

The Distribution of Deoxyribonucleic Acid-Like Ribonucleic Acid in Rat Liver Cells

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The fractionation of total cellular RNA on MAK* columns has proved a useful method of separating D-RNA from r-RNA and t-RNA (Ellem, 1966; Ellem & Rhodes, 1969). The D-RNA can be subdivided into two groups: one eluted towards the end of a NaCl concentration gradient (called Q2 RNA) and the other tenaciously bound to the column after salt elution (called TD-RNA). The latter can be eluted with sodium dodecyl sulphate solution at 35° and then 70°. The function, the relationship to each other and the distribution within the cell of these species of D-RNA are not known. Q2 RNA is known to have a sedimentation coefficient of about 50s (Yoshikawa-Fukada, Fukada & Kawade, 1965), which is much higher than that of TD-RNA (16–18s) (Ellem, 1966). Both of these species are rapidly labelled, and because of their properties it has been suggested that one or both could be m-RNA.

Harris (1964) has suggested that much of the rapidly labelled nuclear RNA is not transferred to the cytoplasm. This has been supported by Shearer & McCarthy (1967) and by Drews, Brawerman & Morris (1968), who showed by DNA-RNA hybridization that the RNA restricted to the nucleus was D-RNA. Drews *et al.* (1968) showed that the cytoplasm of normal liver contains less hybridizable RNA than the nucleus, but that the cytoplasm of neoplastic liver contains as many hybridizable species of RNA as the nucleus. These experiments suggest that more D-RNA is transferred to the cytoplasm in neoplastic liver than in normal liver. To investigate exactly what proportion of D-RNA was being transported to the cytoplasm we have fractionated both nuclear and cytoplasmic RNA from normal liver on MAK columns.

While these experiments were in progress a paper by Miller, Dhont, Vreese & Van Nimmen (1968) has shown that in HeLa cells both Q2 RNA and TD-RNA are transferred into the cytoplasm. In this communication we have only shown the distribution of TD-RNA.

Female albino rats (3 weeks old) of the Wistar strain were injected intravenously at various times with 5 μ C of [5-³H]orotic acid (specific radioactivity

* Abbreviations: MAK, kieselguhr coated with methylated serum albumin; r-RNA, ribosomal RNA; m-RNA, messenger RNA; D-RNA, DNA-like RNA.

6 C/m-mole). They were killed at $\frac{1}{2}$, 2 and 18 hr. after the injection. The liver was removed and immediately placed into cold 0.14 M-NaCl. After thorough washing in cold saline about 0.2 g. was taken and homogenized in 20 ml. of cold 0.14 M-NaCl. Pure nuclei were prepared by the method of Georgiev & Mantieva (1960), which offers a rapid method of obtaining nuclear RNA (Georgiev, 1967). An equal volume of freshly redistilled aq. 90% (w/v) phenol was added and the mixture was shaken at room temperature (approx. 20°) for 5 min. The resulting emulsion was centrifuged at 600g for 5 min. The supernatant aqueous layer contained the cytoplasmic RNA. The interphase layer contained the nuclear RNA, which after being washed was further extracted by the hot-phenol method of Warner, Soeiro, Birnboim, Girand & Darnell (1966) with the modification (Penman, 1968) that chloroform-3-methylbutan-1-ol (99:1, v/v) was used to increase the density of the lower layer.

The purity of the cytoplasmic RNA preparation (Georgiev & Mantieva, 1960) was checked by comparison with RNA prepared from cytoplasm that had been previously separated from nuclei and cell debris by centrifuging through iso-osmotic sucrose. Both preparations of cytoplasmic RNA gave the same results.

The RNA was precipitated with 2 vol. of ethanol at -20° and centrifuged at 1000g for 30 min.

The RNA was placed on an MAK column prepared by the method of Mandel & Hershey (1960) except that the middle layer was omitted. The RNA was first eluted at room temperature (approx. 20°) as one large fraction by means of a steep salt gradient from 0.6 M- to 1.5 M-NaCl. This was called the salt fraction, and it would contain t-RNA, r-RNA species and Q2 RNA. The TD-RNA was then eluted with 0.2% sodium dodecyl sulphate in 0.4 M-NaCl as two fractions, one at 37° and the other at 70°. These fractions, which represented TD-RNA, were pooled. Radioactivity was measured described by Billing, Barbiroli & Smellie (1968). More than 96% of the RNA was recovered from the column.

The results are presented in Table 1. Only a very small proportion of the labelled RNA enters the cytoplasm after a labelling time of 0.5 hr. After 2 hr. the amount entering the cytoplasm increases to

Table 1. Incorporation of [5-³H]orotic acid into rat liver RNA

Rats were killed at $\frac{1}{2}$, 2 or 18 hr. after an intravenous injection of $5 \mu\text{Ci}$ of [5-³H]orotic acid. Nuclear RNA and cytoplasmic RNA were prepared from a weighed amount of liver and were fractionated on MAK columns. The radioactivity in the RNA fractions was measured and the radioactivities of the total nuclear RNA and cytoplasmic RNA are expressed as percentages of the total acid-insoluble radioactivity in the tissue. The radioactivities in the TD-RNA fractions of both nuclear RNA and cytoplasmic RNA were also measured and are expressed as percentages of the total radioactivity in the nuclear RNA and cytoplasmic RNA respectively. Each result represents the mean of three separate experiments.

Time (hr.)	Total acid-insoluble radioactivity (d.p.m./g. wet wt.)	Radioactivity in nuclear RNA (% of total)	Radioactivity in cytoplasmic RNA (% of total)	Radioactivity of TD-RNA (%)		
				In total RNA	In nuclear RNA	In cytoplasmic RNA
$\frac{1}{2}$	113000	98	2	38	39	-
2	227000	74	26	21	28	0
18	326000	53	47	13	25	0

26% of the total cellular RNA and at 18 hr. it rises to 47%. This is the pattern expected from continued RNA synthesis with transport into the cytoplasm.

One of the most striking features of these results is the absence of labelled TD-RNA from the cytoplasm even 18 hr. after the administration of the radioactive precursor. This is not an artifact of the preparation of cytoplasm due to TD-RNA being precipitated with the interphase material, because the same results were obtained with cytoplasm isolated before RNA extraction. This result suggests that normal liver contains a species of D-RNA that is confined to the nucleus. This RNA must also be strongly bound to the chromatin material, because the method of preparation allows nucleoplasmic RNA to be extracted in the 'cytoplasmic' fraction, leaving behind only RNA bound to the chromatin (Georgiev & Mantieva, 1960). These observations suggest that this species of D-RNA is not m-RNA, which from current ideas of protein synthesis would be expected to be transported into the cytoplasm. The possibility cannot be excluded, however, that TD-RNA is present in the cytoplasm in another form that is eluted from the MAK column in the NaCl fraction.

Our results also show that the increased radioactivity in total cellular RNA with time is due to incorporation into t-RNA and r-RNA species. The proportion of TD-RNA in the nuclear fractions falls sharply between 0.5 and 2 hr. and thereafter remains fairly constant. When expressed as a proportion of the total labelled RNA in the cell, however, the value falls continuously from 38% after 0.5 hr. to

21% after 2 hr. and to 13% after 18 hr. These results suggest that TD-RNA has a rapid turnover compared with the other major species of RNA in the cell.

In conclusion it appears that TD-RNA may represent either a mature species of RNA that has a function in the nucleus or a precursor of a species of cytoplasmic RNA that is eluted from MAK columns with salt.

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