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Threonyl-Transfer Ribonucleic Acid Synthetase and the Regulation of the Threonine Operon in *Escherichia coli*

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Two threonine-requiring mutants with derepressed expression of the threonine operon were isolated from an *Escherichia coli* K-12 strain containing two copies of the *thr* operon. One of them carries a leaky mutation in *ilvA* (the structural gene for threonine deaminase), which creates an isoleucine limitation and therefore derepression of the *thr* operon. In the second mutant, the enzymes of the *thr* operon were not repressed by threonine plus isoleucine; the threonyl-transfer ribonucleic acid (tRNA) synthetase from this mutant shows an apparent K_m for threonine 200-fold higher than that of the parental strain. The gene, called *thrS*, coding for threonyl-tRNA synthetase was located around 30 min on the *E. coli* map. The regulatory properties of this mutant imply the involvement of charged threonyl-tRNA or threonyl-tRNA synthetase in the regulation of the *thr* operon.

It has long been established that in the case of amino acid biosynthetic pathways, regulatory signals involve the end product amino acid(s) either singly (univalent control) or in combination (multivalent control). In recent years an extensive body of information has accumulated which implicates various macromolecular components of the translational machinery of protein biosynthesis, in particular cognate aminoacyl transfer ribonucleic acids (tRNA's) and/or their synthetases, in the regulation of some amino acid biosynthetic pathways (2).

The enzymes of threonine biosynthesis are multivalently controlled by threonine and isoleucine (6). Their structural genes are organized as an operon (21). Operator-constitutive mutants (18) and operator-promoter mutants (8) have been recently described. The nature of the regulatory molecules and the mechanism of their interaction with the operator locus in the regulation of expression of the threonine operon remain to be determined. In this paper, we present evidence involving a mutation located around 30 min on the *Escherichia coli* map and establishing a role for threonyl-tRNA synthetase or threonyl-tRNA, or both, in the regulation of the *thr* operon.

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MATERIALS AND METHODS

Media and chemicals. The media and procedures for growth of the cultures were the same as described previously (21). Eosin methylene blue lactose medium was used to screen Lac$^+$ and Lac$^-$ colonies (12). Crystalline sodium penicillin G was from Specia. Ethyl methane sulfonate was obtained from Eastman Kodak Co. All the amino acids were purchased from Merck. Homoserine phosphate was prepared according to the method of Wormser and Pardee (22), using homoserine kinase kindly provided by Benjamin Burr.

L-[^{14}C]threonine was obtained from C.E.A. (France). Bulk tRNA from *E. coli* was kindly provided by F. Chapeville.

Bacterial and phage strains. The strains of *E. coli* K-12 used are described in Table 1. Transduction with P1*vir* was performed according to the method of Lennox (11).

Selection of mutants. The merodiploid strain GT300 was mutagenized with ethyl methane sulfonate, and after penicillin enrichment in the absence of threonine the cells were plated on medium containing 20 mM L-threonine.

Enzyme assays. Cells grown in minimal medium were harvested while still in logarithmic phase and suspended in the following buffers: (i) 10 mM potassium phosphate, pH 7.2, containing 2 mM L-threonine and 0.1 mM L-isoleucine, when the threonine biosynthetic enzymes were assayed; (ii) 10 mM tris(hydroxymethyl)aminomethane, pH 7.3, 10 mM magnesium chloride, 0.1 mM L-threonine, 6 mM 2-mercaptoethanol, 10% glycerol, when threonyl-tRNA synthetase was assayed.

The cells were sonically disrupted and, after centrifugation at 10,000 × *g* for 10 min for the elimina-

TABLE 1. *List of bacterial strains used*

Strain	Sex	Genotype	Origin/reference
DJ105	F⁻	*leu proC trp his ile argH purD met gal ara lac mal str*	J. Miller
GT190	HfrP 4x6	*metLM1005 lysC1004*	(18)
GT189	F⁻	*metLM1005 lysC1004 leu proC trp his str mal gal lac ara*	Ileu⁺ recombinant between GT190 and DJ105
GT300	F′	*metLM1005 lysC1004 str trp his leu proC mal gal lac ara* F101:*thr*⁺ *leu*⁺	F101 from F101/AB2463 transferred to GT189
GT302	F⁺	*metLM1005 lysC1004 str trp his leu proC mal gal lac ara thrS1029* F101:*thr*⁺ *leu*⁺	This paper
GT303	F′	*metLM1005 lysC1004 str trp his leu proC mal gal lac ara ilvA1000* F101:*thr*⁺ *leu*⁺	This paper
GT315	F⁻	*metLM1005 lysC1004 str trp his proC mal gal lac ara ilvA1000*	This paper
F101/AB2463	F′	*argE3 his-4 proA2 leu-6 thr-1 mtl-1 xyl-5 ara-14 galK2 lacY1 recA13 tsx-33 str-31 sup-37* F101:*thr*⁺ *leu*⁺	Adelberg
Gy2615	HfrH	*thi-1 cya*	Mutant 283 (3)
HfrH	Hfr	*str*⁺	F. Jacob

tion of cellular debris, a subsequent centrifugation (105,000 × *g* for 2 h) was performed to assay aspartokinase and homoserine kinase (21).

