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# Transductional Mapping of Gene *trmA* Responsible for the Production of 5-Methyluridine in Transfer Ribonucleic Acid of *Escherichia coli*

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The gene *trmA*, responsible for the production of 5-methyluridine (ribothymidine) in transfer ribonucleic acid, has been located at 79 min on the chromosomal map of *Escherichia coli* K-12. In five-factor crosses the gene order was shown to be *argH-trmA-rif-thiA-metA*. The co-transduction frequency between *argH* and *trmA* was 65%. Furthermore, the *trmA5* mutation was shown to be recessive, in agreement with the notion that the *trmA* gene is the structural gene for the transfer ribonucleic acid (5-methyluridine)methyltransferase.

Transfer ribonucleic acid (tRNA), deoxyribonucleic acid, and ribosomal RNA (rRNA) contain modified nucleosides that are absent from 5*S* rRNA and procaryotic messenger RNA (11). One of the most common methylated nucleosides is 5-methyluridine (ribothymidine) ($m^5U$, abbreviation recommended by the Commission of Biochemical Nomenclature) which has been suggested to occur in one copy per tRNA chain (29). We have previously isolated mutants (*trmA*) that were defective in the production of $m^5U$ in the tRNA (3). These mutants were isolated after heavy mutagenic treatment with ethylmethanesulfonate. In addition to the *trmA* they could contain other mutations that might affect the expression of the methylation defect or modify the consequence to the cell of the loss of $m^5U$. It was therefore necessary to develop pairs of strains that were isogenic except for the *trmA* locus.

The best way to construct isogenic pairs is to isolate true spontaneous revertants. Since $Trm^+$ revertants could not be selected for, we instead had to transduce the *trmA* gene from our original mutants to other strains of *Escherichia coli*. This requires knowledge about the approximate location of the *trmA* gene. By conjugation experiments it was shown to lie between *metB* (78 min) and *ampA* (83 min) on the *E. coli* K-12 chromosomal map (3).

In this paper, the *trmA* gene is shown to be located close to and clockwise from the *argH* gene. In five-factor crosses the gene order was shown to be *argH-trmA-rif-thiA-metA*. The co-transduction frequency between *argH* and *trmA* was 65%. Furthermore, the *trmA5* mutation was shown to be recessive. This result is in agreement with our assumption that the *trmA* gene is the structural gene for the tRNA-($m^5U$)methyltransferase(EC 2.1.1.35; i.e., the tRNA methyltransferase producing $m^5U$).

(A preliminary report of the initial part of this investigation was presented at the Colloquium on tRNA and tRNA Modification in Differentiation and Neoplasia, Washington, D.C., 5-7 October 1970 [4].)

## MATERIALS AND METHODS

**Strains of bacteria and phages.** Several strains of *E. coli* K-12 have been used in this work. Their properties and origin are shown in Table 1. Strain GB1Rif$^R$ is a spontaneous rifampin-resistant mutant of strain AB1932 which was isolated for its ability to grow in the presence of 100 μg of rifampin per ml. Strain GB1-5-751 (*argH, trmA5, thiA, metA*$^+$) is a transductant, obtained from an experiment using strain IB5 (*argH*$^+$*, trmA5, thiA, metA*$^+$) as donor and strain AB1932 (*argH, trmA*$^+$*, thiA*$^+$*, metA*) as recipient. In transduction experiments, either phages P1kc or P1v were used.

**Media and growth conditions.** The minimal medium was made from basal medium E of Vogel and Bonner (28), supplemented with 0.2% glucose, thiamine (1 μg/ml), and the required amino acids (25 μg of the L-epimer per ml). In experiments where transductants were to be analyzed for a thiamine requirement, the medium was supplemented with 0.003 μg of thiamine per ml. Plates were solidified with 1.5% agar. The complete medium used was LB of Bertani (2) supplemented with medium E, thiamine (1 μg/ml), and 0.2% glucose.

**Transduction.** For transduction the procedure of Lennox (16) was followed, but a 27-min period at 37 C was used for deoxyribonucleic acid injection. Multiplicity of infection was either 1.0 or 0.2. Prototrophic transductants among amino acid auxotrophs were selected.

