Tryptophanase Synthesis in *Escherichia coli*: the Role of Indole Replacement in Supplying Tryptophan and the Nature of the Constitutive Mutation *tnaR3*

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The properties of the tryptophanase constitutive mutation *tnaR3* have been investigated. It has previously been reported that mutants carrying *tnaR3* grow poorly on medium that selects for constitutive expression of tryptophanase. Our results now show that this poor growth can be explained by the inability of tryptophanase to catalyse the synthesis of L-tryptophan from indole, pyruvate and ammonia at a rate sufficient to allow normal growth. Improved media for the characterization of tryptophanase constitutive mutants are described. The mutation *tnaR3* rendered tryptophanase synthesis constitutive (at a differential rate that at 37 °C is 30% of the fully induced wild-type) and not further inducible. Diploid studies showed that *tnaR3* is *cis*-dominant, but no evidence was found for any effect in *trans*. In addition to rendering tryptophanase synthesis constitutive, *tnaR3* affects the response of tryptophanase synthesis to different growth temperatures.

INTRODUCTION

Tryptophanase [EC 4.1.99.1; L-tryptophan indole-lyase (deaminating)] is a catabolic enzyme that catalyses the degradation of L-tryptophan to indole, pyruvate and ammonia. In certain circumstances, tryptophanase can also catalyse the synthesis of L-tryptophan in the whole cell. Tryptophanase synthesis is induced both by L-tryptophan and by various analogues of L-tryptophan, and is very sensitive to catabolite repression.

Strains of *Escherichia coli* which have a lesion in tryptophan synthase [EC 4.2.1.20; L-serine hydro-lyase (adding indoleglycerol-phosphate)] can nonetheless grow by using tryptophanase to synthesize L-tryptophan, provided that indole and 5-methyl-DL-tryptophan are included in the medium and that the carbon source is not catabolite repressing (Peck *et al.*, 1971). Thus a *trp*-deleted strain will grow on glycerol/minimal medium supplemented with indole and 5-methyl-DL-tryptophan. It has been hitherto assumed that the role of the 5-methyl-DL-tryptophan was solely to induce tryptophanase synthesis.

Regulatory mutations that affect tryptophanase synthesis have been isolated, either by omission of 5-methyl-DL-tryptophan from the selective media to select constitutive mutants or by the addition of a catabolite-repressing carbon source to select catabolite repression-resistant mutants (Yudkin, 1976; Taylor & Yudkin, 1978). Some mutants are found to have the dual phenotype of constitutivity and catabolite resistance (Ward & Yudkin, 1976). The majority of these regulatory mutations map close to *tnaA*, the structural gene for tryptophanase (Taylor & Yudkin, 1978).

The *tnaA* gene and its putative regulatory region have been sequenced (Deeley & Yanofsky, 1981). The sequence 5' to the coding region shows a number of interesting features. There is a region showing close homology with the binding site for the 3',5'-cyclic AMP-catabolite-sensitive gene activator protein complex (cAMP-CAP) present in the *lac* promoter. This is not surprising

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in view of the absolute dependence of tryptophanase synthesis on the presence of an intact cAMP-CAP system (Ward & Yudkin, 1976). Another striking feature is the long (300 bp) region between the transcription start point and the tryptophanase initiation codon. Deeley & Yanofsky (1982) have speculated on the involvement of some translational mode of control but could find no structure resembling that of the attenuator system found in the *trp*, *phe* and other biosynthetic operons. The same authors have also isolated a number of constitutive and catabolite repression-resistant mutations which map close to *tnaA*; some of these are suppressible by *supC* and yet none are complemented by plasmids carrying the *tnaA* region. Deeley & Yanofsky (1982) suggest that these mutations may be in the gene for a *trans*-acting regulatory protein, but question whether a nonsense mutation in such a protein could overcome the requirement for cAMP-CAP binding.

