Eucaryotic Methionyl Transfer Ribonucleic Acid. Effects of Aminoacylation and of Formylation on Chromatographic Behavior†

Charles E. Samuel,‡ Patrick J. McIlroy, and Jesse C. Rabinowitz*

ABSTRACT: The effects of aminoacylation and of formylation on the chromatographic behavior of bakers' yeast and rat liver methionine transfer ribonucleic acid ($tRNA^{Met}$) on benzoylated DEAE-cellulose (BD-cellulose) have been investigated. Aminoacylation of both the enzymically formylatable ($tRNA_f^{Met}$) and the nonformylatable ($tRNA_m^{Met}$) methionine tRNA of either bakers' yeast or rat liver changes their elution positions with respect to the unacylated forms. Both eucaryotic methionyl-$tRNA_f^{Met}$ species, when formylated with *Escherichia coli* transformylase using 10-formyltetrahydrofolate as the formyl donor, elute at significantly higher salt concentrations than unformylated methionyl-$tRNA_f^{Met}$. It is postulated that aminoacylation and formylation induce conformational changes in the eucaryotic $tRNA^{Met}$ species which, in part, are responsible for the altered chromatographic properties. A simple, rapid method for the purification of $tRNA_f^{Met}$ based on the effect of enzymic formylation has been developed.

Extensive experimentation has led to the conclusion that a specific methionine transfer RNA is involved in the process of polypeptide chain initiation in both procaryotic and eucaryotic organisms. In procaryotic organisms and in the chloroplasts and mitochondria of eucaryotic cells the initiator $tRNA_f^{Met}$ [1] is believed to function in the form of the formylmethionyl ester (Lengyel and Söll, 1969; Marcker and Smith, 1969; Lucas-Lenard and Lipmann, 1971). Recent investigations suggest that an unformylated Met-$tRNA_f^{Met}$ species is the initiator tRNA in the cytoplasm of eucaryotes (Smith and Marcker, 1970; Marcus *et al.*, 1970; Wigle and Dixon, 1970; Housman *et al.*, 1970; Hunter and Jackson, 1971). Whether the difference between procaryote and eucaryote initiation regarding the formyl moiety is a result of differences in the ribosomes, initiation factors, initiator $tRNA^{Met}$, and/or the particular message to be translated remains to be determined.

Activity differences dependent upon the degree of aminoacylation of bacterial tRNA species have been observed in several investigations involving parameters of biological significance such as the inhibition of RNA polymerase (Tissières *et al.*, 1963), the repression of enzyme synthesis (Schlesinger and Magasanik, 1964; Lewis and Ames, 1972), the regulation of RNA synthesis (Böck *et al.*, 1966), the formylation of *Escherichia coli* Met-$tRNA_f^{Met}$ (Schofield, 1970), and the formation of ternary complexes between tRNA, GTP, and factors involved in peptide chain initiation (Rudland *et al.*, 1971) and elongation (Gordon, 1968; Ono *et al.*, 1968). Likewise, proton magnetic resonance (Cohn *et al.*, 1969), sedimentation (Chatterjee and Kaji, 1970), hydrogen exchange (Gantt *et al.*, 1968), circular dichroism (Sarin and Zamecnik, 1965; Adler and Fasman, 1970; Melcher *et al.*, 1971), oligonucleotide binding (Danchin and Grunberg-Manago, 1970), and chromatography (Stern *et al.*, 1969; Samuel and Rabinowitz, 1972) experiments performed with procaryotic tRNA all reveal small changes in the physicochemical properties of tRNA upon aminoacylation which are consistent with the existence of subtle conformational differences between unacylated and aminoacylated tRNA; however, the overall general structure of the tRNA molecule appears to be maintained upon aminoacylation. It has also been reported that the bacterial tRNA involved in the initiation of protein synthesis, $tRNA_f^{Met}$, undergoes a conformational change upon formylation (Stern *et al.*, 1969; Watanabe and Imahori, 1971), and this structural change may be directly related to the ability of formylmethionyl-$tRNA_f^{Met}$ to facilitate the initiation of polypeptide chain biosynthesis.

Because of the unique role fMet-$tRNA_f^{Met}$ plays in the initiation of protein synthesis, we have extended our study of the effects that aminoacylation and formylation have on the chromatographic behavior of bacterial $tRNA^{Met}$ to include $tRNA^{Met}$ from two eucaryotic sources, bakers' yeast and rat liver. In this paper we report that the chromatographic behavior of eucaryotic $tRNA_f^{Met}$ and $tRNA_m^{Met}$ is affected by aminoacylation in a manner similar to that observed previously for bacterial $tRNA^{Met}$ (Samuel and Rabinowitz, 1972); in addition, the formylation of eucaryotic Met-$tRNA_f^{Met}$ catalyzed by *E. coli* transformylase produces a profound change in the chromatographic behavior of the eucaryotic $tRNA_f^{Met}$ comparable to that observed for procaryotic $tRNA_f^{Met}$. The results obtained with yeast and rat liver tRNA are compared to those obtained with *Streptococcus faecalis* R tRNA and are evaluated in terms of possible conformational differences between formylated and unformylated Met-$tRNA_f^{Met}$. The

† From the Department of Biochemistry, University of California, Berkeley, California 94720. *Received May 17, 1973.* This investigation was supported in part by Research Grant A-2109 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

‡ Supported by U. S. Public Health Service Training Grant GM 5 T01 31. Present address: Department of Microbiology and Immunology, Duke University Medical Center, Durham, N. C. 27710.

[1] The abbreviations and trivial names used are: $tRNA^{Met}$, total methionine-accepting tRNA; $tRNA_m^{Met}$, methionine-accepting tRNA which cannot be enzymically formylated; $tRNA_f^{Met}$, methionine-accepting tRNA which can be enzymically formylated; A_{260} unit, the amount of tRNA which when dissolved in 1 ml of solvent has an absorbance of 1.00 at 260 nm; BD-cellulose, benzoylated diethylaminoethylcellulose; TM buffer, 0.01 M Tris-chloride (pH 7.5) and 0.01 M magnesium chloride.

application of the effect of the altered chromatographic elution position mediated by formylation as a simple, rapid method of purification of $tRNA_f^{Met}$ is discussed.

