TISSUE SPECIFIC VARIATION IN THE ISOZYME PATTERN OF THE AP_1 ACID PHOSPHATASE IN MAIZE

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MANY examples of multiple forms of enzymes are now known (WRÓBLEWSKI 1961; KAPLAN 1963; LATNER and SKILLEN 1968). These multiple forms were designated isozymes by MARKERT and MØLLER (1959). Some multiple forms are controlled by alleles of a given gene (ALLEN 1961; ALLEN, MISCH and MORRISON 1963; LEVINTHAL, SINGER and FETHEROLF 1962). Others result from the independent action of more than one gene in some instances with the formation of hybrid enzyme between the products of the two genes as in lactate dehydrogenase (APPELLA and MARKERT 1961; CAHN, KAPLAN, LEVINE and ZWILLING 1962) and in other instances without hybrid enzyme formation (SCHWARTZ 1964a; BECKMAN, SCANDALIOS and BREWBAKER 1964).

Tissue specific variation in an enzyme controlled by a single allele of the E_1 esterase in maize was described by SCHWARTZ (1964b) and ENDO and SCHWARTZ (1966). The NN pH 7.5 esterase synthesized under the control of the E_1^N allele in the seedlings and endosperm showed identical electrophoretic migration rates in starch gel. However, they differed in their inactivation by urea.

Electrophoretic studies of acid phosphatase isozymes in a number of organisms were reviewed by LATNER and SKILLEN (1968) and in maize by BROWN and ALLARD (1969). This paper describes the AP_1 acid phosphatase isozymes in maize. Results will be reported to show the presence of different forms of AP_1 acid phosphatase in the scutellum and the pollen, while both are present in the leaf. In identical genotypes, the single AP_1 isozyme in pollen migrates somewhat faster than the single AP_1 isozyme in the scutellum.

MATERIALS AND METHODS

The tissues used in this investigation were the scutellum, leaf and mature pollen; they were homogenized in distilled water and centrifuged for 20 min at 20,000 $\times$ g in preparation for the electrophoretic assay. A few extracts from other tissues were also tested. The mature pollen was collected in the field during the growing season and stored at — 20°C. Extracts from the scutellum were obtained after the seeds had been soaked in water at 30°C for 16 hr. The extracts were placed in the gels by means of saturated strips of Whatman No. 3MM filter paper. Samples of the leaves were obtained by direct squashing on the filter paper.

Starch gel electrophoresis was performed at pH 8.5 in a sodium borate buffer as described by SCHWARTZ (1960). The acid phosphatase bands were developed by a staining technique based on

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that used for acid phosphatase in Tetrahymena (ALLEN, MISCH and MORRISON 1963). The gels were immersed in a solution of .02 M acetate buffer (pH 4.8) containing 1 mg/ml sodium 1-naphthyl-phosphate (Koch-Light Laboratories Ltd.) and 1 mg/ml Fast Garnet GBC (Sigma Chemical Co.).

The effect of heat and pH on enzyme stability was tested on extracts of pollen, scutellum and mixtures of the two. Heat treatment was given for 20 min at 5°C intervals. The pH of the extracts was adjusted with HCl or NaOH. The adjusted extracts were incubated for 20 min at room temperature before the electrophoretic run. Sodium acetate buffers of various pH levels were used to test the effect of the pH on enzyme activity. The pH was verified in the staining solution after the incubation of the gels.

The following 27 inbred lines were tested in these studies: B-6, B-14, B-37, B-42, B-45, B-48, B-50, B-52, B-54, B-55, B-56, B-57, B-66, CI-31-A and Hy from the Iowa Agricultural Experiment Station; C-103, M-14, N-6, Oh-7, Oh-43, R-105, and R-181-B from the Illinois Agricultural Experiment Station; and K-4, E_1^N/E_1^N, and E_1^S/E_1^S from D. SCHWARTZ at Indiana University. All the inbred lines were grown at Indiana University and later at the Faculty of Agriculture (Hebrew University of Jerusalem), Rehovot, Israel.

RESULTS

Maize plants contain a number of acid phosphatase isozymes controlled by nonallelic genes. Two of these nonallelic isozymes are shown in Figure 1. The enzyme under investigation in this study is in the bands which move the slowest toward the anode at pH 8.5. The gene which specifies this enzyme is designated AP_1. The gene which specifies the faster migrating and more prominent acid phosphatase is designated AP_2. A third locus (AP_3) controls the synthesis of still another acid phosphatase isozyme that migrates faster than AP_2 (EFRON 1969). This enzyme is inhibited by Fast Garnet GBC and therefore is not present in Figure 1.

