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Molecular Cloning and Primary Structure of the *Escherichia coli* Methionyl-tRNA Synthetase Gene

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The intact *metG* gene was cloned in plasmid pBR322 from an F32 episomal gene library by complementation of a structural mutant, *metG83*. The *Escherichia coli* strain transformed with this plasmid (pX1) overproduced methionyl-tRNA synthetase 40-fold. Maxicell analysis showed that three major polypeptides with M_rs of 76,000, 37,000, and 29,000 were expressed from pX1. The polypeptide with an M_r of 76,000 was identified as the product of *metG* on the basis of immunological studies and was indistinguishable from purified methionyl-tRNA synthetase. In addition, DNA-DNA hybridization studies demonstrated that the *metG* regions were homologous on the *E. coli* chromosome and on the F32 episome. DNA sequencing of 642 nucleotides was performed. It completes the partial *metG* sequence already published (D. G. Barker, J. P. Ebel, R. Jakes, and C. J. Bruton, Eur. J. Biochem. 127:449–451, 1982). Examination of the deduced primary structure of methionyl-tRNA synthetase excludes the occurrence of any significant repeated sequences. Finally, mapping of mutation *metG83* by complementation experiments strongly suggests that the central part of methionyl-tRNA synthetase is involved in methionine recognition. This observation is discussed in the light of the known three-dimensional crystallographic structure.

Bacterial aminoacyl-tRNA synthetases have been the object of numerous studies aimed at solving their structure-function relationships (reviewed in references 24 and 28). Among them, *Escherichia coli* methionyl-tRNA synthetase (MTS) benefits from the availability of the crystallographic structure (31) of a fully active fragment with an M_r of 64,000 derived from native MTS (11), a dimer with an M_r of 152,000 (18). The primary structure of this fragment has been recently deduced from the DNA sequence of a truncated form of *metG* (2), and efforts have been made to describe in molecular terms the binding of substrates to the synthetase (4; C. Hountondji, F. Lederer, and S. Blanquet, Biochemistry, in press). The present work describes the cloning of the complete *metG* gene.

Since MTS used for crystallographic and biochemical studies was purified from a merodiploid strain carrying the F32 episome, the intact *metG* gene was isolated from an F32 episomal DNA library.

The MTS primary structure has been completed, allowing us to rule out the occurrence of any significant repeats in the polypeptide chain composing the enzyme, as was previously proposed (8, 9) on the basis of partial amino acid sequence determination.

Finally, the availability of *metG* with large flanking DNA regions gave us an opportunity to study the regulation of its expression and to possibly explain previous physiological results showing that MTS expression was controlled by the level of $tRNA^{Met}$ aminoacylation (10).

MATERIALS AND METHODS

Bacterial strains and growth media. All strains were *E. coli* K-12 derivatives. Genotypes are listed in Table 1. Merodiploid strain EM20031 was used as a source of F32 episomal DNA. Hfr strain JC10240 carrying a Tn*10* transposon inserted in the *srl* operon near *recA* was used to transfer the *recA* marker. PAL1803.1 was isolated as a spontaneous Rif^r derivative of methionine auxotroph strain SB1803 (*metG83*). Conjugation of PAL1803.1 with Hfr strain JC10240 was used to construct PAL1803.3, a *recA* Tet^r derivative. PAL1803.5 (*recA* Tet^s) was selected for loss of tetracycline resistance according to the method of Bochner (7).

Bacteria were grown at 37°C on either LB medium or minimal M9 medium (23) supplemented with 0.4% glucose as carbon source and 200 μg of the required amino acids per ml. When appropriate, tetracycline, chloramphenicol, or rifampin was included at 10, 10, or 200 μg/ml, respectively.

Enzymes. Restriction endonucleases, T4 DNA ligase, calf intestine alkaline phosphatase, DNA polymerase large fragment, and polynucleotidyl-kinase were purchased from Boehringer Mannheim Corp., Bethesda Research Laboratories, or New England Biolabs, Inc. Incubations were carried out as recommended by the suppliers.

DNA purification. Large-scale purification of plasmid DNA was performed after chloramphenicol or spectinomycin amplification according to the method of Clewell (13). The rapid, small-scale extraction procedure of Birnboim and Doly (3) was also used for analytical purposes. Chromosomal DNA of strain AB1111 was purified according to the method of Davis et al. (15).

F32 episomal DNA was purified as follows: a cleared lysate was obtained from 6 liters of culture of EM20031 grown at 37°C to an absorbance value of 1 at an optical density of 650 nm. Chromosomal and episomal DNA was spun down by centrifugation for 1 h at 100,000 × *g*. The pellet was resuspended in 40 ml of 25 mM Tris (pH 8)–10 μM EDTA. After addition of 80 μl of 0.2 N NaOH–1% sodium dodecyl sulfate (SDS) the mixture was submitted to gentle agitation until complete solubilization occurred. Further addition of 60 ml of 3 M sodium acetate (pH 4.8) allowed selective renaturation of the episome. Precipitated chromosomal DNA was eliminated by centrifugation, and the supernatant was phenol extracted twice. DNA was ethanol precipitated and finally purified by banding in cesium chlo-

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TABLE 1. Bacterial strains

Designation	Sex	Genotype	Reference
EM20031	F32	*rpsL dsdC3*/F32 *dsdC*$^+$	(22)
AB1111	F$^-$	*thi-1 leuB6 proA2 hisC3 xyl-5 ara-14 galK2 lacY1 mtl-1 rpsL25* λ$^-$ *supE44*	(17)
SB1803	F$^-$	Same as AB1111, *metG83* (methionine auxotroph)	(6)
PAL1803.1	F$^-$	Same as SB1803, *rpoB* (Rif^r)	This work
PAL1803.3	F$^-$	Same as PAL1803.1, *recA56 srl-300*::Tn*10*	This work
PAL1803.5	F$^-$	Same as PAL1803.3, Tet^s	This work
JC10240	Hfr P045	*ilv-318 recA56 srl-300*::Tn*10 thi-1 relA1 spoT1 rpsE2300 thr-3000*	(14)
CSR603	F$^-$	*thr-1 leuB6 proA2 phr-1 argE3 thi-1 uvrA6 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 rho-33* λ$^-$ *supE44 recA1*	(26)

ride-ethidium bromide equilibrium gradient. The yield was ca. 200 μg of DNA from a 6-liter culture.