Experimental Procedure

Materials. Sodium [^{14}C]formate (53.5 mCi/mmol) was obtained from Schwarz BioResearch. L-[^{14}C]Methionine (57 mCi/mmol) and L-[^{3}H]methionine (290 mCi/mmol) were purchased from Amersham Searle Corporation. DEAE-cellulose was from Schleicher and Schuell, glass fiber filter disks (GF/C, 2.4 cm diameter) were from Whatman, and RNase-free sucrose was from Schwarz/Mann. Bulk unfractionated tRNA prepared from bakers' yeast (lot 40278, $E_{260}^{1\%} = 175$) was purchased from Calbiochem. All other chemicals were of reagent grade. Male Sprague-Dawley rats weighing about 180–200 g each were obtained from Horton Laboratories. A stock culture of *Saccharomyces cerevisiae* X-2180-1A was kindly provided by Dr. C. E. Ballou, Department of Biochemistry, University of California, Berkeley, Calif.

BD-cellulose was prepared according to the procedure of Gillam *et al.* (1967) and contained an average of 2.5 benzoyl residues/mol of anhydroglucose. *l*-10-Formyltetrahydrofolate and *l*-10-[^{14}C]formyltetrahydrofolate were prepared enzymically with crystalline *Clostridium cylindrosporum* formyltetrahydrofolate synthetase as previously described (Samuel *et al.*, 1970).

Growth of Yeast. S. cerevisiae X-2180-1A was grown with vigorous shaking at 30° in medium containing Bacto-yeast extract (1%), Bacto-peptone (2%), and glucose (2%) (Thieme and Ballou, 1970). The doubling time was 84 min, and the cells were harvested during exponential growth.

Preparation of Enzymes. A rat liver enzyme fraction containing methionyl-$tRNA^{Met}$ synthetase activity was prepared by a method based on that of Gonano *et al.* (1971). Male Sprague-Dawley rats were sacrificed, and the livers were quickly excised, washed in 0.25 M sucrose at 4°, frozen in liquid nitrogen, and stored at −90°. For preparation of the enzyme fraction, frozen liver was homogenized with a Brinkman Polytron homogenizer at 4° for 2 min (4 × 30 sec) in Tris-chloride buffer (pH 7.5, 0.10 M) containing magnesium chloride (0.001 M), potassium chloride (0.015 M), and 2-mercaptoethanol (0.20 M) (3 ml of buffer/g of tissue). After centrifugation of the homogenate at 12,000*g* for 15 min and the resulting supernatant fraction at 20,000*g* for 30 min, a high-speed S-150 supernatant solution was obtained by centrifugation in a Spinco Model L-2 65B for 2 hr at 150,000*g*. Two-thirds of the intermediate S-150 supernatant solution was very carefully removed; the upper white lipid material and the pellet were discarded. The S-150 supernatant fraction was then treated with ammonium sulfate, dialyzed, and passed over a DEAE-cellulose column as previously described (Samuel *et al.*, 1972). The resulting enzyme preparation had a 280:260 absorbance ratio of 1.5 and was stored at −90°.

A yeast aminoacyl-tRNA synthetase fraction containing methionyl-$tRNA^{Met}$ synthetase activity was prepared by a modification of the method of Takeishi *et al.* (1967). Freshly harvested washed cells were disrupted by passage through a French pressure cell followed by homogenization in a Waring Blendor for a total of 90 sec (3 × 30 sec). A S-150 supernatant solution was prepared and endogenous tRNA removed as previously described (Samuel *et al.*, 1972). The final enzyme preparation had a 280:260 absorbance ratio of 1.8 and was stored at −90°.

Escherichia coli A_{19} methionyl-$tRNA^{Met}$ synthetase and formylmethionyl-Met-$tRNA_f^{Met}$ transformylase were prepared as previously described (Samuel *et al.*, 1972).

Preparation of Rat Liver tRNA. Bulk unfractionated rat liver tRNA was prepared by a modification of the procedures of Delihas and Staehelin (1966), Fink *et al.* (1968), and Yang and Novelli (1971). Male Sprague-Dawley rats were sacrificed and the livers were immediately excised, washed in 0.25 M sucrose (RNase-free) at 4°, frozen by direct submersion in liquid nitrogen, and stored at −90° until required for use. Approximately 7 g of liver was obtained from each of 20 rats. For extraction of RNA, 130 g of frozen liver tissue was homogenized for 2 min (4 × 30 sec) with a Brinkman Polytron homogenizer at 4° in 260 ml of Tris-chloride buffer (0.01 M, pH 7.5), containing EDTA (0.001 M), sodium chloride (0.10 M), and bentonite (0.4 mg/ml) and in 260 ml of phenol (Mallinckodt) saturated with 0.01 M Tris-chloride (pH 7.5). After shaking the extract for 1 hr at 4° on a reciprocating Eberbach shaker, the aqueous and phenol phases were separated by centrifugation at 14,500*g* for 15 min. The aqueous phase was applied directly to a DEAE-cellulose column (1 ml bed volume/g of tissue) packed at 4° in TM buffer (0.01 M Tris-chloride (pH 7.5) and 0.01 M magnesium chloride) containing 1.0 M sodium chloride, and then equilibrated with TM buffer containing 0.1 M sodium chloride. After application of the sample, the column was washed extensively with 0.1 M sodium chloride–TM until the absorbance at 260 nm decreased to less than 0.1, followed by 0.25 M sodium chloride–TM until the effluent 260-mm absorbance was less than 0.05. The RNA was then eluted with 1.0 M sodium chloride–TM buffer. Fractions with an absorbance at 260 nm greater than 1 were combined and the RNA was recovered by ethanol precipitation and centrifugation. Stripped tRNA was prepared by incubating unfractionated tRNA in 1.8 M Tris-acetate buffer (pH 8.0) for 90 min at 37° as described by Sarin and Zamecnik (1964) and high molecular weight RNA was removed by extraction with cold 1 M sodium chloride prior to recovering the deacylated tRNA by ethanol precipitation and centrifugation. The final precipitate was washed first with 75% ethanol, then with 95% ethanol, dried *in vacuo* at room temperature, and stored under desiccation at −20°. Approximately 67 mg of stripped tRNA was obtained from 130 g of tissue. The final product had a 280:260 absorbance ratio of 0.54, and the absorbance of a 1% solution at 260 nm was 172.

