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The *Escherichia coli fmt* Gene, Encoding Methionyl-tRNA$_f^{Met}$ Formyltransferase, Escapes Metabolic Control

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The genetic organization near the recently cloned *fmt* gene, encoding *Escherichia coli* methionyl-tRNA$_f^{Met}$ formyltransferase (J. M. Guillon, Y. Mechulam, J. M. Schmitter, S. Blanquet, and G. Fayat, J. Bacteriol. 174:4294–4301, 1992), has been studied. The *fmt* gene, which starts at a GUG codon, is cotranscribed with another gene, *fms*, and the transcription start site of this operon has been precisely mapped. Moreover, the nucleotide sequence of a 1,379-bp fragment upstream from *fmt* reveals two additional open reading frames, in the opposite polarity. In the range of 0.3 to 2 doublings per h, the intracellular methionyl-tRNA$_f^{Met}$ formyltransferase concentration remains constant, providing, to our knowledge, the first example of a gene component of the protein synthesis apparatus escaping metabolic control. When the gene fusion technique was used for probing, no effect on *fmt* expression of the concentrations of methionyl-tRNA$_f^{Met}$ formyltransferase or tRNA$_f^{Met}$ could be found. The possibility that the *fmt* gene, the product of which is present in excess to ensure full N acylation of methionyl-tRNA$_f^{Met}$, could be expressed in a constitutive manner is discussed.

N formylation of initiator methionyl-tRNA^{Met} (Met-tRNA$_f^{Met}$) has been shown to strongly stimulate the rate of in vitro protein synthesis from an RNA template (17). The formylation reaction is catalyzed by 10-formyltetrahydrofolate:L-methionyl-tRNA$_f^{Met}$ *N*-formyltransferase (formylase [FMT]). The specificity of the formylation reaction for Met-tRNA$_f^{Met}$ is governed by the sequence of the acceptor stem of tRNA$_f^{Met}$ (11, 18).

The *fmt* gene, encoding FMT in *Escherichia coli*, has been characterized and disrupted (10). *fmt* mutant strains fail to grow at 42°C and exhibit a strong reduction in the cellular growth rate at 37°C (10). Actually, the growth parameters of *E. coli* are particularly sensitive to the intracellular level of FMT, as determined by the construction and study of a set of strains expressing various reduced levels of FMT. Consequently, FMT appears to play an essential role, most likely at the level of translation initiation (10).

In our previous work, *fmt* was proposed to be part of an operon comprising another open reading frame (ORF), *fms*, located upstream from *fmt* (10). Moreover, the translation of *fmt* starts with the unusual codon GUG. Such a non-AUG initiation codon might influence the efficiency of *fmt* translation (3, 16, 27) and serve, in addition, as a regulatory signal (4, 8).

In this study, we further characterize the operon containing the *fmt* gene and, in particular, its control region. The levels of FMT as a function of the cellular growth rate are measured. Moreover, the steady-state levels of expression of a reporter gene, *lacZ*, placed under the control of the transcriptional and translational signals of *fmt*, are measured in cells containing altered concentrations of FMT or tRNA$_f^{Met}$. Finally, the influence of the GUG start codon on the efficiency of *fmt* expression is assayed.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* K-12 strains, plasmids, and phages used in this study are listed in Table 1. General genetic methods were as described by Miller (24). Bacteria were grown either in MOPS minimal medium (25) supplemented with various carbon sources or in Luria-Bertani (LB) medium. When needed, culture media were supplemented with Casamino Acids (final concentration, 0.2% [wt/vol]) or methionine (0.2 mM). Antibiotics were used at concentrations of 50 μg/ml (ampicillin) or 25 μg/ml (chloramphenicol).

Recombinant DNA techniques. General recombinant DNA techniques were as described previously (22, 29, 32). The nucleotide sequence was determined by the dideoxy chain termination method (30) with an ALF DNA sequencer (Pharmacia). For primer extension analysis, the 21-mer oligonucleotide 5′-CACTTCTTCTACCGGTTTAGC-3′, corresponding to the sequence starting 117 bases downstream from the *Xba*I restriction site within the *fms* ORF (10), was synthesized. Two picomoles of this oligonucleotide was 5′ labeled with 10 μCi of [γ-^{32}P]ATP in 10 μl (3,000 Ci/mmol; NEN) and used as a template in primer extension analysis with 150 μg of total RNA extracted from strain JM101Tr(pBS936). The general procedure for this analysis was as described previously (19). The resulting ^{32}P-labeled extension products were analyzed on a 6% polyacrylamide sequencing gel in parallel with DNA sequencing reaction performed with the same oligonucleotide and double-stranded pBS936 DNA as a template.

Enzyme specific activity measurements. tRNA formylation reactions were performed at 25°C with 100-μl assay mixtures as described previously (1, 10). *E. coli* tRNA$_f^{Met}$ (1,500 pmol/A_{260} unit), prepared from an overproducing strain (21), was used as a substrate. Valylation reactions were also performed at 25°C with 100-μl assay mixtures as described previously (10). Unfractionated tRNA from *E. coli* (Boehringer Mannheim) was used as a substrate.