Three alleles of AP_1 which produce enzymes differing in migration rate have been found in maize stocks (Figure 1). The alleles AP_1^F, AP_1^I, and AP_1^S specify the phosphatase isozymes with fast, intermediate, and slow migration rates. At the same time, the faster migrating prominent AP_2 isozymes migrate to the same position, indicating that the AP_1 and AP_2 isozymes are controlled by nonallelic genes. Furthermore, line R-105 has only residual activity of the AP_2 enzyme in the pollen (Figure 1, middle), but the activity of the AP_1 enzyme is not affected.

In scutella and pollen of identical genotypes, single AP_1 isozymes are found in homozygotes. However, the scutellar and pollen isozymes are not identical, and migrate to different positions during electrophoresis. The pollen enzyme migrates somewhat faster than the scutellar enzyme (Figure 2d). These differential migration rates of the scutellar and pollen enzymes are maintained even in incubated mixtures of extracts from both tissues. Leaf tissues of homozygous young seedlings and of older plants show two isozyme bands in approximately equal proportions, as determined from the relative intensity of the two bands (Figure 2a). One of the leaf isozymes appears to be identical to the scutellar isozyme (Figure 2c), and the other leaf isozyme migrates to the position of the pollen isozyme (Figure 2b).

The AP_1 acid phosphatase enzyme behaves as a dimer. Scutella heterozygous

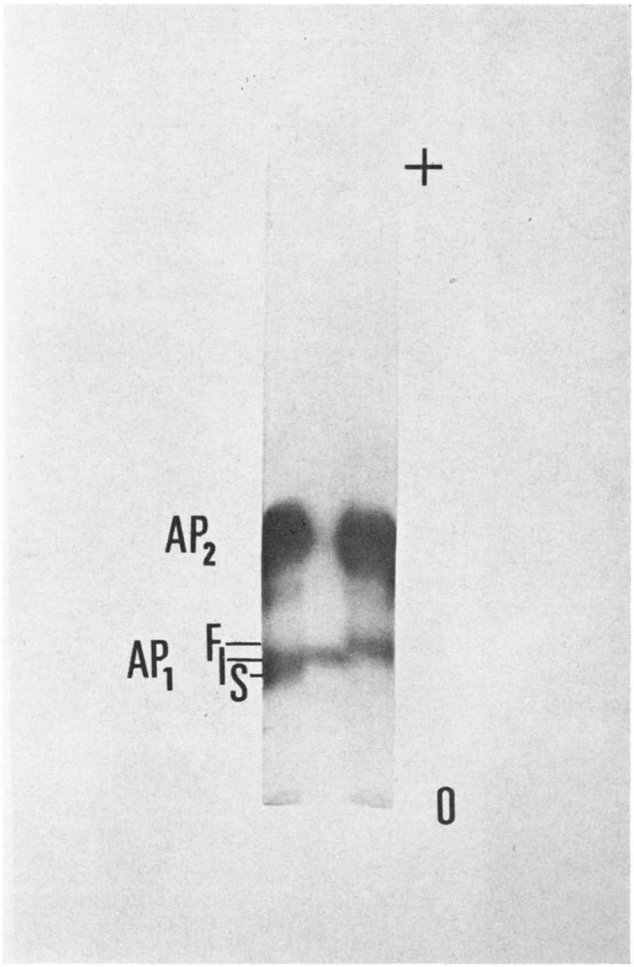

FIGURE 1.—Zymogram of the three acid phosphatase isozymes extracted from mature pollen of maize. Left—AP_1^S/AP_1^S (N-6); middle—AP_1^I/AP_1^I (R-105); right—AP_1^F/AP_1^F (CI-31A).

for AP_1^S/AP_1^F show the presence of an intermediate hybrid isozyme in addition to the autodimers found in the parental homozygotes. Pollen from heterozygous plants lacks the hybrid enzyme. This is to be expected if the enzyme which is present in the mature pollen is synthesized after tetrad formation. A segregation test of AP_1 genotypes in F_2 and testcross families (Table 1) is consistent with the hypothesis that the migration differences for AP_1 are under the control of a single locus with three codominant alleles: AP_1^S, AP_1^I and AP_1^F.

