JOURNAL OF BACTERIOLOGY, Sept. 1988, p. 4147–4152
0021-9193/88/094147-06$02.00/0

Vol. 170, No. 9

Molecular Cloning of the *Escherichia coli miaA* Gene Involved in the Formation of Δ2-Isopentenyl Adenosine in tRNA

JOËL CAILLET* AND LOUIS DROOGMANS†

Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

Received 19 January 1988/Accepted 10 June 1988

Escherichia coli mia **strains were shown to lack Δ2-isopentenylpyrophosphate transferase activity, the first step in the synthesis of the 2-methylthio derivative of 6-(Δ2-isopentenyl) adenosine (ms^2i^6A). A double mutant, *rpsL* (Sm^P) *miaA*, was streptomycin dependent. The wild-type *miaA* gene was cloned by selecting for λ recombinant bacteriophage which eliminated the streptomycin-dependent phenotype and was subsequently recloned into plasmid vectors. The cloned *miaA* gene restored the ms^2i^6A modification to tRNA. The *miaA* gene mapped to 95 min on the *E. coli* map, and we propose the order *mutL-miaA-hflA-purA*.**

The hypermodified nucleoside 6-(Δ2-isopentenyl) adenosine (i^6A) and further modified derivatives are found in position 37 (3′ adjacent to the anticodon) of tRNAs which correspond to codons beginning with a uridine. These modified nucleosides occur in tRNAs from most organisms; they are found in mammals, plants, and microorganisms (28).

In *Escherichia coli*, the 2-methylthio derivative of i^6A (ms^2i^6A) has been characterized in tRNAs specific for phenylalanine, tryptophan, tyrosine, cysteine, leucine, and serine (see reference 28). The biosynthesis of ms^2i^6A occurs at the tRNA level. The first step is the incorporation of the Δ2-isopentenyl group of Δ2-isopentenylpyrophosphate (Δ2-IPP) by the enzyme tRNA (adenosine-37 *N*6) Δ2-isopentenyltransferase (tRNA IPP transferase) (5). The subsequent formation of the 2-methylthio group involves cysteine and *S*-adenosyl-1-methionine and probably occurs in a sequential manner: thiolation of i^6A followed by methylation (1). The *E. coli miaA* mutant (formerly called *trpX*) lacks ms^2i^6A in its tRNAs. The first step in the formation of the hypermodified nucleoside is affected in the mutant since an unmodified adenosine is found in place of ms^2i^6A (10). The mutation causes a derepression of the tryptophan operon because the unmodified $tRNA^{Trp}$ is less able to read the two adjacent UGG codons in the leader sequence. It is presumed that the lack of modification resulting in the destabilization of the codon-anticodon interaction explains this phenomenon (30).

The presence of i^6A or its derivatives in tRNA is not an absolute functional requirement since bacterial mutants whose tRNAs do not contain i^6A grow almost normally. In *Salmonella typhimurium*, the lack of the hypermodified nucleoside 6-(*cis*-4-hydroxy-3-methylbut-3-enyl) adenosine (ms^2io^6A) in tRNA affects the regulation of several operons and has an influence on codon context sensitivity and on translational accuracy. The hydroxylated form of ms^2i^6A has been proposed to be a regulatory element of aerobiosis (see reference 6).

The *MOD5* gene of *Saccharomyces cerevisiae* coding for tRNA IPP transferase has been cloned and sequenced. The same gene codes for both the mitochondrial and cytoplasmic enzymes (9, 22).

An antagonism has been found between *miaA* and certain *rpsL* mutations in *E. coli*; such double mutants require the addition of streptomycin for growth (23). The *rpsL* mutations (formerly called *strA*) can be classified into three groups: they all affect ribosomal protein S12 and result in resistance to streptomycin but they differ as to whether they are just streptomycin resistant (Sm^r) or streptomycin dependent (Sm^d) or streptomycin psuedodependent (Sm^P). In the last case, pseudodependence means that the growth of the mutant is accelerated by the addition of streptomycin. In the presence of an *miaA* allele, this pseudodependence becomes an absolute requirement for streptomycin for growth. This incompatibility is explained by a too high level of translational proofreading in the double mutant, effectively stopping protein synthesis. Growth can be restored by the addition of streptomycin, an antibiotic decreasing the translational proofreading process (8–24).

In this report, we describe the cloning of the *miaA* gene of *E. coli*, coding most likely for the tRNA IPP transferase, by complementation of an *miaA rpsL* (Sm^P) double mutant.

