TENACIOUS BINDING OF THE BULK OF THE DNA-LIKE RNA OF METAZOAN CELLS TO METHYLATED ALBUMIN COLUMNS[1]

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Chromatography on columns of methylated bovine serum albumin adsorbed on Kieselguhr (MAK) is proving very useful as a tool to fractionate nucleic acids. The separation of the broad classes of RNA and DNA is clear, and recovery of undenatured nucleic acids is virtually quantitative [Philipson (1961); Kubinski, et al. (1962)]. Two rapidly labelled RNA fractions (q_1 and q_2) have been resolved from the ribosomal RNA of FL cells [Yoshikawa, et al. (1964)], and the claim has been made that one of them (q_2) represents the messenger RNA of these cells.

We had simultaneously made observations of the rapidly labelled nucleic acids of the E929 strain of L-cells. Elution of the nucleic acids by a concentration gradient of sodium chloride from the MAK yielded results which so closely paralleled those of the Japanese workers as to make their detailed description redundant. However, one of our observations has not been made and seems of considerable importance. It was found that a large part of the rapidly synthesized RNA is

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bound to the MAK too tenaciously to be eluted by the salt gradient. From its labelling characteristics and its composition, it seems to represent the bulk of the cell messenger-or DNA-like RNA.

The L-cells were grown in suspension. The cells had a doubling time of 30-34 hours in Eagle's (1959) modified medium containing 10% calf serum. They were labelled by the addition of P^{32} (as carrier free orthophosphate) at approximately 100 μc/ml. Incubation was terminated by centrifugation at 0°C for 5 minutes at 3000 r.p.m. The cell pellet was lysed by a few minutes shaking in a 2% solution of sodium lauryl sulphate in 0.14M NaCl-0.05M Tris, pH 7.3, containing 10^{-3}M EDTA. The nucleic acids were deproteinized by two shakings at room temperature (20 minutes and 10 minutes) with water saturated phenol. After precipitation of the nucleic acids with 2 volumes of ethanol at -10°C, the pellets were washed with ethanol to remove the residual phenol.

The nucleic acids were then fractionated on MAK columns fashioned after Monier et al. (1962), at 35°C (Kubinski et al. 1962) with a linear gradient of NaCl. Of the RNA which was eluted by NaCl, the most rapidly labelled components corresponded to the q_1 and q_2 peaks of Yoshikawa et al., (1964). Equilibration with isotopic precursor took a little over an hour for q_1, and about 40 minutes for q_2. The nucleotide composition of these two fractions is seen in Table I. Q_1 was ribosomal in type, with a high (59.9%) guanylic + cytidylic content, while q_2 had nearly equal amounts of each nucleotide (G + C = 51.6%). This agrees with the findings of Yoshikawa et al.

However, even after perfusion with 2M NaCl, a large amount of isotope was retained by the column. It could be recovered by elution with 1.5N NH_4OH or by raising the temperature of the column to 90°C

while perfusing it with 1.5M NaCl.* More than 95% of the thermally eluted isotope was precipitated by 10% TCA following the addition of unlabelled ribosomal RNA as carrier. After hydrolysis in 0.3N KOH at 37°C for 20 hours, the P^{32} was quantitatively recovered with the nucleotides of the carrier RNA, separated on Dowex-1-formate (e.g. Hayashi and Spiegelman, 1961). It is therefore, identified as being in RNA (T-RNA). The nucleotide composition of the T-RNA was determined from the distribution of P^{32} among the ribonucleotides. It is clear from Table I that T-RNA resembled DNA rather than ribosomal RNA in its compostion. There was no evidence of compositional heterogeneity among fractions of T-RNA obtained by elution at different temperature ranges.

Table II illustrates the fact that, after short periods of labelling, more P^{32} is incorporated into T-RNA than into the total salt elutable RNA of the cell. Owing to the small amounts of material recovered in these rapidly labelled fractions, the measurement of their specific activities is imprecise. It was clear, however, that T-RNA had a high specific activity, being about 30 times greater than that of ribosomal RNA with periods less than or equal to 30 minutes of labelling, but somewhat less than one half that of the most rapidly labelled fraction (q_2).

RNA with similar properties has been found in chick embryo cells grown in monolayers (Clancy and Ellem, unpublished), and HeLa cells grown in suspension or on glass.

* It is important that the fines be rigorously removed from the Kieselguhr used to make up the column, otherwise the hot saline solution dissolves siliceous matter and produces a high level of ultra-violet absorbing material.

