Purification of *Drosophila* Ribosomal Proteins. Isolation of Proteins S8, S13, S14, S16, S19, S20/L24, S22/L26, S24, S25/S27, S26, S29, L4, L10/L11, L12, L13, L16, L18, L19, L27, 1, 7/8, 9, and 11†

W. Yean Chooi

ABSTRACT: The proteins of *Drosophila melanogaster* embryonic ribosomes were separated into seven groups (A80 through G80) by stepwise elution from carboxymethylcellulose with lithium chloride at pH 6.5 by procedures previously described [Chooi, W. Y., Sabatini, L. M., Macklin, M. D., & Fraser, W. (1980) *Biochemistry 19*, 1425–1433]. Three relatively acidic proteins, S14, S25/S27, and 7/8, have now been isolated from group A80 by ion-exchange chromatography on carboxymethylcellulose eluted with a linear gradient of lithium chloride at pH 4.2. Fractions containing the relatively basic proteins (groups B80 through G80) were further combined into a total of 24 "pools". The criterion for combination was the migration patterns in one-dimensional polyacrylamide gels containing sodium dodecyl sulfate ($NaDodSO_4$) of every fifth fraction from the carboxymethylcellulose column. Each pool contained between 1 and 12 major proteins. Proteins S8, S13, S16, S19, S20/L24, S22/L26, S24, S26, S29, L4, L10/L11, L12, L13, L16, L18, L19, L27, 1, 9, and 11 have now been isolated from selected pools by gel filtration through Sephadex G-100. The amount of each protein recovered from a starting amount of 1.8 g of total 80S proteins varied from 0.2 to 10.8 mg. Five proteins had no detectable contamination, and in each of the others the impurities were no greater than 9%. The amino acid composition of the individual purified proteins was determined. The molecular weights of the proteins were estimated by polyacrylamide gel electrophoresis in $NaDodSO_4$.

Our research concerns the formation of nascent ribosomal RNP particles in the nucleus and the maturation of ribosomes in the cytoplasm. An analysis of the interation between eukaryotic ribosomal RNA and ribosomal proteins is contingent on the isolation and characterization of the molecular components. Although much is known of the chemistry of *prokaryotic* ribosomes, relatively little is known of those from *eukaryotes* [see Wittmann (1974) and Wool (1979) for reviews]. Considerable progress has been made on the purification of rat liver ribosomal proteins (Wool, 1979; Terao & Ogata, 1972; Westermann & Bielka, 1973). *Drosophila melanogaster* is a highly favorable organism for such study because of the availability of considerable quantities of embryos, larvae, and flies at various known developmental stages and also because of the eventual possibilities of the use in such studies of mutants of this most investigated eukaryotic genetic subject. The nucleic acids of *Drosophila* have been extensively studied (Tartof, 1975; White & Hogness, 1977; Laird & Chooi, 1976; Chooi, 1979), but far less information is available about their ribosomal proteins. Pure ribosomal proteins have not been isolated from *Drosophila*.

We have reported previously on the number of proteins found in *Drosophila* ribosomes and on the group fractionation of the 80S[1] proteins by stepwise elution from chromatographic columns containing carboxymethylcellulose (CMC) (Chooi et al., 1980), yielding seven "groups" of proteins. Fractions from this separation have been chosen and combined into "pools" containing between 1 and 12 major proteins. These various pools have now been further fractionated on columns of CMC or Sephadex. We now describe for the first time the purification and properties of 23 of the ~78 *Drosophila* ribosomal proteins.

Materials and Methods

Preparation of Ribosomes. Ribosomes were extracted from *D. melanogaster* embryos as described previously (Sherton & Wool, 1974; Chooi et al., 1980).

Extraction of Ribosomal Proteins. Proteins were extracted from ribosomes with 67% acetic acid, 10 mM Tris-HCl, and 33 mM MgCl as described by Sherton & Wool (1974).

Fractionation of the Total 80S Proteins. The proteins of 1.8 g of the total 80S ribosome (TP80) were separated into seven groups (A80 through G80) by stepwise elution from carboxymethylcellulose (Whatman 52) at 18 °C with LiCl at pH 6.5 as described previously by Collatz et al. (1976) (Figure 1).

The relatively acidic proteins found in group A80 (127 mg) were further resolved in a second carboxymethylcellulose column (2.6 × 30 cm) eluted at 4 °C with 8 L of a linear gradient of 0 to 0.1 M LiCl at pH 4.2. The flow rate was ~50 mL/h. Fractions of 15 mL each were collected.

Selected pools from groups B80 through G80 were resolved by filtration through jacketed columns (1.1 cm × 200 cm) of Sephadex G-100 (Pharmacia). The protein sample (10–20 mg) was dissolved in 8 M urea, incubated for 10 min at 37 °C in 40 mM mercaptoacetic acid, and then dialyzed overnight against 10% acetic acid; the volume of the sample was reduced to ~4 mL by lyophilization if necessary. Chromatography was carried out at 18 °C in a solution containing 40 mM

† From the Department of Biology, Molecular, Cellular, and Development Biology Program, Indiana University, Bloomington, Indiana 47405. *Received Februrary 12, 1980.* This research was supported by a grant from the National Institutes of Health (PHS R01 GM23410-01). This is paper 2 in a series of papers on the purification and characterization of ribosomal proteins.

[1] In the terminology of this paper, "80" (as in 80S and TP80) refers to material from intact *Drosophila* ribosomes of sedimentation 80 S. Proteins identifiable with the larger (60 S) subunit are designated by the prefix "L" (L4, L16, etc.), and those from the smaller subunit (40 S) have the prefix "S" (S8, S14, etc.). The proteins without letter designation (7/8, 1, 9, etc.) were found among 80S proteins but not identified with either ribosomal subunit. The sets of proteins separated by chromatography on carboxymethylcellulose (Chooi et al., 1980) are described as "groups" (as in group A80, B80, etc.); fractions chosen from this procedure because of useful separation of certain proteins are mixed into "pools". This paper describes the fractionation of pools.

