Limitation of Reticulocyte Transfer RNA in the Translation of Heterologous Messenger RNAs†

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ABSTRACT: The effect of various tRNAs on protein synthesis was investigated using a tRNA-dependent cell-free system from Ehrlich ascites cells. Ascites cell tRNA and rabbit liver tRNA were found to promote efficient translation of globin mRNA, oviduct mRNA, and encephalomyocarditis (EMC) viral RNA. In contrast, reticulocyte tRNA participated efficiently only in the translation of globin mRNA; the translation of oviduct mRNA and EMC viral RNA in the presence of reticulocyte tRNA resulted in the synthesis of relatively few large mature proteins and the accumulation of discrete, smaller polypeptides. These results suggest that isoaccepting tRNA species required for the synthesis of ovalbumin and EMC viral protein (but not hemoglobin) are probably functionally absent in reticulocyte tRNA, causing a premature, nonrandom termination of synthesis of these proteins. This provides preliminary evidence that variations in tRNA populations, frequently observed between different cell types, are large enough to define and perhaps regulate the proteins that the cell is capable of synthesizing.

It was suggested earlier that protein synthesis can be regulated at the translation level by the availability of certain species of tRNA (Ames and Hartman, 1963; Itano, 1965; Anderson, 1969). If such mechanisms are operative, alterations in tRNA populations would be expected in systems undergoing shifts in regulatory processes. Changes in the populations of tRNA have been observed during embryonic development, differentiation, neoplasia (Sueoka and Kano-Sueoka, 1970; Borek and Kerr, 1972; Littauer and Inouye, 1973) and in systems under the influence of hormones (Sharma and Borek, 1974). In all cases examined, the changes have been restricted to a few tRNA species and there has been little evidence presented that these changes effect in a discriminatory manner the translation of available mRNAs.

In some cell types there appears to be a correlation in the amino acid acceptor activity of many tRNA species with the amino acid composition of the major protein synthesized. Such a correlation has been observed in the reticulocytes of rabbits (Smith and McNamara, 1971) and sheep (Litt and Kabat, 1972), and silk gland of silkworm during fibroin synthesis (Garel et al., 1971; Delany and Siddiqui, 1975), the lactating mammary gland (Elska et al., 1971), rat fibroblasts during collagen synthesis (Lanks and Weinstein, 1970), bovine lens tissue synthesizing crystallins (Garel et al., 1970), and in rooster liver during estrogen-induced phosvitin synthesis (Mäenpää and Bernfield, 1969). Evidence for the regulation of protein synthesis in eukaryotes by tRNA was provided by studies on hemoglobin synthesis in a cell-free system from rabbit reticulocytes (Gilbert and Anderson, 1970), and by our work on ovalbumin synthesis (Sharma et al., 1973, 1975a, 1976).

In view of the specialization of reticulocytes for the synthesis of hemoglobin as the major protein, tRNA from rabbit reticulocytes was examined for its ability to participate in the translation of other mRNAs in a tRNA-dependent protein synthesizing system that we have developed (Sharma et al., 1975b). Our results show that in the presence of reticulocyte tRNA, oviduct mRNA and EMC[1] RNA are not translated efficiently or with fidelity compared to the translation of these RNAs in the presence of tRNA from rabbit liver or Ehrlich ascites cells. In contrast, the tRNA from reticulocytes, ascites cells, and rabbit liver are equally effective in the synthesis of globin from reticulocyte mRNA. This suggests a functional specialization of reticulocyte tRNA for hemoglobin synthesis (Smith and McNamara, 1971) and also provides evidence that variations in animal cell tRNA populations do have the potential for restricting the translation of certain classes of mRNA within the cell.

Experimental Section

Materials. ^{14}C-amino acid mixture (algal profile) and ribonuclease-free sucrose were obtained from Schwarz/Mann; [^{35}S]methionine was procured from New England Nuclear; creatine kinase was from Boehringer-Mannheim; nucleoside triphosphates were purchased from Sigma Chemical Co.; Oligo(dT)-cellulose (T-2) was a product of Collaborative Research. Extreme care was taken during all manipulations to prevent contamination with adventitious ribonuclease. All glassware was dried overnight at 300 °C and the solutions were made in glass-distilled autoclaved water.