MATERIALS AND METHODS

tRNA isolation and aminoacylation. Crude *E. coli* tRNA was extracted from cells by phenol extraction and DEAE-cellulose column chromatography as described previously (29). Aminoacylation with [^{14}C]phenylalanine was done in a 100-μl reaction mixture containing 2 μM [^{14}C]phenylalanine (525 mCi/mmol), 200 μg of tRNA, 2 μl of an *E. coli* extract (30,000 × *g* supernatant) prepared by the method in reference 29, and buffer (50 mM Trischloride [pH 7.5], 100 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 1 mM dithiothreitol). Incubation was for 10 min at 37°C.

The assay for leucyl-tRNA synthetase was performed in a 1-ml reaction mixture with the buffer described above containing 11.2 μM [^{14}C]leucine (354 mCi/mmol) and 5 μl of enzyme (DEAE-cellulose chromatography fractions, see below). After 5 min of incubation at 37°C, the tRNA was precipitated with trichloroacetic acid, and the mixture was filtered through Whatman GF/C filters. The radioactivity on the filters was measured by scintillation counting.

RPC-5 column chromatography. Crude tRNA charged with [^{14}C]phenylalanine as described above was loaded on top of an RPC-5 column (0.5 by 30 cm). Chromatography was performed as described in reference 10 except that the NaCl, gradient (50 ml) was from 0.4 to 1.5 M NaCl.

Preparation of crude extracts for detection of tRNA IPP transferase activity. Overnight cultures (200 ml of LB medium containing the appropriate antibiotic [21]) were har-

* Corresponding author.
† Present address: Laboratoire de Chimie Biologique, Université Libre de Bruxelles, 1640 Rhode-Saint-Genèse, Belgium.

TABLE 1. *E. coli* strains, phage, and plasmids used

Strains	Genotype and relevant markers	*mutL*	*hflA*	*miaA*	*purA*	*rpsL*	Source or reference
E. coli							
K-12	Prototroph, λ^+ lysogen						R. Maurer
FTP3474	(= DEV15 *mia*::Tn*10*) KL16 *lacZ(UGA) mia*::Tn*10*						E. J. Murgola (14, 23)
RM41	(=Q358) F^- *hsdR*$^-$ *hsdM*$_k^+$ *supE* $\phi 80^r$						R. Maurer (17)
RM42	(=Q359) RM41 P2 lysogen						R. Maurer (17)
Y1R1	*trpR lacZU118 recA*; multistep *recA* derivative of Y1						M. Springer (33)
Y2R1	*trpR lacZU118 recA miaA*; multistep recA derivative of Y2						M. Springer (33)
LOU1	*rpsL* (Sm^p) *miaA* (Sm^p resulting phenotype)						G. C. Kurland (8)
LOU2	LOU1 λ^+ lysogen						This work
ES4	= CGSC 4431 − *tonA2 lacY1* or *lacZ4 tsx-1* or *tsx-70 supE44 gal-6* λ^- *mtl-1 purA45*						CGSC[a] (13, 19, 25)
71/18	Δ(*lac-pro*) *supE thi*/F^- *lacI*^{q} *lacZ*Δ*M15 proA*$^+$*B*$^+$						R. H. Buckingham
71/18 *mutL*	As 71/18 but *mutL*						R. H. Buckingham
JM101	Δ(*lac-pro*) *supE thi*/F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} *lacZ*Δ*M15*						
Lambda phage							
λSE6	Spi^+ Ap^r Kn^r	−	−	−	−	−	R. Maurer (11)
λLOU1–5, 7–14	Spi^- Kn^r	+	+	+	+		This work
λLOU6, 15	Spi^- Kn^r	−	+	+	+		
λLOU16	Spi^- Kn^r					+	
Plasmids							
pBS	Ap^r; Blue Scribe						R. Maurer (31, 34)
pBR322	Ap^r Tc^r						
pJO2	6.6-kb *Sal*I fragment in both orientations in pBR322	−	−	+	−		This work
pJO3							
pJO7	3.5 kb *Sal*I-*Kpn*I fragment in pBS			+			

[a] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

vested by centrifugation, washed with cold 0.9% NaCl, suspended in 2 ml of buffer (50 mM Tris chloride [pH 8], 10 mM $MgCl_2$, 10 mM β-mercaptoethanol), and then ruptured by sonication. The crude extracts were clarified by centrifugation at 10,000 × *g* for 15 min. Protein concentration was determined by the biuret method (15) with bovine serum albumin at the standard; the extracts were then adjusted to 10 mg of protein per ml.

tRNA IPP transferase was measured by the incorporation of Δ3-IPP into unmodified tRNA (from FTP3474).

