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# Transfer Ribonucleic Acid from *Mycoplasma**

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ABSTRACT: Transfer ribonucleic acid from three strains of *Mycoplasma* (*Mycoplasma laidlawii* B, *Mycoplasma gallisepticum* A 5969, and *Mycoplasma sp.* (Kid)) was studied. These transfer ribonucleic acids have sedimentation coefficients similar to that of *Escherichia coli* transfer ribonucleic acid as judged by cosedimentation of *E. coli* and *Mycoplasma* seryl transfer ribonucleic acid. *Mycoplasma* transfer ribonucleic acids contain minor nucleosides in lower amounts than are found in *E. coli* transfer ribonucleic acid. All three *Mycoplasma* strains investigated contain *N*-formylmethionyl transfer ribonucleic acid, which can be formed with formyltetrahydrofolate as formyl donor by both homologous or *E. coli* enzymes.

Chromatography of *M. laidlawii* B transfer ribonucleic acid on benzoylated DEAE-cellulose cleanly separates formylmethionyl transfer ribonucleic acid and methionyl transfer ribonucleic acid. In a transfer ribonucleic acid dependent cell-free amino acid incorporating system from *E. coli* directed by polyuridylic or f2 ribonucleic acid, *Mycoplasma* transfer ribonucleic acids stimulate polypeptide formation.

The smallest free-living cells that have been investigated are pleuropneumonia-like organisms (*Mycoplasma*) (Morowitz, 1966; Klieneberger-Nobel, 1962). *Mycoplasma* cells are much smaller than bacteria and possess a genome about one-quarter as large as *Escherichia coli*. In view of the small size of these organisms, it was of interest to examine more closely their tRNA to see whether it would exhibit special characteristics. The *Mycoplasma* strains used for the study were *Mycoplasma gallisepticum* and *Mycoplasma laidlawii* on which a large amount of biochemical work has been done, and *Mycoplasma sp.* (Kid) whose DNA has an unusually low content of G and C (Bode, 1966).

The present work shows that *Mycoplasma* tRNA has physical properties similar to *E. coli* tRNA. Minor nucleosides are constituents of *Mycoplasma* tRNA; however, they are present in lower amounts than in *E. coli* tRNA (see also Hall *et al.*, 1967). The three *Mycoplasma* strains investigated contain fMet-tRNA. *Mycoplasma* tRNA stimulates polypeptide formation in a tRNA-dependent amino acid incorporating system directed by poly U or f2 RNA.

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## Materials and Methods

*General.* Uniformly labeled L-[$^{14}$C]amino acids were obtained commercially and had the following specific activities (millicuries per millimole): leucine, 248; methionine, 233; phenylalanine, 366; serine, 160; tyrosine, 475; and valine 190. L-[$^{3}$H]Serine had a specific activity of 3.73 Ci/mmole. Formyltetrahydrofolic acid and [$^{3}$H]formyltetrahydrofolic acid were prepared as described previously (Ghosh *et al.*, 1967). f2 RNA was prepared according to Engelhardt *et al.* (1965). BD-cellulose[1] was prepared according to Gillam *et al.* (1967). T2-RNase, snake venom phosphodiesterase, and *E. coli* alkaline phosphatase were obtained from Worthington Biochemical Corp.

*Counting and Scanning of Radioactivity.* Paper disks or paper chromatograms (after cutting into 1-cm strips) were counted in a Packard Tri-Carb liquid scintillation counter (Model 3320). The scintillation medium consisted of 2,5-diphenyloxazole (4 g) and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene (0.1 g) in toluene (1 l.). The counting efficiency for $^{3}$H was 4–6%, and that for $^{14}$C was around 65%.

*Paper Chromatography.* The descending technique was used at room temperature with Whatman No. 1 paper. Solvent systems used were solvent A, isobutyric acid–0.5 M ammonium hydroxide (5:3, v/v); solvent B, isopropyl alcohol–concentrated hydrochloric acid–water (70:15:15, v/v); and solvent C, 1-butanol–glacial acetic acid–water (78:5:17, v/v).

Elution of ultraviolet-absorbing material from paper was carried out with 75 mM ammonium hydroxide. In all cases a corresponding area from a blank chromatogram was also eluted and used as control. Optical density units[2] were measured against the appropriate blanks at neutral pH.

*Paper Electrophoresis.* This was performed on Whatman No. 1 paper with a voltage gradient of 50 V/cm in 0.05 M sodium acetate buffer (pH 3.5). The paper was immersed in Varsol which was water cooled.

*Base Analysis.* The base composition of tRNA was determined by Dowex 50 chromatography following the method of Katz and Comb (1963). The assay was carried out twice (on samples of 20 $A_{260}$ units) for each tRNA, and the average value is given in Table I.

