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Use of a *Ds* Chromosome-Breaking Element to Examine Maize *Vp5* Expression

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To determine the localization of *Vp5* expression in maize, and to further characterize *vp5* mutations in kernels and in other parts of the plant, a genetic technique was utilized to generate mosaic tissues. In particular, advantage is taken of a feature of the *Activator (Ac)*–*Dissociation (Ds)* controlling elements of maize to cause chromosome breakage, allowing phenotypic observation of the lethal *vp5* allele throughout the maize plant. Mosaic tissues result from chromosome breakage at the site of the *Ds* element and subsequent unmasking of a recessive *vp5* allele. The appearance of sectors in both endosperm and leaf tissue indicates that the *Vp5* product behaves in a cell-autonomous fashion. Spectrophotometric analysis of leaf-sector pigments supports this observation.

Carotenoid pigments function as accessory light harvesting pigments in photosynthesis and are the primary photoprotectors of all photosynthetic organisms. In photosynthetic organisms, colored carotenoids protect against photooxidation by quenching highly reactive and damaging singlet oxygen and excited states of chlorophyll (Anderson and Robertson 1960; Mathis and Schenck 1982). In higher plants, carotenoids are additionally found in many nonphotosynthetic tissues (for reviews, see Britton and Goodwin 1982; Goodwin 1976). Carotenoids are also metabolic precursors of the plant hormone, abscisic acid (ABA) (for review, see Creelman and Zeevaert 1984; Koornneef, 1986; Moore and Smith 1985; Robichaud et al. 1980), demonstrating the manifold importance of carotenoids in both photosynthetic and nonphotosynthetic tissues.

The maize carotenoid biosynthetic pathway, deduced from biochemical characterization of mutants altering expression of nuclear encoded genes, is light regulated and plastid localized (Beyer et al. 1985; Horváth et al. 1972; Kreuz et al. 1982; Lutke-Brinkhaus et al. 1982; Mayfield et al. 1986; Robertson et al. 1978). However, mutations blocking the pathway are lethal, precluding mutant analysis throughout development. In addition, limited progress has been made in purification of the biosynthetic enzymes or isolation of genetic loci associated with this pathway, which is a prerequisite for a comprehen-

sive study of the molecular regulation of the pathway in higher plants (Buckner et al. 1990; Dogbo et al. 1988).

In the proposed pathway, the dehydrogenation of the first compound, phytoene, to phytofluene is catalyzed by a phytoene dehydrogenase. Two recessive mutations in maize, *vp5* (mapping to chromosome 1) and *vp2* (mapping to chromosome 5), block this step and therefore accumulate phytoene (Robertson et al. 1978). It is not known if either of these loci encode the phytoene dehydrogenase. In photosynthetic tissue, the absence of colored carotenoids due to these mutations pleiotropically affects the development of the chloroplast and the synthesis of both nuclear and cytoplasmically encoded proteins (Mayfield and Taylor 1984; Robertson et al. 1978). The accumulation of phytoene is manifested by a white kernel phenotype, in contrast to the yellow wild-type color (Figure 1A). The mutations do not prevent kernel development but do have a secondary effect on seed dormancy, an ABA-controlled stage in seed development. In the absence of ABA, as in *vp5* mutants, kernels precociously germinate on the ear at late stages of development. The homozygous recessive mutant seedlings are albino and do not mature.

Reported here is the genetic approach utilized to further characterize the *Vp5* locus of maize. In particular, advantage was taken of a class of nonautonomous-con-