One unit of FMT activity was defined as the amount of enzyme capable of formylating 1 pmol of Met-tRNA$_f^{Met}$ per s in 1 ml of the standard assay mixture. One unit of

TABLE 1. *E. coli* strains, plasmids, and phages used in the present study

Strain, plasmid, or phage	Genotype or marker(s)	Reference or source
K37	*galK rpsL*	23
JM101Tr	*supE thi* Δ(*lac-pro*) *recA56 srl-300*::Tn*10* F′ (*traD36 proA*$^+$*B*$^+$ *lacI*^{q} *lacZ*ΔM15)	12
PAL13TrλFatg	Derivative of JM101Tr; *fmtΔ1*::*kan*; λFatg monolysogen	10
JMλ14	Derivative of JM101Tr; λ14 monolysogen	This work
JMλ14AUG	Derivative of JM101Tr; λ14AUG monolysogen	This work
JMλ15	Derivative of JM101Tr; λ15 monolysogen	This work
PAL14Tr	Derivative of Pal13TrλFatg; λ14 monolysogen	This work
PAL15Tr	Derivative of Pal13TrλFatg; λ15 monolysogen	This work
pBluescript SK$^+$	*bla*	Stratagene
pBSTNAV	*bla*	20
pBStRNA$_f^{Met}$	*bla*; overexpresses tRNA$_f^{Met}$	21
pBStRNA$_m^{Met}$	*bla*; overexpresses tRNA$_m^{Met}$	20
pACYC184	Cm^r	5
pACform	pACYC184 derivative; *fmt-1* (ATG); overexpresses FMT	9
pEBNB	Derivative of pBluescript SK$^+$ having the *Bgl*II and *Nco*I sites between the *Eco*RI and *Bam*HI sites of the polylinker	This work
pBS936	*bla fmt* (contains an 8-kb chromosomal insertion of the *fmt* region)	10
pEform	*bla fmt*′; derivative of pEBNB having the *Bam*HI-*Nco*I fragment of pBS936 inserted between the *Bgl*II and *Nco*I sites of pEBNB	This work
pRS414	*bla* ′*lacZYA*	33
pRS415	*bla lacZYA*	33
pBN14	*bla fmt*::*lacZ* (in frame); derivative of pRS414 having the *Eco*RI-*Bam*HI fragment of pEform inserted between the *Eco*RI and *Bam*HI sites	This work
pBN14AUG	Same as pBN14 but with an AUG codon as the translation start site of *fmt*::*lacZ*	This work
pBN15	*bla fmt*::*lacZ*; derivative of pRS415 having the *Eco*RI-*Bam*HI fragment of pEform inserted between the *Eco*RI and *Bam*HI sites	This work
pE$_1$	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-E$_1$ deletion	This work (see Fig. 2)
pE$_2$	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-E$_2$ deletion	This work (see Fig. 2)
pE$_3$	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-E$_3$ deletion	This work (see Fig. 2)
pE$_4$	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-E$_4$ deletion	This work (see Fig. 2)
pΔX	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-*Xba*I deletion	This work (see Fig. 2)
pΔS	*bla fmt*::*lacZ*; derivative of pBN15 with an *Eco*RI-*Sma*I deletion	This work (see Fig. 2)
λRS45	*i21 lacZYA*	33
λFatg	*fmt-1* (ATG) under the control of the *lac* operon promoter	10
λ15	Derivative of λRS45 obtained by homologous recombination with pBN15	This work
λ14	Derivative of λRS45 obtained by homologous recombination with pBN14	This work
λ14AUG	Derivative of λRS45 obtained by homologous recombination with pBN14AUG	This work

valyl-tRNA synthetase activity was defined as the amount of enzyme capable of aminoacylating 1 pmol of tRNA^{Val} per s in 1 ml of the standard assay mixture.

For measurement of intracellular enzymatic activities in the cultures, two 3-ml cell samples were withdrawn twice during exponential growth, at optical densities at 650 nm of ~0.2 and ~0.5. β-Galactosidase activities in toluene-permeabilized cells were determined as described by Miller (24). For FMT and valyl-tRNA synthetase activity measurements, cells were centrifuged, resuspended in 1 ml of 20 mM Tris-HCl (pH 7.5)–0.1 mM EDTA–0.15 M KCl–10 mM 2-mercaptoethanol, and disrupted by sonication. The values reported represent the average for the two samples.

Protein concentrations in the crude extracts were measured by the technique of Bradford (2) by use of a Bio-Rad protein assay kit with bovine serum albumin as a standard.

tRNA$_f^{Met}$ and *N*-acylmethionyl-tRNA concentrations in vivo. For determination of the concentrations of tRNA$_f^{Met}$ and *N*-acylmethionyl-tRNA in vivo, 250 ml of exponentially growing cells in LB medium was collected by centrifugation and resuspended in 1 ml of 0.3 M sodium acetate (pH 4.8) containing 10 mM EDTA. Thereafter, the procedure was as described previously (10).