In the present paper we examine the properties of *tnaR3* (Taylor & Yudkin, 1978), a mutation that causes constitutive expression of *tnaA* but does not confer resistance to catabolite repression. It was selected as a mutation that allowed a *trp*-deleted strain to grow on glycerol/minimal medium supplemented with indole. *tnaR3* has been shown to map very close to *tnaAts6*, a tryptophanase structural gene mutation, on the side distant from *bglR* (Taylor & Yudkin, 1978). Enzyme assays suggested that it was a high-level constitutive mutation that rendered *tnaA* expression insensitive to the presence of inducer.

We now show, using an improved enzyme assay, that *tnaR3* renders *tnaA* expression about 30% constitutive and indifferent to the presence of L-tryptophan. By constructing suitable strains we have obtained evidence suggesting that *tnaR3* is *cis*-dominant but not *trans*-dominant.

METHODS

Strains of bacteria and phages. These are described in Table 1. The specialized transducing phage λ imm21*tnaA* has been described previously (Borck *et al.*, 1976).

Media. These were as described by Yudkin (1976) and Taylor & Yudkin (1978). The medium used to select for *tnaA*⁺ in *trpAC9* backgrounds was that of Vogel & Bonner (1956) supplemented with 0.2% glycerol, 5 μ g indole ml⁻¹, and 50 μ g 5-methyl-DL-tryptophan ml⁻¹. Other supplements were used at concentrations described in the text.

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

Tryptophanase assay. Samples of culture (0.1–0.5 ml) were added to 1 ml assay buffer (0.1 M-potassium phosphate buffer pH 7.8, 150 μ g pyridoxal phosphate ml⁻¹, 250 μ g bovine serum albumin ml⁻¹, 10 μ M-2-mercaptoethanol, and 100 μ g chloramphenicol ml⁻¹) containing about 10⁹ cells of MY281 (a mutant completely devoid of tryptophanase activity) which had previously been washed in phosphate buffer. The samples were collected in micro test tubes (Eppendorf) and kept on ice until ready for assay. The cells were deposited by centrifugation at 4 °C and resuspended in 1 ml assay buffer. The centrifugation was repeated and the cells were finally resuspended in 1 ml assay buffer. A 0.5 ml sample of each sample was transferred to a glass scintillation vial. Toluene (1 ml) was added to each vial in turn and the samples were shaken at 37 °C. After 5 min preincubation the reaction was started by the addition of 0.1 ml assay mixture (assay buffer containing 10 mg L-tryptophan ml⁻¹). The reaction was stopped by the addition of 3–4 ml Ehrlich's reagent. The colour was allowed to develop for 30 min at room temperature, the samples were transferred to 10 ml plastic centrifuge tubes and the cell debris was removed by centrifugation. The absorbance of each sample at 568 nm was then determined in a stoppered glass cuvette. This assay was found to be linear for assay times of up to at least 45 min, for A_{568} up to at least 0.8 and for sample size up to at least 500 μ l. One unit of tryptophanase is defined as that which will convert 1 nmol L-tryptophan to indole in 1 min. Cell protein concentrations were estimated from the A_{600} at the time of sampling by reference to a standard curve, constructed by drying to constant weight samples of known A_{600} .

Isolation of tnaR3. R803 (*tnaR3* (*at1-gal-bio*)^{del} *trpBE*^{del}) was lysogenized with λ *tnaA* by selecting for immunity to λ imm21 *clear*. One purified lysogen was induced by UV irradiation and the resulting lysate was used to transduce MY281 (*tna2* (*tonB-trpAC9*)^{del}) to Tna⁺. Out of 51 transductants tested, 27 grew on glycerol/indole/minimal medium and had therefore acquired λ *tnaR3*. A putative MY281 (λ *tnaR3*) lysogen was purified and used to prepare a lysate. This lysate was then used to transduce MY281 to Tna⁺. All 850 of these transductants were found, by replication to glycerol/indole/minimal medium, to have the TnaR3 phenotype. The purified λ *tnaR3* was maintained as an R103 (λ *tnaR3*) lysogen.

