clear; the phenomenon is not complicated by the formation of triple ions, as in other cases that we have examined.

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*STUDIES OF TYROSINASE PRODUCTION BY A HETEROCARYON OF NEUROSPORA**

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The tyrosinase of *Neurospora crassa* exists in two forms that differ in their thermostability.[1] This difference is inherited in a simple Mendelian way, the stable form of the enzyme being determined by a gene, T^S, and the labile form by its allele, T^L. The locus is on the right arm of the mating-type chromosome, 18 map units (uncorrected) distal to *adenineless*-35203 but proximal to *albino*-15300. As far as is known at present, the only other property in which the two forms of the enzyme differ is in the activation energy of thermal inactivation, which appears to be higher for the thermolabile form.[2] Measurements of several functional properties, including Michaelis constants for two substrates and relative turnover numbers, have not shown any significant difference between the two enzymes.[2]

Since the T^S-T^L alleles are recognized by a qualitative rather than a quantitative effect on the enzyme, an opportunity was presented for investigating the question of whether the alleles act independently in heterocaryons or whether they interact so as to produce a hybrid form of the enzyme or so as to show dominance of one allele over the other. At the same time it was possible to carry out a test of the hypothesis that tyrosinase synthesis is mediated by self-duplicating cytoplasmic particles. The principle of this test will be explained in connection with the experimental results.

Strains and Methods.—The heterocaryon was produced by fusion of hyphae from T^S and T^L strains carrying, respectively, the mutant genes *adenineless*-35203 and *histidineless*-C140. The strains had previously been made nearly isogenic by six successive backcrosses of *ad*-T^S progeny from the cross *ad*-T^S × *hist*-T^L to the *hist*-

T^L parent. The nutritional markers served to stabilize the heterocaryon on minimal medium and to mark the nuclei in the second part of the experiment. Both homocaryotic components of the heterocaryon were of mating type A. The constitution of the heterocaryon was verified by showing that both original homocaryons could be recovered from a single hyphal tip.[3] Conditions of growth and of extraction and purification of the enzyme were as described previously.[1] Enzyme activities were determined colorimetrically with DL-DOPA as substrate.[1] Thermostability tests were carried out at 59° ± 0.02° C. and pH 6.

Tyrosinase Production by the Heterocaryon.—Measurements of the thermostability of tyrosinase produced by the heterocaryon and by the two parent homocaryons are shown in Figure 1. It is seen that thermal inactivation of the enzyme from the

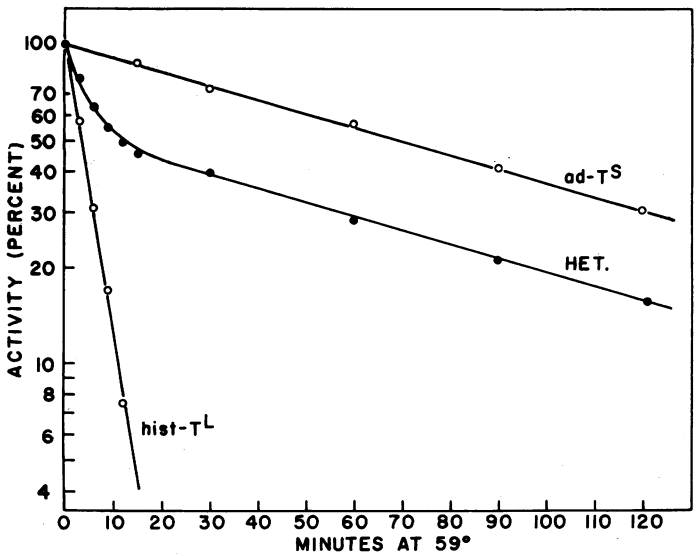

FIG. 1.—Thermal inactivation of tyrosinase from two homocaryotic strains and the heterocaryon between them. The half-life of the enzyme from *ad-T*^{S} is 70 minutes in this experiment; the half-life of the enzyme from *hist-T*^{L} is 3.4 minutes. In the case of the heterocaryon, the curve drawn through the experimental points is the theoretical for a mixture containing 52.3 per cent of tyrosinase of half-life 70 minutes and 47.7 per cent of tyrosinase of half-life 3.4 minutes.

homocaryotic strains is a first-order process, the half-lives of the two forms at 59° being 70 and 3.4 minutes, respectively, in agreement with previous determinations.[1] Inactivation of tyrosinase from the heterocaryon does not follow the first-order course expected in the case of dominance of one allele or of interaction to form an enzyme of intermediate stability. Rather, it closely follows the curve predicted on the basis of a mixture of the two original enzymes. The relative proportions of the two activities are deducible from the first-order tail of the curve, which represents the inactivation of the stable component of the mixture. By extrapolating this portion of the curve back to zero time, the initial amount of thermostable activity can be read off the y-axis; the amount of thermolabile activity and the expected course of the thermal inactivation are then easily derived. In this way it can be

shown that the curve of Figure 1 is that predicted from a mixture containing 52.3 per cent thermostable and 47.7 per cent thermolabile tyrosinase activity.