Isolation of tRNA. Transfer RNA from rabbit liver and rabbit reticulocyte postribosomal supernatant fraction (Gilbert and Anderson, 1970) was isolated as described earlier (Sharma et al., 1975a), except that the 2-propanol precipitation step was omitted. Ehrlich ascites cell tRNA was from 7–8-day old cells. Rabbit reticulocyte whole cell tRNA was extracted at pH 9.0 and the high-molecular-weight RNA was removed by salt precipitation. All tRNA preparations were deacylated and purified on Sephadex G-100.

Extraction of Encephalomyocarditis RNA. Purified virus was from G. D. Searle and Co., England, and the RNA was extracted according to Kerr et al. (1966).

Isolation of Oviduct mRNA. RNA was isolated from ovi-

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[1] Abbreviations used are: DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; ATP, GTP, adenosine and guanosine triphosphates; EMC, encephalomyocarditis.

ducts of laying hens by homogenization in 5 volumes of 50 mM Tris-HCl (pH 8.3), 5 mM EDTA, 75 mM NaCl, 0.5% sodium dodecyl sulfate, and an equal volume of buffer-saturated phenol, in a blender for 1 min at 4 °C. The aqueous phase was reextracted five times with phenol and the RNA was precipitated from the aqueous phase by the addition of 0.1 volume of 20% potassium acetate (pH 5.0) and 2.5 volumes of ethanol. Bulk RNA was used for the isolation of oviduct mRNA by using oligo(dT)-cellulose (Aviv and Leder, 1972).

Isolation of Globin mRNA. Rabbit reticulocyte polysomes were isolated according to Gilbert and Anderson (1970) and globin mRNA was purified by oligo(dT)-cellulose (Aviv and Leder, 1972). In some experiments purified 9S rabbit reticulocyte globin mRNA from G. D. Searle and Co., England, was used.

Isolation of tRNA-Dependent Protein Synthesizing System from Ehrlich Ascites. Ribosomes and ammonium sulfate fractions were freed from tRNA by passing through DEAE-cellulose immediately before addition to the protein synthesizing system (Sharma et al., 1975b).

Assay of Protein Synthesis. The assay mixture contained in a volume of 100 μl: 20 mM Tris-HCl (pH 7.5), 1 mM ATP, 0.2 mM GTP, 6 mM creatine phosphate (all adjusted to pH 7.5), 20 μg of creatine kinase, 0.5 μCi of ^{14}C-amino acid mixture, 0.05 mM of each of the six freshly neutralized nonradioactive amino acids missing from the ^{14}C mixture (or 5 μCi of [^{35}S]methionine and 0.05 mM of each 19 nonradioactive amino acids), 6 mM β-mercaptoethanol, specified amounts of tRNA, KCl, magnesium acetate, mRNA, DEAE-cellulose-passed ribosomes, and ammonium sulfate fraction. All incubations were carried out in duplicate. After incubation for 90 min at 37 °C (a time period for which amino acid incorporation was linear), to determine amino acid incorporation into polypeptides, 0.2 ml of 0.2 N KOH was added and the incubation was continued for an additional 20 min. The tubes were chilled in ice, 1 ml of 5% trichloroacetic acid was added, and the samples were filtered through Whatman GF/C filters, washed 4–5 times with 5% trichloroacetic acid and once with ethanol. The filters were dried and counted in a liquid scintillation counter using 0.4% 2,5-diphenyloxazole, 0.01% 1,4-bis[2-(5-phenyloxazolyl)] benzene in toluene. In reactions where the ovalbumin synthesis was determined by antibody precipitation, duplicate aliquots were processed for immunoprecipitation and other sets of duplicate samples were taken for the determination of amino acid incorporation and sodium dodecyl sulfate gel electrophoresis (Palmiter et al., 1971; Studier, 1973).

