Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4384–4388, June 1986
Genetics

Genetic definition of the translational operator of the threonine-tRNA ligase gene in *Escherichia coli*

(translational regulation/tRNA-related structures)

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ABSTRACT **The *Escherichia coli* gene *thrS* that codes for threonine-tRNA ligase ($tRNA^{Thr}$ ligase, formerly threonine-tRNA synthetase, EC 6.1.1.3) has previously been shown to be negatively autoregulated at the level of translation. Here we describe the use of several *thrS-lac* gene fusions to isolate *cis*-acting regulatory mutations that increase the translation but not the transcription of the *thrS* gene. These mutations lead to a total loss of control of repression and derepression of *thrS*. DNA sequence analysis locates the mutations between 10 and 40 base pairs upstream of the translation initiation codon of *thrS* and more than 100 base pairs downstream of the transcription initiation site. The mRNA region where these mutations are located shares primary and secondary structure homologies with specific parts of several isoacceptor $tRNA^{Thr}$ species. These findings suggest that the ligase regulates its translation by binding to its mRNA at a place that shares some homology with its natural substrate.**

For many years transcription was thought to be the only level at which gene expression was regulated in bacteria. It now appears, however, that a significant number of genes are controlled at the level of translation. Protein-mediated translational regulation was first studied with RNA phages (1), then with bacteriophage T4 (2, 3), and in *Escherichia coli* with the ribosomal protein operons. Among these operons only the following two have been studied in detail genetically: the *L11-L1* and the *L10-L7/L12* operons. In the case of *L11-L1* the translational operator is located near the translation initiation site of *L11*, the first gene of the operon (4). This fits with the intuitive idea that the binding of L1 (the regulatory protein) to the operator inhibits, by steric hindrance, the binding of the ribosomes to the translational initiation site of *L11*. If *L11* is not translated, L1 cannot be translated, since their translation is coupled (5); and the whole operon is thus repressed. In the case of *L10-L7/L12* the situation is more complicated since the translational operator seems to be far away from the translation initiation site of *L10*, which is the first cistron of the operon (6).

This work describes a genetic approach to the study of translational regulation using the threonine-tRNA ligase (formerly the threonine-tRNA synthetase) gene (*thrS*) as model system. The gene *thrS* is located at 38 min on the *E. coli* chromosome and is negatively autoregulated at the translational level as previously shown by the following independent experiments: (*i*) Structural mutants of *thrS* overproduce modified forms of threonine-tRNA ligase ($tRNA^{Thr}$ ligase, EC 6.1.1.3), although thrS mRNA levels are not increased (7). (*ii*) β-Galactosidase synthesized from a *thrS-lac* gene fusion is derepressed in a mutant *thrS* gene background and is repressed when $tRNA^{Thr}$ ligase (synthesized from a plasmid in *trans*) is in excess (8). None of these regulations are seen when β-galactosidase is synthesized from a *thrS-lac* operon fusion (8). (*iii*) *In vitro* experiments show that $tRNA^{Thr}$ ligase synthesis, but not thrS mRNA synthesis, is inhibited in presence of an excess of $tRNA^{Thr}$ ligase in a coupled transcription–translation system (9).

The present paper describes genetic experiments that define the *thrS* translational operator. Throughout this paper we shall employ the operator and repressor terminology always implying translational operator and translational repressor, respectively.

MATERIALS AND METHODS

Strains and Standard Techniques. The *E. coli* K12 strains used in this work are: IBPC5421 (F^-, *thi-1, argE3, lacZ, galK2, mtl-1, xyl-5, tsx-29?, supE44?, rpsL, recA1*), IBPC5311, and IBPC5403 (8). General genetic techniques were as described by Miller (10); general cloning techniques were as described (11, 12).

