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ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE

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The maize R gene is said to show more phenotypic variation than any other locus in higher plants (1). The locus is organized on a modular basis. Individual units -- termed "genic elements" since they function as independent genes -- differ by regulating the presence, intensity and timing of anthocyanin pigmentation in different plant parts. A given allele may comprise only one genic element or, more commonly, an allele comprises a complex of elements, organized as a small gene family. Different numbers and combinations of even a few genic elements gives a large number of possible complexes.

Following molecular cloning of R (2) we concentrated effort initially on a genic element that confers strong pigmentation only to the kernel. The functional limits of this gene (R-sc:124) had been defined genetically by extensive mutagenesis with the transposable element Dissociation (3,4). Subsequently, a set of contiguous probes were prepared from a genomic clone of R-sc:124. This set of probes distinguishes among various R-genic elements, providing physical evidence on gene complexes whose overall organization had been determined genetically. Some surprises were in store, as detailed below. These same tools made it possible to analyze the effect of position of Ds insertion within R-sc:124 on spotting phenotype, germinal reversion rate and frequency of Ds excision. Furthermore, we are now able to address in much more detail some of the unique phenomena of R action, such as a difference in kernel phenotype when certain alleles are transmitted by ovules relative to sperm. The last category of objectives is discussed in the section entitled "updated research plan".

RECENT PROGRESS

Somatic variegation and germinal mutability reflect the position of the transposable element Ds within the R gene. (M. Alleman and J. Kermicle, manuscript in preparation). Sequential mutagenesis of the R-sc:124 allele with the Ac/Ds system of transposable elements was used to generate 42 spotted-aleurone mutants containing inserts of the 2.1 kb element Ds6. The mutations differ only in the position and orientation of Ds6. The patterns of somatic instability of these mutants, resulting from excision of Ds, define a spectrum of aleurone phenotypes ranging from sparse to densely spotted with a distinct discontinuity between groups. The sparsely variegated mutants produced few germinal revertants but relatively many stable null alleles; densely variegated mutants produced many germinal revertants and few stable null derivatives.

Molecular analysis shows the sparsely variegated alleles to be caused by Ds6 insertion within protein coding regions whereas the densely variegated mutants result from insertions in introns or in the flanking regions of the gene. Although the mutability of the alleles varied widely, the excision rate of the Ds6 element was uniform regardless of position or orientation. The excision rate was greater, however, for a mutable R-sc allele involving a different Ds element. The data indicate that, although the total excision rates differs between transposable elements, the somatic and germinal mutability patterns of mutant alleles depends mainly on the position of the transposable element within the gene.

Meiotic instability of the R-r complex arising from displaced intragenic exchange and intrachromosomal rearrangement. (T. P. Robbins, E. Walker, J. L. Kermicle, M. Alleman and S. L. Dellaporta, 1991. Genetics 129:271-283). This collaborative investigation with the Dellaporta laboratory utilized probes derived from the single element alleles R-navajo and R-sc to investigate the structure and instability of the R-r (colored seed;colored plant) complex. Genetic analysis had shown the R-r complex to comprise distinct plant (P) and seed (S) elements arranged in tandem order. A duplication with (P) in one member and (S) in the other is the simplest structure that would account for the observation. Molecular analysis, however, revealed considerable more complexity.

The (P) function is associated with a single sequence within R-r whereas (S) is associated with multiple R segments. Between the two, interspersed with R nonhomologous sequences, lies a truncated R segment (designated "Q"). Unequal crossing over between (P) and (Q) results in loss of (P) function; crossing over between (P) and (S) results in loss of (S) function. Use of restriction site polymorphism to locate the exchange points showed most of the exchange points to occur within R sequences rather than between elements. Localized changes within (P) and (S) are responsible for losses of function that are not associated with exchange of flanking markers. The precise nature of these changes and the arrangements of segments with the (S) subregion are continuing topics of study by the two laboratory groups.

Gametic imprinting in maize in relation to the angiosperm life cycle. (J. L. Kermicle and M. Alleman, 1990. Development 1990 Supplement, 9-14.) In this interpretative paper we draw together evidence indicating differences in the action of genes transmitted via ovules and pollen. Evidence was considered for three levels of genetic manipulation: the entire chromosome set, particular chromosome arms and individual genes. In each case, differences in action are found in the endosperm but in no case has a difference been detected in the embryo. The detection of parental imprinting only in the endosperm points to the few cell divisions during female gameteophyte development as a critical stage. Chromosomally-based variation originating at this time would be preserved by double fertilization, helping to account for the divergent developmental paths followed by the two resulting products.