Construction of episomal gene libraries. An amount of 7 μg of F32 episomal DNA was incubated with 2 U of *Sau*3A for 2 min. The partial digest was then ligated with 2 μg of dephosphorylated *Bam*HI-cut pACYC184. This ligation mixture was used to transform PAL1803.1 competent cells. A total of 1,871 chloramphenicol-resistant transformants were isolated, out of which 1,852 had lost the ability to grow on tetracycline.

Two 15-μg fractions of F32 DNA were digested by 2 and 4 U of *Pst*I, respectively, for 2 h at 37°C and further ligated with 2 μg of dephosphorylated *Pst*I-cut pBR322. The ligation mixture was used to transform PAL1803.1 competent cells. All 1,057 tested clones were recombinants (Tet^r Amp^s).

Identification of plasmid-encoded proteins in maxicells. CSR603 cells, transformed by the appropriate plasmid, were UV irradiated (≃ 50 J/m^2) under agitation and were incubated overnight after addition of 150 μg of cycloserine per ml to kill survivors. Cells were washed with minimal medium, suspended, and labeled for 1 h at 37°C with 10 μCi of L-[^{35}S]methionine (1,200 Ci/mol; C.E.A. Saclay). Medium was eliminated by centrifugation, and lysis was obtained by a freeze-thaw procedure. Samples were then either submitted directly to SDS-polyacrylamide gel electrophoresis analysis or incubated with antibodies, precipitated by protein A-Sepharose (Pharmacia), and washed before heat denaturation and SDS-polyacrylamide gel electrophoresis analysis.

Nick translation and DNA-DNA hybridization. [α-^{32}P] dATP (800 Ci/mmol; New England Nuclear Corp.) labeling of DNA probe by nick translation was carried out according to the method of Davis et al. (15). Bidirectional transfer of DNA from agarose gel to nitrocellulose (BA 85; Schleicher & Schull, Inc.) was performed as described (29). The nitrocellulose filter was incubated with ca. 5 × 10^6 dpm of labeled probe for 16 h at 42°C in hybridization buffer containing 50% formamide.

DNA sequencing. Large DNA restriction fragments purified by agarose gel electrophoresis (low gelling temperature; Sigma type VII) were subsequently digested with *Bst*NI, *Hpa*II, *Sau*3A, *Hin*fI, *Taq*I, or *Bam*HI. Single 5′-labeled fragments were obtained by strand separation on polyacrylamide gel and sequenced by the chemical method of Maxam and Gilbert (21). Sequences were also determined by the dideoxy chain terminator method (27).

General biochemical techniques. To assay levels of MTS activity in *E. coli* cells, crude extracts of late-exponential-growing cultures (10 ml) were prepared by a freeze-thaw procedure, followed by centrifugation at 10,000 × *g* for 5 min. Protein concentrations were determined by the biuret method (20).

Aminoacyl-tRNA synthetase activity was measured by either the amino acid-dependent $^{32}PP_i$-ATP isotopic exchange assay or aminoacylation of tRNA^{Met} (5).

Polyacrylamide gel electrophoresis analysis of cell extracts was performed according to the method of Laemmli (19).

RESULTS

Properties of recombinant plasmids pACSm, pX1, and pX2. Two episomal gene banks were prepared. (i) A *Sau*3A partial digest of F32 episomal DNA was inserted into the *Bam*HI site of plasmid pACYC 184. (ii) A *Pst*I partial digest was inserted into the unique *Pst*I site of pBR322. Competent cells of PAL1803.1 (*recA*$^+$) were transformed with these two plasmid banks. One methionine prototroph transformant was obtained from the *Sau*3A bank, and two were obtained from the *Pst*I bank. Corresponding recombinant plasmids were called pACSm and pX1 and pX2, respectively.

All these purified plasmids were able to transform PAL1803.1 (*recA*$^+$) to methionine prototrophy. In the case of PAL1803.5 (a *recA*$^-$ derivative of PAL1803.1), only pX1 complemented the methionine auxotrophy.

This result suggested that pACSm and pX2 carried only a part of the *metG* gene and were able to rescue the chromosomal mutation by recombination in the *recA*$^+$ strain. Insert sizes of pACSm and pX2 were 1.3 and 3.7 kilobases (kb), respectively. Clearly, pACSm did not have the coding capacity for the 76,000-molecular-weight polypeptide composing MTS.

On the contrary, pX1, which carried a 15-kb insert, was supposed to express an active form of MTS, since it complemented the methionine auxotrophy of the *recA*$^-$ recipient strain.

Overproduction of native MTS. Crude extracts of PAL1803.1 cells harboring pX1, pX2, or pBR322 were submitted to SDS-polyacrylamide gel electrophoresis analysis. The pX1 extract exhibited an intense polypeptide band comigrating with the native subunit (M_r = 76,000) of purified MTS. (Fig. 1A). This polypeptide was absent in extracts from cells carrying pX2 or pBR322.