Construction of λMBXΔ20-28 and λMBXΔL18. These two phages carrying two different *thrS-lac* gene fusions were isolated in the following three steps: (*i*) a simple plasmid carrying a *thrS-lac* gene fusion was constructed; (*ii*) the fusion on the plasmid was deleted or inserted; and (*iii*) the modified fusions were cloned from the plasmids into λ. A 7.08-kilobase (kb) *Eco*RI fragment carrying the *thrS-lac* gene fusion of λMBX1 (8) was inserted in the *Eco*RI site of pBR322 in the orientation where *lac* and *bla* are transcribed in the same direction. The *Hin*dIII site of the resulting plasmid (pMBXR1) was cut and filled-in using the Klenow fragment of DNA polymerase I. The resulting plasmid (pMBΔ2) carries single *Xho* I and *Bst*EII sites. The plasmid pMBΔ20-28 belongs to a set of plasmids derived from pMBΔ2 by creating deletions around the unique *Xho* I site (located within the *thrS* part of the *thrS-lac* gene fusion) using BAL-31. The plasmid pMBΔ2 was linearized with *Xho* I, digested with BAL-31, and recircularized in presence of *Hin*dIII linkers as described (13). The plasmid pMBΔL18 was derived from pMBΔ2 by cutting at the unique *Bst*EII site (located in the stem and loop structure of the operator), filling-in with the Klenow fragment of DNA polymerase I, and religating in presence of *Hin*dIII linkers (CAAGCTTG). This construction should reconstitute two *Bst*EII sites around the *Hin*dIII linker; however, such sites were not found in pMBΔL18. DNA sequencing (described in the legend of Fig. 1) indicated that, although the linker was integrated at the correct place, the filling-in occurred abnormally. The *Eco*RI fragments of pMBΔ20-28 and pMBΔL18 that carry the modified fusions were cloned in the *Eco*RI site of λgt4 (11) in the orientation that reconstitutes the whole *lacZ* gene as described (8).

Selection of the Operator-Constitutive Mutants. An overnight culture (0.1 ml) of IBPC5421(λMBX1)pUB4 or IBPC5421(λMBXΔ20-28)pUB4 was spread on either a minimum

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Abbreviations: bp, base pair(s); O^c, operator constitutive.

A/lactose/arginine plate or on a McConkey/lactose plate (10) both supplemented with ampicillin at 500 μg/ml. On minimum/lactose plates about 100 colonies (called "L" mutants) were found after about 48 hr at 30°C and on McConkey/lactose plates, from 100 to 500 large colonies (called "M" mutants) were found coming out of a thick background after 36–48 hr at 30°C. In the McConkey selection, the large colonies had no specific color on the selection plates; however, the dark red coloration appeared after purification. Independent "L" and "M" mutants were, after purification, thermoinduced, and the resulting lysates used to relysogenize IBPC5421pUB4: in 9 out of 55 cases, the Lac$^+$ phenotype was associated with the phages.

RESULTS

The Cloverleaf-Type Structure at the Beginning of *thrS* Has No Obvious Role in Regulation. tRNA^{Thr} ligase recognizes tRNA as its natural substrate and might thus recognize, as was proposed (9), the cloverleaf-type or tRNA-type structure that the mRNA can form at the beginning of *thrS* (Fig. 1). This recognition (if followed by binding) could repress translation of the thrS mRNA. A straightforward way to test this hypothesis is to determine the effects of deleting the tRNA-type structure on the regulation of *thrS* expression. The phage λMBXΔ20-28, a derivative of λMBX1, carries a 505-base-pair (bp) deletion in *thrS* that eliminates this structure. The left end of the deletion in λMBXΔ20-28 (Fig. 1) is located in such a way that it destroys all the base pairings of the tRNA-type structure as shown in the figure. The effect of the deletion was determined by comparing the regulation of β-galactosidase expression from a wild-type and a deleted *thrS-lac* gene fusion carried by λMBX1 and λMBXΔ20-28, respectively. Since *thrS* is negatively autoregulated, β-galactosidase expression from λMBX1 is derepressed about 3-fold (Table 1, line 1, and ref. 8) in a *thrS1029* background and repressed about a factor of 6 in presence of pUB4, a plasmid that carries *thrS* and overproduces tRNA^{Thr} ligase (Table 2, line 1, and ref. 8). However, β-galactosidase synthesis from λMBXΔ20-28 is still derepressed in a *thrS1029* background (Table 1, line 2) and still repressed in the presence of an excess of tRNA^{Thr} ligase synthesized from pUB4 (Table 2, line 2). For unknown reasons the repression by pUB4 with λMBXΔ20-28 is marginally but reproducibly stronger than with λMBX1 (Table 2). At any rate, β-galactosidase synthesis from λMBXΔ20-28 is regulated in the same manner as the synthesis from λMBX1, which means that the tRNA-type structure at the beginning of *thrS* is not involved in the autoregulation of *thrS* expression.