TABLE 1. *E. coli K-12 strains used for genetic experiments*

| Strains | *trm* allele | Other relevant markers | Source or reference |
|---|---|---|---|
| CP79 | + | *thiA*[a] | (8) |
| IB4 | A4 | *thiA* | (3) |
| IB5 | A5 | *thiA* | (3) |
| IB6 | A6 | *thiA* | (3) |
| IB9 | A9 | *thiA* | (3) |
| IB10 | A10 | *thiA* | (3) |
| AB1932 | + | *argH, metA* | —[b] |
| GB1Rif[R] | + | *argH, rif, metA* | This paper[c] |
| GB1-5-751 | A5 | *argH, thiA* | This paper[c] |
| LMUR | + | *lys, ura, metB, RNaseI⁻ rel* | (26) |
| AB1450 | + | *thiA, ilvD16, argH1, metB1, his-1, strA* | —[d] |
| KLF10/JC1553 | + | KLF10/*argG6, metB1, his-1, leu-6, recA1, strA*. Episome KLF10: PO18 of J4 in *malB* locus covering *metB⁺-argH⁺-thi⁺-malB⁺* | —[d] |
| AB2587 | + | *ilvD188, argH1, his-45, supM20* | —[d] |

[a] According to B. Bachman, strain CP79 is derived from strain W677 (chart 2, reference 1) and thus carries the *thi-1* allele of strain Y10. The same *thi-1* allele is also found in AB1450 (B. Bachman, personal communication). The latter strain has been shown to be auxotrophic for the thiazole moiety of thiamine (T. Kawasaki, and Nose Y., quoted in [15]). Thus, the *thi-1* mutation is likely to have occurred in the *thiA* gene (27).

[b] The *arg* lesion was shown to be *argH* by a feeding test and from genetic data (9, 14, 21). This has also been verified from another laboratory (25).

[c] See Materials and Methods.

[d] Kindly obtained from B. J. Bachman, *E. coli* Genetic Stock Center, Department of Microbiology, Yale University, School of Medicine, New Haven, Conn.

**Scoring of Rif, Thi, and Trm phenotypes.** ArgH⁺ or MetA⁺ transductants were transferred into 0.5 ml of selective medium containing a low level of thiamine (0.003 μg/ml) and incubated at 37 C overnight in microculture containers consisting of 25 wells (Bertani, personal communication; see also reference 6). The low level of thiamine was used to prevent carry over of thiamine when replica plating was made. After incubation, drops were diluted in 0.9% NaCl by using a replicator consisting of 25 steel needles. Replica plating was then performed on plates containing selective medium (control plates) and plates lacking the metabolite to be tested. In this way even the *thiA* marker was easily scored. Determination of the Rif phenotype can be misjudged. We found that many rifampin-sensitive (Rif[S]) clones could grow on plates containing rich medium and 100 μg of rifampin per ml with or without ethylenediaminetetraacetic acid most probably owing to an inoculum effect. The Rif phenotype was determined by transferring colonies to plates that contained selective medium and 100 μg of rifampin per ml. Those colonies that were able to grow on rifampin-containing plates were further restreaked on such plates. Only clones which still were able to grow when restreaked were designated rifampin resistant (Rif[R]). In fact, all clones, initially judged as Rif[R] on selective medium containing rifampin were found to grow when restreaked.

The Trm phenotype was scored in the following way (suggested by L. Isaksson, Uppsala, Sweden). Cells from colonies on plates containing selective medium (so-called control plates, see above) were grown in 2 ml of rich medium. The cultures were incubated overnight, washed once in 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.0, containing 10 mM magnesium acetate, and suspended in 200 μl of the same buffer supplemented with lysozyme (200 μg/ml) and deoxyribonuclease I from beef pancreas (1× crystallized, 20 μg/ml). The suspension was frozen and thawed three times, incubated for 5 min at 37 C, and finally placed in a boiling water bath for 5 min. These preparations were assayed for methyl-accepting activity of the RNA by adding 50 μl of a fivefold-concentrated assay mixture containing *S*-adenosyl-L-[*methyl*-$^{14}$C]methionine (52 or 58 mCi/mM; 2.6 to 2.9 nmol/ml final reaction volume) and 5 μl of enzyme extract, prepared as described below. The final concentration of the assay mixture is as described in the companion paper (5). The mixture was incubated for 2 h at 30 or 37 C. The reaction was then stopped with 2 ml of 0.01 M $La(NO_3)_3$ in 0.5 M $HClO_4$. The precipitates were washed directly on glass-fiber filter paper (Whatman GF/C) with 10 ml of cold 5% trichloroacetic acid and 20 ml of acetone. The incorporation into RNA from a Trm⁻ culture was five to 10 times that from a Trm⁺ culture, which was between 100 to 200 counts/min. Each experiment always included duplicates of known Trm⁺ and Trm⁻ cells as controls; representative incorporations in such controls are given in the text footnotes to Table 6.