The proteins expressed by plasmids pX1 and pX2 were further identified in a maxicell experiment. Plasmid pX1 directed the synthesis of three major polypeptides with M_rs of 76,000, 37,000, and 29,000. Only the polypeptide with an M_r of 76,000 was immunoprecipitated by specific antibodies directed against native MTS purified from merodiploid strain EM20031 carrying the F32 episome. Plasmid pX2 also encoded the above pX1 polypeptide with an M_r of 37,000 and a minor species with an M_r of 52,000 also immunoprecipi-

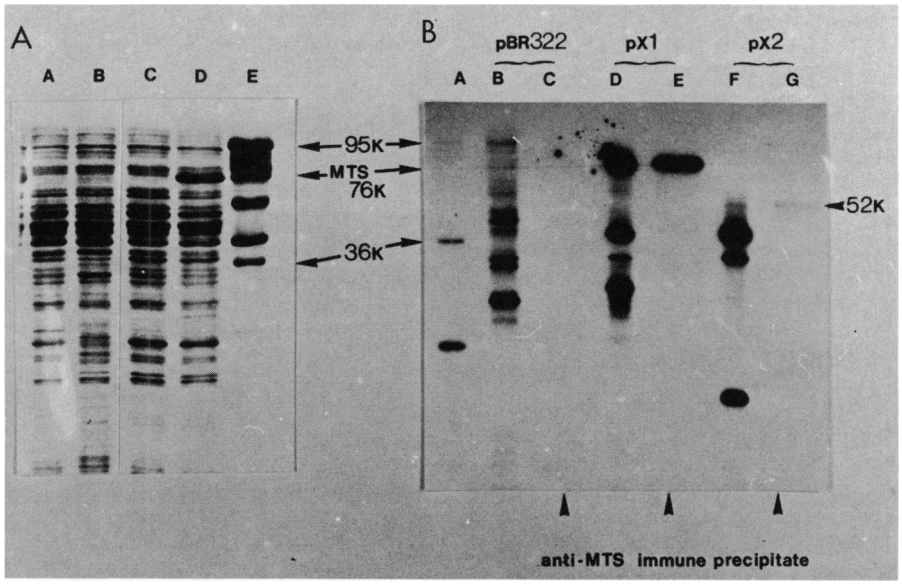

FIG. 1. Identification of the proteins expressed from pX1 plasmid. Electrophoretic analyses were performed on 12.5% SDS-polyacrylamide gel according to the method of Laemmli (19). (A) Overproduction of MTS. Each slot was loaded with crude cell extract containing 40 μg of protein, and bands were visualized with Coomassie blue. Lanes: A, PAL1803.1 transformed with pBR322; B, EM20031; C, PAL1803.1 transformed with pX2; D, PAL1803.1 transformed with pX1; E, molecular weight markers, including purified MTS. (B) Identification of the proteins expressed by recombinant plasmids after [^{35}S]methionine pulse-labeling of UV-irradiated CSR603 cells transformed with pX1, pX2, or pBR322 (maxicells). Samples equivalent to 2×10^5 dpm were loaded on the gel directly or after immune precipitation by anti-MTS antibodies. After electrophoresis, the gel was treated for fluorography and exposed to Kodak X-Omat film at −70°C for 48 h. Lanes: A, molecular weight markers; B, pBR322 extract; C, pBR322 immunoprecipitate; D, pX1 extract; E, pX1 immunoprecipitate; F, pX2 extract; G, pX2 immunoprecipitate.

tated by the anti-MTS specific antibodies. This indicated that a truncated protein, retaining antigenic determinants of native MTS, was synthesized from pX2.

To confirm that the protein overproduced by the pX1-carrying strains was native MTS, the following experiments were undertaken. Comparative activity measurements were carried out on crude cell extracts of AB1111 (*metG*$^+$), the PAL1803.1 parental strain, transformed by pX1 or pBR322. The pX1-harboring cells overproduced MTS 40-fold with respect to cells harboring pBR322 (Table 2). Strain AB1111 (*metG*$^+$) or derived strain PAL1803.5 (*metG83 recA56*) transformed by pX2 or pACSm did not show an increase of the methionine-dependent ATP-PP$_i$ exchange activity. This result was in agreement with the observation that only pX1 was able to complement the *recA metG* mutants.

TABLE 2. MTS activity in cell extracts of strains carrying the studied plasmids[a]

Strain(Plasmid)	ATP-PP$_i$ exchange activity		Aminoacylation activity	
	U	Ratio to AB1111 (pBR322)[b]	Units	Ratio to AB12111 (pBR322)[b]
AB1111(pBR322)	340	1.0	12	1
AB1111(pX1)	13,200	38.5	490	41
AB1111(pX2)	220	0.6	10	0.8
PAL1803.5(pX1)	8,210	24.0	ND	
PAL1803.5(pX2)	<5			
PAL1803.5(pX2)	<5			

[a] MTS activity was followed in crude extracts of AB1111 (*metG*$^+$ *recA*$^+$) and PAL1803.5 (*metG83 recA56*) cells carrying the various plasmids indicated in the Table. Units correspond to 1 pmol of [^{32}P]ATP synthesized per mg of protein per s at 25°C and to 1 pmol of L-[^{14}C]methionyl-tRNA formed per mg of protein per s at 25°C for ATP-PP exchange activity and aminoacylation activity, respectively. ND, Not determined.

[b] Activity levels in the extracts are compared with that of AB1111(pBR322).

Taken together, these results strongly suggested that plasmid pX1 encoded native MTS. The integrity of the cloned *metG* gene was further established by DNA hybridization, restriction analysis, and sequencing.

MTS activity in the crude extract of pX1-carrying strains was fully inhibited by anti-MTS specific antibodies. Homogeneous MTS purified from the merodiploid strain EM20031 carrying the F32 episome and extract of cells carrying pX1 were titrated in parallel by the antibodies. Identical titration curves (Fig. 2) indicated that the pX1-encoded enzyme and the purified MTS had the same antigenic determinants.

Finally, MTS overproduced by the pX1-harboring strains and purified to homogeneity by affinity chromatography (M. Fromant, Ph.D. thesis, Université de Paris VII, Paris, France, 1982) was shown to be a dimer with an M_r of 152,000, indistinguishable from the native enzyme.

Restriction mapping and DNA hybridization. Restriction maps of pACSm, pX1, and pX2 were established (Fig. 3). Maps showed that the pX1 insert consisted of three *Pst*I fragments, 3.5, 9, and 2.4 kb long. The 3.5-kb fragment was also present in the pX2 insert, together with a small 200-base-pair (bp) *Pst*I fragment. All three plasmids shared a common region ca. 0.8 kb long, in which part of the *metG* gene should be located. The restriction map of this common region, around the unique *Hin*dIII site, was identical to that deduced from the partial *metG* sequence already solved (2). By comparison with this sequence, the start of the *metG* gene could be assigned on the pX1 map, 820 bp upstream of the *Hin*dIII site.

