Rapidly Labelled Ribonucleic Acid from Rat Liver

STUDIES IN THE ATTACHMENT TO RIBOSOMES AND STIMULATION OF THE INCORPORATION OF AMINO ACIDS INTO POLYPEPTIDES IN CELL-FREE SYSTEMS

By B. R. WILKINSON* AND K. S. KIRBY

*Chester Beatty Research Institute, Institute of Cancer Research:
Royal Cancer Hospital, Fulham Road, London, S.W. 3*

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1. Rapidly labelled RNA from rat liver, either as a complex with DNA (m-RNA-DNA) or with ribosomal RNA (m-RNA-RNA) binds to ribosomes in the polysome region. No binding could be demonstrated with ribosomal RNA or native DNA from *Bacillus subtilis*. 2. With ribosomes from rat liver, *Escherichia coli* or hepatoma the m-RNA-DNA stimulated incorporation of amino acids with rat-liver ribosomes only, whereas the m-RNA-RNA complex was effective with ribosomes from *E. coli* or the hepatoma. 3. Polyuridylic acid was effective as messenger RNA with all three ribosomes but much greater stimulation was obtained with ribosomes from *E. coli* and the hepatoma. 4. The degree of incorporation of phenylalanine with polyuridylic acid and ribosomes from a hepatoma was decreased by about 50% when ribosomal RNA was present.

Rapidly labelled RNA has been isolated either associated with DNA (Kidson, Kirby & Ralph, 1963) or with ribosomal RNA (Kirby, 1965; Wilkinson & Kirby, 1966). The presence of the bulk of this RNA from rat liver in the nuclear fraction 20 min. after injection of [³H]orotic acid and the complete inhibition of its synthesis by the action of actinomycin D pointed to an RNA synthesized rapidly on a DNA template and indicative of messenger RNA (Brenner, Jacob & Meselson, 1961). The extent to which this material is equivalent to messenger RNA can be examined by further fractionations, by studying the ability to bind to ribosomes and to stimulate the incorporation of amino acids into polypeptides.

Henshaw, Bojarski & Hiatt (1963), Revel & Hiatt (1964) and Brawerman, Gold & Eisenstadt (1963) have demonstrated that rapidly labelled RNA from rat liver will bind to ribosomes and can stimulate the incorporation of amino acids into polypeptide with a system from *Escherichia coli*. Their preparations of rapidly labelled RNA were different from those we have used and we have similar results with bacterial systems. We have extended these experiments to the use of mammalian ribosomes and report the differential effects of the two preparations of rapidly labelled RNA with different types of ribosomes.

* Present address: Department of Genetics, University of Hawaii, Honolulu, Hawaii 96822.

EXPERIMENTAL

Materials

The following commercial sources were used: ATP and CTP (Sigma Chemical Co., St Louis, Mo., U.S.A.), GTP, UTP and polyuridylic acid (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.), creatine kinase (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.), deoxyribonuclease (2× crystallized; Worthington Biochemical Corp., Freehold, N.J., U.S.A.), ribonuclease (Armour and Co., Eastbourne, Sussex), creatine phosphate and tetraethylammonium hydroxide (25%) (British Drug Houses Ltd., Poole, Dorset).

Methods

Isolation of ribosomes. Free ribosomes were obtained from *E. coli* by the method of Nirenberg & Matthaei (1961). Ribosomes from the transplantable hepatoma and microsomes and RNP† particles from rat liver were prepared as described previously (Wilkinson & Kirby, 1966). The combined pellets obtained after centrifuging for 3 hr. at 105 000g were dispersed in 4 ml. of 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl and 0.1 mM-magnesium acetate by gentle stirring for 45 min. at 2°. Any undissolved material was then removed by centrifuging at 10 000g/10 min./2°.

