Initiation of Polysome Formation in Mouse Sarcoma 180 Ascites Cells. Utilization of Cytoplasmic Messenger Ribonucleic Acid*

Se Yong Lee,† Velibor Krsmanovic, and George Brawerman†

ABSTRACT: Incubation of mouse sarcoma 180 ascites cells in the absence of nutrients leads to conversion of the polysomes into ribosomal monomers apparently free of peptidyl tRNA and mRNA. Addition of amino acids to the starved cells results in rapid polysome formation. In cells prelabeled with uridine in the presence of a low level of actinomycin D, starvation leads to the accumulation in the cytoplasm of heterodisperse radioactive material ranging from 25 S to at least 160 S. This material seems to serve as precursor to polysomal mRNA, as indicated by its apparent incorporation into polysomes during recovery. No nuclear mRNA appears to be utilized in the early stages of recovery, since these are not affected by pretreatment with a large dose of actinomycin. The heterodisperse material contains RNA with a distribution of S values similar to that of polysomal mRNA. Two different size classes of this material yield RNA with the same distribution of S values. The RNA in this material is associated with other components which cause it to be retained on Millipore filters, even in the presence of 0.5 M KCl. The high rates of sedimentation of these complexes are not caused by nonspecific binding of proteins during cell lysis. Starvation appears to affect a specific initiation step. Characteristics of the early stages of polysome formation suggest that the sensitive step might involve an activation of the initiation site on mRNA

Studies of the nature of messenger RNA in eukaryotic cells indicate that it occurs in the cytoplasm in association with proteins or other components. Rapidly labeled cytoplasmic particles with sedimentation values ranging from 20 to 90 S have been detected in various cell types (Spirin *et al.*, 1964; Perry and Kelley, 1966; Infante and Nemer, 1968; Kafatos, 1968; Henshaw and Loebenstein, 1970). These high sedimentation values, as well as buoyant densities far lower than those of free RNA, have been attributed to the presence of large amounts of proteins bound to the RNA. Pulse-labeled material released from polysomes by EDTA treatment has been found to have similar characteristics (Perry and Kelley, 1968; Cartouzou *et al.*, 1968; Henshaw, 1968; Lee and Brawerman, 1971). It has been postulated that mRNA is transported into the cytoplasm in the form of nucleoprotein particles, and that these are incorporated as such into the polysome structure (Spirin, 1966; Henshaw, 1968). It has not been possible, however, to obtain evidence for the uptake of these structures into polysomes (Latham and Darnell, 1965). The study of the mRNA-containing particles has also been hampered by the presence in the same extracts of pulse-labeled RNA in polysomal structures.

Mouse ascites tumor cells provide an experimental system favorable for the study of mRNA–ribosome interaction. It has been shown by Hogan and Korner (1968) that addition of amino acids to Landschütz ascites cells leads to rapid polysome formation, and that this process is not affected by high doses of actinomycin D. Sarcoma 180 ascites cells, used in the present study, proved to be equally sensitive to changes in amino acid supply. When incubated in the absence of nutrients, these cells rapidly lost their polysomes. Both mRNA complexes and ribosomal monomers accumulated in the starved cells. Addition of amino acids led to very rapid polysome formation. Because of the rapidity of the process, the utilization of preformed cytoplasmic mRNA could be easily observed. This report describes the process of polysome formation and some of the characteristics of the RNA-containing particles that become incorporated into the polysomes.

Experimental Section

Tumor Cells. Mouse sarcoma 180 ascites cells were kindly supplied by Dr. Sartorelli (Department of Pharmacology, Yale University, New Haven, Conn.). They were maintained by weekly transfer of about 1×10^7 cells into the peritoneum of male ICR albino mice. The cells were usually used for experiments 5–7 days after inoculation.

Incubation. The peritoneal fluid was diluted four- to fivefold with warm Krebs bicarbonate buffer (120 mM NaCl–5 mM KCl–2.5 mM $CaCl_2$–1 mM KH_2PO_4–1 mM $MgSO_4$–0.2% $NaHCO_3$, pH 7.8–8.0). The cells were collected by centrifugation at 400*g* for 0.5 min and washed 3 times with warm Krebs buffer. For starvation and recovery experiments, the cells were then suspended in Krebs buffer to a density of approximately 1×10^7 cells/ml and incubated with gentle agitation at 37° for 30 min. Recovery was initiated by addition of glucose to a 1% final concentration, bovine serum (1 ml per 10 ml of culture), and a complete mixture of L-amino acids to the following final concentrations (expressed in mg/l.): Ala, 9; Arg, 105; Asn, 15; Asp, 13; Cyss, 32; Gln, 294; Glu 14; Gly, 8; His, 31; Ile, 52; Leu, 52; Lys, 58; Met, 15; Phe, 32; Pro, 12; Ser, 11; Thr, 48; Try, 19; Tyr, 47.