In the series of inbred lines tested, AP_1^S was found to be the most common allele and AP_1^I the least common. There is complete correlation of the leaf, scutellum and pollen isozyme bands in the various genotypes. All plants which were classified as AP_1^S/AP_1^S by showing the slower migrating band in the scutellum show also the slowest migrating band in the pollen and the slowest

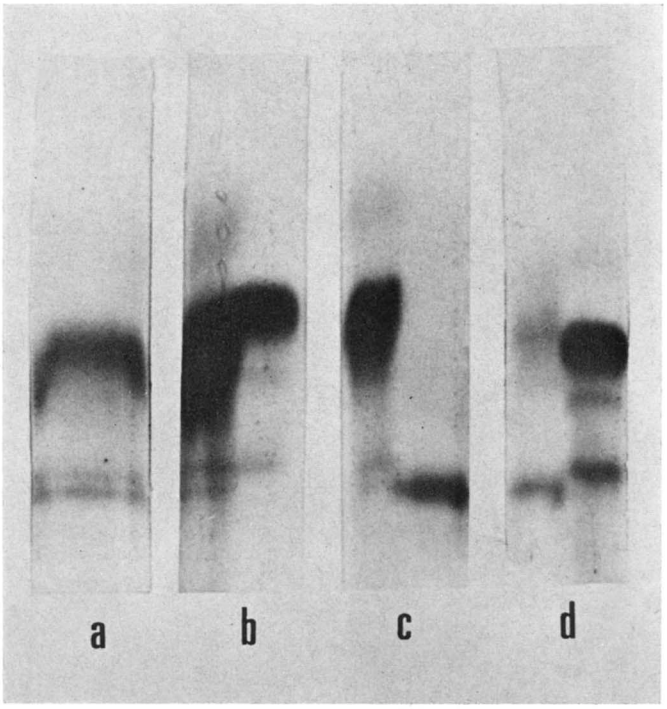

FIGURE 2.—Zymograms of the acid phosphatase isozymes from leaf, scutellum and mature pollen of homozygous AP_1^S/AP_1^S plants of the inbred line N-6. (a) Extract of leaf, showing two multiple bands of the AP_1 enzyme. (b) Extracts of leaf (left) and pollen (right). (c) Extracts of leaf (left) and scutellum (right). (d) Extracts of scutellum (left) and pollen (right).

double band in the leaf. Plants of the genotype AP_1^F/AP_1^F, which form the fastest migrating band in the scutellum, also show the fastest band in the pollen as well as the most rapidly migrating double band in the leaf. AP_1^I/AP_1^I plants form isozymes of intermediate migration rate in all tissues. This complete correlation would not be expected if the scutellar and pollen isozymes were specified by different nonallelic genes.

TABLE 1

Segregation and goodness-of-fit test in F_2 and testcross families in crosses involving the AP_1^S and AP_1^F alleles

AP_1 Cross	Tissue	Genotypic Classes			χ^2	Probability
		SS	*SF*	*FF*		
$S/F \times S/F$	Pollen	20	45	25	1.06†	.50–.70
$S/F \times S/F$	Scutellum	32	56	36	1.42†	.30–.50
$S/F \times S/S$	Pollen	29	28	..	.01‡	.90–.95
$S/F \times F/F$	Pollen	..	19	21	.10‡	.70–.80

† Tested for goodness of fit to 1:2:1
‡ Tested for goodness of fit to 1:1

Another test was conducted to determine whether the two bands in the leaf are under the control of a single gene. If the two bands are under the control of two nonallelic genes, each with three alleles specifying isozymes with different migration rates (i.e., the slower migrating band controlled by an AP_x gene and the faster migrating band by an AP_y gene), some strains should have bands with different distances between them, if the genes were not very tightly linked. For example, if the AP_x isozyme were of the S type and the AP_y of the F type, the distance between the two bands would increase. Extracts of leaves from each of about 600 lines of maize were tested in electrophoresis. From each line three different plants were tested. In all lines the distance between the two bands remained the same, indicating that the two bands are controlled by the same gene.

