Solomon, I., and Bloembergen, N. (1956), *J. Chem. Phys. 25*, 261.

Suelter, C. H., Singleton, R., Jr., Kayne, F. J., Arrington, S., Glass, J., and Mildvan, A. S. (1966), *Biochemistry 5*, 131.

Supniewski, J. V., and Salzberg, P. L. (1948), Organic Syntheses, Blatt, A. H., Ed., Collect. Vol. I, New York, N. Y., Wiley, p 46.

Tietz, A., and Ochoa, S. (1958), *Arch. Biochem. Biophys. 78*, 477.

Utter, M. F., and Keech, D. B. (1963), *J. Biol. Chem. 238*, 2603.

Villafranca, J. J., and Mildvan, A. S. (1972), *J. Biol. Chem. 247*, 3454.

Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E. (1968), *J. Chem. Phys. 48*, 3831.

Developmental Biochemistry of Cottonseed Embryogenesis and Germination. Preferential Charging of Cotton Chloroplastic Transfer Ribonucleic Acid by *Escherichia coli* Enzymes†

William C. Merrick‡ and Leon S. Dure III§*

ABSTRACT: Aminoacyl-tRNA synthetases from *E. coli* can charge about 40% of the tRNA of cotton cotyledons that contain about 10% chloroplastic tRNA, can charge about 50% of the tRNA from cotyledons that have germinated several days, and contain about 40% chloroplastic tRNA, and can charge over 70% of the tRNA from partially purified chloroplasts of this tissue. Thus these enzymes show a greater extent of recognition for chloroplastic tRNA species than for cytoplasmic species. When this heterologous charging is examined for each amino acid, the charging of all isoaccepting species can be demonstrated for arginine, histidine, lysine, and methionine, and the charging of only chloroplastic species can be demonstrated for leucine, isoleucine, and tyrosine. The charging of none of the species for alanine, glutamine, and proline is observed. The charging of some species from both sources is indicated for the other amino acids, except for tryptophan whose *E. coli* synthetase was inactive. No instance of misacylation was found in this system. A mixture of cytoplasmic and chloroplastic synthetases from cotton cotyledons, on the other hand, charge about 50% of *E. coli* tRNA. All the $tRNA^{Ala,Ile,\text{ and }Val}$ of *E. coli* is charged by the cotton enzymes but no $tRNA^{Gln}$. Varying amounts of the tRNA for the other amino acids are charged by the cotton synthetases. There is no overlapping between the synthetases from the two sources that recognize all the isoacceptors from the heterologous source. That is, the bacterial synthetases recognize all the cotton isoacceptors for four amino acids, yet the cotton synthetases for the same isoacceptors do not recognize all the bacterial isoacceptors for these amino acids. Similarly, cotton synthetases recognize all the bacterial isoacceptors for three amino acids, but the bacterial enzymes that recognize these species fail to react with all the corresponding isoacceptors from cotton. The bearing these data have on the possible origin of chloroplasts is discussed.

Currently, there is a great deal of interest in the biochemical relationship between bacteria and the organelles of higher organisms. With this in mind, we have examined the extent to which the aminoacyl-tRNA synthetases of *Escherichia coli* can aminoacylate the tRNA of cotton cotyledons with each of the 20 amino acids. We have previously determined the amount of chloroplastic tRNA for several amino acids that is present in cotton cotyledons at various stages of development and maturation, and have identified by column chromatography the number and relative levels of chloroplastic and cytoplasmic isoaccepting tRNA species for several amino acids during the development of this tissue (Merrick and Dure, 1972). With this background, we have been able to determine the relative capability of *E. coli* enzymes for charging specific cytoplasmic and chloroplastic tRNA species from cotton.

We have also determined the amount of *E. coli* tRNA that can be acylated by a mixture of cotton cotyledon cytoplasmic and chloroplastic aminoacyl-tRNA synthetases.

Experimental Procedures

Materials

Transfer RNA and aminoacyl-tRNA synthetases were prepared from cotton cotyledons and partially purified chloroplasts by routine procedures as previously described (Merrick and Dure, 1972). Cotyledon tRNA was prepared from cotyledons at several developmental stages, and a crude mixture of aminoacyl-tRNA synthetases was prepared from 5-day germinated, greened cotyledons to ensure a high concentration of chloroplast synthetases. Transfer RNA from *E. coli* W was purchased from Schwarz BioResearch, and a crude preparation of aminoacyl-tRNA synthetases from *E. coli* B was prepared by the method of Kelmers *et al.* (1965).

The source and specific radioactivity of the 20 amino acids used were as previously reported (Merrick and Dure, 1972).

† From the Department of Biochemistry, University of Georgia, Athens, Georgia 30601. *Received August 24, 1972.* This paper is the fifth in this series. This investigation was supported in part by Atomic Energy Commission Contract No. SRO-AT-(38-1)-638 and Grant No. GB-16632 from the National Science Foundation.

‡ Present address: National Heart Institute, National Institutes of Health, Bethesda, Md.

§ Recipient of U. S. Public Health Service Career Development Award No. GM-35309-05.

In addition [^{3}H]methionine, which was used in the "double label" experiment presented in Figure 4, was obtained from New England Nuclear, and had a specific radioactivity of 3470 mCi/mmol.