Labeling Experiments. In most cases, cells harvested from the peritoneal cavity were washed and incubated for 30 min in Krebs buffer supplemented with 0.04 μg/ml of actinomycin D. This level of actinomycin was used to prevent labeling of ribosomal RNA (Penman *et al.*, 1968). Glucose, bovine serum, and amino acids were added together with uridine-5-*t* (New England Nuclear, Boston, Mass., 20 Ci/mmole) to a final con-

* From the Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut. *Received July 27, 1970.* This work was supported by a U. S. Public Health Service Research Career Program Award (GM-K3-3295) from the National Institute of General Medical Sciences (to G. B.) and a research grant from the U. S. Public Health Service (GM-11527).

† Present address: Department of Biochemistry–Pharmacology, Tufts University School of Medicine, Boston, Mass. Address correspondence to G. B.

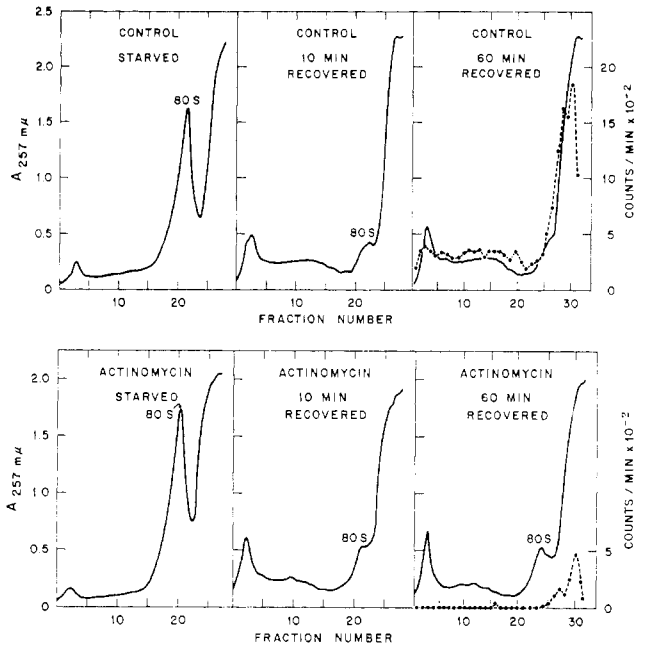

FIGURE 1: Effect of a high dose of actinomycin D on polysome formation in starved cells. Cells were pretreated as described in the Experimental Section for the labeling experiments, but without radioactive uridine. For the last 10 min of preincubation in complete medium, the level of actinomycin in one cell suspension was raised to 10 μg/ml. In subsequent operations, actinomycin concentrations were maintained at 0.04 μg/ml for control cells and 10 μg/ml for treated cells. After starvation, recovery was initiated in the presence of 5 μCi/ml of uridine-*t*. Fractions for radioactivity measurements were taken only from the 60-min sample: solid line, recorder tracing of ultraviolet absorbancy at 257 mμ; dashed line, acid-insoluble radioactivity.

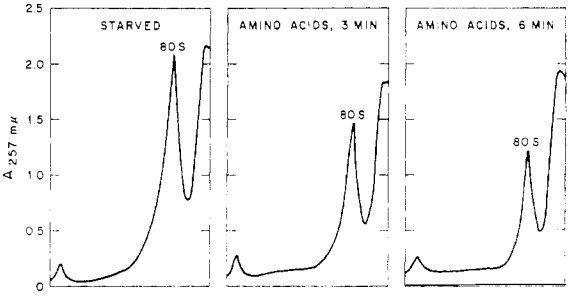

FIGURE 2: Early stages of the recovery process. Cells incubated for 30 min in Krebs buffer were supplemented with amino acids, glucose, and bovine serum. Samples were taken at indicated times and just prior to addition of nutrients.

