SEPARATION OF RAPIDLY LABELED RNA OF ANIMAL CELLS INTO DNA-TYPE AND RIBOSOMAL RNA-TYPE COMPONENTS

M. Yoshikawa, T. Fukada and Y. Kawade

Institute for Virus Research, Kyoto University, Kyoto, Japan

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The validity of the messenger RNA hypothesis (1) has been well supported by evidence from a variety of experiments in bacterial systems (2-5). The messenger RNA is in general distinguished from other cellular RNA's by its high turnover rate and a base composition similar to DNA. In animal cells, however, the characteristics of messenger RNA have not been so clearly defined, although metabolically active RNA which is rapidly labeled by radioactive precursors has been detected and studied in various animal cells (6-12). The results of Scherrer et al. (11, 12) appear to be especially significant in that they obtained the rapidly labeled RNA of growing HeLa cells in the form of two molecular species with sedimentation constants of 45 S and 35 S as estimated by sucrose density gradient centrifugation. Both of these were found to have a base composition similar to ribosomal RNA (rRNA), and were shown to be essentially composed of rRNA precursor by "chase" experiments, although a small amount of metabolically unstable fraction was also detected in them. From our studies on the rapidly labeled RNA of growing FL cells (an established cell line derived from human amnion), we can now demonstrate its separation into two components by using the methylated albumin column (13), one having a base composition similar to DNA and the other similar to rRNA. Thus, the present techniques provide a means of isolating what is likely to be the messenger RNA of animal cells. Such a DNA-like RNA fraction has been found in some animal cells (14, 15), but it should be noted that in our study the RNA appeared to be of a very large size and might represent the undegraded state.

FL cells were grown in monolayers in 500 ml Roux bottles with 50 ml of a phosphate-free Eagle's medium (16) containing double strength amino acids and vitamins and 10% calf serum. Labeling of nucleic acids was accomplished by adding P^{32} of varying activities, depending on the length of the labeling period, together with 0.25 μM/ml each of the four nucleosides. After labeling, the cells were suspended in 0.14 M NaCl-0.01 M tris buffer at pH 8.1 containing 5×10^{-4} M EDTA. Nucleic acids were extracted by the sodium dodecyl sulfate (0.5%)-phenol method at 0-4°C. Under these conditions, 95% of RNA and 90% of DNA could be extracted, and no significant fractionation of labeled RNA such as described by Sibatani et al. (17, 14) seemed to take place.

The nucleic acid thus obtained was fractionated on a methylated albumin column according to Mandell and Hershey (13). The resolution of the two components of animal cell rRNA is poorer than in the case of bacterial rRNA (18-20), but could be much improved by keeping the column at 30-35°C (20). A typical chromatogram is shown in Fig. 1. Soluble RNA, DNA and rRNA were eluted at about 0.5, 0.75 and 0.95 M NaCl, respectively, and the rapidly labeled RNA appeared later than rRNA. With the 30 min. pulse-labeled material, two radioactive peaks were observed at about 1.05 and 1.15 M NaCl, which we call q_1 and q_2, respectively (Fig. 1). On longer labeling, the q_2 peak soon became small compared to q_1, and the q_1 peak shifted progressively toward rRNA. After 24 hours (the generation time of the cell) of labeling, the radioactivity almost paralleled the absorbance profile of rRNA.

Since the methylated albumin column separates RNA molecules mainly according to their size, q_1 and q_2 may be supposed to have larger sizes than rRNA. This was shown to be the case by sucrose density gradient centrifugation of RNA recovered from the chromatographic fractions. As seen in Fig. 2, two radioactive peaks were observed ahead of rRNA, which are most likely to correspond to the q_1 and q_2 RNA. Their sedimentation constants were estimated to be about 40 and 50 S, respectively.

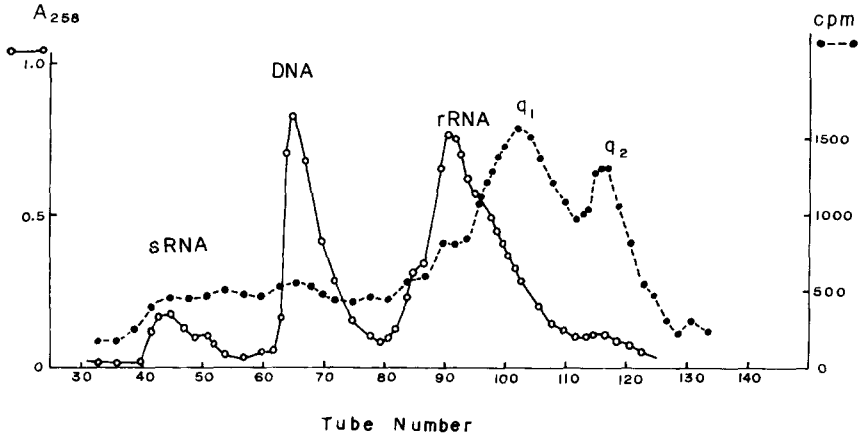

Fig. 1. Chromatographic analysis of nucleic acids of FL cells labeled by P^{32} (20 μC/ml medium) for 30 min. Chromatography was performed according to Mandell and Hershey (13) but at 30°C. Elution was done by a NaCl gradient containing 0.01 M phosphate buffer at pH 6.7 and 5×10^{-4} M EDTA, with a flow rate of 3 ml/tube/10 min.