Isolation of O^c Mutants. To locate the *thrS* translational operator, we devised a general way of isolating O^c mutants in *thrS*. The parental strain, IBPC5421 (a nonpolar *lacZ, recA* strain) was monolysogenized with either λMBX1 (this phage does not carry *lacY* that is provided by the chromosome) or its derivative, λMBXΔ20-28, described in the preceding paragraph. Both monolysogens are phenotypically Lac$^+$ but when transformed by pUB4 (the plasmid that overproduces tRNA^{Thr} ligase), they become phenotypically Lac$^-$ (the corresponding β-galactosidase values are given in Tables 2 and 3). We isolated from these Lac$^-$ strains independent spontaneous Lac$^+$ mutants. Since we wanted O^c-type mutations on the *thrS-lac* gene fusions, we screened for phage-linked mutations by inducing the Lac$^+$ mutants and relysogenizing IBPC5421 pUB4. In 9 cases out of 55, the Lac$^+$ phenotype was linked to the phages. The striking feature of this selection is that all the phage-linked mutants are, as shown in the next paragraph, neither derepressible nor repressible translational up mutants—i.e., they are exactly the type of O^c mutants we were looking for.

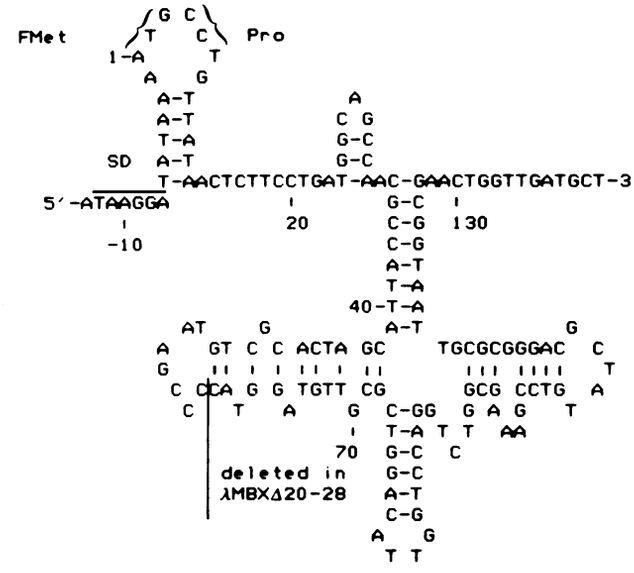

FIG. 1. Cloverleaf-type structure at the beginning of *thrS*. In this figure and in the subsequent Fig. 2, although RNA structures are described, all sequence determinations were done on DNA. To indicate this fact, we show RNA sequences with thymidine instead of uridine. The secondary structure shown is that proposed and discussed (9). The Shine–Dalgarno sequence (SD) is overlined. The first two amino acids (fMet-Pro) of *thrS* and the left end of the Δ20-28 deletion (59 bp on the 3′ side of the adenosine of the *thrS* ATG) are also indicated. All the nucleotides located to the right of the deletion boundary are absent in λMBXΔ20-28. All the sequencing was done using the chain-termination method (18). The ends of the Δ20-28 deletion were sequenced to the right and left from the *Hin*dIII linker inserted at the deletion locus. Sequencing to the left (or upstream) was done on a M13mp8 recombinant (19) carrying a *Pst* I–*Hin*dIII insert. Sequencing to the right (or downstream) was done with M13mp8 carrying a *Bam*HI–*Hin*dIII insert.