Our interest in imprinting derives from the finding that some R alleles confer solid coloration to the aleurone layer of the endosperm when transmitted maternally, but weak and patchy coloration when transmitted paternally. In this paper we summarized the evidence showing the difference to be transmitted chromosomally. Maternal and paternal forms of R retained their distinction during endosperm development even when present in the same nucleus. Gene activity states evidently need not be reset each cell cycle but can be transmitted miotically, contributing to cell heredity and helping to account for stability of the differentiated state.

UPDATED RESEARCH PLAN

The principal objectives of the proposal dated July, 1989, remain intact. Attention will be given here only to situations where the objectives either have been supplemented or the approach modified in light of subsequent progress.

Many combinations of recessive colorless-seed variants (r) recombine to produce the occasional full color derivative. For some time we have been aware that the pattern of intragenic recombination was dependent on the particular combination of r alleles used. Combinations involving any one of a number of Ds insertion alleles with a wild-type allele that confers only plant color (r-r) produce R's mostly in association with recombination of flanking markers. Such R "crossover" producing combinations are to be compared with pairwise combinations of the Ds insertion alleles, which most frequently produce R derivatives having a parental combination of flanking markers (putative gene conversions). The difference was attributed to the inserts themselves (5). Recently we learned that stable recessive alleles resulting from transposition of Ds retained their characteristic pattern of recombination, calling into question the earlier interpretation. More likely, it now appears, difference in the progenitor chromosomes lying outside the region defined by the mutant sites governs the pattern of recombination. By identifying crossovers in the immediate R region, parental origin of the flanking regions can be varied, thereby testing dominance of the effect and whether the critical region is 5' or 3' of R. Using restriction site polymorphisms as markers provides a much finer level of resolution than previously possible.

Recovery of wild-type (R) from r r heterozygous combinations is as whole kernels, never as multikernel events or as aleurone sectors. It is as though recombination is occurring only at meiosis. The only circumstance where aleurone spotting has been observed is when the two mutants are present in the same chromosome as members of a tandem duplication. To test whether the effect of cis arrangement is essential for recombination, members of the duplication have been separated and are being tested in trans. Similarly, combinations that proved stable in trans are being introduced into the duplication to test stability in cis. Preliminary observations suggest somatic recombination is occurring preferentially if not exclusively between closely linked r sequences.

Molecular approaches can now be applied to some of the unique phenomena of R action where genetic analysis alone had reached a state of diminishing return. One example will suffice. The R-stippled allele reduces the action of sensitive alleles in heterozygotes. This effect differs from the "co-suppressing" effect of transgenes on the indigenous wild-type gene in petunia (reviewed in ref. 1), in that the effect of R-st on homologous sequences is heritable. R-stippled's effect is associated with duplicated R segments since stepwise reduction in segment number through unequal crossing over leads to reduction and then loss of its transallelic effect. Our ability to determine the organization of this properly in R-st has been restricted by an inability to distinguish between the duplicated regions. Recently, restriction site differences near the ends of most of the segments were identified. This knowledge is being applied to a set of unequal crossover and terminally truncated derivatives of R-st in order to associate particular duplication members and combinations of them with its transallelic effect.

LITERATURE CITED

1. Dooner, H. K., Robbins, T. P., Jorgenson, R. A. 1991. Genetic and developmental control of anthocyanin biosynthesis. Annu. Rev. Genet. 25:173-199.

2. Dellaporta, S. L., Greenblatt, I., Kermicle J. L., Hicks, J. B., Wessler, S. R. 1988. Molecular cloning of the maize R-nj allele by transposon tagging with Ac. In 18th Stadler Genet. Symp., ed. J. P. Gustafson, R. Appels, eds., pp. 263-282. New York: Plenum.

3. Kermicle, J. L. 1980. Probing the component structure of a maize gene with transposable elements. Science 208:1457-1459.

4. Kermicle, J. L., Alleman, M., Dellaporta, S. L. 1989. Sequential mutagenesis of a maize gene using the transposable element Dissociation. Genome 31:712-716.

5. Dooner, H. K., Kermicle, J. L. 1986. The transposable element Ds affects the pattern of intragenic recombination at the Bz and R loci in maize. Genetics 113:135-143.

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