Further studies on nuclear and cytoplasmic ribonucleic acids*

In previous reports[1,2], it was shown that nuclear ribonucleic acid (nRNA) of calf thymus could be fractionated into two classes differing in extractability and metabolic activity. Metabolic heterogeneity of nRNAs has been reported also from several laboratories[3–8]. The nucleotide composition of $nRNA_1$ (extractable with neutral phosphate buffer) is different from that of $nRNA_2$ (residual RNA), but is indistinguishable from that of microsomal RNA (MsRNA)[1,2]. Both $nRNA_1$ and MsRNA could be purified as ribonucleoproteins which are not distinguishable from each other with respect to the electrophoretic mobility and RNA content[1,2].

It is the purpose of this communication to describe further studies on the nRNAs and cytoplasmic RNAs in some tissues of the rabbit and rat with special reference to the relation of $nRNA_1$ to MsRNA.

First, the incorporation of inorganic ^{32}P *in vivo* into nuclear and cytoplasmic RNAs and deoxyribonucleic acid (DNA) of albino-rabbit appendix, thymus, and liver has been examined. It is seen from Fig. 1 that the overall uptake of ^{32}P by RNA and DNA is highest in appendix, intermediate in thymus, and lowest in liver. In appendix and thymus, $nRNA_2$ has the highest specific activity, while there is little difference between the activities of $nRNA_1$, MsRNA and DNA in these tissues. The activities of MsRNA and DNA in the liver are very low compared with those of appendix and thymus. From these observations, it may be concluded that no simple precursor–product relationship exists between $nRNA_1$ and MsRNA in the case of appendix and thymus tissues so far as RNA-phosphorus is concerned. As uptake of ^{32}P by DNA can safely be taken as a measure of the average rate of mitotic activity of the cell, the results rather suggest that a large part of the isotopic labelling in MsRNA as well as in $nRNA_1$ is the result of the mitotic doubling of each RNA, because the specific activities of MsRNA, $nRNA_1$ and DNA increase at nearly the same rate after the injection of the isotope. The tentative conclusion from these observations would be that upon mitosis, the bulk of the MsRNA is not derived from $nRNA_1$, but may be synthesized independently in the cytoplasm, although the possibility of $nRNA_2$ being the precursor of MsRNA after some structural changes cannot be excluded.

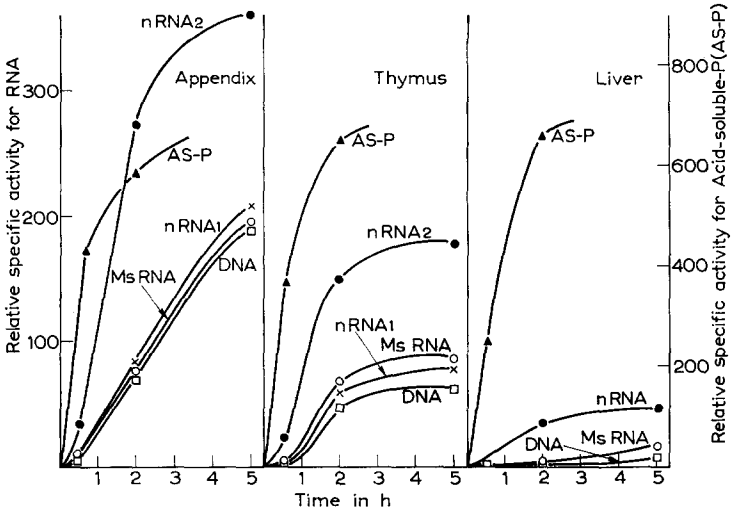

Fig. 1. Time course of ^{32}P uptake *in vivo* by RNAs of rabbit tissues. Rabbit weighing about 2 kg received 0.6 mC ^{32}P by intravenous injection. Animals were killed at time intervals indicated, and appendix, thymus and liver were taken out. Nuclear and cytoplasmic fractions were obtained as described previously[2,3] in the case of appendix and thymus. Citric acid nuclei were prepared in the case of liver for the preparation of nRNA. RNA and DNA were isolated according to the method of AMANO *et al.*[10] for the radioactivity measurements. Relative specific activity = $\frac{\text{counts/min/mg P}}{\text{total injected counts/min}} \cdot 10^6$.