Whatman DEAE-32- and CM-32-celluloses[1] were obtained from H. Reeve Angel, and Takadiastase RNase T_1 (E.C.-2.7.7.26) was obtained from Worthington.

Methods

Determinations of the Per Cent of Cotton and E. coli tRNA Charged by Cotton and E. coli Synthetases. The amount of the total tRNA of the several tRNA preparations that was charged by enzymes from the two sources was determined by measuring the extent of incorporation of radioactive amino acids into aminoacyl-tRNA as described previously (Merrick and Dure, 1972). From 0.5 to 2 A_{260} units of tRNA were acylated with the individual [^{14}C]- or [^{3}H]amino acids by the two enzyme preparations in 0.25-ml reaction mixtures. The assay system was optimized for each amino acid with respect to the concentration of ATP, amino acid, and synthetase preparation required to give complete charging within 20–40 min. The optimum ATP concentration ranged between 0.001 and 0.005 M and the concentration of amino acid and of the cotton synthetase preparation used was as previously reported (Merrick and Dure, 1972). The concentration of the *E. coli* synthetase preparation used ranged between 0.07 and 0.9 mg of protein per 0.25 ml of reaction mixture. Since we were interested only in determining the amount of tRNA charged by the two enzyme preparations, no attempt was made to assay each amino acid at the pH value that would give the maximum rate of charging with each of the enzyme preparations. It has been reported by Jacobson and others [see review by Jacobson (1971)] that it is often possible to obtain heterologous charging by using extreme or unusual reaction conditions, and that this sort of charging may not represent intrinsic recognition of tRNA by synthetase. Thus, in this work the reaction conditions we have used for the assays of heterologous charging were the same that were found to give rapid homologous charging.

Determination of the Isoaccepting tRNA Species Charged by the Two Enzyme Preparations. In order to more closely examine the individual isoaccepting tRNA species that were charged in the heterologous systems, the reaction mixtures in several instances were scaled up to give between 20,000 and 60,000 cpm of acylated tRNA. After incubation at 30° for a length of time sufficient to completely aminoacylate the tRNA with the given amino acid, the reaction mixture was applied to a DEAE-23 cellulose column (0.9 × 11 cm) equilibrated with 0.01 M sodium acetate, pH 4.5, 0.01 M $MgCl_2$, 0.01 M mercaptoethanol, and 0.3 M NaCl. The column was washed with this buffer until all free [^{14}C]amino acid and protein were eluted from the column. The [^{14}C]aminoacyl-tRNA was eluted with the same buffer made 1.0 M in NaCl. The eluate (5–10 ml) was diluted with 1 vol of distilled water and 60 A_{260} units of yeast nucleate carrier nucleic acid were added. The [^{14}C]-aminoacyl-tRNA along with the carrier nucleic acid were precipitated by the addition of 2.5 vol of cold 95% ethanol. This precipitate was dissolved in 1–2 ml of 0.01 M sodium acetate (pH 5.5)–0.001 M EDTA. Takadiastase RNase T_1 was added to this solution (150 enzyme units/A_{260} unit) and the solution incubated for 30 min at 37°. Additional RNase T_1 was added at this point (60 enzyme units/A_{260} unit) and the incubation continued for an additional 60 min. The digestion mixture was applied to a DEAE-32 cellulose column (1.0 × 24 cm) equilibrated with 0.01 M sodium acetate, pH 4.5. Radioactivity that was not retained by this column (positively charged [^{14}C]aminoacyl oligonucleotides) was applied to a CM-32-cellulose column of similar size equilibrated with the same buffer. The DEAE-cellulose column was eluted with a linear gradient (300 ml–300 ml) of 0.0–0.4 M NaCl that was 0.01 M sodium acetate, pH 4.5, and the CM-32-cellulose column was eluted with a linear gradient (100 ml–100 ml) of 0.0–0.2 M NaCl also buffered at pH 4.5 with 0.01 M sodium acetate. Elution was carried out at 30 ml/hr and 1-ml fractions were collected. Each fraction was mixed with 10 ml of a scintillation mixture designed for counting aqueous samples and its radioactivity determined. The counting efficiency of this method is 75% for ^{14}C and 25% for ^{3}H.

The elution profile of this column was monitored at 260 nm with an ISCO flow-through column monitor and the completeness of the RNase T_1 digestion was verified by the characteristic elution profile at 260 nm of the digested carrier yeast nucleate that was the source of the bulk of the eluting oligonucleotides. The RNase T_1 hydrolysis was performed at pH 5.5 to minimize the amino acid discharge from tRNA.