Operation of Benzoylated DEAE-Cellulose Columns. Benzoylated DEAE-cellulose column chromatography was performed essentially as described by Gillam *et al.* (1967). All operations were carried out at room temperature. Columns were packed as outlined (Gillam *et al.*, 1967), and equilibrated with 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M magnesium chloride and 0.4 M sodium chloride. The tRNA to be analyzed was applied to the column in the above buffer, and the column was then washed briefly with the same buffer solution. Elution was effected by a positive sodium chloride gradient in 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M magnesium chloride as indicated under the respective figure legends. Upon completion of the gradient, the column was washed with a 2 M sodium chloride solution in 30% ethanol. Gradients were routinely generated with an ISCO Dialagrad Model 380 gradient programmer (Lincoln, Neb.), and salt concentrations were determined with a Radiometer conductivity meter (Copenhagen, Denmark). The sodium chloride concentration required for elution of a particular $tRNA^{Met}$ species as reported in Table I was found to vary

TABLE I: Elution Position of tRNA^{Met} Species from BD-Cellulose.

tRNA^{Met} Species	Sodium Chloride Concentration[a]		
	Yeast	Rat Liver	*S. faecalis* R[b]
tRNA$_f^{Met}$	0.49	0.57	0.58
Met-tRNA$_f^{Met}$	0.62	0.60	0.65
fMet-tRNA$_f^{Met}$	0.76	0.73	0.79
tRNA$_m^{Met}$	0.63	0.71	0.67
Met-tRNA$_m^{Met}$	0.82	0.75	0.76

[a] In 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M magnesium chloride. [b] Data from Samuel and Rabinowitz, 1972.

0.02 M or less between individual chromatography experiments under constant conditions.

Assay of Column Fractions for Methionine and Formyl Acceptance Activity. When unacylated stripped bulk tRNA was chromatographed, the tRNA contained in column fractions was recovered by ethanol precipitation and assayed for methionine acceptance activity with enzymes prepared from *E. coli* and either yeast or rat liver and for formyl acceptance activity with enzyme prepared from *E. coli*. The reaction mixture (50 μl) for assaying methionine acceptance contained Tris-chloride buffer (pH 7.6, 0.10 M), ATP (4 mM), magnesium chloride (15 mM), potassium chloride (10 mM), 2-mercaptoethanol (20 mM) L-[^{14}C]methionine or L-[^{3}H]methionine (0.075 mM), tRNA fraction, and enzyme. For assaying formyl acceptance, the reaction mixture contained Tris-chloride buffer (pH 7.4, 0.1 M), ATP (4 mM), magnesium chloride (15 mM), potassium chloride (10 mM), 2-mercaptoethanol (20 mM), L-[^{12}C]methionine (0.20 mM), *l*-10-[^{14}C]formyltetrahydrofolate (0.025 mM), tRNA fraction, and enzyme. Incubation was for 10 min at 30° for aminoacylation and for 15 min at 30° for transformylation. The reactions were stopped by addition of cold 10% trichloroacetic acid and the precipitates collected on glass fiber filters. The assay tubes were rinsed with 5% trichloroacetic acid and the washes were decanted onto the filters, which were subsequently counted for radioactivity in 10 ml of Bray's solution (Bray, 1960) with a Nuclear-Chicago Mark I liquid scintillation counter.

Analysis of Double Labeled Columns. When tRNA^{Met} samples labeled with either a radioactive methionyl residue or radioactive formyl group were chromatographed, the column fractions were assayed by precipitating the tRNA directly by the addition of 0.25 volume of 50% trichloroacetic acid and analyzing for radioactivity by the filter disk technique as outlined above. All tritium and ^{14}C double label calculations were performed with the aid of programs written for an Olivetti Programma 101.

Preparation of Enzymically Aminoacylated and of Enzymically Formylated tRNA$_f^{Met}$ and tRNA$_m^{Met}$. Fractionated tRNA^{Met} species were aminoacylated with methionine and, where indicated, enzymically formylated as described under the standard assays for methionine and formyl acceptances except for the following modification: resolved tRNA$_f^{Met}$ or tRNA$_m^{Met}$, radioactively labeled or [^{12}C]methionine, and [^{14}C]- or [^{12}C]formyltetrahydrofolate were present in the incubation mixture as indicated under the respective figure descriptions. Transfer RNA was isolated from the reaction mixtures by phenol extraction and repeated ethanol precipitation.

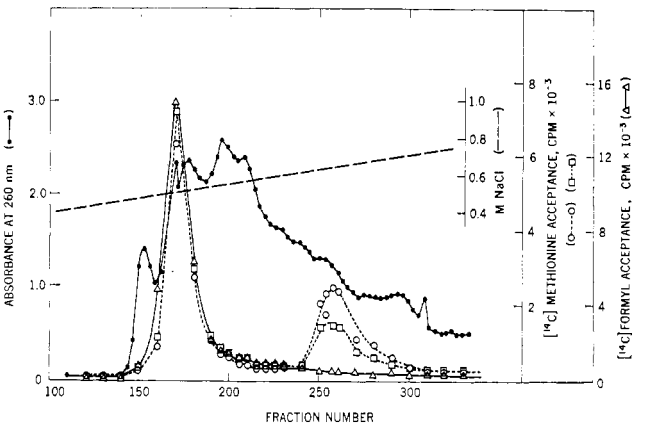

FIGURE 1: Chromatography of uncharged bulk bakers' yeast tRNA on BD-cellulose. tRNA (~1025 A_{260} units) was applied to a 1.2 × 75 cm column of BD-cellulose and chromatographed as described under Experimental Procedure. Elution was with a positive sodium chloride gradient running from 0.4 to 1.0 M sodium chloride in 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M magnesium chloride. Fractions (3.0 ml) were collected and the flow rate was 37.8 ml/hr. Methionine acceptance was assayed with aminoacyl-tRNA synthetase prepared from yeast (O- - -O) and from *E. coli* (□- - -□). Formyl acceptance was assayed with *E. coli* transformylase (△—△).

