

Characterization of Messenger-Like Ribonucleic Acid from *Saccharomyces cerevisiae* by the Use of Chromatography on Methylated Albumin-Kieselguhr

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Chromatography on methylated albumin-kieselguhr of RNA from *Saccharomyces cerevisiae* was used to separate stable RNA from a tenaciously bound DNA-like RNA fraction. The tenaciously bound RNA, which was eluted with a dilute solution of sodium dodecyl sulphate, was characterized as messenger-like RNA by its sedimentation behaviour, nucleotide composition, lack of methylated bases and labelling kinetics. Chromatography of purified ribosomal RNA indicated a minor contamination of the tenaciously bound fraction with ribosomal RNA. On the other hand, a large portion of pulse-labelled polyribosomal RNA from protoplasts of *Saccharomyces cerevisiae* was tenaciously bound to the columns. The 'chase' of isotopic label from the messenger-like RNA was found to be retarded during inhibition of protein synthesis both by cycloheximide and by starvation for a carbon source.

Previous attempts to study mRNA in yeast have been hampered by a combination of several unique characteristics of this organism. First, when total cell extracts are employed, the rapid labelling kinetics of mRNA are obscured because even after very brief labelling periods most of the label appears in ribosomal precursor RNA and rRNA (Retel & Planta, 1967; de Kloet, 1966; Taber & Vincent, 1969). This high rate of rRNA synthesis is facilitated both by the comparatively large fraction (about 2%) of the yeast genome coding for rRNA (Retel & Planta, 1968; Wintersberger, 1967) and the rapid conversion of ribosomal precursor RNA into mature rRNA (Taber & Vincent, 1969). Secondly, although polyribosomes provide an opportunity to study a subcellular fraction enriched in mRNA, the quantitative extraction of yeast polyribosomes has been possible only through the use of yeast protoplasts formed by enzymic digestion of the cell wall (Duell, Inoue & Utter, 1964; Hutchison & Hartwell, 1967). However, protoplast formation requires subjecting the yeast to abnormal physiological conditions for extended periods, thus precluding the study of mRNA synthesis and decay during completely normal growth conditions. During mechanical disruption of whole yeast cells, apparently any force sufficient for disruption of the cell wall is also sufficient to cause appreciable damage to the labile polyribosome complex. Alternatively it might be proposed that this polyribosome degradation results from a nor-

mally high intracellular ribonuclease activity that is decreased either by inactivation or leaching-out during formation of protoplasts.

In this investigation total RNA from mechanically disrupted yeast cells was fractionated by MAK* chromatography with a step-gradient elution procedure designed to separate the stable RNA from an unstable fraction tentatively identified with mRNA. When nucleic acids from metazoan cells are chromatographed on MAK columns, most of the nucleic acid species are eluted, and separated, by a sodium chloride gradient. However, a large portion (about 80%) of the rapidly labelled RNA, which is DNA-like in base composition and has a sedimentation profile similar to polyribosomal mRNA, is tenaciously bound to the column (Ellem, 1966; Ewing & Cherry, 1967; Lingrel, 1967; Ellem & Rhode, 1968). This fraction, termed TD-RNA, can be eluted with a dilute solution of sodium dodecyl sulphate (Roberts & Quinlivan, 1969; Ellem, 1966). In this paper an analogous fraction of RNA from yeast was tested with regard to its base composition, sedimentation properties and labelling kinetics. In addition, the decay of label in this fraction was tested under several different growth conditions. The chromatographic behaviour of purified rRNA and pulse-labelled polyribosomal RNA from yeast protoplasts was also investigated.

In contrast with metazoan cells, yeast cells

* Abbreviation: MAK, methylated albumin-kieselguhr.

provide an opportunity to study mRNA in a homogeneous eukaryotic cell culture with a short generation time. Moreover, as with bacteria, the effects of different growth rates and restrictive growth conditions can be conveniently studied.

MATERIALS AND METHODS

Organism and growth conditions. The haploid aUr4 (S2112D) strain of *Saccharomyces cerevisiae* (obtained from Dr R. K. Mortimer, University of California, Berkeley, Calif., U.S.A.) was used in all experiments. This strain is auxotrophic for uracil and histidine. Except where otherwise specified, a modification of the synthetic media described by Roman (1956) was used. Uracil and histidine were present at 10 μ g/ml to meet the deficiency of the auxotrophic strain. Cultures were grown at 30°C in 1 litre cylinders with agitation provided by constant aeration. The generation time under these conditions was about 4 h. Isotope incorporation, and nucleic acid extractions were carried out with mid-log phase cultures (about 10⁷ cells/ml). Transfer of cells was accomplished by collection on membrane filters.