Construction of R128 (tnaR3 tnaAts6). This construction was performed in two stages. We first isolated a *tnaR3 tna*⁻ derivative by using localized mutagenesis. A P1 lysate prepared on MEB67 (*tnaR3 bglR* (*tonB-trpAC9*)^{del}) was mutagenized with hydroxylamine as described previously (White & Yudkin, 1979). This was used to transduce R103 (*tnaR3 bglR*⁺) to growth on salicin (*bglR*). The transductants were then replicated to

Table 1. *Strains of bacteria used in this work*

All strains are *E. coli* K12 derivatives. The specialized transducing phage λ imm21*tna* (here called λ *tna*) was the gift of W. J. Brammar. λ *tna2* was constructed by M. D. Yudkin by homogenotization. The construction of λ *tnaR3* is described in the text. To avoid confusion the *tna* alleles carried by λ *tna* in strains lysogenic for this phage and its derivatives are described fully.

Strain	Genotype	Source
W3110	Prototroph	C. Yanofsky
AC9	(<i>tonB-trpAC9</i>) ^{del}	C. Yanofsky
MY281	<i>tna2</i> (<i>tonB-trpAC9</i>) ^{del} Val ^R	This laboratory
MY355	<i>tna2 trpR trpBE</i> ^{del} (<i>attλ-gal-bio</i>) ^{del} Val ^R	This laboratory
MY634	<i>tnaP2 thr his arg</i> (<i>tonB-trpAC9</i>) ^{del} <i>cya855 crp868 lac gal xyl man rpsL supE</i> Val ^R	This laboratory
MY1396	<i>tnaA106</i> Val ^R	This laboratory
D3	<i>tnaR3 metE thr his arg cysG</i> (<i>tonB-trpAC9</i>) ^{del} <i>xyl man rpsL supE</i> Val ^R	This laboratory
ts6	<i>tnaAts6 metE thr his arg</i> (<i>tonB-trpAC9</i>) ^{del} Val ^R <i>bglR</i>	This laboratory
HVT7	<i>tna2 thr his arg</i> (<i>tonB-trpAC9</i>) ^{del} <i>xyl man rpsL supE bglR</i>	This laboratory
HVT25	<i>tna2 metE</i> (<i>tonB-trpAC9</i>) ^{del} Val ^R <i>bglR</i>	This laboratory
R100	<i>metE</i> (<i>tonB-trpAC9</i>) ^{del}	HVT25 $\times$ P1(W3110)
R103	<i>tnaR3 metE</i> (<i>tonB-trpAC9</i>) ^{del}	HVT25 $\times$ P1(D3)
R126	<i>tnaAts6 metE</i> (<i>tonB-trpAC9</i>) ^{del}	HVT25 $\times$ P1(ts6)
R128	<i>tnaR3 tnaAts6 metE</i> (<i>tonB-trpAC9</i>) ^{del}	See text
R200	<i>metE</i>	R100 $\times$ P1(W3110)
R203	<i>tnaR3 metE</i>	R103 $\times$ P1(W3110)
R225	<i>tna2 metE</i> Val ^R <i>bglR</i>	HVT25 $\times$ P1(W3110)
R226	<i>tnaAts6 metE</i>	R126 $\times$ P1(W3110)
R228	<i>tnaR3 tnaAts6 metE</i>	R128 $\times$ P1(W3110)
R303	<i>tnaR3 metE</i> (λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺)	R203 $\times$ λ <i>tna</i>
R325	<i>tna2 metE</i> Val ^R <i>bglR</i> (λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺)	R225 $\times$ λ <i>tna</i>
R326	<i>tnaAts6 metE</i> (λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺)	R226 $\times$ λ <i>tna</i>
R328	<i>tnaR3 tnaAts6 metE</i> (λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺)	R228 $\times$ λ <i>tna</i>
R403	<i>tnaR3 metE</i> (λ <i>tna2</i>)	R203 $\times$ λ <i>tna2</i>
R500	<i>metE</i> (λ <i>tnaR3 tnaA</i> ⁺)	R200 $\times$ λ <i>tnaR3</i>
R525	<i>tna2 metE</i> Val ^R <i>bglR</i> (λ <i>tnaR3 tnaA</i> ⁺)	R225 $\times$ λ <i>tnaR3</i>
R803	<i>tnaR3 trpR trpBE</i> ^{del} (<i>attλ-gal-bio</i>) ^{del} Val ^R	MY355 $\times$ P1(R103)
RA110	<i>tnaP2 metE</i> (<i>tonB-trpAC9</i>) ^{del} Val ^R	HVT25 $\times$ P1(MY634)
MEB65	<i>tnaAts6 metE</i> (<i>tonB-trpAC9</i>) ^{del} <i>bglR</i>	R126 $\times$ P1(HVT7)
MEB67	<i>tnaR3 metE</i> (<i>tonB-trpAC9</i>) ^{del} <i>bglR</i>	R103 $\times$ P1(HVT7)
MEB70	<i>tnaR3 tnaA112 metE</i> (<i>tonB-trpAC9</i>) ^{del} <i>bglR</i>	See text
MEB86	<i>tnaA106</i> (<i>tonB-trpAC9</i>) ^{del} Val ^R	AC9 $\times$ P1(MY1396)