*Identification of Minor Nucleosides in tRNA.* Minor nucleosides from a tRNA digest (with snake venom phosphodiesterase and bacterial alkaline phosphatase) were identified by their ultraviolet spectra and their elution position on Dowex 50, as described by Uziel *et al.* (1968). Ribothymidylic acid was detected by two-dimensional paper chromatography in solvents A and B of a T2 RNase digest of 25 $A_{260}$ units of tRNA. In this system ribothymidylic acid is separated from the four major mononucleotides (Nishimura *et al.*, 1967).

*Organisms and Culture Media.* The three *Mycoplasma* strains, *M. laidlawii* B, *M. gallisepticum* A 5969, and *M. sp.* (Kid), used in this study were kindly given by Dr. H. Morowitz. They were grown to late-log phase at 37° in 2-l. flasks without shaking, in the following media: beef heart (Morowitz and Terry, 1969) medium (*M. laidlawii*) and tryptose (Razin *et al.*, 1965) medium (*M. gallisepticum* and Kid). *E. coli* K12 (strain CA244; Brenner and Beckwith, 1965) was grown in nutrient broth and harvested in late-log phase.

TABLE I: Base Composition of Unfractionated Kid and *E. coli* tRNA.[a]

| | Kid | *E. coli* (CA 244) |
|---|---|---|
| U + rT + ψ | 24.3 | 22.0 |
| G | 29.2 | 32.0 |
| A | 22.0 | 19.5 |
| C | 24.6 | 26.2 |

[a] For details, see Materials and Methods.

*tRNA and AA-tRNA Synthetases.* Frozen, washed *Mycoplasma* cells were suspended in a buffer containing 0.01 M magnesium acetate, 0.001 M Tris-chloride (pH 7.5), 0.2 M β-mercaptoethanol, and 0.1% sodium dodecyl sulfate. The mixture was shaken with eight-tenths volume of phenol for 45 min. The upper phase was reextracted with five-tenths volumes of phenol for 5 min. The remaining steps were as described by Zubay (1962) including a final DEAE-cellulose step. The yield of tRNA was 0.5–2 mg/g wet weight of cells.

The mixture of *Mycoplasma* AA-tRNA synthetases (free from tRNA) was prepared as previously described for *E. coli* AA-tRNA synthetases (Söll *et al.*, 1967) and was stored in 50% glycerol at −20°.

*E. coli tRNA and AA-tRNA Synthetases.* Unfractionated tRNA and AA-tRNA synthetases were prepared as reported previously (Söll *et al.*, 1967). Pure *E. coli* $tRNA^{Val}$ (specific activity of 1700 pmoles/$A_{260}$) was a gift from Mr. L. Johnson. Purified $tRNA^{Ser}$ (specific activity of 1450 pmoles/$A_{260}$) was kindly given by Dr. K. L. Roy.

*Assay for Amino Acid Acceptor Activity.* Unless otherwise mentioned, the incubation mixture contained per milliliter: 1 mg of tRNA, 50 μmoles (for *Mycoplasma* AA-tRNA synthetases), or 100 μmoles (for *E. coli* AA-tRNA synthetases) of sodium cacodylate (pH 7.2), 10 μmoles of magnesium acetate, 10 μmoles of potassium chloride (80 μmoles of ammonium chloride was added for *Mycoplasma* AA-tRNA synthetases where appropriate), 2 μmoles of ATP, AA-tRNA synthetase preparation, and radioactive amino acids (2–4 nmoles, of specific activities stated above). After incubation at 37°, aliquots were used for assay of acid-precipitable radioactivity with the filter paper technique (Hoskinson and Khorana, 1965).

*Formation of fMet-$tRNA^{fMet}$.* In addition to the compounds described above, the reaction mixture contained 2.5–5.0 nmoles of [$^{3}$H]formyltetrahydrofolate or formyltetrahydrofolate.

*Sedimentation of AA-tRNA.* Zone sedimentation was performed as described by Burgi and Hershey (1963). A linear concentration gradient of 20–80% deuterium oxide (v/v) in 0.01 M sodium acetate (pH 5)–0.005 M magnesium chloride was used. The sample of AA-tRNA in 0.1 ml of aqueous buffer was layered on a 5.2-ml gradient and spun in an SW65 rotor of a Spinco L2-65B at 5° and 65,000 rpm for 9.5 hr. The polyallomer tube was punctured and three drop fractions were

[1] Abbreviation used is: BD-cellulose, benzoylated DEAE-cellulose.

[2] One optical density unit, $A_{260}$, is defined as that amount of material per ml of solution which produces an absorbance of 1 in a 1-cm light path at 260 nm.

collected into scintillation vials and counted for radioactivity in Bray's solution (Bray, 1960).