Ribosomes and RNP particles were characterized by determination of sedimentation coefficients on the Spinco model E ultracentrifuge at 29 500 rev./min. for 20 min. by using schlieren optics. Studies were also made on sucrose

† Abbreviation: RNP, ribonucleoprotein.

gradients (5–20%) in 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl and adjusting the Mg^{2+} as required. This gradient was used as the hepatoma ribosomes gave a reasonably sharp peak under the conditions used (Fig. 1) and were well separated from the rat liver microsomes fraction (Fig. 2). A portion (1 ml.) of ribosome dispersion was layered over 23.8 ml. of gradient which was then centrifuged at 23 000 rev./min. for 2 hr. at 5° (Spinco model L centrifuge). Fractions were collected and extinctions at 260 m μ and 280 m μ were measured. Studies on the ratio of free to bound ribosomes were carried out with 3 ml. of 50% sucrose in the same salt mixture at the bottom tube (cf. Henshaw, Bojarski & Hiatt, 1963). Tumour ribosomes consisted of 58% of RNA, determined by the orcinol reaction (Dische & Schwartz, 1937) and 42% of protein (method of Lowry, Rosebrough, Farr & Randall, 1951).

Attachment of rapidly labelled RNA to ribosomes. The

Fig. 1. Centrifugation in sucrose-density gradient (5–20% sucrose in 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, pH 7.6, with Mg^{2+} , as shown below), of ribosomes from hepatoma tissue. All readings are at 260 m μ . —, With 0.1 mM- Mg^{2+} ; ----, with 10 mM- Mg^{2+} . Centrifugation was in the Spinco model L centrifuge (SW 25.1 rotor) at 24 000 rev./min. for 2 hr.

Fig. 2. Centrifugation in sucrose-density gradient (5–20% sucrose in 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, 10 mM- Mg^{2+} , pH 7.6) of microsomes from rat liver (----) and ribosomes from hepatoma tissue (—). Centrifugation was as described for Fig. 1.

RNA (either as m-RNA-RNA or m-RNA-DNA, 1 mg.) was dissolved in 10 mM-sodium acetate (0.5 ml.) and mixed with an equal volume of ribosomes or RNP particles dispersed in 50 mM-tris-HCl, pH 7.6, 60 mM-KCl, 5 mM-NaCl, 0.1 mM-magnesium acetate. After shaking gently the Mg^{2+} was adjusted to 10 mM by the addition of 100 mM-magnesium acetate. This concentration was used for comparison of these results with those of the studies in the stimulation of the incorporation of amino acids. The mixture remained at 2° or 37° for 30 min. and was then layered over a sucrose (5–20%) gradient in the same salt-buffer mixture as above with 10 mM- Mg^{2+} . After centrifuging for 2 hr. the tubes were pierced at the bottom, about 30 fractions were collected, each was mixed with 1 ml. of 0.5% EDTA and the extinctions were read at 260 m μ .

Extraction of RNA from sucrose gradients. The fractions were then heated 100° for 10 min., cooled and saturated with butan-2-ol and each fraction was shaken with 1 ml. of a mixture of butan-2-ol (100 ml.), 2,2'-diethyldihexylamine (11.7), acetic acid (2.0 ml.) and 8-hydroxyquinoline (0.5 g.). After the phases had separated 0.5 ml. of top phase was mixed with 10 ml. of scintillation fluid and the radioactivity determined with a Tri-Carb Liquid Scintillation Spectrometer. When radioactively labelled RNA was used (either ribosomal or rapidly labelled RNA) 95–100% was found in the top phase as long as the final Na^+ (or K^+) concentration was below 50 mM.

To label the RNA associated with microsomes it was necessary to inject 600–800 μ C of [3H]orotic acid/rat 20 min. before removing the liver for the preparation of RNP particles.

Attachment of DNA was studied with DNA from *Bacillus subtilis* labelled with [3H]thymidine. Denaturation was carried out by heating a solution of this preparation at 100° for 10 min. and cooling rapidly.

Ribosomal RNA was labelled by injecting 200 μ C of [3H]orotic acid/rat 18 hr. before removal of the liver and isolation of the RNA by method 1 (Kirby, 1965). Analysis by sucrose-gradient centrifugation showed that the extinction and radioactive profiles were identical.

Interaction with ribosomes was examined by dissolving the nucleic acid (1 mg.) in 10 mM-sodium acetate (1 ml.), mixing with the ribosomal dispersion, adjusting the Mg^{2+} to 10 mM and analysing by sucrose-density centrifugation.