Homoserine dehydrogenase I (HDHI), aspartokinase I, and homoserine kinase activities were measured as described previously (16, 21). The assay for threonine synthase takes advantage of an accessory threonine deaminase activity of this enzyme and measures the rate of formation of α-ketobutyrate (7, 18, 19).

Threonyl-tRNA synthetase was assayed by determining the transfer of L-[^{14}C]threonine to acceptor tRNA essentially according to Calendar and Berg (4), with some modifications (1).

Threonine deaminase was assayed by measuring the rate of formation of α-ketobutyrate (7) according to Szentirmai and Umbarger (20).

Protein concentration was determined by the biuret method (9).

RESULTS

Regulatory properties of the mutants. To select for mutations located outside of the *thr* operon, a strain carrying two copies of the *thr* operon was mutagenized. Among the threonine-requiring mutants isolated, two were analyzed. Data presented in Table 2 show that the specific activity of HDHI is at least four times higher in the mutants than in the parental strain grown under the same conditions (with 5 mM L-threonine). The threonine inhibition of HDHI is identical to that of the parental strain.

The threonine requirement of one of the mutants, GT303, is in fact due to a leaky mutation in the *ilvA* structural gene, which codes for threonine deaminase. The strain has barely detectable threonine deaminase activity, and this results in a limitation in isoleucine, which is produced by this enzyme from threonine; thus the strain could grow either in the presence of threonine or in the presence of isoleucine. In this mutant, HDHI was derepressed compared with the parental strain when the cells were grown in the presence of 5 mM L-threonine (Table 2). However, similar specific activities for HDHI are obtained when the parental strain and the mutant are grown in the presence of 5 mM L-isoleucine (results not shown).

TABLE 2. *HDH and threonine deaminase activities of the threonine-requiring mutants and the parental strains*

Strain[a]	Sp act (nmol of product formed/min/mg of protein)	
	HDHI	Threonine deaminase
GT189	36	43
GT300	110	45
GT302	1,280	47
GT303	410	<2

[a] All cultures were grown in the presence of 5 mM L-threonine.

In the other mutant, GT302, all enzymatic activities of the threonine operon were derepressed about 10-fold compared with the parental strain grown under the same conditions (Table 3). Threonine deaminase was unaffected in this mutant (Table 2).

Localization of the mutation in strain GT303. Strain GT303 was first cured of its episome by the acridine orange technique (12). The cured organism, GT315, shows properties similar to those of strain GT303.

TABLE 3. *Levels of the threonine biosynthetic enzymes in GT189, GT302, Rev 1, and Rev 2*

Strain[a]	Sp act (nmol of product formed/min/mg of protein)			
	Aspartokinase I	HDHI	Homoserine kinase	Threonine synthase
GT189	18	80	35	<0.6
GT302	177	1,075	462	7
Rev 1	ND[b]	100	ND	ND
Rev 2	ND	230	ND	ND

[a] See footnote to Table 2.
[b] ND, Not determined.

P_1 phage grown on strain GT315 was used to transduce a Cya$^-$ strain GY2615, and Cya$^+$ (Lac$^+$) transductants were selected. The Ile$^-$ mutation of strain GT315 was found to be 60% cotransducible with *cya*, as is the *ilvADE* operon.

Threonyl-tRNA synthetase activities of strain GT189 and strain GT302: kinetic parameters. The threonyl-tRNA synthetase activities of strains GT189 and GT302 are presented in Table 4. Clearly, strain GT302 has no activity when the threonine concentration in the assay is 0.2 mm. The V_{max} values determined by a Lineweaver-Burk plot are identical for both parental and mutant strains when saturating concentrations of threonine are used. The $K_{m_{app}}$ for threonine of the synthetase from strain GT189 was 89 μM, of the order of that found by other authors (15) for the wild-type enzyme. In contrast, the apparent affinity of the same enzyme extracted from the mutant GT302 was 200-fold higher (16 mM) (Fig. 1).

Localization of the mutation in strain GT302. Strain GT302 was cured by taking advantage of its thermosensitivity to 41°C, exhibited by the merodiploid but not by the haploid strains. The frequency of survival at 41°C on complete medium was approximately 10^{-8}, and virtually all survivors were cured. Interrupted mating, using HfrH and the selected markers proline and tryptophan, placed the mutation about 2 min away from the tryptophan marker. More precise genetic mapping is in progress.