0006-2960/80/0419-3469$01.00/0

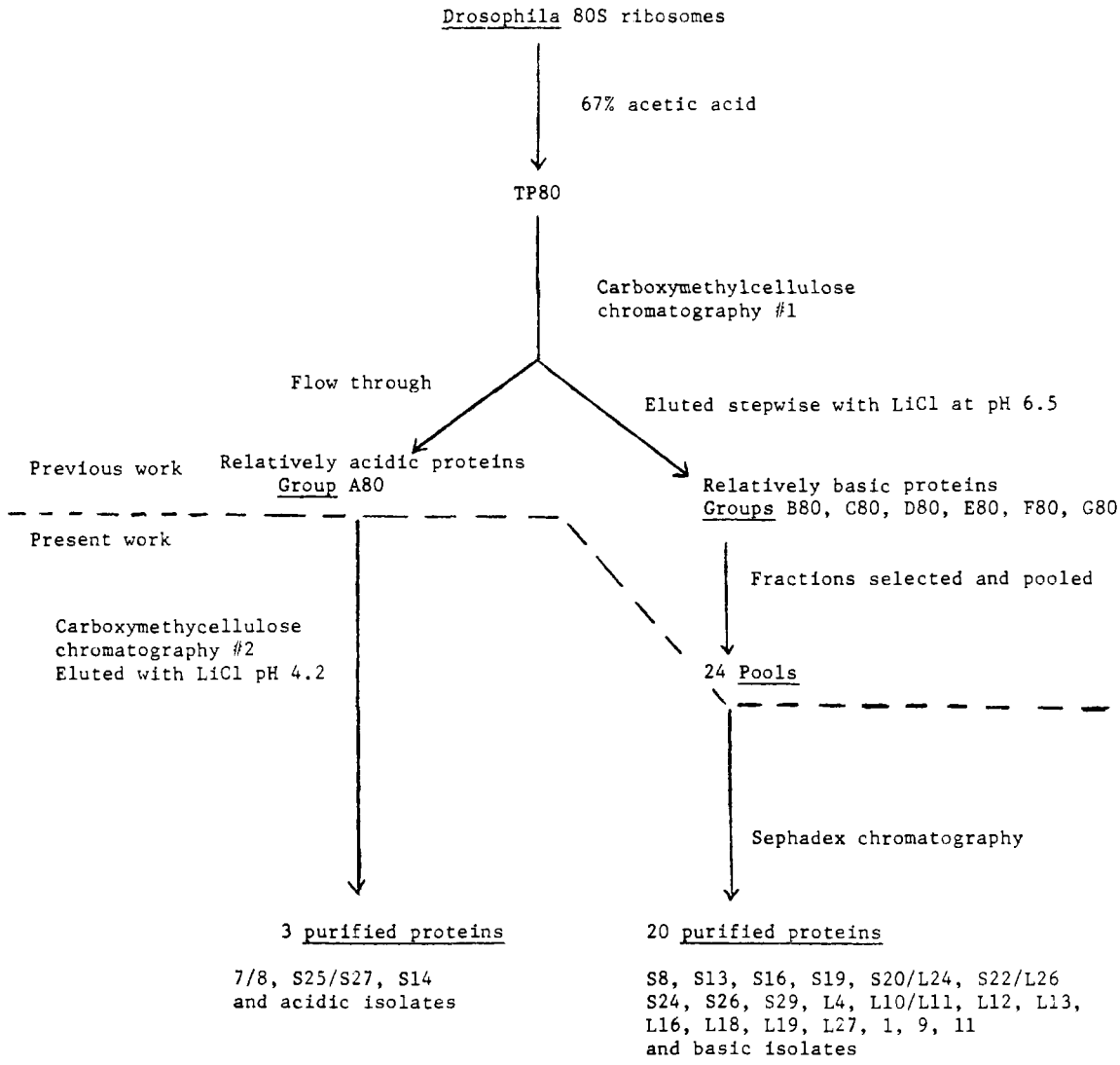

FIGURE 1: Flow sheet of the sequence of steps used in the purification of ribosomal proteins. See Materials and Methods for details.

mercaptoacetic acid and 10% acetic acid. The elution of the proteins was monitored either by continuously recording the absorption at 280 nm or by determining the absorption at 280 nm of each fraction.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Ribosomal proteins were identified by micro two-dimensional gel electrophoresis (Collatz et al., 1976) as follows. The protein in a given column fraction sample was precipitated with 15% trichloroacetic acid (Cl_3AcOH), collected by centrifugation in a microfuge, and dissolved in 8 M urea before electrophoresis in the first dimension. Identification of the ribosomal protein(s) in a fraction was facilitated by analyzing each sample alone on one-half of the second dimension gel slab and in the presence of a small amount of TP80 on the other half. Examination of the sample alone allowed one to estimate the number of proteins in the fraction and to assess the purity of each. The small amount of TP80 provided a background pattern that facilitated identification of the proteins in the sample.

Determination of Purity and Molecular Weights of Isolated Proteins. The molecular weight and purity of isolated proteins were estimated from one-dimensional polyacrylamide gels containing $NaDodSO_4$ by using the procedure of Laemmli (1970) except that the concentration of acrylamide was 15%. The molecular weight of the ribosomal protein isolated was estimated by comparison of the migration distance of the ribosomal protein with respect to the migration distances of the known molecular weight markers analyzed simultaneously in the same gel. The standard molecular weight markers used were bovine albumin (66 200), chicken egg albumin (45 000), bovine erythrocyte carbonic anhydrase (31 000), horse skeletal muscle myoglobin (17 200), and horse heart cytochrome *c* (12 400). The extent of contamination of the isolated proteins was determined by scanning the photographic negatives of the gels with a densitometer Helena Quick Scan RD) after staining with Commassie Blue [0.1% Commassie Brilliant Blue R (Sigma), 60% water, 30% methanol, and 10% acetic acid). The proportion of the total absorption that was not derived from the main band was taken as the percentage of contamination.