Amino acid-incorporation studies. Cell sap was obtained from rat liver for studies with mammalian systems. The liver was homogenized in 2 vol. of cold 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl and 10 mM-magnesium acetate. The Mg^{2+} was so adjusted to remove any free m-RNA from the system. Particles were removed by centrifuging first at 12 000g for 10 min. and then, after making mm with respect to GSH, at 105 000g/3 hr. The supernatant fraction was used directly or dialysed against 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl and 10 mM-magnesium acetate and mm-GSH. The preparations were generally used immediately but could be stored for 1–2 weeks at –15°.

Cell sap from *E. coli* was prepared either as the S30 fraction of Nirenberg & Matthaei (1961) or freed from ribosomes by centrifuging at 105 000g/3 hr.

Composition of the cell-free systems. The various cofactors were dissolved in 60 mM-KCl, 5 mM-NaCl, 50 mM-tris-HCl, pH 7.6. The composition of the system was: ribosomes (or RNP particles), 0.3 ml. (6 mg. of RNA/ml.); cell sap (0.2 ml.); 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl (0.1 ml.); ATP

(10 μ moles/ml.) + GTP (2.5 μ moles/ml.) + CTP (7 μ moles/ml.) + creatine phosphate (80 μ moles/ml.) (0.1 ml.); creatine kinase, 0.1 ml. (0.1 mg./ml.); GSH, 0.1 ml. (100 μ moles/ml.); amino acid (1.2 mg. each except the radioactive one, 5 ml.) (0.05 ml.); radioactive amino acid (0.05 ml. of a solution containing 10 μ C/ml.).

Preincubation was carried out by mixing ribosomes (or RNP particles) containing about 2.5 mg. of RNA, with cell sap and incubating at 37°/60 min. The mixture was cooled, dialysed overnight against two changes of 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, 10 mM-Mg²⁺ and 6 mM-mercaptoethanol (500 ml. each time). The m-RNA-DNA (200 μ g.) or m-RNA-RNA (1 mg.) in 1 ml. of 10 mM-sodium acetate then replaced the tris-HCl-KCl-NaCl mixture. Polyuridylic acid (50 μ g./system) was also used as a messenger RNA with [¹⁴C]phenylalanine; 60 mM-KCl can be replaced by 190 mM-ammonium acetate or 160 mM-NH₄Cl (Gilbert, 1963). The systems were incubated at 35° for 60 min.

The composition of the cell-free system from *E. coli* was that of Nirenberg & Matthaei (1961).

Radioactive assay. Amino acid incorporation was stopped by adding an equal volume of a cold solution of unlabelled amino acid and 2 vol. of 20% (w/v) trichloroacetic acid. The mixture was left at 2° for 30 min., the precipitate centrifuged off and washed twice with 20% trichloroacetic acid. The sediment was mixed with 5% (w/v) trichloroacetic acid, heated at 90° for 15 min., cooled, centrifuged off and washed four times with 5% trichloroacetic acid; recovery each time was achieved by centrifugation. The tubes were finally drained, wiped dry and the sediment was dissolved in 25% tetraethylammonium hydroxide (0.2 ml.). A portion was mixed with the scintillation fluid and the radioactivity determined in a Tricarb liquid scintillation spectrometer.

Modification of m-RNA-RNA. Conditions in which the secondary structure of ribosomal RNA was lost (Kirby, 1965) were examined to determine the effect of this on the stimulatory capacity of the RNA.

mm-Potassium acetate, 2-methoxyethanol-water (1:4) or mM-triethylammonium acetate was used to modify the RNA (4 mg.), which was dissolved in 4 ml. of each of the mixtures and then heated at 70° for 30 min. The solution was cooled quickly, evaporated to dryness and redissolved in either 0.4 ml. of water (after treatment with potassium acetate and triethylammonium acetate) or 0.4 ml. of mM-potassium acetate (after treatment in aqueous methoxyethanol).

RESULTS

RNP particles and ribosome preparations. The RNP particles isolated in the presence of 10 mM-Mg²⁺ showed two major peaks on ultracentrifugation (110s and 80s), but considerable heterogeneity was present. In mM-Mg²⁺ the particles were mainly 80s and the dissolution was largely reversible if the Mg²⁺ was returned to 10 mM. However, if the particles were separated or treated in 0.1 mM- or 0.01 mM-Mg²⁺ and then examined at 10 mM-Mg²⁺ non-specific aggregation or even precipitation took place. This was particularly pronounced if the particles were pre-treated with 0.5% EDTA.