*Chromatography of M. laidlawii tRNA on BD-cellulose.* *M. laidlawii* tRNA (575 $A_{260}$ units) in water was adsorbed on a column (1 × 84 cm) of BD-cellulose equilibrated with a solution containing 0.2 M sodium chloride, 0.05 M sodium acetate (pH 5), and 0.01 M magnesium chloride. The column was then eluted at room temperature with a linear gradient (0.4–1.5 M) of sodium chloride (total volume of the gradient was 1 l.) containing 0.05 M sodium acetate (pH 5), 0.01 M magnesium chloride, and 0.04% sodium azide. Fractions (9 ml) were collected at a flow rate of 18 ml/hr. At the end of the salt gradient (at fraction 110), a gradient (0–20%) of ethanol (total volume, 500 ml) containing 1.5 M sodium chloride, 0.05 M sodium acetate (pH 5), 0.01 M magnesium chloride, and 0.04% sodium azide was applied to elute the remaining nucleotidic material. Fractions (7.5 ml) were collected at a flow rate of 22.5 ml/hr. Selected fractions were dialyzed against water and then assayed for acceptor activity with *E. coli* AA-tRNA synthetases.

*Amino Acid Incorporating System.* The preparation of a tRNA-dependent, cell-free amino acid incorporating system from *E. coli* K12 directed by poly U or by f2 RNA was described previously (Söll, 1968; Söll and RajBhandary, 1967). The actual incubation conditions were as follows. (a) Poly U experiment: the reaction mixture contained per milliliter: 50 μmoles of Tris-chloride (pH 7.8), 10 μmoles of magnesium acetate, 50 μmoles of ammonium chloride, 1 μmole of ATP, 0.25 μmole of GTP, 5 μmoles of phosphoenolpyruvate, 25 μg of phosphoenolpyruvate kinase, 1.25 μCi of [$^{14}$C]phenylalanine, 15 $A_{260}$ units of ribosomes, 1.0 $A_{260}$ unit of poly U, about 1.2 mg of supernatant protein, and the indicated amount of tRNA. (b) F2 experiment: the reaction mixture contained per milliliter: 75 μmoles of Tris-chloride (pH 7.8), 7.5 μmoles of magnesium acetate, 50 μmoles of ammonium chloride, 6 μmoles of β-mercaptoethanol, 1 μmole of ATP, 0.25 μmole of GTP, 5 μmoles of phosphoenolpyruvate, 25 μg of phosphoenolpyruvate kinase, 5 μmoles of formyltetrahydrofolate, 2 μCi of [$^{14}$C]valine with the other 19 [$^{12}$C]amino acids (75 nmoles each), 18 $A_{260}$ units of ribosomes, 5.9 $A_{260}$ units of f2 RNA, about 50 μg of initiation factor proteins, 1 mg of supernatant protein, and the indicated amount of tRNA.

After incubation at 37°, polypeptide synthesis was assayed by acid insolubility and was carried out on filter paper as described previously (Morgan *et al.*, 1966) with 5% trichloroacetic acid.

## Results

The DNA of the Kid strain of *Mycoplasma* has a very low G and C content of 24.9% (Bode, 1966). Thus, it was an interesting question whether such a base composition is also reflected in the Kid tRNA. As seen from Table I, Kid tRNA has a more usual base composition. A recent investigation of Kid DNA by Ryan and Morowitz (1969) revealed a small section of the genome (about 1.4% of the total) to have a G and C content similar to the one found in Kid tRNA. It is therefore possible that such regions contain the genes for tRNA. This view is strengthened by the finding that such DNA sections hybridize with Kid tRNA (Ryan and Morowitz, 1969).

*Minor Bases in Mycoplasma tRNA.* tRNA from a wide variety of organisms contains a large number of minor nucleosides. However, Hall *et al.* (1967) noted the almost complete absence of minor bases in the total RNA (a mixture of tRNA, rRNA, and mRNA) of a certain *Mycoplasma* strain (M-880). We searched for minor bases in our *Mycoplasma* tRNA preparations using two-dimensional paper chromatography of T2 RNase digests of tRNA, and ion-exchange chromatography of nucleosides derived by enzymatic digestion of tRNA (see Materials and Methods). Table II lists some of the minor bases found in *M. laidlawii* and in Kid tRNA. The total amount of these bases present in *Mycoplasma* tRNA is lower than in *E. coli* tRNA. In addition, Kid tRNA appears to have a much lower content of minor bases than *M. laidlawii* tRNA. The less frequent occurrence of minor bases in *Mycoplasma* tRNA did not permit their exact quantitation to be carried out because of the limited amount of tRNA available. Furthermore, possible contamination of tRNA by rRNA fragments or by 5S RNA, which accompany tRNA in the usual chromatographic procedures, can obscure the results. However, it was seen from our qualitative determinations that pseudouridine, 4-thiouridine, and $N^7$-methylguanosine occur more frequently than other minor bases. Moreover, ribothymidine and 2′-*O*-methyl nucleosides were present in very low amounts in our Kid tRNA preparation. Two minor bases found in unfractionated tRNA from a variety of organisms, isopentenyladenosine and its methylthio derivative, are potent cytokinins (Helgeson, 1968). Such compounds promote growth and differentiation in certain plant cells. Hydrolysates of *M. laidlawii* and *M. gallisepticum* tRNAs are cytokinin active (D. J. Armstrong, unpublished data). However, no activity is found in hydrolysates of Kid tRNA (D. J. Armstrong, unpublished data). This again may indicate a lower amount of minor bases in the Kid tRNA.