RNase T_1 specifically cleaves polynucleotides at the site of guanine residues producing guanosyl 3′-phosphate at the 3′ termini (Sato-Asano, 1959). Thus, digesting [^{14}C]aminoacyl-tRNA with this enzyme produces only one radioactive oligonucleotide fragment from each aminoacyl-tRNA molecule—the fragment containing the amino acid. The nucleotide length and composition of this fragment depend upon the position of the guanine residue nearest the CpCpA amino acid terminus of the molecule. Isoaccepting tRNA species that produce different aminoacyl oligonucleotides in the digestion can be distinguished and their relative concentrations measured if the [^{14}C]aminoacyl oligonucleotides are chromatographed on DEAE- or CM-cellulose at pH 4.5 *without* urea. This pH is used to produce about 0.5 net positive charge on cytosine residues and a slight net positive charge on adenine residues. Urea is omitted in this procedure to allow maximum interaction between the nucleoside residues and the column matrix, which promotes the separation of aminoacyl oligonucleotides that differ in nucleotide composition but not in nucleotide number. There is obviously a serious limitation to this technique in ascertaining the number and relative levels of isoaccepting tRNA species. Isoaccepting species that differ in nucleotide composition only in other parts of the polynucleotide chain, including the anticodon region, will generate the same aminoacyl oligonucleotide fragment upon digestion with RNase T_1 and thus not be distinguished by this technique. Transfer RNA from germinated, greened cotyledons was used as the cotton tRNA for digestion and chromatography in order to visualize a substantial amount of the chloroplastic tRNA species.

Results

Charging of Cotton tRNA by E. coli Synthetases. In Table I the extent to which *E. coli* synthetases can charge tRNA prepared from cotton cotyledons at different developmental stages with each of the 20 amino acids is given. These values are compared in this table with the relative amount of tRNA present in the several preparations that can be charged with cotton synthetases. The total amounts of each tRNA preparation that can be charged with synthetases from the two

[1] Abbreviations used are: CM-cellulose, carboxymethylcellulose; DEAE-cellulose, diethylaminoethylcellulose.

TABLE I: Per Cent of Cotton tRNA Charged with Each Amino Acid by Cotton and *E. coli* Synthetases.

Amino Acid	Source of tRNA: Young Embryo Cotyledons	Dryseed Cotyledons	Green Cotyledons	Chloroplasts
All tRNA Species Charged by *E. coli* Synthetases				
Arginine				
Cotton	9.0	8.7	9.3	9.5
E. coli	9.2	9.0	9.5	9.8
Histidine				
Cotton	3.4	3.3	3.7[a]	3.6
E. coli	3.4	3.3	3.7[a]	3.5
Lysine				
Cotton	5.2	5.3	3.6[a]	4.0
E. coli	5.3	5.4	3.6[a]	4.0
Methionine				
Cotton	3.5	3.3	4.7[a]	5.8
E. coli	3.5	3.4	4.9[a]	5.8
Only Chloroplastic tRNA Species Charged by *E. coli* Synthetase				
Leucine				
Cotton	10.0	9.8	11.2[b]	11.1
E. coli	0.5	0.5	3.2[b]	7.2
Isoleucine				
Cotton	3.2	3.2	3.3[b]	4.4
E. coli	0.5	0.5	1.7[b]	3.6
Tyrosine				
Cotton[c]	2.7	2.7	2.7	2.7
E. coli	0.3	0.3	1.0	2.3
No tRNA Species Charged with *E. coli* Synthetase[e]				
Alanine				
Cotton	5.1	5.1	4.8	4.7
E. coli	0	0	0	0
Glutamine				
Cotton	0.2	0.2	0.2	0.2
E. coli	0	0	0	0
Proline				
Cotton	4.6	4.6	4.1	3.2
E. coli	0	0	0	0
Some Chloroplastic and Some Cytoplasmic tRNA Species Charged by *E. coli* Synthetase				
Asparagine				
Cotton	1.4	2.5	1.3	2.1
E. coli	1.0	1.5	1.2	2.1
Aspartic acid				
Cotton	6.8	6.5	6.1	5.0
E. coli	1.0	1.0	1.1	2.9
Cysteine				
Cotton	0.8	0.7	0.7	0.9
E. coli	0.3	0.3	0.4	0.6
Glutamic acid				
Cotton	2.0	2.1	2.2	3.0
E. coli	0.2	0.5	0.4	1.4
Glycine				
Cotton	10.0	10.1	9.7	8.6
E. coli	2.9	2.9	3.1	4.5
Phenylalanine				
Cotton	4.6	4.6	5.0[d]	6.7
E. coli	1.5	1.4	3.5[d]	6.4
Serine				
Cotton	3.4	3.2	3.8	4.1
E. coli	1.5	1.4	2.0	3.5
Threonine				
Cotton	5.6	5.6	5.4	4.7
E. coli	1.8	1.8	2.6	3.5
Valine				
Cotton	9.0	8.9	8.8	6.6
E. coli	5.0	5.2	6.3	6.0
***E. coli* Synthetase(s) Not Demonstrable[f]**				
Tryptophan				
Cotton	2.1	1.9	2.3	1.9
Totals				
Cotton	92.6	92.3	92.9	93.1
E. coli	37.9	38.4	48.2	67.2
E. coli % of cotton	41	42	52	72

[a] See Figure 1. [b] See Figure 2. [c] Cotton synthetase(s) unstable, values estimated from *E. coli* charging. [d] See Figure 3. [e] *E. coli* synthetases active, see Table II. [f] *E. coli* synthetase(s) inactive, see Table II.