Determination of radioactivity. The specific conditions for the incorporation of radioisotopes are described in the legends of the Figures. A Beckman liquid-scintillation spectrometer was used for the radioassays. Portions of aqueous samples were mixed with a scintillant containing 4 g of 5-(4-biphenyl)-2-(4-*tert.*-butylphenyl)-1-oxa-3,4-diazole (Packard Instrument Co., Downers Grove, Ill., U.S.A.), 80 g of naphthalene/l, 400 ml of methylcellosolve/l and 600 ml of toluene/l. Isotopically labelled compounds were purchased from the New England Nuclear Corp., Boston, Mass., U.S.A.

Extraction of high-molecular-weight RNA from whole cells. Yeast cells, that had been freeze-dried to dryness, were disrupted in the cold in a Mickle tissue disintegrator (H. Mickle, Gomshall, Surrey, U.K.) for 1 min with 3 mm diameter glass beads. Microscopic examination revealed that 90% of the cells were broken reproducibly by this method. The disrupted cells were suspended in an equal mixture of water-saturated phenol and buffer containing 0.1 M-potassium acetate, 0.1 M EDTA, 5% (w/v) sodium dodecyl sulphate and 5% (w/v) bentonite pretreated as described by Fraenkel-Conrat, Singer & Tsugita (1961). RNA was extracted from this mixture by stirring at 2°C for 5 h. The aqueous phase was then dialysed extensively against 0.1 M-potassium acetate, and after sedimentation of a small amount of potassium dodecyl sulphate, the RNA was precipitated by adding 2 vol. of 95% (v/v) ethanol at -10°C. The ethanol precipitate was dissolved in appropriate buffers at the concentrations required. No special attempt was made to remove the small amount of DNA that might be present in RNA prepared in this manner, since yeast cells contain 50 times as much RNA as DNA (Wehr & Parks, 1969; Chargaff & Zamenhof, 1948). Further, significant contamination of the tenaciously bound fraction with DNA is excluded by the rapid labelling and 'chase' characteristics of this fraction (see the Results section).

For extraction of RNA from ribosomes, the cells were disrupted as described above, and the cell debris was sedimented at 20000 rev./min for 10 min in a Spinco model L ultracentrifuge with the no. 40 angle head. The

ribosomes, contained in the supernatant, were then sedimented by centrifuging for 5 h at 40000 rev./min. The rRNA was extracted from the resulting pellet as described above.

Isolation of polyribosomal mRNA from protoplasts. Protoplasts were formed by digestion of the cell wall with glucylase (Endo Laboratories Inc., Garden City, N.J., U.S.A.) essentially as described by Hutchison & Hartwell (1967). The protoplasts were incubated for 10 min with [³H]uracil, after which time cycloheximide was added to prevent polyribosome breakdown (Hartwell & McLaughlin, 1968, 1969). The cells were lysed by freezing and thawing in 1% Brig-58 (Atlas Powder Co., Wilmington, Del., U.S.A.), followed by extraction for 10 min at 2°C. After centrifugation to remove the cell debris, the supernatant, containing the polyribosomes, was layered on a 10-30% (w/v) sucrose gradient, and centrifugation was carried out for 1 h in the Spinco model L ultracentrifuge at 35000 rev./min with the SW39 rotor at 5°C. RNA was extracted from the pooled polyribosome fractions by the cold phenol method described above.

MAK column chromatography. The columns were prepared by the method of Osawa & Sibatani (1966), which is a simplified version of the more standard procedure of Mandell & Hershey (1960). The columns were 1.2 cm in diameter with displacement volumes of about 10 ml. Columns of this size were found to be sufficient for adsorption of up to 2 mg of RNA. The elution rate of 0.25 ml/min was produced by a water head of about 30 cm. E_{260} was determined in 5 ml fractions that were collected by using a Beckman model 32 fraction collector. Radioactivity was determined in 0.5 ml samples of these fractions. Elution was carried out at 30°C.

The RNA samples were adsorbed to the columns in small volumes of 0.2 M-NaCl in potassium phosphate buffer (pH 6.7), followed by passage of 20-30 ml of the same buffer to wash through low-molecular-weight contaminants. This was followed by a salt-pH gradient that was established by passing 150 ml of 1.5 M-NaCl, pH 9.0, into 150 ml of 0.2 M-NaCl, pH 6.7, contained in the mixing vessel. This gradient was interrupted after the passage of 175 ml, as this was found to be sufficient for complete elution of the bulk nucleic acids. Water (25 ml) was then passed through the columns to avoid subsequent precipitation of potassium dodecyl sulphate. No nucleic acids (or radioactivity) were eluted by the water. Final elution of the tenaciously bound RNA was accomplished by passing 20 ml of a 0.1% (w/v) sodium dodecyl sulphate solution. Radioassay of the column residue showed that essentially all of the radioactivity was eluted by this procedure.