salicin/indole/5-methyl-DL-tryptophan medium to detect Tna⁻ colonies. We found 21/5000 transductants that failed to grow on this medium. Three were eventually found to be *tna*. One of these mutants, MEB70, lacked detectable tryptophanase activity. Tna⁺ revertants of MEB70 were found to have retained their tryptophanase-constitutive phenotype.

MEB70 (*tnaR3 tna*⁻) was transduced to growth at 30 °C on glycerol/indole/5-methyl-DL-tryptophan, supplemented with 20 μ l L broth per plate, with P1 grown on MEB65 (*tnaR*⁺ *tnaAts6*). This procedure allows the selection of both Tna⁺ and Tna^{ts6}. Transductants were replicated to glycerol/indole/minimal medium supplemented with 50 μ g L-cysteine ml⁻¹ and incubated at 30 °C for 7 d. The L-cysteine was added to stimulate growth of constitutive strains on glycerol/indole medium (see Results). The prolonged incubation of the replicas was to allow any *tnaR3 tnaAts6* recombinants to grow. We obtained 19 *tnaR3* recombinants out of 750 colonies replicated. Of these one grew on glycerol/indole/5-methyl-DL-tryptophan at 30 °C but not at 40 °C. This recombinant failed to grow on glycerol/indole/minimal medium at any temperature tested. When tested on glycerol/indole medium supplemented with 50 μ g *S*-benzyl-L-cysteine ml⁻¹ (which we had previously found to enhance growth of constitutive strains on glycerol/indole medium but to have no stimulatory effect on inducible strains – see Results) it behaved as expected for a *tnaR3 tnaAts6* derivative. That is, it grew on glycerol/indole/5-methyl-DL-tryptophan or glycerol/indole/*S*-benzyl-L-cysteine media at 30 °C but not at 40 °C. Strain R126 (*tnaR*⁺ *tnaAts6*), on the other hand, grew at 30 °C on glycerol/indole/5-methyl-DL-tryptophan medium but not on glycerol/indole/*S*-benzyl-L-cysteine medium, and at 40 °C grew on neither medium. Both strains grew on glycerol/indole medium supplemented with L-tryptophan at both 30 °C and 40 °C, and R103 (*tnaR3 tnaA*⁺) grew on all these media at both 30 °C and 40 °C. Subsequent enzyme assays demonstrated the thermolabile constitutive expression of tryptophanase in strain R228 (the *trp*⁺ derivative of R128) – see Results.

RESULTS

Mapping of tnaR3

Taylor & Yudkin (1978) have shown that the mutation *tnaR3* is more than 98% co-transduced by P1 phage with *tnaAts6*, a tryptophanase structural gene mutation, and suggested that it was on the side of *tnaA* distant from *bglR*. We have confirmed this conclusion by mapping *tnaR3* against *tnaA106* which, of all the *tnaA* mutations isolated by White & Yudkin (1979), maps furthest from *bglR*.