sources are given at the bottom of the table and show that, whereas over 90% of the tRNA in each preparation is acylatable by the cotton synthetases, the amount acylatable by *E. coli* synthetases is not the same for each preparation. The bacterial synthetases charge approximately the same amount of the tRNA of young embryo cotyledons and dry seed cotyledons. Although the metabolic activities and developmental status of cotyledons from these two stages are quite different, we have not found any change in the pool of tRNA acceptors nor isoacceptors between them (Merrick and Dure, 1972); consequently the near identity of the amount of tRNA in these two preparations that is charged by the bacterial enzymes is not surprising. However, there is roughly a 20% increase in the amount of tRNA chargeable by *E. coli* synthetases when tRNA from germinated cotyledons is used. Furthermore, there is an additional large increase in the amount of tRNA charged when tRNA from partially purified chloroplasts is used. We have estimated that chloroplast tRNA comprises about 8–10% of the total tRNA of young embryo and dry seed cotyledons, about 40% of the tRNA from germinated cotyledons, and about 75% of the tRNA from partially purified chloroplasts (Merrick and Dure, 1972). From this it can be inferred that the *E. coli* synthetases can charge a substantial number of cytoplasmic

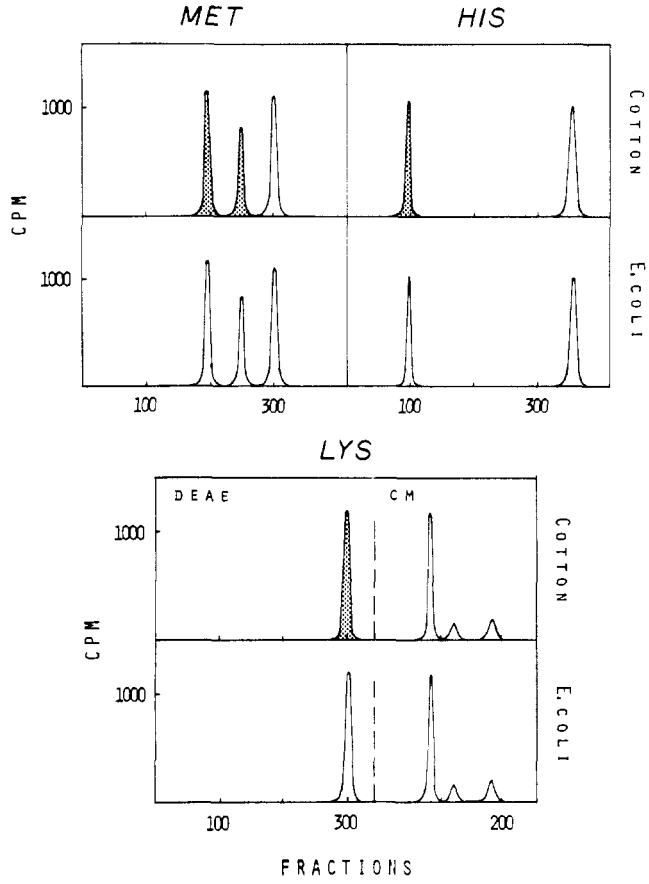

FIGURE 1: Radioactivity elution profiles of [^{14}C]methionyl, [^{14}C]-*histidyl*, and [^{14}C]*lysyl* oligonucleotides from DEAE- and CM-cellulose columns. Transfer RNA from germinated, greened cotton cotyledons was charged by cotton synthetases and *E. coli* synthetases, the charged tRNA digested with RNase T_1, and the resultant oligonucleotide mixtures were chromatographed as described under Methods. The synthetase source is indicated on the right side of each set of profiles. Shaded elution peaks represent aminoacyl oligonucleotides from chloroplastic aminoacyl-tRNA.

tRNA species, but that they can react with a much larger number of chloroplastic species.

Table I, third column, gives the data obtained with tRNA from germinated, greened cotyledons; however, the same data were obtained with tRNA from germinated, etiolated cotyledons, which again was expected, since we have shown that the amplification of chloroplast tRNA that occurs during the first few days of germination occurs in dark grown plants as well as those exposed to light (Merrick and Dure, 1972).

When the amount of tRNA from each of these preparations that can be acylated by the bacterial synthetases is examined individually for each amino acid, the extent of heterologous recognition falls into several categories. These categories are shown by the groupings of the amino acids in Table I. In the first category, comprised of arginine, histidine, lysine, and methionine, all the isoaccepting tRNA species appear to be acylatable by the *E. coli* enzymes since the amount of each tRNA preparation charged by the bacterial enzyme preparation is the same as that charged by the cotton enzyme preparation. To demonstrate this, the isoaccepting species for three of these (histidine, lysine, and methionine) were visualized by the DEAE- and CM-cellulose column chromatography of RNase T_1 digests of tRNA acylated with these amino acids by the homologous and heterologous enzymes. The elution profiles from these columns of the [^{14}C]aminoacyl oligonucleotides produced by the digestion are presented in Figure 1. It is apparent in this figure that the bacterial enzymes acylate the same isoaccepting species as do the homologous enzymes. The elution peaks that we have found to represent chloroplastic species (Merrick and Dure, 1972) are shaded in this and following figures. We have previously shown that the chloroplastic $tRNA^{Met}$ species that produces the methionyl oligonucleotide that elutes second from the DEAE column is the chloroplastic $tRNA_f^{Met}$, and that it can be formylated to produce formylmethionyl-tRNA by the *E. coli* transformylase also (Merrick and Dure, 1971).