TABLE II: Minor Nucleosides found in *Mycoplasma* tRNA.[a]

| Kid[b] | *M. laidlawii*[c] |
|---|---|
| Ribothymidine | Ribothymidine |
| Pseudouridine | Pseudouridine |
| 4-Thiouridine | 4-Thiouridine |
| $N^1$-Methyladenosine | $N^2$-Methylguanosine |
| $N^6$-Methyladenosine | 5-Methylcytidine or |
| $N^7$-Methylguanosine | $O^2$-methylcytidine |
| | $N^6$-Methyladenosine |
| $N^2$-Methylguanosine | $N^1$-Methyladenosine |
| | $N^7$-Methylguanosine |

[a] For details, see Materials and Methods. [b] Unfractionated Kid tRNA was used. [c] tRNA of fraction 43 (Figure 6) was used.

*Thermal Denaturation of Kid and E. coli tRNA.* Since the qualitative work on the base composition of Kid tRNA indicated a much lower content of minor bases than is found in *E. coli* tRNA, the physical characteristics of Kid tRNA were examined. Thermal denaturation curves of unfractionated Kid tRNA and *E. coli* tRNA were determined in the presence of $Mg^{2+}$ and are shown in Figure 1. Although the melting

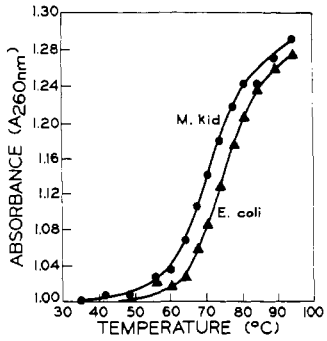


FIGURE 1: Thermal denaturation curve of unfractionated Kid and *E. coli* tRNA. Measured in 0.01 M sodium cacodylate (pH 7.2) and 0.005 M magnesium acetate and 0.2 M sodium chloride. Correction for the expansion of water due to heating was not made.

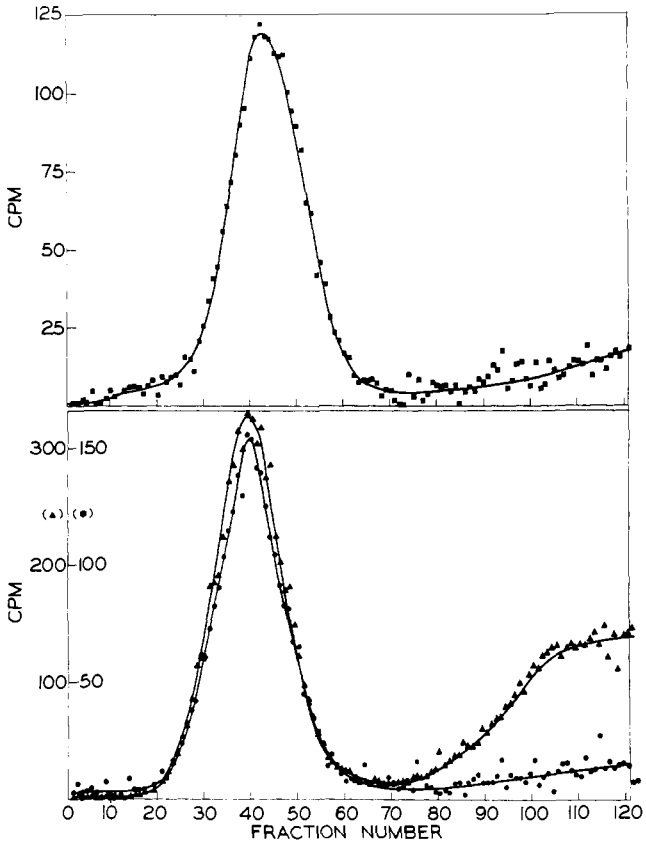


FIGURE 2: Sedimentation of Kid [$^3$H]Ser-tRNA (top) and of a mixture of Kid [$^3$H]Ser-tRNA (—●—) and purified *E. coli* [$^{14}$C]Ser-tRNA (—▲—) (bottom). For details, see Materials and Methods. The $^{14}$C radioactivity in the fractions 100–120 is free [$^{14}$C]serine which was present in the [$^{14}$C]Ser-tRNA preparation.

temperature of Kid tRNA is about 3° lower than that of *E. coli* tRNA, this may be due to the lower G and C content (by 4.4%) of Kid tRNA (see Table I) and not be a reflection of the lower amount of minor bases present. The extent of hyperchromicity is similar for both tRNAs which indicates a similar degree of secondary structure.