To obtain highly radioactive bulk RNA fractions for the labelling kinetics studies, a simplified elution procedure was followed. Instead of the salt gradient described above, the tRNA and rRNA were eluted in a single step with 30 ml of 1.5 M-NaCl, pH 9.0. This drastic steepening of the gradient was found to have no effect on the total amount of material eluted by this step. Further, this simplification obviated the use of the fraction collector: the two fractions of interest were collected in graduated cylinders. Exactly 1 mg of RNA was adsorbed to each column. Thus, the radioactivity, shown in Figs. 7, 9, 10 and 11, represents the total radioactivity (c.p.m./mg of input RNA) eluted by the 1.5 M-NaCl and the sodium

dodecyl sulphate as determined from 0.5ml samples of each. In any given pulse or chase experiment, isotope incorporation or dilution was carried out in a single culture, and samples were removed at the designated times for RNA extraction and MAK chromatography.

Nucleotide analysis. Nucleotide analyses were performed by electrophoresis essentially as described by Davidson & Smellie (1952) with modifications for isotopic compositional analysis. Yeast cells were incubated for 20min with [^{32}P]phosphoric acid ($10\mu\text{Ci/ml}$) and total RNA was extracted as described above. After chromatography on MAK columns, the salt-eluted (rRNA) and sodium dodecyl sulphate-eluted (TD-RNA) fractions were dialysed against water and freeze-dried. The freeze-dried samples were hydrolysed in 0.5ml of 0.3M-KOH at 37°C for 18h. After neutralization with HClO_4 , marker nucleotides were added and the samples were concentrated by evaporation. Samples were applied to Whatmann 3MM paper strips mounted in a Spincopaper electrophoresis cell (model R, Durrum type). Electrophoresis was continued for 2h with a potential of 20V/cm. The nucleotide bands were identified and marked under u.v. light. The dried bands were then cut out and transferred to scintillation vials for the radioassay.

RESULTS

Fig. 1 shows a typical MAK column profile of pulse-labelled yeast RNA that was eluted with a salt gradient followed by sodium dodecyl sulphate. The first peak represents low-molecular-weight con-

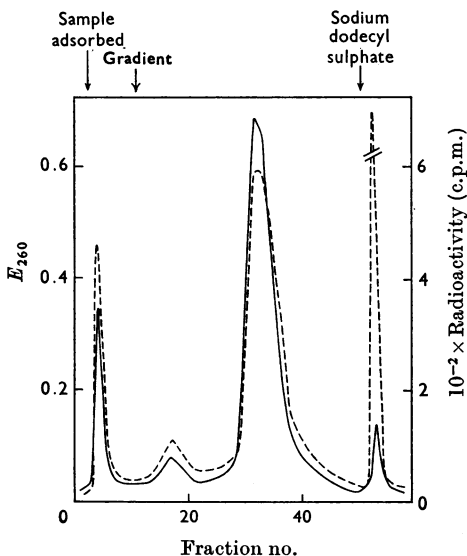


Fig. 1. Typical MAK column chromatogram of pulse-labelled yeast RNA. Cells were pulsed for 2min with [^{14}C]uracil ($10\mu\text{Ci/ml}$). The peak c.p.m. in the sodium dodecyl sulphate fraction was 1992. —, E_{260} ; ----, radioactivity.

taminants that are not adsorbed to the column. As with RNA from metazoan cells, the tRNA precedes, and is clearly separated from the rRNA, whereas the ribosomal species are not separated from each other. Just after the rRNA an area of slightly higher specific radioactivity that probably represents ribosomal precursor RNA is eluted. A sharp peak with high specific radioactivity results from subsequent elution with sodium dodecyl sulphate.

To estimate the amount of contamination of the sodium dodecyl sulphate fraction with rRNA, cells were labelled for 3h with [^3H]uracil, and RNA extracted from purified (sedimented) ribosomes was chromatographed on MAK columns. The results of this experiment (Fig. 2) show that the bulk of the labelled rRNA is eluted by the salt gradient, with only about 5% of the label being eluted by sodium dodecyl sulphate.

