SHORT COMMUNICATION

Complementation Analysis of Eleven Tryptophanase Mutations in *Escherichia coli*

By M. K. WHITE AND M. D. YUDKIN

Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford OX1 3QU

(Received 9 May 1979)

Nine independent mutants deficient in tryptophanase activity were isolated. Each mutation was transferred to a specialized transducing phage that carries the tryptophanase region of the *Escherichia coli* chromosome. The nine phages thus produced, and a tenth carrying a previously characterized tryptophanase mutation, were used to lysogenize a bacterial strain harbouring a mutation in the tryptophanase structural gene and also a suppressor of polarity. In no case was complementation observed; we conclude that there is no closely linked positive regulatory gene for tryptophanase.

INTRODUCTION

The tryptophanase of *Escherichia coli* catalyses the reversible reaction:

The enzyme is encoded by the *tna* gene, the expression of which is inducible and subject to severe catabolite repression. Mutations to constitutivity are known, and these are very closely linked to *tna*, in a locus called *tnaR* (Taylor & Yudkin, 1978); however, the nature of *tnaR* is unknown.

Tryptophanase has much in common with D-serine deaminase. Both enzymes are catabolic and dependent on pyridoxal phosphate, they catalyse similar reactions, both are induced by their substrates and both suffer catabolite repression. Mutations leading to the constitutive synthesis of D-serine deaminase are known, and these are very closely linked to the structural gene for the enzyme, in a locus called *dsdC* (Bloom & McFall, 1975). However, *dsdC* is also the site of *trans*-recessive mutations that lead to loss of D-serine deaminase, and this fact and other lines of evidence have led to the conclusion that *dsdC* is a positive regulatory gene (Bloom *et al.*, 1975).

We wondered whether *tnaR* might be a positive regulatory gene for *tna*. If it were, one would expect to be able to isolate tryptophanase-deficient mutants of *E. coli*, the mutations in which would lie in *tnaR* rather than *tna*. A mutation of this sort could be recognized by its ability to complement a mutation in *tna*.

METHODS

Organisms. All bacteria were *E. coli* K12 of mating type F⁻. A strain carrying *rho103* and a strain carrying the amber mutations *lacZU118* and *trpE9914* were kindly given by Dr C. Yanofsky. Other strains were from our laboratory collection.

Media. These were as described by Yudkin (1976) and Taylor & Yudkin (1978).

Lysogenizing with λ imm21*tna* and displacement of λ imm21*tna* by λ . These techniques have been described by Taylor & Yudkin (1978).

Mutagenesis of phage P1. This was done as described by Tully & Yudkin (1977).

Other genetic techniques and the assay of tryptophanase. These were as described by Yudkin (1976).

RESULTS

Isolation of new tryptophanase-deficient mutants

We used the technique of directed mutagenesis (Hong & Ames, 1971) to isolate mutants deficient in tryptophanase. This technique involves growing generalized transducing phage on a donor containing a marker for which there is a positive selection and which is closely linked to the gene in which mutations are desired, and transducing this marker into a suitable recipient after the phage preparation has been mutagenized *in vitro*. Some of the transductants thereby acquire mutations in the desired gene. We chose *bglR*, which is about 70% cotransducible with *tna* and *tnaR*, as the marker for which selection could be made. We therefore grew phage P1 on MY711 (*tna*⁺ *bglR*), mutagenized the preparation with hydroxylamine (Tully & Yudkin, 1977), and used it to transduce MY1386 (*trp*^{del} *AC9* Val^R *tna*⁺) to growth on salicin/minimal agar supplemented with tryptophan. (*bglR* strains can grow on salicin as sole source of carbon; *bglR*⁺ strains cannot.) We replicated the transductants to salicin/minimal agar supplemented with indole and 5-methyltryptophan. (*tna*⁺ *trp*^{del} strains can use indole as a source of tryptophan when expression of the *tna* gene is induced with 5-methyltryptophan; *tna trp*^{del} strains cannot.) We isolated and tested all colonies that grew on salicin/tryptophan medium but not on salicin/indole/5-methyltryptophan medium and in this way obtained nine tryptophanase-deficient mutants out of about 15000 transductants. The mutations in these nine strains are called *tna101* to *tna109*.

Transfer of the mutations to a specialized transducing phage

We wanted to test all the new mutations for ability to complement a mutation (*tnats6*) that is known to lie in *tna*, the structural gene for the enzyme. In order to do so, we transferred each of them to the specialized transducing phage λ imm21*tna*⁺ (Borck *et al.*, 1976), so that we could then lysogenize the *tnats6* strain with the set of *tna* phages. The method of recombining each mutation into λ imm21*tna*⁺ was as follows.