*Sedimentation of Mycoplasma tRNAs.* In order to compare the sedimentation properties, *M. laidlawii*, *M. gallisepticum*, and Kid tRNA were charged with [$^3$H]serine by *M. laidlawi.* AA-tRNA synthetases. Each of these *Mycoplasma* [$^3$H]Ser-tRNAs was cosedimented with *E. coli* [$^{14}$C]Ser-tRNA (a pure single species) in a deuterium oxide–water gradient in the preparative ultracentrifuge. In all cases, the mixture of tRNAs ran together, which indicates no large differences in the sedimentation coefficients of the tRNAs. An example is seen in Figure 2. [$^3$H]Ser-tRNA Kid sediments alone (top) and together with [$^{14}$C]Ser-tRNA *E. coli* (bottom). This figure shows that sedimentation of the Kid tRNA alone is not influenced by the addition of the other tRNA. The same is true for *E. coli* tRNA (data not shown). As a test of the sensitivity of the sedimentation experiments, a sample of *E. coli* 5S[$^{32}$P]RNA was cosedimented with the *E. coli* tRNA. The ratio of the distances sedimented by 5S RNA and [$^{14}$C]Ser-tRNA was 1.09. The peaks were separated by 8 fractions out of a total of 120.

*Mycoplasma AA-tRNA Synthetases.* The purification of aa-tRNA synthetases from *M. laidlawii* and Kid was performed analogously to the preparation of these enzymes from *E. coli.* Very soon it became obvious that their stability was somewhat different from *E. coli* AA-tRNA synthetases, and that they expressed optimal activity at lower buffer concentrations. The mixture of AA-tRNA synthetases thus obtained was tested for its ability to charge homologous and heterologous tRNA. For example, Kid AA-tRNA synthetase and *E.coli* AA-tRNA synthetase were used to charge unfractionated Kid tRNA and a pure species of *E. coli* tRNA$^{Val}$, with [$^{14}$C]-valine (Figure 3). It is clear that both enzymes charge the heterologous tRNA. Another example is presented in Figure 4 where *M. laidlawii* or *E. coli* AA-tRNA synthetases were used to charge unfractionated *M. laidlawii* tRNA and a pure species of *E. coli* tRNA$^{Ser}$. In both cases, the *Mycoplasma* AA-tRNA synthetases charged the tRNA to a lesser extent than *E. coli* AA-tRNA synthetase, regardless of the origin of the tRNA. This lack of complete charging could be completely corrected by the addition of ammonium chloride to the reaction mixture. The stimulatory effect of ammonium chloride on the extent of charging of Kid tRNA with [$^{14}$C]valine by the homologous AA-tRNA synthetase is seen in Figure 5. A brief survey of the charging ability of the Kid AA-tRNA synthetases showed that many of them require ammonium chloride for maximum charging of homologous or of heterologous tRNA. It was also found that potassium ions will substitute for ammonium. A similar observation on the stimulation by am-

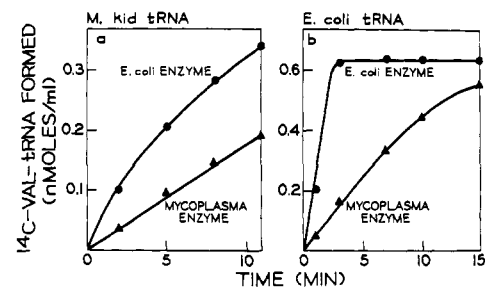


FIGURE 3: Attachment of [$^{14}$C]valine to (a) unfractionated Kid tRNA and (b) to pure *E. coli* tRNA$^{Val}$ by Kid and *E. coli* AA-tRNA synthetases. Reaction a contained 9.9 $A_{260}$/ml of Kid tRNA. Reaction b contained 0.4 $A_{260}$/ml of tRNA. For details, see Materials and Methods.

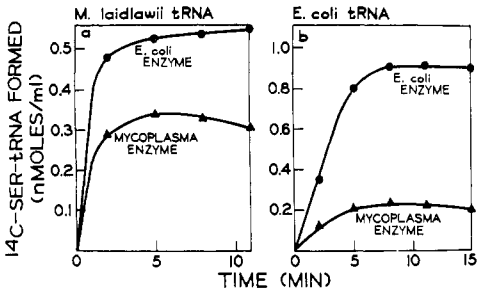


FIGURE 4: Attachment of [$^{14}$C]serine to (a) unfractionated *M. laidlawii* tRNA and (b) to a purified *E. coli* tRNA$^{Ser}$ species, by *M. laidlawii* and *E. coli* a-tRNA synthetases. Reaction a contained 14.3 $A_{260}$/ml of tRNA. Reaction b contained 0.60 $A_{260}$/ml of tRNA. For details, see Materials and Methods.