Determination of Amino Acid Composition. The isolated individual ribosomal proteins (1–20 nmol of each protein) were hydrolyzed for 24 and 72 h under vacuum in sealed tubes containing constant-boiling, twice-distilled, 6 N HCl at 110 °C. After hydrolysis, the HCl was removed under vacuum and the hydrolysate was dissolved in sodium citrate dilution buffer (Beckman), pH 2.2, containing 50 nmol of α-aminoguanidinopropionic acid and 50 nmol of norleucine as internal standards. The amounts of the amino acids in each hydrolysate were determined with a Beckman 121 M analyzer. Except for Thr, Ser, Val, Leu, and Ile, the amounts (mole percent) of the amino acids in each protein were derived from an av-

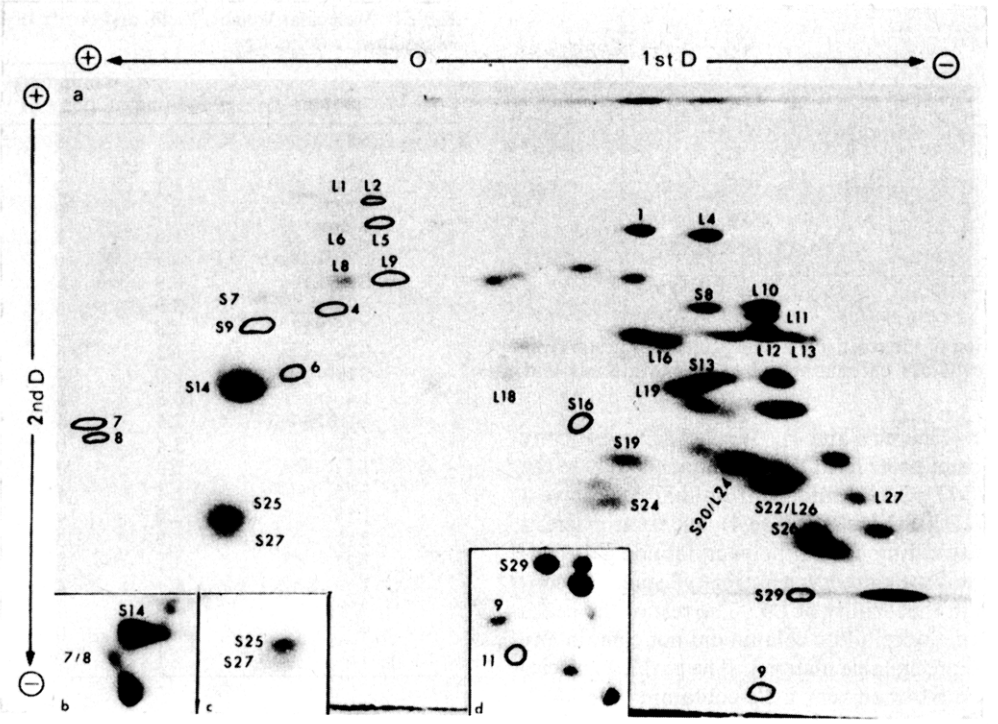

FIGURE 2: (a) Micro two-dimensional electrophoresis of the acidic (left) and the basic (right) ribosomal proteins. The drawn-in circle at the top indicates the point of origin (sample application). Some of the spots in the gel were clearly visible to the eye but are barely discernible or cannot be seen in the photograph. The purified proteins that are described in this communication are named in this photograph. (b) Proteins 7/8 are more readily observed when the time of electrophoresis is reduced to 40 min. (c) Proteins S25/S27 are clearly resolved as two separate spots when the two-dimensional gels are run according to the method of Kaltschmidt & Wittmann (1970). (d) The lower molecular weight proteins, e.g., 9 and 11, are consistently seen when the two-dimensional gels are performed according to the method of Kaltschmidt & Wittmann (1970). These proteins are only occasionally seen in the micro two-dimensional gels run according to the procedure described by Collatz et al. (1976).

erage of the 24- and 72-h estimates. The concentration of Thr and Ser was taken as the zero time value determined by extrapolation of the 24- and 72-h estimates. The concentration for Leu, Val, and Ile was obtained from the 72-h estimate to assure complete hydrolysis. Trp and Cys were not determined.

Results and Discussion

Group Fractionation of 80S Proteins. The sequence of steps undertaken in the purification of *Drosophila* ribosomal proteins described in this paper is shown in Figure 1. The *Drosophila* ribosomal proteins in the seven groups (A80–G80) from the CMC column were identified by micro two-dimensional polyacrylamide gel electrophoresis and by one-dimensional polyacrylamide electrophoresis in gels containing $NaDodSO_4$ (Collatz et al., 1976; Laemmli, 1970). The procedures and nomenclature of the proteins identified in each group were similar to those reported previously (Chooi et al., 1980).

In the following discussion, we have designated two isolated proteins as S20/L24 and S22/L26 because it is not certain whether each designated pair is a single polypeptide or two distinct proteins. (They could not be unambiguously resolved by two-dimensional polyacrylamide gel electrophoresis.) However, the configuration of each spot had originally suggested that it contained two proteins, probably one from the small subunit and one from the large subunit (Chooi et al., 1980). Three other isolated proteins are designated as L10/L11, S25/S27, and 7/8 because the two spots in each pair migrate very close to each other in the two-dimensional gels although the centers of each of the spots are not coincident. When isolated, each of these proteins forms a single spot in two-dimensional gels and a single band after one-dimensional electrophoresis in gels containing $NaDodSO_4$. Therefore, we cannot yet be sure if the protein isolated is one or the other of the designated pair or a mixture of the two.

Isolation of Acidic Proteins. The proteins that do not bind to carboxymethylcellulose at pH 6.5 (group A) were identified by two-dimensional electrophoresis as L1, L2, L5, L6, L8, L9, S7, S9, S14, S25/S27, 4, 6, and 7/8 (Figure 2). Poor staining and variability in concentration in different TP80 preparations were consistently encountered. Hence, the identification and characterization of these relatively acidic proteins were difficult. Of the ~15 acidic proteins, 5 (S7, S9, S14, and S25/S27) stained intensely but only when large amounts were analyzed by two-dimensional polyacrylamide gels. In addition, the higher molecular weight proteins (L1, L2, L5, L6, L8, and L9) frequently formed streaks rather than discrete spots in two-dimensional gels, a problem that could not be alleviated with higher concentrations of mercaptoacetic acid and removal of aggregates by centrifugation prior to loading of the sample. At this time it is not certain which of the ~15 relatively acidic proteins are truly ribosomal structural proteins, which are factors transiently associated with the ribosomes, and which are contaminants that have no functional role in protein synthesis. In this paper we describe an attempt to purify and characterize some of these proteins as a first step in resolving these three possibilities.

About 127 mg of A80 was applied to a column of carboxymethylcellulose and eluted with a linear gradient of 0–0.1 M LiCl at pH 4.2 (Figure 3). The identity of proteins was confirmed by taking samples from fractions at the beginning, in the middle, and toward the end of every peak and analyzing them by one-dimensional polyacrylamide gels in $NaDodSO_4$ and by two-dimensional polyacrylamide gels in urea (Figure 4). The purity was assessed by one-dimensional gel electrophoresis in $NaDodSO_4$ as described under Materials and Methods. Three peaks contained only single proteins, S14,

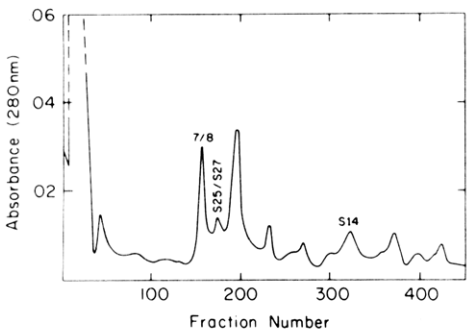

FIGURE 3: Separation of the relatively acidic proteins in group A80 by carboxymethylcellulose chromatography. See Materials and Methods for details.

