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Mutations in *Escherichia coli* that Relieve Catabolite Repression of Tryptophanase Synthesis. Tryptophanase Promoter-like Mutations

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SUMMARY

From a strain lacking adenyl cyclase and the catabolite-sensitive gene activator protein, two mutants were isolated that can synthesize tryptophanase. Each mutation is extremely closely linked to the tryptophanase structural gene. The mutations differ from one another in the rate of synthesis of tryptophanase that they permit in the genetic background in which they were isolated; they differ from one another and also from the wild type in the maximum rate of synthesis of tryptophanase that they permit in a genetic background with intact adenyl cyclase and catabolite-sensitive gene activator protein. Both mutations appear to lie in the tryptophanase promoter.

INTRODUCTION

The accompanying paper (Yudkin, 1976) shows that the synthesis of tryptophanase in wild-type *Escherichia coli* is absolutely dependent on an intact cyclic AMP–catabolite-sensitive gene activator (c-AMP–CGA) protein system. Nevertheless, from mutants that lack both adenyl cyclase and the CGA protein it is possible to isolate revertants that are able to synthesize tryptophanase. We describe two such revertants and present evidence that suggests that the mutations responsible for the revertant phenotype are in the promoter for the tryptophanase structural gene.

METHODS

Genetic symbols. These are as in Taylor & Trotter (1972), and include *crp* (the structural gene for the catabolite-sensitive gene activator protein), *cya* (the structural gene for adenyl cyclase), and *tna* (the structural gene for tryptophanase). Ind$^+$ strains are capable of using indole as a source of tryptophan in the absence of 5-methyltryptophan; Val^R strains are valine-resistant.

Genetic methods, and differential rate of tryptophanase synthesis. These were as described by Yudkin (1976).

Bacteria. Strain MY571 (F$^-$*trp*^{del} *AC9*) was from Dr C. Yanofsky, strain MY618 (F$^-$ *crp868 metB strA*) from Dr J. Scaife, and strain MY613 (F$^-$ *thr leu his arg trp* Ind$^+$ *cysG lac Y gal xyl mal man strA*) from Dr M. Jones-Mortimer. Strain MY281 (F$^-$ *trp*^{del} *AC9 tna* Val^R) was constructed from strain MY571 by the method described by Yudkin (1976).

Strain MY283 (F$^-$ *thr his arg trp*^{del} *AE-tonB*^{del} *lac Y gal xyl mal man strA* Val^R *cya855 crp868*) was constructed in the following way. Spontaneous *tonB* deletions of strain MY613 were selected (Gottesman & Beckwith, 1969), and an Ind$^-$ derivative was purified; this was shown to carry a deletion of all the *trp* structural genes (Jackson & Yanofsky, 1972). This strain was transduced to *leu*$^+$ and then to *cya855* Val^R (Yudkin, 1976); the resulting strain is

MY279. To introduce *crp*⁻ we made use of three facts (our unpublished work): (i) neither *cya*⁻ nor *crp*⁻ strains are able to synthesize tryptophanase in L-broth; (ii) *cya*⁻ strains synthesize tryptophanase and give a positive test for indole when grown overnight in L-broth supplemented with 1 mM-c-AMP, whereas *crp*⁻ strains do not; (iii) *crp* can be readily co-transduced with *cysG*. We therefore transduced strain MY279 with phage P1 grown on strain MY618, selected *cysG*⁺ recombinants, and retained one that was unable to make tryptophanase in the presence of c-AMP; this is strain MY283. We were able to recover both *cya*⁻ and *crp*⁻ from a transducing lysate of phage P1 grown on the strain.

Media. These were as described previously (Yudkin, 1976). L-Threonine, L-histidine and L-arginine (50 μg of each/ml) were added to minimal media for all strains that required these amino acids. 'Minimal medium' is to be taken as including these amino acids when they are required.

RESULTS AND DISCUSSION

Isolation and properties of the revertants

Strain MY283 carries a deletion of all the *trp* structural genes. To grow on minimal medium containing indole and 5-methyltryptophan it must therefore make tryptophanase (Yudkin, 1976). However, as it carries mutations in the structural genes for adenyl cyclase and the CGA protein, it will be able to make tryptophanase only by acquiring a means of expressing *tna* that does not depend on the c-AMP–CGA system.