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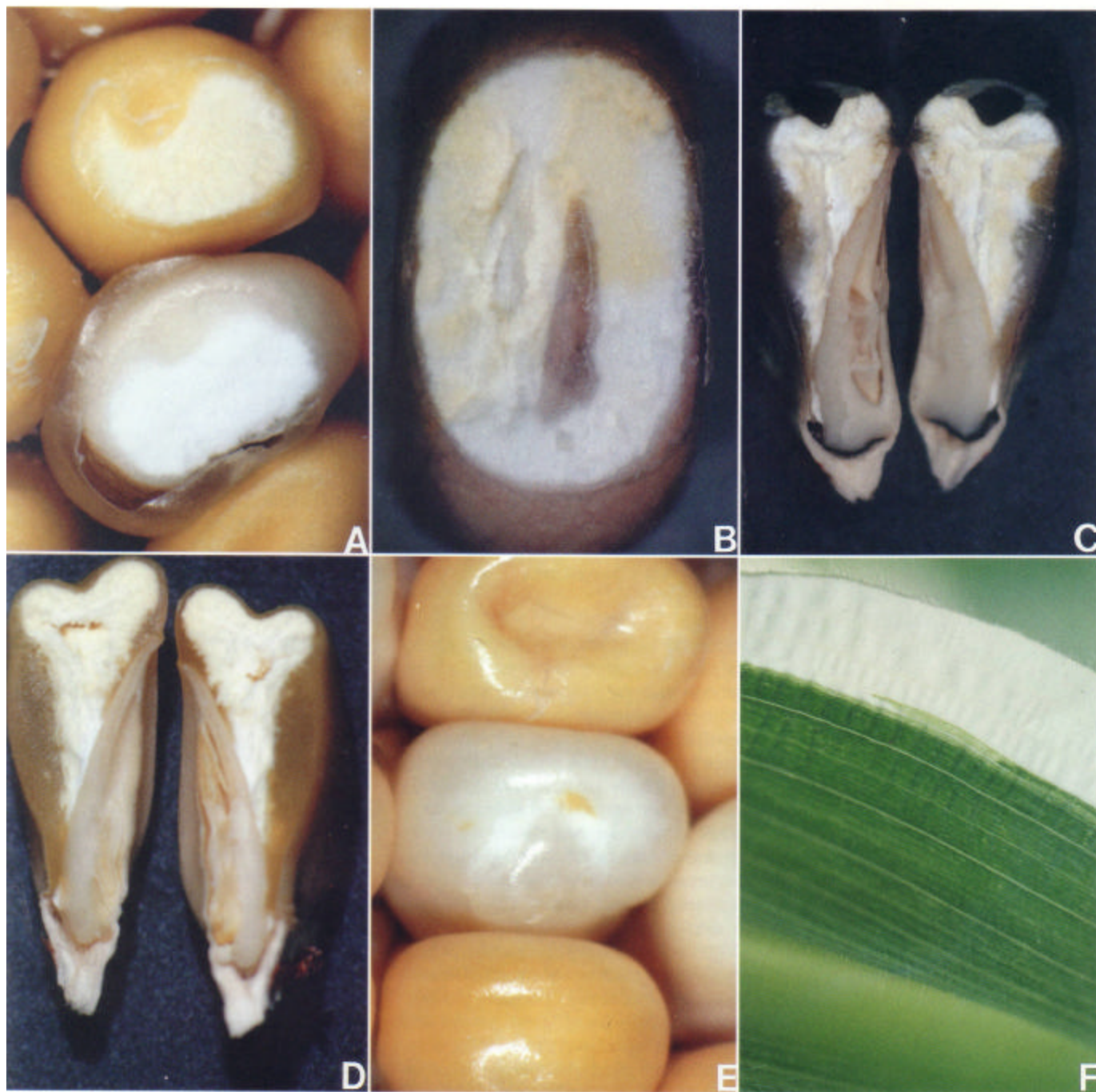


Figure 1. Kernel and leaf phenotypes: (A) Kernels from an ear segregating the standard *up5* homozygous mutant white kernels and nonmutant yellow kernels. Kernel tops have been removed to provide a view of the endosperm; (B) Top view of kernel exhibiting chromosome breakage on *1S*, produced by cross-pollination of a *up5* heterozygous stock by a line containing a *Ds* chromosome-breaking element on *1S* and an *Ac* at the *P50* locus on *1S*. The top of the kernel has been removed to provide a clear view of the endosperm. Coloration of the aleurone layer of the endosperm results from anthocyanin pigmentation. White sectors are due to chromosome breakage and unmasking of the recessive, *up5* allele; (C) The kernel shown in (B) was subsequently transversely sliced; (D) Transverse section of yellow kernel shown in (A); (E) A *Mutator*-induced *up5* mutant kernel. The white kernel exhibits yellow revertant sectors due to transposition of a transposable element away from the *Up5* locus; (F) Leaf sectors observed in a plant grown from a kernel with a genotype similar to that shown in 1B.