In earlier experiments (Table 5) the Trm⁻ property was screened among *metA⁺-thiA⁺* transductants as follows. Individual colonies were grown in 2 ml of rich medium overnight and five such cultures were combined. Cells were washed, and total RNA (rRNA, 5S

rRNA, and tRNA) was prepared by freezing and thawing in the presence of lysozyme and deoxyribonuclease (1× crystallized), followed by phenol extraction. The RNA, which thus originated from five different clones, were precipitated twice with ethanol and assayed for methyl group acceptor activity (level 1). Those RNA preparations that had methyl group acceptor activity were then chosen, and the component strains were tested individually (level 2). Those showing incorporation were cloned three times and tested again (level 3). Details of this procedure have been described before (3).

**Preparation of enzyme extract, used for determining Trm phenotype.** Three grams of washed frozen cells of strain LMUR or frozen spheroplasts of strain AB1932 were thawed and suspended in 10 ml of cold 50 mM Tris-hydrochloride buffer (pH 7.5). The suspension was made 5 mM in 2-mercaptoethanol, 1 mM in magnesium acetate, and 10% in ethylene glycol and then electrophoretically purified deoxyribonuclease I from beef pancreas was added (2 $\mu$g/ml). The cells or spheroplasts were disintegrated according to French and Milner (10). The extract (11 ml) was incubated at 37 C for 10 min and then diluted with 12 ml of 50 mM Tris-hydrochloride buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 10 mM magnesium acetate, and 10% ethylene glycol. The solution was centrifuged at 20,000 × *g* for 15 min, the pellet was discarded, and the supernatant was centrifuged at 270,000 × *g* for 1 h and the ribosomal pellet was discarded. Streptomycin (20 mg/ml) was added to the supernatant, and the precipitation was removed by centrifugation. After a 40 to 70% ammonium sulfate fractionation, the enzyme preparation was dialyzed for 16 h against 10 mM Tris-hydrochloride buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, and 10% ethylene glycol. The enzyme extract was made 50% in ethylene glycol and stored at −20 C. Such an enzyme preparation has an absorbancy at 280 nm-to-absorbancy at 260 nm ratio of about 1.0, which indicates a nucleic acid content of about 3%. Protein concentration was about 8 mg/ml, measured by the method of Lowry et al. (17) using bovine serum albumin as standard. The tRNA($m^5U$)methyltransferase is rather stable under these conditions and retains 50% of its activity during about 3 months of storage.

**Determination of the recessiveness of the trmA5 mutation.** Strain GB1-5-751 (*argH-trmA5-thiA-metA*$^+$) was grown overnight in medium LB at 37 C. Strain KLF10/JC1553 (JC1553: *argG6, metB, his, leu, recA, str*$^r$ and carrying episome KLF10 which covers the region *metB*$^+$-*argH*$^+$-*trmA*$^+$-*thiA*$^+$-*malB*$^+$) was grown at 37 C overnight in medium E supplemented with glucose, thiamine, leucine, arginine, and histidine, but lacking methionine. Strains GB1-5-751 and KLF10/JC1553 were grown in LB medium for two generations and then mixed at a cell density of $2 \times 10^8$ cells/ml in a ratio of 1:1. After 60 min of incubation, samples were taken out and plated on agar containing medium E and glucose. Cells of GB1-5-751 that had acquired the episome were then selected (frequency of about $10^{-5}$). Colonies were transferred into 3 ml of the selective medium (medium E and glucose) and grown ovenight. Part of each culture (2.5 ml) was tested for the Trm property as described above.