Accession number. The DNA sequence described in this paper has been assigned GenBank-EMBL accession number X65946.

RESULTS AND DISCUSSION

Nucleotide sequence of the chromosomal region upstream from *fms*. The *fmt* gene could be the 5′-distal cistron of an operon since (i) its putative ribosome binding site (RBS) overlaps the translation stop codon of another ORF, of 167 amino acids, *fms*, which also is preceded by an RBS; (ii) no putative transcription initiation site on the DNA sequence can be recognized immediately upstream from *fmt*; and (iii) *fmt* is followed by a sequence that has dyad symmetry and a stretch of T residues and resembles a rho-independent transcription terminator (28).

To search for the promoter(s) responsible for the transcription of both *fms* and *fmt*, we determined the DNA sequence of the 1,379-bp *Bam*HI-*Xba*I DNA fragment located upstream from *fms* (10). Analysis of the DNA sequence (Fig. 1) revealed two additional ORFs, in the opposite polarity compared with *fms* and *fmt*. The first ORF, called *smf*, starts 130 bp upstream from the *fms* start codon, on the opposite DNA strand. It encodes 311 amino acids and is preceded by a putative RBS. The second ORF, called

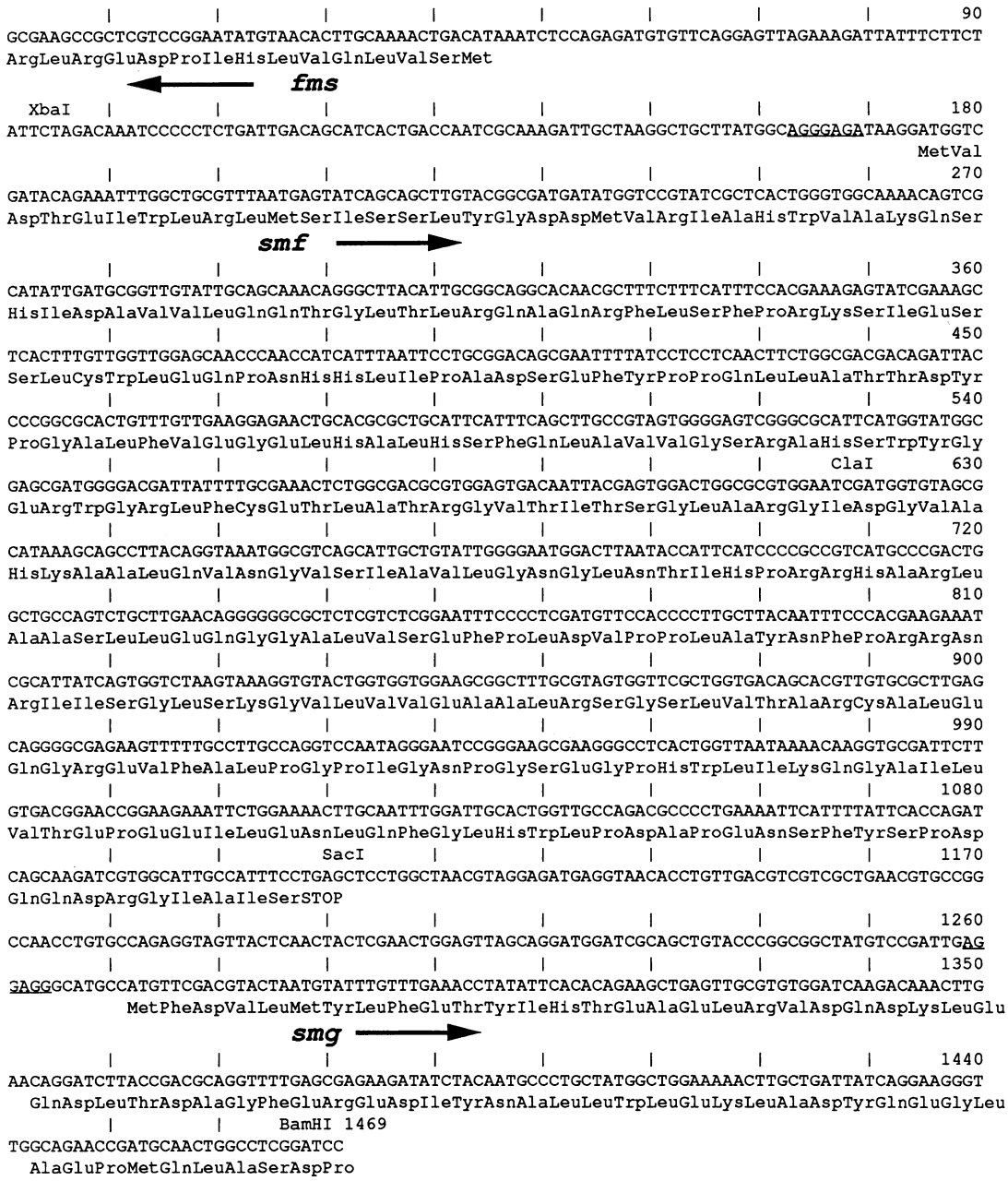


FIG. 1. Nucleotide sequence of the 1,379-bp DNA fragment located upstream from *fms*. The nucleotide sequences of both strands of the *Xba*I-*Bam*HI DNA fragment located upstream from *fms* (see Fig. 2) were determined by the dideoxy chain termination method (30). The DNA sequence of one strand and the translated amino acid sequences deduced from *smf* and *smg* are shown. Putative RBSs are underlined. Restriction sites used for cloning into M13mp18 or M13mp19 also are indicated.