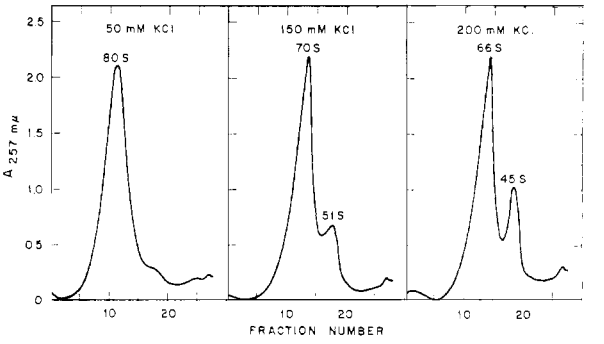

FIGURE 3: Dissociation of ribosomes from starved cells into subunits. Ribosome suspensions in 50 mM Tris-HCl, pH 8.0–1 mM $MgCl_2$–5 mM β-mercpatoethanol and indicated concentrations of KCl were centrifuged through linear 10–30% sucrose gradients supplemented with the same ingredients. Centrifugations were in the SW 39 Spinco rotor at 35,000 rpm for 50 min. Sedimentation values were determined by comparing positions of peaks to that of 80S monomer in 50 mM KCl (difference in positions of 80S monomer and 66S subunit in 200 mM KCl is the equivalent of 3 fractions).

centration of 5 μCi/ml, and the incubation was continued for 30 min. The cells were next harvested and washed 3 times with warm Krebs buffer, then subjected to the starvation treatment in the presence of 0.04 μg/ml of actinomycin.

In some early experiments the cells were prelabeled directly in the peritoneal cavity by injecting first a 1-ml mixture of amino acids and glucose as described by Hogan and Korner (1968), in addition to 0.7 μg of actinomycin. This was followed 30 min later by a second injection containing 50 μCi of uridine-*t* and 0.08 μg of actinomycin in 2 ml of the amino acids and glucose mixture. One hour later, the cells were harvested as usual. The latter labeling procedure was subsequently discontinued, as the *in vitro* labeling proved far more effective.

Cell Lysis and Preparation of Polysomes. Cell samples were brought quickly to 0°, collected by centrifugation at 500*g* for 1 min, and washed once with ice-cold 1% NaCl. All operations were carried out rapidly at 0–4°. The cells were next washed twice with a hypotonic solution consisting of 10 mM Tris-HCl, pH 7.6, 10 mM KCl, and 1 mM $MgCl_2$. The swollen cells were lyzed by gentle suspension in 2.5 volumes of 50 mM Tris, pH 7.6–130 mM KCl–6.5 mM $MgCl_2$–6.5 mM β-mercaptoethanol–0.13% Triton X-100–13% sucrose. The lysate was centrifuged at 500*g* for 5 min to remove the nuclear fraction, and the supernatant used for polysome analysis.

For preparation of polysomes, the lysate was centrifuged at 17,000*g* for 10 min and 4-ml portions of the supernatant were layered over 5 ml of 20% sucrose in TKM (50 mM Tris, pH 7.6–50 mM KCl–1 mM $MgCl_2$) and centrifuged in the Spinco rotor 40 at 36,000 rpm for 40 min. This last step yields polysomes nearly free of subunits and monomers (Lee and Brawerman, 1971).

Ribosomes from starved cells were obtained by centrifugation of 9-ml portions of the 17,000*g* supernatant over 2 ml of 20% sucrose in TKM at 49,000 rpm for 1 hr. The ribosomal and polysomal pellets were suspended in TKM and stored in liquid N_2.

Zone Centrifugations. For analyses of polysome distribution, 0.1- to 0.2-ml samples of cytoplasmic extracts were layered over 4.5 ml of 10–30% linear sucrose gradients in TKM. A 0.5-ml cushion of 2 M sucrose was placed at the bottom of the centrifuge tubes to minimize sedimentation of the heavy polysomes into the pellet (Hogan and Korner, 1968). Centrifugations were at 45,000 rpm for 25 min in the SW 50 Spinco rotor, unless otherwise indicated. After centrifugation, the solution was pumped from the bottom of the tube through a Uvicord (LKB, Stockholm, Sweden) ultraviolet monitor. The S values listed in the figures are for identification purposes only. The value for the ribosomal monomer was set arbitrarily at 80 S.

For radioactivity measurements, fractions from the gradients were precipitated with ice-cold 10% trichloroacetic acid in the presence of 50 μg of carrier RNA or protein. The precipitates were collected on glass fiber filters, washed with 0.1% trichloroacetic acid, dried, and placed in toluene scintillation mix (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-

