the radioactivity was counted for incorporation of ^{14}C. Usually incorporation of ^{14}C into ferritin ranged from 50 to 120 counts per minute per milliliter of incubation mixture, whereas radioactivity incorporated into total liver protein was between 30,000 and 100,000 counts per minute per milliliter of incubation mixture. In order to eliminate variations of incorporating capacity from one polysome preparation to another, the uptake into ferritin has been expressed as a percentage of total incorporation of amino acid radioactivity into peptides (Table 1). The difference in ferritin labeling between the two polysome populations is consistent in all five comparisons of free and total polysome populations and averages 65 percent greater activity with the free population. This is not due to a difference in release of completed peptide chains from the two polysome populations; examination of peptides released under the conditions of incubation (*7*) showed that 39 percent of the labeled peptide was released from the free polysomes during incubation, and 43 percent from the total polysome preparation.

In order to verify that the activity observed in the ferritin fraction is not due to a radioactive contaminant, ferritin samples were isolated by the unmodified method of Drysdale and Munro (*4*) after incubation of the cell-free system with free polysomes. The ferritin was then treated with 1 percent sodium lauryl sulfate at 70°C in order to dissociate ferritin into its subunits. The free subunits were separated from the parent ferritin with a polyacrylamide gel (*8*). The protein bands were then eluted and examined for radioactivity. This treatment resulted in dissociation of a substantial part of the original ferritin into subunits which were found to contain a considerable proportion of the radioactivity of the ferritin applied to the gel. Because of the difficulty of measuring the protein content of the subunits in the gel, it was not possible to obtain more than a rough indication that the amount of radioactivity migrating with the subunits was proportional to the amount of protein in this fraction. The presence of activity in the separated subunits strongly suggests that the radioactivity associated with the original isolated ferritin must have taken the form of ferritin protein.

This evidence of preferential synthesis of ferritin by free polysomes prompted us to examine the relative efficiencies of free and total polysome populations in the synthesis of serum albumin, a secreted protein. The conditions of incubation of free and total polysomes were similar to those used for studying ferritin biosynthesis. After an hour of incubation, albumin was isolated with acid ethanol followed by immunological precipitation; coprecipitation of nonspecific proteins was minimized by initial treatment with chick serum albumin and then antiserum to chick albumin. Incorporation of ^{14}C-amino acids into albumin was expressed as a percentage of total counts of radioactivity incorporated into peptides by the cell-free system. The results of two such experiments (Table 1) show that, in contrast to ferritin, albumin is less effectively synthesized by free polysomes than by the mixture of free and attached polysomes. Consequently, our evidence favors the view that free polysomes and membrane-attached polysomes synthesize different types of protein, and is compatible with the hypothesis that the free polysomes are associated with formation of proteins retained in the cell, whereas the attached polysomes make secreted proteins.

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Alcohol Dehydrogenase in Maize: Genetic Basis for Multiple Isozymes

Abstract. *There are two distinct sets of alcohol dehydrogenase isozymes from maize, but they have subunits in common. Induced mutations at the* (Adh_1) *locus altered the migration rate of both Set I and Set II isozymes.*

Maize zymograms show two distinct well separated sets of alcohol dehydrogenase (ADH) isozymes (*1*). Intense slow migrating isozymes have been designated as the Set I bands and the less intense bands which migrate to a more anodal position as Set II. Inheritance of the Set I and Set II ADH isozymes is completely correlated. In homozygotes, single ADH bands appear in one of three positions in the Set I and Set II regions. The position of

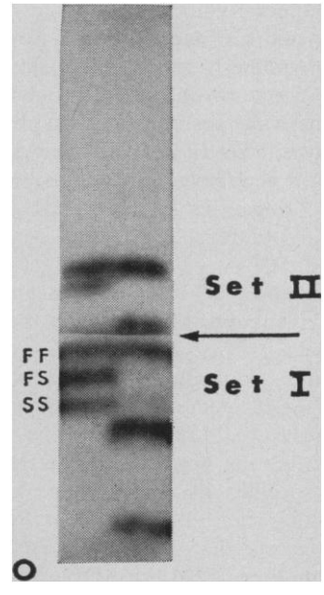

Fig. 1. Zymograms of Adh_1^F heterozygous with Adh_1^S (left) and with a mutant allele obtained by EMS treatment of Adh_1^S (right). Note the altered positions of both the Set II and Set I bands in the EMS-induced mutant. The Set II bands are much fainter than the Set I bands. In order to show all the bands clearly in a single photograph the gel was cut in two in the region between the two sets of bands (indicated by the arrow), and the upper half of the gel was developed for a longer period to intensify the Set II bands; *O* designates the origin, and the anodal pole is at the top. Materials used for electrophoresis were scutella from kernels which had been soaked in water for 48 hours, germinated 24 hours, and then soaked again in water for another 24 hours. The starch-gel electrophoretic and zymographic procedures were as described (*4*).

the Set II band is always correlated with the position of the band in the Set I region. Plants with the fastest migrating Set I isozyme (ADH^C) also show the fastest migrating Set II band. The same is true for the slow (ADH^S) and intermediate (ADH^F) migrating Set I and Set II isozymes. This correlation is complete and no exceptions have been found in thousands of samples analyzed to date. In heterozygotes, a hybrid isozyme appears in the Set I region but not in Set II although the Set I and Set II enzymes do not differ significantly in size.