smg, starts 164 bp downstream from the stop codon of *smf* and also is preceded by an RBS. It does not end within the cloned fragment. A comparison of the amino acid sequence deduced from *smf* or of the incomplete one deduced from *smg* with those of other proteins in the NBRF data bank (release 31) did not reveal any significant resemblance.

Transcription of the *fms-fmt* operon. The occurrence of the *smf* and *smg* cistrons, in the opposite polarity compared with *fms* and *fmt*, suggested that the transcription start site of the putative *fms-fmt* dicistronic operon could be located in the intergenic region between *fms* and *smf*. For testing of this idea, the *Bam*HI-*Nco*I fragment of plasmid pBS936, which contains the four ORFs, was cloned between the *Eco*RI and *Bam*HI sites of plasmid pRS415 to yield pBN15 (Table 1), in which the transcription of the *lacZ* gene is under the control of any promoter possibly occurring on the cloned fragment.

Six derivatives of plasmid pBN15 with deletions of various lengths originating from the *Eco*RI site were constructed thereafter. Four *Eco*RI-*Eco*RI deletions were obtained between the *Eco*RI site of pBN15 (corresponding to the *Bam*HI site of *smg*; Fig. 2) and four *Eco*RI sites (E_1, E_2, E_3, and E_4) created in the inserted DNA fragment. The corresponding

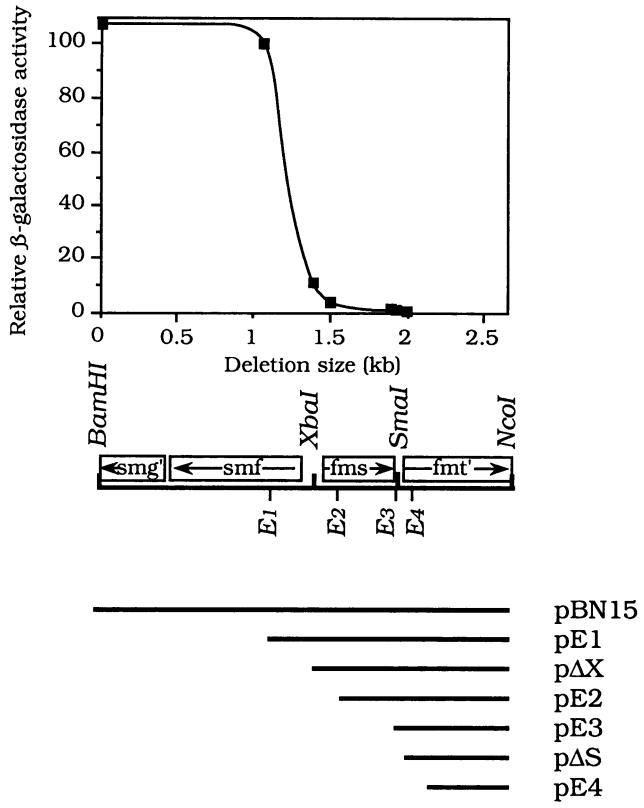


FIG. 2. *fmt* is cotranscribed with *fms*. Various deletions in plasmid pBN15, carrying the *fmt*::*lacZ* operon fusion (Table 1), were constructed (bottom). Four oligonucleotides, O_1 (5'-CGCAG GAATTCAATGATGGTT), O_2 (5'-GTGAATGCAGGAATTCAGC GT), O_3 (5'-AACAACAACGAATTCGTCAGA), and O_4 (5'-GTAC ACCTGAATTCGCAGCGCGTC), complementary to the indicated positions (E_1, E_2, E_3, and E_4, respectively) in the *fms-fmt* region (middle), were synthesized, as was another oligonucleotide, O_5 (5'-TTCTTGTGGACGCAGGGAAACA), corresponding to a sequence located downstream from the *Nco*I restriction site within the *fmt* gene. O_5 and either O_1, O_2, O_3, or O_4 were used to amplify by the PCR the corresponding regions on plasmid pBS936. The polymerase chain reaction products were restricted with the following combinations of restriction enzymes: *Eco*RI and *Xba*I (O_1-O_5 amplification), *Eco*RI and *Sma*I (O_2-O_5 and O_3-O_5 amplifications), and *Eco*RI and *Nco*I (O_4-O_5 amplification). Each resulting DNA fragment was inserted between the corresponding sites in plasmid pBN15, yielding pE_1, pE_2, pE_3, and pE_4. The bottom part of the figure represents the portion of the *Bam*HI-*Nco*I region remaining in the indicated plasmids. Plasmids pΔS and pΔX were obtained after restriction of pBN15 with *Eco*RI-*Sma*I (pΔS) or *Eco*RI-*Xba*I (pΔX), filling in with the Klenow fragment of DNA polymerase I, and recircularization. Note that the *Eco*RI site on plasmid pBN15 is located immediately upstream from *Bam*HI. The β-galactosidase activities in JM101Tr cells harboring each of the studied plasmids were determined as described by Miller (24). Bacteria were grown in MOPS minimal medium supplemented with Casamino Acids and ampicillin. Samples of the culture were withdrawn during exponential growth. β-Galactosidase activity measurements were systematically obtained from two samples and corrected for the average activity found in JM101Tr(pE_4) (328 ± 40 U). The β-galactosidase activities shown at the top of the figure are expressed as percentages of the activity measured for JM101Tr(pE_1) (9,304 ± 200 U). Data are plotted as a function of the sizes of the various deletions created in the studied plasmids.