Preparation of Chemically Formylated tRNA$_m^{Met}$. Fractionated tRNA$_m^{Met}$ aminoacylated with methionine was chemically formylated by a modification of the procedure of Gillam *et al.* (1968). Formic acid was used in place of phenoxyacetic acid to prepare the formyl ester of *N*-hydroxysuccinimide which was concentrated *in vacuo*, but not crystallized. An approximate tenfold excess of the *N*-hydroxysuccinimide ester of formic acid was added with continuous stirring at 0° to a solution of Met-tRNA$_m^{Met}$ in 0.1 M triethanolamine chloride buffer (pH 4.3) containing 0.01 M magnesium chloride. The pH of the reaction mixture was adjusted to 8.0 with 1 N sodium hydroxide. After incubation at 0° for 1 hr, acetic acid was used to adjust the pH of the reaction mixture to 4.5, and the tRNA was recovered by ethanol precipitation. The extent of formylation was estimated after alkaline hydrolysis by electrophoresis in pH 3.5 pyridine–acetic acid buffer on Whatman No. 3MM paper.

Results

Chromatography of Stripped tRNA. The chromatographic fractionation on BD-cellulose of bakers' yeast tRNA (Figure 1) and of rat liver tRNA (Figure 2) stripped of amino acids yields two separate isoaccepting methionine tRNA species when eluate tRNA fractions are assayed with the homologous eucaryotic aminoacyl-tRNA synthetase enzyme preparation. In both cases of eucaryotic tRNA^{Met} resolution, the peak of methionine accepting activity which elutes first is capable of serving as a substrate for enzymic formylation catalyzed by the procaryotic transformylase prepared from *E. coli* using 10-formyltetrahydrofolate as the formyl donor after aminoacylation with methionine, whereas the second peak of methionine accepting activity cannot be enzymically formylated under these conditions. Confirming the results of RajBhandary and Ghosh (1969) and Petrissant *et al.* (1970), respectively, aminoacyl-tRNA synthetase prepared from *E. coli* catalyzes the aminoacylation of yeast tRNA$_m^{Met}$ less efficiently than the aminoacylation of yeast tRNA$_f^{Met}$ (Figure 1), and rat liver tRNA$_m^{Met}$ is not charged to any detectable extent by the *E.*

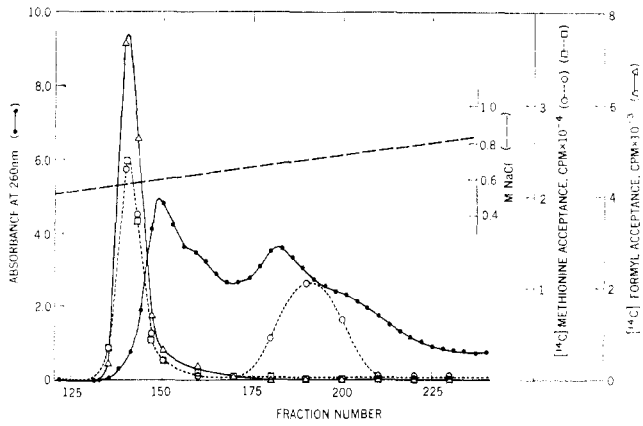

FIGURE 2: Chromatography of uncharged bulk rat liver tRNA on BD-cellulose. tRNA (~775 A_{260} units) was applied to a 1.2 × 62 cm column of BD-cellulose and chromatographed as described under Figure 1, except that the flow rate was 43.9 ml/hr. Methionine acceptance was assayed with aminoacyl-tRNA synthetase prepared from rat liver (O- - -O) and from *E. coli* (□- - -□). Formyl acceptance was assayed with *E. coli* transformylase (△—△).

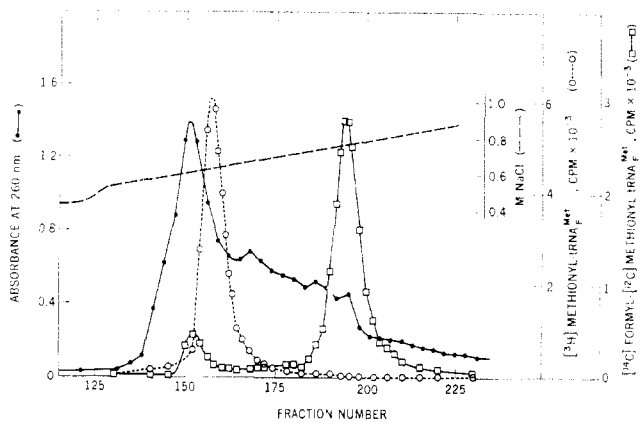

FIGURE 4: Chromatography of rat liver [^{3}H]methionyl-$tRNA_f^{Met}$ and [^{14}C]formyl[^{12}C]methionyl-$tRNA_f^{Met}$ on BD-cellulose. A sample containing purified $tRNA_f^{Met}$ charged with [^{3}H]methionine but not formylated, purified $tRNA_f^{Met}$ charged with [^{12}C]methionine and formylated enzymically with 10-[^{14}C]formyltetrahydrofolate, and uncharged unfractionated carrier tRNA was chromatographed on a 1.0 × 38 cm column of BD-cellulose as described under Figure 3, except that the flow rate was 21.9 ml/hr.

coli enzyme under conditions where $tRNA_f^{Met}$ is readily aminoacylated (Figure 2). The relative order in which the unacylated yeast and rat liver methionyl-tRNA species elute, $tRNA_f^{Met}$ before $tRNA_m^{Met}$, is similar to that observed for the methionyl-tRNA species prepared from several procaryotic and eucaryotic organisms (Seno *et al.*, 1968; Takeishi *et al.*, 1968; Hayashi *et al.*, 1969; Smith and Marcker, 1970; Jackson and Hunter, 1970; Samuel and Rabinowitz, 1972).

Chromatography of Methionyl-tRNA and Formylmethionyl-tRNA. When either bakers' yeast or rat liver $tRNA_f^{Met}$ aminoacylated with methionine is enzymically formylated, its elution position on BD-cellulose is dramatically changed relative to that of the unformylated aminoacylated $tRNA_f^{Met}$ species. The marked shift in the elution positions of the Met-$tRNA_f^{Met}$ species to higher salt concentrations upon formylation is illustrated by cochromatography of [^{3}H]methionyl-$tRNA_f^{Met}$ and [^{14}C]formyl[^{12}C]methionyl-$tRNA_f^{Met}$ prepared from either yeast (Figure 3) or rat liver (Figure 4) $tRNA_f^{Met}$.