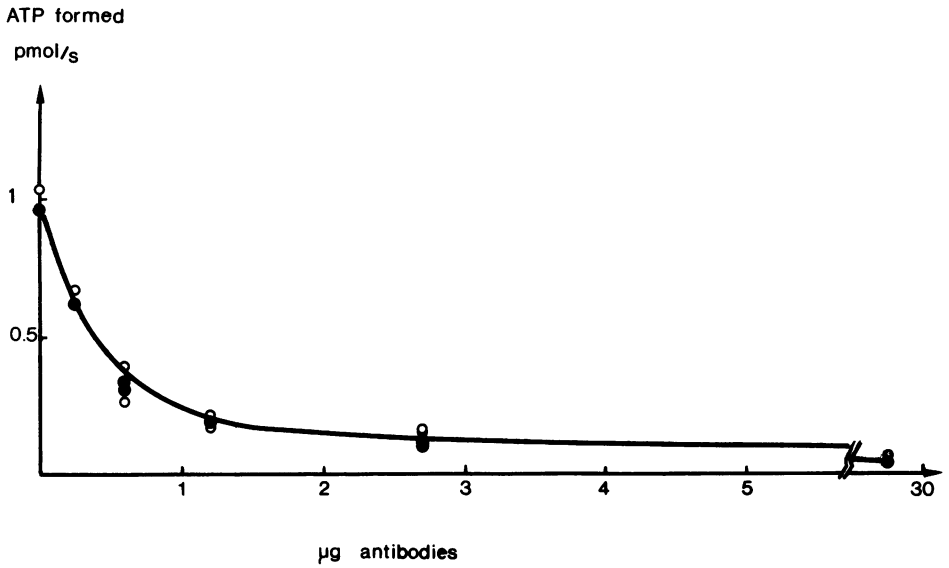

FIG. 2. Titration of purified MTS and of crude extract from cells carrying pX1 by anti-MTS specific antibodies. An amount of 25 μl of either 2.5 nM purified MTS (from merodiploid strain EM20031) or diluted PAL1803.1(pX1) extract containing 0.15 μg of total proteins was incubated with increasing amounts of purified anti-MTS antibodies in standard assay buffer and assayed for $^{32}PP_i$-ATP isotopic exchange activity. These conditions insured identical activity of samples in the absence of antibodies. Activity is plotted as a function of the added antibodies (μg). Open circles correspond to pX1 extract activity, and closed circles correspond to purified MTS.

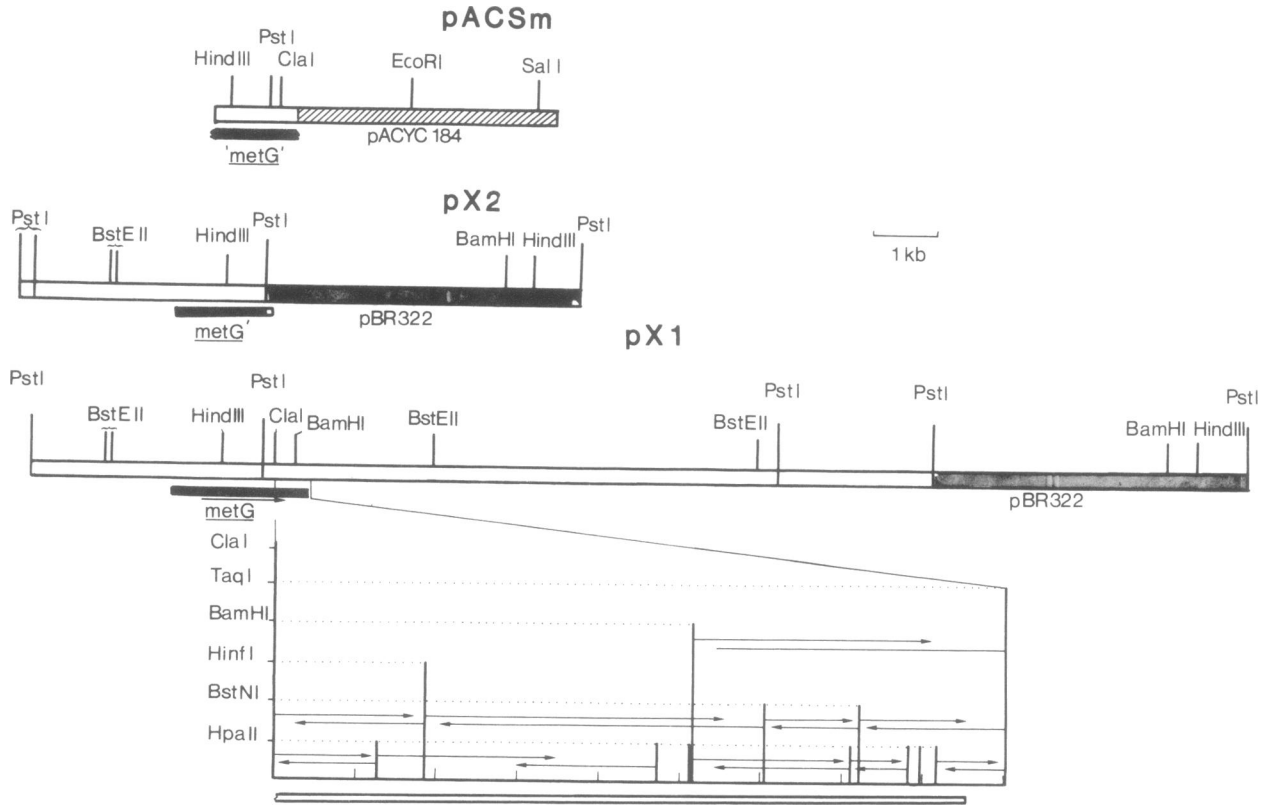

FIG. 3. Restriction maps of pACSm, pX1, and pX2 and sequencing strategy. All three plasmids were from independent cloning experiments. Insert sizes are 1.3 kb for pACSm, 3.7 kb for pX2, and 15 kb for pX1. The hatched boxes represent DNA of the vector plasmids. Some restriction sites within the vectors were omitted for the sake of clarity. The sequenced *ClaI-TaqI* fragment is shown at the bottom of the figure. 5'-^{32}P-labeled single-stranded DNA fragments were sequenced by the chemical method. Horizontal arrows starting from the cleavage sites show the nucleotides sequenced on each of the two DNA strands.