rolling (transposable) elements, termed *Dissociation (Ds)* elements, which were first characterized by McClintock (1946, 1947, 1951). These particular *Ds* elements, likely to have a unique physical structure (Döring et al. 1989), provide sites for chromosome breakage mediated by a transacting autonomous-controlling element, *Activator (Ac)*. Chromosome breakage

during somatic development results in variegated tissue when such chromosome-breaking elements, residing on the appropriate chromosome arm, are introduced into lines heterozygous for the locus of interest. Chromosome breakage allows for phenotypic observation of the lethal *up5* allele in both kernels and whole plants.

Materials and Methods

Plant Materials and Growth

Kernels photographed came from maize plants grown under typical summer field conditions and harvested 30–40 days after pollination. I extracted pigments from greenhouse plants grown under supplemental lighting. Maize lines, containing

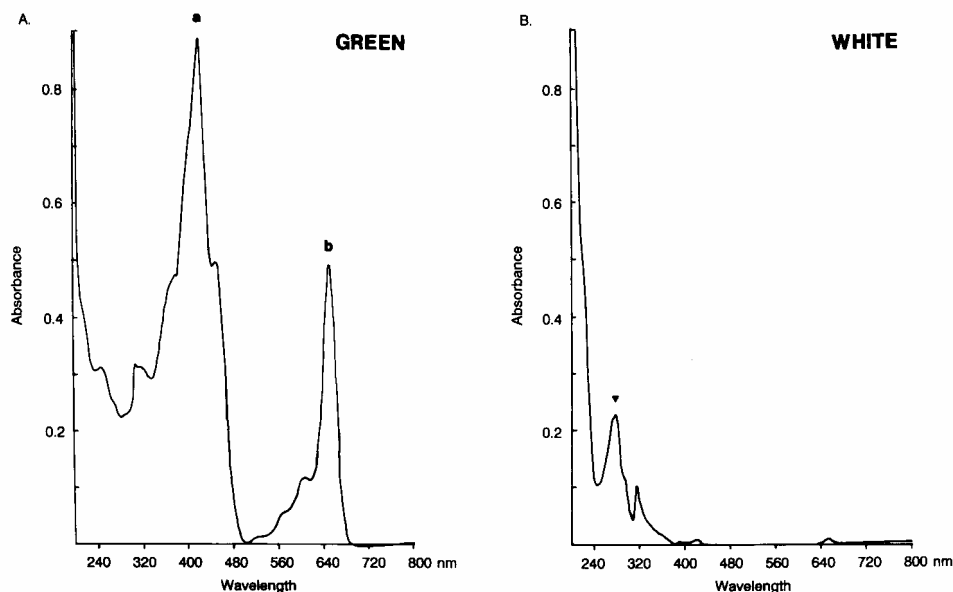


Figure 2. Spectrophotometric analysis of pigments in leaf tissue exhibiting chromosome breakage and subsequent unmasking of the recessive *vp5* allele: (A) Green leaf sample. a = carotenoids and b = chlorophyll; (B) White sector from the same leaf as in A. The arrow points to the maximum absorbance of the noncolored carotenoid precursor, phytoene.

dek1 or the *Ds* chromosome breaker (*Ds-4*) on chromosome 1S, were developed by and obtained from Dr. M. G. Neuffer (University of Missouri, Columbia, Missouri). The chromosome breaking stock was of the genotype *Vp5 P^{uv}(Ac) Dek1 Ds-4/Vp5 p dek1, A C R*. The exact position of *Ds-4* on chromosome 1S (short arm of chromosome 1) has not been defined (Neuffer 1986). I used this stock as the pollen parent and crossed it onto a *vp5* heterozygous stock obtained from the Maize Genetics Stock Center. The *Mutator*-induced *vp5* seed was obtained from Dr. Donald Robertson (University of Iowa, Ames, Iowa). Kernels shown in Figure 1E were produced by self-pollination of this latter stock.