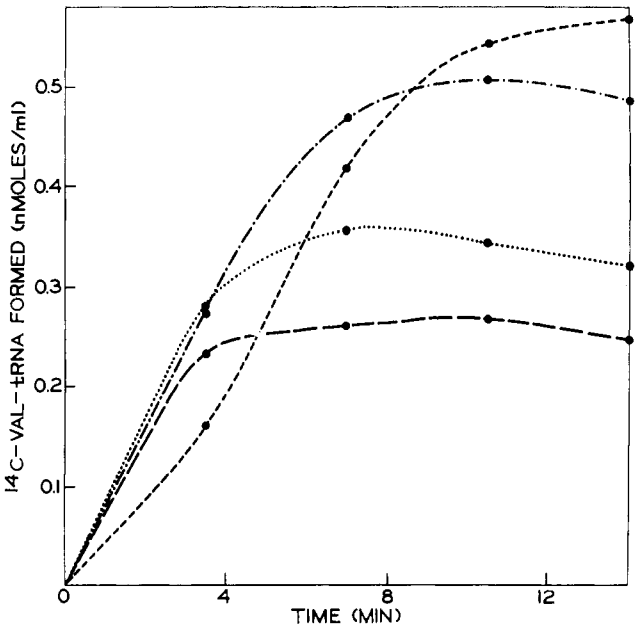


FIGURE 5: Effect of ammonium chloride on charging of Kid tRNA with [$^{14}$C]valine by Kid AA-tRNA synthetases. The concentration of Kid tRNA was 7.2 $A_{260}$/ml. The usual reaction mixtures had the following concentrations of ammonium chloride. (— — — —) 0 M, (·····) 0.04 M, (—·—·—·) 0.08 M, and (- - - - - -) 0.14 M. The extent of charging observed in this experiment in the presence of 0.14 M ammonium chloride was the same as seen when only *E. coli* AA-tRNA synthetases were used for acylation.

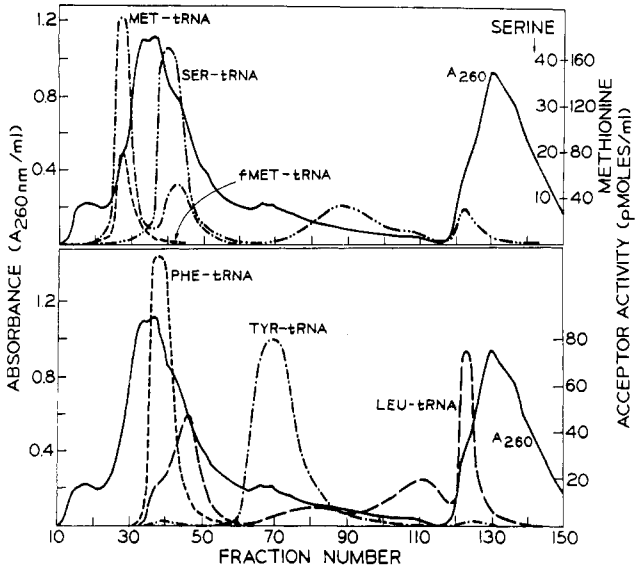


FIGURE 6: BD-cellulose chromatography of *M. laidlawii* tRNA. For the sake of clarity the tRNA$^{fMet}$ distribution is drawn on half-scale compared with the tRNA$^{Met}$ distribution. For details, see Materials and Methods.

monium chloride of the extent of charging of yeast tRNA$^{fMet}$ with the homologous synthetase was recently reported (Ghosh and RajBhandary, 1969).

*Fractionation of M. laidlawii tRNA on BD-cellulose.* After chromatography on BD-cellulose, *M. laidlawii* tRNA showed an elution pattern (Figure 6) similar to that of *E. coli* tRNA (Roy and Söll, 1968). The large peak in the salt gradient contains most of the acceptor RNAs. The second peak which was eluted from the column with alcohol contained a sizeable amount of nucleotidic material. Upon further examination this material was found to contain deoxyribooligonucleotides. This indicates the existence of a powerful deoxyribonuclease in *M. laidlawii* which degrades DNA to fragments which contaminate a usual tRNA preparation. The presence of such a nuclease was also noted by J. Ryan and H. Morowitz (unpublished data). Only a little tRNA$^{Ser}$ and tRNA$^{Leu}$ are present in the second peak, while all tRNA$^{Tyr}$ and tRNA$^{Phe}$ elute much earlier, in contrast to the behavior of the same *E. coli* tRNAs (Roy and Söll, 1968). Most interesting is the observation that methionine acceptor activity separates well into two peaks. Clark and Marcker (1966) have shown that out of at least two species of methionine tRNAs in *E. coli*, only one can be formylated (to fMet-tRNA$^{fMet}$). The same is true in the case of yeast tRNA (Takeishi *et al.*, 1968; Ghosh and RajBhandary, 1969). When the column fractions were checked for incorporation of radioactivity from [$^{3}$H]formyltetrahydrofolate in the presence of [$^{12}$C]methionine, a remarkable coincidence between the distribution of formyl acceptor activity and the first peak of methionine acceptor activity was observed. This suggests the presence of tRNA$^{fMet}$ in *M. laidlawii*.