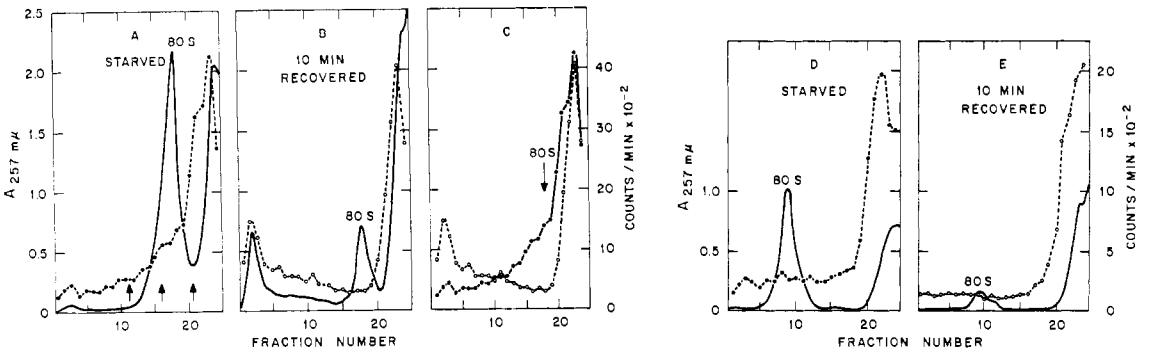

FIGURE 4: Rapidly labeled RNA in starved and recovered cells. Cells were prelabeled and subjected to starvation and recovery treatments as described in the Experimental Section. Starved cells were incubated in Krebs buffer for 40 min; recovered cells, 30 min in Krebs and 10 min in complete medium. Cytoplasmic extracts centrifuged at 41,000 rpm for 30 min in 4A and 4B, and for 95 min in 4D and 4E. Cushions of 2 M sucrose were used in 4A and 4B. Figure 4C represents superimposed radioactivity profiles of 4A and 4B.

phenyloxazolyl)]benzene per l. of toluene). They were counted in a Packard Tri-Carb liquid scintillation spectrometer (background for H^3: 10 counts per min).

Results

Appearance of Polysomes in Starved Cells. Incubation of sarcoma cells in the absence of nutrients for 30 min leads to the nearly complete disappearance of polysomes from the cytoplasm (Figures 1, 2). Cells prior to the starvation treatment had a full complement of polysomes when previously incubated in complete medium. Addition of amino acids, glucose, and bovine serum to the starved cell suspension brings about the rapid conversion of the resulting ribosomal monomers back into polysomes. Ten minutes after the addition of nutrients, the monomer peak has virtually disappeared (Figure 1). The totality of the ribosomes remains mobilized in the polysomes for at least 1 hr. The early stages of polysome formation can be seen in Figure 2. A significant amount of conversion of monomers to polysomes can be detected within 3 min after addition of nutrients. The disappearance of monomers is progressive, but small polysomes do not accumulate. Thus the intermediate stages of recovery are characterized by the gradual uptake of monomers into large polysomal aggregates, rather than the formation of increasingly larger polysomes as suggested by Hogan and Korner (1968).

The rate of polysome formation, as well as the extent of polysome depletion, tended to vary somewhat. Cells which had been incubated in complete medium prior to the starvation treatment usually showed the greatest amount of residual polysomes and the highest rate of recovery (compare Figures 1 and 2). In all cases, however, the great majority of ribosomes was converted into polysomes within 10 min.

As with Landschütz ascites cells (Hogan and Korner, 1968), amino acid supply appeared to be the critical factor for the recovery process in S-180 cells. Bovine serum, although used routinely, was not needed. Addition of glucose alone had no effect on polysome formation.

It has been reported by Kerr *et al.* (1966) that Krebs ascites cells incubated in the absence of nutrients yield ribosome preparations with relatively little capacity for *in vitro* polypeptide synthesis, and that active ribosomes can be obtained from the same cells after a short incubation in complete medium. The parallelism between these variations and the changes in polysome distribution reported here indicate that the protein-synthesizing capacity of the ribosome preparations was a function of their polysome content.

State of Ribosomes in Starved Cells. The disappearance of polysomes during the starvation treatment could be due either to fragmentation of polysomal mRNA or to completion of a translation cycle and prevention of reinitiation. In the first case the monomers should still contain mRNA fragments and nascent polypeptide chains, while in the second case they should be free. These two states of polysomes can be distinguished by the relative susceptibility to dissociation into subunits. It has been shown by Lawford (1969) that rat liver ribosomes obtained by puromycin treatment of polysomes (and consequently free of peptidyl tRNA) are dissociated into subunits at a KCl concentration of 150 mM in the presence of 1 mM Mg^{2+}. Untreated polysomes, or ribosomes obtained by RNase treatment, were not dissociated under these conditions. Figure 3 shows that exposure of the S-180 monosomes from starved cells to 200 mM KCl leads to complete dissociation into subunits. Some dissociation of the monomers is also apparent in the presence of 150 mM KCl. S-180 polysomes were not affected by these treatments. Thus it appears that the ribosomal monomers in starved cells do not have any attached peptidyl tRNA. As is shown below, no mRNA appears to be bound to these ribosomes either.