We have explained (*1*) the ADH zymogram patterns by postulating the existance of two *Adh* genes. The Adh_1 gene is polymorphic, and dimers composed of two ADH_1 subunits appear as bands in the Set I region. The Adh_2 gene is considered to be represented by only a single allele, and it specifies an enzymatically inactive, but more negatively charged, polypeptide. It was hypothesized that Set II isozymes are heterodimers composed of an active ADH_1 and an inactive ADH_2 subunit. This hypothesis readily explains the correlation between the Set I and Set II isozyme band positions and the absence of a hybrid Set II isozyme in heterozygotes. In heterozygotes, such as Adh_1^S/Adh_1^F, only the $ADH_1^S \cdot ADH_2$ and $ADH_1^F \cdot ADH_2$ Set II bands can be formed.

Scandalios (*2*) proposed an alternative explanation. He considers that the two sets of isozyme bands are independently controlled by two closely linked *Adh* genes. According to his nomenclature, Adh_2 specifies the major slow migrating isozyme (our Set I), and Adh_1 specifies the weak, more anodal migrating set of isozymes (our Set II). He suggests that the ADH_2 isozymes are dimers, but that the ADH_1 isozymes either exist as monomers or are dimers with polymerization restricted to identical subunits.

This explanation was previously considered but we held it to be unlikely because of (i) the complete correlation in the migration rates of the Set I and Set II isozymes in Adh_1^S, Adh_1^F, and $Adh_1^{C(t)}$ genotypes; (ii) the occurrence of a fourth allele, $Adh_1^{C(m)}$, which specifies isozymes at the same Set I and Set II positions as $Adh_1^{C(t)}$ but with much reduced activity in both regions; and (iii) the fact that in homozygotes the duplicate gene $Adh_1^{\overline{FC}}$ produces isozymes in the F and C positions in both the Set I and Set II regions.

If the two sets of ADH isozymes are under the independent control of two linked genes as proposed by Scandalios, induced mutations that alter the migration rate of one isozyme should not affect the position of the second ADH band. However, according to the hypothesis proposed by this author, mutations at the Adh_1 locus which change the charge of the Adh_1 subunit should alter the migration rates of both the Set I and Set II bands since both are composed entirely or partially of ADH_1 subunits.

The Adh_1^S homozygous kernels were treated with ethyl methanesulfonate (EMS) according to the procedure of Briggs *et al.* (*3*), planted in the field and the plants were pollinated by homozygous Adh_1^F plants. The F_1 kernels were screened electrophoretically for mutants of the Adh_1^S allele.

Results indicated that the Set I and Set II isozymes have subunits in common. Four mutations at the Adh_1^S locus were found which produced an isozyme with an altered migration rate in the Set I region. In every case there was a concomitant and correlated change in the position of the Set II band (Fig. 1). If the two ADH isozymes were independently controlled by different genes, these results would require simultaneous, correlated mutations in both genes —a highly unlikely condition. Numerous mutations were recovered which resulted in the elimination of the ADH^S band in both the Set I and Set II regions but these are not critical to the argument since they could represent aberrations which deleted closely linked loci. The Adh_2 mutations which should alter the migration rate of only the Set II isozyme were not found in this study.

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Auditory Sequence: Confusion of Patterns Other Than Speech or Music

Abstract. *Accurate perception of temporal order is essential for many auditory tasks. Yet the temporal pattern of four successive sounds (for example, hisses, buzzes, and tones) could not be recognized even when the duration of each sound was considerably longer than either the average phoneme in normal discourse or the notes of melodies. Although each of the stimuli was perceived, their order remained frustratingly elusive.*

Realization of the importance of sequence recognition to normal auditory function led Hirsh (*1*) to state that any theory of auditory perception requires ". . . the concepts of sequence and temporal pattern to play the same role that Gestalt or form or shape has played in visual perception." The ease and assurance with which temporal order is perceived are especially obvious for speech and music, and it has been assumed that this ability could be applied to sequences of other sounds as well.

However, during a psychophysical experiment involving a repeated sequence of three successive sounds (1000-hz tone, broad-band noise, 600-hz tone), we found that, although listeners perceived each of the separate sounds clearly, they could not tell the order in which they occurred, even with continued listening. It was impossible to tell if the low-pitch tone followed the noise or the high-pitch tone. Yet the duration of each sound was 200 msec, considerably longer than the 70 to 80 msec for the average speech sound in normal discourse, and the 50 msec required for perceiving the sequence of successive notes in music (*2*).

Previous studies on perception of the order of three or more different sounds have involved only speech or music. However, when pairs of electrically generated sounds (for example, a tone and a buzz) were presented, subjects identified which of the two sounds came first (stimulus duration, as short as 20 msec) (*3*). The difficulties which our subjects had in recognizing sequences of three arbitrarily selected sounds suggested that we were not