Preliminary Notes

Metabolic interrelationships between soluble and microsomal RNA in rat-liver cytoplasm

In the present communication we wish to report the results of some experiments *in vitro*, suggesting a metabolic transfer of radioactivity from soluble polynucleotides of the cell sap to microsomal ribonucleoprotein particles and *vice versa*, under conditions which are known to favour amino acid incorporation into these particles. Both processes require ATP.

Transfer of radioactivity from s-RNA to m-RNA. When pH-5 enzymes (*cf.* HOAGLAND *et al.*[1]) obtained from rat liver labelled *in vivo* with radioactive inorganic phosphate were incubated with a 15,000 × *g* liver supernatant for different periods of time in the presence of ATP and PGA, the ribonucleoprotein particles isolated from the microsomes by means of deoxycholate (*vide infra*) become labelled. This labelling is completed in about 5 min. Omission of ATP and PGA from the reaction mixture results in a decreased transfer, whereas the specific activity of microsomal RNA drops even further in the presence of 0.02 *M* arsenate, showing a definite requirement for ATP.

These preliminary experiments do not establish the polynucleotide nature of the precursor in this reaction process. Essentially identical results were obtained, however, with s-RNA, isolated from pH-5 enzymes by means of zone electrophoresis on starch. As was shown in a previous communication[2], pH-5 enzymes contain s-RNA as a free polynucleotide which migrates in the electric field as a narrow and distinct zone, leaving behind protein, ATP and AMP.

Inorganic phosphate travels at about the same rate as s-RNA. Following electrophoresis (4.5 V/cm, 16 h, phosphate buffer, pH 7.4, I, 0.025) radioactive s-RNA was eluted from the starch segments and dialysed against 10 l distilled water for about 16 h in order to remove free inorganic phosphate. The dialysed RNA was incubated in varying amounts with 15,000 × *g* liver supernatant in the presence of ATP and PGA (see legend of Fig. 1). Microsomes isolated from the incubation mixture by centrifugation at 105,000 × *g* for 30 min were solubilized in 0.5 % Na-deoxycholate according to LITTLEFIELD *et al.*[3] and centrifuged for another 90 min at 105,000 × *g*. The sedimented particles were extracted with a 10 % NaCl solution at 100° and RNA was precipitated by 3 vol. cold ethanol. RNA was plated and counted in a gas flow counter, the amount being determined by reading absorbance at 260 mμ following hydrolysis in 5 % $HClO_4$.

Fig. 1 shows that RNA isolated from the microsomal particles becomes labelled almost in a linear fashion with increasing concentrations of labelled s-RNA. Very recently HULTIN AND VON DER DECKEN[4] reported a similar metabolic transfer of soluble polynucleotides, which they obtained in a different way (phenol extraction).

Abbreviations: AMP, GMP, CMP, and UMP, adenylic, guanylic, cytidylic, and uridylic acid; ATP, adenosine triphosphate; PGA, 3-phosphoglyceric acid; s-RNA, soluble RNA; m-RNA, microsomal RNA; Tris, tris(hydroxymethyl)aminomethane.

Transfer of radioactivity from m-RNA to the soluble fraction. Liver microsomes were isolated from rats treated previously with radioactive inorganic phosphate, and resuspended in the homogenisation medium described by LITTLEFIELD AND KELLER[5]. The suspension was centrifuged for 2 min at about 500 × g and the supernatant was incubated with soluble enzymes supplemented with ATP and PGA for different periods of time (see legend Fig. 2). The soluble enzymes were obtained by dialysing a 105,000 × g liver supernatant for 20 h against 10 l 0.01 *M* Tris buffer, pH 7.4. Following incubation microsomes were removed by centrifugation at 105,000 × g for 2 h. 1 mg carrier RNA (from yeast) was added to 5 ml of the supernatant and RNA was isolated in the conventional way[1].

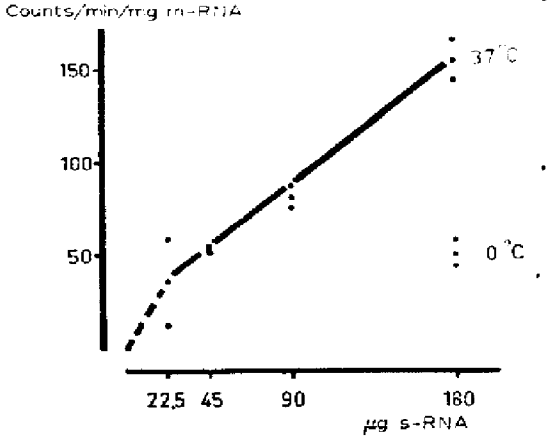

Fig. 1. Transfer of label from [^{32}P]s-RNA to m-RNA. Incubation mixture: 6 ml of 15,000 × *g* liver supernatant (non-radioactive), 10 μmoles ATP, 80 μmoles PGA and [^{32}P]s-RNA in the amounts as indicated. Total vol., 11 ml. Incubation in air for 10 min at 0° or 37°. Each point represents the result of one incubation experiment, the circles (○) indicating the average of two duplicates.