Another part (0.5 ml) of each culture was tested for ability to transfer the episome. This was performed in the following way. Strain AB1450 (*ilv, metB, argH, thi, his, strA*) was grown overnight in medium LB and the cells were washed in 0.9% NaCl. About $1.5 \times 10^9$ cells were transferred to plates containing medium E, glucose isoleucine, valine, histidine, and streptomycin. Cells to be tested for ability to transfer the KLF10 episome were inoculated on small areas of these plates and on plates without AB1450 cells. Ability to transfer was then recorded as growth on plates with these cells. It was also checked that growth of AB1450 alone on these plates did not occur. Thus, growth indicated that the episome of KLF10, covering at least *metB-argH-trmA-thi*, was transferred.

## RESULTS

We have isolated mutant strains of *E. coli* that have little or no $m^5U$ in their tRNA (3). They are designated *trmA*. Since the mutation that had occurred in mutant strain IB5 (*trmA5*) resulted in a total loss of $m^5U$ in the tRNA, we decided to start the genetic analysis by mapping the allele *trmA5* carefully. Initial transduction experiments indicated that this allele was located between *argH* and *thiA*. This region also includes the *rif* gene (27). To map the *trmA* gene in relation to the *rif* gene, we isolated a spontaneous rifampin-resistant mutant of AB1932 that was able to grow in the presence of 100 $\mu$g of rifampin per ml. In a transductional cross this strain GB1Rif$^R$ (*argH, trmA*$^+$, *rif, thiA*$^+$, *metA*) was used as recipient and strain IB5 (*argH*$^+$, *trmA5, rif*$^+$, *thiA, metA*$^+$) was used as a source of P1v lysate. MetA$^+$ and ArgH$^+$ transductants were selected separately. The analysis of 177 MetA$^+$ transductants is given in Table 2. The results suggest the gene order *trmA5-rif-thiA-metA*. Among all the MetA$^+$ transductants none was ArgH$^+$. When 153 ArgH$^+$ transductants were analyzed (Table 3), all five genes involved segregated. Only four of the possible 16 groups were lacking. The data in Table 3 suggest that the gene order is *argH-trmA5-rif-thiA-metA*. We thus conclude that the *trmA5* allele lies between the *argH* and *rif* genes.

Data from the transduction experiments mentioned above were combined with data from a similar transduction experiment in which strain AB1932 was recipient. Altogether, 476 Met$^+$ and 301 Arg$^+$ transductants were analyzed, and the co-transduction frequencies be-

TABLE 2. *Mapping of the trmA gene by P1 transductions: properties of MetA⁺ transductants in a five-factor cross*[a]

| Progeny genotype | | | | | No. of colonies | % of total scored |
|---|---|---|---|---|---|---|
| *argH* | *trmA* | *rif* | *thiA* | *metA* | | |
| R | D | D | D | D | 40 | 23 |
| R | R | D | D | D | 46 | 26 |
| R | D | R | D | D | 7 | 4 |
| R | R | R | D | D | 23 | 13 |
| R | D | D | R | D | 1 | 0.6 |
| R | R | D | R | D | 7 | 4 |
| R | D | R | R | D | 7 | 4 |
| R | R | R | R | D | 46 | 26 |

[a] Phage P1v was grown on strain IB5 (*argH*$^+$, *trmA5*, *rif*$^+$, *thiA*, *metA*$^+$). Recipient was strain GB1Rif$^R$ (*argH*, *trmA*$^+$, *rif*, *thiA*$^+$, *metA*). MetA$^+$ transductants were selected, and the different unselected markers were analyzed as described in Materials and Methods. D, Donor phenotype; R, recipient phenotype.