Immunoprecipitation of Ovalbumin by Monospecific Rabbit Antiovalbumin Serum. Ovalbumin synthesis was determined in aliquots of the assay mixture after incubation for 90 min at 37 °C by specific immunoprecipitation with antiserum against purified ovalbumin (Palmiter et al., 1971). Aliquots (25 μl) of the reaction mixture were adjusted to 230 μl in plastic microfuge tubes (Beckman) by the addition of 15 mM NaCl in 10 mM sodium phosphate (pH 7.5), to this were added 10 μl of ovalbumin (5 μg), 50 μl of ovalbumin antiserum (antibody excess), and 20 μl of 10% sodium deoxycholate. The contents of the tubes were mixed and incubated overnight at 37 °C. The precipitates were collected by centrifugation in Beckman microfuge (Model 152) and were washed 3–4 times with 10 mM sodium phosphate (pH 7.5) containing 150 mM NaCl, 1% deoxycholate, and 1% Triton X-100 (Sharma et al., 1973). The pellet was dissolved and counted. Correction for nonspecific trapping of radioactivity by the immunoprecipitate was made by carrying out precipitation reactions in identical aliquots of the reaction mixture in the presence of 5 μg of bovine serum albumin and 50 μl of bovine albumin antiserum. The immunoprecipitate from assay mixtures with oviduct mRNA usually contained 3000 to 6000 cpm compared to 600–800 cpm of nonspecific trapping. Ovalbumin synthesis was reproducibly quantified by this procedure. Ovalbumin specific radioactivity was not precipitated from incubations in which rabbit globin mRNA or encephalomyocarditis viral RNA were translated. The specificity of the precipitation reaction was also checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the antibody–antigen precipitate (Palmiter et al., 1971). The radioactivity in the antibody–antigen precipitate from estrogen-stimulated chick oviduct explants migrated as a single peak with the mobility of ovalbumin.

TABLE I: Translation of Oviduct mRNA in the Presence of tRNAs from Different Sources.[a]

tRNA Source	^{14}C Amino Acid Incorporation (cpm)	Ovalbumin Synthesis (cpm)	Ovalbumin Synthesis, % of Amino Acid Incorporation
None	1 800	0	0
Ehrlich ascites cells	56 700	21 400	37.7
Rabbit reticulocyte	33 500	7 200	21.5
Rabbit liver	62 700	26 100	41.6
Yeast	7 400	250	3.4
E. coli	7 950	0	0

[a] The details for protein synthesis were the same as described under Experimental Section. The reaction mixture contained DEAE-cellulose passed ribosomes (0.45 A_{260} unit), ammonium sulfate fractions (0.12 A_{260} unit), 3 mM magnesium acetate, 60 mM KCl, oviduct mRNA (5 μg), and 5 μg of different tRNAs. Endogenous amino acid incorporation (in absence of added mRNA) has been subtracted from these values. Background amino acid incorporation was 9000–11 000 cpm for incubations containing Ehrlich ascites, reticulocyte, and liver tRNAs. In assays containing yeast and *E. coli* tRNAs, endogenous amino acid incorporation was 2500–3000 cpm.

Results

In the reconstituted tRNA-dependent protein synthesizing system from Ehrlich ascites cells, addition of rabbit reticulocyte tRNA supported oviduct mRNA-directed protein synthesis to a lesser extent than the tRNA from Ehrlich ascites cells or rabbit liver (Table I). The difference observed with reticulocyte tRNA was more pronounced when the specific protein synthesis was measured by immunoprecipitation of the newly synthesized protein with monospecific antiserum to ovalbumin (Table I). The reduced effectiveness of reticulocyte tRNA in ovalbumin synthesis was not due to suboptimal amounts of tRNA added to the reaction mixture. With different tRNA preparations, protein synthesis reached saturation at approximately 5 μg of tRNA/assay mixture (Figure 1). Similar results were obtained regardless of whether the reticulocyte tRNA was isolated from whole cells or from a postribosomal supernatant fraction. Both sources of reticulocyte tRNA were used to investigate the possible effect of a preferential loss of certain tRNA species from the postribosomal supernatant (Smith and McNamara, 1974) from which the tRNA used in the earlier experiments was isolated. The results shown in Table I and Figure 1 were repeated using three different preparations of tRNA isolated from postribosomal supernatant fraction and whole reticulocytes. The apparent extent of ovi-

duct mRNA translation varied somewhat depending upon the preparations of ribosomes and ammonium sulfate fractions used in the assays; however, in all cases a significant reduction in translation with reticulocyte tRNA was observed compared to liver or ascites cell tRNA. Reproducible results with different tRNA preparations required the use of protein synthesizing systems that showed at least a 20–50-fold stimulation in amino acid incorporation following the addition of tRNA.

