ON THE POSSIBLE IDENTITY OF ENZYME REQUIREMENT IN THE TRANSFER OF sRNA AND AMINO ACIDS TO RIBOSOMES

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It has been shown previously (von der Decken and Hultin, 1958, Hultin and von der Decken, 1959) that in vivo-labeled sRNA* prepared from rat liver is transferred to the ribonucleoprotein particles of rat liver microsomes. Similar results have also been obtained by Hoagland (1958) and Bosch, Bloemendal and Sluyser (1959). The sRNA molecules are specific for individual amino acids and current evidence strongly suggests that the various amino acids are transferred by sRNA to the ribosomes. The question of how the ribosomes direct the alignment of amino acids in a specific sequence has been clarified recently. Thus, messenger RNA has the ability to attach itself to the ribosomes and there to determine the sequence of amino acids (Risebrough, Tissières and Watson, 1962). Very little is known, however, about a possible interaction between sRNA and messenger RNA when the amino acids are lined up on the ribosomal template during the process of peptidization. One link in this complex system, the enzymic relationship between the transfer of sRNA and of amino acids from sRNA to ribosomal particles, will be described in this paper.

Experimental. All preparations were carried out at 4°C.

1. Preparation of ^{32}P-sRNA. Sprague Dawley rats (180 g) were injected

* Abbreviations: PEP, phosphoenol pyruvate; TCA, trichloroacetic acid; sRNA, soluble or transfer RNA; ^{32}P-sRNA, sRNA labeled in vivo with ^{32}P-phosphate.

with ^{32}P-phosphate (1 mc) and decapitated after about 15 hrs. A liver homogenate was prepared in 0.15 M KCl and centrifuged for 1 hr at 105 000 g. The middle part of the supernatant (the cell sap) was sucked off and precipitated with solid $(NH_4)_2SO_4$ at 55% saturation. After 10 min. the precipitate was collected by centrifugation and the supernatant again precipitated with $(NH_4)_2SO_4$ at 85% saturation. Both of the $(NH_4)_2SO_4$ precipitates were dissolved in water, extracted with distilled phenol, and the RNA of the water phase was precipitated in 70% ethanol. The collected precipitates were dissolved and dialyzed against water over night. The dialysates were then centrifuged for 90 min. at 105 000 g.

2. Enzyme fractionation. Cell sap was prepared from 10 g of rat liver as above and treated with 0.1% protamin-sulfate for 1 hr. After removal of the precipitate, $(NH_4)_2SO_4$ was added as above. The precipitates were dissolved in water, the $(NH_4)_2SO_4$ displaced by 0.01 M K-phosphate buffer, pH 7.2 using Sephadex G-25 columns. Hydroxylapatite-cellulose (Serva Entwicklungslabor, Heidelberg) was equilibrated with 0.01 M K-phosphate buffer, pH 7.2. The proteins were adsorbed on the hydroxylapatite columns (1.3 x 4 cm) and eluted stepwise with K-phosphate buffer as described by Herbert (1959). Aliquots of 1.5 ml were collected. The three samples of each buffer concentration showing the highest extinction at 280 mμ were dialyzed over night against 0.25 M sucrose, 0.035 M tris, pH 7.8, 0.025 M KCl and 0.01 M $MgCl_2$ (medium A).

3. Method of incubation. (a) Transfer of ^{32}P-sRNA. Ribonucleoprotein particles prepared according to Rendi and Hultin (1960) from 0.5 to 0.7 g of rat liver were incubated in a volume of 0.6 ml with about 30 μg of ^{32}P-sRNA (about 600 c.p.m.) and with the eluates of the columns as shown in Fig. 1. Cofactors were added in concentrations as follows: ATP 1.25 mM, GTP 0.2 mM, GSH 10 mM, PEP 12.5 mM and pyruvate kinase 30 μg/ml. After incubation for 7 min. at 35°C, the incubation mixture was diluted to 11 ml with medium B (as medium A but with 0.005 M $MgCl_2$) and centrifuged for 60 min. at 105 000 g. The particulate fraction was rinsed with medium B, re-

suspended in the same medium, and precipitated in 5% ice-cold TCA. The precipitates were washed three times with TCA, and the RNA was extracted at 90°C in 5% TCA and determined by the method of Mejbaum (1939). The radioactivity was measured with an end window counter (Tracerlab).

(b) Transfer of ^{14}C-amino acids. sRNA was labeled with ^{14}C-amino acids as described by von der Decken and Campbell (1962). As a source of amino acids, a ^{14}C-labeled algal protein hydrolysate, extensively purified by hydrolysis and column chromatography, was used. Incubation conditions were the same as under (a), except that the ^{32}P-sRNA was replaced by sRNA-bound ^{14}C-amino acids (about 20 μg of RNA, 550 c.p.m.). After incubation for 3 min. at 35°C the proteins were precipitated in 5% TCA, extracted with hot (90°) TCA and lipid solvents and the radioactivity was measured at infinite thinness.