We first grew phage P1 on each of the nine new mutants and used the phage preparations to transduce MY678 [*trpR trp*^{del} *BE9*(*att* λ -*gal-bio*)^{del}] to valine resistance. Since valine resistance is about 20% cotransducible with *tna* (Yudkin, 1976), we were able to recover tryptophanase-deficient derivatives of MY678 from each transduction. We lysogenized these with λ imm21-*tna*⁺ which, since the host is deleted for *att* λ , can be integrated only near the chromosomal *tna* locus. We then induced the nine lysogens by ultraviolet irradiation. We expected that each of the resulting lysates would include two types of transducing particles – λ imm21*tna*⁺, and λ imm21*tna* containing, as a result of recombination, the tryptophanase mutation present in the lysogen. [Since the transducing phage carries *tnaR* (R. M. Edwards, unpublished work), it would be able equally well to acquire a mutation in this locus if it were this that had originally led to the tryptophanase-deficient phenotype of the new mutants.]

We then used each of these unpurified lysates to lysogenize the homologous mutant strain in which the mutation was first identified. Each lysogeny mixture would be expected to contain both heterogenotes, e.g. *tna101*(λ imm21*tna*⁺), and homogenotes, e.g. *tna101*(λ imm21*tna101*), apart from bacteria that had not been lysogenized. The second of these classes can be recognized by the fact that although they are lysogens, i.e. immune to λ imm21*cI*, they are Tna⁻, i.e. unable to use indole as a source of tryptophan in the presence of 5-methyltryptophan. We purified lysogenic homogenotes from each of the nine mixtures and induced them. Phage was purified from each lysate by two serial platings on the homologous mutant strain, and by growth on the same strains we obtained high-titre stocks of nine phages of genotypes λ imm21*tna101* to λ imm21*tna109*.

We proved that each of these phages carried the bacterial *tna* region, since each was able to recombine with several *tna* mutants to yield Tna⁺ transductants. However, when each

Table 1. *Tryptophanase activity of cultures grown at 40 °C*

Each strain was grown from a single colony in glycerol/minimal medium supplemented with L-tryptophan ($100\text{ }\mu\text{g ml}^{-1}$) at $40\text{ }^{\circ}\text{C}$. When the cultures reached an A_{600} of 0.1 to 0.3, duplicate samples were taken into chloramphenicol (final concn $100\text{ }\mu\text{g ml}^{-1}$) and the bacteria were harvested by centrifugation. The cell pellets were washed twice and assayed for tryptophanase by the method of Yudkin (1976). This procedure involves the conversion of tryptophan to indole, and the assay of the indole by formation of a 4-dimethylaminobenzaldehyde complex which absorbs at 568 nm. The results are expressed as the absorbance of this complex at 568 nm divided by the absorbance of the bacterial culture at 600 nm. This fraction is proportional to the tryptophanase activity per mg cell mass.

Strain	A_{568}/A_{600}
W3110 <i>tna</i> ⁺ <i>rho103</i>	1.33
W3110 <i>tnats6 rho103</i>	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna</i> ⁺)	0.24
W3110 <i>tnats6 rho103</i> (λ)	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna101</i>)	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna102</i>)	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna103</i>)	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna104</i>)	0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna105</i>)	< 0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna106</i>)	< 0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna107</i>)	< 0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna108</i>)	< 0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna109</i>)	< 0.02
W3110 <i>tnats6 rho103</i> (λ imm21 <i>tna2</i>)	< 0.02

was used to lysogenize a strain carrying its own homologous mutation, the resulting lysogens (presumed to be homogenotes) all proved to be *Tna*⁻.

We also used a tenth λ imm21*tna* phage, λ imm21*tna2*. This contains the mutation *tna2* and has been described previously (see Taylor & Yudkin, 1978). Before the present experiments it had not been proved conclusively whether *tna2* was located in the structural gene for tryptophanase.

Construction of diploid strains for complementation tests

The mutation *tnats6* renders tryptophanase activity temperature-sensitive in cell-free extracts and has been proved to lie in the structural gene for the enzyme (Taylor & Yudkin, 1978). We chose this as the reference mutation against which to test the ten other mutations for complementation, and transferred it to strain W3110 by cotransduction with *bgIR*.