The second category in Table I is comprised of those amino acids (leucine, isoleucine, and tyrosine) whose chloroplastic tRNA species only appear to be recognized by the *E. coli* synthetases. Table I shows that only a very small amount of the tRNA for these amino acids in the preparations from ungerminated cotyledons is charged by the bacterial enzymes, that a great deal more is charged in the preparation from germinated cotyledons, and that most of the tRNA for these amino acids is charged in the preparation of chloroplast tRNA. It should be pointed out that the cotton enzyme preparation does not acylate $tRNA^{Tyr}$ species, presumably because of the lability of the tyrosyl-tRNA synthetases. Consequently, the amount of $tRNA^{Tyr}$ present in the cotton tRNA preparations is estimated here from the amount of acylation obtained with the *E. coli* synthetase preparation, assuming that the bacterial enzymes recognize only the chloroplastic species as suggested by the data in Table I. The specificity of the bacterial enzymes for chloroplastic species alone is demonstrated for leucine and isoleucine in Figure 2. In this figure, which gives the elution profiles of the RNase T_1 digests of leucyl- and isoleucyl-tRNA produced by the two enzyme preparations, it is apparent that only those species that we have designated as chloroplastic species (Merrick and Dure, 1972) are acylated by the *E. coli* synthetases. Notice that we have concluded that the leucyl oligonucleotide eluting first from the column is produced by two isoaccepting $tRNA^{Leu}$ species, one cytoplasmic and one chloroplastic (shaded). Our basis for this stems from two observations. (a) The relative size of this peak is large when tRNA from embryonic cotyledons or roots is used or conversely when tRNA from chloroplasts is used (see Merrick and Dure, 1972) suggesting that a cytoplasmic and a chloroplastic $tRNA^{Leu}$ produce the same leucyl oligonucleotide on RNase T_1 digestion in this case. (b) Elution profiles from this column of leucyl oligonucleotides produced by the RNase T_1 digests of tRNA acylated by the *E. coli* enzyme preparation show only a very small amount of leucyl oligonucleotide in this region when tRNA from enbryonic cotyledons is used, a much larger amount when tRNA from germinated cotyledons is used (shown in Figure 2), and an amount almost equal to that obtained with the cotton enzyme preparation when chloroplastic tRNA is used. This indicates that of the two $tRNA^{Leu}$ species that produce the leucyl oligonucleotide that elutes in this position, the *E. coli* enzyme preparation charges only one, which is chloroplastic in origin.

The third category is comprised of tRNA for nine amino acids in which the bacterial enzymes apparently recognize some of the tRNA species from each source. The data in Table I suggest that all of the chloroplastic species and some of the cytoplasmic species of $tRNA^{Asn,Phe,Ser,Thr,\text{ and }Val}$ are recognized by the *E. coli* synthetases in that most of the tRNA for these amino acids from partially purified chloroplasts is acylated by this enzyme preparation, but that too high an

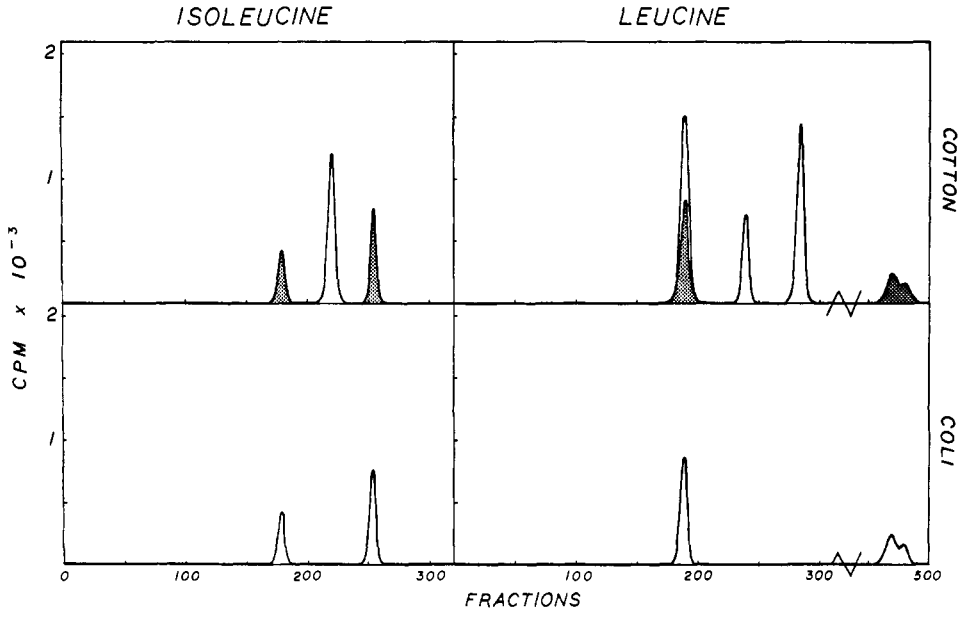

FIGURE 2: Radioactivity elution profiles of [^{14}C]isoleucyl and [^{14}C]leucyl oligonucleotides from DEAE-cellulose columns.