The molecular-weight distribution of pulse-labelled RNA in the sodium dodecyl sulphate fraction was determined by zonal centrifugation on sucrose gradients (Fig. 3). The sucrose profile of the radioactivity from the sodium dodecyl sulphate fraction shows a broad peak, with most of the radioactivity sedimenting slightly slower than the 18S rRNA. The relatively low E_{260} profile shows the characteristic 28S and 18S rRNA peaks with essentially no correspondence between the E_{260} and radioactivity profiles.

To further identify the sodium dodecyl sulphate fraction with mRNA, the chromatographic behaviour of pulse-labelled yeast polyribosomal RNA was investigated. Polyribosomal RNA was extracted from yeast protoplasts as detailed in the Materials and Methods section. Fig. 4 shows the sucrose profile of polyribosomal RNA from proto-

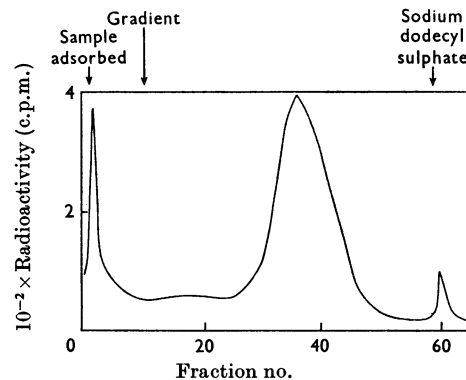


Fig. 2. MAK column chromatogram of long-term labelled rRNA extracted from sedimented ribosomes. Cells were incubated for 3h with [^3H]uracil and RNA was extracted from the ribosomal pellet.

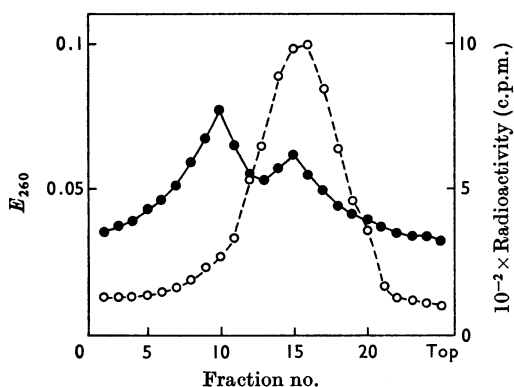


Fig. 3. Sucrose-density-gradient sedimentation profile of RNA eluted from a MAK column with sodium dodecyl sulphate. Yeast cells were labelled for 5 min with [^{14}C]uracil and the RNA was chromatographed on an MAK column. The sodium dodecyl sulphate fractions were collected, dialysed against water and freeze-dried. After dissolving in a small volume of water, the samples were layered on a 5–25% (w/v) sucrose gradient prepared in buffer containing 0.1M-potassium acetate–0.1M-EDTA, pH5.9. Centrifugation was carried out for 4.5 h in the Spinco model L ultracentrifuge at 39000 rev./min with the SW39 rotor at 5°C. Three-drop fractions, collected from the bottom of the tube, were diluted to 2 ml for determination of E_{260} (●). Three-drop fractions from another tube were added to scintillant for the radioassay (○).

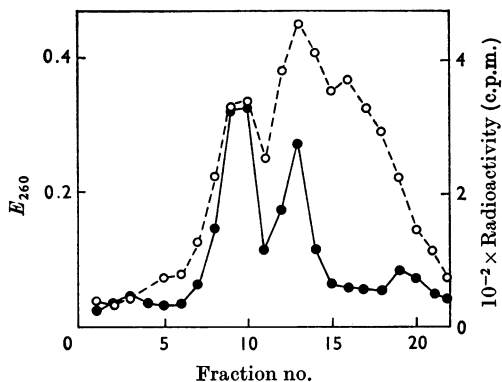


Fig. 4. Sucrose-density-gradient sedimentation profile of rapidly labelled polyribosomal RNA from yeast protoplasts. Centrifugation and collection of the fractions was carried out as described in Fig. 3. ●, E_{260} ; ○, radioactivity.

plasts that were pulsed with [^3H]uracil for 10 min. Although a significant amount of label was incorporated into rRNA during the 10 min pulse, the characteristic high specific radioactivity peak of mRNA may be seen in fractions 14–18 (Fig. 4). These

