NUCLEOTIDE COMPOSITION OF VARIOUS RIBOSOMES

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Recent research on ribosomes indicates that the RNA in the ribosomes, which represents about 60-80% of the total RNA in the cell, may carry the genetic information related to the sequences of the amino acids in soluble proteins to be synthesized in the particles. Thus, the nucleotide composition of these well-defined organelles may provide some information about coding. Furthermore, it would be more informative to obtain the nucleotide composition of ribosomes which synthesize only one kind of protein as in the case of ribosomes isolated from reticulocytes which synthesize mostly hemoglobin (Schweet, et al., 1958) (Borsook, et al., 1952). This report presents data on the nucleotide composition of the RNA in the ribosomes of pea seedling stems, rabbit reticulocytes, and reticulocytes of sheep of two hemoglobin types.

Methods. Rabbit reticulocytes were produced according to the method of Borsook et al., (1952). Sheep blood samples were obtained and the hemoglobin classified by electrophoretic mobility on starch (Shreffler, 1960). Sheep were injected subcutaneously at 2-day intervals for 6 days with a 5% phenylhydrazine solution at the rate of 0.4 g/100 lb body weight. The hematocrit dropped to 12% where it leveled off 10 to 11 days after commencing treatment. Injections were then resumed at 0.25 g/100 lb at 2-day intervals until the reticulocyte count reached 40% of the circulating red cells. A total of 1.6 g phenylhydrazine/100 lb were injected.

Sheep ribosomes (in solution C) and rabbit ribosomes (in solution A) prepared according to Ts'o and Vinograd (in press), and pea ribosomes (in

solution C) (Ts'o, et al., 1956) were hydrolyzed in 0.3N KOH for 18 hr at 37°C (Davidson and Smellie, 1952). After precipitation at pH 1 by perchloric acid the supernatant was adjusted to pH 3.5. Approximately 0.5 mg of RNA nucleotides contained in 0.4 ml was applied as a band 8 cm from one end of a strip of water-washed Whatman 3 mm paper, 56 x 7.6 cm, and nucleotide separation (Markham and Smith, 1952) (Davidson and Smellie, 1952) was carried out at 29 V/cm, 7°C and pH 3.5, 0.04M citrate buffer. The leading band, UMP, moved approximately 38 cm in 5 hours. Blank strips were run simultaneously for background.

Nucleotide regions and the corresponding blank regions were cut from the strips and were rolled in heavy aluminum foil, hung in 12 ml conical centrifuge tubes and centrifuged for 1 min at 2000 rpm in an International Clinical centrifuge. The cutouts were then saturated by adding water to the lower portion and recentrifuged with the lower portion oriented toward the top of the centrifuge tube. This process was repeated 3 times and the eluant made to 5 ml. Nucleotide recovery was 97% as determined by phosphorous analysis by the method of Allen (1940) modified for quantities of 0.2 to 2.5 μg (Markham, 1955).

Procedures for extraction of RNA from ribosomes and for measurement of $O.D._{260\ m\mu}$ versus temperature profile study were previously described (Helmkamp and Ts'o, 1961).

Results and discussion. The nucleotide composition of pea seedlings given in Table I appears to be similar to that of white clover (Lyttleton, 1960) and of E. coli (Spahr and Tissieres, 1959), but differs significantly from that of the reticulocytes which synthesize mainly hemoglobin.

Two types of sheep hemoglobin, which are determined genetically by a single gene have been reported (Evans et al., 1956). We have attempted to study the relationship between the nucleotide ratio of the ribosomes isolated from these two types of reticulocytes to the amino acid composition of hemoglobin they synthesize. The published data of amino acid analyses of sheep

Table 1. Ribonucleotide composition of ribosomes from plant and animal sources.