Identification of modified nucleotides in tRNA. Complete digestion of tRNA with RNase T_2 and phosphorylation of nucleoside 3′-phosphate with T4 polynucleotide kinase and [γ^{32}P]ATP followed by treatment with nuclease P1 were performed as described previously (26). Cellulose thin-layer plate chromatography of the resulting ^{32}P-labeled nucleotides was performed with the following solvent system: the first dimension was developed with isobutyric acid-concentrated NH_4OH-water (66:1:33, vol/vol/vol); the second dimension was developed with 0.1 M sodium phosphate (pH 6.8)-solid $(NH_4)_2SO_4$-isopropanol (100:60:2, vol/wt/vol). The ^{32}P-labeled nucleotides were detected by autoradiography.

Construction of *E. coli* genomic library. Wild-type strain K-12 (see Table 1) chromosomal DNA was partially digested with *Sau*3A as described previously (20), and 12- to 18-kilobase (kb) fragments were extracted from agarose gel (32). They were ligated with *Bam*HI-cleaved λSE6 (11). After in vitro packaging (Promega Biotec packaging extracts used as recommended by the supplier), the titers of the bacteriophage were determined on RM41 and RM42 (see Table 1). Selection for recombinant phage was done by amplification of the library on RM42 (a P2 lysogen strain on which only recombinant λ phage grow). The library was constructed during the Advanced Bacterial Genetics Course in 1986 at Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

General DNA techniques. Preparation of DNA, agarose gel electrophoresis, and standard recombinant DNA techniques were done as described in references 7, 20, and 27. Restriction enzymes were used as recommended by the suppliers.

Bacteriological methods. Bacterial strains, bacteriophage λ, and plasmids used are listed in Table 1. Streptomycin resistance and dependence were tested on LB plates containing 100 μg of streptomycin per ml. Bacteriological media are described in reference 21.

The *purA*$^+$ alleles on λLOU phage were tested by the growth of lysogens of ES4 on minimal A medium in the absence of added purine. *mutL* was tested by the frequency of appearance of spontaneous streptomycin-resistant mutants.

RESULTS AND DISCUSSION

***E. coli miaA* mutant has no tRNA IPP transferase activity.** To detect the activity of tRNA IPP transferase in crude extracts, we prepared tRNA from strains FTP3474 and Y1R1 (Table 1). tRNA from strain FTP3474 which carries a Tn*10* insertion in *mia* totally lacked ms^2i^6A, whereas tRNA from strain Y1R1 was totally modified. Crude extracts of strains Y2R1 (*miaA*) and Y1R1 were made and tested for tRNA IPP transferase activity. It is known that Δ2-IPP is incorporated into tRNA, whereas Δ3-IPP is not (18); however, we used this latter form radioactively labeled because it is the only one commercially available. To be incorporated into tRNA, the Δ3-IPP must first be converted into Δ2-IPP by the enzyme Δ3-IPP isomerase and then covalently linked to tRNA by the enzyme tRNA IPP transferase, resulting in i^6A-tRNA.

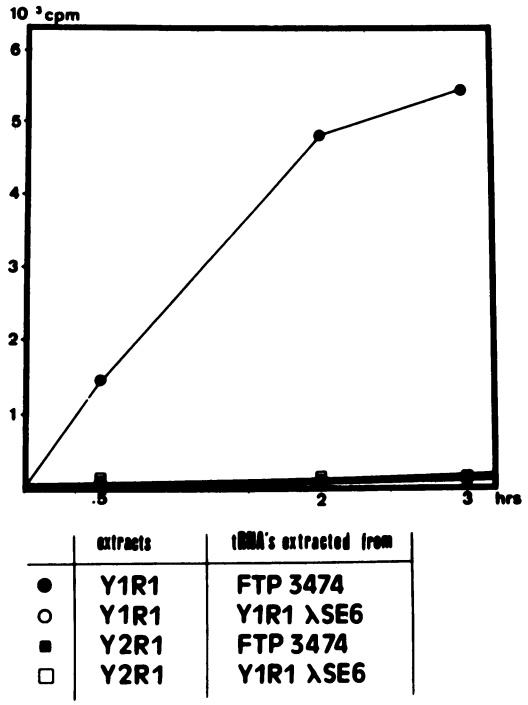

FIG. 1. Kinetics of Δ3-IPP incorporation into tRNAs. tRNAs were extracted and crude extracts were prepared from *miaA* mutant strains (FTP3474, Y2R1) or *miaA* wild-type strains (Y1R1, Y1R1 λSE6) as described in the text and tRNA IPP transferase activity was determined as described below. The incubation mixture (100 μl) contained 50 mM Tris chloride (pH 8), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 50 μg of tRNA, 2.5 mM Δ3-IPP (56 mCi/mmol), and 40 μl of a crude extract. The mixture was incubated at 37°C, and after different periods of time, the reaction was stopped by phenol extraction. The tRNA in the aqueous phase was trichloroacetic acid precipitated, the sample was filtered through Whatman GF/C filters, and the radioactivity retained on the filters was measured by scintillation counting.