The AP_1-controlled isozymes from the scutellum and the pollen are identical in their heat stability and differ from the AP_2 enzyme (Figure 3). In both the AP_1^S/AP_1^S (N-6) pollen extract (bottom) and the mixture of AP_1^S/AP_1^S (N-6) scutellar extract with AP_1^I/AP_1^I (R-105) pollen extract (top), the activity of the AP_1 isozymes starts to fall at 50°C and completely disappears at 60°C. The AP_2 pollen enzyme still exhibits some activity at 65°C. Similarly, the scutellum and the pollen forms have identical pH stability (Figure 4), and their activity is affected to the same degree by the pH of the staining solution (Figure 5). Quantitative measurements in pollen extract with a densitometer have shown that the optimum pH for the AP_1 enzyme is somewhat higher (4.9) than that for the AP_2 enzyme (4.7). At pH levels above the optimum, enzyme activity is more sharply reduced than at levels below the optimum.

The enzyme forms of the scutellum and the pollen have the same "retardation coefficients" (SMITHIES 1962), thus eliminating degree of polymerization as the reason for the differences in migration rates. In 10% starch concentration the scutellar band migrates to a distance equal to 90.1% of that of the pollen enzyme, while in 15% starch this proportion is 90.5%.

DISCUSSION

BROWN and ALLARD (1969) described three codominant alleles in maize, $Phos_4^S$, $Phos_4^N$ and $Phos_4^F$, controlling phosphatase isozymes with different migration rates. It seems that the $Phos_4$ gene is probably not the same as the AP_1 gene, since the AP_1-controlled isozyme is not active at the alkaline pH (7.5) in which the $Phos_4$ isozymes were tested. Furthermore, BROWN and ALLARD (1969) reported that there is a single band controlled by $Phos_4$ in the seedling, while two bands were found to be controlled by the AP_1 gene in this study.

All the results presented in this paper are consistent with the hypothesis that despite the differences in migration rates, the scutellar and the pollen AP_1 enzymes are controlled by the same gene. Therefore, the conclusion which might be drawn from this study is that the AP_1 acid phosphatase can exist in two forms with different electrophoretic mobilities. The form which the enzyme takes depends on the tissue in which it is synthesized. The leaf is composed of many cell types. Since the analysis was not performed on single cells, both enzyme forms might exist in the same cell, or any one cell type in the leaf

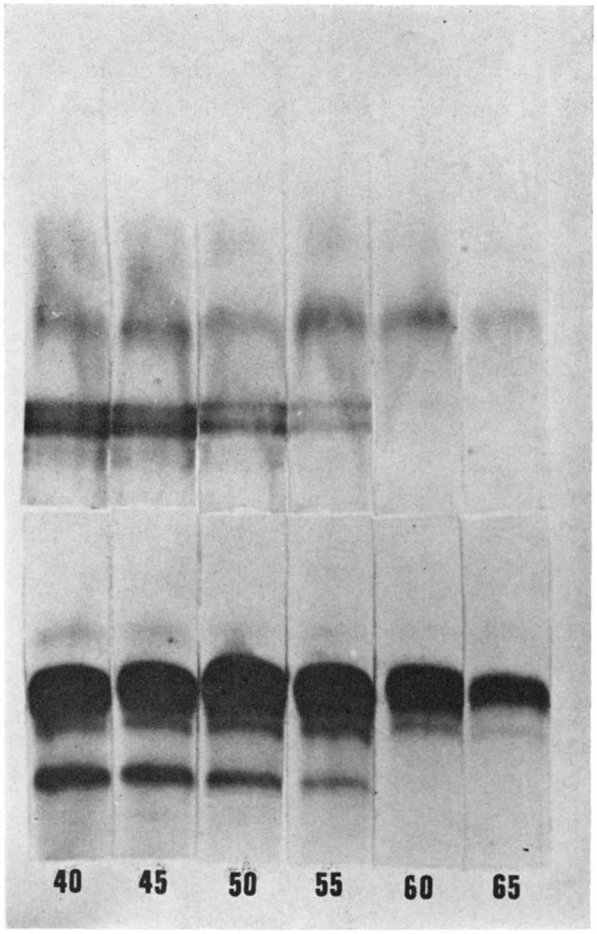

FIGURE 3.—Effect of temperature on the stability of the acid phosphatase isozymes of maize. Bottom—extract of pollen from the inbred line N-6 (AP_1^S/AP_1^S). Top—Mixture of extracts from the scutellum of the inbred line N-6 (AP_1^S/AP_1^S) and pollen of the inbred line R-105 (AP_1^I/AP_1^I).

could contain one or the other enzyme. It is difficult to distinguish between the two alternatives, but the former appears more likely. If only one enzyme form is found per cell, one might expect that the relative proportions of the two enzymes would vary in plant parts which differ strikingly in structure. However, the relative intensity of the two bands in the doublet is identical in the roots, coleoptile, leaves, and the first stem internodes of young seedlings.