amount of the tRNA from ungerminated cotyledons is acylated for the recognition to be confined to chloroplastic species alone. The validity of this interpretation is demonstrated in Figure 3 for $tRNA^{Phe}$ species. The elution profiles of RNase T_1 digests of phenylalanyl-tRNA formed by each of the enzyme preparations show that the *E. coli* synthetases acylate both of the chloroplastic $tRNA^{Phe}$ species and one of the two cytoplasmic $tRNA^{Phe}$ species. Again we have concluded that two $tRNA^{Phe}$ species produce a single phenylalanyl oligonucleotide on RNase T_1 digestion in the same manner as we did with leucine. The isoaccepting species of $tRNA^{Asn,Ser\ and\ Thr}$ from cotton produce only one aminoacyl oligonucleotide upon RNase T_1 digestion (Merrick and Dure, 1972), and thus cannot be used to further substantiate our interpretation of the charging by *E. coli* synthetases of these acceptors.

The bacterial enzymes apparently acylate some but not all of the species of $tRNA^{Asp\ and\ Gly}$ from both sources. This is suggested by the data in Table I that show that too much of the tRNA from ungerminated cotyledons is charged for the charging to be restricted to chloroplast species, yet not enough of the tRNA from partially purified chloroplasts is charged to indicate the charging of all the chloroplast species for these two amino acids. The data in Table I also suggest that some but not all of the $tRNA^{Cys\ and\ Glu}$ from chloroplasts and probably none of the cytoplasmic species for these two amino acids are charged by the bacterial enzymes, since the charging percentages are consistently low but increase somewhat with tRNA from the chloroplast preparation.

The tRNA species for alanine, glutamine, and proline constitute another category in that none of these species are charged by the *E. coli* synthetases. The bacterial synthetases for these amino acids are active as shown by the ability of the *E. coli* enzyme preparation to acylate *E. coli* tRNA with these amino acids (see below and Table II). In contrast, tryptophanyl-tRNA synthetase activity cannot be demonstrated in the *E. coli* synthetase preparation with *E. coli* tRNA, presumably because of its lability; consequently the extent of its recognition of cotton $tRNA^{Trp}$ could not be determined.

Charging of E. coli tRNA by Cotton Synthetases. The extent to which a mixture of cytoplasmic and chloroplastic aminoacyl-tRNA synthetases from green cotton cotyledons can acylate *E. coli* tRNA is given in Table II. This table shows that under our reaction conditions over 90% of the *E. coli* tRNA can be charged by *E. coli* enzymes even with the tryptophanyl-tRNA synthetase inactive. Somewhat over 50% of this tRNA is shown in this table to be acylatable by cotton enzymes. Here again, the recognition of tRNA species by the heterologous enzymes falls into several categories. The data show that all of the species of $tRNA^{Ala,Ile,\ and\ Val}$ are charged by the cotton enzymes, that none of the $tRNA^{Gln}$ species are charged, and that some of the species for all the other amino acids are charged, except tyrosine which cannot be assayed because of the lability of the cotton tyrosyl-tRNA synthetases. Only a very small amount of cotton $tRNA^{Gln}$ is charged by the cotton enzymes, hence the failure to detect any acylation of *E. coli* $tRNA^{Gln}$ by the cotton enzymes may be due to the lability of the cotton glutaminyl-tRNA synthetases rather than to an intrinsic inability of these molecules to react.

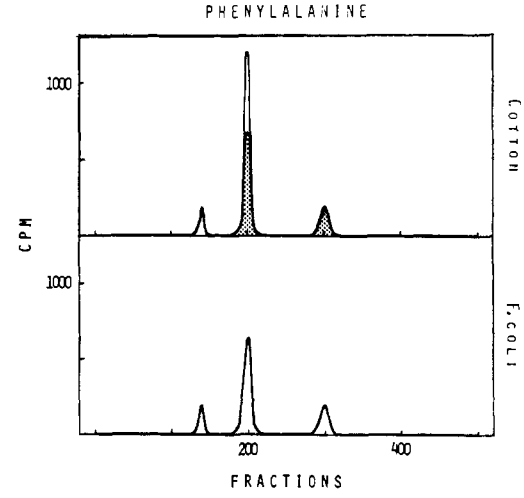

FIGURE 3: Radioactivity elution profiles of [^{14}C]phenylalanyl oligonucleotides from DEAE-cellulose columns.

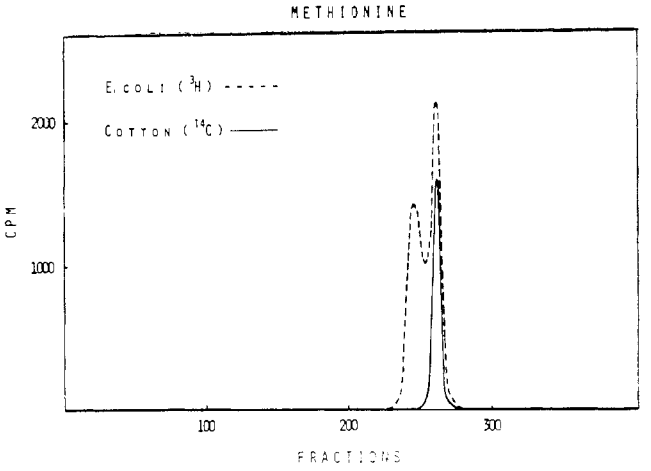

FIGURE 4: Radioactivity elution profiles of [^{14}C]- and [^{3}H]methionyl oligonucleotides from a DEAE-cellulose column. [^{14}C]Methionyl-tRNA formed by cotton synthetases from *E. coli* tRNA and [^{3}H]methionyl tRNA formed by *E. coli* synthetases from *E. coli* tRNA were digested with RNase T_1, and the resultant oligonucleotide mixtures were chromatographed together on the column.

