Change in Sedimentation Profile of Protein Synthesizing Particles
Observed After Protein Synthesis

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Protein synthesizing particles from rat liver sedimented more slowly after incubation under protein synthesis conditions. This change paralleled the degree of leucine incorporation which is stimulated by added RNA. Both effects seemed to be specific.

During our efforts to develop a cell free protein synthesizing system from a eukaryotic source, we concentrated on the properties of the protein synthesizing particles. These particles were prepared similar to *E. coli* ribosomes \(^1,^2\) (see Table 1), but they sedimented faster. When studying the sedimentation behavior of these particles we observed an unexpected change in their sedimentation profile following protein synthesis. Without incubation under protein synthesis conditions, the particles sedimented fast (\(>500\) s: fig. 1 b). The sedimentation profile changed when the particles were incubated together with nuclear RNA (size below 70 s: fig. 1 a) under standard protein synthesis conditions (fig. 1 c). This effect occurred with total nuclear RNA from rat liver. After fractionation by sucrose gradient centrifugation (fig. 2), RNA fraction C which had the highest specific radioactivity after a \(^{32}P\) pulse, caused the most distinct change. This same RNA fraction C stimulated leucine incorporation best (Table 1). In contrast to this, fraction A of nuclear RNA had no capacity to stimulate protein synthesis and caused no change in the sedimentation profile.

The fact that only RNA C, but not the other fractions of nuclear RNA and not T4 RNA caused the described sedimentation change, suggests that this change might be due to a specific interaction of RNA with the protein synthesizing particles. Earlier we reported that the interaction of messenger RNA with ribosomes is specific\(^2,^3\). The specific influence of RNA fraction C on the sedimentation of protein

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\text{Table 1. Stimulation of leucine incorporation into TCA precipitable material by ribonucleic acids in a cell free system from rat liver. Cell free system: 15—20 g liver were excised from two 120—140 g heavy Sprague Dawley rats immediately after sacrifice and after cooling by injecting cold isotonic saline into the portal vein. The livers were minced by scissors and homogenized in 2 vol. Tris HCl 0.05 M pH 7.6, 0.025 M KCl, 0.01 M MgCl}_2, 0.25 M sucrose (TSS) in a Potter Elvehjem homogenizer. The postmitochondrial supernatant (15000 \(g\) min) was centrifuged at 40000 rpm (Spinco L 50) for 2 hours. The clear supernatant was used as enzyme fraction, the pellet was suspended in TSS and dialyzed against 6.010.0
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\(^1\) P. Traub and W. Zillig, Hoppe-Seyler's Z. physiol. Chem. 343, 246 [1966].

SEDIMENTATION OF PROTEIN SYNTHESIZING PARTICLES

Fig. 1. Sedimentation profiles of protein synthesizing particles. Sucrose gradients 5—50% in H2O or TS buffer (see table 1), SW 39 Spinco L 50, 25000 rpm 30 min. a) nuclear RNA only, b) cell free incubation mixture after 30 min incubation without added RNA, c) incubation mixtures which had been incubated with either RNA C (fig. 2) o—o (gradient C), or RNA B △—△, RNA A □—□ (gradient A), or T4 RNA ×—×. T4 bacteriophages and E. coli ribosomes served as markers. Compiled from 14 similar experiments. Incorporated 14C leucine sedimented with the active particles. Total nuclear RNA in the incubation mixture produced a similar profile as the one in gradient C.

synthesizing particles, the fast synthesis of this RNA fraction as shown by the high specific radioactivity, and the ability of RNA C to stimulate leucine incorporation, may indicate that this RNA is mRNA.

The observed change in the sedimentation behavior of protein synthesizing particles could be explained by a release of active substructures like, for instance, polysomes. This possibility is supported by electronmicrographs 4. Such protein synthesizing substructures isolated by sucrose gradient centrifugation (fig. 1 c, fraction 13 of gradient C) appear indeed polysome-like. The particles without active protein synthesis (in the bottom fractions of fig. 1 c gradient A) display clotty material.

On the other hand, the change in sedimentation could be caused by a swelling or unfolding of the particles in the process of protein synthesis. This would be consistent with the observation that inorganic phosphate has also influence on the sedimentation of these particles 5.

Fig. 2. Sucrose gradient of nuclear RNA from rat liver. 300 µg nuclear RNA in TS buffer was applied to a 5—30% linear sucrose gradient and centrifuged in a SW 39 at 38000 rpm for 6 hours. ×—× = optical density at 260 nm, o⋯o = cpm Cerenkov radiation when the rats had received 250 µci 32P ortho-phosphate i.p. each 3.5 hours prior to sacrifice. Catalase served as marker. Preparation of nuclear RNA: After freezing and thawing, the nuclei were suspended in 2 vol 0.03 M sodium phosphate pH 6.8, 0.15 M NaCl, 0.3 M sodium deoxycholate. After treatment with 80% phenol at 20 °C, the phenol phase and interphase were reextracted with the same buffer at 65 °C for 20 min. The aqueous phase was rephenolized and RNA precipitated by ethanol. This RNA is referred to as nuclear RNA. After freezing and thawing, the peak fractions A, B, C were pooled and RNA precipitated. RNA was, then, dissolved in 0.05 M Tris pH 7.6 for use in the cell free system. T4 RNA was isolated from T4 am 50 ts endolysin infected E. coli K12 20 min after infection at 37 °C.

5 Other authors had also observed a stimulation of endogenous protein synthesis by phosphate as we did: H. G. Schweiger, S. Rapoport, and F. Scholzel, Hoppe-Seyler's Z. physiol. Chem. 306, 33 [1956].
6 P. Herrlich and N. Lang, Hoppe-Seyler's Z. physiol. Chem. 348, 1377 [1967].