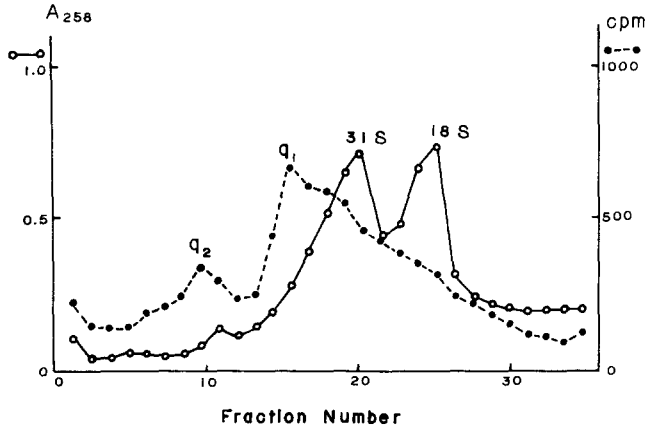

Fig. 2. Sucrose density gradient centrifugation of RNA labeled for 30 min. The labeled RNA in the Chromatographic fractions shown in Fig. 1 was collected by ethanol precipitation together with rRNA added as carrier, and, after phenol treatment (to remove contaminating methylated albumin released from the column), centrifuged on a linear sucrose gradient (5–20%) containing 0.1 M NaCl, 0.1 mM $MgCl_2$ and 0.01 M acetate buffer at pH 5.2, at 36,000 rpm for 210 min. in a Hitachi SW40 rotor.

The base compositions of various RNA were determined by P^{32} counts incorporated into the four nucleotides. The results are given in Table 1. It can be seen that the rRNA has more guanine and cytosine than adenine and uracil, as is also found with other mammalian rRNA's (21). The DNA in contrast is rich in adenine and thymine. The composition of q_1 was found to agree fairly well with that of rRNA. q_2 has a very different base composi-

Table 1. The base composition of FL cell RNA and DNA (mole %).

	Adenine	Uracil (Thymine)	Guanine	Cytosine
rRNA (18 S)	19.4	19.4	34.1	27.5
rRNA (31 S)	17.9	18.8	37.9	28.1
q_1 RNA, Expt. 1	17.5	22.9	31.4	28.4
" 2	17.8	22.7	31.7	28.1
q_2 RNA, Expt. 1	30.0	30.6	20.3	19.6
" 2	29.7	30.3	19.9	19.4
DNA	27.7	30.5	20.5	21.3

rRNA: P^{32} was given to FL cells for 24 hours and the RNA extracted and fractionated by the methylated albumin column. The two rRNA components were then separated by zone centrifugation on a sucrose gradient, collected, and hydrolyzed with 0.3 N KOH at 37°C for 18 hours. The resulting nucleotides were separated on a Dowex 1 (formate) column (Osawa, S., Takata, K. and Hotta, Y., Biochim. Biophys. Acta 28, 271 (1958)), and the radioactivity in the four nucleotide fractions was determined. The average of three determinations on a single sample is given.

q_1 and q_2 RNA: RNA from 30 min. labeled cells was fractionated as in Fig. 1, several fractions centering around the q_1 and q_2 peaks were pooled separately, and the RNA was collected by ethanol precipitation. To reduce cross contamination, each was further subjected to sucrose density gradient centrifugation, and the material in the region corresponding to q_1 and q_2 of Fig. 2 was recovered (1/2-2/3 of the total radioactivity recovered). The RNA was then analyzed as above with cold carrier RNA added. The results in two independent experiments are given.

DNA: DNA obtained from the column chromatography was purified by RNase treatment, then hydrolyzed with 95% formic acid at 175°C (sealed tube) for 30 min. The resulting bases were then separated by paper chromatography and estimated by ultraviolet absorption. The average of three determinations on one sample is given.

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tion which is strikingly similar to DNA. The q_1 and q_2 RNA's must therefore be of entirely different nature.

The rapidly labeled RNA was thus shown to be separated into two components, q_1 and q_2, with the size and base composition distinct from each other. The "chase" experiments done in the presence of Actinomycin D also indicated marked difference between the two, q_2 being metabolically much more unstable. An obvious working hypothesis is that q_1 is the precursor of rRNA and q_2 the messenger RNA of these cells. Our results are in harmony

with those of Scherrer et al. (12), if their 45 S RNA is assumed to correspond to our q_2 grossly contaminated by q_1. Metabolic and other studies of these RNA's now in progress will be reported in detail elsewhere.

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