the *bz2-mu1* allele plus some additional analyses. The primary trick used in the *bronze-2* cloning was to hybridize all phage containing *Mu* homology with Northern blots containing just two lanes: mRNA prepared from purple (B P1) or green (b p1) husk tissues. Only one phage showed differential hybridization, and this contained the *bz2-mu1* allele. Will we be so lucky a second time? As pessimists, all of our other strategies are designed to eliminate *Mu*-hybridizing bands on genomic Southern blots as *a2* candidates. We could find a band that co-segregates unambiguously with the mutable phenotype. We think that the *a2* mutables will contain a known element type; we have already found lines with low *Mu1.4* or *Mu1.7* copy numbers. **We would appreciate samples of *Mu3*, *4*, *5*, *6*, etc. to check copy number thoroughly.**

We will prepare Southern blots of genomic DNA restricted with C-methylation insensitive enzymes that also cut outside the *Mu* elements, i.e. *BclI*, *SphI*, etc., and hybridize with pA/B5 or SD2 (*Mu1.7*-specific). We will discount bands [1] if found in some but not all spotted K from a single ear or [2] if also found in the tester or in non-spotted sister kernels. After phage are obtained from a library, we will determine the size of *BclI* fragments in the inserts and set aside as unlikely any phage containing fragments that match the unlikely list from tests 1 & 2. We will thus focus on just a few candidates. One more possibility: Our *a2-mu1* isolate included sister mutable kernels; two of these have been outcrossed for 5 generations. If the two original K shared 30 *Mu* inserts, after 5 generations there should be only 1 - 3 fragments in common. We'll check these stocks very carefully!

PROPERTIES OF AN ACTIVE MUTATOR LINE PLACED IN CULTURE

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The long term goal of these studies is to introduce marked *Mu* elements into an active Mutator cell line to attempt to dissect the regulation of *Mu* element behaviors. Several preliminary studies are in progress to test the feasibility of this approach. Our strategy and results are summarized below:

1. Embryos from reciprocal crosses of A188 X Active Mutator (*bz2-mu1* marker gene) yield rapidly growing callus at a high frequency. Some of these cultures are embryogenic: one plant has already been regenerated and self-pollinated. Because of prior crosses, both the Active Mutator plant and the A188 inbred line had the same A188 cytoplasm. The A188 nuclear contribution was about 60%.

2. Examining 18 pools of 3 callus cultures each and 22 individual, independent cultures, we find that the *Hin*fI sites in the termini of *Mu1.4* and *Mu1.7* elements usually remain unmodified over at least a six month period in culture. Only two callus cultures have been found in which there is masking of a few *Hin*fI sites in these size classes of *Mu* elements; both of these cultures had an A188 maternal parent.

3. We have demonstrated that viable protoplasts can be prepared from the Active Mutator callus cultures using conditions slightly modified from those used with Black Mexican Sweet suspension cultures. The Mutator protoplasts can be successfully electroporated and actively express DNA constructs; in fact, expression is generally higher than the standard Black Mexican Sweet tissue culture line. Because the protoplasts are smaller, a higher voltage is required to successfully introduce DNA.

Future studies will utilize a variety of *Mu* insertions into marker genes with readily scored or selected traits in attempts to develop a transient assay for *Mu* excision. The Mutator protoplasts will also be used to study the impact of *Mu* insertion on reporter gene expression in an active Mutator line compared to the non-Mutator Black Mexican Sweet line.

Sequence Analysis and a Putative Germinal Revertant of the *bz2-mu1* Allele of *Zea mays*

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We have determined the nucleotide sequence of the mutable *bz2-mu1* allele of maize; this sequence comprises the entire *mu1* element with about 1 kbp of *bz2* flanking sequence. The *mu1* element is 1370 bp long and is bounded by 9 bp direct repeats with the sequence 5' GCCAGACAC 3'. It is approx. 99% homologous to the *Mu* element from *Adh1S3034* (Barker *et al. Nucl. Acids Res.* 12: 5955) with most of the differences being base pair changes or single insertions/deletion events. Of particular note, however, is that the final 7 bp of the right terminal inverted repeat was not observed in the cloned sequences.

By removing the *mu1* and 9bp repeat sequences we have reconstructed the putative wild-type *Bz2* allele. Preliminary evidence suggests that all of the *Bz2* transcribed region is shown in the sequence presented. Using single stranded probes against Northern blots, we have deduced the size of the *Bz2* transcript in purple tissues (approx. 840 bases) and the direction of transcription. There is no long open reading frame (ORF) in the *Bz2* sequence capable of coding for an 840 base transcript indicating the likelihood of intron(s) being present in the gene.