TABLE 3. *Mapping of the trmA gene by P1 transduction: properties of ArgH⁺ transductants in a five-factor cross*[a]

| Progeny genotype | | | | | No. of colonies | % of total scored |
|---|---|---|---|---|---|---|
| *argH* | *trmA* | *rif* | *thiA* | *metA* | | |
| D | D | D | D | D | 10 | 7 |
| D | D | D | D | R | 16 | 10 |
| D | D | D | R | D | 5 | 3 |
| D | D | D | R | R | 13 | 9 |
| D | D | R | D | D | 0 | |
| D | D | R | D | R | 4 | 3 |
| D | D | R | R | D | 5 | 3 |
| D | D | R | R | R | 60 | 39 |
| D | R | D | D | D | 0 | |
| D | R | D | D | R | 3 | 2 |
| D | R | D | R | D | 2 | 1 |
| D | R | D | R | R | 3 | 2 |
| D | R | R | D | D | 0 | |
| D | R | R | D | R | 0 | |
| D | R | R | R | D | 3 | 2 |
| D | R | R | R | R | 29 | 19 |

[a] Phage P1v was grown on strain IB5 (*argH*$^+$, *trmA5*, *rif*$^+$, *thiA*, *metA*$^+$). Recipient was strain GB1Rif$^R$ (*argH*, *trmA*$^+$, *rif*, *thiA*$^+$, *metA*). ArgH$^+$ transductants were selected, and the different unselected markers were analyzed as described in Materials and Methods. D, Donor phenotype; R, recipient phenotype.

tween the genes *argH*, *metA*, *trmA*, *rif*, and *thiA* are summarized in Fig. 1. The *trmA5* allele lies clockwise and very close to the *argH* gene. The co-transduction frequency between the *argH* and *trmA5* allele was 65%. The gene order mentioned earlier is in agreement with the data given in Fig. 1.

**Gene localization of different trmA alleles.** Of the seven mutant strains (IB1, IB4, IB5, IB6, IB8, IB9, and IB10) originally isolated, all were deficient in $m^5U$ in the tRNA, but to a varying degree (3). We wanted to learn whether several genes were involved in the $m^5U$ deficiency in the tRNA, or whether all mutations occurred in the same region. Therefore, phage P1 was grown on four of the isolated mutants, namely, strains IB4, IB6, IB9, and IB10. Strain AB1932 or GB1Rif$^R$ was used as a recipient. Tables 4 and 5 show that the mutations in the four strains are localized in the same area as the *trmA5* allele. However, when strain IB10 was used as donor, the co-transduction frequency between the *argH*$^+$ and *metA*$^+$ genes was unexpectedly high. This observation does not influence the conclusion about the common location of the different *trmA* alleles.

**Dominance or recessiveness of the trmA5 mutations.** If the *trmA* gene is the structural gene for the tRNA($m^5U$)methyltransferase, it should be recessive. Extracts from *trmA* mutants show no tRNA($m^5U$)methyltransferase activity when measured with $m^5U$-deficient tRNA's as substrates (3). These and other results suggest that the tRNA($m^5U$)methyltransferase was mutated. The dominance or recessiveness of the *trmA5* mutation was determined in order to strengthen such a hypothesis. Strain GB1-5-751, which is *argH-trmA5-thiA-metA*$^+$, was crossed with strain KLF10/JC1553, which carries an episome covering the region *metB*$^+$-*argH*$^+$-*trmA*$^+$-*thi*$^+$-*malB*$^+$, with *malB*$^+$ as the origin of the episome. Cells of strain GB1-5-751, which could grow without arginine and thiamine and thus had acquired the episome, were selected. Such cells were grown in

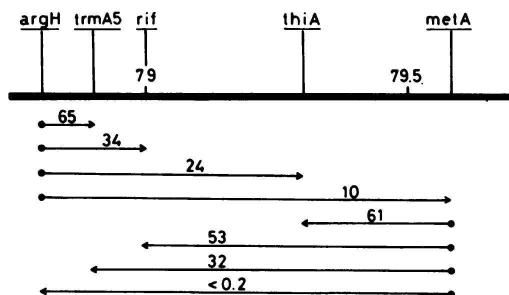


FIG. 1. *Genetic map of the trmA region, with time indications and position of earlier known genes according to Taylor and Trotter (27). The co-transduction frequencies given in percent are from Table 4.*

TABLE 4. *Mapping of trmA6 and trmA10 by P1 transduction*[a]

| Donor | | Recipient | No. of transductants tested | Co-transduction frequencies (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | *trm* allele | | | *argH* | *trmA* | *rif* | *thi* | *metA* |
| IB5 | A5 | AB1932 and | 476 | <0.2 | 32 | 53 | 61 | * |
| | | GB1Rif$^R$ | 301 | * | 65 | 34 | 24 | 10 |
| IB6 | A6 | AB1932 | 59 | * | 61 | ND | 34 | 3 |
| IB10 | A10 | GB1Rif$^R$ | 95 | <1 | 28 | 41 | 60 | * |
| | | | 31 | * | 52 | 48 | 13 | 48 |