Ribosomes from hepatomas were isolated without the use of detergent. They were obtained in high yield and appeared mainly as 80s particles when isolated in the presence of 0.1 mM-Mg²⁺. Dimerization took place when Mg²⁺ was increased to 10 mM (Fig. 1). This was reversible by lowering the Mg²⁺ concentration and there was no tendency to non-specific aggregation. A comparison of the microsomes isolated from rat liver with the ribosomes from the hepatoma is made in Fig. 2.

Attachment studies. (a) Endogenous m-RNA-RNP particles isolated with 10 mM-Mg²⁺ were associated with rapidly labelled RNA and this was removed if the Mg²⁺ was decreased to 0.1 mM (Fig. 3a and b). Essentially a similar result was obtained by incubation in the presence of cell sap. After 10 min. the particles were largely monomers although some aggregation occurred. We have shown previously (Wilkinson & Kirby, 1966) that no rapidly labelled RNA is released from the nucleus into the cytoplasmic fraction by extraction of the RNA samples and fractionation by sucrose-density gradient centrifugation.

(b) Exogenous RNA: rapidly labelled RNA attached to RNP particles and ribosomes in the presence of 10 mM-Mg²⁺. The attachment of m-RNA-DNA to RNP particles and m-RNA-RNA

Fig. 3. Centrifugation in sucrose-density gradient (5–20% sucrose in 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, 10 mM-Mg²⁺, pH 7.6) of RNP particles from rat liver. Centrifugation was as described for Fig. 1. —, $E_{260\text{ m}\mu}$; ----, radioactivity. (a) Immediately after preparation; (b) after incubation at 37° for 10 min.

Fig. 4. Centrifugation in sucrose-density gradient (5–20% sucrose in 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, 10 mM-Mg²⁺, pH 7.6) to demonstrate the attachment of m-RNA to ribosomes or RNP particles. Centrifugation was as described for Fig. 1. —, $E_{260} \text{ m}\mu$; ----, radioactivity. (a) Attachment of m-RNA-DNA to RNP particles from rat liver, 30 min. at 37° before centrifugation. (b) Attachment of m-RNA-RNA to ribosomes from hepatoma tissue.

Fig. 5. Centrifugation in sucrose-density gradient (5–20% sucrose, 50 mM-tris-HCl, 60 mM-KCl, 5 mM-NaCl, 10 mM-Mg²⁺, pH 7.6) to demonstrate the attachment of denatured DNA from *B. subtilis* to RNP particles from rat liver. The DNA was incubated with RNP particles for 30 min. at 2° before centrifugation. Centrifugation was as described for Fig. 1. —, $E_{260} \text{ m}\mu$; ----, radioactivity.

to ribosomes from the hepatoma are shown in Fig. 4(a) and (b). The attachment was stable at 2° (Fig. 4b) but, at 37°, even though there was some breakdown of the RNP particles, the attachment remained.

(c) DNA: native DNA from *B. subtilis* did not attach to RNP particles, but after denaturation showed a non-specific attachment (Fig. 5).

(d) Transfer RNA attached to RNA only in the presence of enzymes and source of energy.

(e) Ribosomal RNA, either native or after denaturation at 70° for 10 min. in 10 mM-sodium acetate, showed no attachment to ribosomes from the hepatoma or to RNP particles.

Studies on the incorporation of amino acids. The activity of polyuridylic acid in stimulating polypeptide formation from [¹⁴C]phenylalanine is shown in Table 1. The incorporation stimulated by polyuridylic acid is equivalent to about 0.150 μmole of [¹⁴C]phenylalanine/mg. of RNA in the system.

In an *E. coli* S30 system 0.36 μmole of phenylalanine/mg. of RNA was incorporated with K⁺ in the system and 0.65 μmole /mg. of RNA when NH₄⁺ was present (cf. Gilbert, 1963).