A potential germinal revertant of the *bz2-mu1* allele was also recovered as a purple sector in an ear from a *bz2-mu1* lineage. Southern blot analysis of DNA derived from purple seed has established that the flanking restriction sites are identical to those found in the progenitor allele and that the transposable element is no longer present. In addition, the restriction fragments of the revertant allele are slighty smaller (50-100 bp) than the progenitor indicating a deletion of part of the *Bz2* sequence likely occurred as a consequence of excision.

Investigating the Effect of Robertson's *Mutator* Insertions on *Adh* Gene Expression Using a Transient Assay System

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Robertson's *Mutator* is a line of maize that contains a family of transposable elements. The first *Mutator* element cloned and characterized was an insertion of 1.4 kbp in the first intron of *Adh1;* this element was called *Mu1.* Also, two additional independent 1.4 kbp *Mu* element insertions were recovered and characterized in *Adh1* intron 1. Each of these *Mu* insertions decreased *Adh* gene expression to different levels in a position-dependent fashion.

In an effort to elucidate the mechanisms by which gene expression is decreased, we have attempted to recreate the insertion mutations in plasmid constructs using the firefly luciferase gene to assess expression. The constructs consist of the *Adh1* promoter, intron 1 with and without *Mu* insertions, the luciferase coding region, and the nopaline synthase (NOS) 3' region. The constructs were then introduced into BMS protoplasts by electroporation and tested for luciferase expression. We will show that expression levels of *Mu*-containing constructs in the transient assay are similar to the enzyme levels of the mutant *Adh1* alleles *in vivo.*

Effect of nitrogen deprivation on the rate of transposable element excision.

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The dramatic effects of "genomic stress" on the activity states of maize transposable elements have been well established. We are interested in determining whether more subtle environmental stresses can also affect transposable element activity. With this in mind, we have introduced mutable alleles of the maize genes that determine plant color (*Bz, Bz2, C2*) into a *B* (booster of anthocyanin) background. In the presence of *B*, fully colored sectors resulting from transposon excision can be observed on the sheath. The size, number, and position of sheath sectors enables us to predict the timing and frequency of excision. Precise Mu excision in the aleurone is apparently limited to very late in development, producing a pattern of small, fairly uniform spots. We have also observed this pattern of Mutator spotting in the anther. It is this lack of variability in the timing of excision that makes Mutator- generated alleles especially useful for our investigations. We have, however, also looked at the excision of *Spm* from *c2*, an event which apparently occurs at random throughout aleurone development.

Our results with the *B c2Spm* line suggest that growth on low nitrogen stimulates excision of *Spm* from *c2*. The frequency of "long" stripes (arbitrarily defined as those stripes extending from node to node) doubled in plants grown with 1/10 standard nitrate and no NH_4^+, while "short" stripes (less than 1 node in length) tripled in these nitrogen-deprived plants.

Unfortunately, the excision of *Mu* from mutable alleles of *bz* and *bz2* could not be determined unambiguously in our lines. We did find, however, that nitrogen deprivation strongly affected both the intensity and distribution of pigment in both *bz* and *Bz* plants. We are currently working on the construction of alternative *B* lines carrying Mutator alleles of *bz, bz2*, and *c2*.

Utilization of genetic markers to study the genetic control of Mutator activity and the distribution of *Mu* elements in the genome

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The most intriguing aspect of Mutator (*Mu*) activity is that it has not been explained by the segregation of one or more Mendelian units. The observation of cases where a given ear is composed of an active and an inactive sector is also perplexing, because one conclusion from this observation is that *Mu* activity isn't the result of genetic segregation. Moreover, when an active Mutator is crossed to a non-Mutator line most progeny are active.

Quantitative traits show a similar problem: it is difficult to detect single Mendelian units affecting one character, and it is common to observe more than one phenotype for a given genotype. The major problems are that these traits are strongly affected by environmental factors and are usually polygenic. A recent approach to overcome the complexity of quantitative traits has been to associate these traits to characters showing simple inheritance.

The aims of this project, which is in progress, are:
1) to test whether *Mu* activity can be correlated with the segregation of simple Mendelian genes (morphological and RFLP markers). For this purpose an active Mutator line, whose somatic activity can be assayed at the bronze2 locus, was crossed to the Mangelsdorf's tester which carries a morphological marker on each chromosome. On selfing, we can determine if *Mu* activity segregates preferentially with any linkage group. This analysis may provide an answer to whether the loss of somatic instability results from genetic segregation, or occurs in a random selection of genotypes. It might enable us to map to linkage groups, the autonomous factor(s) responsible for activity.
2) to use genetic markers in order to determine the distribution of multicopy genes, such as the *Mu* elements, within the genome. For example, if one allele of a marker is significantly associated with a higher *Mu* element copy number than the other allele, this will suggest the presence of a linked *Mu* element cluster, whose copy number and location can be estimated by studying additional close-by markers.

