JOURNAL OF BACTERIOLOGY, Apr. 1976, p. 547-549

Vol. 126, No. 1
Printed in U.S.A.

Evidence For Autogenous Regulation of *Pseudomonas putida* Tryptophan Synthase

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Received for publication 30 December 1975

Studies of a *trpA* mutant constitutive for tryptophan synthase production support the hypothesis of autogenous regulation (R. F. Goldberger, 1974; A. R. Proctor and I. P. Crawford, 1975) of the *Pseudomonas putida trpAB* loci.

Mutants blocked early in the tryptophan pathway of *Pseudomonas putida (trpE, D, F,* and *C* loci*)* cannot produce indole-3-glycerol phosphate (InGP), the inducer of tryptophan synthase (EC 4.2.1.20, L-serine hydrolyase [adding indole]) (TS) (Fig. 1). They maintain an uninduced basal TS level which is inadequate to allow utilization of 5 μg of indole per ml as a source of tryptophan. At higher indole concentrations (>10 μg/ml), sufficient InGP is generated from indole by the basal level of TS (InGP ⇄ indole activity) to cause TS induction. Consequently, early blocked Trp⁻ mutants have induced TS levels and grow well when given high indole concentrations. Two types of "indole-utilizer" mutations occur commonly in "early-blocked" Trp⁻ mutants which enable them to grow on low indole concentrations (1). Both types are closely linked to the two TS structural genes, *trpA* and *trpB*. One type *(trpX)* causes constitutive production of TS. The second type occurs in the *trpA* gene and results in both the loss of TS-A activity and the induction of TS (as measured by TS-B activity) by lower concentrations of indole than are required in strains lacking this mutation (7).

We previously proposed and discussed the hypothesis that the alpha-chain of *P. putida* TS is an integral part of the regulatory mechanism which governs induction of the *trpAB* gene pair by InGP, i.e., it is autogenously regulated (7) (Fig. 2). Indole per se is not normally recognized as an effector molecule for TS induction (7), and the hypothesis stipulates that in the *trpA* type of indole-utilizer mutant, an alteration in the TS alpha-chain changes the repressor molecule so that either InGP or indole can be recognized as an effector for TS induction.

A strain studied by Crawford and Gunsalus (1), *trpC911A801* (previously *trpS11i1*), was obtained as a spontaneous indole utilizer in the early-blocked strain, *trpC911* (previously *trpS11*). As it lacks InGP → indole activity, it corresponds to the second *(trpA)* type of indole utilizer. In the experiments of Crawford and Gunsalus (1), however, this strain appeared to exhibit a constitutive level of TS six or seven times higher than the low basal level of TS in the parental early-blocked strain, *trpC911*. We confirmed the sevenfold increase of TS in *trpC911A801* (Table 1, lines 1 and 2).

It should be noted that previous studies indicate coordinate regulation of *trpA* and *trpB* (1, 3, 6). Furthermore, the ability of *trpC911A801* to grow on indole clearly indicates that high TS-B activity is present; this could only result from induction of a missense alpha-chain when the beta-chain is induced (Fig. 1). Therefore, we will refer to induction of TS where only TS-B activity was measured. Other *trpA*-type indole-utilizer mutants such as *trpA508* have been studied (7), and these commonly occurring mutants do not exhibit altered basal TS levels. Hence, *trpA801* exhibits properties of both *trpA* and *trpX* indole-utilizer mutants. Revertants of *trpA801* were obtained and characterized to eliminate the possibility that mutations at both of these loci were simultaneously selected in this novel strain.