* Supported by grants from The Rockefeller Foundation and for Fundamental Scientific Research of the Ministry of Education awarded to the "Cooperative Research on The Nucleic Acids".

Such negative data led us to examine whether the renewal of MsRNA in non-mitotic tissue, such as adult liver, was supported by a supply of $nRNA_1$ from the nucleus. The time course of incorporation of ^{32}P into different RNAs of adult-rat liver was followed. In this experiment, nuclei were isolated in 2.2 *M* sucrose following the method of CHAUVEAU *et al.*[11]. Here again, the uptake of ^{32}P by $nRNA_2$ (57% of nRNA in amount) is greater than uptakes in the other fractions. What is noticeable in this tissue is the fact that the rate of incorporation of ^{32}P into $nRNA_1$ (43% of nRNA) is much higer than that into MsRNA. However, analyses of nucleotide composition of $nRNA_1$ and MsRNA do not simply support the idea that $nRNA_1$ as such is the precursor of MsRNA in this tissue. In Table I are listed the nucleotide compositions of $nRNA_1$, $nRNA_2$ and MsRNA of rabbit appendix and rat liver. In the case of appendix, $nRNA_1$ and MsRNA have practically the same composition, whereas $nRNA_2$ is different from $nRNA_1$ and MsRNA. These observations are in good accord with those obtained on calf-thymus RNAs[1,2]. In the case of rat liver, however, the nucleotide composition of MsRNA is significantly different from that of $nRNA_1$, which is rather similar to that of $nRNA_2$. It is not known whether $nRNA_1$ in this tissue is a mixed population of more than one RNA, one of which might correspond to the $nRNA_1$ of mitotic tissues.

TABLE I

NUCLEOTIDE COMPOSITION OF NUCLEAR AND CYTOPLASMIC RNAS OF RABBIT APPENDIX AND RAT LIVER

The nucleotide composition was determined by Dowex-1-formate ion-exchange chromatography after the alkaline hydrolysis of each fraction as described previously[2].
Molar ratio, adenylic acid = 10.0

RNA	*Guanylic*	*Cytidylic*	*Uridylic*	*Purine / pyrimidine*
Rabbit appendix				
MsRNA	19.0	15.8	10.8	1.09
$nRNA_1$*	18.8	15.8	10.8	1.08
$nRNA_1$**	19.2	16.3	10.6	1.09
$nRNA_2$*	14.0	14.4	12.0	0.91
Rat liver				
MsRNA	19.0	14.9	10.6	1.14
$nRNA_1$**	15.6	13.7	12.8	0.97
$nRNA_1$***	15.8	11.4	12.7	1.07
$nRNA_2$**	13.7	13.6	11.4	0.95

* Prepared from the nuclei isolated in 0.25 *M* sucrose-0.004 *M* $CaCl_2$.
** Prepared from the nuclei isolated in 2.2 *M* sucrose according to the method of CHAUVEAU, MOULÉ, AND ROUILLER[11].
*** Prepared from the nuclei isolated in 2.2 *M* sucrose[11], using animals starved for 10 days, and refed protein-rich diet for 1 day.

Although the data reported here do not exclude a possibility that a part of MsRNA is derived from $nRNA_1$ (or $nRNA_2$), circumstantial evidence leads us to consider another role of nRNAs in nuclear metabolism rather than that of the precursor of cRNA. In this connection, it might be noted that there have been no conclusive experiments so far, to demonstrate the transfer of nRNA into cytoplasm. Even the well-known experiments by GOLDSTEIN AND PLAUT[9] on amoebae do not seem to us as decisive as it would appear, because the possibility that acid-soluble phosphorus compounds of implanted labelled nucleus are involved in the labelling of host-cytoplasmic RNA was not excluded.

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Received February 18th, 1957