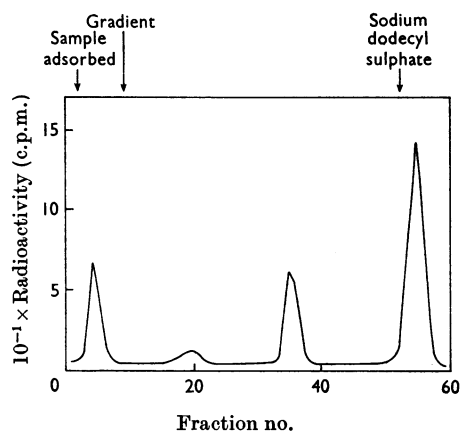


Fig. 5. MAK column chromatogram of rapidly labelled polyribosomal RNA taken from fractions 14–18 of the sucrose gradient shown in Fig. 4. These fractions were combined, dialysed against 0.2M-NaCl buffer, and adsorbed on the MAK column.

fractions were then combined and chromatographed on an MAK column (Fig. 5). The elution profile shows that a large portion (about 75%) of the radioactive polyribosomal mRNA is, in fact, tenaciously bound to the column, and is subsequently eluted by sodium dodecyl sulphate. Although Fig. 5 also shows salt-eluted peaks of tRNA and rRNA, this would be expected since the sucrose fractions that were pooled also contained some of these RNA species.

Although the MAK column chromatograms clearly show that rRNA is quantitatively eluted by the salt gradient, it is not readily apparent where the ribosomal precursor RNA is eluted. When RNA from higher organisms is eluted from MAK columns with a salt gradient such as the one used here, the ribosomal precursor is eluted slightly after the rRNA (see, e.g., Ellem, 1966). Since yeast ribosomal precursor is also rapidly labelled, the principal concern in this investigation was the extent to which the sodium dodecyl sulphate fraction might be contaminated with this RNA species. Since mRNA is not methylated (Moore, 1966), pulsing the cells with methyl-labelled methionine should result in the labelling of all RNA species except mRNA and 5S RNA. The MAK column chromatogram of RNA from yeast cells that were pulsed with [$Me\text{-}^3\text{H}$]methionine is shown in Fig. 6. This chromatogram shows that essentially all of the methyl-labelled RNA is eluted by the salt gradient. The unusually high amount of radioactivity in the tRNA region could be due to the presence of labelled methionine-charged tRNA. The small

radioactivity peak trailing the rRNA probably represents ribosomal precursor RNA.

To further characterize the sodium dodecyl sulphate fraction, the labelled nucleotide composition of this RNA was determined. RNA from cells that were labelled for 20 min with [^{32}P]-phosphate was adsorbed on an MAK column and elution was carried out as usual. The radioactivity elution profile was similar to the profiles obtained when cells were pulsed with radioactive uracil. The labelled base composition of the peak salt-eluted fraction (rRNA) and of the peak sodium dodecyl sulphate-eluted fraction was determined. The results of this experiment, together with some base ratios determined by other workers are summarized in Table 1. This table shows that the composition

of the sodium dodecyl sulphate-eluted RNA closely resembles the composition of yeast DNA and pulse-labelled RNA from yeast polyribosomes. Moreover, the nucleotide composition of the rRNA based on this isotopic determination closely resembles the composition determined by direct methods.

In the labelling kinetics experiments the simplified elution procedure, described in the Materials and Methods section, was followed. The kinetics of [^{14}C]uracil incorporation into TD-RNA was used to determine the rate of synthesis of this species during normal growth conditions. Fig. 7 shows the

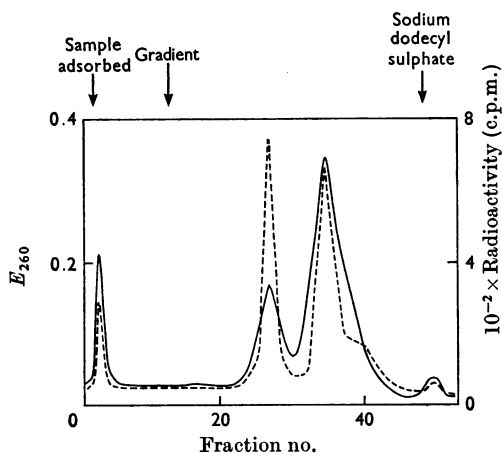


Fig. 6. MAK column chromatogram of RNA from cells that were pulsed for 5 min with [$M\epsilon$ - ^3H]methionine (1.0 $\mu\text{Ci/ml}$). —, E_{260} ; ----, radioactivity.