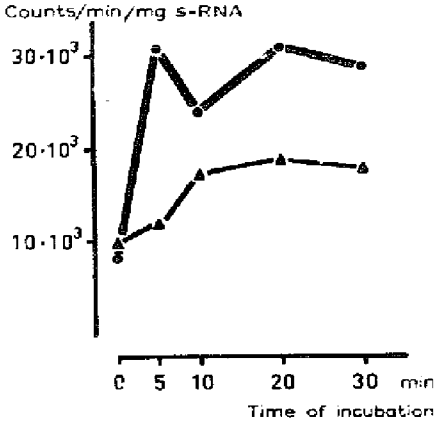

Fig. 2. Transfer of soluble polynucleotides from ^{32}P-labelled microsomes to the medium. Incubation mixture: ^{32}P-labelled microsomes (containing 72 mg protein, 2.7 mg RNA), dialysed 105,000 g liver supernatant (containing 140 mg protein, 0.55 mg RNA), 5 μmoles ATP and 40 μmoles PGA. Total vol., 5 ml. Incubation in air at 37°. ● In the presence of 5 μmoles ATP and 40 μmoles PGA. ▲ In the presence of 100 μmoles K-arsenate and absence of ATP and PGA.

When the microsomes were incubated in the presence of ATP and PGA the specific activity of s-RNA in the medium increased about 3-fold in 5 min, then remaining constant (Fig. 2). The radioactive s-RNA was non-dialysable and yielded radioactive AMP, GMP, UMP and CMP on alkaline hydrolysis and isolation of the nucleotides on Dowex I (formate). The release of soluble polynucleotides from the microsomes was considerably less when the incubation mixture was supplemented with K-arsenate instead of PGA and ATP. Similar curves were obtained when the medium contained 0.01 *M* fluoride in addition to 0.02 *M* arsenate.

Summarizing it may be said that a metabolic relationship seems to exist between soluble and microsomal RNA in rat-liver cytoplasm, which reveals itself by energy-dependent transfers of poly- or oligonucleotides in both directions.

Thanks are due to Dr. P. EMMELOT for valuable discussions and to Miss M. DE VRIES and Miss G. VAN DER WENDE for skilled technical assistance.

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Received April 3rd, 1959

* Laboratory for Inorganic Chemistry of the University of Amsterdam, working under the auspices of The Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

Enzymic cleavage of phosphoramidic acid

The chemical hydrolysis of phosphoramidate has been thoroughly investigated by RATHLEV AND ROSENBERG[1]. Their results indicate that at pH values above 4 the rate of hydrolysis is proportional to the concentration of the anion. Phosphoramidate has been suggested as a metabolic intermediate both by RATHLEV AND ROSENBERG[1] and by SPECK[2], but no evidence for its participation in metabolic sequences has been presented. In view of its chemical reactivity[3] and the recent evidence for metabolic activity of adenylic-5'-phosphoramidate[4], a reinvestigation of possible enzymic reactions of phosphoramidate was undertaken.

Sonic extracts prepared from *Escherichia coli* (Crookes strain), grown on a mineral-salts medium with succinate as the sole carbon source, catalyzed a rapid evolution of NH_3 from phosphoramidate. Boiled extracts were without catalytic activity. Treatment of the *E. coli* extracts with protamine sulfate followed by fractionation with $(NH_4)_2SO_4$ and rigorous dialysis of the fractions revealed at least two separate enzyme systems capable of catalyzing the release of NH_3 from phosphoramidate. Fraction I, which was precipitated by $(NH_4)_2SO_4$ below 0.5 saturation, was shown to require a divalent metal (Mg^{++} or Mn^{++}) and a sulfhydryl compound (cysteine or glutathione) to achieve a maximum rate of NH_3 release (Table I). The optimum pH for activity of Fraction I was 7.4. Paper chromatography of a reaction mixture containing Fraction I, phosphoramidate, cysteine and Mg^{++} with *n*-propanol–NH_4OH–H_2O (6:3:1) as a developing solvent revealed inorganic phosphate and phosphoramidate as the only phosphate-containing compounds present. It is assumed from these results that the reaction catalyzed by Fraction I is the cleavage of phosphoramidate to phosphate and NH_3. Even with the mildest conditions phosphate and phosphoramidate cannot be differentiated colorimetrically since molybdate catalyzes a very rapid hydrolysis of the latter[1].

Another *E. coli* fraction, Fraction III, precipitated between 0.58 and 0.9 saturation with $(NH_4)_2SO_4$, was also shown to catalyze a rapid evolution of NH_3 from phosphoramidate, while the intermediate fraction (0.5 to 0.58 saturation) was low in catalytic activity. The rate of NH_3 release catalyzed by Fraction III was maximal at pH 5.1 and was not stimulated by the addition of either divalent metals or reducing