Pigment Extraction and Analysis

Total leaf pigments were extracted from mature plants. Tissue from equivalent amounts of green or white sectors was homogenized in acetone using a Dounce homogenizer. I added an equal volume of petroleum ether, centrifuged samples at 15,000 × *g*, then transferred the organic phases to new tubes, dried them down, and resuspended them in twice the original volume of petroleum ether. Throughout the procedure, I protected samples from exposure to light. Sample absorbance readings were scanned between 200 and 800 nm in a dual-beam Perkin Elmer spectrophotometer. Preliminary identification of pigment classes was made according to Scolnik et al. (1987).

Results

To determine the localization of *Vp5* expression in maize, and to further characterize *vp5* mutations in kernels and in other parts of the plant, a genetic technique was used to generate tissues that exhibit a mixture of both mutant and wild-type phenotypes within one plant organ. This approach eliminated the need to first isolate new mutations containing transposable element insertions at the *Vp5* locus and bypassed the problems associated with obtaining mature tissue from a lethal mutant. A maize line heterozygous for a standard recessive *vp5* allele, which maps to chromosome 1S (short arm of chromosome 1), was crossed by a line (kindly provided and developed by Dr. M. G. Neuffer) containing a *Ds* chromosome-breaking element on chromosome 1S. An *Ac* on the chromosome carrying the *Ds* breaker mediates breaks at the site of the *Ds*, eliminating all genes distal to the *Ds*. This was previously tested by Dr. Neuffer, and confirmed by this author, through crossing this *Ds* chromosome breaker line by a stock heterozygous for the lethal recessive, *dek1* (*defective kernel*) mutation, which maps to chromosome 1S. Chromosome breakage causes unmasking of the *dek1* mutation, which is manifested by the segregation of defective kernels and kernels that exhibit *dek1* sectors on the ear (Neuffer 1986). The *dek1* kernels and/or sectors are easily distinguished by their lack of anthocyanin

pigmentation, which is a pleiotropic effect of this mutation (Neuffer and Sheridan 1980). Similarly, pollination of a stock heterozygous for *vp5* by this *Ds*-chromosome breaker stock leads to unmasking of the recessive *vp5* allele as a consequence of chromosome breakage of the *Ds*-containing chromosome arm. Lineages of cells derived from such a break are hemizygous for the recessive *vp5* allele and exhibit a white endosperm phenotype, whereas lineages of cells arising from nonbroken arms express the wild-type, yellow endosperm phenotype. The resulting sectorized kernel is shown in Figure 1B,C. These variegated kernels obtained by somatic events of chromosome breakage contrast with the stable phenotypes of the yellow wild type and white, *vp5*, standard recessive kernels (Figure 1A,D). The white sectors (Figure 1B,C) are large in size, indicating chromosome breaks that occurred early in somatic development of the endosperm. In comparison, Figure 1E shows a kernel that exhibits transposition of a transposable element away from the *Vp5* locus late in somatic development, giving rise to small, yellow revertant sectors.

I planted kernels obtained from the above crosses to obtain whole plants in which to observe the variegated phenotype. Whereas large sectors were observed in kernels, an indication of chromosome breakage occurring early in somatic development, leaf sectors were typically small and not evident until late in plant development. Only one in 10 plants exhibited sectors that were large enough for biochemical analysis. An unusually large white sector spanning all leaf layers is shown in Figure 1F. This white sector continues along both leaf edges for almost the entire length of the leaf. Adjacent to the white sector is a region that contains a light-green sector representing chromosome breakage in cells of only some leaf layers. The dark-green portion of the leaf actually contains many tiny sectors, representing chromosome breakage late in development. Other large sectors were usually no more than 2–3 cm in length and 2–3 mm in width on a single leaf and spanned some or all leaf layers. White sectors spanning all cell layers, as shown in Figure 1F, are useful for biochemical or molecular analysis. Pigments were extracted from equal amounts of tissue from white sectors or from the surrounding green tissue to analyze pigment composition spectrophotometrically. Figure 2A shows the analysis of pigments in the green portion of the leaf tissue. As expected,

peaks corresponding to chlorophyll (approximately 652 nm) and colored carotenoids (peak maxima at 380, 420, and 450 nm) are present. One white sector, which traversed cells in all leaf layers, was approximately 2 mm wide and extended the length of the leaf (not shown). Figure 2B shows a spectrophotometric analysis of pigments obtained from part of this white sector. The chlorophyll peak (at 652 nm) is almost entirely absent, and, in addition to a minute peak (at 420 nm) corresponding to absorption by colored carotenoids, there are peaks corresponding to the carotenoid precursor phytoene (peak maxima at 278 and 310, with shoulders at 270 and 290 nm) present in this *vp5* mutant issue.