S25/S27, and 7/8 (Figures 3 and 4). We had some difficulty in the identification of proteins in the peak immediately to the right of the S25/S27 peak. Material from that peak gave a distinct band in $NaDodSO_4$ gels (Figure 4), but electrophoresis of that sample in two-dimensional polyacrylamide gels containing urea was not satisfactory; a "streaky" spot was consistently obtained in the vicinity of L5. The rest of the peaks from the carboxymethylcellulose column did not contain any trichloroacetic acid precipitable material. The purified proteins 7/8, S25/S27, and S14 had very little contamination (4, 2, and 3%, respectively; Table I).

Isolation of Basic Proteins. The relatively basic proteins (groups B80 through G80) were eluted by LiCl at pH 6.5 from the carboxymethylcellulose column. The chromotypes obtained were similar to those previously reported (Chooi et al., 1980). Small samples of every fifth fraction from the ion-exchange column were analyzed by one-dimensional polyacrylamide gel electrophoresis in $NaDodSO_4$ in order to fractionate further the proteins in these groups. Fractions showing similarities in their migration patterns in $NaDodSO_4$ gels were pooled; 24 pools were obtained from groups B80 through G80. The

Table I: Molecular Weight, Yield, and Purity of Isolated Ribosomal Proteins

protein	yield (mg)	contamination (%)	av M_r $\times 10^{-3}$
S8	4	5	36.4
S13	1.8	6	28.7
S14	4.1	3	17.2
S16	0.2	0	24.7
S19	1.8	8	20.9
S20/L24	8.7	0	20.5
S22/L26	0.9	8	20.0
S24	3.9	8	19.8
S25/S27	2.8	2	13.0
S26	1.3	6	20.4
S29	1.0	4	17.7
L4	10.8	5	60.0
L10/L11	7.5	4	35.5
L12	5.4	0	34.5
L13	7.3	9	35.5
L16	1.4	4	28.0
L18	2.0	4	29.5
L19	1.8	3	26.0
L27	3.1	3	21.7
1	5	4	61.0
7/8	4.8	4	15.5
9	1.7	0	15.5
11	3.4	0	11.5

identity of the proteins in each pool was established by two-dimensional gel electrophoresis with results similar to those reported earlier (Chooi et al., 1980).

The analysis of the proteins in each pool by one-dimensional electrophoresis on $NaDodSO_4$ gels usually revealed mixtures. The various pools were then shown to contain between 1 and 12 major proteins as determined by two-dimensional gel electrophoresis. Since the identity of the proteins in each pool was established unequivocally by two-dimensional gels, we are able to identify the protein or proteins of a pool corresponding to each band in the $NaDodSO_4$ gels. Each $NaDodSO_4$ band

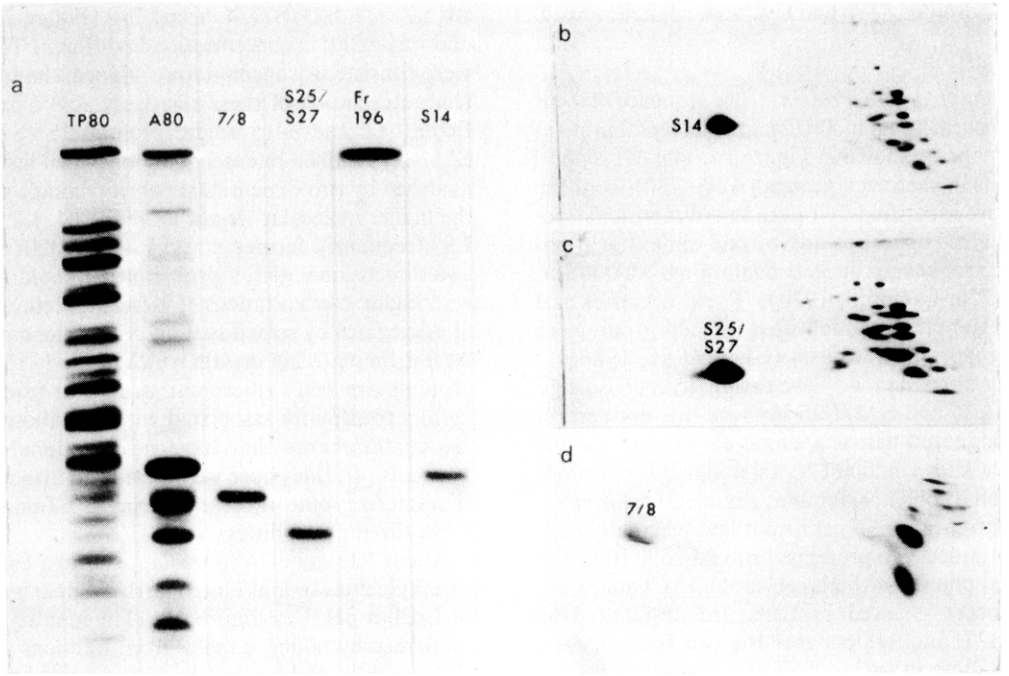

FIGURE 4: (a) Polyacrylamide gel electrophoresis in $NaDodSO_4$ of TP80, group A80 proteins, purified acidic proteins (7/8, S25/S27, and S14), and proteins from fraction (Fr) 196 of the carboxymethylcellulose column (see Figure 3). (b) Micro two-dimensional polyacrylamide gel electrophoresis of the purified protein S14 (left) and the relatively basic proteins from TP80 (right). See Figure 2 for the relative orientation of the acidic and the basic proteins run in the same second dimension slab gel. (c) Micro two-dimensional polyacrylamide gel electrophoresis of the purified protein S25/S27 (left) and the relatively basic proteins from TP80 (right). (d) Micro two-dimensional gel electrophoresis of the purified protein 7/8 (left) and the relatively acidic proteins from TP80 (right).