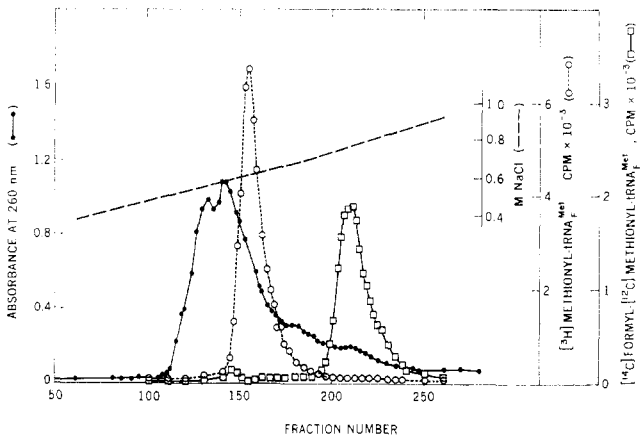

FIGURE 3: Chromatography of bakers' yeast [^{3}H]methionyl-$tRNA_f^{Met}$ and [^{14}C]formyl[^{12}C]methionyl-$tRNA_f^{Met}$ on BD-cellulose. A sample containing purified $tRNA_f^{Met}$ charged with [^{3}H]-methionine but not formylated, purified $tRNA_f^{Met}$ charged with [^{12}C]methionine and formylated enzymically with 10-[^{14}C]formyltetrahydrofolate, and uncharged unfractionated carrier tRNA was chromatographed on a 1.0 × 44 cm column of BD-cellulose as described under Figure 1 except that 2.0-ml fractions were collected and the flow rate was 25.3 ml/hr.

Recent studies with *S. faecalis* R tRNA established that, when analyzed on BD-cellulose, unformylated Met-$tRNA_f^{Met}$ elutes well before Met-$tRNA_m^{Met}$, whereas fMet-$tRNA_f^{Met}$ elutes slightly after Met-$tRNA_f^{Met}$; thus, in the case of this bacterium, the net effect of formylation is an apparent inversion of the elution order of the isoaccepting methionine tRNA species, $tRNA_f^{Met}$ and $tRNA_m^{Met}$ (Samuel and Rabinowitz, 1972). In order to establish what effect enzymic formylation of eucaryotic Met-$tRNA_f^{Met}$ has on the elution order of the isoaccepting methionine tRNA species, unformylated [^{14}C]-methionyl-$tRNA_f^{Met}$ and [^{12}C]formyl[^{14}C]methionyl-$tRNA_f^{Met}$ were cochromatographed with [^{3}H]methionyl-$tRNA_m^{Met}$. As shown in Figure 5, the retardation of yeast Met-$tRNA_f^{Met}$ upon formylation did not reverse the elution order of $tRNA_f^{Met}$ with respect to $tRNA_m^{Met}$. Formylation of rat liver Met-$tRNA_f^{Met}$ likewise did not invert the elution order with respect to Met-$tRNA_m^{Met}$ (Figure 6), although the elution positions of rat liver fMet-$tRNA_f^{Met}$ and Met-$tRNA_m^{Met}$ (Figure 6) were of closer proximity than the respective elution positions of yeast fMet-$tRNA_f^{Met}$ and Met-$tRNA_m^{Met}$ (Figure 5).

The ionic strength required to elute the various forms of yeast and rat liver $tRNA^{Met}$ from BD-cellulose under the conditions employed in this investigation are summarized in Table I. In order that meaningful comparisons could be made between these values and those results recently reported from this laboratory on *S. faecalis* R $tRNA^{Met}$ (Samuel and Rabinowitz, 1972), the same batch of BD-cellulose was employed in all experiments and identical chromatography conditions were maintained because the salt concentration required for elution of a given tRNA species varies with the temperature of column operation, buffer composition, and degree of benzoyl substitution of the BD-cellulose used[2] (Gillam *et al.*, 1967; Henes *et al.*, 1969).

Discussion

In spite of the knowledge that has been accumulated on the primary structure of the $tRNA^{Met}$ species from *E. coli*

[2] C. E. Samuel and J. C. Rabinowitz, unpublished observations.

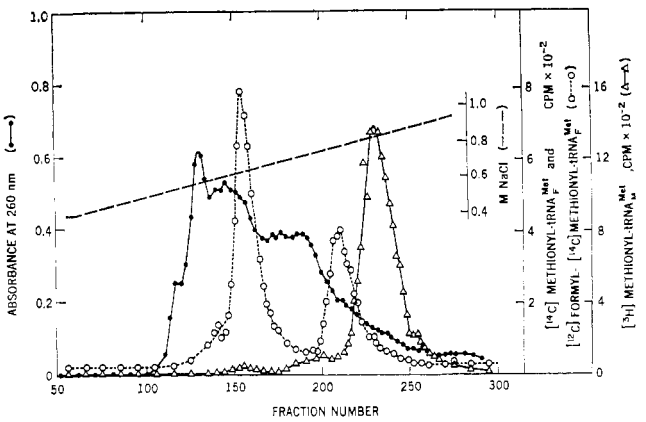

FIGURE 5: Chromatography of bakers' yeast [^{14}C]methionyl-tRNA$_f^{Met}$, [^{12}C]formyl[^{14}C]methionyl-tRNA$_f^{Met}$, and [^{3}H]methionyl-tRNA$_m^{Met}$ on BD-cellulose. A sample containing purified tRNA$_f^{Met}$ charged with [^{14}C]methionine but not formylated, purified tRNA$_f^{Met}$ charged with [^{14}C]methionine and formylated enzymically with 10-[^{12}C]formyltetrahydrofolate, purified tRNA$_m^{Met}$ charged with [^{3}H]methionine, and uncharged carrier tRNA was chromatographed on a 1.0 × 40 cm column of BD-cellulose as described under Figure 3, except that the flow rate was 31.4 ml/hr. As in previous experiments, the order of elution of the tRNA$_f^{Met}$ derivatives is methionyl-tRNA$_f^{Met}$ (peak at fraction number 155) followed by the formylmethionyl-tRNA$_f^{Met}$ (peak at fraction number 211).