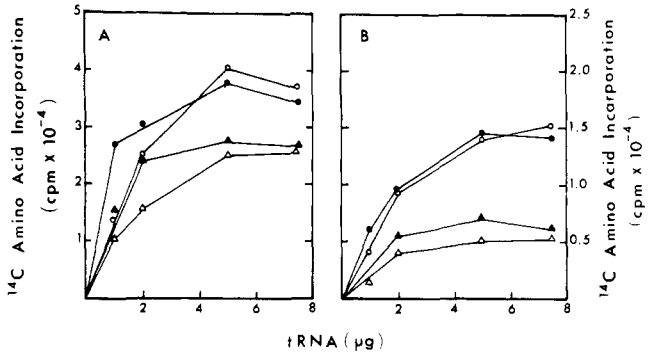

FIGURE 1: Stimulation of protein synthesis by oviduct mRNA in a tRNA-dependent protein synthesizing system. The details for protein synthesis were the same as described for Table I. Oviduct mRNA (5 μg) was translated with specified amounts of tRNA from Ehrlich ascites cells (●), rabbit liver (○), whole reticulocytes (▲), or reticulocyte supernatant fraction (△). (A) Stimulation of amino acid incorporation; (B) ovalbumin synthesis as measured by specific immunoprecipitation.

The products of cell-free protein synthesis in the presence of oviduct mRNA and ascites cell or rabbit liver tRNA were analyzed by sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. These gel patterns showed a prominent peak of radioactivity with the mobility expected of ovalbumin synthesized in vitro (Figure 2 A,D); the in vitro synthesized ovalbumin migrates slightly faster than the ovalbumin synthesized in chick oviducts, presumably because it lacks carbohydrate moiety (Rhoads et al., 1971). However, the sodium dodecyl sulfate-polyacrylamide gel pattern of proteins synthesized in the presence of reticulocyte tRNA did not show a characteristic sharp peak of radioactivity for ovalbumin (Figure 2 B,C). Ovalbumin antibody precipitates of the reaction mixture containing ascites cell or rabbit liver tRNA migrated with the mobility of ovalbumin synthesized in vitro on the gels and constituted 75–80% of the total radioactivity; the remaining 20–25% of the radioactivity migrated faster than ovalbumin (Figure 3 A,D). In contrast, antibody precipitates of the reaction mixture containing reticulocyte tRNA showed only 35–45% of the radioactivity with the characteristics of ovalbumin synthesized in vitro (Figure 3 B,C); the remaining 55–65% of the radioactivity migrated faster than ovalbumin. The faster moving components probably represent nascent incomplete ovalbumin polypeptides that were still capable of

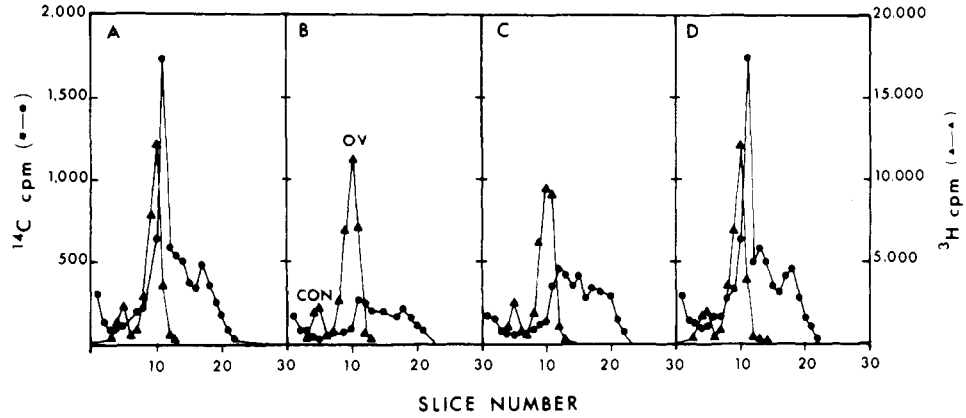

FIGURE 2: Sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of proteins synthesized with oviduct mRNA and tRNA from different sources. Aliquots (25 μl) of the reaction mixture, following 90-min incubation, were precipitated with 5% trichloroacetic acid; the precipitates were washed twice with 5% trichloroacetic acid, mixed with 5 μl of ^{3}H-labeled conalbumin and ovalbumin as internal standard (synthesized in oviduct magnum explants), and coelectrophoresed (Palmiter et al., 1971). Oviduct mRNA (5 μg) was translated with 5 μg of tRNA from Ehrlich ascites cells (A), reticulocyte supernatant fraction (B), whole reticulocytes (C), and rabbit liver (D). ^{3}H-labeled conalbumin and ovalbumin (▲—▲); ^{14}C-labeled proteins synthesized with oviduct mRNA (●—●). The mobility of conalbumin (CON) and of ovalbumin (OV) is indicated.