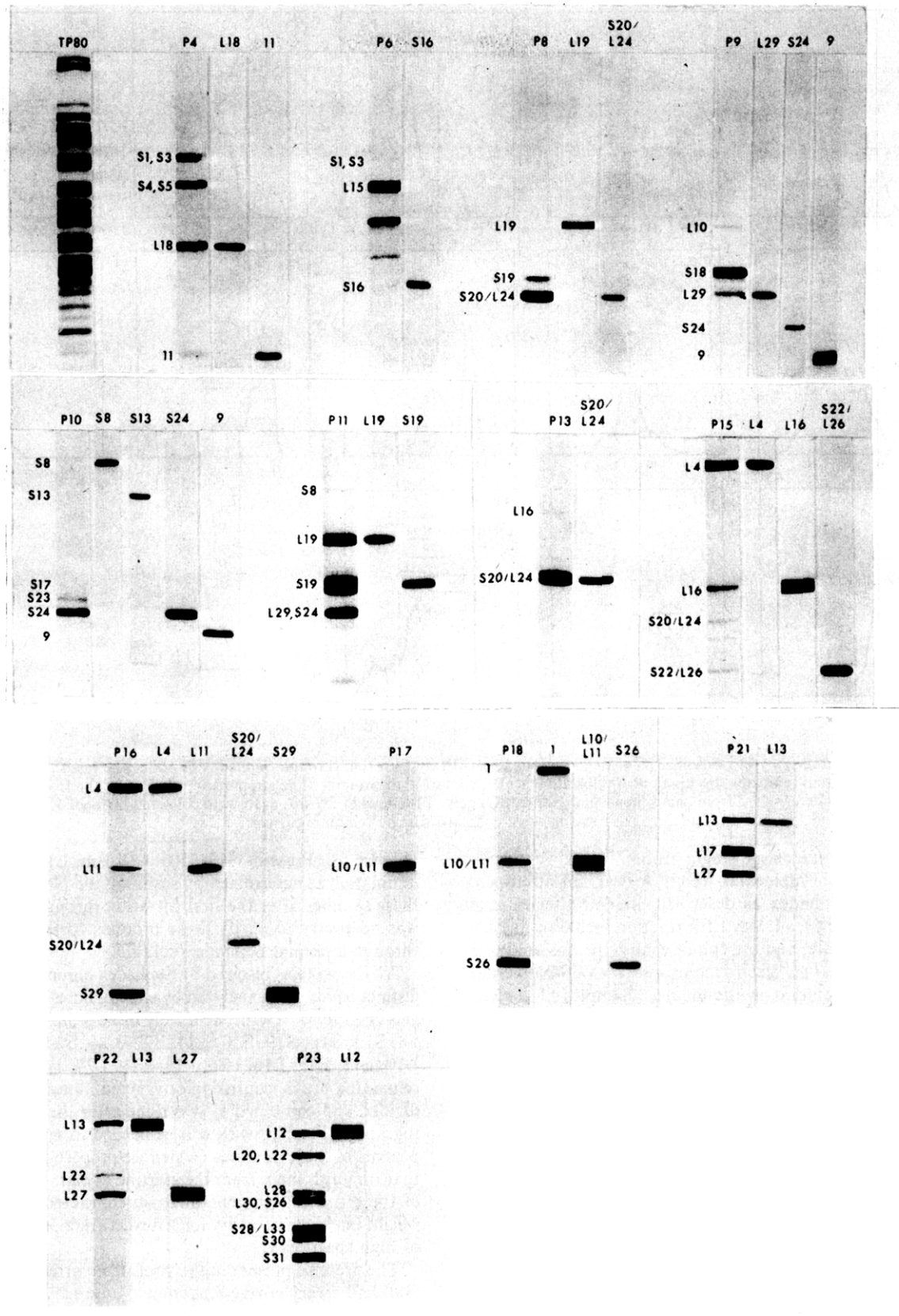

FIGURE 5: Polyacrylamide gel electrophoresis in $NaDodSO_4$ of ribosomal proteins in individual pools and the respective purified proteins from each pool. Proteins were purified by Sephadex chromatography as described under Materials and Methods.

generally contained one or two proteins. Therefore, by a combination of one-dimensional and two-dimensional gels, we were able to determine the number of proteins in each pool, to establish their identity, and to estimate their relative concentrations and their molecular weight distributions. This information was used in rapid screening of a large number of pools for the further fractionation in Sephadex.

Each of 13 pools (4, 6, 8, 9, 10, 11, 13, 15, 16, 18, 21, 22, and 23) was chromatographed on Sephadex (Figure 5). These pools were selected on the basis that they contained fewer proteins (four on the average), that at least some of the migration bands in $NaDodSO_4$ gels contained only single pro-