When rapidly labelled RNA associated with DNA (m-RNA-DNA) was used, a factor of stimulation of 1.6 was found with RNP particles from rat liver and none at all with ribosomes from *E. coli* or from the transplantable hepatoma (O'Sullivan & Kirby, 1964). However, when rapidly labelled RNA was associated with ribosomal RNA (m-RNA-RNA) factors of stimulation of 1.83 and 1.42 respectively were found with ribosomes from *E. coli* and from the transplantable hepatoma. This m-RNA-RNA had no effect when tested with RNP particles from rat liver. The stimulatory activity was absent if the rapidly labelled RNA was degraded with ribonuclease before testing. Ribosomal RNA prepared by method 1 (Kirby, 1965), in which no rapidly labelled RNA was present, had no stimulatory activity with any type of ribosome tested.

When heated at 70° ribosomal RNA loses much of its secondary structure (Kirby, 1965). The effect of heating under different conditions on the stimulation of the incorporation of [¹⁴C]leucine into polypeptides is shown in Table 2. These results represent an incorporation of about 6 μmoles of [¹⁴C]leucine/mg. of RNA.

DISCUSSION

Methods. While the basic methods are the same as those described previously (cf. Nisman & Pelmont, 1964) the extraction of RNA from ribosomes with butan-2-ol and 2,2'-diethyldihexylamine provides a rapid and quantitative system. It is a modification of that described earlier by Kidson *et al.* (1963). It is essential to have ethylenediaminetetra-acetate present and to heat the ribosomes to 100° to denature the protein before the extraction is carried out. If the Na⁺ concentration

Table 1. *Stimulation of the incorporation of [¹⁴C]phenylalanine into polypeptide by action of polyuridylic acid with ribosomes from transplantable hepatoma*

Treatment of ribosomes	Addition	Counts/min./system	Phenylalanine incorporated (μmoles)
No preincubation	None	4375	0.330
After preincubation	None	87	
	Polyuridylic acid	5327	0.373
	Polyuridylic acid + ribosomal RNA	2462	
	Polyuridylic acid + DNA	4428	

Table 2. *Effect of different treatments on m-RNA-RNA used for incorporation system with ribosomes from transplantable tumour*

The factor of stimulation is shown in parentheses after counts/min. The parentheses in the first column of the third, fourth and fifth rows indicate that the RNA was heated to 70° with the compound named, as described in the Methods section, before it was added to the incorporating system.

Addition	Rat liver m-RNA-RNA (counts/min./system)	m-RNA-RNA from transplantable tumour (counts/min./system)
None	429	429
m-RNA-RNA	608 (1.42)	616 (1.44)
m-RNA-RNA (potassium acetate)	627 (1.46)	651 (1.52)
m-RNA-RNA (2-methoxyethanol)	631 (1.47)	667 (1.56)
m-RNA-RNA (triethylammonium acetate)	603 (1.41)	640 (1.50)

is increased the amount of amine and acetic acid should be doubled. The dissolution of the final trichloroacetate-protein precipitate in tetraethylammonium hydroxide is also advantageous, in that solution, which is rapid, can be added directly to the scintillation fluid and requires only a short time for adaptation to the dark.

Ribosomes. Monomeric ribosomes (80s) have been obtained in good yield from a transplantable hepatoma without the use of detergent. Similar ribosomes have been obtained from transplantable sarcomas and it has been reported that other tumours provide a good yield of monomeric ribosomes (Petermann, Mizen & Hamilton, 1956; Petermann, Hamilton & Mizen, 1954). The ribosomes described have about the same activity as those from *E. coli* with a polyuridylic acid-phenylalanine system. The activity may be related to the relatively high RNA content of these ribosomes. The compositions of different ribosomes have been summarized by Arnstein (1964). It should be noted that RNA from tobacco-mosaic virus was almost inactive in stimulating the incorporation of amino acids with ribosomes from the hepatoma. This is analogous to the failure of tobacco-mosaic-virus RNA to act as messenger with ribosomes from rabbit reticulocytes (Nisman & Pelmont, 1964).

Attachment studies. Endogenous m-RNA is

attached to polysomes and released by lowering Mg^{2+} or by preincubation (Schaechter, 1963). Rapidly labelled RNA, as m-RNA-RNA or m-RNA-DNA, attached to RNP particles and to tumour ribosomes in the presence of 10mM- Mg^{2+} . Native DNA did not attach to ribosomes, neither did native or denatured ribosomal RNA. However, if DNA was first denatured a non-specific attachment occurred.