Table I

Nucleotide Composition[a] of L-cell RNA Fractions Relative to Adenylic Acid as 10.0

Nucleic Acid	Uridylic (or Thymidylic)	Cytidylic	Guanylic	G + C%	No. of Analyses
ribosomal RNA[b]	11.7 ± 0.4[f]	16.6 ± 0.3	17.5 ± 0.4	61.1	(6)
q_1 RNA[c]	10.9	14.5	16.7	59.9	(2)
q_2 RNA[c]	11.9	11.5	11.9	51.6	(2)
50-60° RNA[d]	11.2	10.5	12.0	51.5	(2)
60-70° RNA	12.3	9.5	10.3	47.0	(3)
70-80° RNA	11.5	10.7	10.5	49.6	(1)
DNA[e]	9.3	6.9	6.8	42-44	

a) Analyses were performed by Dowex-1-formate ion exchange chromatography as in eg. Hayashi and Spiegelman (1961). The composition of all RNA samples was based on the distribution of P^{32} amongst the nucleotides of their alkaline hydrolysate in the presence of carrier ribosomal RNA from Ehrlich ascites tumour cells. The composition of the carrier RNA was found to be A = 10.0, U = 11.5 ± 0.4, C = 16.3 ± 0.2, G = 18.1 ± 0.3 from 14 analyses, based on the optical density of the recovered nucleotides.

b) Time of exposure of cells to P^{32} did not influence composition of ribosomal RNA and figures are means of RNA labelled for 1 and 6 hrs.

c) q_1 and q_2 RNA obtained by column chromatography of nucleic acids labelled for 40 minutes since they were still well resolved after this length of time. After 45 minutes labelling, the amount of isotope entering ribosomal RNA makes it difficult to discriminate q_2 and especially q_1 from it.

d) T-RNA samples labelled for 3 hours and 6 hours. No significant differences in composition were found either between cells labelled for different times less than 6 hours, or between fractions of T-RNA eluted over different ranges above 50°C.

e) DNA base analysis of mouse ascites tumour from Colter et al. (1962). The G + C% figures are the range found by Sueoka (1961) and Schildkraut et al. (1962).

f) ± Standard Error.

This T-RNA represented a small fraction of the cell RNA (approximately 10% of the optical density of the total RNA recovered from the columns with L-cells and HeLa cells). Since it was rapidly labelled and resembled DNA in its composition, the most reasonable hypothesis concerning its nature is that it represents the main bulk of the messenger or DNA-like RNA of the metazoan cell.

Table II

Amount of Isotope Incorporated and Specific Activity of the Rapidly Labelled RNA Fractions of L-cells During Short Exposures to P^{32}.

Labelling Time	Isotope incorporated (as total cps.) into: DNA		RNA	q_1RNA	q_2RNA	Total salt eluted RNA	T-RNA[d]
15 (mins.)	22.9[a]	(1.9)[b]	4.7 (0.4)	9.1[c] --	23.0 (37)	36.8(2.9)	30.9(17)
30 (mins.)	102	(8.5)	19.6 (1.6)	59.1 --	69.1(100)	148 (14)	235 (43)
45 (mins.)	410	(34)	265 (21)	106 --	72.6(181)	443 (29)	826 (82)

a) Total c.p.s. in pooled fractions; corrections for actual and expected recovery from the columns have been made.
b) Specific activity expressed as c.p.s. ÷ Total optical density of fraction.
c) Owing to superimposition of the radioactivity of this fraction with the descending limb of the optical density of the ribosomal RNA, no estimate of the actual amount of this fraction can be made, and hence its specific activity cannot be estimated.
d) RNA eluted from the MAK columns after the saline gradient has terminated at 1.5M NaCl, by heating to 90°C--see text.

It is interesting to recall that DNA which has been rendered single-stranded during the process of incorporation into transformed bacteria (Lacks, 1962), also binds tenaciously to MAK columns. Hershey et al. (1963) also found that partial denaturation of the double helices by stirring solutions slower than at shearing speeds, caused the DNA to bind to MAK so as to resist salt elution. We have found that melting DNA in 0.3M NaCl-0.03M Na citrate at 100°C for 15 minutes and rapid chilling resulted in its quantitative binding to MAK so as to resist salt elution. The single stranded DNA could be eluted by either alkaline washing or hot 1.5M NaCl. These parallels between the bonding strength of single stranded DNA and DNA-like T-RNA may imply similarities in their structure differentiating them from ribosomal RNA.

Preliminary rechromatography of T-RNA suggests that it has undergone some change as a result of the heating. If reapplied to a fresh column in 0.4M NaCl, more than 90% of it is retained. Most

of this is eluted over a broad range of salt concentration by a salt gradient to 1.5M NaCl. Only about 30 to 40% is retained tenaciously by the MAK. Work is in progress to clarify this and to examine the T-RNA for other characteristics of messenger RNA.

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