All these observations showed that pACSm carried an internal part of *metG* and that pX2 contained the regulatory region of *metG* and 70% of the structural gene. By examining the sequence described by Barker et al. (2) it could be concluded that pX2 encoded an hybrid protein with the first 475 codons of *metG* and 9 codons from pBR322 fused after the *Pst*I site, accouting well for the polypeptide with an M_r 52,000 observed in the maxicell experiment. However, this hybrid species was inactive, as shown by ATP-PP_i exchange activity measurements in mutant strains carrying the pX2 plasmid.

Since pX1 was obtained by cloning after partial digestion of the F32 episome, it was necessary to control (i) that the environment of the *metG* gene in plasmid pX1 was identical to that on the episome, i.e., that the two *Pst*I fragments carrying *metG* on pX1 (3.5 and 9 kb, respectively) were also adjacent on the episome and (ii) that the *metG* region was the same on the F32 episome and on the chromosome.

Partial restriction map of the F32 episome around the *metG* locus was determined by DNA-DNA hybridization. Plasmid pACSm, obtained independently of pX1, carried an internal part of *metG* (Fig. 3) and was used as probe. F32 DNA was cleaved by *Bst*EII, *Pst*I, or *Pst*I plus *Hin*dIII. After agarose gel electrophoresis, DNA fragments were transferred to nitrocellulose and hybridized to ^{32}P-labeled pACSm. The *Pst*I digest of F32 (Fig. 4A, lane 4) showed two DNA fragments hybridizing with the probe (3.5 and 9 kb). These two fragments comigrated with those carrying *metG* on plasmid pX1 (Fig. 4A, lane 2). However, digestion by *Pst*I plus *Hin*dIII revealed that the above episomal 3.5-kb *Pst*I-*Pst*I band corresponded in fact to two comigrating, distinct *Pst*I-*Pst*I fragments. One *Pst*I-*Pst*I fragment was not cleavable by *Hin*dIII, whereas the other was, yielding a 2.8-kb fragment (Fig. 4A, lane 3), as expected from the pX1 restriction map. Since the vector plasmid pACYC184 did not hybridize with the F32 episome (data not shown), it could therefore be concluded that the episome carried a second region homologous to the pACSm insert. The presence of this second region on the episome was confirmed by pACSm hybridization to a *Bst*EII digest of F32 DNA. The physical map of pX1 (Fig. 3) indicated that the entire *metG* gene was carried by a single 5.5-kb *Bst*EII-*Bst*EII fragment. This fragment was present on the hybridization pattern of F32 digested by *Bst*EII (Fig. 4A, lane 5). However, another hybridizing band of 10 kb was revealed, which should correspond to the second region discussed above.

This second region was not present on the *E. coli* chromosome, since the hybridization pattern of pACSm with AB1111 chromosomal DNA cleaved by *Bst*EII (Fig. 4B) only showed the 5.5-kb fragment.

DNA sequencing. The DNA sequence of the 3′ terminal region of *metG* was elucidated, thus completing the primary structure of the enzyme. The determination of the sequence of a 451-bp *Cla*I-*Taq*I fragment confirmed the last 91 nucleotides of the partial DNA sequence already published (2) and showed that the open reading frame of *metG* terminated 25 bp before the *Taq*I site.

As demonstrated previously (11), limited proteolysis of native MTS yields a 64,000-molecular-weight active monomeric fragment. The sequences of the peptides obtained by complete tryptic digestion of this 64,000-molecular-weight fragment have been published (2). However, seven peptides could not be aligned on the partial amino acid sequence shown by Barker et al. (2) and were predicted to correspond to the unresolved C-terminal region of MTS. The present 3′-terminal sequence of *metG* (426 bp) enabled us to identify six of these peptides. They covered more than 38% of the C-terminal portion of MTS (Fig. 4).

The pX1 sequence corresponding to the first 191 nucleotides of *metG* was also solved. It confirmed the location of the 5′ terminus of the gene. The complete sequence of the *metG* accounted for a molecular ratio of 76,127, in good