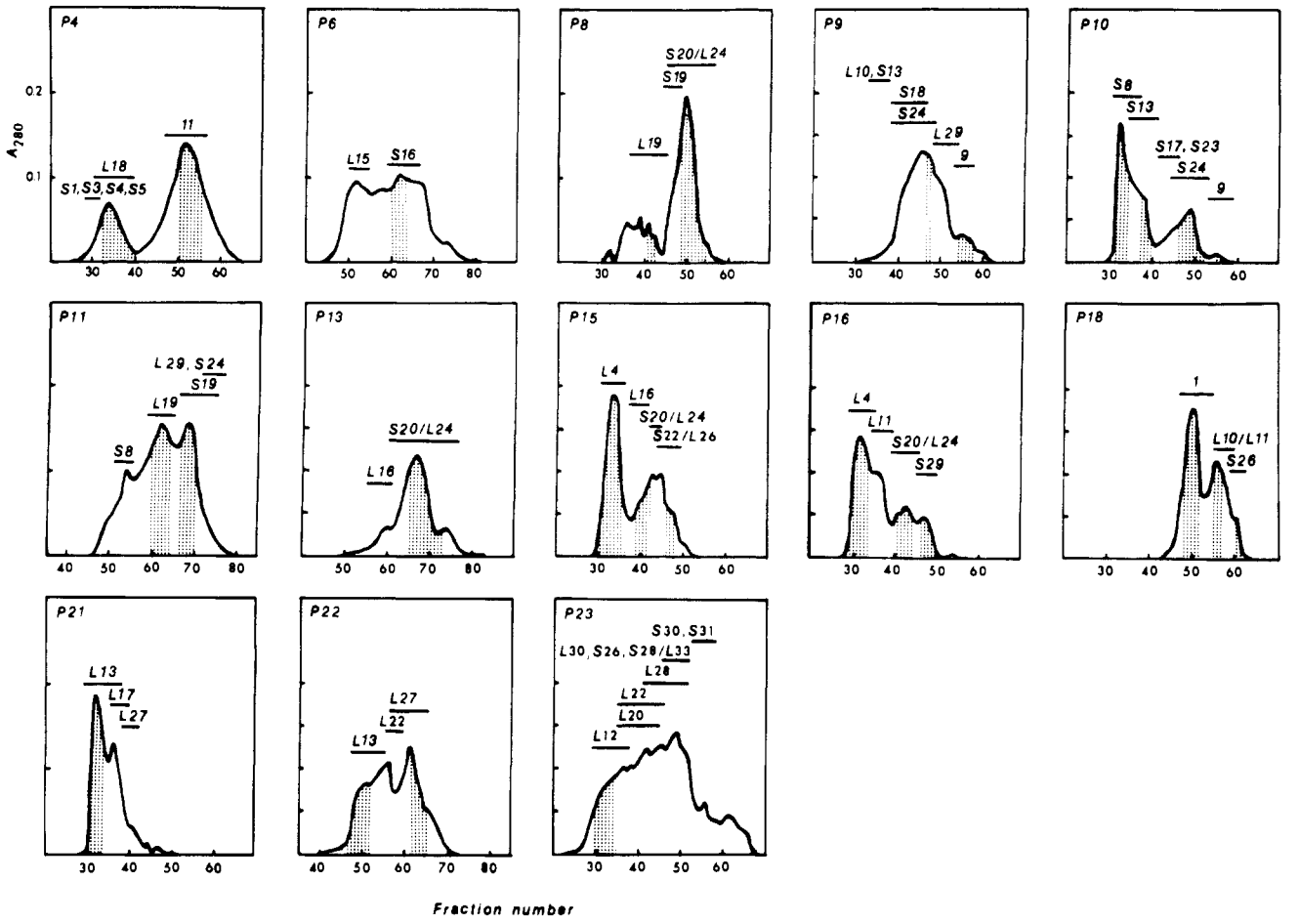

FIGURE 6: Chromatography of ribosomal proteins in various pools (P) by filtration through Sephadex G-100. The length of the line under the designated protein indicates the range of the fractions which contain that protein. The stippled area indicates the fractions containing purified proteins as determined from one-dimensional $NaDodSO_4$ gels. Fractions of 3.3 mL each were collected for pool 4, 1.5-mL fractions were collected for pools 6 and 11, and 2.5-mL fractions were collected for the remaining pools.

teins, and that some of the single protein bands were sufficiently different in molecular weight to warrant attempted separation in Sephadex as described under Materials and Methods (Figure 6). Every fifth fraction including the beginning, the middle, and the end of each peak was analyzed by one-dimensional gel electrophoresis in $NaDodSO_4$ in order to identify the proteins in the eluate. Samples of a given fraction, two samples of TP80 (one at each side of the gel), and molecular weight standard markers (see Materials and Methods) were run simultaneously in adjacent lanes in the same $NaDodSO_4$ gel. To determine if we had indeed isolated a single protein, we compared the migration pattern of each band in $NaDodSO_4$ gels of samples after filtration through Sephadex with the original identification pattern of protein(s) assigned to each $NaDodSO_4$ band of the particular pool (Figure 5). It there was any ambiguity, a sample of the isolated protein was further analyzed in two-dimensional gels.

Figure 6 shows the typical separation of proteins from 13 selected pools. In many cases, isolated proteins were eluted in a single peak: e.g., 11 (pool 4), L18 (pool 4), S16 (pool 6), S8 (pool 10), S24 (pool 10), L19 (pool 11), S19 (pool 11), S20/L24 (pool 13 and 16), L4 (pool 15 and 16), S29 (pool 16), 1 (pool 18), L10/L11 (pool 18), L13 (pool 21), and 9 (pool 9 and 10). On occasion, more than one protein was found at the trailing edges of the peak. Some proteins (S13, S22/L26, S26, L12, L16, and L27) were eluted from only portions of discrete peaks. This may be partly because these proteins are represented in lower proportions in their respective pools: e.g., S13 (pool 10), L16 (pool 15), and L12 (pool 23) (Figure 5). Proteins S13, L16, L18, and L19 had 20% contamination as determined by scanning the $NaDodSO_4$ gels of these samples after the first filtration through Sephadex. It was necessary to purify these proteins further by filtration through a second Sephadex column.

All the proteins resolved by Sephadex chromatography form distinct bands when analyzed by electrophoresis on $NaDodSO_4$ gels (Figure 5). Twenty relatively basic proteins were isolated: S8, S13, S16, S19, S20/L24, S22/L26, S24, S26, S29, L4, L10/L11, L12, L13, L16, L18, L19, L27, 1, 9, and 11. The remaining peaks, consisting of varying mixtures, can be subdivided and combined as was done after the CMC chromatography. Further work will be needed to separate the components of these mixtures (which we propose to cell "isolates" to distinguish them from the starting pools). The similarities of these proteins in general basic character and molecular weight imply the necessity for a further fractionation procedure of high specificity.

The proteins present in the pools from groups B80 through G80 had a very strong tendency to aggregate. Aggregation was probably attributable to disulfide bond formation since the aggregates seemed to be dispersed only by treatment with large amounts of mercaptoacetic acid. We found that proteins were eluted in a random fashion unless (1) samples were treated with at least 40 mM mercaptoacetic acid just prior to loading onto Sephadex columns and (2) 40 mM mercaptoacetic acid was included in the elution buffer. No adjustments were made for the spontaneous oxidation of the mercaptoacetic acid that occurred during the running period of