To create a nutritional selection for revert-

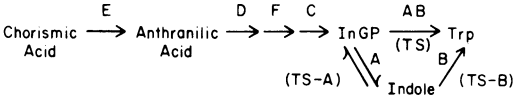

FIG. 1. *The tryptophan biosynthetic pathway with the corresponding trp gene designations shown above each enzymatic step (arrow). TS of P. putida is an $\alpha_2\beta_2$ tetramer which catalyzes the three reactions illustrated in the triangular scheme above. A mutation in either trpA (alpha-chain) or trpB (beta-chain) causes loss of the TS activity. Most trpA mutants lose both TS and TS-A activities, retaining TS-B activity, and similarly trpB (beta-chain) mutants retain only TS-A activity. All three activities are lost if either chain is not produced, or if either chain is so severely altered as to prevent proper tetramer formation.*

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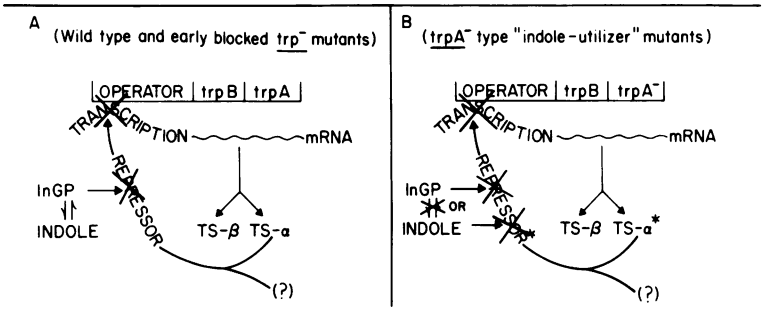

FIG. 2. *Autogenous regulation model for TS induction in P. putida. A negative control scheme is shown to illustrate the proposed involvement of the trpA gene product (alpha-chain) in regulation of the trpAB operon. (A) We propose that the TS alpha-chain constitutes the repressor molecule (or [?] a part thereof), which negatively controls transcription of the trpAB operon ("X" through repressor). (B) Indole-utilizing mutants in the trpA gene, such as trpA508, alter the alpha-chain (*) and hence the repressor (*), such that indole per se as well as InGP is recognized as an effector molecule for TS induction.*

TABLE 1. *Specific activity of tryptophan synthase in extracts of various trp mutant strains*[a]

Strain	Growth condition	
	No inducer[b]	Anthranilate[c]
trpC911	6	5
trpC911A801	42	44
trpE602A801-53	30	2,739
trpE602A⁺-53R	5	25
trpE602thr-605	5	40
trpE602	4	32
trpE602A508	4	675

[a] Cells were harvested from late exponential phase cultures grown in minimal medium (8) containing 0.2% glucose and indicated supplements. Extracts were prepared (3) and assayed for protein (5) and activity in the conversion of indole to tryptophan (3) in the presence of added alpha subunit. Specific activity = units of activity (micromoles/minute) × 10^4 per mg of protein.

[b] Medium supplemented with 4 μg of L-tryptophan per ml.

[c] Medium supplemented with 40 μg of anthranilate per ml and 4 μg of L-tryptophan per ml.

ants of *trpA801*, *trpC911* was exchanged by transduction for *trpE602* as illustrated in Fig. 3. Revertants of *trpA801* will grow on anthranilic acid in *trpE602A801*, but not in *trpC911A801*, since *trpC911* blocks conversion of anthranilic acid to InGP (Fig. 1). Revertants of *trpA801* were obtained as described in Fig. 3. All *trpA801* revertants lose both the ability to grow on low levels of indole (5 μg/ml) (Fig. 3) and the TS constitutivity, reverting to the low basal level of early-blocked Trp⁻ mutant strains. This result is illustrated in Table 1 for *trpE602A801* (isolate 53) and its *trpA⁺* revertant, *trpE602A⁺-53R*. These experiments eliminate the possibility that the TS constitutivity of *trpC911A801* could be due to an unrecognized *trpX* mutation.

The maximal TS level induced in *trpE602A801* by addition of anthranilic acid (40 μg/ml) as a source of the TS inducer, InGP, is about four times higher than that obtained by the same treatment of *trpE602A508* (Table 1). Apparently, *trpA801* results in hyperinducibility of TS by InGP, a result that would not be expected if *trpA801* merely allowed the production in the cell, somehow, of a small amount of InGP. When strains *trpC911A801* and *trpE602A801* were grown with 40 μg of indole per ml and 4 μg of L-tryptophan per ml, TS was induced six- and ninefold, respectively, above the basal levels shown for these strains in Table 1. Since both strains lack TS-A activity and cannot metabolize indole to the TS inducer, InGP, then *trpA801*, like *trpA508* and other *trpA*-type indole-utilizer mutants (7), appears to confer the ability to recognize indole per se as an inducer of TS.