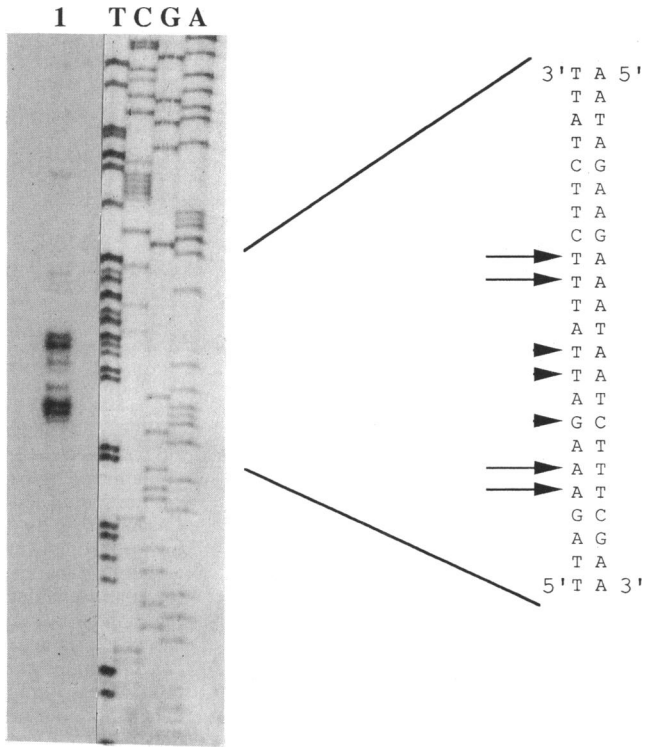
deletion plasmids were called pE_1, pE_2, pE_3, and pE_4 (Fig. 2). The two other deletions were constructed by taking advantage of the single *Sma*I and *Xba*I sites on pBN15 to yield plasmids pΔS and pΔX, respectively (Fig. 2).

The β-galactosidase activities expressed in *lacZ* strain JM101Tr containing each of the above-described plasmids were then measured. As shown in Fig. 2, the β-galactosidase activity measured with pBN15 dropped by a factor of 7 upon deletion of the *Eco*RI-*Xba*I fragment (plasmid pΔX), whereas it remained unmodified with the *Eco*RI-E_1 deletion (plasmid pE_1). Moreover, the 20-fold reduction in β-galactosidase activity in JM101Tr(pE_2), compared with that in JM101Tr(pBN15), was nearly identical to that obtained with any of the three plasmids, pE_3, pΔS, and pE_4, that carry larger deletions than pE_2. These results demonstrated that the inserted *Bam*HI-*Nco*I DNA fragment contained one or several promoters located between E_1 and *Xba*I, upstream from *fms* (Fig. 2), and responsible for the transcription of both *fms* and *fmt*.

For precise mapping of the transcription start site of the *fms-fmt* operon, primer extension analysis of total RNA from strain JM101Tr(pBS936) was undertaken by use of reverse transcriptase (Fig. 3). Four bases, clustered within a 10-base region, were identified as transcription start sites of the *fms-fmt* operon. In agreement with the results of this mapping experiment, sequences matching the consensus sequence for the *E. coli* transcription promoters were found upstream from the above-described transcription initiation region. The corresponding −10 and −35 sequences are boxed in Fig. 3. It is interesting to note that the −35 box is located upstream from the *Xba*I site, whereas the −10 one overlaps this restriction site. This localization is in agreement with the observation that, in the above-described experiment, *lacZ* transcription was more strongly stimulated when plasmid pΔX rather than plasmid pE_2 was used.

Finally, the promoter of the *smf* gene was mapped in a similar way (data not shown). Transcription of this gene starts at a single A located downstream from the transcription start sites of the *fms-fmt* operon (Fig. 3). Consequently, the mRNAs for *smf* and *fms-fmt* overlap.