Properties of the O^c Mutants. The different mutant phages

Table 1. Effect of the *thrS* alleles on β-galactosidase synthesis from λMBX1 and its derivatives

Fusion	β-Galactosidase, units per OD_{650} of bacteria		Derepression (*thrS1029*/wt)
	Wild type	*thrS1029*	
λMBX1 (wt)	407 (±12)	1283 (±37)	3.15
λMBXΔ20-28 (internal deletion)	238 (±21)	926 (±35)	3.9
λMBXM1-9 ($G_{-32}\rightarrow A$)	3174 (±92)	3208 (±276)	1.01
λMBXM1-11 ($G_{-32}\rightarrow T$)	2863 (±44)	2914 (±278)	1.01
λMBXΔL18 (*in vitro* insertion)	934 (±65)*	912 (±35)	0.97

The β-galactosidase levels are expressed as "Miller" units per OD_{650} of bacteria (10). The results are averages of at least four β-galactosidase measurements at OD_{650} between 0.2 and 0.5. The numbers in parentheses are SD for each value. Monolysogenic derivatives of wild-type IBPC5421 [IBPC5311 for the value followed by (*)], and *thrS1029* (IBPC5403) strains were obtained as described (8). Bacteria were grown in Mops/glucose medium (20) supplemented with arginine and threonine each at 50 μg/ml for several generations at 30°C before β-galactosidase measurements were made. Doubling times are always about 100 min. wt, Wild type.

Table 2. Effect of an excess of $tRNA^{Thr}$ ligase on β-galactosidase synthesis from λMBX1 and its derivatives

Fusion	β-Galactosidase, units per OD_{650} of bacteria		Repression (pUC8/pUB4)
	pUC8 (vector)	pUB4 (*thrS*)	
λMBX1 (wt)	496 (±27)	78 (±16)	6.3
λMBXΔ20-28 (internal deletion)	281 (±29)	20.6 (±1.7)	13.6
λMBXM1-9 (G_{-32}→A)	3161 (±548)	2424 (±136)	1.3
λMBXM1-11 (G_{-32}→T)	2510 (±119)	2546 (±232)	1
λMBXΔL18 (*in vitro* insertion)	1146 (±97)*	815 (±69)*	1.4

The β-galactosidase levels are expressed as "Miller" units per OD_{650} of bacteria (10), and values in parentheses are SDs. Complementary explanations are as in the legend of Table 1 with the exception that the growth medium was supplemented with a mixture of all amino acids (20) and with ampicillin that was added at a concentration of 100 μg/ml every 2 hr during growth. Doubling times are always about 80 min. wt, Wild type.
*IBPC5311 derivative.

to which the Lac^+ phenotype was found to be linked were used to lysogenize, as single copies, isogenic wild-type and *thrS* strains. The first parental phage, λMBX1, is derepressed about 3-fold in a *thrS* background (Table 1, line 1). The two mutant phages derived from λMBX1, called λMBXM1-9 and λMBXM1-11, synthesize β-galactosidase at the same level in a wild-type and a mutant *thrS* background (Table 1, lines 2 and 3). The second parental phage λMBXΔ20-28 is derepressed about 4-fold in a *thrS* background (Table 4, line 1) whereas its seven derivatives (grouped in five classes by DNA sequence, see later) have lost the property of being derepressed in a *thrS* background (Table 4).

Monolysogenic derivatives of a wild-type host with the parental and mutant phages were transformed with pUC8 and pUB4 (the pUC8 derivative that carries *thrS* and overproduces $tRNA^{Thr}$ ligase). β-Galactosidase synthesized by λMBX1 is repressed by a factor of about 6 in the presence of pUB4 (Table 2, line 1), whereas the synthesis from its two derivatives (λMBXM1-9 and M1-11) is no longer repressed in *trans* by pUB4 (Table 2). β-Galactosidase synthesized by λMBXΔ20-28 is repressed by a factor of about 14 by pUB4, whereas the synthesis from its seven derivatives is no longer repressed (Table 3). These results show that all the mutants we isolated have simultaneously lost both properties of being derepressed in a *thrS* background and of being repressed in the presence of pUB4—i.e., they have properties typical of O^c mutants.