There are a number of possible explanations for the results described in this paper. Studies of multiple molecular forms of enzymes (KITTO, WASSERMAN and KAPLAN 1966) have shown that the same polypeptide can exist in a number of conformational states. The AP_1 enzyme can be considered to have two stable conformational forms. The conformational forms taken by the enzyme may be

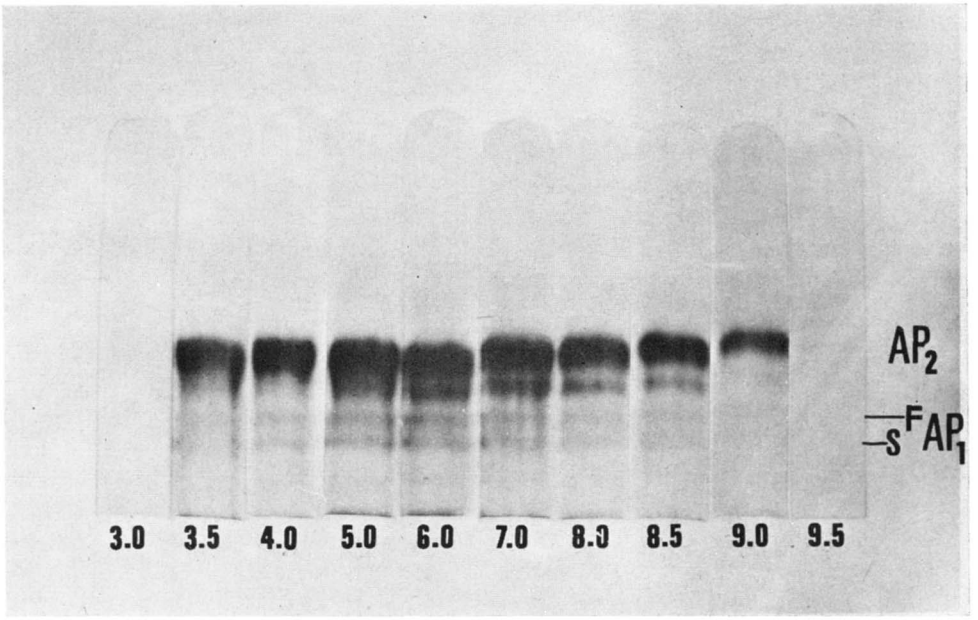

FIGURE 4.—Effect of pH on the stability of the acid phosphatase isozymes of maize in a mixture of extracts from the scutellum of the inbred line N-6 (AP_1^S/AP_1^S) and pollen of the inbred line CI-31A (AP_1^F/AP_1^F). The pH was adjusted 20 minutes before the electrophoretic run. Staining was performed in the same staining solution at pH 4.8.