Spontaneous revertants of GT302 cured of its episome. Spontaneous revertants were isolated from the cured strain, which shows properties similar to those of strain GT302.

The kinetic properties of the threonyl-tRNA synthetase of two of the revertants were determined (Table 4). The enzyme of one of them (Rev 1) has a $K_{m_{app}}$ for threonine that is sixfold less, and a V_{max} that is sixfold less, than that of the parental strain. For the other revertant the K_m was 60-fold greater, and the V_{max} was the same. In Rev 1, expression of the threonine operon was the same as that of the wild type (Table 3). The HDH specific activity of extracts from Rev 2 grown in the presence or in the absence of threonine was the same as that from GT189 grown in the absence of threonine (Table 3).

Other revertants were threonine excretors, their HDHI being desensitized towards threonine. Thus, overproduction of threonine saturates the altered threonyl-tRNA synthetase, phenotypically correcting the effect of the mutation. Such a phenomenon has been reported in the case of the histidine operon (17).

DISCUSSION

By using a technique used for identification of regulatory components coded for by genes located outside of operons, two mutants concerned with the regulation of the threonine operon were isolated. Both bear regulatory modifications as shown by the derepression of the threonine operon. One of them, strain

TABLE 4. *Threonyl-tRNA synthetase activity of GT189, GT302, Rev 1, and Rev 2*

Strain	Concn (M) of threonine in the threonyl-tRNA synthetase assay	Sp act of threonyl-tRNA synthetase (nmol of product formed/min/mg of protein)
GT189	2×10^{-4}	0.85
GT302		<0.01
Rev 1		0.24
Rev 2		0.03
GT302	1.6×10^{-2}	0.45

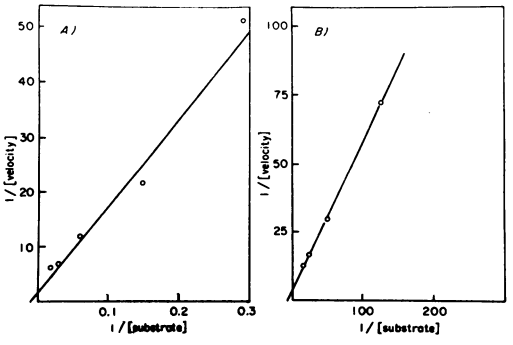

FIG. 1. *Determinations of K_m for threonine of threonyl-tRNA synthetase in crude extracts of strain GT189 (A) and GT302 (B). Values for 1/[velocity] are given in nanomoles/minute per milligram of protein; values for 1/[substrate] are given in micromolarity for L-threonine.*

GT303, which showed derepressed expression of the threonine biosynthetic enzymes when grown in the presence of threonine but not in the presence of isoleucine, probably carries a leaky mutation in *ilvA*, the structural gene for threonine deaminase. Two arguments are in favor of such an explanation: a very low level of the biosynthetic threonine deaminase is found in this mutant, and the mutation lies close to the *ilvADE* region. Derepression of the threonine operon occurs even in the presence of excess threonine, due to a limitation of isoleucine biosynthesis. The phenotype of strain GT303 confirms the concept of multivalent regulation of the threonine operon by threonine plus isoleucine (6).

The other mutant, GT302, has a high level of threonine biosynthetic enzymes compared with the parental strain under all conditions of growth. The measurements of threonyl-tRNA synthetase activity in the mutant showed an increased K_m for threonine of about 200-fold over that of the parental strain. Furthermore, the threonyl-tRNA synthetase from the strain cured of its episome retained the high K_m for threonine; the regulatory modification of expression of the *thr* operon observed in the mutated merodiploid is still present in the cured strain. Both properties are affected concomitantly in revertant Rev 1. The $K_{m_{app}}$ for threonine of the threonyl-tRNA synthetase of strain Rev 2, though reduced, was still 60-fold greater than that of the wild type; therefore, the regulation of expression of the *thr* operon remained altered.

A threonyl-tRNA synthetase mutant with about 10-fold decreased affinity for threonine has already been described by Nass and Thomale (14). In their mutant, the decrease in affinity does not result in the derepression of the enzymes of the threonine operon. In the same study, other mutants of the threonyl-tRNA synthetase were investigated. None of them exhibits a derepressed *thr* operon. Some of them, however, are derepressed in the presence of borrelidin, a compound acting as an inhibitor of the threonyl-tRNA synthetase (13). These authors postulate a role for the threonyl-tRNA synthetase in the regulation of the threonine operon. From our studies, it can be concluded that threonyl-tRNA synthetase is indeed involved in this regulation either by itself or by its product, charged threonyl-tRNA.

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