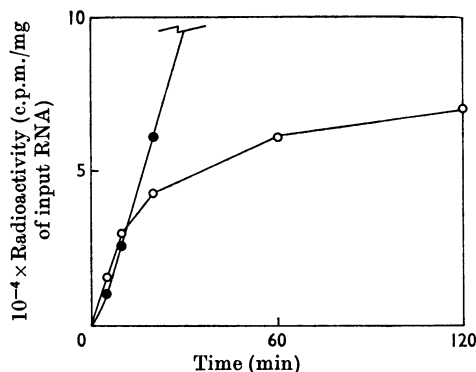


Fig. 7. Kinetics of [^{14}C]uracil incorporation into MAK column RNA fractions during normal growth conditions. At zero time [^{14}C]uracil was added (0.1 $\mu\text{Ci/ml}$). Unlabelled uracil was present at 5 $\mu\text{g/ml}$. The ordinate values represent the total radioactivity in the given fraction as detailed in the Materials and Methods section. The 60 and 120 min values for the salt-eluted RNA were found to lie on the near-straight line established by the earlier points. ●, Salt-eluted; ○, sodium dodecyl sulphate-eluted.

Table 1. Nucleotide composition of yeast RNA eluted from an MAK column in comparison with determinations made by other workers

See the Materials and Methods section for details of the base-ratio determination.

Source of nucleic acid	Nucleotide composition (mol/100 mol)			
	A	T (U)	G	C
This experiment:				
Peak salt-eluted fraction (rRNA)	25.6	28.4	28.2	18.0
Peak sodium dodecyl sulphate-eluted fraction (TD-RNA)	31.0	32.6	18.5	18.4
Yeast rRNA*	26.4	28.4	28.2	17.0
Yeast DNA†	31.5	33.7	17.6	17.2
Pulse-labelled polyribosomal mRNA from yeast protoplasts‡	31.0	32.0	16.0	21.0

* Schweizer, Mackechnie & Halvorson (1969).

† Fukahara (1967).

‡ Hartlief & Koningsberger (1968).

distribution of [^{14}C]uracil in the stable (salt-eluted) RNA and TD-RNA at increasing incorporation times. The incorporation of isotope into the stable RNA is almost linear throughout the time-course of the incorporation. This would be expected if the lag due to dilution of precursor pools was negligible. On the other hand, the incorporation into TD-RNA, although rapid initially, declines considerably over the 2h labelling period. A curve of this type would be expected to result from incorporation into a rapidly turning-over molecular species such as mRNA. If it is assumed that this fraction is nearly saturation-labelled at 2h, the half-life of this species can be graphically estimated to be approximately 20 min.

A delay in the incorporation due to the presence of a large intracellular precursor pool would significantly change the above estimate. The incorporation into the salt-eluted fraction was nearly linear even after brief labelling periods. However, since this fraction contains both tRNA and rRNA, rapid initial labelling of tRNA (or the terminal adenosine of tRNA) might compensate for a lag in the labelling of rRNA, yielding what appears to be linear incorporation. To investigate this possibility, cells were incubated with [^{14}C]uracil and samples were taken from the culture over a 1h period. RNA was extracted, and the 28S, 18S, and 4-5S species were separated by sucrose-density-gradient centrifugation. The relative specific radioactivity of each species was determined for each labelling period. The results of this experiment (Fig. 8) show that although the tRNA is labelled slightly before the rRNA, the labelling of both species becomes linear after 10 min. A delay of this order would not significantly affect the interpretation of Fig. 7.

To investigate the decay kinetics of the TD-RNA, 'chase' experiments were carried out under several different physiological conditions. Fig. 9

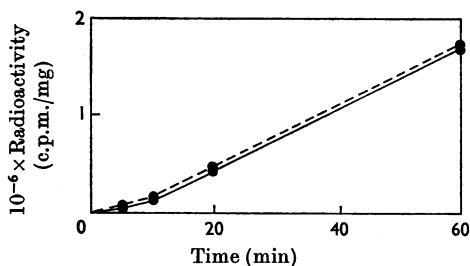


Fig. 8. Kinetics of incorporation of [^{14}C]uracil into rRNA and tRNA, which were separated by zonal centrifugation on a sucrose gradient. Incorporation of [^{14}C]uracil was carried out as described in Fig. 7. Centrifugation was carried out as described in Fig. 3. The peak 18S and 28S rRNA fractions were combined to obtain the values shown for rRNA. ●—●, rRNA; ●---●, tRNA.

shows the results of such an experiment carried out under normal growth conditions. The radioactivity is rapidly 'chased' out of the TD-RNA with complex, but nearly first-order decay kinetics: a half-life of about 15 min can be estimated from this curve. The specific radioactivity of the salt-eluted fraction shows a slow near-linear decline over the 2h period as would be expected for a stable radioactive species that is constantly being diluted with non-radioactive material.