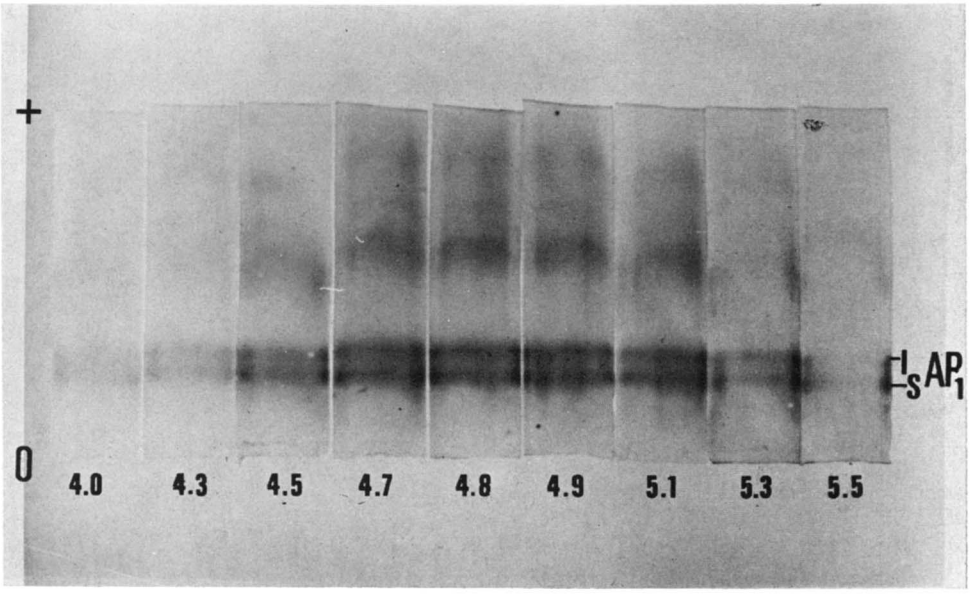

FIGURE 5.—Effect of pH on the activity of the acid phosphatase isozymes from a mixture of extracts from scutellum (AP_1^S/AP_1^S–N-6) and pollen (AP_1^I/AP_1^I–R-105).

determined by the cellular environment during synthesis, and result in different folding configurations in pollen and scutellum. This scheme was proposed by SCHWARTZ (1964b) to explain the tissue-specific differences in urea sensitivity of the E_1^N esterase in maize. The factors which influence the type of folding may be absent in the leaf tissue, so that the enzyme exists in both stable conformations.

A second but less likely possibility is along the lines established by ETZLER and LAW (1967) to explain "dominance" for alkaline phosphatase and leucine aminopeptidase in chicken. The pollen and scutellar enzymes may differ by the presence or absence of a charged group conjugated to the protein. Accordingly, the two tissues would differ in that only one of them would contain an enzyme which attaches the charged group to the acid phosphatase molecule: a pollen enzyme attaching a negatively charged group or a scutellar enzyme attaching positively charged group. The weakness in this scheme consists of the fact that the concentration of the second enzyme in the leaf would have to be so limited that only one-half of the acid phosphatase molecules are acted upon, and both enzyme forms exist. Of course, this argument would not hold if each cell type of the leaf contains only one enzyme form. Neuraminidase had no effect on the migration rate of either the pollen or the scutellar forms of the enzyme. A somewhat similar explanation may be based upon a supposition of Moss and KING (1962). They suggested that the different bands of alkaline phosphatase in the same human tissue might be complexes with different proteins, but actually fractions of the same enzyme. According to this model, the pollen and scutellar enzymes may be bound to two different proteins, one of which is present in the scutellum but not in the pollen, and the other in the pollen but not in the scutellum. Both proteins are present in the leaf, so that the enzyme can be bound to both. This model eliminates the weakness of the previous scheme, since it does not require limited concentrations of a second enzyme. However, since the two enzyme forms have identical retardation coefficients, the two proteins which bind with the enzyme should not differ considerably in their size.

At present it is difficult to decide which of the three schemes discussed is the most probable. Attempts are now being made to determine the basis of the different migration rates of the AP_1 isozymes in the different tissues of the maize plant. It should be taken into consideration that, even though it is not likely, the possibility of two very closely linked genes cannot be completely ruled out. An unequivocal critical test would require an induced mutation of one allele to another, e.g., AP_1^S to AP_1^I, to see if the mutation alters the isozymes in all tissues. Attempts to induce such mutation by ethyl methanesulfonate are presently under way.

I wish to express my gratitude to DR. D. SCHWARTZ, in whose laboratory this work was initiated, for his kind hospitality and many helpful suggestions.

SUMMARY

The AP_1-controlled acid phosphatase enzyme, one of a number of acid phosphatase enzymes present in maize plants, has been investigated by means of

starch gel electrophoresis. The AP_1 locus specifies the slowest moving acid phosphatase isozymes at pH 8.5. Three alleles, AP_1^S, AP_1^I and AP_1^F, which specify the AP_1 acid phosphatase isozymes with slow, intermediate and fast migration rates, respectively, have been found. For each genotype, single nonidentical AP_1 isozymes were found in scutellum and pollen of homozygous plants. The pollen isozyme migrated somewhat faster than the scutellar isozyme. Both forms are present in the leaf. The two isozymes were identical in their heat and pH stability and in their pH optima. It was suggested that the two isozymes are controlled by a single gene. Possible explanations for the results obtained are discussed.

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