*Evidence for the Existence of fMet-tRNA in Mycoplasma. M. laidlawii, M. gallisepticum,* and Kid tRNA were charged with [$^{3}$H]formyltetrahydrofolate in the presence or absence of [$^{12}$C]methionine by *M. laidlawii* and *E. coli* extracts. The results in Table III indicate clearly that in the presence of [$^{12}$C]-methionine, the tRNAs can be formylated by either *Mycoplasma* or *E. coli* enzymes (see also Figure 6). The limited extent of formylation by the *Mycoplasma* enzymes is not caused by insufficient aminoacylation of the tRNA, since charging with [$^{14}$C]methionine is equally good with homologous or heterologous AA-tRNA synthetases (Table III). This is reminiscent of the properties of crude yeast extracts which convert only a small fraction of yeast Met-tRNA$^{fMet}$ into fMet-tRNA (Ghosh and RajBhandary, 1969). With any of the other 19 amino acids, no incorporation of radioactivity from [$^{3}$H]formyltetrahydrofolate occurred with *E. coli* or *Mycoplasma* enzymes. Thus only Met-tRNA$^{fMet}$ is enzymatically formylated.

The [$^{3}$H]fMet-tRNA from the above experiment was isolated. Hydrolysis with alkali followed by paper electro-

TABLE III: Charging Mycoplasma tRNAs with [$^{12}$C]Methionine and [$^{3}$H]Formyltetrahydrofolate or with [$^{14}$C]Methionine (cpm/50 $\mu$l).[a]

| Source of Enzyme | tRNA ($A_{260}$/ml) *M. laidlawii* (11.4) −[$^{12}$C]Met | *M. laidlawii* (11.4) +[$^{12}$C]Met | *M. gallisepticum* (6.3) −[$^{12}$C]Met | *M. gallisepticum* (6.3) +[$^{12}$C]Met | Kid (7.2) −[$^{12}$C]Met | Kid (7.2) +[$^{12}$C]Met |
|---|---|---|---|---|---|---|
| | | | Expt I | | | |
| *M. laidlawii* | 71 | 295 | 63 | 293 | 111 | 386 |
| *E. coli* | 135 | 2929 | 146 | 2552 | 138 | 4531 |
| | +[$^{14}$C]Met | | +[$^{14}$C]Met | | +[$^{14}$C]Met | |
| | | | Expt II | | | |
| *M. laidlawii* | 4681 | | 4174 | | 10957 | |
| *E. coli* | 4320 | | 5935 | | 12807 | |

[a] For details, see Materials and Methods. Incubation time was 15 min.

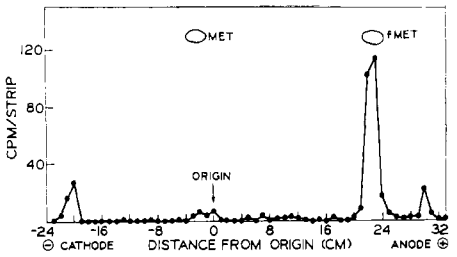


FIGURE 7: Paper electrophoresis of an alkali hydrolysate of *M. laidlawii* [$^{3}$H]fMet-tRNA. [$^{3}$H]fMet-tRNA (500 cpm) formed by *M. laidlawii* extracts was isolated and freed from acid-soluble radioactivity by extensive dialysis. It was then evaporated to dryness and treated with 0.1 N KOH (50 $\mu$l). After 15 min at 37° the solution was neutralized with $HClO_4$ and the precipitate was centrifuged off. The supernatant was subjected to paper electrophoresis at pH 3.5 for 2 hr. The electropherogram was cut into 1-cm strips and counted. Methionine and *N*-formylmethionine were used as markers.

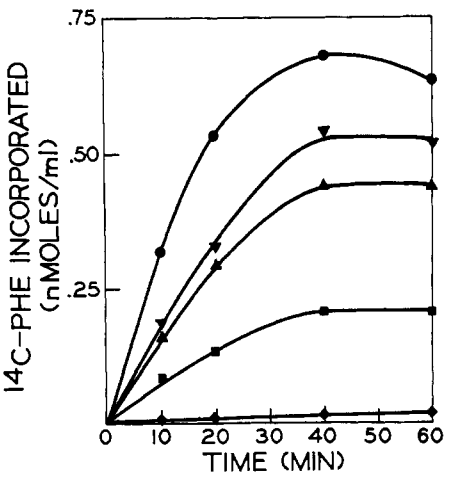


FIGURE 8: Poly U directed polyphenylalanine formation with *Mycoplasma* and *E. coli* tRNAs. (—●—●—) Kid tRNA (38 $A_{260}$/ml), (—▼—▼—) *E. coli* tRNA (38 $A_{260}$/ml), (—▲—▲—) *M. laidlawii* tRNA (38 $A_{260}$/ml), (—■—■—) *M. gallisepticum* tRNA (39 $A_{260}$/ml), and (—◆—◆—) no tRNA added. For details, see Materials and Methods.

phoresis of the hydrolysate showed that the $^{3}$H incorporated into tRNA traveled as far as formylmethionine (Figure 7). Material eluted from the electropherogram and chromatographed in solvent C showed an $R_F$ value similar to that of *N*-formylmethionine. The above experiments indicate the existence of fMet-tRNA in the three *Mycoplasma* strains.