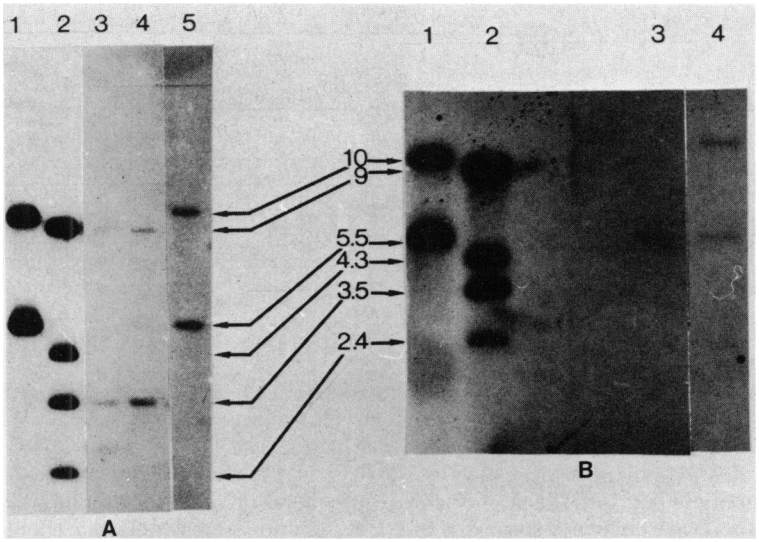

FIG. 4. Identification by DNA-DNA hybridization of the DNA fragments carrying *metG* on plasmid pX1, the F32 episome, and the AB1111 chromosome. After digestion with appropriate endonucleases, restriction fragments were electrophoresed on a 0.75% agarose gel. DNA fragments were transferred to nitrocellulose and hybridized in situ with 5×10^6 dpm of ^{32}P-labeled pACSm. Autoradiography of the filter revealed fragments with sequences homologous to the *metG* region carried on pACSm. (A) ^{32}P-labeled pX1 cleaved by *Bst*EII (lane 1) or *Pst*I (lane 2) was used as a marker. Also shown is the F32 episome digested by *Pst*I plus *Hin*dIII (lane 3), *Pst*I (lane 4), or *Bst*EII (lane 5). (B) ^{32}P-labeled pX1 cleaved by *Bst*EII (lane 1) or *Pst*I (lane 2) was used as a marker. Also shown is the AB1111 chromosome digested by *Bst*EII (lane 3) and the F32 episome digested by *Bst*EII (lane 4).

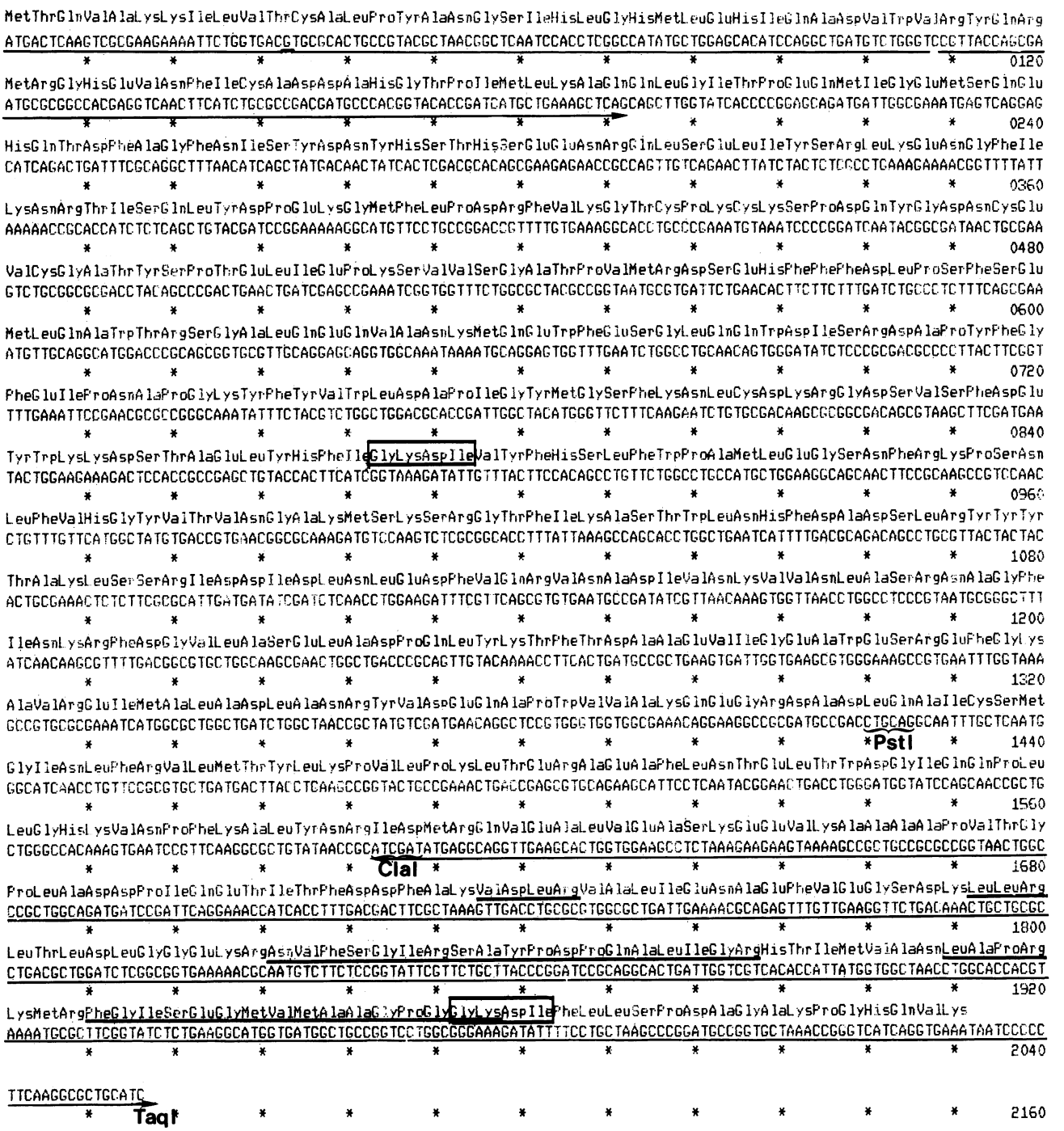

FIG. 5. Complete sequence of *metG*. The partial *metG* sequence of Barker et al. (2) is combined with the DNA sequences obtained in this study (underlined nucleotides of the figure). Underlined amino acids show the peptides reported by Barker et al. (2), which can be assigned on the 142 C-terminal residues of MTS. The repeated tetrapeptide is boxed.

agreement with recent measurements (2, 16). The amino acid composition deduced from the DNA sequence agreed well with that obtained from acid hydrolysis of the purified protein (11). The longest duplicated sequence was a tetrapeptide, Gly-Lys-Asp-Ile, boxed in Fig. 5.