Source	U	G	A	C
Pea stem	.220 ± .014	.314 ± .011	.243 ± .020	.223 ± .007
Rabbit reticulocytes	.182 ± .006	.345 ± .014	.186 ± .010	.287 ± .012
Sheep reticulocytes				
No. 1 (HbII)	.180 ± .004	.310 ± .006	.188 ± .005	.322 ± .003
No. 2 (HbI)	.193 ± .007	.327 ± .006	.178 ± .005	.302 ± .013
No. 3 (HbI)	.173 ± .003	.344 ± .008	.180 ± .006	.303 ± .002

Date expressed as fraction of total nucleotides recovered as calculated from phosphorus normalized to 1.000 ± average of deviations from the mean. All analyses are the mean of 3 determinations.

hemoglobin I and II, (Van der Helm, et al., 1957) indicated a difference of amino acids composition between them of probably 30-36 amino acids out of the 590 amino acids assumed to be in the sheep hemoglobin. The differences are in alanine (6), aspartic acid (4), glutamic acid (4), glycine (10) and probably are in tyrosine (2) serine (4) and threonine (4) also. The first nucleotide analysis of the reticulocyte ribosomes from two sheep, no. 1 and no. 2, (sheep no. 1 produced exclusively hemoglobin II, and sheep no. 2 produced exclusively Hb I) indicated possibly significant differences of nucleotide composition especially in C and G. Analysis of the nucleotide composition of another sheep having only Hb I (sheep no. 3) showed differences between sheep of the same hemoglobin type, namely Hb I. This difference, representing the variation due to preparation of reticulocyte ribosomes from different individuals, is much larger than the variation between analyses of a given sample. Because of this difficulty, we can only draw the general conclusion that the nucleotide composition of these two types of sheep reticulocyte ribosomes are quite similar, but cannot establish statistically

significant differences between these particles which may be related to the difference in amino acid composition of hemoglobin.

It was noted that the ribosomal RNA from sheep contains 63-64% "G + C", while the ribosomal RNA from peas contains only 53% "G + C". Fig. 1 illustrates

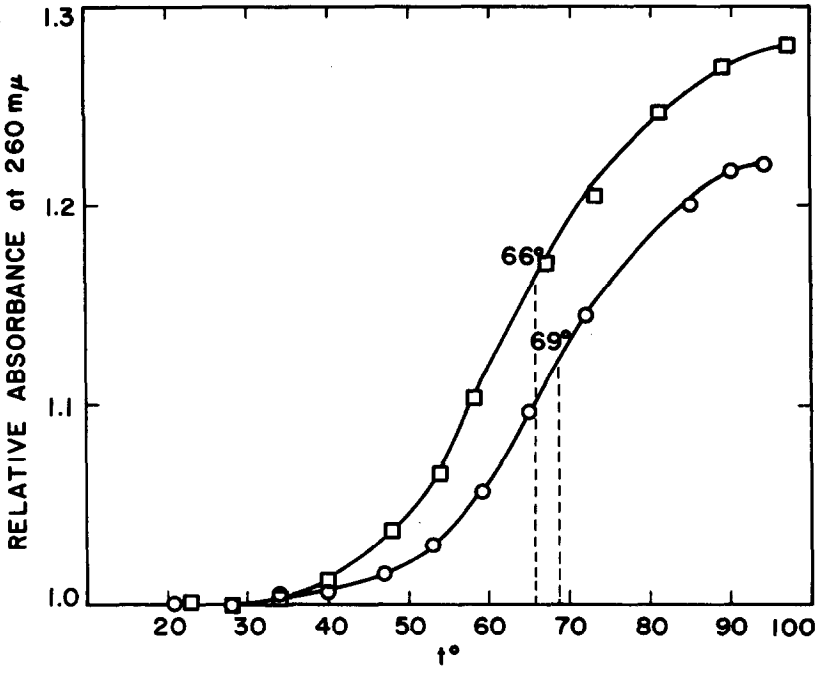

Fig. 1. Relative absorbancy at 260 mμ versus temperature of sheep (-o-o-) and pea RNA (-□-□-) in 0.1M NaCl, 0.1M Na-acetate, 1 x 10^{-3}M $MgCl_2$, pH 5.5.

the relative absorbance at 260 mμ versus temperature curves of these two RNAs. Interestingly, the T_m (temperature at the 50% increase of the total hypochromic effect) of the sheep (No. 2) RNA (69°C) is higher than that of the pea RNA (66°C). The magnitude of the increase of the T_m (3°C) is also similar to that for DNA having a difference of "G + C" to "A + T" of 9.5% (Marmur and Doty, 1959). The magnitude of the hypochromic effect of the sheep RNA having high "G + C" is also lower than that of the pea RNA as expected from the DNA results (Marmur and Doty, 1959). The above data are

suggestive that base pairing similar to that in DNA may also occur in RNA.

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