Cells of strain MY283 were harvested from stationary culture in L-broth, washed twice in 0·9 % NaCl and plated on glucose–minimal medium with indole and 5-methyltryptophan. After two days at 37 °C, colonies appeared at about 1 to $2/10^{10}$ cells plated. Two strains, each arising from a separate broth culture of strain MY283, were purified and named MY627 and MY634. They are similar in the following respects: they make colonies in 1 to 2 days at 37 °C on glucose–minimal medium with indole and 5-methyltryptophan, or on the same medium supplemented with gluconate; they fail to grow on glycerol–tryptophan–minimal or on arabinose–tryptophan–minimal medium; they make β-galactosidase, when induced, at the same very low rate as the strain MY283 from which they are derived. They differ in their rate of synthesis of tryptophanase: in glucose–tryptophan–minimal medium strain MY627 makes 15 units/mg protein and strain MY634 makes 41 units. (Strain MY283 makes less than 1 unit/mg protein.)

Genetic characterization

The above results suggest that the mutations in strains MY627 and MY634 permit the expression of *tna* notwithstanding the *cya*⁻ and *crp*⁻ mutations, but do not allow other catabolite-sensitive systems to be expressed. Since the c-AMP–CGA protein system is thought to work by interaction with the promoter, and since it is known that some *lac* promoter mutations permit *lac* to be expressed in a *cya*⁻ or *crp*⁻ background (Arditti, Grodzicker & Beckwith, 1973), an obvious possibility is that strains MY627 and MY634 contain tryptophanase promoter mutations. To test this idea, we did a number of transductions designed to show whether the mutations are located near *tna*.

Phage P1 grown on strain MY634 was used to transduce strain MY571 to grow on glucose–minimal medium supplemented with indole and 5-methyltryptophan and with gluconate. [Strain MY571 cannot normally grow on this medium because it carries a *trp* deletion and because the severe catabolite repression imposed by the glucose and gluconate prevents the synthesis of sufficient tryptophanase to convert indole to tryptophan (Yudkin, 1976); since strain MY634 is able to grow in these circumstances we presumed that it would be able

to donate this ability to strain MY571.] By replicating the transductants to medium supplemented with valine we showed that 26 % had become valine-resistant, and by replicating to glycerol–tryptophan–minimal medium we showed that 7 % had inherited *cya*⁻ from the donor.

In a similar way we showed that the mutation that permitted tryptophanase synthesis in strain MY627 was 19 % co-transducible with valine resistance and 4 % co-transducible with *cya*.

These results show that the two mutations are close to the *ilv* locus in which valine resistance maps, on the opposite side of *ilv* from *cya*, and they suggest that the mutations may be very near to *tna* (see Fig. 1 of Yudkin, 1976).

To determine the linkage to *tna*, we used the phage preparation grown on strain MY634 to transduce strain MY281 (*trp*^{del} *tna*⁻) to Tna⁺. Recombinants were selected on indole–5-methyltryptophan–minimal medium with glycerol as carbon source (on which *trp*^{del} strains can grow provided that they are Tna⁺); they were then streaked on to the same medium and on to indole–5-methyltryptophan medium with glucose and gluconate, to determine whether they had also inherited the mutation that permits expression of *tna* in the presence of these carbon sources. Of 117 transductants tested, all were able to grow on the latter medium. (When we repeated this experiment using phage P1 that had been grown on another revertant strain that is indistinguishable from strain MY634 we found that 399 out of 401 transductants tested were able to grow on glucose–gluconate–minimal medium with indole and 5-methyltryptophan.) Similarly, 102 out of 102 Tna⁺ recombinants obtained by transducing strain MY281 with phage P1 grown on strain MY627 were able to express *tna* in the presence of glucose plus gluconate.

At first sight these results suggest that the mutations that permit strains MY627 and MY634 to express *tna* are more than 99 % co-transducible with the *tna* structural gene. However, since the mutations in strains MY627 and MY634 are co-transducible to a slight extent with *cya*⁻, selection of Tna⁺ transductants on medium containing glycerol as sole source of carbon will have excluded a small fraction of potential transductants (those few that have inherited *cya*⁻ as well as *tna*⁺ from the donor). In spite of this slight bias in the selection, we think it safe to conclude that the mutations in strains MY627 and MY634 are extremely closely linked with *tna*. This conclusion is compatible with their being in the tryptophanase promoter.