***metG* rescue experiments.** The SB1803 strain carried an allele of *metG* encoding a mutant MTS with a 67-fold increased K_m for methionine and an unaltered K_m for tRNA and ATP (1). It was therefore reasonable to conclude that the mutation affected an amino acid involved in methionine recognition. This mutation should lie in the part of *metG* carried by pACSm because this plasmid rescued methionine auxotrophy in a *recA*$^+$ *metG83* strain. To map more precisely the mutation *metG83*, the following experiments were designed. Plasmid pX2 was submitted to limited cleavage by *Eco*RV. After ligation, deleted plasmids were used to transform PAL1803.5. Transformants were selected for tetracycline resistance, and plasmids were characterized by restric-

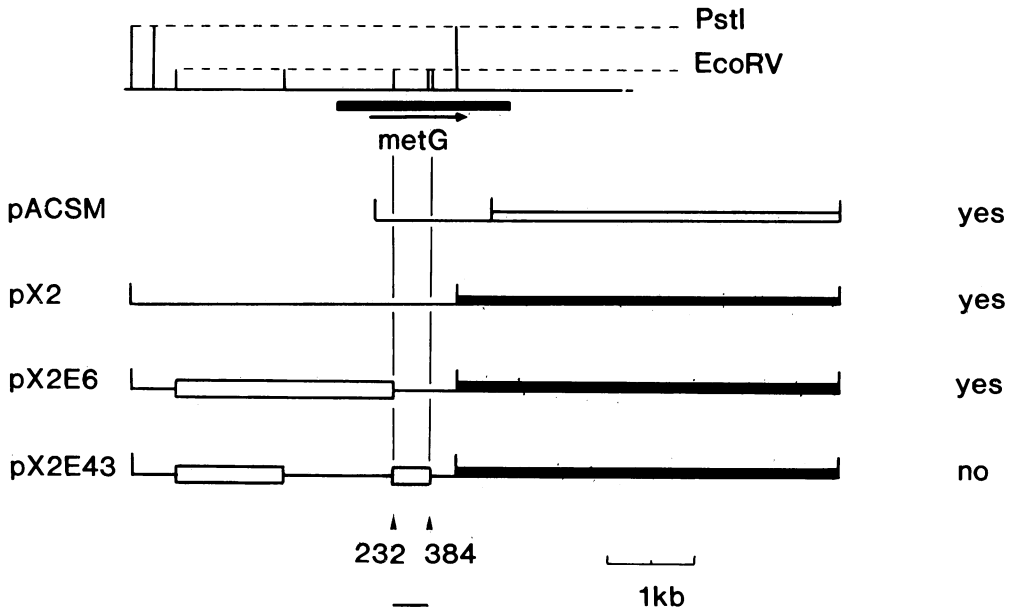

FIG. 6. Mapping of the *metG83* mutation. Restriction maps of pACSm and pX2 and of the deleted plasmids pX2E6 and pX2E43 are superimposed. The numbers correspond to positions of deletion endpoints in the amino acid sequence of MTS. Plasmids pACSm, pX2, and pX2E6 yielded Met^+ recombinants in strain SB1803 (*metG83 recA*$^+$), whereas pX2E43 did not.

tion mapping after small-scale purification (Fig. 6). Plasmid pX2 and the derived plasmids pX2E6 and pX2E43 were unable to complement the methionine auxotrophy of PAL1803.5 (*metG83 recA*$^-$). When introduced into the *recA*$^+$ *metG83* strain SB1803, pX2 and pX2E6 produced Met^+ recombinants at a frequency of 2×10^{-2}, significantly higher than the spontaneous *metG83* reversion rate ($\simeq 10^{-6}$). On the contrary, pX2E43 did not produce Met^+ recombinants. Therefore, the mutation on the SB1803 chromosome should lie in a region common to both pX2 and pX2E6 but not to pX2E43. This region was a 462-bp *Eco*RV fragment corresponding to residues 232 to 384 in the center of the MTS amino acid sequence (Fig. 6).

DISCUSSION

Plasmid pX1 carried three *Pst*I-*Pst*I episomal fragments and had *metG* complementation activity in a *recA*$^-$ background. Maxicell analysis showed that three major polypeptides with M_rs of 76,000, 37,000, and 29,000 were encoded by pX1. The 76,000-molecular-weight polypeptide had the same M_r as the promoter of MTS and could be immunoprecipitated by anti-MTS antibodies.

Fine mapping of *metG* was achieved by restriction analysis of the 15-kb insert of pX1. A DNA sequence of 642 nucleotides confirmed previous data from Barker et al. (2). It revealed a 2,031-bp open reading frame, accounting for a polypeptide with an M_r of 76,127 and corresponding to the structural part of *metG*.

DNA-DNA hybridization experiments showed that around *metG* the restriction map of pX1 was identical to those of episome F32 and the *E. coli* chromosome. This was confirmed by comparison of plasmid pX1 and plasmid pLC20-25 from the Clarke-Carbon chromosomal bank (12). The latter plasmid expressed MTS (25) and was able to transform PAL1803.5 to methionine prototrophy. We verified by restriction mapping that the pLC20-25 insert (9 ± 1 kb) overlapped 4.5 kb of pX1, including the *metG* gene (data not shown).

The hybridization experiments revealed that the F32 episome carried a second region homologous to *metG*. Repeated attempts to clone this region by complementation of *metG* strains were unsuccessful, suggesting that this homologous sequence did not carry another active *metG* copy. This result possibly reflects a partial *metG* duplication on the episome, which could be the result of the sex factor integration or excision process near the *metG* locus.

Previous work showed that 130 residues could be removed by limited proteolysis from the C terminus of the MTS protomer (11). The resulting fragment of 550 residues (64,000 molecular weight) retained full specificity and activities but no longer dimerized. This monomeric fragment was crystallized (30), and its crystal structure at 0.25 nm has been recently reported (31).

The MTS protomer is a biglobular molecule composed of three domains: the two N-terminal domains (residues 1 to 110 and 111 to 246) form the first globule, whereas the C-terminal domain (residues 284 to 480) forms the second globule. The most striking feature of three-dimensional structure is the presence in the N-terminal domain of a mononucleotide-binding fold or "Rossman fold," believed to be the binding site for ATP. The *metG83* rescue experiments described above strongly suggested that some residues between positions 232 and 384, close to or into the C-terminal domain, were essential for methionine recognition and thus participated to the active site of the enzyme. This conclusion is in agreement with recent affinity labeling studies which identify Lys 335 as the major residue labeled by 3'-oxydized $tRNA_f^{Met}$ (Hountondji et al., in press). Together these observations suggest that the C-terminal domain of the 64,000-molecular-weight MTS is also involved in the catalytic mechanism.