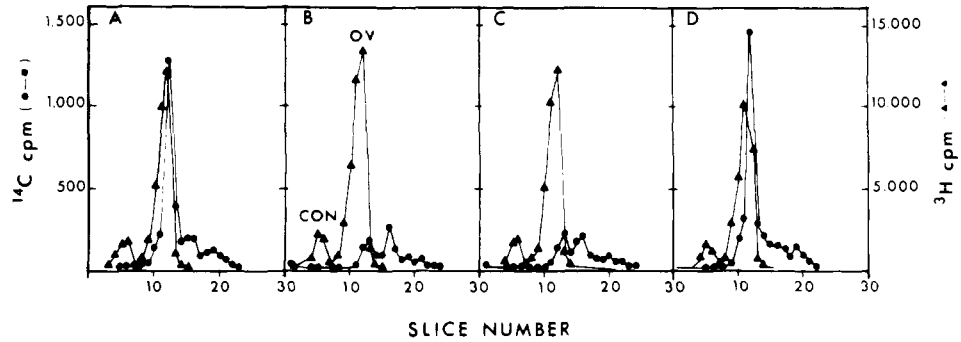

FIGURE 3: Sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of ^{14}C-labeled proteins precipitated with antiserum to ovalbumin. Antibody precipitation was carried out in 25-μl aliquots of the reaction mixture of protein synthesis after 90 min of incubation. Antibody precipitates were washed twice and mixed with 5 μl of ^{3}H-labeled standard consisting of conalbumin and ovalbumin, and coelectrophoresed (Palmiter et al., 1971). Oviduct mRNA (5 μg) was translated with 5 μg of tRNA from Ehrlich ascites cells (A), reticulocyte supernatant fraction (B), whole reticulocyte (C), and rabbit liver (D). ^{3}H-labeled standard (▲—▲); ^{14}C-labeled proteins precipitated with ovalbumin antiserum (●—●).

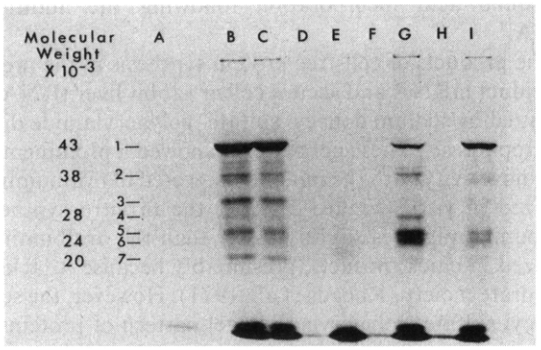

FIGURE 4: Autoradiography of [^{35}S]methionine-labeled products of cell-free translation in response to oviduct mRNA and different tRNAs. Oviduct mRNA (5 μg) was added with 5 μg of tRNA from different sources. The conditions for cell-free protein synthesis are described under Table I. Aliquots (25 μl) of the reaction mixtures were precipitated with 5% trichloroacetic acid; the precipitates were washed twice with 5% trichloroacetic acid, solubilized (Palmiter et al., 1971), and electrophoresed on sodium dodecyl sulfate–10% polyacrylamide slab gels (Studier, 1973) for 18 h at 20 V. Following electrophoresis, the gels were stained with Coomassie brilliant blue for 1 h and destained by 5% methanol–7% acetic acid. The gels were dried under vacuum and autoradiographed using Kodak blue x-ray film. (A) Ascites cell tRNA; (B) ascites cell tRNA + oviduct mRNA; (C) rabbit liver tRNA + oviduct mRNA; (D) yeast tRNA; (E) yeast tRNA + oviduct mRNA; (F) reticulocyte tRNA; (G) reticulocyte tRNA + oviduct mRNA; (H) yeast + reticulocyte tRNA; (I) yeast + reticulocyte tRNA + oviduct mRNA. The molecular weights indicated on the left are apparent molecular weights. Band 1 is ovalbumin.

interacting with the ovalbumin-specific antibody.

The results obtained with reticulocyte tRNA in ovalbumin synthesis were not due to the inactivation of reticulocyte tRNA species during isolation, since these tRNA species were as effective as ascites and rabbit liver tRNA in supporting the translation of globin mRNA (Table II). Saturation for amino acid incorporation was reached with 5 μg of tRNA from the different sources and the in vitro synthesized proteins comigrated with rabbit globin following electrophoresis on sodium dodecyl sulfate–polyacrylamide disc gels (data not shown). These results differ somewhat from those of Gilbert and Anderson (1970) who observed in a tRNA-dependent protein synthesizing system from rabbit reticulocytes that more globin synthesis took place in the presence of saturating concentrations of rabbit reticulocyte tRNA than with rabbit liver tRNA. This difference may be a reflection of the fact that the reticulocyte protein synthesizing system, in contrast to the ascites cell system, is a homologous system for globin mRNA translation and may contain aminoacyl-tRNA synthetases or other factors that function most efficiently with reticulocyte tRNA.