An alternative possibility is that in each mutant the *tna* gene has been duplicated and one copy has become translocated and attached to a promoter that is independent of the c-AMP–CGA protein system. This suggestion is incompatible with the following facts: (i) By plating out the mutant strains on nutrient agar and replicating several thousand colonies to glucose medium with indole and 5-methyltryptophan we showed that our mutants were stable, whereas duplication–translocation events in *E. coli* are characterized by instability (Hill *et al.* 1969; Jackson & Yanofsky, 1973); (ii) the co-transducibility between our mutations and valine resistance is the same as that between *tna* and valine resistance in the wild type (contrast the much decreased linkage between *cysB* and the translocated *trp* genes described by Jackson & Yanofsky, 1973); (iii) the mutations are extremely closely linked to *tna*: if there were two copies of the *tna* gene in the mutants, transduction would frequently separate *tna*⁺ from the mutations that lead to catabolite insensitivity. We think our results are best explained by assuming that the promoter of the *tna* gene has mutated in strains MY627 and MY634. We designate these mutated promoters *tnaP1* and *tnaP2* respectively.

Table 1. *Differential rates of tryptophanase synthesis in cya⁻ crp⁻ and cya⁺ crp⁺ backgrounds*

Strain	Genotype	Differential rate of tryptophanase synthesis (enzyme units/mg protein)	
		Glycerol–minimal medium	Glucose–minimal medium
MY571	*trp*^{del} *tnaP*⁺ *cya*⁺ *crp*⁺	173	13
MY627	*trp*^{del} *tnaP1 cya*⁻ *crp*⁻	—*	15
MY281/627	*trp*^{del} *tnaP1 cya*⁺ *crp*⁺	57	31
MY634	*trp*^{del} *tnaP2 cya*⁻ *crp*⁻	—*	41
MY281/634	*trp*^{del} *tnaP2 cya*⁺ *crp*⁺	105	42

* Because of the absence of the c-AMP–CGA protein system, strains MY627 and MY634 do not grow in glycerol–minimal medium.

Expression of the mutations in a cya⁺ crp⁺ background

We purified one recombinant from each of the transductions in which strains MY627 and MY634 were the donors and strain MY281 was the recipient. By measuring differential rates of tryptophanase synthesis in these recombinants, we were able to study the expression of *tnaP1* and *tnaP2* in a strain that had an intact c-AMP–CGA protein system. The results are presented in Table 1, together with results of similar experiments in strain MY571 – the isogenic strain with a wild-type *tna* region.

tnaP1, when placed in a *cya⁺ crp⁺* background, is evidently far less susceptible to catabolite repression than a wild-type promoter; moreover, it promotes expression of *tna* in glycerol–minimal medium at only about one-third of the wild-type rate. *tnaP2*, when placed in a *cya⁺ crp⁺* background, retains more sensitivity to catabolite repression, but in glycerol–minimal medium it still promotes expression at only 60 % of the wild-type rate. Thus both promoter mutations change the rate of expression of *tna* whether they are in a *cya⁻ crp⁻* or in a *cya⁺ crp⁺* background.

Neither of our two mutants corresponds closely to any of the large number of *lac* promoter mutants that have been described. *lac* promoter mutations fall into three general classes: (*a*) mutations that permit greatly diminished expression of *lac* in a *cya⁺ crp⁺* background but the same rate of expression as *P⁺* in a *cya⁻ crp⁻* background (Beckwith, Grodzicker & Arditti, 1972); (*b*) mutations that permit the wild-type rate of expression in a *cya⁺ crp⁺* background but a much higher rate of expression than *P⁺* in a *cya⁻ crp⁻* background (Arditti *et al.* 1973); (*c*) mutations that allow a lower rate of expression than *P⁺* in either a *cya⁺ crp⁺* or a *cya⁻ crp⁻* background (Hopkins, 1974). Our mutants most nearly resemble *lac* mutants of class (*b*), which were isolated by a procedure analogous to ours; but (unlike those) our mutations clearly affect the maximum level of gene expression in a *cya⁺ crp⁺* background. Beckwith *et al.* (1972) suggested that the *lac* promoter can be divided into two sites, of which one interacts with the CGA protein and is altered in mutants of class (*a*), and the other interacts with the RNA polymerase and is altered in mutants of classes (*b*) and (*c*). Our results do not provide evidence for a clear demarcation between two such sites for the tryptophanase promoter, since the mutations abolish dependence on the c-AMP–CGA system and also diminish the maximum level of gene expression. Our mutations could be deletions covering both putative sites, but deletions of that sort would probably have a more drastic effect on the maximum level of *tna* expression. Again, our strains MY627 and

MY634 may each carry two mutations; they were, however, isolated without the use of a mutagen. In any case the *lac* promoter is perhaps not very similar to the tryptophanase promoter, since *lac* is expressed at 2 % of the wild-type rate even in *cya*$^-$ *crp*$^-$ strains (Beckwith *et al.* 1972), whereas the corresponding figure for *tna* is less than 0·1 %.

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