[a] Phage P1v was grown on each of the donors listed, which possess the indicated *trmA* allele. They are all *argH*$^+$, *rif*$^+$, *thiA*, and *metA*$^+$. Recipient was either strain AB1932 (*argH*, *trmA*$^+$, *rif*$^+$, *thiA*$^+$, *metA*) (donor strains IB5 and IB6) or strain GB1Rif$^R$ (donor strains IB5 and IB10), which is a spontaneous rifampin-resistant mutant of strain AB1932. In the case of strain IB5, data from transduction experiments with either strain AB1932 (299 MetA$^+$ and 148 ArgH$^+$ transductants) or strain GB1Rif$^R$ (177 MetA$^+$ and 153 ArgH$^+$ transductants) are combined. Thus, the co-transduction frequency between *argH*$^+$-*rif*$^+$ and *metA*$^+$-*rif*$^+$ is based only on data obtained with the recipient strain GB1Rif$^R$. *, Indicates the selected markers; ND, not determined.

TABLE 5. *Mapping of the trmA4 and trmA9 alleles by transduction*[a]

| Donors | *trm* allele | Selected markers | | | |
|---|---|---|---|---|---|
| | | *metA*$^+$-*thiA*$^+$ | | *argH*$^+$-*thiA*$^+$ | |
| | | No. of colonies tested | % Trm$^-$ | No. of colonies tested | % Trm$^-$ |
| IB4 | A4 | 180 | 2–9 | 4 | 0 |
| IB5 | A5 | 225 | 2–6 | 32 | 56 |
| IB9 | A9 | 188 | 2–3 | 7 | 71 |

[a] Phage P1kc was grown on each of the donors listed, which possess the indicated *trm* alleles, and they are all *metA*$^+$, *thiA*, and *argH*$^+$. Recipient in all experiments was strain AB1962, which is *argH*, *thiA*$^+$, and *metA*. The highest percentage of co-transduction between MetA$^+$ and Trm$^-$ is on level 1 of screening and the lowest is on level 3 (see Materials and Methods). The *argH*$^+$-*thiA*$^+$ transductants were tested individually for the Trm$^-$ property. All *metA*$^+$-*thiA*$^+$ transductants found were Arg$^-$, and all *argH*$^+$-*thiA*$^+$ transductants were Met$^-$.

TABLE 6. *Dominance/recessive test for trmA mutation*[a]

| Methyl group incorporation level (counts/min) | No. of colonies | Ability to transfer (%) |
|---|---|---|
| 150 | 58 | 100 |
| 150–200 | 14 | 100 |
| 200–300 | 2 | 100 |
| 300–350 | 1 | 100 |
| 350 | 0 | |

[a] Strain GB1-5-751 (*argH-trmA5-thiA-metA*$^+$) was incubated with strain KLF10/JC1553. This strain carried the episome KLF10 which covers the *metB* to *malB* region and which is *metB*$^+$-*argH*$^+$-*thiA*$^+$-*malB*$^+$ (origin). Selection was made for cells of GB1-5-751 which had acquired the episome, and 75 colonies were picked and tested as described in Materials and Methods. Control experiments with five samples of strain GB1-5-751 (Trm$^-$) incorporated 1,024 to 1,341 counts/min (average, 1,120 counts/min), and five samples of strain GB1-5-41 (Trm$^+$) incorporated 93 to 124 counts/min (average, 112 counts/min).

selective medium. The presence of the episome (ability to simultaneously transfer *metB*$^+$, *argH*$^+$, and *thiA*$^+$) was tested in one part of each culture, and in the other part the Trm phenotype was determined. All of the colonies tested contained the episome KLF10, which indicated that they were *trmA*$^+$/*trmA5* heterozygotes (Table 6). Such clones had at the same time become Trm$^+$.