Resolution of the Heterocaryon.—To test the theory that production of the enzyme is mediated by self-duplicating cytoplasmic particles, the heterocaryon was resolved into its genetic components by cutting off and subculturing hyphal tips; a fraction of such tips can be expected to be homocaryotic by chance. Since there are no effective crosswalls in the hyphae of *Neurospora*, the genetically unlike nuclei of a heterocaryon share a common cytoplasm. On the hypothesis of self-perpetuating cytoplasmic agents, homocaryotic cultures derived in the manner described might therefore be expected to resemble the parent heterocaryon in their tyrosinase production, rather than the original homocaryotic strains.

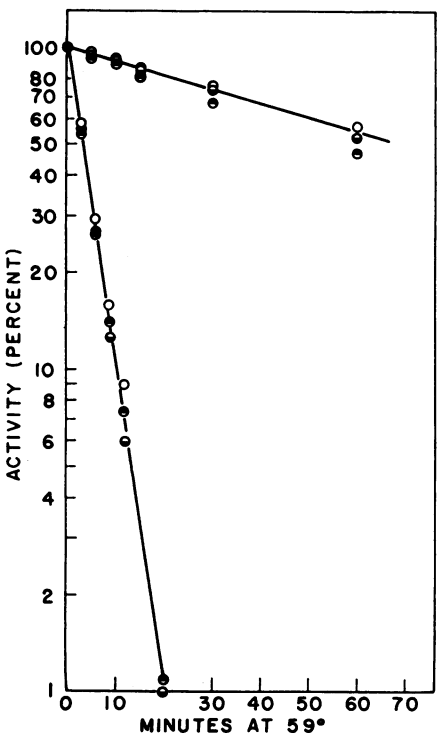

FIG. 2.—Thermal inactivation of tyrosinase from six homocaryons obtained by asexual resolution of an *ad-*T^S*/hist-*T^L heterocaryon. The curves are drawn to fit half-lives of 70 and 3.1 minutes, respectively.

Of 1,220 hyphal tips taken, 638 survived on transfer to minimal medium supplemented with adenine and histidine. Of these, 6 proved to be homocaryotic, as shown by the fact that they failed to grow on minimal medium. Transfer to medium supplemented with adenine or histidine showed that 3 of the isolates were adenineless (and therefore T^S) and 3 were histidineless (therefore T^L). These isolates were next grown in large cultures, and their tyrosinase was extracted, purified, and tested. The results, plotted in Figure 2, show that all the homocaryons produced only one form of tyrosinase, and this in accordance with the genetic constitution of the nucleus. The presence of 1 per cent of thermostable activity in the thermolabile preparations would have been detected, or the presence of 10 per cent of labile activity in the stable preparations. The half-lives determined for the six enzyme preparations were 3, 3.4, and 3.5 minutes for the enzyme from the histidineless cultures and 55, 69, and 74 minutes for the enzyme from the adenineless cultures. The stable form is generally more variable than the labile form in thermostability tests, owing to the fact that it is influenced to a greater extent by impurities in the preparations.[1] The effective impurity seems to be principally tyrosine, traces of which are apparently released by a proteolytic enzyme which contaminates some of the tyrosinase preparations. Like other tyrosinases, *Neurospora* tyrosinase is inactivated in reacting with its substrates, and the presence of tyrosine in the preparations causes an apparent decrease in the thermostability of the stable form; the labile form is hardly affected, since it is thermally inactivated too rapidly at 59° to engage in many catalytic cycles.

Discussion.—The absence of allelic interaction in the synthesis of tyrosinase by the heterocaryon parallels findings which have been made in the genetically similar case of sickle-cell anemia in man. It has been found that sickle-cell heterozygotes produce both the normal and the abnormal form of hemoglobin.[4] The same thing is true for the β_1- and β_2-lactoglobulins of cattle, which, on the basis of gene-frequency analysis of the data of Aschaffenburg and Drewry,[5] appear to be determined by a pair of allelic genes. It remains to be seen whether this will turn out to be a general rule for genes governing the structure of proteins, as would be expected on the template theory of gene action.

The failure to detect cytoplasmic transmission of tyrosinase-forming capacity in hyphal tip transfers is consistent with the known Mendelian inheritance of the thermostability difference. In order to account for the rarity of known cases of cytoplasmic inheritance compared with chromosomal heredity, it has been suggested that plasmagenes may be subject to decay in the germ line.[6] The present experiments lend no support to this idea, in so far as it draws a distinction between the germ line and the soma. Decay of the hypothetical plasmagenes in our case must be presumed to take place in asexual as well as sexual generations. It may be pointed out that the present experiments are not subject to the interpretation that persistence of the hypothetical plasmagenes depends on the presence of particular chromosomal genes (as in the case of kappa in *Paramecium*[7]), since both *T*-alleles support tyrosinase synthesis.

In view of the fact that the RNA (ribonucleic acid) of tobacco mosaic virus has recently been shown to possess genetic properties,[8] the continued failure to detect genetic activity in the cytoplasm—which contains RNA—in anything approaching the variety of activities associated with the chromosomes poses an interesting problem. One possible answer is that two structurally different forms of RNA exist, one capable of self-duplication and the other not.[9]

Summary.—A heterocaryon between two strains carrying, respectively, the alleles T^S and T^L, governing the thermostability of tyrosinase, was found to produce both forms of the enzyme. These alleles thus appear to act independently in the synthesis of the enzyme. The heterocaryon was resolved by means of hyphal tip transfers, without going through the sexual stage. No evidence was found for cytoplasmic transmission of tyrosinase-forming ability.

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