Utilization of Cytoplasmic mRNA for Polysome Formation. Because of the rapidity of the recovery process, it seemed likely that mRNA already present in the starved cells was being utilized for polysome formation. In order to examine this possibility, cells prelabeled with uridine-*t* in the presence of a low level of actinomycin were subjected to the starvation treatment. Cytoplasmic extracts of the treated cells contained heterodisperse radioactive material with a very wide range of S values (Figure 4A, D). There was no indication of labeled RNA associated either with the monomers or the free subunits. After a 10-min recovery period, a large portion of the radioactivity was associated with the newly formed polysomes (Figure 4B). Superimposition of the radioactivity profiles from starved and recovered cells indicates an apparent shift to the polysome region of radioactive material ranging from 25 S to at least 160 S. The heterogeneous nature of this material is clearly illustrated in Figure 4D,E which shows the complete absence of distinct radioactivity peaks.

Effect of Actinomycin D on Polysome Formation. Although the above results suggest that the prelabeled cytoplasmic material is utilized for polysome formation, the possibility

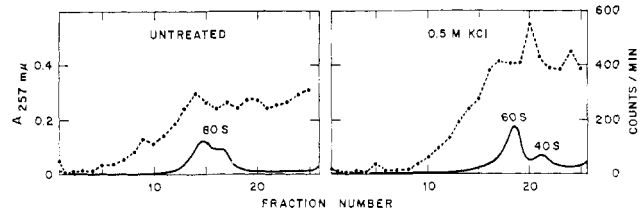

FIGURE 5: Effect of KCl on sedimentation behavior of labeled fractions from starved cells. Fractions 12–20, obtained by zone centrifugation as in Figure 4A (see outside arrows), were pooled. Samples of pooled material were recentrifuged in SW 25 Spinco rotor at 22,000 rpm for 5.5 hr. For the KCl treated sample, the sucrose gradient also contained 0.5 M KCl.

remains that the labeled RNA appearing in the polysomes might be derived from the nucleus. In order to examine this possibility, the recovery experiment was performed under conditions designed to avoid mRNA transport from the nucleus. It has been shown by Latham and Darnell (1965) and by Penman *et al.* (1968) that in HeLa cells exposed to a high level of actinomycin D (10 μg/ml), entry of prelabeled nonribosomal RNA into polysomes ceases 10–15 min after addition of the drug. This is presumably due to exhaustion of the mRNA pool within this time period. In the present study, S-180 cells were pretreated with the same level of actinomycin for considerably longer periods of time in order to ensure that mRNA transport from the nucleus is no longer significant at the time polysome formation is initiated.

In one experiment, the cells were exposed to 10 μg/ml of actinomycin for the last 10-min period of an incubation in complete medium and for the subsequent 30-min starvation period. As can be seen in Figure 1, polysome formation in the cells exposed to the high level of actinomycin was virtually as rapid and effective as in the control cells. An effect of actinomycin became evident only after a 60-min recovery period, when monomers began to reappear in treated cells (Figure 1). This provides an indication that mRNA eventually becomes limiting in the cytoplasm of treated cells. Labeled uridine, added at the time recovery was initiated, failed to appear in the polysomes of the cells exposed to actinomycin (Figure 1). This shows that the amount of drug used in these experiments was sufficient to block mRNA formation. A more extensive pretreatment with actinomycin (30 min in complete medium and 30 min in starvation conditions) had as little effect on the recovery process as that shown in Figure 1.

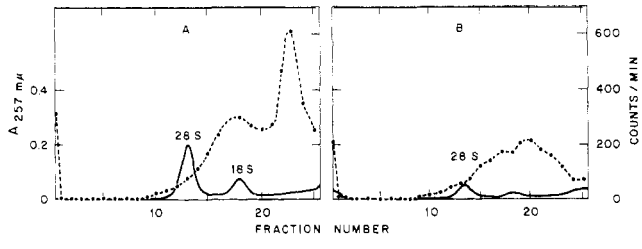

FIGURE 6: Sedimentation characteristics of RNA derived from heavy and light fractions from starved cells. Fractions 12–16 and 17–20, obtained by zone centrifugation as in Figure 4A (see arrows), were pooled separately. Samples of the resulting two fractions were treated with 0.5% sodium dodecyl sulfate by very brief exposure to 37° followed by chilling in ice. Samples were next layered over sucrose gradients in TKM and centrifuged at 22,000 rpm for 16 hr: A, slowly sedimenting fraction; B, rapidly sedimenting fraction.