Influence of the GUG translation initiation start site on the expression of *fmt*. The effect on the translation of *fmt* of the occurrence of the unusual GUG translation initiation start site was evaluated by use of the gene fusion technique. Advantage was taken of the *Nco*I restriction site to fuse in frame the *fmt* gene to the *lacZ* coding sequence in plasmid pRS414 to yield pBN14 (Table 1). The region upstream from the *fmt*::*lacZ* in-frame fusion in plasmid pBN14 extends up to the *Bam*HI site within the *smg* ORF. The expression of β-galactosidase from plasmid pBN14 depends therefore on both the transcription and the translation signals of *fmt*. The *fmt*::*lacZ* GUG translation initiation codon in pBN14 was changed to an AUG one by site-directed mutagenesis (31), yielding pBN14AUG. The wild-type and mutated *fmt*::*lacZ* fusions were then transferred into phage λRS45 by homologous recombination in vivo to yield λ14 and λ14AUG, respectively. Each λ phage was used to lysogenize *lacZ* strain JM101Tr. The β-galactosidase activities produced from the corresponding monolysogens (strains JMλ14 and JMλ14AUG) were measured. From the results shown in Table 2, it appeared that changing the GUG translation initiation codon into an AUG one resulted in a 2.2-fold increase in the expression of the *fmt*::*lacZ* fusion. Such a moderate effect is similar to those previously measured for either the *lacZ* gene or the *cya* gene from *E. coli*, in which



TTTCTGTATCGACCATCCTTATCTCCCTGCCATAAGCAGCCTTAGCAATCTTTGCGATTGGTCAGT

GluThrAspValMet

XbaI

GATGCTGTCAATCAGAGGGGGATTTGTCTAGAATAGAAGAAATAATCTTTCTAACTCCTGAACACA

- 35 - 10

TCTCTGGAGATTTATGTCAGTTTTGCAAGTGTTACATATTCCGGACGAGCGGCTTCGCAAAGTTGCT

MetSerValLeuGlnValLeuHisIleProAspGluArgLeuArgLysValAla

FIG. 3. Fine mapping of the *fms-fmt* promoter. The 21-mer oligonucleotide 5'-CACTTCTTCTACCGGTTTAGC-3' was 5' labeled with [γ-^{32}P]ATP and used in primer extension analysis with total RNA extracted from strain JM101Tr(pBS936) or from strain JM101Tr as a control. The labeled extension product (lane 1) was analyzed on a 6% sequencing gel in parallel with a DNA sequencing reaction performed with the same oligonucleotide and double-stranded pBS936 plasmid DNA as a template (lanes T, C, G, and A). The autoradiogram is shown in the upper left part of the figure. The corresponding DNA sequence is shown in the upper right part, together with arrows indicating the mapped transcription start sites. Mapping of the *smf* promoter was performed in an analogous manner with O_1 as an extension primer (data not shown). The lower part of the figure indicates the locations of the various transcription start sites (vertical arrows) in the *smf-fms* intergenic region and the directions of transcription (horizontal arrows). The indicated ORFs are those of *smf* (top) and *fms* (bottom). The RBS of *fms* is underlined. Putative −10 and −35 consensus sequences for the transcription of *fms-fmt* are boxed. Bases identical to the consensus sequence proposed by Rosenberg and Court (28) are boldfaced.

initiation codons were changed into GUG or AUG and GUG, respectively (16, 27).

Control of the expression of the *fmt* gene. Because of the participation of FMT in protein synthesis, its cellular concentration may be governed by metabolic regulation, i.e., a general regulatory mechanism in *E. coli*, yet not fully understood at the molecular level, which couples the concentrations of components of the biosynthetic machinery with cell growth rate. Such a coupling has been firmly established for ribosomes and tRNAs (reviewed in reference 14) and also has been demonstrated to occur for several aminoacyl-tRNA synthetases as well as initiation factors IF_1, IF_2, and IF_3 (reviewed in reference 7; see also references 6 and 26).

Variations in growth rate were obtained by use of various culture media containing different carbon sources. Cells were harvested at the exponential growth stage, and crude extracts were prepared for enzymatic activity measurements. Surprisingly, the FMT specific activity remained

TABLE 2. Influence of the *fmt* initiation codon (GUG or AUG) and of the cellular concentration of FMT or of $tRNA_f^{Met}$ on the expression of various *fmt*::*lacZ* fusions in vivo