In yeast and many higher organisms the antibiotic cycloheximide has been shown to inhibit specifically protein synthesis, apparently by blocking the transfer of amino acids from aminoacyl-tRNA to growing polypeptide chains (Lin, Mostreller & Hardesty, 1966; de Kloet, 1966; Siegal & Sisler, 1964). In yeast protoplasts cycloheximide inhibits polyribosome degradation, but allows continued RNA synthesis (de Kloet, 1966; Hartwell & McLaughlin, 1969). Accordingly, the disappearance of radioactivity from the TD-RNA fraction was investigated during inhibition of protein synthesis by cycloheximide. Fig. 10 shows that the 'chase' of label from the TD-RNA is almost completely inhibited in the presence of cycloheximide. The minor decrease in radioactivity could be accounted for by continued synthesis and utilization of unlabelled precursors: this would apply

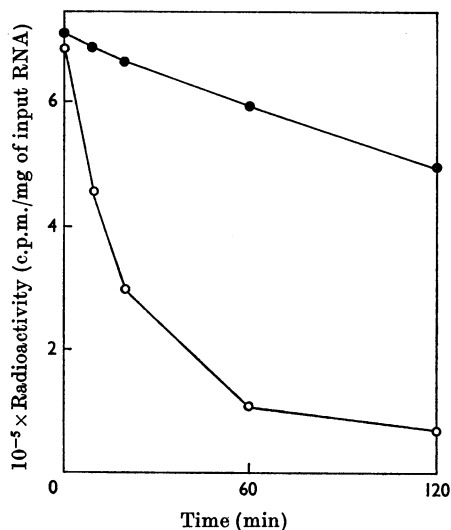


Fig. 9. 'Chase' of [^{14}C]uracil from MAK column RNA fractions under normal growth conditions. Cells were incubated for 15 min with [^{14}C]uracil ($0.2 \mu\text{Ci/ml}$ and $0.7 \mu\text{g/ml}$) and at zero time unlabelled uracil was added ($100 \mu\text{g/ml}$). The ordinate values represent the total radioactivity in the given fraction as detailed in the Materials and Methods section. ●, Salt-eluted; ○, sodium dodecyl sulphate-eluted.

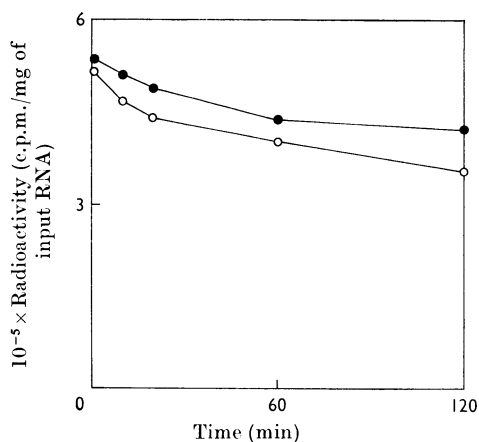


Fig. 10. 'Chase' of [^{14}C]uracil from MAK column RNA fractions in the presence of $25\ \mu\text{g}$ of cycloheximide/ml. Except for the addition of cycloheximide at zero time, the experiment was carried out under the conditions described for Fig. 9. ●, Salt-eluted; ○, sodium dodecyl sulphate-eluted. Separate incorporation experiments showed that protein synthesis was completely and abruptly inhibited by cycloheximide at this concentration, whereas RNA synthesis continued at normal rates for 1 h.

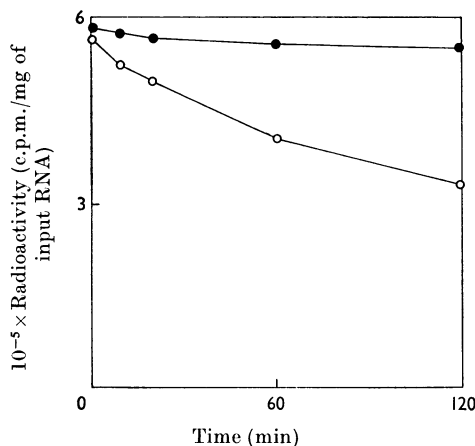


Fig. 11. 'Chase' of [^{14}C]uracil from MAK column RNA fractions during glucose starvation. At zero time the cells were transferred to glucose-deficient media. Except for the absence of glucose in the media, this experiment was carried out under the conditions described in Fig. 9. ●, Salt-eluted; ○, sodium dodecyl sulphate-eluted. Separate isotope incorporation experiments showed that both protein synthesis and RNA synthesis were completely and abruptly inhibited in the absence of glucose.

to both the salt-eluted and the TD-RNA. Based on this experiment it appears that the breakdown of the TD-RNA is dependent on continued protein synthesis.