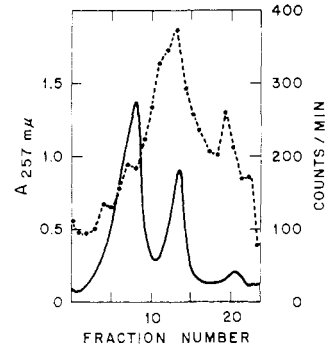

FIGURE 7: Sedimentation characteristics of polysomal RNA. Polysomes, prepared from cells labeled *in vivo*, were treated with sodium dodecyl sulfate and centrifuged through a 5–20% linear sucrose gradient in 10 mM Tris-HCl, pH 7.6, at 49,000 rpm for 3 hr.

The above results strongly suggest that the initial conversion of monomers into polysomes is not dependent on supply of mRNA from the nucleus. It appears, therefore, that the heterodisperse material accumulated in the starved cells serves as precursor to polysomal mRNA.

Characteristics of the Heterodisperse Cytoplasmic RNA of Starved Cells. Material recovered from a 30S–150S sedimentation zone (as indicated in Figure 4A) resediments with about the same S values (Figure 5). Radioactivity remaining near the top of the sucrose gradient apparently represents contamination by the slowly sedimenting component evident in Figure 4A. The ribosomal monomer is partially resolved into two components after recentrifugation (Figure 5). This may represent either dissociation or dimer formation.

Sodium dodecyl sulfate treatment of the 30–150S material releases radioactive RNA with far lower S values. Heavy and light portions of this material (divided as indicated in Figure 4A) show about the same size distribution of radioactive RNA (Figure 6). Both fractions comprise heterogeneous RNA with a broad peak overlapping the 18S ribosomal peak. The lighter fraction (Figure 6A) also contains a more homogeneous slowly sedimenting component. This is probably derived from the contaminating light material evident in Figure 5. The size distribution of labeled RNA in Figure 6 is very similar to that of radioactive RNA from purified polysomes (Figure 7).

The sharp reduction in S values after sodium dodecyl sulfate treatment suggests that the heterogeneous material contains RNA bound to other components. The data in Table I show that most of the labeled material is retained on Millipore filters, as would be expected of ribonucleoprotein particles (Infante and Nemer, 1968). Exposure to 0.5 M KCl has relatively little effect on the retention of the apparent complexes. Free RNA extracted from the particles is also retained at low ionic strength in the presence of ribosomes, but very little of it is bound to the filters in the presence of 0.5 M KCl.

The S values of the heterodisperse material are somewhat reduced in the presence of 0.5 M KCl. The effect of KCl on the sedimentation of the particles, however, is somewhat obscured by the tendency of RNA to form aggregates at high ionic strength. RNA isolated from the 30S–150S material, when centrifuged in the presence of 0.5 M KCl, showed some increase in S values, but they remained considerably lower than those shown in Figure 5.

Baltimore and Huang (1970) reported on the occurrence in HeLa cells of proteins which have the capacity to bind non-

specifically to RNA molecules and cause them to sediment at high rates. The resulting artificial complexes, in contrast to the ones described in this report, are unstable at high ionic strength. In order to verify whether such nonspecific binding of proteins contributes to the high S values of the S-180 particles, starved cells prelabeled as in Figure 4 were lysed in the presence of unlabeled rat liver nuclear RNA in amounts nearly equivalent to those of the total cellular RNA. The added RNA would have been expected to adsorb the majority of the binding factors (see Baltimore and Huang, 1970) and lead to a reduction in the S values of the labeled particles. The sedimentation profile of the heterodisperse material, however, remained unchanged. This indicates the absence of any nonspecific binding of proteins to the mRNA during cell lysis.

TABLE I: Retention of the mRNA Complexes and of Free RNA on Millipore Filters.[a]

	Total Radioactivity (cpm)	Radioactivity Retained on Filters	
		0.05 M KCl	0.5 M KCl
Polysomal RNA	2223		71 (3)
Polysomal RNA + ribosomes	2223	1551 (70)	224 (10)
Heterodisperse material	1073	1171 (100)	968 (90)

[a] Samples were diluted to 3 ml with ice-cold 50 mM Tris-HCl, pH 7.6, and 1 mM $MgCl_2$ in the presence of the appropriate KCl concentrations, and of 0.75 A_{260} unit of S-180 ribosomes when indicated. The solutions were filtered slowly through Millipore filters (HA 0.45 μ) and washed with the same solutions. Heterodisperse material was obtained by zone centrifugation of cell lysate as indicated in Figure 4, except that a longer centrifugation time was used in order to avoid contamination by slowly sedimenting radioactive material. Polysomal RNA (560 cpm/μg) was prepared from labeled polysomes by phenol extraction in the cold in the presence of 0.5% sodium dodecyl sulfate and 0.1 M Tris-HCl, pH 7.6. Values for total radioactivity were obtained from trichloroacetic acid precipitates on Millipore filters. Values in parentheses represent per cent of total radioactivity in samples.