The differences observed with reticulocyte tRNA in the translation of oviduct mRNA could be due to the presence of small amounts of one or more rate-limiting tRNA species in reticulocyte tRNA which are required for the translation of oviduct mRNA. To test this possibility, reticulocyte tRNA was supplemented with yeast tRNA in the assay mixture. Yeast tRNA by itself did not significantly stimulate amino acid incorporation with oviduct mRNA (Table I); this was expected, since not all yeast tRNA species can be charged by mammalian aminoacyl-tRNA synthetases (Aviv et al., 1971; Jacobson, 1971). The polypeptides synthesized using oviduct mRNA and various tRNAs in the in vitro system were examined by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis (Figure 4). When ascites tRNA or liver tRNA was replaced by reticulocyte tRNA, there was less synthesis of ovalbumin

TABLE II: Stimulation of Amino Acid Incorporation in the Presence of Globin mRNA and EMC Viral RNA.

tRNA Source	^{14}C Amino Acid Incorporation (cpm)	
	Globin mRNA	EMC RNA
None	132	3 800
Ehrlich ascites cells	43 300	177 100
Rabbit reticulocyte (postribosomal supernatant)	53 300	94 200
Rabbit reticulocyte (whole cell)	45 700	106 500
Rabbit liver	48 900	164 000
Yeast	—	6 300
E. coli	—	3 500

[a] The details for protein synthesis were the same as described for Table I. EMC RNA (3 μg) was translated at 5 mM magnesium acetate, 100 mM KCl, and globin mRNA (2.5 μg) at 3 mM magnesium acetate and 60 mM KCl. Background amino acid incorporation was 4000–5000 cpm at 5 mM magnesium acetate, 100 mM KCl, and under the conditions of globin mRNA translation 6500–10 000 cpm. (—) Indicates not determined.

(slot G, band 1), the relative amount of polypeptide 3 was reduced, and discrete polypeptides smaller than ovalbumin (bands 4, 5, and 6) accumulated. Addition of yeast tRNA (5 μg) to assay mixtures containing 5 μg of reticulocyte tRNA (slot I) resulted in the increase in protein synthesis, and the electrophoretic pattern of synthesized proteins resembled more closely the patterns of proteins produced with ascites cell tRNA (slot B) or rabbit liver tRNA (slot C).

The ability of reticulocyte tRNA to support protein synthesis directed by a heterologous mRNA also was tested using EMC viral RNA. EMC virus replicates in ascites cells and its translation in ascites cell-free system has been well studied (Aviv et al., 1971; Boime et al., 1971; Boime and Leder, 1972; Kerr et al., 1972). In a cell-free system from Krebs ascites cells EMC RNA directs the synthesis of 12–14 polypeptides, ranging in molecular weight from 20 000 to 140 000. Most of these polypeptides are the result of premature nonrandom termination of translation (Boime and Leder, 1972; Kerr et al., 1972). However, the proteins synthesized in vitro contain sequences characteristic of both virion capsid (Boime and Leder, 1972; Kerr et al., 1972) and EMC virus-specific proteins synthesized in infected cells (Kerr et al., 1972). Reticulocyte tRNA was approximately 50% less effective than ascites cell tRNA in stimulating amino acid incorporation with EMC RNA (Table II). Varying the amount of reticulocyte tRNA from 2 to 10 μg in the assay mixture did not affect the results; saturation of amino acid incorporation in the presence of EMC RNA was reached with 5 μg of reticulocyte tRNA (data not shown). An examination by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis of the viral RNA-specific polypeptides synthesized in vitro using reticulocyte tRNA (Figure 5, slot F) showed quantitative and qualitative differences from the polypeptides synthesized with ascites cell tRNA (Figure 5, slot B). Polypeptide 6 appears to be absent from the incubations containing reticulocyte tRNA (slot F), polypeptides 8 and 9 were reduced, polypeptide 10 was more prominent, and there were two diffuse bands corresponding to polypeptide 11 in slot B. Supplementation of reticulocyte tRNA with yeast tRNA resulted in an increase in protein synthesis, and the electrophoretic pattern of synthesized po-

