THE REGULATORY MECHANISM OF ENZYME SYNTHESIS IN THE TRYPTOPHAN BIOSYNTHETIC PATHWAY OF ESCHERICHIA COLI K-12

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The researches of Yanofsky and his colleagues (Yanofsky, C. and Crawford, I. P., 1959; Yanofsky, C. and Lennox, E. S., 1959; Yanofsky, C., 1960) have demonstrated that the enzyme proteins in the tryptophan biosynthetic pathway are determined by the closely linked structural genes, designated as A, B, ... anth.

It has been revealed by Monod and Cohen-Bazire (1953) that the addition of tryptophan to the culture of bacteria immediately inhibits the further formation of these enzymes. Moreover, Cohen and Jacob (1959) have isolated the mutant of E. coli, in which the synthesis of all these enzymes is not repressed by tryptophan. This mutant (R_{try}) is interpreted as the result of the mutation in the regulator gene. All the results described above suggest that the structural genes in the system of tryptophan, as in the case of lactose (Jacob and Monod, 1961), form a genetic unit, an operon, and that they would be coordinated through an operator gene.

Recently, a temperate bacteriophage has been isolated and designated as ϕ80 by one of the authors (Matsushiro, A., 1961). By cross- or P1-transduction-experiments, it has been established that this prophage is closely linked to try loci on the chromosome of E. coli K-12. The phage ϕ80 is able to transduce the try loci specifically; that is, only the character try^+ is transduced in this strain. This transduction is quite similar to Gal-transduction by phage lambda

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(Morse, Lederberg and Lederberg, 1956). An advantage of using phage ϕ80 consists in the fact that man can analyze more clearly the regulatory mechanisms of the enzyme synthesis in tryptophan pathway.

The purpose of this communication is to report the results of such experiments.

As in the case of Gal-λ-transducing system, most of $\underline{try}^+$ transductants are heterogenotes. The heterogenotes, after UV-induction, liberate HFT-phages containing both active ϕ80 and defective transducing particles, designated as ϕ80$\underline{dt}$. One can classify ϕ80$\underline{dt}$ obtained from independent origin into following two types. The first is able to transduce the character $\underline{try}^+$ to all the $\underline{try}^-$ mutants, implying that all the structural genes of the $\underline{try}$ operon would be incorporated into the genome of this type of ϕ80$\underline{dt}$. The second type of ϕ80$\underline{dt}$ would be deprived of $\underline{anth}$ locus which determines the structure of the enzyme protein of the anthranilic synthetase (As-ase), as it is incapable of transducing the character $\underline{anth}^+$ to the $\underline{anth}^-$ mutants which are unable to synthesize the enzyme As-ase. The latter type of ϕ80$\underline{dt}$, which is hereafter called ϕ80$\underline{dt}$ $\underline{anth}^{del}$, possesses the remarkable properties. In the heterogenotic transductants, which could be represented by the symbol* A^-B^+ $\underline{anth}^+$/ex A^+B^+ $\underline{anth}^{del}$, the synthesis of the B protein of the tryptophan synthetase (Ts-ase) is repressed only $\underline{partially}$ by tryptophan (see Fig. 1(b)), while that of the A protein is not repressed by this amino acid. In this case the synthesis of the As-ase seems to be repressed completely (see Fig. 1(b)).

In the heterogenote which carries the first type of ϕ80$\underline{dt}$, A^-B^+ $\underline{anth}^+$/ex A^+B^+ $\underline{anth}^+$, the synthesis of these proteins is completely repressed as in the wild type (see Fig. 1(a)). The effect of deletion in $\underline{anth}$ locus is, therefore, quite comparable to that of constitutive mutation, O^c in the system of lactose (Jacob et al., 1960; Jacob and Monod, 1961). It affects only the genes in $\underline{cis}$ position, but not those in $\underline{trans}$ position. It is pleiotropic because it affects simultaneously A and B proteins in the Ts-ase and As-ase.

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* determinants on the bacterial chromosome/ex determinants carried by the phage

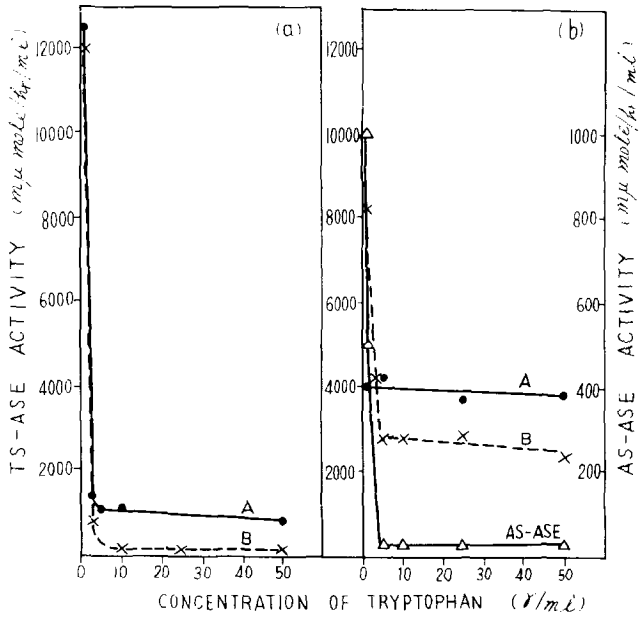

Fig. 1. The enzyme synthesis in various strains

The fresh bacterial culture was grown in the synthetic medium supplemented with casein hydrolysate (0.4%) and excess of DL-tryptophan (100γ/ml). Washed cells were regrown in the same fresh medium containing variety of tryptophan concentrations (0, 1, 3, 5, 10, 25, 50γ/ml), permitting the enzyme synthesis for 1 hr. Each sample was separately centrifuged and resuspended in tris-buffer (pH 7.6) containing pyridoxal phosphate and GSH (10^{-4}M). They were disrupted for 10 minutes with sonic oscillator (10 kc) and spun down. Each supernatant of them was used for the enzyme assay. The determination of the Ts-ase was carried out by the method of Yanofsky (1955), and that of the As-ase was by the method of Srinivasan (1954), and Moyed (1960). a) heterogenote A^-B^+ $anth^+$/ex A^+B^+ $anth^+$ b) heterogenote A^-B^+ $anth^+$/ex A^+B^+ $anth^{del}$.

Thus one can imagine that, on the basis of the hypothesis of Jacob and Monod (1961), an operator will be included in the anth locus or closely linked to the anth locus. And the loss of affinity to the repressor, which will be caused by the deletion of the operator, would lead to the non-repressible synthesis of the enzyme protein in the tryptophan pathway.

Detailed reports of this work are now in preparation.

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