All the mutants we isolated synthesize increased levels of β-galactosidase. At this point it was essential to determine whether this increase is due to an increase of transcription or translation of the *thrS-lacZ* hybrid genes. To answer this question, we measured the levels of lacZ mRNA synthesized from a subset of mutant phages integrated as monocopies in Δ*lac* hosts by hybridization of *in vivo*-labeled RNA to a single-stranded M13 DNA probe carrying 1.8 kb of *lacZ* DNA. The level of lacZ mRNA synthesized from λMBXΔ20-28 is the same as that of its two derivatives, λMBXΔ20-28L6-8 and M6-1 (Table 5, lines 1–3), although the β-galactosidase level of the mutants is 10- and 5-fold higher, respectively, than that of the wild type (Table 4). Similarly the *lacZ* mRNA level synthesized from λMBX1 is the same as that of its derivative λMBXΔL18 (Table 5), although the β-galactosidase level in that mutant is increased more than 2-fold (Table 1). The phage λMBXΔL18 carries an *in vitro*-derived insertion that will be described later.

Nucleotide Sequence Changes in the O^c Mutants. The regulatory region of *thrS* is shown in Fig. 2*A* in a form that indicates a possible secondary structure of the thrS mRNA in front of the structural gene. The mRNA start is located 163 nucleotides in front of the ATG for *thrS*—i.e., to the 5′ side of the sequence shown in the figure (J. F. Mayaux and G. Fayat, personal communication). All the translational operator mutations are located between −10 and −40 (+1 is the adenosine of the *thrS* initiation codon ATG). Most mutations are point mutations; however, there are two exceptions: M2-2 is a 9-bp duplication that causes a sequence change on the 5′ side of the Shine–Dalgarno sequence (also called ribosome recognition sequence), and L18 is an insertion synthesized *in vitro* to be described later. We believe that this clustered set of seven different mutations partially define, at least genetically, the *thrS* operator.

At this level it is essential to point out that the operator mutants were not genetically mapped before they were sequenced. This raises the question of the correspondence between the sequenced mutations and the phenotypes. Our belief that the sequenced defects correspond to the O^c-type properties of our spontaneous mutants is based on the following facts. (*i*) Most of the mutants were sequenced from the *thrS-lac* gene boundary of the fusions up to the −50 region of the *thrS* promoter and showed no additional base changes. (*ii*) We isolated by *in vitro* mutagenesis a construct in which there is a single mutation in the operator and showed that the regulation was lost in a way analogous to the other (*in vivo*) mutations. The phage λMBXΔL18 carries a mutation synthesized by ligating a *Hin*dIII linker in a filled-in *Bst*EII site located inside the operator locus. In contrast to λMBX1 (the

Table 3. Effect of an excess of $tRNA^{Thr}$ ligase on β-galactosidase synthesis from λMBXΔ20-28 and its derivatives

Fusion	β-Galactosidase, units per OD_{650} of bacteria		Repression (pUC8/pUB4)
	pUC8 (vector)	pUB4 (*thrS*)	
λMBXΔ20-28	281 (±29)	20.6 (±1.7)	13.6
λMBXΔ20-28L3-5	1047 (±108)	781 (±25)	1.3
λMBXΔ20-28M6-1 (G_{-40}→A)	1098 (±40)	984 (±133)	1.1
λMBXΔ20-28L5-4	1715 (±167)	1475 (±96)	1.1
λMBXΔ20-28L6-8 (G_{-32}→A)	2153 (±103)	2014 (±121)	1.07
λMBXΔ20-28M1-2 (T_{-31}→A)	2139 (±28)	1900 (±62)	1.12
λMBXΔ20-28M4-11 (C_{-16}→T)	2067 (±281)	1195 (±50)	1.7
λMBXΔ20-28M2-2 (duplication)	1610 (±161)	1606 (±75)	1.0

The β-galactosidase levels are expressed as "Miller" units per OD_{650} of bacteria (10), and values in parentheses are SDs. Complementary explanations are as in the legend of Table 2.