Bacterial strain[a]	Plasmid[b]	β-Galactosidase activity	Overexpressed macromolecule
JMλ14		27 ± 3	
JMλ14AUG		60 ± 3	
JMλ14	pACYC184	25 ± 4	
JMλ14	pACform	28 ± 4	FMT
JMλ14	pBSTNAV	26 ± 3	
JMλ14	$pBStRNA_f^{Met}$	24 ± 3	$tRNA_f^{Met}$
JMλ14	$pBStRNA_m^{Met}$	22 ± 3	$tRNA_m^{Met}$
PAL14Tr		25 ± 3	
PAL14Tr	pACYC184	28 ± 3	
PAL14Tr	pACform	23 ± 4	FMT
PAL14Tr	$pBStRNA_f^{Met}$	18 ± 4	$tRNA_f^{Met}$
PAL14Tr	$pBStRNA_m^{Met}$	23 ± 3	$tRNA_m^{Met}$
JMλ15		226 ± 10	
PAL15Tr		230 ± 15	
PAL15Tr	pACYC184	237 ± 25	
PAL15Tr	pACform	200 ± 20	FMT
PAL15Tr	$pBStRNA_f^{Met}$	217 ± 25	$tRNA_f^{Met}$
PAL15Tr	$pBStRNA_m^{Met}$	241 ± 20	$tRNA_m^{Met}$

[a] Monolysogenic derivatives of the λ14 or λ14AUG (*fmt*::*lacZ* in-frame fusions) or λ15 (*fmt*::*lacZ* operon fusions) prophages integrated in strain JM101Tr were isolated, yielding strains JMλ14, JMλ14AUG, and JMλ15, as were λ14 or λ15 lysogens of PAL13TrλFatg (expressing a low level of FMT activity), yielding strains PAL14Tr and PAL15Tr (Table 1). β-Galactosidase activities from several independent lysogens were shown to vary as multiples of the lowest measured value that was considered to come from a monolysogen.

[b] Derivatives of the various strains containing the indicated plasmids were constructed. Depending on the carried plasmid, cells were grown in the presence of ampicillin ($pBStRNA_f^{Met}$ and $pBStRNA_m^{Met}$) or chloramphenicol (pACYC184 and pACform).

insensitive to the variations in growth rate (Fig. 4). In the same experiment, the valyl-tRNA synthetase specific activity, which is subject to metabolic regulation through transcriptional activation, was also assayed as a control.

The occurrence of a constant level of FMT in the cell regardless of growth parameters may reflect the occurrence of a retroactive control of the expression of *fmt*, involving either FMT itself or formyl–Met-$tRNA_f^{Met}$, the product of the reaction catalyzed by FMT. For the purpose of measuring whether the cellular concentration of FMT or initiator $tRNA_f^{Met}$ could influence the expression of *fmt*::*lacZ* fusions, the monolysogenic strain JMλ14, expressing an *fmt*::*lacZ* in-frame fusion, was transformed with various plasmids: $pBStRNA_f^{Met}$, $pBStRNA_m^{Met}$, pBSTNAV, pACYC184, and pACform (Table 2). Neither 50-fold overexpression of FMT activity nor 5-fold overexpression of $tRNA_f^{Met}$ modified the β-galactosidase activity expressed from the fusion, compared with that from the controls, $pBStRNA_m^{Met}$ and pBSTNAV (Table 2). Similar results were obtained with a JM101Tr derivative carrying a single copy of λ15, which bears an *fmt*::*lacZ* operon fusion, i.e., for which β-galactosidase transcription is under the control of the *fmt* promoter (Table 2).

Next, the effect of a reduced intracellular FMT concentration on the expression of *fmt*::*lacZ* fusions was measured. For this purpose, strain PAL13TrλFatg, in which the intracellular FMT concentration is reduced 100-fold (10), was lysogenized with a single copy of either λ14 (PAL14Tr) or λ15 (PAL15Tr). As shown in Table 3, in PAL13TrλFatg cells, because of the limiting intracellular FMT concentration, the extent of Met-$tRNA_f^{Met}$ N acylation was lowered by

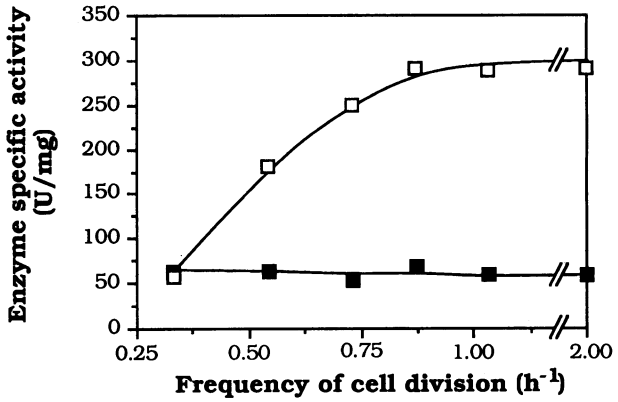


FIG. 4. Cellular level of FMT as a function of *E. coli* growth rate. K37 cells were grown at 37°C in MOPS minimal medium (25) containing 0.4% glucose, 0.4% glycerol, or 0.4% acetate as the carbon source and supplemented with either methionine (0.2 mM) or Casamino Acids (0.2%). LB medium was also used to obtain the highest growth rate. One unit of FMT (or valyl-tRNA synthetase) activity was defined as the amount of enzyme capable of formylating (or aminoacylating) 1 pmol of Met-$tRNA_f^{Met}$ (or $tRNA^{Val}$) per s in the standard assay. Protein concentrations in the crude extracts were determined by the technique of Bradford (2). The FMT concentrations in the crude extracts, given in units per milligram of total protein, are plotted as a function of the number of doubling events per hour measured for exponentially growing cultures. Symbols: ■, FMT specific activity; □, valyl-tRNA synthetase specific activity.