In several cases it is possible to predict from the data in Table II the number of *E. coli* isoaccepting species that the cotton enzymes acylate. For example, the cotton enzymes charge about one-half of the $tRNA^{Met}$ present in the *E. coli* tRNA preparation. Two $tRNA^{Met}$ species can be demonstrated in the *E. coli* tRNA preparation by the DEAE-cellulose chromatography of RNase T_1 digested [^{14}C]methionyl-tRNA (Merrick and Dure, 1972). From this it appears that the cotton synthetases acylate one of these two species. This is demonstrated to be the case in Figure 4 which shows the elution profiles of the cochromatography of [^{14}C]- and [^{3}H]methionyl oligonucleotides derived from methionyl-tRNA produced by the cotton and *E. coli* synthetases respectively. Only one of the two $tRNA^{Met}$ species is shown to have been charged by the cotton enzymes. It is interesting that the species acylated by the cotton enzyme preparation can be identified as the *E. coli* $tRNA_f^{Met}$ (Merrick and Dure, 1972), and that the cotton chloroplastic methionyl oligonucleotide derived from chloroplastic $tRNA_f^{Met}$ elutes at the identical position from the DEAE-cellulose column (Merrick and Dure, 1971, 1972). This fact indicates that the cotton chloroplastic $tRNA_f^{Met}$ has the nucleotide composition, CAACCA, at its 3′-terminus as does the *E. coli* $tRNA_f^{Met}$.

TABLE II: Per Cent of *E. coli* tRNA Charged with Each Amino Acid by Cotton and *E. coli* Synthetases.

Amino Acid	Source of Synthetases	
	E. coli	Cotton
All Species Charged		
Alanine	2.3	2.3
Isoleucine	3.5	3.5
Valine	7.5	7.7
Some Species Charged		
Arginine	7.6	2.1
Asparagine	1.5	0.4
Aspartic acid	3.6	2.7
Cysteine	0.9	0.4
Glutamic acid	4.6	2.6
Glycine	6.1	5.0
Histidine	3.3	1.1
Leucine	10.1	6.7
Lysine	6.3	3.5
Methionine	6.1[a]	3.2[a]
Phenylalanine	6.0	1.8
Proline	4.3	1.1
Serine	5.7	4.0
Threonine	5.1	2.6
Tryptophan	[b]	0.8
No Species Charged		
Glutamine	3.9	0
Cotton Synthetase not Demonstrable		
Tyrosine	2.9	
Total	91.3	51.5

[a] See Figure 4. [b] *E. coli* synthetase not demonstrable.

Discussion

In view of the apparent similarity between bacterial and chloroplastic rRNA and ribosomes (Loening and Ingle, 1967), the similarity in the response of the two protein synthesis systems to inhibitors (Smillie *et al.*, 1971), and their joint requirement for formylmethionyl-tRNA for the initiation of protein synthesis (Schwartz *et al.*, 1967), it is not surprising that the bacterial aminoacyl-tRNA synthetases recognize more chloroplastic tRNA species than cytoplasmic tRNA species. This observation indicates that a greater number of recognition sites remain functional between bacterial enzymes and chloroplast tRNA than between these enzymes and cytoplasmic tRNA. This might suggest that the evolutionary time distance between the divergence of chloroplast-originating organisms and bacteria is less than that between the divergence of bacteria from the organisms that were the progenitors of the nucleus of higher plants. This interpretation assumes chloroplast capture by the organisms that gave rise to higher plants (Margulis, 1969). However, an alternative view that accounts for the lower interaction between cytoplasmic tRNA and *E. coli* enzymes by assuming an accelerated rate of change in the evolution of the plant nucleus is also tenable.

It would be interesting to know whether cotton cytoplasmic or chloroplastic enzymes are chiefly responsible for the 50% acylation of *E. coli* tRNA that we observe. We have separated cotton cytoplasmic and chloroplastic synthetases for isoleucine and leucine and examined their separate recognition of *E. coli* tRNA species. In the case of isoleucine, both synthetases appear to charge all the bacterial $tRNA^{Ile}$, whereas, in the case of leucine, the chloroplastic enzyme acylates about two-thirds of the bacterial $tRNA^{Leu}$ and the cytoplasmic enzyme about one-third.[2]

It seems possible that the measurement of the extent of heterologous charging between critically selected procaryots and organelle and cytoplasmic components of eucaryots may prove useful in suggesting phylogenetic relationships in much the same way that comparisons of macromolecule sequences have proven useful.

[2] Unpublished data.

