*ON A MECHANISM OF SUPPRESSOR GENE REGULATION OF TRYPTOPHAN SYNTHETASE ACTIVITY IN NEUROSPORA CRASSA**

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There is abundant evidence that the potential of a cell to synthesize a specific enzyme is primarily under the control of a single genetic locus.[1, 2] However, repeated instances have been found in which modifier genes, called "suppressors," exert a profound effect on enzyme activity in certain mutant cells.[3-8] It is known, for example, that specific suppressor mutations are capable of partially restoring enzyme activity in mutants which lack this particular activity.[3, 5] These suppressor genes are distinct from the original enzyme locus, and, as is often the case, there is no linkage between the two loci.[3] Furthermore, the suppressor genes do not seem to be duplications of the original enzyme locus, for the suppressors often act in combination with certain mutant alleles only.[3] Consequently, it seems quite clear that a number of genetic regions may be capable of controlling the appearance of a single enzyme activity.

This paper is concerned with a discussion of the nature of suppressor gene control and, in particular, with whether it exists at the level of enzyme formation, either qualitative or quantitative, or of enzyme function. The basic problem would seem to hinge on the relative contributions of the original enzyme locus and the suppressor loci to the structure of the enzyme. If it were to be found in a given instance that the responsibility for the determination of enzyme structure residues entirely at a single locus, then an explanation of suppressor gene action would have to be sought in terms of those factors which may regulate the expression of enzyme activity.

An effort will be made here to review some of the results obtained by workers in several laboratories, who have been studying a particular mutant-suppressor system, the *td* system in *Neurospora crassa* and *Escherichia coli.* Some new evidence will be presented which suggests that, in at least one case in *Neurospora*, suppressor gene action is not concerned with providing genetic "information" essential for the synthesis of a structurally normal enzyme.

Experiments with *Neurospora* and *E. coli* have shown that certain tryptophan-requiring (*td*) mutants which are unable to utilize indole for growth and which lack tryptophan synthetase activity form, instead, a protein (CRM)[9] which is antigenically similar to the enzyme.[10-12] Although immunological experiments[13] have failed to reveal differences between the CRM proteins of different *td* mutants, recent enzymatic studies[14-16] suggest that characteristic differences may be present. It has been found that the CRM proteins of certain *td* mutants of *E. coli*[12,15] and *Neurospora*[16-18] are able to catalyze indole formation from indole glycerol phosphate as well as to synthesize indole glycerol phosphate from indole and triose phosphate. However, such CRM proteins are unable to catalyze a reaction with L-serine in the step necessary for tryptophan synthesis.[15, 16, 18] In some of the *td* mutants the CRM proteins have lost all their enzymatic activity, failing even to react with indole or indole glycerol phosphate.[16] It appears most likely, there-

fore, that the CRM proteins in the *td* mutants represent a spectrum of characteristic genetically altered tryptophan synthetase molecules in which one or more catalytic properties of the normal enzyme have been modified or lost.[11, 14–16]

The relationship between *td* mutant suppressibility and the presence of a CRM protein is of particular interest. One might reasonably ask whether the action of a suppressor gene which restores tryptophan synthetase activity in a certain *td* mutant is contingent on the presence of a CRM protein. While *td*-suppressor mutations have not been demonstrated in every CRM-forming mutant, all the suppressible mutants of *Neurospora* so far tested form a CRM protein.[16, 19] Those mutants which do not form CRM appear to be unsuppressible.[10, 11, 16, 19] Mutant strains which contain low levels of tryptophan synthetase activity following the introduction of a specific suppressor gene,[3] also continue to form large quantities of CRM.[10, 16] These results suggest that only a partial "repair" of damage to the enzyme system has occurred as a result of suppressor gene action. Some recent work with *E. coli* on the contrary, suggests that CRM may not be required for suppressibility.[20] However, it should be emphasized that the absence of CRM does not preclude the synthesis of other aberrant proteins lacking the necessary determinant groups for immunological cross-reaction with tryptophan synthetase.

It is clear from the work of Yanofsky and Bonner[3] that suppressor genes which are effective in partially restoring tryptophan synthetase activity in the *td* mutants of *Neurospora* exhibit a striking allele specificity, that is to say, a particular suppressor gene may affect one or more alleles of a series but not others of the same series. It may be inferred from such specificity relationships that certain suppressor loci contain "information" relevant to the structure of the enzyme. Indeed, these studies offer one of the strongest arguments for such an interpretation. Subsequent experiments[21] have disclosed a somewhat anomalous situation in which five "specific" suppressor genes were found to affect the same *td* allele. Oddly enough, no linkage has been observed between the *td* locus and any of these suppressor genes, and the five suppressor genes themselves appear to be randomly distributed in the genome. Contrary to expectation, tryptophan synthetase prepared from wild type or from any one of several suppressed mutant strains exhibited similar biochemical properties.[3] These results strongly suggest that several genes, located in widely different chromosomal regions, may be capable of "repairing" a damaged enzyme-forming system sufficiently to permit the cell to form enzyme which seems indistinguishable from the wild-type protein.