Incorporation of amino acids. Early experiments showed that with m-RNA-DNA and RNP particles from rat liver very little stimulation of amino acid incorporation was observed unless microsomes were isolated in 0.01mM- Mg^{2+} . At that Mg^{2+} concentration no endogenous m-RNA remained with the RNP particles; preincubation was unnecessary and the factor of stimulation was somewhat variable. Barondes, Dingman & Sporn (1962) and Hoagland & Askonas (1963) have also observed stimulation of incorporation in rat liver systems. The RNP particles with polyuridylic acid as a messenger gave a factor of stimulation for phenylalanine of only 4.0, an indication that this is potentially a poor system compared with that from *E. coli*. A similar result has been obtained by Campbell, Cooper & Hicks (1964) with RNP particles and polyuridylic acid. With ribosomes from *E. coli* a good stimulation is obtained with polyuridylic acid, but none with a m-RNA-DNA

preparation, even if previously treated with deoxyribonuclease. It should be noted that Brawerman *et al.* (1963) and DiGirolamo, Henshaw & Hiatt (1964) have obtained a stimulation with rapidly labelled RNA from liver and *E. coli* system. However, their methods of preparation were different from those described, and it should be particularly noted that DiGirolamo *et al.* found a marked inhibitory effect of oligodeoxyribonucleotides.

Tumour ribosomes were isolated as these were reported to be monomeric and required no treatment with detergent (Petermann *et al.* 1956). O'Neal & Griffin (1963) had found ribosomes from ascites tumour to be more effective with m-RNA than ribosomes from liver. A good yield of monomeric ribosomes, which showed no tendency to non-specific aggregation at 10mM-Mg²⁺, could be isolated from a transplantable hepatoma. These particles gave a good factor of stimulation for phenylalanine with polyuridylic acid (500–1200) but practically no stimulation with m-RNA–DNA. Because of this it was decided to investigate rapidly labelled RNA associated with ribosomal RNA. This particular preparation showed no capacity for stimulation of amino acid incorporation with RNP particles but gave extremely consistent stimulation with ribosomes from *E. coli* or from the hepatoma. However, although the factor of stimulation was low (equivalent to about 15 μ moles of leucine, or about 150 μ moles of amino acids if there were about 10% of leucine in the polypeptide), it was found that ribosomal RNA in which no rapidly labelled RNA was present inhibited the incorporation of phenylalanine by polyuridylic acid by about 50%. Since ribosomal RNA did not bind to ribosomes it is likely that the inhibition was due to the rapidly labelled RNA binding to ribosomal RNA in the presence of 10mM-Mg²⁺. The effect of heating the m-RNA–RNA complexes was not very significant (Table 2), and indeed heating in 10mM-Na⁺ and then cooling increases the association of rapidly labelled RNA with ribosomal RNA (Wilkinson, 1965).

The reason for the difference between m-RNA–RNA and m-RNA–DNA is not completely clear but, apart from the differences in the nucleic acids, the former preparation is completely free from protein whereas some protein is always associated with the m-RNA–DNA. The protein may aid the binding to RNP particles, which, having been prepared with detergent, are deficient in protein compared with the tumour ribosomes (cf. Campbell *et al.* 1964). The rapidly labelled RNA in the m-RNA–RNA preparation may be in such a firm complex with the ribosomal RNA that there is little tendency to transfer it to RNP particles, which contain the same ribosomal RNA. It is clear

that the presence of RNA and DNA in these produces a degree of complexity in these investigations and further studies in rapidly labelled RNA after separation from other components are required. However, the binding to ribosomes and the stimulation of the incorporation of amino acids are in agreement with the results of other workers (Barondes *et al.* 1962; Hoagland & Askonas, 1963; DiGirolamo *et al.* 1964) that a proportion of the rapidly labelled RNA from rat liver is of the nature of messenger RNA. It seems likely that tumour ribosomes will be of considerable help in further investigations on the activities of different rapidly labelled RNA in promoting polypeptide biosynthesis.

The reason for the high proportion of free ribosomes in transplantable tumours is unknown, but Noll, Staehelin & Wettstein (1963) have suggested that polyribosomes may reflect the stability of the m-RNA. Transplantable tumours should therefore have m-RNA of a high rate of turnover and since the m-RNA of this tumour has been characterized (Wilkinson & Kirby, 1966) the hypothesis can be examined experimentally.

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