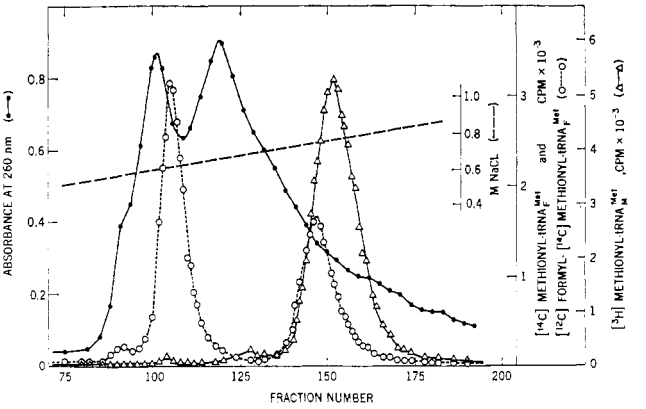

FIGURE 6: Chromatography of rat liver [^{14}C]methionyl-tRNA$_f^{Met}$, [^{12}C]formyl[^{14}C]methionyl-tRNA$_f^{Met}$, and [^{3}H]methionyl-tRNA$_m^{Met}$ on BD-cellulose. A sample containing purified tRNA$_f^{Met}$ charged with [^{14}C]methionine but not formylated, purified tRNA$_f^{Met}$ charged with [^{14}C]methionine and formylated enzymically with 10-[^{12}C]-formyltetrahydrofolate, purified tRNA$_m^{Met}$ charged with [^{3}H]-methionine, and uncharged carrier tRNA was chromatographed on a 1.0 × 38 cm column of BD-cellulose as described under Figure 3, except that the flow rate was 19.0 ml/hr. As in previous experiments, the order of elution of the tRNA$_f^{Met}$ derivatives is methionyl-tRNA$_f^{Met}$ (peak at fraction number 105) followed by the formylmethionyl-tRNA$_f^{Met}$ (peak at fraction number 146).

(Dube *et al.*, 1968; Cory and Marcker, 1970) and yeast (Simsek and RajBhandary, 1972), the basic problem of the relationship of structure to function and specificity has not yet been solved. Structural changes occurring upon aminoacylation and possibly upon formylation of various tRNA species appear to be intricately connected with the mediation and regulation of various biological processes (Tissières *et al.*, 1963; Schlesinger and Magasanik, 1964; Böck *et al.*, 1966; Ono *et al.*, 1968; Gordon, 1968; Schofield, 1970; Rudland *et al.*, 1971; Lewis and Ames, 1972).

Transfer RNA fractionation on BD-cellulose depends predominantly upon hydrophobic and ionic interactions (Gillam *et al.*, 1967, 1968); however, all of the principles underlying separation of tRNAs on BD-cellulose are not fully understood, and it is evident that three-dimensional structure is definitely involved in the chromatography of tRNAs (Stern *et al.*, 1969; Zachau, 1969). The slightly higher salt concentrations required to elute the aminoacylated tRNA$_f^{Met}$ and tRNA$_m^{Met}$ species from BD-cellulose than the respective unacylated methionine tRNA species under comparable conditions (Table I) may be the result of a conformational difference between unacylated and aminoacylated tRNA (Sarin and Zamecnik, 1965; Gantt *et al.*, 1968; Cohn *et al.*, 1969; Stern *et al.*, 1969; Danchin and Grunberg-Manago, 1970; Chatterjee and Kaji, 1970; Adler and Fasman, 1970; Melcher *et al.*, 1971). However, increased interaction of the aminoacylated tRNA^{Met} species with the benzoylated DEAE-cellulose due to the hydrophobic nature of the methionine side chain may also be a contributing source responsible for the difference in elution position, although the additional positive charge present in the α-amino group of methionine would tend to weaken the ionic interactions between the 3′-terminal region of the aminoacylated tRNA and the column resin.

It is surprising that a moiety as small as the formyl group in comparison to the total size of the transfer RNA molecule has such a profound effect on the chromatographic behavior of tRNA^{Met}. In view of the large number of negatively charged phosphodiester linkages present in RNA, it is difficult to visualize how a net decrease of one positive charge due to the acylation of the free α-amino group of methionine would have such a significant effect on the interaction of the tRNA with the BD-cellulose explicable solely on electrostatic interactions. Indeed, the change in elution position upon formylation is even greater when compared to tRNA$_f^{Met}$ than to Met-tRNA$_f^{Met}$, although the apparent net charge is the same for tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$. Experiments carried out with two reverse phase column systems, RPC-2 (Epler *et al.*, 1970) and RPC-3 (Shugart *et al.*, 1969a,b), methylated albumin silicic acid (Leder and Bursztyn, 1966; Stern *et al.*, 1969), and BD-cellulose (Samuel and Rabinowitz, 1972), have unequivocally established that the chromatographic property of formylated methionyl-tRNA is different than that of unformylated methionyl-tRNA. However, the only tRNA used in the above investigations was from either procaryotic organisms or eucaryotic organnelles where initiation of protein synthesis is believed to require formylation of the Met-tRNA$_f^{Met}$. Our results obtained with yeast and rat liver tRNA$_f^{Met}$ indicate that the profound change in chromatographic behavior upon formylation of Met-tRNA$_f^{Met}$ previously observed for *S. faecalis* R tRNA$_f^{Met}$ on BD-cellulose (Samuel and Rabinowitz, 1972) is not unique for tRNA from procaryotic sources, but instead is a common phenomenon of both procaryotic and eucaryotic tRNA$_f^{Met}$ alike. The vast difference in chromatographic behavior of fMet-tRNA$_f^{Met}$ from Met-tRNA$_f^{Met}$ on BD-cellulose is most readily explained in terms of a conformational difference between unformylated and formylated Met-tRNA$_f^{Met}$.