Our recent experiments with a temperature-sensitive tryptophan-requiring mutant of *N. crassa*, strain td_{24}, have provided some information on this important and perplexing aspect of gene-enzyme relationships. Strain td_{24} requires tryptophan for growth at 25° C., but it grows slowly without tryptophan above 30° C., and at this temperature also forms a slight amount of active tryptophan synthetase.[3] In all instances this mutant forms large quantities of a CRM protein.[11, 14] It has been possible to obtain highly *active* tryptophan synthetase from this mutant grown at 25° C., by suitable fractionation of crude *inactive* extracts.[14] A considerable quantity of CRM is present in both the crude inactive preparations and the active fraction. An inorganic inhibitor which completely inhibits the fractionated mutant enzyme but which has no effect on the wild-type enzyme at comparable concentrations can be isolated from the inactive td_{24} preparations. Whether this

inhibitor is associated with an organic component in vivo is not known. The inhibitor can be found in other *td* mutants and also in wild-type strains. To date, the effects of the inhibitory material can best be duplicated by zinc.[19]

It appears from studies with strain td_{24} that gene mutation has in this instance resulted in the formation of an altered, metal-sensitive enzyme. The active enzyme which is obtained by the fractionation of crude inactive extracts appears to represent a conversion of CRM to active tryptophan synthetase by the dissociation of a metal-protein complex.[19]

The metal-sensitivity difference between the wild-type and td_{24} tryptophan synthetase preparations has provided one approach to the study of suppressor gene action. If a suppressor gene actually provides "information" essential for the synthesis of an enzyme possessing normal structure, then tryptophan synthetase formed in mutant td_{24} carrying the suppressor-24 (su_{24}) gene should be inhibitor-resistant, like the wild-type enzyme. If, on the other hand, the su_{24} gene in some manner converts an altered enzyme from an inactive to an active state without changing the basic structure of the protein, then the suppressed and the unsuppressed mutant enzymes might show similar inhibitor sensitivity. The results of a number of inhibitor experiments are summarized in Table 1. The data seem to favor the interpretation that suppressor action is based on control at the functional rather than the enzyme-forming level. Partially purified preparations of tryptophan synthetase from suppressed mutant strains td_{24} su_{24} and td_3 su_3 (a strain which is cross-suppressible with td_{24})[3] appear to be metal-sensitive, and in this manner resemble tryptophan synthetase from the unsuppressed mutant, td_{24}.

TABLE 1*

THE SENSITIVITY OF WILD-TYPE, *td* MUTANT, AND *td*-SUPPRESSED MUTANT TRYPTOPHAN SYNTHETASES TO ASHED td_{24}-INHIBITOR AND TO ZINC

Partially Purified Tryptophan Synthetase	Concentration Range for 50 Per Cent Inhibition of Enzyme Activity	
	td_{24} Inhibitor (ml.)	Zinc Acetate (Molar Concentration of Zn^{++})
Strains:		
td_{24}	0.05 to 0.1	5×10^{-5} to 1×10^{-4}
td_{24} su_{24}	0.05 to 0.1	5×10^{-5} to 1×10^{-4}
td_3 su_3	0.05 to 0.1	5×10^{-5} to 1×10^{-4}
5256A (wild-type)	0.5 to 1.0	5×10^{-4} to 1×10^{-3}
5297a (wild-type)	0.5 to 1.0	5×10^{-4} to 1×10^{-3}
C-84 (histidine$^-$)†	0.5 to 1.0	5×10^{-4} to 1×10^{-3}

* Extracts were purified using protamine sulfate, ammonium sulfate, and alumina gel (see n. 11). The enzyme activity in all the preparations was adjusted to a comparable level. In all the procedures, triple-distilled water was employed. Ashed inhibitor was prepared from strain td_{24} by boiling an aliquot of a 25–80 per cent ammonium sulfate fraction of the td_{24} extract, centrifuging and discarding the precipitate, and ashing the supernatant solution. The ashed material was taken up in distilled water and brought to the volume of the original sample. Enzyme preparations from the different strains were treated with the ashed inhibitor or with zinc for 5 minutes in an ice bath. The substrate mixture, consisting of saturating concentrations of indole, L-serine, and pyridoxal phosphate (see n. 24), was added, and the tubes containing the complete system were incubated for 1 hour at 37° C. The reaction was then stopped with 0.2 ml. of 5 per cent sodium hydroxide, and the amount of indole that had disappeared in the control and the inhibitor or zinc-containing samples was measured by the *p*-dimethyl aminobenzaldehyde reaction (see n. 24).

† D. Hogness and H. K. Mitchell, *J. Gen. Microbiol.*, *11*, 401, 1954.

These findings suggest that in some instances suppressor gene action in the *td* system may be concerned with the control of factors such as metal and coenzyme availability or the concentration of metal-binding agents. The results of current studies[19] clearly indicate that the synthesis, stability, and activity of tryptophan synthetase can be markedly influenced by the concentration of copper and zinc

and of pyridoxal phosphate and by the presence of chelating agents. Certainly, the regulation of such factors in vivo may be influenced by many genes, and it would be reasonable to suppose that suppressor genes may be effective in such ways. In the case of mutant td_{24}, where the effect of a suppressor gene can be effectively mimicked in vivo by increasing the temperature and in vitro by fractionation, the suppressor gene does not appear to supply information concerning the structure of the enzyme. The latter role would seem to be reserved for the *td* locus itself.

Summary.—Evidence has been presented that suppressor gene action may in certain cases be concerned with the expression of enzyme activity rather than with enzyme formation. In the *Neurospora* tryptophan synthetase system, the suppressor gene does not appear to provide "information" necessary for the synthesis of a structurally normal enzyme.

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