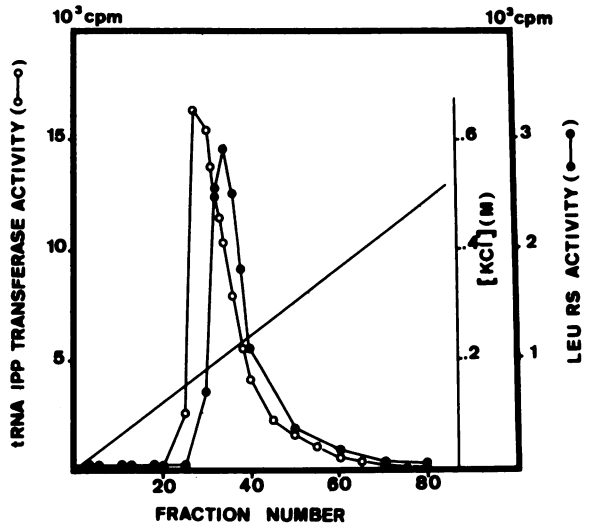

FIG. 2. DEAE-cellulose column chromatography elution profile of leucyl-tRNA synthetase activity (LeuRS) and tRNA IPP transferase activity of fractions complementing an *miaA* strain crude extract (the fractions alone do not exhibit any tRNA IPP transferase activity [values not included in the figure]). For separation of Δ3-IPP isomerase and tRNA IPP transferase by DEAE-cellulose column chromatography, the JM101 pJO3 strain was grown overnight in 4 liters of LB medium containing 100 μg of ampicillin per ml. The cells were pelleted by centrifugation, washed with cold 0.9% NaCl, and suspended in 60 ml of the starting chromatographic buffer (50 mM potassium phosphate [pH 6.5], 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 10% glycerol). The cells were disrupted by sonication, and the cell debris was discarded by centrifugation at 8,000 × *g* for 1 h. The supernatant was adjusted to 250 ml in the starting chromatographic buffer and loaded on the top of a DEAE-cellulose column (1.5 by 20 cm). After being washed with the starting buffer, the adsorbed material was eluted with a 500-ml KCl gradient (0 to 0.6 M) made up in the same buffer. The flow rate was 30 ml/h; 5-ml fractions were collected and tested for leucyl-tRNA synthetase as described in Materials and Methods and for incorporation of radioactive Δ3-IPP in tRNA; this latter test was performed as described in the legend to Fig. 1 in the presence of crude tRNA extract from an *miaA* strain and in the presence or absence of 40 μl of an *mia* strain crude extract.

Figure 1 shows the results obtained for the incorporation of radioactivity from Δ3-IPP into tRNA. Using an extract from strain Y1R1 (*miaA*$^+$), we observed an incorporation of Δ3-IPP into the unmodified tRNA, whereas no incorporation was observed with the fully modified tRNA. This indicates that the enzymatic activity observed in vitro is involved in the formation of i^6A in tRNA since ms^2i^6A-modified tRNA is not a substrate. Moreover, this activity is associated with the *miaA* gene since an extract from strain Y2R1 (*miaA*) did not catalyze the incorporation of Δ3-IPP, whatever the tRNA used. The indicates that the *miaA* mutation affects either Δ3-IPP isomerase or tRNA IPP transferase.

To determine which of these two enzymes is lacking in an *miaA* strain, we separated these two enzymes by DEAE-cellulose column chromatography. The behavior of *E. coli* tRNA IPP transferase on this chromatographic material has already been reported: this enzyme coelutes with leucyl-tRNA synthetase (5).

Figure 2 shows the elution profile of leucyl-tRNA synthetase and of the activity complementing an *miaA* extract for the incorporation of Δ3-IPP into tRNA. A strain carrying a plasmid which complements the *miaA* mutation was in fact used for this experiment. Since none of the fractions alone promoted any Δ3-IPP incorporation into tRNA, Δ3-IPP isomerase and tRNA IPP transferase activities were separated by this method. When we combined the fractions complementing the *miaA* extract with the pool passed through the column during loading and washing operations, we observed a restoration of the incorporation activity. Thus, the other activity necessary for Δ3-IPP incorporation into tRNA is in the flowthrough.

Since it was shown that the enzyme tRNA IPP transferase coelutes with leucyl-tRNA synthetase, then the activity into the flowthrough must be the Δ3-2-IPP isomerase. Thus, we conclude that it is the tRNA IPP transferase which is lacking in the *miaA* mutant.