P1 grown on MEB67 (*tnaR3 bglR trpAC9*) was used to transduce MEB86 (*tnaA106 bglR⁺ trpAC9*) to Tna⁺ with selection on glycerol/indole/5-methyl-DL-tryptophan. The *tnaA⁺* transductants were replica plated to glycerol/indole plates to identify *tnaR⁺* recombinants. Out of 5000 *tnaA⁺* transductants, 13 (0.3%) were *tnaR⁺*. Of these 13 recombinants 9 were *bglR* (donor type). Since the expected frequency of co-transduction between *bglR* and *tnaA* is 70%, we conclude that only a single cross-over event is required to give *tnaR⁺ tnaA⁺ bglR* recombinants and hence that the order of these three markers is *tnaR3-tnaA106-bglR*.

Behaviour of strain R103 on selective media

We found that strain R103 (*tnaR3 trpAC9^{del}*) grew much more slowly on glycerol/indole/minimal medium than on the same medium supplemented with 5-methyl-DL-tryptophan, or with L-tryptophan. Yet Taylor & Yudkin (1978) had reported that the mutation *tnaR3* renders tryptophanase synthesis constitutive at about 80% of the fully induced wild-type rate. It was therefore difficult to understand why R103 should grow better on glycerol/indole/5-methyl-DL-tryptophan medium than on glycerol/indole medium.

We could readily isolate derivatives of strain R103 which grew well on glycerol/indole medium; these proved to be resistant to catabolite repression as well as being higher-level constitutives. Moreover, the catabolite-resistant promoter mutant RA110 (*tnaP2 trpAC9^{del}*) (Ward & Yudkin, 1976) which is also constitutive for tryptophanase synthesis, grew better than R103 on glycerol/indole medium despite the fact that it is a lower-level constitutive.

These observations implicate catabolite repression as a cause of the difference in growth rates between R103 growing on glycerol/indole/5-methyl-DL-tryptophan and glycerol/indole media. Catabolite repression might result from a restriction in growth imposed by limitation of tryptophan; but the question still remains why tryptophan should become limiting in the first place. We suggest that the ability of tryptophanase to synthesize L-tryptophan is enhanced in the presence of 5-methyl-DL-tryptophan. This could be explained if 5-methyl-DL-tryptophan were an allosteric activator of tryptophanase, but no such interaction has been reported. We favour the hypothesis that the reaction leading to L-tryptophan on glycerol/indole medium is different to that taking place in the presence of 5-methyl-DL-tryptophan. It is known (Snell, 1975) that tryptophanase will catalyse replacement reactions of the type:

and that the kinetic parameters for this reaction are more favourable to the synthesis of L-tryptophan than are those for the reverse of the normal tryptophanase reaction (see Introduction) which we have hitherto assumed to be the route of L-tryptophan synthesis under these selective conditions.

To test this hypothesis we examined a number of different compounds that are substrates for the replacement reaction but do not induce tryptophanase, to see whether they would stimulate growth of R103 on glycerol/indole medium. The data presented in Table 2 show that some of these compounds do indeed have such an effect. One of these substrates, S-methyl-L-cysteine, even allowed strain AC9 (*tnaR⁺ trpAC9*) to grow on glycerol/indole medium. We assume that this is because S-methyl-L-cysteine is such a good substrate for the replacement reaction that even uninduced levels of tryptophanase can provide sufficient L-tryptophan for growth under these conditions. Another compound, S-benzyl-L-cysteine, allowed the clearest distinction to be made between constitutive and non-constitutive strains on glycerol/indole medium.

Table 2. Growth of R103 and AC9 on glycerol/indole/minimal medium supplemented with replacement-reaction substrates

Strains R103 (*tnaR3 trpAC9^{del}*) and AC9 (*tnaR⁺ trpAC9^{del}*) were streaked on glycerol/minimal medium supplemented with indole at 5 µg ml⁻¹ and potential replacement-reaction substrates at 50 µg ml⁻¹. Growth was scored after 48 h incubation at 37 °C. ++, Colonies about 1 mm diameter; + + + +, colonies about 3 mm diameter; —, no growth visible.