A few clones incorporated a significantly higher amount of methyl groups than the control, which has the Trm$^+$ phenotype. The recipient strain GB1-5-751 is not *recA*, and therefore it is likely that in some cells some of the tested cultures contained a few cells in which recombination with the episome had occurred. Such cells are therefore haploid for the *trmA* gene. However, the majority (78%) of the colonies tested had become Trm$^+$ and had acquired the episome. Thus, they were *trmA*$^+$/*trmA5* heterozygotes. These results strongly suggest that the *trmA* mutation is recessive.

## DISCUSSION

All the results in Tables 2 and 3 and in Fig. 1 are most easily reconciled with the gene order *argH-trmA5-rif-thiA-metA*, in which the order of the earlier known genes are in agreement with

Taylor and Trotter (27). Using the same recipient, strain AB1932, and selecting for $MetA^+$ transductants, Jasper et al. (14) obtained a 12% co-transduction frequency between *metA* and *argH*. When we selected for $MetA^+$ transductants, we found no co-transduction between *metA* and *argH* (<1/476), but when selecting for $ArgH^+$ transductants we obtained about 10% co-transduction between this gene and *metA* (Fig. 1). The discrepancy between these results is unclear to us.

The *trmA* gene is responsible for the formation of $m^5U$ in the tRNA. Three additional genes, *bfe*, *glyT*, and *supM* (*Sup*15B), are known to be located in the region *argH-rif* (7, 13, 14, 22). Two of these are concerned with the biosynthesis of tRNA, namely, *glyT*, the structural gene for $tRNA^{GLY}_{GGA/G}$ and *sup15B* allelic to the *supM* mutation, mediating the ability to read the UAG (ochre) codon (13, 22). A strain carrying the *supM* mutation (AB2587) had the normal level of $m^5U$ in its tRNA (results not shown). Thus the *trmA* and *supM* genes are not identical.

The locations of a few genes coding for other enzymes involved in tRNA modification are known. In *Salmonella typhimurium*, *hisT* mutants are defective in the enzyme responsible for the production of pseudouridine in the anticodon stem of tRNA (23). Furthermore, mutants defective in the biosynthesis of 7-methylguanosine (*trmB*) and 2-thio-5-methyl-aminomethyluridine (*trmC*) have been isolated, and the corresponding genes have been located close to *proA* and *thyA*, respectively (20). Mutants defective in rRNA methylation have been isolated by us, but so far they have not been mapped (3). Mutation to kasugamycin resistance (*ksgA*) has been shown to affect the formation of 6-dimethyladenine in rRNA (12, 24). The *ksgA* gene lies close to 1 min on the chromosomal map of *E. coli* and is thus not located in the vicinity of the *trmA* gene (24). Marinus and Morris (19) have isolated mutants defective in the production of 6-methyladenine or 5-methylcytosine in the deoxyribonucleic acid. These mutations lie at 65 and 37.5 min, respectively, and, thus, far from the *trmA* mutation (18). Thus, no other known mutation that affects a modifying enzyme lies near the *trmA* gene at 79 min.

The only methylated compound found to differ in both the originally isolated mutants and in transductants was $m^5U$ in the tRNA (3, 5). Enzyme extracts from $Trm^-$ transductants as well as from the originally isolated mutants contained no tRNA($m^5U$)methyltransferase activity when the substrate was tRNA from the same strain or from other $Trm^-$ strains (3, 5). Under the same conditions enzyme extract from $Trm^+$ cells produced $m^5U$ in vitro. Furthermore, when enzyme extracts from $Trm^+$ and $Trm^-$ cells were mixed, the specific activity of the tRNA($m^5U$)methyltransferase was not more influenced than expected from dilution by $Trm^-$ extract (results not shown). Genetic experiments have shown that the same gene is likely to be mutated in strains IB4, IB5, IB6, IB9, and IB10 (Tables 4 and 5). The *trmA5* mutation was shown to be recessive (Table 6), and from the biochemical data mentioned above it is likely that all the originally isolated mutants are mutated in the same gene, which might then be the structural gene for the tRNA($m^5U$)methyltransferase. We have therefore tentatively designated that gene *trmA* and the alleles *trmA4*, *trmA5*, *trmA6*, *trmA9*, and *trmA10* from strains IB4, IB5, IB6, IB9, and IB10, respectively. Data presented here are in agreement with the suggestion that there is only one tRNA($m^5U$)methyltransferase in *E. coli* (3).

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