Discussion

As shown in Figure 1B,C, pollination of a maize line heterozygous for *vp5* by a line containing the *Ds* chromosome-breaking element on chromosome 1S resulted in kernel endosperm that displayed white sectors on a yellow background, as opposed to a diffuse color that might have been expected if *Vp5* encoded a diffusible gene product expressed in only some cells. White sectors were also observed in leaf tissue derived from germination of the sectorized kernels. These results suggest that the *Vp5* gene product behaves in a cell-autonomous fashion in both endosperm and leaves. That is, a diffusible product would have been expected to traverse cell boundaries, preventing the manifestation of variegated tissues. However, sectors were evident in both kernels and leaf tissue. Biochemical analysis of white leaf sectors revealed an absence of colored carotenoids and chlorophyll, supporting the notion that the *Vp5* product does not diffuse from the nonmutant tissue to alleviate the pleiotropic effects of the *vp5* mutation on the accumulation of chlorophyll and development of chloroplasts in the white leaf sectors. This lack of diffusion was evident both across one cell layer and between leaf cell layers. The small amount of chlorophyll found in the white sector is consistent with the fact that, even in homozygous recessive albino leaves, such low levels of chlorophyll are evident (Robertson et al. 1978). An absence of sectors in the embryo tissue suggests that chromosome breakage in embryo tissue occurs very late in development, as found in leaf tissue, and sectors are therefore not readily observed.

A chromosome-breaking transposable

element proved useful in analyzing *Vp5* expression in maize endosperm and leaves. The sector size and number is different in leaf, endosperm, and embryo tissue, as shown here. The utility of this technique depends on obtaining observable sectors. These genetic stocks will be valuable for future molecular analysis of DNA, mRNA, and protein in wild-type and mutant sectors. For example, the mutant tissue contains a gene deletion that would be useful in characterization and verification of a cloned gene encoding *Vp5*. Such mosaic tissue is also useful for in situ hybridization studies. This genetic technique is particularly valuable in the study of carotenoid biosynthesis, as many nuclear mutations result in lethal albino phenotypes, which cannot otherwise be studied at the whole plant level. Furthermore, as a prelude to cloning of the *Vp5* locus, identification of transposable element-tagged *vp5* mutable kernels depends on being able to distinguish yellow revertant sectors on a white mutant background. As it is not known where and how carotenoids are sequestered in the endosperm, it is possible that somatic reversion due to excision of a transposable element might give rise to a light yellow or diffuse pattern, much like a *y1* phenotype (Mangelsdorf and Fraps 1931; Randolph and Hand 1940). A diffuse phenotype would probably be difficult to spot due to natural variations in endosperm color and would be less useful in further studies of *Vp5* action. The results described here suggest that new transposable element-insertion mutations at *Vp5* can be identified by screening for kernels having yellow and white sectors—white sectors represent a transposable element inserted at *Vp5*, and yellow sectors represent the excision and transposition of the element away from *Vp5*. This will facilitate the cloning of the *Vp5* locus by transposon tagging (reviewed in Hake et al. 1989). In fact, after initiating this study using the *Ds* breaker, I obtained a mutable *vp5* allele, as shown in Figure 1E.

The genetic approach described here can be similarly used for rapidly testing the feasibility of screening for other transposon-tagged genes. A strategy for cloning a gene that encodes an unknown product is to tag the gene with a transposable element. Transposable element-insertion mutations are distinguished from point mutations by screening for variegated phenotypes. Loss of chromosome arm conditioning a phenotype due to loss of a cell-autonomous function will be manifested as an easily distinguishable varie-

gated phenotype in a heterozygous background. Likewise, this approach is of general utility for characterizing the expression of lethal alleles of other essential genes.

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