Two lines of evidence suggest the altered chromatographic behavior induced by formylation of yeast and rat liver Met-tRNA$_f^{Met}$ most probably is not a reflection of the conformation necessary for the initiation of protein biosynthesis, but instead is a reflection of the conformation of peptidyl-tRNA. First, the initiation of polypeptide chain biosynthesis in the cytoplasm of eucaryotic organisms has been demonstrated to occur with unformylated Met-tRNA$_f^{Met}$ (Smith and Marcker, 1970; Marcus *et al.*, 1970; Wigle and Dixon, 1970; Housman *et al.*, 1970; Hunter and Jackson, 1971). Second, the methio-

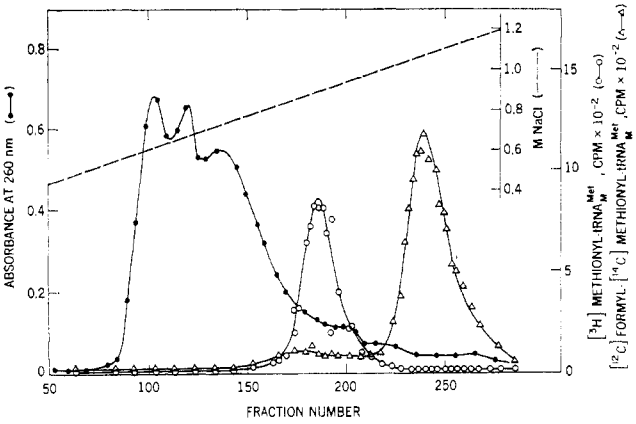

FIGURE 7: Chromatography of bakers' yeast [^{3}H]methionyl-tRNA$_m^{Met}$ and [^{12}C]formyl[^{14}C]methionyl-tRNA$_m^{Met}$ on BD-cellulose. A sample containing purified tRNA$_m^{Met}$ charged with [^{3}H]methionine but not formylated, purified tRNA$_m^{Met}$ charged with [^{14}C]methionine and formylated chemically with the *N*-hydroxysuccinimide ester of [^{12}C]formic acid, and uncharged unfractionated carrier tRNA was applied to a 1.0 × 39 cm column of BD-cellulose and chromatographed as described under Experimental Procedure. Elution was with a positive sodium chloride gradient running from 0.4 to 1.25 M sodium chloride in 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M magnesium chloride. Fractions (2.0 ml) were collected, and the flow rate was 34.6 ml/hr.

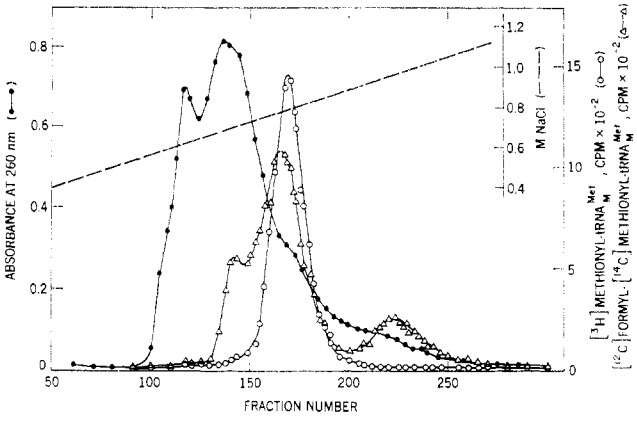

FIGURE 8: Chromatography of rat liver [^{3}H]methionyl-tRNA$_m^{Met}$ and [^{12}C]formyl[^{14}C]methionyl-tRNA$_m^{Met}$ on BD-cellulose. A sample containing purified tRNA$_m^{Met}$ charged with [^{3}H]methionine but not formylated, purified tRNA$_m^{Met}$ charged with [^{14}C]methionine and formylated chemically (30%) with the *N*-hydroxysuccinimide ester of [^{12}C]formic acid, and uncharged unfractionated carrier tRNA was chromatographed on a 1.0 × 40 cm column of BD-cellulose as described under Figure 7, except that the flow rate was 31.7 ml/hr.

nine tRNA species from both yeast and rat liver that functions in peptide elongation but not initiation, tRNA$_m^{Met}$, when chemically formylated with the formyl ester of *N*-hydroxysuccinimide, chromatographs differently from unformylated Met-tRNA$_m^{Met}$. The shift in elution position of Met-tRNA$_m^{Met}$ upon chemical formylation to higher salt concentrations was verified by cochromatography of [^{3}H]methionyl-tRNA$_m^{Met}$ and [^{12}C]-formyl[^{14}C]methionyl-tRNA$_m^{Met}$ prepared from either yeast (Figure 7) or rat liver (Figure 8) tRNA$_m^{Met}$. In the case of rat liver tRNA$_m^{Met}$, but not yeast tRNA$_m^{Met}$, a variable amount of chemically formylated Met-tRNA$_m^{Met}$ eluted earlier than unformylated Met-tRNA$_m^{Met}$ rather than later (Figure 8) and probably reflects acylation of a nucleoside present in rat liver tRNA but absent in yeast tRNA (Friedman, 1972).

Woese (1970) has proposed a reciprocating ratchet model for ribosome movement during messenger RNA translation whereby the anticodon loop of Met-tRNA$_f^{Met}$ and other aminoacyl-tRNAs is rearranged from a "Hodgson–Fuller" structure to a "Fuller–Hodgson" structure upon formylation of Met-tRNA$_f^{Met}$ or formation of peptidyl-tRNA. The conformational changes of rat liver and yeast Met-tRNA$_f^{Met}$ implied from our chromatographic studies may reflect such a particular conformation of peptidyl-tRNA rather than a conformation of tRNA$_f^{Met}$ required for the initiation of polypeptide chain biosynthesis. The relaxation kinetic studies of the early melting transition of *E. coli* tRNA$_f^{Met}$ recently reported by Cole and Crothers (1972) establish that the tertiary structure of tRNA$_f^{Met}$ can be broken in steps, thus indicating that the tRNA can undergo conformational changes such as presumably would be required in going from an aminoacyl conformation (Met-tRNA) to a peptidyl conformation (fMet-tRNA).

The profound change in chromatographic behavior displayed by tRNA$_f^{Met}$ upon enzymic formylation has been utilized in the development of a simple, rapid purification technique for tRNA$_f^{Met}$. Chromatography on BD-cellulose of bulk rat liver or *S. faecalis* R tRNA containing unformylated tRNA$_f^{Met}$ followed by chromatography of tRNA$_f^{Met}$ in the formylated state yields tRNA$_f^{Met}$ preparations with ~1665 pmol of methionine acceptance per A_{260} unit of tRNA.[3] This value compares quite favorably to the value obtained for "100%" pure tRNA$_f^{Met}$ from *E. coli* K-12, 1615 pmol of methionine per A_{260} unit of tRNA.[4] The purification technique has the advantage of providing a rapid method for the isolation of large quantities of pure tRNA$_f^{Met}$ not requiring chemical modification of the tRNA or elaborate fractionation equipment, utilizes a column packing with a "long-life" resolving capacity, and appears to be applicable to any methionyl-tRNA, procaryotic or eucaryotic, that can be enzymically formylated.