Table 4. Effect of the *thrS* alleles on β-galactosidase synthesis from λMBXΔ20-28 and its derivatives

Fusion	β-Galactosidase, units per OD_{650} of bacteria		Derepression (*thrS1029*/wt)
	Wild type	*thrS1029*	
λMBXΔ20-28	238 (±21)	926 (±35)	3.9
λMBXΔ20-28L3-5	1381 (±184)	1041 (±72)	0.75
λMBXΔ20-28M6-1 ($G_{-40}\rightarrow A$)	1283 (±155)	1341 (±123)	1.04
λMBXΔ20-28L5-4	2469 (±337)	1786 (±137)	0.72
λMBXΔ20-28L6-8 ($G_{-32}\rightarrow A$)	2152 (±31)	2042 (±113)	0.94
λMBXΔ20-28M1-2 ($T_{-31}\rightarrow A$)	2441 (±354)	2246 (±240)	0.92
λMBXΔ20-28M4-11 ($C_{-16}\rightarrow T$)	1793 (±79)	1841 (±160)	1.02
λMBXΔ20-28M2-2 (duplication)	2092 (±235)	2072 (±163)	0.99

The β-galactosidase levels are expressed as "Miller" units per OD_{650} of bacteria (10), and values in parentheses are SDs. Complementary explanations are as in the legend of Table 1. wt, Wild type.

parental phage), the expression of β-galactosidase from λMBXΔL18 is not derepressed in a *thrS* strain and not repressed in presence of an excess of $tRNA^{Thr}$ ligase synthesized from pUB4 (Tables 1 and 2). These results and the fact that the level of lacZ mRNA synthesized from λMBXΔL-18 is the same as that from λMBX1 (Table 5), although the β-galactosidase level is more than doubled, shows that the single hit *in vitro* mutant behaves similarly to the *in vivo* mutants.

DISCUSSION

The first set of experiments described in this paper prove that the cloverleaf- or tRNA-type structure at the beginning of the *thrS* gene is not in fact involved in the autoregulation of *thrS* expression, contrary to earlier suggestions (9). It is, however, still possible that this structure is involved in the derepression of *thrS* expression induced by threonine starvation (21). Although the location on the mRNA proved not to be what was suggested earlier, such a tRNA-type operator might be involved in the regulation of *thrS* expression (see below).

Our second set of experiments describe the isolation and characterization of *thrS* regulatory mutants selected using *thrS-lac* gene fusions. The mutants were selected for increased expression, under conditions where β-galactosidase synthesis from the *thrS-lac* gene fusions was repressed by an excess of $tRNA^{Thr}$ ligase in the cell. All the isolated mutants are characterized by: (*i*) an increased level of translation without any increase in transcription and (*ii*) a total loss of control—i.e., a constitutive expression of the *thrS-lac* hybrid gene. The mutations causing these effects are all grouped within 30 bp just in front of the Shine–Dalgarno sequence of *thrS* (Fig. 2). Thus, these mutations partially define the operator of *thrS*.

Although the eight independently selected constitutive point mutations are found at five loci within 30 bp in front of the Shine–Dalgarno sequence, it is possible that the operator extends further upstream on the mRNA. The operator does not, however, extend more than 59 nucleotides downstream of the translation initiation site, since we find that these regions can be deleted without any effect on the control.

Constitutive mutations in *thrS* are located next to the Shine–Dalgarno sequence that places *thrS* in the same category as the majority of the translationally controlled genes for which the operator is located next to or overlaps with the translation initiation site of the controlled gene. The present constitutive mutations are unique in this category in that they were directly selected by *in vivo* methods. Constitutive mutations of a translational operator have been isolated only in two cases in which the operator is found next to the translation initiation site: in the case of the T4 *rIIB* gene, which is negatively controlled at the translational level by the *regA* gene product, and in the case of the *L11-L1* operon. In the former case, previously isolated mutants in the translation initiation site of *rIIB* were found to escape to *regA* gene product control (3). In the latter case, constitutive mutations were isolated *in vitro* at four different loci in a region of the mRNA where the secondary structure rather than the primary structure seems to be essential for regulation (4).