Table II: Amino Acid Composition of Purified Proteins[a]

protein	S8	S13	S14[b,c]	S16[b]	S19	S20/L24	S22/L26	S24	S25/S27[b,c]	S26	S29	L4	L10/L11	L12	L13	L16	L18	L19	L27[b]	1	7/8[c]	9	11
Lys	14.9	13.0	10.3	12.0	11.6	8.6	14.0	11.3	7.3	11.4	11.8	9.7	13.7	16.4	20.1	11.0	8.2	11.6	13.7	14.8	8.9	10.0	7.5
His	1.0	1.8	4.7	4.7	3.1	1.5	2.5	2.2	3.1	2.6	2.2	2.2	2.1	2.6	1.4	2.6	1.2	2.6	3.5	4.1	0.4	4.4	0.5
Arg	2.7	5.3	3.0	10.6	8.0	8.3	7.0	9.7	6.9	7.9	10.2	10.6	7.4	8.6	6.8	10.8	6.3	8.7	10.6	7.9	2.5	7.6	12.4
Asp	9.5	7.2	12.9	6.9	8.1	8.3	6.5	9.7	15.6	7.1	8.3	7.8	6.7	6.7	7.0	7.9	8.3	8.5	4.9	6.7	10.8	4.3	7.0
Thr	3.8	4.3	2.5	3.2	4.1	4.0	3.5	7.2	4.6	4.5	3.5	3.5	3.5	3.7	5.1	3.9	4.6	5.1	5.0	6.2	2.7	4.8	4.1
Ser	5.9	6.3	5.0	3.2	5.8	8.1	9.7	5.8	6.8	6.5	5.3	3.5	4.2	5.3	4.0	4.8	6.4	6.0	4.3	5.9	7.0	4.7	3.3
Glu	7.0	8.6	10.3	10.4	9.3	6.6	9.7	9.1	10.2	9.3	10.5	7.0	10.1	7.9	5.1	11.9	11.2	10.6	8.7	7.8	12.9	9.0	16.8
Pro	5.8	6.9	4.3	5.5	6.2	5.1	3.8	4.0	3.1	4.3	4.4	4.5	5.4	5.0	7.0	4.8	5.5	4.7	5.1	3.9	3.7	16.6	4.7
Gly	10.5	7.0	7.6	7.7	9.3	14.3	8.9	8.8	9.6	9.9	9.4	15.0	5.5	7.2	6.0	7.2	7.9	11.9	8.7	7.9	9.4	7.6	10.5
Ala	21.6	15.1	9.2	7.2	5.0	9.7	7.6	6.8	8.1	8.7	7.2	10.6	8.0	9.1	18.6	8.9	9.1	7.5	12.8	8.4	14.8	6.9	4.4
Val	4.5	8.5	10.5	8.5	6.2	6.9	7.8	7.3	6.9	8.5	5.6	8.3	10.3	7.1	4.1	5.2	10.4	4.0	6.8	4.9	9.3	5.0	11.0
Met	0.4	1.0	0.5	0.2	3.4	2.0	0.8	1.1	1.9	1.5	1.1	0.7	1.5	1.8	1.7	3.8	1.8	1.6	1.5	5.5	1.8	2.3	1.8
Ile	4.1	4.4	5.5	6.2	5.4	5.5	5.5	5.8	8.6	5.0	4.9	3.6	5.3	3.4	3.9	4.6	6.7	4.9	3.7	5.5	4.3	2.4	4.2
Leu	4.3	6.0	11.0	6.7	8.2	5.8	8.3	5.3	4.1	6.4	9.9	8.0	9.1	8.5	5.6	7.7	8.3	6.7	4.6	5.2	8.0	8.9	9.4
Tyr	2.1	2.0	1.0	3.1	2.6	2.1	2.7	2.8	2.5	2.9	2.6	1.8	3.0	3.2	2.2	2.3	2.0	2.4	3.4	2.0	1.9	2.2	0.8
Phe	1.9	2.6	1.7	3.9	3.7	3.2	1.7	3.1	0.7	3.5	3.1	3.2	4.2	3.5	1.4	2.6	2.1	3.2	2.7	3.3	1.6	3.3	1.6

[a] The values for amino acids are in mole percent. [b] The values for amino acids were derived from 24 h hydrolysis. [c] Relatively acidic proteins.

the column. The elution buffer in the reservoir was deliberately kept at a minimum volume and refilled with freshly made buffer as needed to preserve a reducing eluant.

Yield and Purity of Isolated Proteins. The amount of the individual purified proteins obtained from 1.8 g of TP80 varied from 0.2 to 10.8 mg (Table I). The yield appeared to be dependent on several factors: for example, the quantity of starting material in the pools and the number of steps taken to achieve satisfactory separation. The purity of the isolated proteins determined as described under Materials and Methods varied from 91 to 100%. Five proteins showed no detectable contamination while the impurities in the others listed above were no greater than 9%.

Molecular Weight. The molecular weight of each of the purified ribosomal proteins was determined as described under Materials and Methods (Figures 4 and 5 and Table I).

Amino Acid Composition. (*a*) *Basic Proteins.* The amino acid composition of the isolated acidic and basic proteins was determined (Table II). Only the amino acids listed in the table were taken into account in calculating the composition. The general pattern of the proteins isolated can be seen to be similar, though the exact composition of each protein is unique.

(*b*) *Acidic Proteins.* The amino acid composition of the purified acidic proteins (7/8, S14, and S25/S27) is shown in Table II. There has been considerable discussion in the literature [for a review, see Wool (1979)] of proteins that appear to be both structurally and functionally conserved in such diverse organisms as *Escherichia coli* (L7/L12), rat liver (L40/L41), yeast (A1/A2), and *Artemia* (E17/EL12). One of our pairs (7/8) seems to be related in electrophoretic mobility to these proteins. When the difference indexes (Metzer et al., 1968) between 7/8 and these possibly related heterologous proteins were compared, 7/8 appears disparate. To add to this confusion, pairs shown to be structurally and/or functionally related have also been shown to have markedly different amino acid compositions (Tsurugi et al., 1978). It seems that meaningful statements will have to await direct comparisons in terms of function and immunological relationships.

Acknowledgments

I am grateful to Drs. Frank Putnam and Frank Gurd for aid with the amino acid analyses, to Beth Dolliver and Mike Macklin for valuable technical assistance, to Linda M. Sabatini for some assistance with the acidic protein isolation, and to Dean Fraser for critical comments of the manuscript.

References

Chooi, W. Y. (1979) *Chromosoma 74*, 57–74.

Chooi, W. Y., Sabatini, L. M., Macklin, M. D., & Fraser, W. (1980) *Biochemistry 19*, 1425–1433.

Collatz, E., Lin, A., Stöffler, G., Tsurugi, K., & Wool, I. G. (1976) *J. Biol. Chem. 251*, 1808–1816.

Kaltschmidt, E., & Wittmann, H. G. (1970) *Anal. Biochem. 251*, 2867–2975.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Laird, C. D., & Chooi, W. Y. (1976) *Chromosoma 58*, 193–218.

Metzer, H., Shapiro, M. B., Mosimann, J. E., & Vinton, J. E. (1968) *Nature (London) 219*, 1166–1168.