Molecular cloning of *miaA* gene. It has been shown that certain combinations of *miaA* and *rpsL* mutations can generate a dependence on streptomycin for growth (23). We used such a streptomycin-dependent strain, LOU1 (*rpsL* [Sm^P] *miaA*; Table 1) to screen a lambda library for phage which eliminated the streptomycin dependence of the double mutant (i.e., allowed growth on streptomycin-free medium). An *E. coli* chromosomal DNA library was constructed in λSE6 as described in Materials and Methods. This vector confers kanamycin resistance and is capable of growing lysogenically as a phasmid in an immune host. Samples from three independently amplified batches of the initial library were used to infect LOU2 (LOU1 λ$^+$ lysogen; Table 1) at a

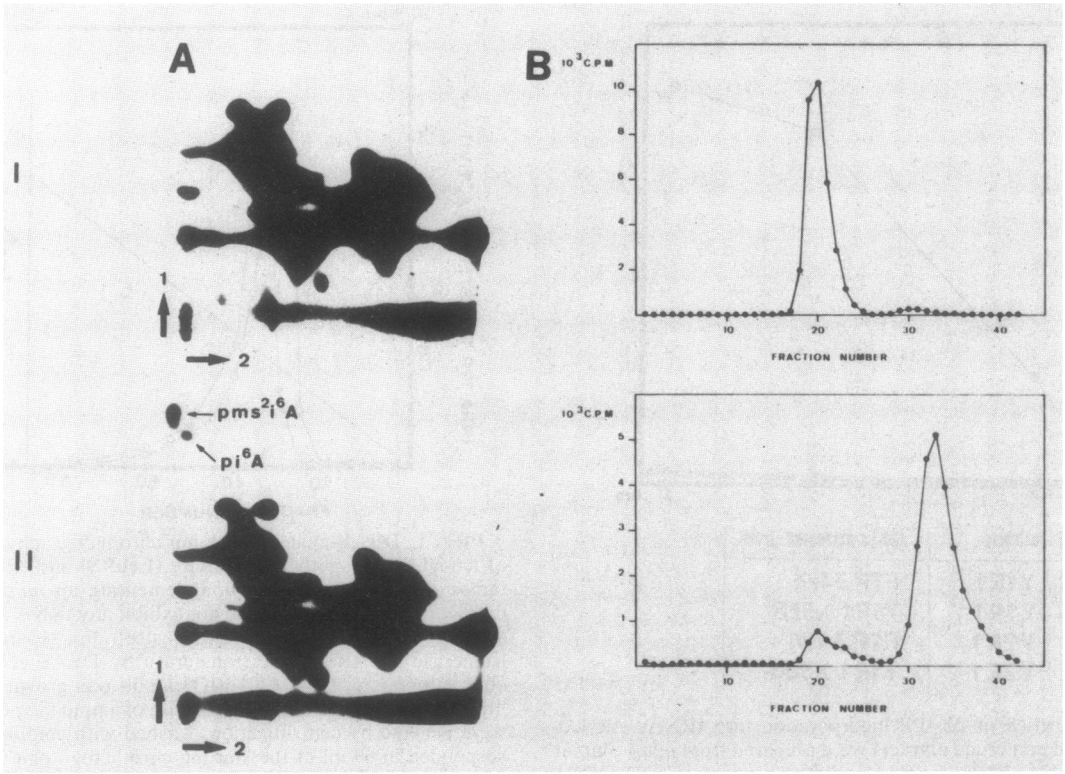

FIG. 3. (A) Cellulose thin-layer plate chromatography autoradiograms of ^{32}P-labeled nucleotides of tRNAs of Y2R1 λSE6 (I) and Y2R1 λLOU7 (an *miaA*-complementing phage) (II). (B) RPC-5 column chromatography elution profiles of Phe-tRNA^{Phe} extracted from Y2R1 λSE6 (top) and Y2R1 λLOU7 (bottom). Experiments were done as described in Materials and Methods.

multiplicity of infection of 0.1. Of a total of 10^7 phage screened, approximately 600 recombinant lambda phage were found to be double lysogens which permitted growth of LOU2 on rich-medium plates containing kanamycin (25 μg/ml) but no streptomycin. A total of 182 lysogens were picked and patched on LB plates containing kanamycin plus or minus streptomycin. Only 1 of the 182 lysogens was found to confer streptomycin sensitivity. This phage, λLOU16, most probably carries the wild-type *rpsL* allele since *rpsL* mutations are recessive. Lysates from 15 of the complemented Kn^r Sm^r lysogens of LOU2 were obtained by UV irradiation. These lysates also contain λ$^+$ and are called λLOU1 to λLOU15.