Five out of the nine *thrS* constitutive mutations selected *in vivo*, change either guanosine −32 or thymidine −31 and, strikingly, the same two nucleotides, guanosine and thymidine, form the constant part of the anticodons of all three $tRNA^{Thr}$ sequences (Fig. 2*A*). The suggested analogy between the region of the thrS mRNA where guanosine −32 and thymidine −31 are located, and the anticodon loops of the $tRNA^{Thr}$ is further supported by the fact that the Ninio–Gouy computer program, optimized to recognize tRNA-type secondary structures (22), does in fact place guanosine −32 and thymidine −31 in a loop. The structure of the thrS mRNA shown in Fig. 2*A* was derived using the Zucker computer program (ref. 15; see the legend of Fig. 2 for details). The interesting point is that the stem and loop structure in front of *thrS* located between thymidine −49 and adenosine −13 in

Table 5. lacZ mRNA levels synthesized from λMBX1 and λMBXΔ20-28 and their derivatives

Origin of [^{3}H]RNA	Input RNA, cpm	lac mRNA bound, cpm	% mRNA bound
λMBXΔ20-28	1.03×10^6	128 (±15)	1.24 (±0.14)
λMBXΔ20-28L6-8 ($G_{-32}\rightarrow A$)	0.97×10^6	140 (±13)	1.43 (±0.13)
λMBXΔ20-28M6-1 ($G_{-40}\rightarrow A$)	1.03×10^6	136 (±16)	1.32 (±0.15)
λMBX1 (wt)	1.3×10^6	99 (±6)	0.76 (±0.05)
λMBXΔL18 (*in vitro* insertion)	1.18×10^6	99 (±3)	0.84 (±0.03)

The values indicated in the third column (lac mRNA bound) are given with blanks subtracted. The blanks are the number of cpm of [^{3}H]RNA retained on the filters with M13mp8 DNA instead of M13mp8*lacI4* DNA (7). The blanks are between 13 and 15 cpm. Each value is an average of three determinations, and the SD from the average is indicated in parentheses. Monolysogens of IBPC5311 were grown in Mops/glucose media (20) supplemented with arginine at 50 μg/ml and labeled for 90 sec with [^{3}H]uridine as described (7). RNA extraction and filter hybridizations were as in ref. 7. The cpm on the filters were always checked to respond linearly to the input [^{3}H]RNA.