To test further the relationship between the TD-RNA decay and the rate of protein synthesis, a 'chase' experiment was carried out during general metabolic inhibition produced by starvation for a carbon source (glucose in this case). Fig. 11 shows that the breakdown of the labelled TD-RNA is significantly retarded in comparison with the rapid breakdown observed under normal growth conditions (Fig. 9). The breakdown is not, however, inhibited as completely as it is in the presence of cycloheximide.

DISCUSSION

Since it was apparent from the initial column chromatograms of briefly labelled RNA that the bulk of the rapidly labelled fraction was not eluted by the salt gradient, no special attempt was made to further resolve the salt-eluted species. When RNA from metazoan cells is fractionated on MAK columns, a minor portion of the rapidly labelled RNA (the Q_2 RNA) is eluted by the salt gradient although it is only partially resolved from the ribosomal and ribosomal precursor RNA (Ellem, 1966; Yoshikawa-Fukada, Fukada & Kawade, 1965). In a study of rapidly labelled rat liver RNA

(Kunz, Niessing, Schnieders & Sekeris, 1970), a preliminary note regarding the tenacious binding of rapidly labelled yeast RNA to MAK columns supports the present findings.

In contrast with the briefly labelled RNA, long-term labelled rRNA (from sedimented ribosomes) is quantitatively eluted by the salt gradient (Fig. 2). Minor contamination of the ribosomal pellet with mRNA could account for the small peak of radioactivity eluted by the sodium dodecyl sulphate in this case. Similarly, methyl-labelled RNA, presumably containing no mRNA, is quantitatively eluted by the salt gradient (Fig. 6).

The yeast TD-RNA was shown to have several characteristics of mRNA. The sucrose-density-gradient profile of briefly labelled TD-RNA appears as a broad 12S–20S peak (Fig. 3) similar to the profile of pulse-labelled polyribosomal mRNA (Fig. 4). When such polyribosomal mRNA is chromatographed separately, the major portion of it is tenaciously bound to the column (Fig. 5). Although pulse-labelled RNA from yeast polyribosomes has been previously characterized as a polydisperse 12S–23S fraction (Hartlieb & Koningsberger, 1968) the same workers found that the β -galactosidase mRNA had a sedimentation coefficient of about 28S.

The yeast TD-RNA was further characterized as mRNA by its close similarity in nucleotide

composition to yeast DNA and to pulse-labelled polyribosomal mRNA from yeast protoplasts. In contrast, isotopic nucleotide analysis of total yeast RNA from cells that were pulsed with ^{32}P for 5 min shows that this RNA contains only 40% that resembles DNA whereas 60% resembles rRNA (Kitazume & Ycas, 1963).

One of the important advantages of this MAK column fractionation procedure over the isolation of polyribosomal mRNA from yeast protoplasts is that the turnover of mRNA can be investigated under normal physiological conditions. Thus, from the kinetics of [^{14}C]uracil incorporation, and the 'chase' of label from the TD-RNA, a half-life of 15–20 min was estimated (Figs. 7 and 9). Fig. 9 further shows that even after brief labelling periods a large fraction of the label is in stable RNA. This has also been shown to be the case in *Escherichia coli*, where mRNA is synthesized at half the rate of stable RNA (Salser, Janin & Levinthal, 1968).

The decay of labelled TD-RNA was also studied during inhibition of protein synthesis both by cycloheximide, and by glucose starvation (Figs. 10 and 11). In both cases the decay of this fraction was significantly retarded. The results are in general agreement with studies of the decay of bacterial mRNA. When protein synthesis is inhibited either by chloramphenicol, or amino acid starvation in *E. coli*, the mean life of mRNA is considerably increased (Forchhammer & Kjeldgaard, 1967). When energy metabolism is blocked by anaerobiosis in *Bacillus subtilis*, the decay of rapidly labelled RNA, as well as the decay of β -galactosidase messenger is significantly retarded (Fan, Higa & Levinthal, 1964; Nakada & Fan, 1964). The decreased breakdown of messenger during the absence of protein synthesis apparently results from some protection given to the messenger by the intact polyribosome complex (Fan *et al.* 1964; Nakada & Fan, 1964; Gilbert, 1963).

In most studies involving the characterization of mRNA, or estimates of its turnover rate, interpretive difficulties have resulted from an inadequate chemical or physical separation of the total mRNA from stable RNA. On the basis of the results of this paper, and other studies involving meta-zoan nucleic acids, MAK column chromatography appears to be one of the more effective means of separating mRNA from other nucleic acid species.

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