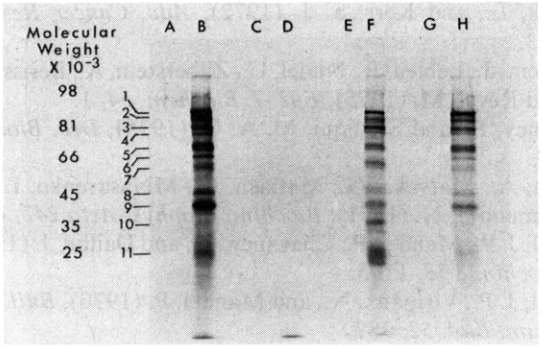

FIGURE 5: Autoradiography of [^{35}S]methionine-labeled products of cell-free translation in response to EMC RNA and different tRNAs. EMC RNA (3 μg) was translated at 5 mM magnesium acetate, 100 mM KCl, and 5 μg of different tRNA. Other details are described under Figure 4. (A) Ascites cell tRNA; (B) ascites cell tRNA + EMC RNA; (C) yeast tRNA; (D) yeast tRNA + EMC RNA; (E) reticulocyte tRNA; (F) reticulocyte tRNA + EMC RNA; (G) yeast + reticulocyte tRNA; (H) yeast + reticulocyte tRNA + EMC RNA.

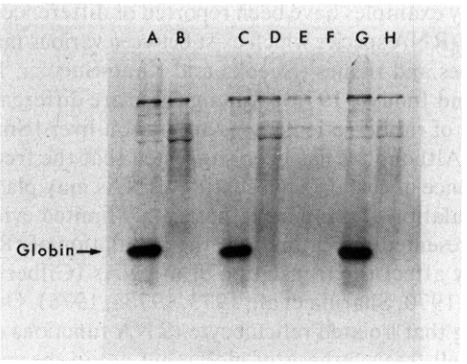

FIGURE 6: Autoradiography of [^{35}S]methionine-labeled products of cell-free protein synthesis in response to globin mRNA and different tRNAs. Details for the translation of globin mRNA (3 μg) with 5 μg of tRNA have been described under Table II. Electrophoresis was on sodium dodecyl sulfate–12.5% polyacrylamide slab gels. (A) Ascites cell tRNA + globin mRNA; (B) ascites cell tRNA; (C) reticulocyte tRNA + globin mRNA; (D) reticulocyte tRNA; (E) yeast tRNA + globin mRNA; (F) yeast tRNA; (G) yeast + reticulocyte tRNA + globin mRNA; (H) yeast + reticulocyte tRNA.

lypeptides (slot H) resembled more closely the pattern of polypeptides synthesized in the presence of ascites cell tRNA.

Products of the cell-free translation of globin mRNA were analyzed by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis and were found to migrate almost exclusively as a single radioactive band with the mobility of globin (Figure 6), using either ascites cell tRNA (slot A) or reticulocyte tRNA (slot C) in the system. Addition of yeast tRNA with reticulocyte tRNA had no effect on the product of translation of globin mRNA (slot G).

Discussion

Protein synthesis in a tRNA-dependent system should not take place efficiently or with fidelity unless the added tRNA contains all isoaccepting species required by the mRNA being translated and unless these tRNAs can be charged by the aminoacyl-tRNA synthetases present in the system. In our system the tRNA from *E. coli* or yeast did not support translation of oviduct mRNA (Table I, Figure 4) or EMC RNA (Table II, Figure 5, and Aviv et al., 1971), due at least partially to the fact that many of the tRNA species from these sources cannot be aminoacylated using mammalian enzymes (Aviv et al., 1971; Jacobson, 1971). In addition, however, *E. coli* tRNA, which was charged using *E. coli* aminoacyl-tRNA synthetases, did not support translation of EMC RNA (Aviv et al., 1971) and caused miscoding in the synthesis of hemoglobin (Hunter and Jackson, 1970), presumably because not all isoaccepting tRNAs needed for the translation of these mRNAs were present in the *E. coli* tRNA population. Our results indicate that most reticulocyte tRNA species are successfully aminoacylated by the ascites cell enzymes as evidenced by the efficient synthesis of globin in the presence of reticulocyte tRNA (Figure 6, slot C). However, the possibility exists that there are a few of these species that are needed for the synthesis of nonglobin proteins and that can be charged by the reticulocyte, but not the ascites cell, aminoacyl-tRNA synthetases. The inability of reticulocyte tRNA to participate effectively in the translation of oviduct mRNA and EMC viral RNA also could be due to the absence of, or modification to, an inactive form of isoaccepting tRNA species necessary for the translation of these mRNAs. These explanations would be in accord with the data of Smith and McNamara (1971, 1974) who have found an unusual tRNA distribution in rabbit reticulocytes and postulated a specialization of reticulocyte tRNA for hemoglobin synthesis.