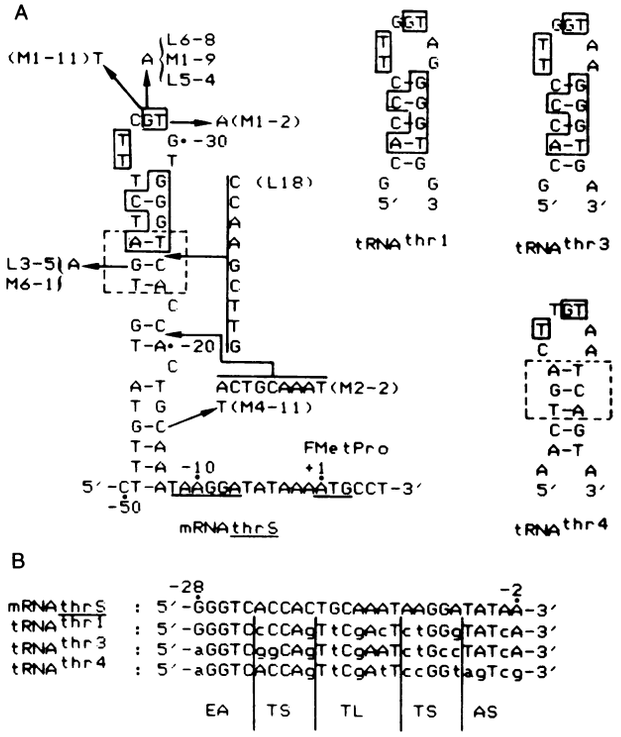

FIG. 2. (*A*) Secondary structure of the thrS mRNA and of the anticodon stem and loop of the three sequenced tRNA^{Thr}s (14). (*B*) Primary structure homologies between the thrS mRNA and the three tRNA^{Thr}s. The secondary structure shown for the mRNA of *thrS* was obtained with the Zucker program (15) asking guanosine −32 and thymidine −31 to be unpaired. The Shine–Dalgarno sequence and the initiation codon of *thrS* are underlined. The operator-constitutive (O^c) mutations are indicated with their respective names. In the case of the *L18* mutation the insert could have the sequence C(CAAGCTTG)$_n$, where *n* is the number of times the *Hin*dIII linker was inserted in this locus. The number *n*, although very probably equal to 1, because of the way the linkers were digested after blunt ligation, was not determined since sequencing in this case was done to the right and to the left from the *Hin*dIII site (see below). In *A* the homologies between the thrS mRNA and the tRNA found by looking first at secondary and then at primary structure, are shown boxed either continuously or with dashed lines. In *B* the tRNA nucleotides homologous to mRNA nucleotides are indicated with upper case letters, nonhomologous nucleotides are indicated with lower case letters. EA, extra arm; TS, Tψ stem; TL, Tψ loop; and AS, acceptor stem. The homologies in *B* were found using several computer programs. The first is a simple program where a window of a chosen size is taken on the second sequence and moved under the first sequence; if the homology for a given window is higher than a chosen value, the homology is edited. With such a program, the best homologies, when more than 3 kb of DNA downstream of the transcription initiation site of *thrS* is examined, are the homologies shown. The same homologies are found using a program based on the local homology algorithm of Smith and Waterman (16) and also using the Kanehisa program based on the Lipman–Wilbur algorithm (17). All the sequencing was done using the chain-termination method (18). The *L18* insertion (a *Hin*dIII linker in the *Bst*EII site in front of *thrS*) was sequenced to the right and left from the *Hin*dIII linker using the same strategy as for Δ20-28 (described in the legend of Fig. 1). The *M1-9* and *M1-11* mutations were sequenced using M13mp18 (19) recombinants carrying the 508-bp *Hpa* I fragments (containing the start of *thrS*) from λMBXM1-9 and λMBXM1-11 inserted into the *Hin*dII site of the polylinker. All the other mutations were sequenced using M13mp8 recombinants carrying *Pst* I–*Hin*dIII fragments (where *Pst* I is in front of the *thrS* promoter and *Hin*dIII is at the locus of the Δ20-28 deletion) from different mutant derivatives of λMBXΔ20-28.

Fig. 2*A* has striking homologies with the anticodon stem and loop structure of tRNA^{Thr1} and tRNA^{Thr3} (boxed in Fig. 2*A*). The stem of the same secondary structure in front of *thrS* also shares some homology with the anticodon stem of tRNA^{Thr4} (in dashed-line boxes in Fig. 2*A*).

Independent of the secondary structure, several computer programs indicate that some homology exists between the −28 to −2 region of the thrS mRNA and the three tRNA^{Thr} sequences (Fig. 2*B*). The homologies with respect to the tRNAs concern the regions extending from the extra arm to the beginning of the amino acid acceptor stem (Fig. 2*B*). The homologies between the genetically defined operator locus of *thrS* and the tRNA^{Thr} sequences suggests that tRNA^{Thr} ligase regulates its translation by binding to the operator locus on the thrS mRNA just in front of the translation initiation site. The operator, which could fold as the stem and loop structure shown in Fig. 2, and the existence of the mutation M2-2, a 9-bp duplication, which increases the distance between this stem and loop structure and the Shine–Dalgarno sequence (Fig. 2*A*), suggests that the distance between these two elements is essential for regulation.

An interaction between the operator and tRNA^{Thr} ligase is, furthermore, suggested by the isolation of *thrS* structural mutants that repress the operator constitutive mutants described in this paper (M.S., unpublished experiments). Among these mutants, some repress *thrS* expression from its wild-type operator with a 100-fold increase in efficiency. These super-repressors most probably bind the wild-type operator with an increased affinity, and they can thus be used to simplify RNA protection experiments, which may provide a useful test of the model suggested by our genetic results.

We are grateful to L. Gold, G. Stormo, T. Schneider, and P. Dessen for fruitful discussions; to J. F. Mayaux, G. Fayat, and S. Blanquet for unpublished data; and to E. Brody for reading the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique, from the Institut National de la Recherche Médicale, from the Fondation pour la Recherche Médicale, and from E. I. du Pont de Nemours and Company.

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