Results. Fig. 1 shows the pattern of enzyme activity of the various fractions obtained after column chromatography. The transfer of ^{32}P-sRNA was closely paralleled by the transfer of ^{14}C-amino acids from sRNA measured by incorporation of ^{14}C-label into protein. From each $(NH_4)_2SO_4$ precipitate at least one fraction could be isolated which was active in catalyzing the transfer of both ^{32}P-sRNA and ^{14}C-amino acids. Two sRNA preparations, derived from the two $(NH_4)_2SO_4$ precipitates were used. Both kinds of RNA were transferred to the particulate fraction by the active eluates to about the same extent. The degree of purification of the most active eluates, the 0.2 M-eluate of 55% $(NH_4)_2SO_4$ and the 0.1 M-eluate of the 55-85% $(NH_4)_2SO_4$ was at least 15 and 7 fold, respectively. The transfer catalyzed by these eluates was tested for its dependency on cofactors. Table 1 summarizes some of the results obtained. Addition of PEP and pyruvate kinase alone did not increase the transfer of sRNA above the level of the control system which was incubated in the absence of high energy compounds. The transfer was increased after addition of ATP and GTP to the nucleoside triphosphate generating system. However, when GTP alone together with PEP and pyruvate kinase were added the transfer was

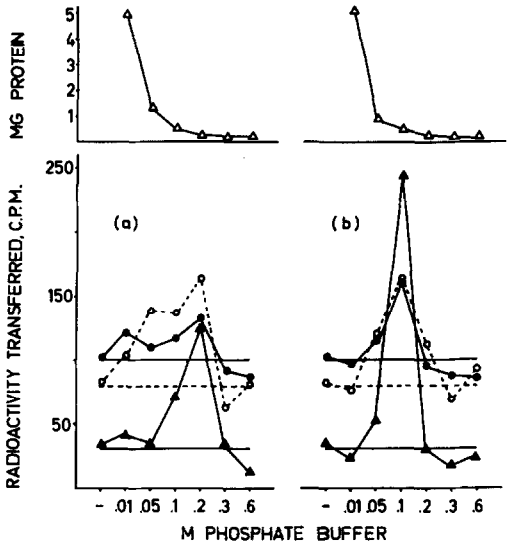

Fig. 1. Distribution of enzyme activities specific for transfer of ^{32}P-sRNA and sRNA-bound ^{14}C-amino acid to ribonucleoprotein particles in a rat liver system. (a) Eluates from hydroxylapatite column of 55% $(NH_4)_2SO_4$ precipitate. (b) Eluates of 55-85% $(NH_4)_2SO_4$ precipitate.

▲ ——— ▲ Transfer of ^{14}C-amino acid.

o - - - - o Transfer of ^{32}P-sRNA of 55% $(NH_4)_2SO_4$ precipitate.

● ——— ● Transfer of ^{32}P-sRNA of 55-85% $(NH_4)_2SO_4$ precipitate.

△ ——— △ Amount of protein in mg of eluates from hydroxylapatite columns added to the incubation system.

Table 1

Energy dependent transfer of ^{32}P-sRNA to ribonucleoprotein particles in a rat liver system. The control system contained ribonucleoprotein particles (0.5 mg RNA), ^{32}P-sRNA of 55-85% $(NH_4)_2SO_4$ precipitate (30 µg, 600 c.p.m.) and the transfer enzyme (0.2 M-eluate of 55% $(NH_4)_2SO_4$ precipitate, 180 µg protein, or 0.1 M-eluate of 55-85% $(NH_4)_2SO_4$ precipitate, 420 µg protein).

Additions to Control System	0.2 M-Eluate, 55% $(NH_4)_2SO_4$	0.1 M-Eluate, 55-85% $(NH_4)_2SO_4$
	C.p.m./mg RNA	
None	86	84
PEP, pyruvate kinase,	100	96
" " ATP	97	146
" " ATP, GTP	144	160
" " GTP	202	260

increased even more. This indicates a strong dependency on GTP of the transfer reaction. It was known (Hultin and von der Decken, 1961) that omission of ATP in the generating system does not decrease the transfer of ^{14}C-amino acids to protein either provided GTP is present in the system.

No definite answer could be obtained concerning the effect of GSH on the transfer of ^{32}P-sRNA. Some of the enzyme preparations showed a strong dependency on GSH in contrast to others which were not affected by this agent. However, the transfer of ^{14}C-amino acids with the same enzyme preparations was in all cases stimulated by GSH.

A further relationship between the transfer of ^{32}P-sRNA and the transfer of ^{14}C-amino acids to the ribosomes was noted in mixed rat-Escherichia coli incubation systems. It had been observed that ^{14}C-amino acids bound to rat liver sRNA were transferred to a very limited extent to ribosomes of E. coli in the presence of rat liver enzymes. Similar results have been reported by Rendi and Ochoa (1962) for the transfer of ^{14}C-L-leucine. In the present experiments it could be shown that not only the incorporation of ^{14}C-amino acids into protein but also the transfer of rat liver ^{32}P-sRNA to the ribosomes by enzymes from rat liver or E. coli was almost negligible. These results suggest a double specificity; on one side between sRNA and transfer enzymes, and on the other between transfer enzymes or sRNA and ribosomes.

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