An interesting feature of our data is the fact that *different* groups of aminoacyl-tRNA species are completely acylated by the heterologous enzymes. That is, the *E. coli* synthetases recognize all the species of cotton tRNA for arginine, histidine, lysine, and methionine, whereas the cotton synthetases which react with these homologous species do not recognize all the species of *E. coli* tRNA for these four amino acids. Rather, the cotton synthetases recognize all the species of *E. coli* tRNA for alanine, isoleucine, and valine, yet the bacterial synthetases for these amino acids do not react with the corresponding set from cotton. This observation suggests that the recognition interaction is not necessarily identical in homologous and heterologous systems, since the enzymes from both sources that recognize identical tRNA species from one source do not in turn recognize all the same tRNA species from the second source. Other examples of this phenomenon have been cited by Jacobson (1971), and it must be taken into account by any theory of tRNA-synthetase recognition.

References

Jacobson, K. B. (1971), *Progr. Nucl. Acid Res. Mol. Biol. 11*, 461.

Kelmers, A. D., Novelli, G. D., and Stulberg, M. P. (1965), *J. Biol. Chem. 240*, 3979.

Loening, U. E., and Ingle, J. (1967), *Nature (London) 215*, 363.

Margulis, L. (1969), *J. Geol. 77*, 606.

Merrick, W. C., and Dure, L. S. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 641.

Merrick, W. C., and Dure, L. S. (1972), *J. Biol. Chem. 247*, 7988.

Sato-Asano, K. (1959), *J. Biochem. (Tokyo) 46*, 31.

Schwartz, J., Meyer, R., Eisenstadt, J., and Brawerwan, G. (1967), *J. Mol. Biol. 25*, 571.

Smillie, R. M., Bishop, D. G., Gibbons, G. C., Graham, D., Grieve, A. M., Raison, J. K., and Reger, B. J. (1971), *in* Autonomy and Biogenesis of Mitochondria and Chloroplasts, Boardman, N. K., Linnane, A. W., and Smillie, R. M., Ed., Amsterdam, North-Holland Publishing Co., p 422.

Protein Synthesis in Chicken Muscular Dystrophy†

Barbara-Anne Battelle‡ and James R. Florini*

ABSTRACT: Activities of ribosome preparations from muscles of normal and dystrophic chickens of various ages were compared. Ribosomes from breast muscles of newly hatched dystrophic chickens were significantly less active in protein synthesis than those from muscles of normal chickens, but this difference was reversed in older animals. The differences in activities could not be explained by elevated RNase activity or by differential loss of polysomes during the isolation procedure. We suggest that the lower activity of breast muscle ribosomes from young dystrophic chickens was caused by a defect in the ribosomes themselves. The basis for the increase in activity of muscle ribosomes from older animals is not clear, but it is not due to increased collagen synthesis. We conclude that the progressive loss of protein from dystrophic muscles cannot be explained by an inability of ribosomes from these muscles to efficiently synthesize protein. However, preliminary qualitative comparisons of proteins synthesized in normal and dystrophic chicken breast muscles showed that there may be changes in the types of soluble proteins synthesized in muscles of older dystrophic chickens.

Baieve and Florini (1970) have reported that a substantial decrease in RNA synthesis (measured in intact muscles or in isolated nuclei) occurs in young chickens with muscular dystrophy. On the basis of assays with Mg^{2+} and Mn^{2+} in the incubation medium, it was suggested that these differences could be attributed to changes in activity or amount of enzyme involved in synthesis of nonribosomal RNA; the enzyme for rRNA synthesis was relatively unaffected by the disease. If this apparent decrease in polymerase II activity leads to lesser availability of mRNA to the ribosomes, then it might be expected that ribosomes from dystrophic muscle would contain fewer polysomes and be less active in protein synthesis than ribosomes from normal muscle. However, studies in which the protein synthetic activity of normal and dystrophic chicken muscle were compared *in vivo* and in intact muscles *in vitro* (Weinstock *et al.*, 1969) showed dystrophic muscle to be more active in protein synthesis than normal muscle.

In an attempt to reconcile the apparently conflicting results of studies of RNA and protein synthesis in dystrophic muscle, we have compared the protein synthetic activity of isolated ribosome preparations (assayed in the transfer reaction) from breast muscles of normal and dystrophic chickens of various ages. We found that ribosomes from breast muscles of newly hatched dystrophic chickens were less active in protein synthesis than ribosomes from breast muscles of comparable normal chickens, but that this difference was reversed with ribosomes from older chickens. The lower activity of ribosomes from young dystrophic animals was not attributable to a lack of mRNA but rather to some defect in the ribosomes themselves. The cause of the increased activity of ribosomes from older dystrophic chickens was not clear, but it was not associated with an increased synthesis of collagen. Preliminary

† From the Department of Biology, Syracuse University, Syracuse, New York 13210. *Received September 19, 1972.* A preliminary report of this work was presented at the meetings of the American Society of Biological Chemists, San Francisco, Calif. (Battelle *et al.*, 1971). This investigation was in partial fulfillment of requirements for a Ph.D. degree (Battelle, 1972), and was supported by a grant from the Muscular Dystrophy Associations of America.

‡ Present address: Department of Neuropathology, Harvard Medical School, Boston, Mass. 02115.