The importance of the C-terminal domain in the catalysis is further suggested by the analysis of the products encoded by plasmid pX2. One of them was shown to be a 52,000-molecular-weight polypeptide immunoprecipitated by anti-MTS antibodies. However, although this 52,000-molecular-weight polypeptide corresponded to the N-terminal structured part

(475 residues) of the 64,000-molecular-weight active MTS (550 residues) in the crystal structure (31), this product was inactive and was unable to complement the *metG83* mutation. It can therefore be concluded that the last 75 residues, which are thought to be disordered in the crystal, either participate directly to catalysis or are required for active structuration of the N-terminal domain.

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LITERATURE CITED

1. **Ahmed, A.** 1973. Mechanism of repression of methionine biosynthesis in *Escherichia coli*. I. The role of methionine, *S*-adenosyl methionine, and methionyl-tRNA in repression. Mol. Gen. Genet. **123**:299–324.
2. **Barker, D. G., J. P. Ebel, R. Jakes, and C. J. Bruton.** 1982. Methionyl-tRNA synthetase from *E. coli*: primary structure of the active crystallized tryptic fragment. Eur. J. Biochem. **127**:449–457.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Blanquet, S., P. Dessen, and G. Fayat.** 1979. Methionyl-tRNA synthetase from *E. coli*: structure-functions relationships of a dimeric enzyme with repeated sequences, p. 281–294. *In* T. Abelson, P. A. Schimmel, and D. Söll (ed.), tRNA structure, properties and recognition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Blanquet, S., G. Fayat, and J. P. Waller.** 1974. The mechanism of action of methionyl-tRNA synthetase from *E. coli*. Eur. J. Biochem. **44**:343–350.
6. **Blumenthal, T.** 1972. P1 transduction: formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. Virology **47**:76–85.
7. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
8. **Bruton, C. J.** 1979. Probing the substructure, evolution and interactions of aminoacyl-tRNA synthetase, p. 47–68. *In* J. D. Smith and J. Celis (ed.), Nonsense mutations and tRNA suppressors. Academic Press, London.
9. **Bruton, C. J., R. Jakes, and G. L. E. Koch.** 1974. Repeated sequences in methionyl-tRNA synthetase from *E. coli*. FEBS Lett. **45**:26–28.
10. **Cassio, D.** 1975. Role of methionyl-transfer ribonucleic acid in the regulation of methionyl-transfer ribonucleic acid synthetase of *Escherichia coli* K-12. J. Bacteriol. **123**:589–597.
11. **Cassio, D., and J. P. Waller.** 1971. Modification of methionyl-tRNA synthetase by proteolytic cleavage and properties of the trypsin modified enzyme. Eur. J. Biochem. **20**:283–300.
12. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *E. coli* genome. Cell **9**:91–99.
13. **Clewell, D. B.** 1972. Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
14. **Csonka, L. N., and A. J. Clark.** 1980. Construction of an Hfr strain useful for transferring *recA* mutations between *Escherichia coli* strains. J. Bacteriol. **143**:529–530.
15. **Davis, R. W., J. R. Roth, and D. Botstein.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Dessen, P., S. Blanquet, G. Zaccai, and B. Jacrot.** 1978. Anticooperative binding of initiator $tRNA^{Met}$ to methionyl-tRNA synthetase from *Escherichia coli*: Neutron scattering studies. J. Mol. Biol. **126**:293–313.
17. **Garrick-Silversmith, L., and P. E. Hartman.** 1970. Histidine requiring mutants of *Escherichia coli* K12. Genetics **66**:231–239.
18. **Koch, G. L. E., and C. J. Bruton.** 1974. The subunit structure of methionyl-tRNA synthetase from *Escherichia coli*. FEBS Lett. **40**:180–182.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature (London) **227**:680–695.
20. **Lowry, G. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. **193**:265–275.
21. **Maxam, M. and W. Gilbert.** 1980. Sequencing end-labeled DNA with base specific chemical cleavage. Methods Enzymol. **65**:499–550.
22. **McFall, E.** 1967. Position effect on dominance in the D-serine deaminase system of *Escherichia coli* K-12. J. Bacteriol. **94**:1989–1993.
23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Morgan, S., and D. Söll.** 1978. Regulation of the biosynthesis of amino acid tRNA ligases and of tRNA. Prog. Nucleic Acid Res. Mol. Biol. **21**:181–207.
25. **Neidhart, F. C., V. Vaughn, T. A. Phillips, and P. L. Bloch.** 1983. Gene-protein index of *Escherichia coli* K-12. Microbiol. Rev. **47**:231–284.
26. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. D. Clarke, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148**:45–62.
27. **Sanger, F., A. R. Coulson, B. G. Barell, A. J. H. Smith, and B. Roe.** 1980. Cloning in single stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–172.
28. **Schimmel, P., and D. Söll.** 1979. Aminoacyl-tRNA synthetases: general features and recognition of transfer RNA. Annu. Rev. Biochem. **48**:601–648.
29. **Smith, G. E., and M. D. Summers.** 1980. Bidirectional transfer of DNA and RNA to nitrocellulose or diazobenyzloxymethyl-paper. Anal. Biochem. **109**:123–129.
30. **Waller, J. P., J. L. Risler, C. Monteilhet, and C. Zelwer.** 1971. Crystallization of trypsin-modified methionyl-tRNA synthetase from *Escherichia coli*. FEBS Lett. **16**:186–188.
31. **Zelwer, C., J. L. Risler, and S. Brunie.** 1982. Crystal structure of *Escherichia coli*. Methionyl-tRNA synthetase at 2.5 Å resolution. J. Mol. Biol. **155**:63–81.