We now anticipated a possible difficulty in interpretation that might arise if the result of a complementation test appeared negative. The straightforward interpretation of such a result would be that the two mutations that failed to complement were in the same cistron. However, another possibility is that the two mutations are in different cistrons but in the same transcription unit, and that the failure to complement is due to polarity of the more promoter-proximal mutation. To avoid this difficulty, we introduced into W3110 *tnats6* the polarity-suppressing mutation *rho103* (Korn & Yanofsky, 1976) by cotransduction with valine resistance (to which *rho* is very closely linked.) That the resulting strain W3110 *tnats6 rho103* did in fact contain the *rho* mutation was proved by the fact that phage P1 grown on it could suppress the polarity of two separate nonsense mutations (*trpE9914* and *lacZU118*) in the same strain.

We lysogenized W3110 *tnats6 rho103* with each of the ten λ imm21*tna* phages that we have described. We also lysogenized the strain with λ imm21*tna*⁺ (to serve as a positive control), and we made W3110 *tnats6 rho103*(λ) by displacing λ imm21*tna*⁺ from the last-named strain with wild-type λ .

Results of the complementation tests

Haploid strains containing *tnats6* synthesize negligible quantities of active tryptophanase when grown in minimal medium with tryptophan at 40 °C (Taylor & Yudkin, 1978). We therefore grew our strains at 40 °C and measured the activity of tryptophanase, expecting that complementation would lead to the appearance of active enzyme. From the results given in Table 1, several conclusions are apparent.

(1) Strain W3110 *tnats6 rho103* contains less than 2 % of the tryptophanase activity of the isogenic *tna*⁺ strain.

(2) Lysogeny of this strain with λ *imm21tna*⁺ increases the tryptophanase activity more than 10-fold. We conclude that, as expected, λ *imm21tna*⁺ complements *tnats6*. [We have repeatedly observed that various *tna*(λ *imm21tna*⁺) strains make less tryptophanase than *tna*⁺ strains. We believe that *tna* on the prophage is transcribed from a bacterial promoter, as its expression is induced by tryptophan or 5-methyltryptophan, and subject to catabolite repression, like that of *tna* in the normal location; since the latter is very close to the origin of replication of the chromosome, there will be more copies per cell of the chromosomal *tna* than of λ *tna*, and this fact may in part account for the different rates of expression.]

(3) When λ *imm21tna*⁺ is removed by displacement with λ the resulting lysogen makes almost no heat-stable tryptophanase.

(4) None of the ten λ *imm21tna* lysogens makes heat-stable tryptophanase; hence none of the ten mutations complements *tnats6*.

DISCUSSION

Since the complementation tests were done in the presence of the polarity suppressor *rho103*, the failure of the ten mutations tested to complement *tnats6* cannot, in all probability, be explained by polarity. Although alternative explanations (such as a *cis*-acting regulatory gene) have not been rigorously excluded, we think it is reasonable to conclude that the ten mutations are, like *tnats6*, in the tryptophanase structural gene, and hence that none of them are in *tnaR*. However, *tnaR* is extremely close to *tna* (more than 98 % cotransducible with phage P1) and one would have therefore expected it to be a target for mutagenesis by the method used.

The significance of the results can be discussed in the following way. Suppose that *tnaR* is a positive regulatory gene and that it *can* be mutated to give tryptophanase deficiency; what is the probability that out of ten tryptophanase-deficient mutants none should be in *tnaR*?

To assess this probability we make two assumptions. First, we assume that the mutability of *tnaR* per unit length is the same as that of *tna*. (We note that mutations leading to loss of activity of the positive regulator can readily be obtained in such systems as *araC*, *cysB*, *dsdC* and *malT*.) Secondly, we assume that the molecular weight of the (monomeric) protein hypothetically encoded by *tnaR* is 28000. [This figure is chosen as equal to the smallest of three known bacterial regulatory proteins: *araC* (28000; Wilcox *et al.*, 1974), *lacI* (38000; Gilbert & Müller-Hill, 1970) and *cysB* (39000; D. M. Mascarenhas, unpublished work).] The molecular weight of the monomer encoded by *tna* is 56000 (London & Goldberg, 1972). Then, the probability that ten out of ten mutations leading to tryptophanase deficiency should all lie in *tna* is

$$\left(\frac{56000}{56000 + 28000}\right)^{10} = \left(\frac{2}{3}\right)^{10} = 0.017$$

Hence, if the assumptions we have made are correct, the probability that *tnaR* encodes a

positive regulatory protein but that we have failed to find negative mutations in *tnaR* is less than 2%. We conclude that it is unlikely that *tnaR* is a positive regulatory gene, unless it is very small or immutable.

We are indebted to Mr R. M. Edwards for valuable suggestions and for allowing us to cite some of his experimental results.

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