28%. In such strains, with a low FMT level, β-galactosidase activity remained unmodified, compared with that in control strains JMλ14 and JMλ15 (Table 2).

Transformation of either PAL14Tr or PAL15Tr by an FMT-overproducing plasmid, pACform, resulted in a 5,000-fold increase in the FMT content. As shown in Table 2, the β-galactosidase activities in strains PAL14Tr(pACform) and PAL15Tr(pACform) were unmodified, compared with those in PAL14Tr and PAL15Tr, respectively.

The above-described experiments showed that the expression of the *fmt*::*lacZ* protein and operon fusions was insensitive to variations in the intracellular FMT concentration in a 5,000-fold range (Table 2). Taken together, these results strongly indicate that the expression of the *fmt* gene is

TABLE 3. Effect of the intracellular FMT concentration on the extent of N acylation of $tRNA_f^{Met}$

Bacterial strain[a]	Growth rate (min)[b]	Total $tRNA_f^{Met}$ concn (%)[c]	FMT concn (%)[d]	Met-$tRNA_f^{Met}$ N acylation (%)[e]
JM101Tr	38	100 ± 8	100 ± 5	81 ± 5
JM101Tr($pBStRNA_f^{Met}$)	35	496 ± 10	93 ± 5	80 ± 5
PAL13TrλFatg	155	113 ± 8	1.0 ± 0.5	58 ± 3

[a] Cells were grown in LB medium. FMT specific activity, $tRNA_f^{Met}$ concentration, and Met-$tRNA_f^{Met}$ N acylation were determined as described in reference 10.

[b] Doubling times of the cultures.

[c] The concentration of $tRNA^{Val}$ also was measured to standardize the $tRNA_f^{Met}$ content of these tRNA preparations. A value of 100% corresponded to 0.48 pmol of $tRNA_f^{Met}$ per pmol of $tRNA^{Val}$.

[d] A value of 100% corresponded to 51 U/mg.

[e] The extent of Met-$tRNA_f^{Met}$ N acylation is given as a percentage of the corresponding measured total $tRNA_f^{Met}$ concentration.

insensitive to variations in either intracellular FMT or intracellular $tRNA_f^{Met}$ concentrations or to the extent of N acylation of Met-$tRNA_f^{Met}$.

Conclusions. The present study shows first that *fmt* is cotranscribed with *fms*, a gene encoding a putative protein sharing sequence identities with zinc metallopeptidases (10). Substitution of an AUG for the GUG translation start codon of *fmt* only slightly increases the expression of the *fmt* product.

Second, the intracellular expression of FMT is not sensitive to metabolic control. To our knowledge, this fact makes FMT the first example of a component of the cellular machinery of protein biosynthesis that escapes control by cell growth rate.

Finally, using the gene fusion technique, we failed to show control of the expression of *fmt* mediated by either the extent of N acylation of Met-$tRNA_f^{Met}$ or the intracellular FMT or $tRNA_f^{Met}$ concentration. Although one cannot exclude the possibility that an effector not tested in the present study has an effect on FMT expression, the expression of the *fmt* gene appears to be constitutive.

The data in Table 3 show that, with a 100-fold decrease in cellular FMT activity, the extent of N acylation of Met-$tRNA_f^{Met}$ is reduced only by 30%. Moreover, when the $tRNA_f^{Met}$ concentration is increased fivefold in wild-type *E. coli* cells, the proportion of formyl-Met-$tRNA_f^{Met}$ to total $tRNA_f^{Met}$ remains unchanged (Table 3). These data indicate that the number of FMT molecules normally present in an *E. coli* cell is in large excess with respect to the number required to ensure full N formylation of Met-$tRNA_f^{Met}$. Notably, an extract obtained from 3×10^7 bacteria grown in LB medium allowed the N formylation of 0.18 pmol of Met-$tRNA_f^{Met}$ per s in the standard assay. If one assumes a turnover number of 20 s^{-1} in the reaction catalyzed by FMT (15), the number of FMT molecules in one bacterial cell can be estimated to be on the order of 10^2. This number is significantly smaller than those of the other proteins involved in the initiation of translation, including methionyl-tRNA synthetase ($\geq 10^3$ molecules per cell [13]) or valyl-tRNA synthetase (as deduced from the data in Fig. 4, with the assumption of a turnover number of 1 s^{-1}). The synthesis in *E. coli* of the non-rate-limiting number of 10^2 FMT molecules could therefore be at a negligible energetic cost, compared with the energy expended for the synthesis of the other translational factors. This idea may explain why the cell has no advantage in regulating *fmt* expression, while tight control of the expression of other proteins of the translational apparatus appears beneficial (reviewed in reference 7).

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