Abstract

The fate of Mutator elements introduced into maize protoplasts by electroporation

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Chimeric gene constructs containing either intact Mu1.4 or Mu1.7 elements were introduced into maize protoplasts by electroporation to determine if either type of element encodes sufficient information to support transposition. Transformed calli were recovered containing from one to several copies of the integrated plasmid. The structure of the integrated DNA from individual transformants was determined by Southern analysis as soon as sufficient callus became available (about 4 months). Selected transformants were periodically re-tested over a period of 30 months for evidence of changes in the fragment pattern and methylation state. Events displaying the correct hybridization pattern were observed at low frequency early in the growth of the culture. Particular nucleotides in the introduced elements were methylated but the rate of modification varied with the individual transformant. Particular components in the chimeric construct have a strong impact on the number of integrated copies suggesting that transposable element copy number can be manipulated in this system.

Molecular Analysis of the Distribution of Selected Elements of the Mutator System in Stocks from Different Mutator-induced Y1 Mutants in Maize.

T. L. Kelson, B. Buckner, and D. S. Robertson

We are interested in studying the incidence of mutation induced by Mutator elements other than Mu1. Over 40 Mutator-induced Y1 mutant families were initially screened to determine the copy number of Mu1-hybridizing elements. Each family contained between 5-20 copies of Mu1-hybridizing elements. Eight of these families were chosen for further study because of the high ratio of Mu1.7 to Mu1 elements as demonstrated by the intensity of bands on autoradiographs after digestion with HinfI, electrophoresis, and Southern blotting. Heterozygous seeds (Y1/y1-Mum) were planted and outcrossed to a standard y1 stock (y1/y1). The progeny seeds of this cross were of two types: Y1/y1 (yellow) and y1-Mum/y1 (white). DNAs isolated from seedlings grown from both types of these seeds were digested with BamHI, a restriction enzyme which cuts outside of Mu1 and Mu1.7 elements, electrophoresed, and Southern blotted to nylon membranes. These segregation analyses were done by separately hybridizing each blot with radioactively labeled Mu1, Mu1.7, and Mu3 specific fragments.

Martha G. James, Philip Stinard, Donald S. Robertson, and Joan Stadler.
Regeneration of a putative Mu-loss embryogenic callus line. Iowa State University, Ames, Iowa, 50011.

Plants were regenerated from a hybrid H99/Mutator embryogenic callus line for which the Mutator (Mu^2) parent was a putative Mu-loss plant. Primary regenerants (R_0) were self-pollinated and the progeny plants (R_1) outcrossed reciprocally with a standard line (Q60). F_1 progeny from two of these reciprocal crosses were then planted and self-pollinated. As in Robertson's standard test for Mutator activity (Robertson, 1978), progeny ears were examined for the appearance of new seedling mutants. A new mutant frequency of 8% was observed when one of the R_1 plants was crossed as a female. No new seedling mutants were observed when this plant was crossed as a male, or from either cross of the other R_1 plant. HinfI digests of the DNA from the mutant seedlings and their wildtype siblings showed the Mu elements to be modified at the HinfI sites. Similar digests of DNA from various tissues of two other primary regenerants (R_0 somaclones) showed the Mu elements in the immature ear of one of the regenerants to mimic the mixed HinfI modification state of the Mu elements in the callus line. Other tissues examined had Mu elements which were modified at these sites. These preliminary findings have encouraged us to expand the number of crosses and examination of Mu element modification state in these regenerants to determine if an inactive Mutator system has been reactivated.

Mu-INDUCED MUTANT ALLELES BEING STUDIED

Name:
Lab:

Gene Studied	Type of Mu Insert	Type of Analysis (summarize briefly)

Additional Comments
(Briefly discribe your interest in this gene, somatic mutability pattern, Mu orientation, etc):

OBSERVATIONS ON CHANGES IN Mu ACTIVITY

Name:
Lab:

Type of Cross	Assay(s) for Mu Activity	Activitated/Inactivated and to what extent

Additional Comments
(Modification/methylation status of Mu elements or reporter gene, etc):

TRANSPOSON TAGGING AND GENE ISOLATION

Name:
Lab:

Gene of Interest	Tagged?	Number of Mutants	*Mu* Copy Number	Segregating *Mu* Band (Type of *Mu* Element)

Additional Comments
(Please indicate problems encountered in cloning):