*Incorporation Studies with Mycoplasma tRNAs.* Since the *Mycoplasma* tRNAs display differences in the content of minor bases compared with *E. coli* tRNA, it was of interest to see whether they participate in protein synthesis in a tRNA-dependent *E. coli* amino acid incorporating system. With poly U as mRNA, polyphenylalanine formation was brought about by tRNA from all three *Mycoplasma* strains (Figure 8), though at different levels. Although the same amount of unfractionated tRNA was used, the content of $tRNA^{Phe}$ in the various tRNA preparations may have been different. The observed incorporation with *Mycoplasma* tRNAs was not due to contaminating tRNA in the incorporating system, since the control shows almost no peptide formation. Using f2 RNA as messenger (Figure 9), both[3] *M. gallisepticum* and Kid tRNAs stimulated polypeptide formation at low $Mg^{2+}$ concentrations. This indicates that *Mycoplasma* tRNAs can replace homologous tRNA in an *E. coli* system directed by natural mRNA.

## Discussion

*Mycoplasma* is an interesting group of organisms. In view of their small size which approaches the minimum theoretical size for a self-replicating organism (Morowitz, 1966), one may

[3] Our preparation of *M. laidlawii* tRNA contained some inhibitor of protein synthesis since this preparation and also a mixture of it with *E. coli* tRNA did not support polypeptide formation in this experiment.

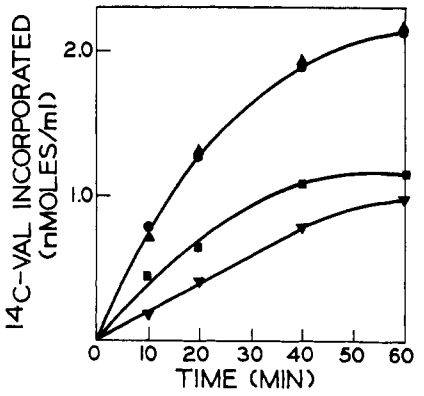


FIGURE 9: f2 RNA-directed amino acid incorporation with *Mycoplasma* and *E. coli* tRNAs. (—▲—) *E. coli* tRNA (7.8 $A_{260}$/ml) + Kid tRNA (9.9 $A_{260}$/ml), (—●—) *E. coli* tRNA (7.8 $A_{260}$/ml), (—■—) Kid tRNA (9.9 $A_{260}$/ml), and (—▼—) *M. gallisepticum* tRNA (8.5 $A_{260}$/ml). The value of the control tube (no tRNA was subtracted (1.0 nmole/ml at 60-min incubation). For details, see Materials and Methods.

ask whether the various cellular macromolecules possess any special properties.

On examination of the tRNAs from three strains of *Mycoplasma*, we found that they show physical properties very similar to those of *E. coli* tRNA (*e.g.*, sedimentation and melting behavior). *Mycoplasma* tRNA is also charged by *E. coli* aa-tRNA synthetases and can also substitute for *E. coli* tRNA in *in vitro* protein synthesis in an *E. coli* cell-free amino acid incorporating system directed by synthetic and natural mRNAs. The observed presence of fMet-$tRNA_1$ in all three *Mycoplasma* strains examined may suggest the involvement of formylmethionine in peptide-chain initiation in *Mycoplasma* just as in other procaryotic organisms (Lengyel and Söll, 1969).

The most interesting observation concerns the amount of minor nucleosides present in *Mycoplasma* tRNA. In contrast to the results reported for the total RNA of *Mycoplasma* M-880 (Hall *et al.*, 1967), we detected a variety of minor nucleosides as constituents of *Mycoplasma* tRNAs. However, such minor nucleosides occur much less frequently in *Mycoplasma* tRNA than in *E. coli* tRNA. The tRNA of the Kid strain has a particularly low content of minor nucleosides. (This tRNA contains less than 1 residue of ribothymidine/80 nucleotides.) Obviously, it will be interesting to see what the minimum amount of minor nucleosides in a functional tRNA can be. An answer to this question may be obtained by base analysis and by biochemical studies of pure Kid tRNA species. Such tRNA species lacking some of the modified nucleosides may be promising substrates for studying the biosynthesis of tRNA and of its minor nucleoside constituents.

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