Supplement	Strain . . .	Growth	
		AC9	R103
5-Methyl-DL-tryptophan		+ + + +	+ + + +
L-Phenylalanine		—	—
S-Methyl-L-cysteine		++	+ + + +
S-Ethyl-L-cysteine		—	+ + + +
S-Benzyl-L-cysteine		—	+ + + +
L-Cysteine		—	+ + + +

These findings lead us to suggest that the selection used by Taylor & Yudkin (1978) in the selection of *tna* constitutive mutants, namely the plating of *tnaA⁺ trpAC9* strains on glycerol/indole medium, was too severe. This might account for the apparently low frequency of constitutive mutants obtained. In addition, the fact that all of the second-step mutations leading to improved growth of constitutive mutants on glycerol/indole medium resulted in the strains' becoming resistant to catabolite repression implies that this selection will introduce a bias against ordinary constitutive mutations. Indeed it may well be that the only reason why Taylor & Yudkin isolated some constitutive mutants that were not resistant to catabolite repression was that their selections were made from Cys⁻ strains and were therefore performed in medium supplemented with L-cysteine. L-Cysteine is another substrate for the replacement reaction and Table 2 shows that L-cysteine stimulates the growth of R103 on glycerol/indole medium.

Tryptophanase expression in R203

The mutation *tnaR3* renders tryptophanase expression constitutive. Taylor & Yudkin (1978) have reported that in the absence of L-tryptophan, *tnaR3 tnaA⁺* strains synthesize tryptophanase at a differential rate that is 80% of the fully induced wild-type and that this rate of synthesis is unaffected by the addition of L-tryptophan. We now wish to amend these findings in two respects.

(a) The previous observations were made with a whole-cell assay for tryptophanase. When we used toluene-treated cells for the assay we found that the differential rates we obtained for wild-type strains fully induced for tryptophanase were five to ten times higher. The constitutive rate of tryptophanase synthesis obtained for strain R203 (*tnaR3 trp⁺*) was unaffected by the change in assay. We suspect that L-tryptophan uptake is rate-limiting in the whole-cell assay of the fully induced wild-type (Edwards & Yudkin, 1982).

(b) The addition of L-tryptophan to cultures of R203 growing on glycerol/minimal medium represses tryptophanase synthesis by about 20% (data not shown). We interpret this as self-catabolite repression (Katz & Englesberg, 1971) of tryptophanase synthesis by the pyruvate released in the tryptophanase reaction [a combination of glycerol and pyruvate as carbon source is known to repress tryptophanase synthesis severely (McFall & Mandelstam, 1963; Sato *et al.*, 1969)]. In support of this hypothesis we found that repression of tryptophanase synthesis by L-tryptophan in R203 did not occur when pyruvate replaced glycerol as sole carbon source. Furthermore, the repeated addition of small amounts of pyruvate to cultures of R203 grown in glycerol/minimal medium could mimic the repression of tryptophanase synthesis by L-tryptophan (data not shown).

Using the amended assay described in Methods, we found that strain R200 (*tnaR⁺ tnaA⁺*), when grown at 37 °C in minimal medium containing 1% pyruvate as carbon source and 100 µg L-tryptophan ml⁻¹, synthesized tryptophanase at a differential rate of 1000 to 1100 units (mg protein)⁻¹. When grown in the same medium with or without tryptophan, strain R203

Table 3. *Differential rates of tryptophanase synthesis*

The cultures were grown at 30 °C in minimal medium supplemented with 1% pyruvate. Where indicated, cultures were induced for tryptophanase synthesis by the addition of 500 µg L-tryptophan ml⁻¹.

Strain	Genotype	Differential rate of tryptophanase synthesis [units (mg protein) ⁻¹]	
		- Tryptophan	+ Tryptophan
R200	<i>tnaR</i> ⁺ <i>tnaA</i> ⁺	< 20	415
R325	<i>tnaR</i> ⁺ <i>tna2</i> /λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺	< 20	320
R203	<i>tnaR3</i> <i>tnaA</i> ⁺	208	220
R403	<i>tnaR3</i> <i>tnaA</i> ⁺ /λ <i>tnaR</i> ⁺ <i>tna2</i>	183	203
R525	<i>tnaR</i> ⁺ <i>tna2</i> /λ <i>tnaR3</i> <i>tnaA</i> ⁺	137	154
R303	<i>tnaR3</i> <i>tnaA</i> ⁺ /λ <i>tnaR</i> ⁺ <i>tnaA</i> ⁺	220	544
R500	<i>tnaR</i> ⁺ <i>tnaA</i> ⁺ /λ <i>tnaR3</i> <i>tnaA</i> ⁺	174	656

(*tnaR3 tnaA*⁺) synthesized tryptophanase at a differential rate of 290 units (mg protein)⁻¹. Hence our revised estimate of the degree of constitutivity conferred by *tnaR3* is about 30% of the fully induced wild-type.

