Preliminary Notes

Some aspects of the relation between nuclear and cytoplasmic ribonucleic acids*

The hypothesis that nuclear ribonucleic acid (nRNA) is the precursor cytoplasmic RNA (cRNA)[1] is mainly based on the observations that isotopic precursors are incorporated at a much higher rate into nRNA than into any fraction of cRNA. BARNUM *et al.*[2,3], however, demonstrated in their tracer experiments that nRNA does not satisfy the theoretical requirements of the precursor of cRNA. Another objection to this hypothesis is derived from the differences in average nucleotide composition between nRNA and cRNA[4,5]. However, neither of these objections seems to be conclusive, since the heterogeneity of RNA might obscure the situation. Autoradiographic evidence recently presented by GOLDSTEIN AND PLAUT[6] using amoeba clearly demonstrated that at least a part of the cRNA is derived from the nucleus.

Evidence has been presented showing that in the calf thymus, there are at least two kinds of RNA in the nucleus differing in extractability[7,8] and metabolic activity. $nRNA_1$ is associated with a protein fraction (Fraction I) which can be readily extracted with neutral phosphate buffer from nuclei isolated in sucrose. $nRNA_2$ may be obtained from the residue of the phosphate extract by dissolving in 1 *M* NaCl, followed by high speed centrifugal sedimentation (Fraction II). The diffusable nature of Fraction I suggests the possibility that the $nRNA_1$ in this fraction might represent the precursor of cRNA[7]. This is further examined in the present communication.

Calf thymus nuclei and the nuclear protein fractions were prepared by the method described by ALLFREY *et al.*[7,8]. The cytoplasmic fraction was obtained from the tissue homogenate in a sucrose-$CaCl_2$ medium by centrifuging at 2,000 × *g* for 8 min. Microsomes were spun down at 90,000 × *g* for 60 min from the cytoplasmic fraction from which the mitochondrial fraction had previously been removed by centrifugation at 7,500 × *g* for 20 min. The acid-treated, lipid-free powder of each fraction was treated with 0.5 *N* KOH at 37° C for 20 h. After precipitation of proteins and DNA by acid, the supernatant was neutralized and the RNA mononucleotides were quantitatively separated on a Dowex-1-formate column, using a modified "formic acid system" of HURLBERT *et al.*[9]. The nucleotide composition of RNA's from different fractions of calf thymus tissue is given in Table I. It is clear that $nRNA_2$ is characterized by a higher uridylic, and a lower guanylic acid content as compared with that of cRNA. The point of interest however lies in the fact that $nRNA_1$ reveals practically the same nucleotide composition as the RNA of cytoplasm or microsomes.

Ribonucleoprotein isolated from Fraction I by means of streptomycin[10,**] and from microsomes are not distinguishable with respect to their electrophoretic mobility, and RNA content

TABLE I

NUCLEOTIDE COMPOSITION OF RNA'S FROM DIFFERENT NUCLEAR AND CYTOPLASMIC FRACTIONS OF CALF THYMUS TISSUE

(Molar ratio, adenylic = 10.0)

Fraction	*Guanylic*	*Cytidylic*	*Uridylic*	*Purine/pyrimidine*
Whole tissue	17.3	13.5	11.4	1.11
Sucrose nuclei	15.7	13.5	12.5	0.99
Fraction I (nucleus)	18.0	14.1	10.1	1.16
Fraction II (nucleus)	15.1	13.4	12.5	0.97
Cytoplasmic fraction	17.9	14.4	10.3	1.13
Microsomes	17.6	14.6	10.1	1.12

* 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". We wish to express our indebtedness to Dr. A. E. MIRSKY and Dr. V. G. ALLFREY for their kind suggestions and advice in this work.

** Kindly furnished through the courtesy of Dr. Y. KOTAKE of the Meiji Seika Kaisha Ltd., Tokyo.

(about 55% by weight). These observations together with the nucleotide analyses in Table I suggest that $nRNA_1$ in Fraction I is now on its way out into the cytoplasm as a *ribonucleoprotein*.

It is one of the requirements for the precursor-product relationship that, in the early phases of the reaction, the incorporation of isotope into the precursor RNA must occur at a higher rate

TABLE II

INCORPORATION OF ^{32}P *in vivo* INTO RNA'S OF DIFFERENT FRACTIONS OF CALF THYMUS 3 HOURS AFTER INTRAVENOUS INJECTION OF 15 mc ^{32}P

Fraction	*Specific activity (counts/min·mg RNA-P)*					
	Mixed nucleotides	*Mixed* nucleotides (calculated)*	*Adenylic*	*Guanylic*	*Cytidylic*	*Uridylic*
Fraction I (nucleus)	1360	1370	990	2700	685	960
Fraction II (nucleus)	3330	3500	3800	3260	3910	3100
Cytoplasmic fraction	850	880	866	905	710	1140
Microsomes	880		-		-	-

* Calculated from the specific activity of mononucleotides.

than into the product RNA. However, this rule is not applicable if the product RNA independently turns over. A preliminary experiment on the incorporation *in vivo* of ^{32}P into RNA's*** of calf thymus has been carried out. It is clearly seen from Table II that $nRNA_2$ is the most active site of ^{32}P incorporation among all fractions examined. It is also obvious that ^{32}P incorporates into $nRNA_1$ almost twice as actively as into cRNA. However, examination of the data on the incorporation into individual mononucleotides indicated that the situation is not at all simple. The higher specific activity of the mixed nucleotides of $nRNA_1$ over cRNA is not due simply to a higher activity of all mononucleotides, but to an unusually high specific activity of the guanylic acid. Several possible explanations may be given for the relation between $nRNA_1$ and cRNA. One is that, despite the apparent chemical similarities of these two RNA's, they are synthesized independently. It is also possible, however, that in connection with some cytoplasmic activities such as protein synthesis, cRNA turns over independently from $nRNA_1$, even if the latter serves as the sole precursor of cRNA. Further experiments on this matter are now in progress.

SYOZO OSAWA
KENZO TAKATA
YASUO HOTTA

Biological Institute, Faculty of Science, Nagoya University, Chikusa-Ku, Nagoya (Japan)

[1] R. JEENER AND D. SZAFARZ, *Arch. Biochem.*, 26 (1950) 54.
[2] C. P. BARNUM AND R. A. HUSEBY, *Arch. Biochem.*, 29 (1950) 7.
[3] C. P. BARNUM, R. A. HUSEBY AND H. BERMUND, *Cancer Research*, 13 (1953) 880.
[4] J. N. DAVIDSON AND R. M. S. SMELLIE, *Biochem. J.*, 52 (1952) 594, 599.
[5] D. ELSON, L. W. TRENT AND E. CHARGAFF, *Biochim. Biophys. Acta*, 17 (1955) 362.
[6] L. GOLDSTEIN AND W. PLAUT, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 874.
[7] V. G. ALLFREY, A. E. MIRSKY AND S. OSAWA, *Nature*, 176 (1955) 1042.
[8] V. G. ALLFREY, A. E. MIRSKY AND S. OSAWA, *J. Gen. Physiol.*, 40 (1957) 451.
[9] R. B. HURLBERT, H. SCHMITZ, A. F. BRUMM AND V. R. POTTER, *J. Biol. Chem.*, 209 (1954) 23.
[10] K. TAKATA AND S. OSAWA, *Biochim. Biophys. Acta*, 24 (1957) 207.

Received June 11th, 1957

*** Thanks are due to Dr. A. SIBATANI of the Yamaguchi Medical School who kindly informed us of his unpublished procedure for the isolation of RNA for radioactivity measurements.