Our results clearly demonstrate that a single, revertible point mutation in *trpA* simultaneously causes the following: (1) loss of TS-A activity; (ii) constitutive production of TS at seven times the basal level; (iii) hyperinducibility of TS by InGP, the normal inducer; and (iv) recognition of indole per se as an effector molecule in TS induction. The finding that a mutation in *trpA* can cause constitutivity and hyperinducibility of TS implicates the alpha-chain as an integral part of the regulatory mechanism which governs induction of *trpA* and *trpB* by InGP. We conclude that the unusual properties of *trpA801* are consistent with and provide further support for the hypothesis (Fig. 2) of autogenous regulation of tryptophan synthase in *P. putida* (7).

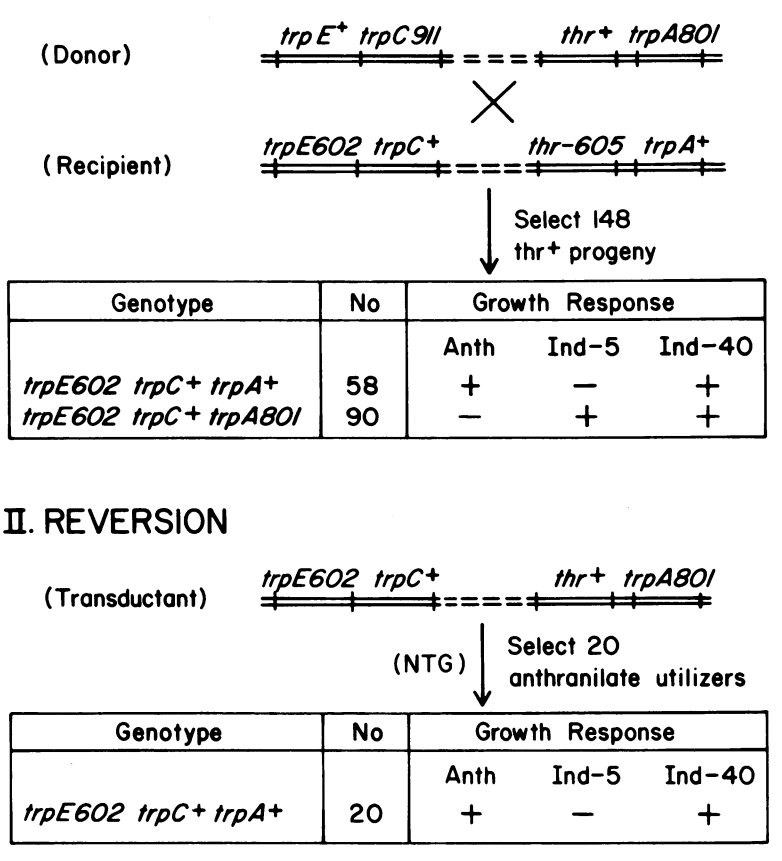

FIG. 3. *Selection of trpA801 revertants. Linked markers are represented on unbroken line segments (see references 3, 6). I. The donor, recipient, and progeny genotypes from a pf16 transduction performed according to the method of Maurer and Crawford (6). Growth response was determined on plates of minimal medium containing: Anth, 10 μg of anthranilic acid per ml; Ind-5, 5 μg of indole per ml; Ind-40, 40 μg of indole per ml. II. Twenty representative trpE602A801 transductant colonies were purified, retested for growth requirements, grown for 16 h in L-broth (4) containing 1 μg of N-methyl-N'-nitro-N-nitrosoguanidine per ml, then spread on Anth plates (about 10^7 cells/plate). A single revertant (anthranilate-utilizing) colony was picked from each and tested for growth response as described above.*

This research was aided by a postdoctoral fellowship grant no. PF-806 awarded to A. R. Proctor from the American Cancer Society, and Public Health Service research grant GM 19098 from the National Institute of General Medical Sciences.

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