Dominance studies with λtna

tnaR3 is *cis*-dominant. The fact that we were able to construct derivatives of λ*tna* that carried *tnaR3* and that transduced *tnaR*⁺ strains to constitutivity (see Methods) strongly suggests that the *tnaR* locus is carried on λ*tna*. In diploids of the type *tnaR*⁺ *tna2*/λ*tnaR3* *tnaA*⁺ we found that tryptophanase was constitutive and not further inducible (see Table 3) regardless of whether the *tnaR3* allele was carried on the λ*tna* or on the bacterial chromosome. We conclude that *tnaR3* is at least *cis*-dominant.

tnaR3 is not *trans*-dominant. The results described above suggest that *tnaR*⁺ is not dominant to *tnaR3*. We now ask whether *tnaR3* is dominant to *tnaR*⁺. The assay results shown in the last two lines of Table 3 show that in diploids of the type *tnaR*⁺ *tnaA*⁺/λ*tnaR3* *tnaA*⁺ there is both an inducible and a constitutive component to the expression. This implies that the effect of *tnaR3* is not to render *tnaA* expression constitutive in *trans*. It is possible, however, that some form of co-dominance is operating, i.e. that when *tnaR*⁺ and *tnaR3* are both present, expression from both *tnaA* genes is partly constitutive and partly inducible. The alternative explanation is that constitutive expression can occur only from the *tnaA* gene in *cis* to *tnaR3*. To distinguish between these two possibilities we constructed a strain in which *tnaR3* was in *cis* to the temperature-sensitive lesion *tnaA*ts6 (see Methods), and introduced λ*tnaR*⁺ *tnaA*⁺ into this strain to form a diploid (R328) of the type *tnaR3 tnaA*ts6/λ*tnaR*⁺ *tnaA*⁺. We then tested for any *trans*-dominance of *tnaR3* by looking for constitutive expression of tryptophanase at 42 °C. Since *tnaA*ts6 renders tryptophanase inactive at this temperature, constitutive expression should only be observed if *tnaR3* were making the *tnaA*⁺ allele partially constitutive in *trans*. We therefore assayed the diploid (R328) along with suitable controls at 42 °C in the presence or absence of L-tryptophan (Table 4). We consistently failed to find any evidence for constitutive expression at 42 °C in the diploid R328.

It remained a possibility, however, that there was a low level of heat-stable constitutive expression in this strain caused by a weak *trans* effect of *tnaR3*. We ruled out this possibility by performing assays that utilized a larger sample of cells (5 ml) and that were correspondingly more sensitive. The data presented in Table 5 show that for R328 the differential rate of tryptophanase synthesis at 42 °C in the absence of L-tryptophan is no greater than the sum of expression from R326 and R228. We therefore conclude that *tnaR3* has no effect on *tnaA* expression in *trans*.

We were surprised to find that the rate of synthesis of tryptophanase was strongly affected by temperature even in the wild-type R200. Tryptophanase synthesis in R203 (*tnaR3*) is much less

Table 4. *Differential rates of tryptophanase synthesis*

The cultures were grown in minimal medium with 1% pyruvate as carbon source. A single colony of each strain was picked into 10 ml of the minimal medium which was then divided for overnight incubation at 30 °C and 42 °C. The overnight cultures were used to inoculate fresh media at the appropriate temperature. Where indicated (+) the cultures were induced for tryptophanase expression by the addition of 500 µg L-tryptophan ml⁻¹.