Any functional absence of a tRNA species needed for protein synthesis, whether caused by lack of transcription, modification, or aminoacylation of the tRNA, should lead to a nonrandom premature termination of translation. Such a termination accompanied by the accumulation of incomplete polypeptide chains has been reported for the translation of globin mRNA and mengovirus RNA by extracts of interferon-treated L cells (Content et al., 1975). A similar premature termination of protein synthesis would explain the effect of reticulocyte tRNA on the translation of oviduct mRNA (Figure 4, slot G) in which smaller polypeptides (bands 4, 5, and 6) appear to accumulate at the expense of larger polypeptides (ovalbumin and band 3), in comparison to translation using ascites cell tRNA or liver tRNA (Figure 4, slots B and C). EMC viral RNA-directed protein synthesis also showed a preferential accumulation of smaller polypeptides with reticulocyte tRNA compared with ascites cell tRNA (Figure 5). In both instances, translation of oviduct mRNA or EMC viral RNA in the presence of reticulocyte tRNA supplemented with yeast tRNA (which by itself is unable to support protein synthesis in this system) resulted in a pattern of newly synthesized polypeptides that resembled more closely that of polypeptides synthesized in the presence of ascites cell tRNA or liver tRNA. This suggests that one or more species of tRNA, missing from reticulocyte tRNA and required for the translation of these mRNAs, was supplied by the yeast tRNA.

The supposition of the complete absence of critical tRNA species essential for ovalbumin synthesis would not explain the small amount of ovalbumin that is synthesized in vitro in the presence of reticulocyte tRNA (Figure 3B,C). It is possible that the ascites cell protein synthesizing system, although tRNA dependent, contains sufficient residual ascites cell tRNA to supplement the reticulocyte tRNA, with a small amount of the critical tRNA species, and permit limited ovalbumin synthesis. Another possibility is that alternate isoaccepting tRNA species can substitute occasionally and inefficiently for the putative missing species and thus permit some ovalbumin to be made. Either of these possibilities would be consistent with the observation that the limited synthesis of nonglobin proteins, in the presence of reticulocyte tRNA, cannot be overcome by the addition of excess reticulocyte tRNA to the system (Figure 1).

Many examples have been reported of differences in isoaccepting tRNA species which exist between various mammalian cell types and tissues (Sueoka and Kano-Sueoka, 1970; Littauer and Inouye, 1973). Among these are differences in the tRNAs of rabbit reticulocytes and rabbit liver (Smith et al., 1974). Although it has been suggested that the frequency of occurrence of certain isoaccepting tRNAs may play a role in the regulation of protein synthesis, only limited evidence has been presented indicating that the population of tRNAs can actually affect the translation of mRNAs (Gilbert and Anderson, 1970; Sharma et al., 1973, 1975a, 1976). Our results, showing that isolated reticulocyte tRNA functions efficiently in the cell-free synthesis of globin but not in the synthesis of ovalbumin or EMC viral proteins, provides additional evidence that tRNA has the potential for differentially restricting protein synthesis in certain cell types. This conclusion is valid, however, only if our results are due to the reticulocyte tRNA itself and not due to a specific inactivation or contamination of the tRNA during isolation. The following observations support our belief that the restriction of in vitro protein synthesis by reticulocyte tRNA is not an artifact of tRNA isolation. (1) The limitation was observed with tRNA isolated from whole reticulocytes and from high-speed supernatant fractions of reticulocytes by using the same isolation procedures used for the isolation of ascites cell tRNA and rabbit liver tRNA. (2) The reticulocyte tRNA was purified by procedures that yield a product with no detectable contamination (Sharma et al., 1975a). (3) The reticulocyte tRNA functioned well in the translation of globin mRNA, showing that any inactivation of some labile tRNA species or contamination must be of a highly selective and unusual type. (4) The supplementation of reticulocyte tRNA with yeast tRNA restored the ability of the extracts to translate ovalbumin mRNA and EMC viral RNA, indicating that the reticulocyte tRNA did not contain selective inhibitors of protein synthesis.

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