To characterize the cloned gene which eliminates the streptomycin dependence in the *miaA rpsL* (Sm^p) double mutant, we tested other phenotypic traits which are associated with the *miaA* mutation. The *miaA* mutation causes a derepression of the *trp* operon which can be detected by the absence of growth at 42°C on a minimal medium plate containing glucose, 5 μg of indole per ml, 100 μg of 2-amino-5-methylbenzoic acid per ml, 50 μg of cysteine per ml, 40 μg of leucine per ml, and 40 μg of methionine per ml. Thus, the strain Y2R1 does not grow on this medium, whereas Y1R1 does. Y2R1 lysogenic for the 15 λLOU phage exhibited an *miaA*$^+$ phenotype. This strongly suggests that the complementing gene present on the recombinant lambda phage is specific for *miaA* and could be either the structural gene or a suppressor of the *miaA* mutation.

The tRNAs extracted from the *miaA* mutant strains complemented with the recombinant phage were analyzed for the presence of ms^{2}i^{6}A in tRNA. The lack of ms^{2}i^{6}A causes a characteristic shift during RPC-5 column chromatography of tRNA—the undermodified tRNA elutes at a lower salt concentration (2). Figure 3B shows the elution profiles of tRNA^{Phe} extracted from Y2R1 λSE6 (*miaA*) and subsequently charged with [^{14}C]phenylalanine (Fig. 3B, top) compared with that of Y2R1 λLOU7, the same strain lysogenized with an *miaA*-complementing phage (Fig. 3B, bottom). The Phe-tRNA^{Phe} from this latter strain eluted at a higher salt concentration. The presence of the λLOU7 phage resulted in the restoration of the ms^{2}i^{6}A modification and i^{6}A intermediary. Finally, the modified nucleotides in tRNAs extracted from these strains were analyzed by thin-layer chromatography as described in Materials and Methods. The autoradiograms of Fig. 3AII show spots corresponding to both ms^{2}i^{6}A- and i^{6}A-labeled nucleotides in total tRNA of Y2R1 λLOU7.

The *mia*::Tn*10* allele of FTP3474 was tested with phage λLOU7. The tRNAs extracted from FTP3474 λLOU7 and analyzed by RPC-5 column chromatography showed the characteristic elution profile of ms^{2}i^{6}A-modified tRNAs, whereas tRNAs extracted from FTP3474 λSE6 were unmodified. Thus, the λLOU7 phage carries a gene which complements this second *mia* mutation. Subclones (see below) of λLOU7 also complemented both mutations and produced approximately equal levels of overproduction of the Δ3-IPP incorporation (Fig. 4). Thus, it would seem likely that the *miaA* gene is carried by λLOU7 since it complements both a Tn*10* insertion and an ochre mutation.

Localization of *miaA* gene within λLOU DNA. To localize more precisely the *miaA*-complementing gene on the cloned DNA fragment in λLOU phages, we subcloned it from the λ vector to the multicopy plasmid pBR322, selecting for fragments which complement the streptomycin dependence phe-

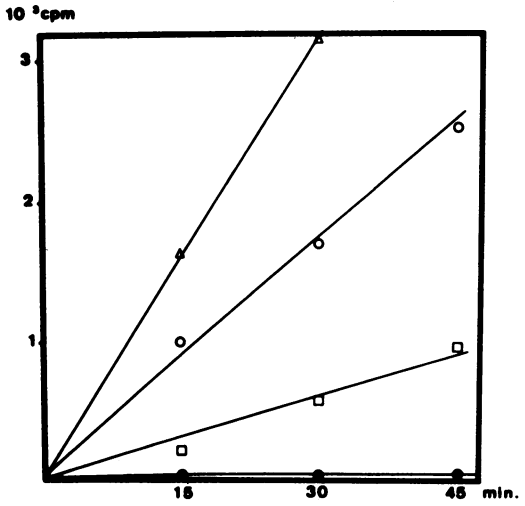

FIG. 4. Kinetics of Δ3-IPP incorporation into undermodified tRNA by crude extracts from Y2R1 pBR322 (●) and FTP3474 pBR322 (●), Y1R1 pBR322 (□), Y2R1 pJO3 (○), and FTP3474 pJO3 (△). Y2R1 and FTP3474 are *miaA* mutant strains; Y1R1 carries *miaA*$^+$; pJO3 carries the *miaA* gene. The experiments were done as described in the legend to Fig. 1, using 40 μl of crude extracts at 10 mg/ml.

notype at 30°C. λLOU7 DNA digested with *Sal*I and ligated with *Sal*I-digested pBR322 plasmid DNA was used to transform strain LOU1 (16). A complementing plasmid, pJO3 (Fig. 5), was shown to carry a 6.6-kb *Sal*I fragment (pJO2 carries this *Sal*I fragment in the other orientation). The second step in these subcloning experiments was to reduce