Discussion

The heterodisperse RNA-containing structures described in this report are apparently released from polysomes during starvation and reutilized for polysome formation after addition of amino acids. They resemble the rapidly labeled cytoplasmic particles described by various investigators (Spirin *et al.*, 1964; Perry and Kelley, 1966; Infante and Nemer, 1968; Kafatos, 1968; Henshaw and Loebenstein, 1970). The present results, therefore, provide strong evidence for the suggestion that these particles serve as precursors to polysomal mRNA.

The heterogeneity of the presumed cytoplasmic mRNA appears to be caused by the presence of varying amounts of other components bound to the RNA. This is indicated by the sharp reduction in sedimentation values of the labeled material after sodium dodecyl sulfate treatment, and by the very similar size distribution of the RNA released from two different size classes of this material. The complexes are relatively little affected by exposure to 0.5 M KCl, and their high rates of sedimentation are not due to nonspecific attachment of binding factors during cell lysis. These characteristics are rather similar to those of the pulse-labeled material released from rat liver polysomes by EDTA treatment (Lee and Brawerman, 1971).

The behavior of polysomes during starvation and recovery indicates the occurrence of a regulatory process controlling polysome formation. Amino acid deprivation leads to polysome breakdown, with the accumulation in the cytoplasm of free ribosomes and mRNA. A specific initiation step appears to be affected. Polysome breakdown cannot be attributed simply to depletion of some amino acids, since this could be expected to slow down translation and thus preserve the polysomes. Most of the components required for initiation of polysome formation appear to be functional in the starved cells. This is indicated by the rapidity of the recovery process. During the early stages of recovery, the gradual disappearance of monosomes is accompanied by the appearance of large polysomal aggregates. Such behavior would not be expected if either ribosome activation or formation of initiator aminoacyl-tRNA were the rate-limiting step. In the latter case, small polysomes would accumulate as additional ribosomes slowly add to the mRNA. The recovery pattern shown in Figure 2 could be explained by an increasing availability of mRNA molecules to fully competent ribosomes. In this manner, mRNA molecules made available would become rapidly saturated with ribosomes as translation proceeds. This interpretation, however, must be reconciled with the apparent presence of mRNA in the starved cells. We propose that the initiation site on mRNA is not available for ribosome attachment in starved cells, and that the rate-limiting step during recovery is the activation of this site. Attachment of a protein to the initiation site could provide a molecular basis for such a control mechanism. Further experimental evidence will be required, however, to determine the exact nature of the process involved in the interruption of polysome formation during starvation.

Acknowledgments

The authors gratefully acknowledge the very able technical assistance of Mrs. Mary Ann Krutz during the course of this investigation.

References

Baltimore, D., and Huang, A. S. (1970), *J. Mol. Biol. 47*, 263.
Cartouzou, G., Attali, J-C., and Lissitzky, S. (1968), *Eur. J. Biochem. 3*, 284.
Henshaw, E. C. (1968), *J. Mol. Biol. 36*, 401.
Henshaw, E. C., and Loebenstein, J. (1970), *Biochim. Biophys. Acta 199*, 405.
Hogan, B. L. M., and Korner, A. (1968), *Biochim. Biophys. Acta 169*, 129.
Infante, A. A., and Nemer, M. (1968), *J. Mol. Biol. 32*, 543.
Kafatos, F. C. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 1251.
Kerr, I. M., Cohen, N., and Work, T. S. (1966), *Biochem. J. 98*, 826.
Latham, H., and Darnell, J. E. (1965), *J. Mol. Biol. 14*, 13.
Lawford, G. R. (1969), *Biochem. Biophys. Res. Commun. 37*, 143.

Lee, S. Y., and Brawerman, G. (1971), *Biochemistry*, in press.
Penman, S., Vesco, C., and Penman, M. (1968), *J. Mol. Biol. 34*, 49.
Perry, R. P., and Kelley, D. E. (1966), *J. Mol. Biol. 16*, 255.
Perry, R. P., and Kelley, D. E. (1968), *J. Mol. Biol. 35*, 37.
Spirin, A. S. (1966), *in* Current Topics in Developmental Biology, Vol. 1, Monroy, A., and Moscona, A. A., Ed., New York, N. Y., Academic, p 1.
Spirin, A. S., Belitsina, N. V., and Ajtkhozhin, M. A. (1964), *Zh. Obshch. Biol., 25*, 321.

Transfer Ribonucleic Acids in Rat Liver and Morris 5123 Minimal Deviation Hepatoma*

Fabio Gonano,† Vincenzo P. Chiarugi, Giuseppe Pirro, and Marina Marini

ABSTRACT: Twenty aminoacyl-tRNAs from the "minimal deviation" hepatoma Morris 5123 have been compared on RPC-II chromatography with normal rat liver aminoacyl-tRNA.