Strain	Genotype	Tryptophan	Differential rate of tryptophanase expression [units (mg protein) ⁻¹]			
			30 °C		42 °C	
			–	+	–	+
R226	<i>tnaR</i> ⁺ <i>tnaAts6</i>		< 20	166	< 20	< 20
R228	<i>tnaR3 tnaAts6</i>		95	87	< 20	< 20
R326	<i>tnaR</i> ⁺ <i>tnaAts6/λtnaR</i> ⁺ <i>tnaA</i> ⁺		< 20	291	< 20	75
R328	<i>tnaR3 tnaAts6/λtnaR</i> ⁺ <i>tnaA</i> ⁺		104	589	< 20	158

Table 5. *Tryptophanase synthesis in strain R328*

The medium used was as described in the legend to Table 4. The cultures were grown at 42 °C in the absence of inducer and 5 ml samples were taken periodically for assay.

Strain	Genotype	Differential rate of tryptophanase synthesis [units (mg protein) ⁻¹]
R228	<i>tnaR3 tnaAts6</i>	3.3
R326	<i>tnaR</i> ⁺ <i>tnaAts6/λtnaR</i> ⁺ <i>tnaA</i> ⁺	1.0
R328	<i>tnaR3 tnaAts6/λtnaR</i> ⁺ <i>tnaA</i> ⁺	4.4

Table 6. *Effect of growth temperature on the apparent degree of constitutivity conferred by tnaR3*

Single colonies of R203 (*tnaR3 tnaA*⁺) and R200 (*tnaR*⁺ *tnaA*⁺) were picked into minimal medium supplemented with 1% pyruvate. Each culture was divided into three for overnight incubation at 30 °C, 37 °C and 42 °C. The cultures were then diluted into fresh medium at the appropriate temperature. The cultures of R200 were induced by the addition of 500 µg L-tryptophan ml⁻¹.

Growth temperature	Differential rate of tryptophanase synthesis [units (mg protein) ⁻¹]	
	R200 (+ Tryptophan)	R203 (– Tryptophan)
	30 °C 37 °C 42 °C	455 1000 79
		227 290 149

sensitive to temperature. Table 6 shows the apparent degree of constitutivity exhibited by R203 at different temperatures. We have no convincing explanation for this effect but it does not affect the foregoing argument about the lack of *trans*-dominance of *tnaR3*.

DISCUSSION

Taylor & Yudkin (1978) found that plating a *trp*-deleted *tnaA*⁺ strain on glycerol/indole/minimal medium yields spontaneous *tna* constitutive mutants at a very low frequency (< 10⁻¹⁰). We have previously taken this result to mean that control of tryptophanase synthesis does not operate by a simple repressor mechanism. Our finding that the above selection is more severe

than we had supposed perhaps explains the low frequency at which constitutive mutants were isolated.

The mutation *tnaR3* renders tryptophanase synthesis constitutive at a differential rate 30% of that observed for the fully induced wild-type at 37 °C. It is *cis*-dominant but does not render a *tnaA* allele constitutive in *trans*. It is situated very close to *tnaA* on the side of *tnaA* distant from *bglR*. These findings are consistent with *tnaR3* being either an operator mutation that prevents binding of a repressor protein (compare *lacO^c*) or else a mutation rendering tryptophanase synthesis independent of a positive regulator (compare *araF^c*). It is interesting to note that *araF^c* mutants readily undergo second-step events to give the catabolite-insensitive *araX^c* mutants (Colome *et al.*, 1977). However, it cannot be altogether ruled out that *tnaR* is the gene for a regulator (RNA or protein), which acts only in *cis* because of restrictions of stability or diffusion.

An intriguing feature of this study was the finding that *tnaA* expression is temperature sensitive in *tnaR⁺* but not in *tnaR3* strains. We have no convincing explanation of this finding.

The results presented in this paper should lead to an improved selection for tryptophanase constitutive mutants. For example, glycerol/indole/minimal medium supplemented with L-cysteine or S-benzyl-L-cysteine will allow the growth of constitutive mutants but not of the wild-type. We hope that the elucidation of the selections used to isolate regulatory mutants may lead to the isolation of mutations that will improve our understanding of the mechanism of regulation of tryptophanase synthesis, which at present remains obscure.

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