FIG. 5. Restriction map of λLOU7, *miaA*-complementing recombinant phage, and subcloning of the *miaA* gene in pBR322. pJO3 carries a 6.6-kb *Sal*I fragment selected by complementation of an *miaA rpsL* (Sm^p) double mutant (streptomycin dependent), and pJO7 carries a 3.5-kb *Sal*I-*Kpn*I fragment. The line marked by an asterisk shows the physical position of polypeptides encoded by the *hflA* locus (4) represented by closed boxes; arrows indicate the direction of transcription of these genes. The position of the *miaA* gene is indicated by a closed box whose boundaries are not determined. The *miaA* gene covered the *Bam*HI site because the 2.5-kb *Sal*I-*Bam*I fragment subcloned in a plasmid was not obtained by selecting for streptomycin dependence complementation. The *purA* and *mutL* loci are indicated by thick lines.

the size of the fragment carrying the *miaA*-complementing gene. The 6.6-kb *Sal*I fragment was digested with *Kpn*I, and the two resulting fragments of 3.1 and 3.5 kb were cloned into Blue Scribe digested with *Kpn*I and *Sal*I. pJO7, the 3.5-kb *Sal*I-*Kpn*I fragment, complemented the streptomycin dependence of LOU1 at 30°C (Fig. 4).

Genetic mapping of *miaA* gene. The *mia*::Tn*10* gene has been mapped at 95 min on the *E. coli* chromosome (14), and the *miaA1* mutation has been mapped at 96 min on the *S. typhimurium* chromosome counterclockwise of *purA* with the gene order *mutL-miaA-purA* (12). The *E. coli* linkage map (3) shows at 95 min the gene order *hfl-mutL-purA*. The λLOU phage were tested for the presence of *purA* and *mutL* as described in Materials and Methods. All the λLOU phages which carried *miaA* (λLOU1 to 15) also carried *purA*. The subcloned 6.6-kb fragment of pJO3, however, did not complement *purA*. The 71/18 *mutL* strain produced spontaneous streptomycin-resistant colonies at a frequency of 10^{-7} to 10^{-8}, whereas for a wild-type strain (71/18), the frequency was 10^{-9}. The presence of the wild-type *mutL* allele on the λLOU phage was detected by the reduction in the frequency of appearance of spontaneous streptomycin-resistant bacteria in 71/18 *mutL* λLOU lysogens compared with that of the wild-type strain. Thirteen of the 15 λLOU phage carried *mutL*.

Recently, the cloning of the *hflA* locus (94.5 min on the *E. coli* chromosome) has been reported (4). Comparison of the published *hflA* restriction map with that of λLOU7 showed that these clones overlap and allowed us to localize *miaA*, *hflA*, and *purA* on λLOU7. All these data are summarized in Fig. 5. Moreover, preliminary experiments indicated the presence of *hflA* on all the λLOU phage (data not shown). The pJO3 recombinant plasmid did not carry the *hflA* gene whole locus and did not complement an *hflA* mutation. Thus, we suggest the following gene order: *miaA-hflA-purA* at 95 min on the *E. coli* chromosome. The results of the experiments described above concerning *mutL* are in agreement with the mapping of these genes in *S. typhimurium*, and although it has not been formally proved, we propose the mapping of *mutL* counterclockwise of *miaA* by analogy.

ACKNOWLEDGMENTS

We gratefully acknowledge M. Grunberg-Manago for support. R. H. Buckingham, M. Springer, and J. A. Plumbridge for helpful discussions, and B. Bachmann, G. C. Kurland, R. Maurer, and E. J. Murgola for the gift of strains.

This work was supported by grants from the Centre National de la Recherche Scientifique (UA 1139), from the Fondation pour la Recherche Médicale, from E.I. du Pont de Nemours & Co., and from Paris VII University.

LITERATURE CITED

1. **Agris, P. F., D. J. Armstrong, K. P. Schäfer, and D. Söll.** 1975. Maturation of a hypermodified nucleoside in transfer RNA. Nucleic Acids Res. **2**:691–698.
2. **Arnold, H. H., R. Raettig, and G. Keith.** 1977. Isoaccepting phenylalanine tRNAs from *Bacillus subtilis* as a function of growth conditions. Differences in the content of modified nucleosides. FEBS Lett. **73**:210–214.
3. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
4. **Banuett, F., and I. Herskowitz.** 1987. Identification of polypeptides encoded by an *Escherichia coli* locus (*hflA*) that governs the lysis-lysogeny decision of bacteriophage λ. J. Bacteriol. **169**: 4076–4085.
5. **Bartz, J. K., L. K. Kline, and D. Söll.** 1970. N^6-(Δ2-Isopentenyl) adenosine: biosynthesis *in vitro* in transfer RNA by an enzyme purified from *Escherichia coli*. Biochem. Biophys. Res. Com-