Sherton, C. C., & Wool, I. G. (1974) *Methods Enzymol. 30*, 506–539.

Tartof, K. D. (1975) *Annu. Rev. Genet. 9*, 355–385.

Terao, K., & Ogata, K. (1972) *Biochim. Biophys. Acta 285*, 473–482.

Tsurugi, K., Collatz, E., Todokoro, K., Ulbrich, N., Lightfoot,

H., & Wool, I. G. (1978) *J. Biol. Chem. 253*, 946–955.
Westermann, P., & Bielka, H. (1973) *Mol. Gen. Genet. 126*, 349–356.
White, R. I., & Hogness, D. S. (1977) *Cell 10*, 177–192.
Wittmann, H. G. (1974) in *Ribosomes* (Nomura, M., Tissieres, A., & Lengyel, P., Eds.) pp 93–114, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Wool, I. G. (1979) *Annu. Rev. Biochem. 48*, 719–754.

Isolation and Sequence Analysis of Two Major Leucine Transfer Ribonucleic Acids (Anticodon Mm-A-A) from a Rat Tumor, Morris Hepatoma 5123D†

Erika Randerath,* Ramesh C. Gupta, Harold P. Morris, and Kurt Randerath

ABSTRACT: The nucleotide sequences of two major $tRNA^{Leu}$ species (anticodon Mm-A-A) isolated from Morris hepatoma 5123D were determined by a combination of a newly developed thin-layer readout sequencing method [Gupta, R. C., & Randerath, K. (1979) *Nucleic Acids Res. 6*, 3443–3458] and additional ^3H- and ^{32}P-labeled derivative methods entailing chromatographic fingerprinting and base-specific enzymatic cleavages. The nucleotide sequence of the two hepatoma $tRNA_{Mm\text{-}A\text{-}A}^{Leu}$ species, one of which has U and the other of which has A in position 50 at the tip of the long extra arm, is pG-U-C-A-G-m^2G-A-U-G-(m^2)G-C-(ac^4)C-G-A-G-U-G-G-D-C-ψ-A-A-G-G-C-m_2^2G-C-C-A-G-A-C-U-Mm-A-A-m^1G*-ψ-ψ-C-U-G-G-L-(ψ)U-C-C-G-U or A-A-U-G-G-A-G-m^5C-G-U-G-G-G-T-ψ-C-G-m^1A-A-U-C-C-C-A-C-U-U-C-U-G-A-C-A-C-C-A_{OH}. These are the first leucine tRNA sequences from higher eukaryotes that have been determined. Noteworthy features of the mammalian leucine tRNAs are the presence of ψ in the β region of the D loop and the occurrence of three unknown hypermodified nucleosides (Mm, m^1G*, and L) in positions 35, 38, and 45, respectively. m^1G* was converted to m^1G by treatment with alkali. Sequencing gels indicated that the parent base of the 2′-O-methylated nucleoside Mm may be a pyrimidine, probably a C derivative, as indicated by the chromatographic behavior of nucleotides containing Mm. The presence of a pyrimidine in the wobble position would be consistent with the anticodon sequence Mm-A-A and the leucine codons U-U-G and U-U-A. The occurrence of a hypermodified nucleoside, L, in the first position of the long extra arm appears unusual; thus far the only modified nucleoside found in this position is Um in eukaryotic serine tRNAs. Since all tRNAs with a long extra arm sequenced to date have a pyrimidine in this position, L is likely to be a pyrimidine, probably a U derivative, as inferred from chromatographic data.

The number of mammalian tRNAs sequenced to date is rather small. Only 11 of the ~110 tRNAs whose primary structures have been elucidated are of mammalian origin [see Gauss et al. (1979) for a compilation of sequenced tRNAs]. Although tumor tRNAs have been known for a number of years to exhibit alterations of their column chromatographic elution profiles [e.g., Taylor et al. (1967), Baliga et al. (1969), Gallo & Pestka (1970), and Volkers & Taylor (1971)] as well as their base compositions [e.g., Randerath & Randerath (1973), Randerath, E., et al. (1974), and Chia et al. (1976)] when compared with their normal counterparts, little information on the structure of tRNAs of neoplastic origin is available. So far, only four such tRNAs have been sequenced, i.e., $tRNA_f^{Met}$ (Piper & Clark, 1974), $tRNA_4^{Met}$ (Piper, 1975a), and $tRNA_1^{Val}$ (Piper, 1975b) of mouse myeloma cells and $tRNA^{Asn}$ (Roe et al., 1979) of the Walker 256 carcinosarcoma. The latter tRNA is the only tumor tRNA sequenced to date that was compared directly with its counterpart in normal host tissue, albeit not the tissue of origin of the tumor. This comparison showed a difference in the wobble position of the anticodon, with Q being present in liver and G in tumor $tRNA^{Asn}$ (Roe et al., 1979). In an attempt to elucidate the structural differences between hepatoma and liver tRNAs, we have as a first step recently purified several tRNAs from Morris hepatoma 5123D (Morris & Wagner, 1968) and report here on the sequences of two major leucine tRNAs from this tumor. These are the first leucine tRNAs from a higher eukaryote that have been sequenced.

Experimental Procedures

Materials. Hepatoma 5123D was originally chemically induced in the laboratory of Dr. H. P. Morris (Morris & Wagner, 1968) and serially transplanted intramuscularly in female Buffalo rats. The tumor used in the present work was from generation 123. Tissue was excised quickly from etherized rats immediately after bleeding and stored at −70 °C. BD-cellulose was from Schwarz/Mann, acrylamide and methylenebis(acrylamide) were from Bio-Rad, and poly(A,C,-G,U) was from Miles Laboratories. The sources of materials used for sequence analysis have been indicated previously (Gupta & Randerath, 1979; Randerath et al., 1979, 1980; Gupta et al., 1979).

Crude tRNA. Crude tRNA was isolated from 800 g of tumor by phenol extraction at pH 4.5 (0.14 M NaOAc buffer), followed by adsorption to DEAE-cellulose, essentially as described by Roe (1975). Reextraction of the first phenol phase with NaOAc buffer increased the recovery of tRNA almost twofold. About 650 μg of crude tRNA was obtained per g of tumor.

† From the Department of Pharmacology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030. *Received January 29, 1980.* This work was supported by U.S. Public Health Service Grants CA-10893(P8) and CA-13591 to K.R. and American Cancer Society Grant NP-315 to E.R.

0006-2960/80/0419-3476$01.00/0