References

Adler, A. J., and Fasman, G. D. (1970), *Biochim. Biophys. Acta 204*, 183.

Böck, A., Faiman, L. E., and Neihardt, F. C. (1966), *J. Bacteriol. 92*, 1076.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Chatterjee, S. K., and Kaji, H. (1970), *Biochim. Biophys. Acta 224*, 88.

Cohn, M., Danchin, A., and Grunberg-Manago, M. (1969), *J. Mol. Biol. 39*, 199.

Cole, P. E., and Crothers, D. M. (1972), *Biochemistry 11*, 4368.

Cory, S., and Marcker, K. A. (1970), *Eur. J. Biochem. 12*, 177.

Danchin, A., and Grunberg-Manago, M. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 9*, 327.

Delihas, N., and Staehelin, M. (1966), *Biochim. Biophys. Acta 119*, 385.

Dube, S. K., Marcker, K. A., Clark, B. F. C., and Cory, S. (1968), *Nature (London) 218*, 232.

Epler, J. L., Shugart, L. R., and Barnett, W. E. (1970), *Biochemistry 9*, 3575.

Fink, L. M., Goto, T., Frankel, F., and Weinstein, I. B. (1968), *Biochem. Biophys. Res. Commun. 32*, 963.

[3] C. L. Murray, P. J. McIlroy, C. E. Samuel, and J. C. Rabinowitz, unpublished observations.

[4] A. D. Kelmers, personal communication.

Friedman, S. (1972), *Biochemistry 11*, 3435.
Gantt, R. R., Englander, S. W., and Simpson, M. V. (1968), *Biochemistry 8*, 475.
Gillam, I., Blew, D., Warrington, R. C., von Tigerstrom, M., and Tener, G. M. (1968), *Biochemistry 7*, 3459.
Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M. (1967), *Biochemistry 6*, 3043.
Gonano, F., Chiarugi, V. P., Pirro, G., and Marini, M. (1971), *Biochemistry 10*, 900.
Gordon, J. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 179.
Hayashi, H., Fisher, H., and Söll, D. (1969), *Biochemistry 8*, 3680.
Henes, C., Krauskopf, M., and Ofengand, J. (1969), *Biochemistry 8*, 3024.
Housman, D., Jacobs-Lorena, M., RajBhandary, U. L., and Lodish, H. F. (1970), *Nature (London) 227*, 913.
Hunter, A. R., and Jackson, R. J. (1971), *Eur. J. Biochem. 19*, 316.
Jackson, R., and Hunter, T. (1970), *Nature (London) 227*, 672.
Leder, P., and Bursztyn, H. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 1579.
Lengyel, P., and Söll, D. (1969), *Bacteriol. Rev. 33*, 264.
Lewis, J. A., and Ames, B. N. (1972), *J. Mol. Biol. 66*, 131.
Lucas-Lenard, J., and Lipmann, F. (1971), *Annu. Rev. Biochem. 40*, 409.
Marcker, K. A., and Smith, A. E. (1969), *Bull. Soc. Chim. Biol. 51*, 1453.
Marcus, A., Weeks, D. P., Leis, J. P., and Keller, E. B. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1681.
Melcher, G., Paulin, D., and Guschlbauer, W. (1971), *Biochimie 53*, 43.
Ono, Y., Skoultchi, A., Klein, A., and Lengyel, P. (1968), *Nature (London) 220*, 1304.
Petrissant, G., Boisnard, M., and Puissant, C. (1970), *Biochim. Biophys. Acta 213*, 223.
RajBhandary, U. L., and Ghosh, H. P. (1969), *J. Biol. Chem. 244*, 1104.
Rudland, P. S., Whybrow, W. A., and Clark, B. F. C. (1971), *Nature (London), New Biol. 231*, 76.
Samuel, C. E., D'Ari, L., and Rabinowitz, J. C. (1970), *J. Biol. Chem. 245*, 5115.
Samuel, C. E., Murray, C. L., and Rabinowitz, J. C. (1972), *J. Biol. Chem. 247*, 6856.
Samuel, C. E., and Rabinowitz, J. C. (1972), *Anal. Biochem. 47*, 244.
Sarin, P. S., and Zamecnik, P. C. (1964), *Biochim. Biophys. Acta 91*, 653.
Sarin, P. S., and Zamecnik, P. C. (1965), *Biochem. Biophys. Res. Commun. 20*, 400.
Schlesinger, S., and Magasanik, B. (1964), *J. Mol. Biol. 9*, 670.
Schofield, P. (1970), *Biochemistry 9*, 1694.
Seno, T., Kobayashi, M., and Nishimura, S. (1968), *Biochim. Biophys. Acta 169*, 80.
Shugart, L., Chastain, B., and Novelli, G. D. (1969a), *Biochim. Biophys. Acta 186*, 384.
Shugart, L., Chastain, B., and Novelli, G. D. (1969b), *Biochem. Biophys. Res. Commun. 37*, 305.
Simsek, M., and RajBhandary, U. L. (1972), *Biochem. Biophys. Res. Commun. 49*, 508.
Smith, A. E., and Marcker, K. A. (1970), *Nature (London) 226*, 607.
Stern, R., Zutra, L. E., and Littauer, U. Z. (1969), *Biochemistry 8*, 313.
Takeishi, K., Nishimura, S., and Ukita, T. (1967), *Biochim. Biophys. Acta 145*, 605.
Takeishi, K., Ukita, T., and Nishimura, S. (1968), *J. Biol. Chem. 243*, 5761.
Thieme, T. R., and Ballou, C. E. (1970), *Biochem. Biophys. Res. Commun. 39*, 621.
Tissières, A., Bourgeois, S., and Gros, F. (1963), *J. Mol. Biol. 7*, 100.
Watanabe, K., and Imahori, K. (1971), *Biochem. Biophys. Res. Commun. 45*, 488.
Wigle, D. T., and Dixon, G. H. (1970), *Nature (London) 227*, 676.
Woese, C. (1970), *Nature (London) 226*, 817.
Yang, W. K., and Novelli, G. D. (1971), *Methods Enzymol. 20*, 44.
Zachau, H. G. (1969), *Angew. Chem., Int. Ed. Engl. 8*, 711.