mun. **40**:1481–1487.
6. **Björk, G. R.** 1987. Modification of stable RNA, p. 719–736. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarber (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
7. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Diaz, I., M. Ehrenberg, and G. C. Kurland.** 1986. How do combinations of *rpsL*$^-$ and *miaA*$^-$ generate streptomycin dependence? Mol. Gen. Genet. **202**:207–211.
9. **Dihanich, M. E., D. Najarian, R. Clark, E. C. Gillman, N. C. Martin, and A. K. Hopper.** 1987. Isolation and characterization of *MOD5*, a gene required for isopentenylaton of cytoplasmic and mitochondrial tRNAs of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:177–184.
10. **Eisenberg, S. P., M. Yarus, and L. Soll.** 1979. The effect of an *Escherichia coli* regulatory mutation on transfer RNA structure. J. Mol. Biol. **135**:111–126.
11. **Elledge, S. J., and G. C. Walker.** 1985. Phasmid vectors for identification of genes by complementation of *Escherichia coli* mutants. J. Bacteriol. **162**:777–783.
12. **Ericson, J. H., and G. C. Björk.** 1986. Pleiotropic effects induced by modification deficiency next to the anticodon of tRNA from *Salmonella typhimurium* LT2. J. Bacteriol. **166**: 1013–1021.
13. **Eriksson-Grennberg, K. G.** 1968. Resistance of *Escherichia coli* to penicillins. II. An improved mapping of the *ampA* gene. Genet. Res. **12**:147–156.
14. **Gallagher, P. J., I. Schwartz, and D. Elsevier.** 1984. Genetic mapping of *pheU*, an *Escherichia coli* gene for phenylalanine tRNA. J. Bacteriol. **158**:762–763.
15. **Gornall, A. G., C. J. Bandawill, and M. M. David.** 1949. Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. **177**:751–766.
16. **Hanahan, D.** 1983. Studies on transformation of *E. coli* with plasmids. J. Mol. Biol. **166**:557–580.
17. **Karn, J., S. Brenner, L. Barnett, and G. Cesarini.** 1980. Novel bacteriophage λ cloning vehicle. Proc. Natl. Acad. Sci. USA **77**: 5172–5176.
18. **Kline, L. K., F. Fittler, and R. H. Hall.** 1969. N^6 (Δ^2 isopentenyl) adenosine. Biosynthesis in transfer ribonucleic acid *in vitro*. Biochemistry **8**:4361–4371.
19. **Liberfarb, R. M., and V. Bryson.** 1970. Isolation, characterization, and genetic analysis of mutation genes in *Escherichia coli* B and K-12. J. Bacteriol. **104**:363–375.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Miller, J. H. (ed.).** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Najarian, D., M. E. Dihanich, N. Martin, and A. K. Hopper.** 1987. DNA sequence and transcript mapping of *MOD5*: features of the 5′ region which suggest two translational starts. Mol. Cell. Biol. **7**:185–191.
23. **Petrullo, L. A., P. J. Gallagher, and D. Elseviers.** 1983. The role of 2-methylthio *N*6 isopentenyladenosine in readthrough and suppression of nonsense codons in *Escherichia coli*. Mol. Gen. Genet. **190**:289–294.
24. **Ruusala, T., D. Andersson, M. Ehrenberg, and G. C. Kurland.** 1984. Hyperaccurate ribosomes inhibit growth. EMBO J. **3**: 2575–2580.
25. **Siegel, E. L., and J. J. Ivers.** 1975. *mut-25*, a mutation to mutator linked to *purA* in *E. coli*. J. Bacteriol. **121**:524–530.
26. **Silberklang, M., A. M. Gillum, and V. L. Raj Bhandary.** 1979. Use of *in vitro* ^{32}P labelling in the sequence analysis of non radioactive tRNAs. Methods Enzymol. **54**:58–109.
27. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Sprinzl, M., T. Hartmann, F. Meissner, J. Moll, and T. Vorderwülbecke.** 1987. Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res. **15**:r53–r188.
29. **Stern, R., and U. Z. Littauer.** 1971. *In* G. L. Cantoni, and D. R. Davies (ed.), Procedures in nucleic acids research. Harper & Row, Publishers, Inc., New York.
30. **Vacher, J., H. Grosjean, C. Houssier, and R. H. Buckingham.** 1984. The effect of point mutations affecting *Escherichia coli* tryptophan tRNA on anticodon-anticodon interactions and on UGA suppression. J. Mol. Biol. **177**:329–342.
31. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
32. **Vogelstein, B., and D. Gillepsie.** 1979. Preparation and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**:615–619.
33. **Yanofsky, C., and L. Soll.** 1977. Mutations affecting $tRNA^{Trp}$ and its charging and their effects on regulation of transcription termination at the attenuator of the tryptophan operon. J. Mol. Biol. **113**:663–677.
34. **Zagursky, R. J., and M. L. Berman.** 1984. Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. Gene **27**:183–191.