The analysis of the chromatographic profiles of individual aminoacyl-tRNAs showed a complete similarity between tRNAs for 17 amino acids. Of the remaining three, one, $tRNA^{Asn}_{5123}$ was displaced with respect to the corresponding one of rat liver, $tRNA^{Glu}_{5123}$ showed only one peak of acceptor activity, while $tRNA^{Glu}_{rat\ liver}$ had a second peak which eluted at a higher salt concentration. The most interesting feature was a new $tRNA^{Phe}$ present in Morris 5123 hepatoma. Its coding properties have been extensively tested Using random polynucleotides and oligonucleotide triplets as templates for ribosome binding measurements, no differences were found between the two $tRNA^{Phe}_{5123}$, rat liver $tRNA^{Phe}$, and *E. coli* $tRNA^{Phe}$. Hybridization experiments using the $tRNA^{Phe}$ specific for Morris 5123 hepatoma and rat liver DNA indicate that there is a specific complementarity between their base sequences. The reasons for the chromatographic differences between tRNAs of hepatoma and rat liver are discussed in terms of differences in methylation of some bases which are not involved in the codon or aminoacyl-tRNA synthetase recognition site.

The attention of many authors has been focused recently on the possible role of tRNA in the regulatory processes of the cell. The chromatographic behavior of all species of tRNAs has been analyzed by different methods. Comparisons have been made between species, organs, and tissues (Holland *et al.*, 1967; Taylor *et al.*, 1967, 1968), between cytoplasm and organelles (Buck and Nass, 1968, 1969; Fournier and Simpson, 1968), during mammalian virus infection (Subak-Sharpe *et al.*, 1966), and after bacterial virus infection (Sueoka *et al.*, 1966; Pollack, 1966; Waters and Novelli, 1967; Hung and Overby, 1968), at various stages of differentiation (Lee and Ingram, 1967; Yang and Comb, 1968; Vold and Sypherd, 1968; Anderson and Cherry, 1969), in different growth conditions (Doi *et al.*, 1968; Heyman *et al.*, 1967; Wettstein and Stent, 1968; Yang *et al.*, 1969), and under hormonal action (Agarwal *et al.*, 1969). Many different tumors have been examined: leukemia P-388 (Morton and Rogers, 1965), ethionine-induced hepatomas (Axel *et al.*, 1967; Ortwerth *et al.*, 1968), plasma cell tumors (Yang and Novelli, 1968a,b; Mushinsky and Potter, 1968, 1969), and Novikoff ascites tumors (Baliga *et al.*, 1968, 1969; Goldman *et al.*, 1969).

In most of the above-mentioned studies, both quantitative and qualitative differences were observed between some of the aminoacyl-tRNAs.

Many hypotheses have been postulated about the significance of the experimental data, but without clear correlations between chromatographic patterns, coding properties, and functional conditions of the cells, no single hypothesis has been firmly substantiated.

In this paper we compared, by reverse phase chromatography (Weiss and Kelmers, 1967), aminoacyl-tRNAs obtained from rat liver and so-called "minimal deviation hepatoma," Morris 5123 (Wu, 1967). As we pointed out in a preliminary report (Gonano and Chiarugi, 1969), by direct comparison with the normal tissue from which the tumor has been originated, this system potentially can give us information about relatively early modifications of a malignant tissue. Whether the tRNA plays any role in the malignant transformation of the hepatic cell or the tumor transformation affects the properties of the normal cell tRNA, it is possible to detect alterations in the chromatographic pattern of the tRNAs.

In this report we present evidence that a difference has been observed for asparaginyl-, glutamyl-, and phenylalanyl-tRNA, between Morris 5123 hepatoma and rat liver. The coding properties and the origin of the new $tRNA^{Phe}$ Morris have been studied by oligonucleotide triplet-induced binding to ribosomes and by DNA–RNA hybridization techniques. No significant differences have been found between the two $tRNA^{Phe}_{Morris}$ species.

Materials and Methods

Animals and Tissues. Morris 5123 and 5123c were obtained by serial transplantations in the inbred Buffalo strain of rats. The tumor was in a solid form, encapsulated, and was

* From the Institute of General Pathology (F. G., G. P., and M. M.), University of Modena, Modena, Italy, and the Institute of General Pathology (V. P. C.), University of Firenze, Florence, Italy. *Received July 21, 1970.* This work has been supported by grants from the Italian Research Council (C. N. R.) to both institutes.

† To whom inquiries should be addressed at the Istituto di Patologia Generale, 41100 Modena, Italy.