Relations between Latent Membrane-bound and Active Polyribosomal Messenger Ribonucleoprotein Particles in Dormant and Developing *Artemia* Embryos *

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Encysted embryos of the brine shrimp, *Artemia*, are in a state of deep dormancy, characterized by the lack of metabolic activity and the absence of polyribosomes. Latent mRNA in the form of messenger ribonucleoprotein (mRNP) particles are stored in the cytoplasm, in great part associated directly with endoplasmic membranes. After hydration, protein synthesis is rapidly activated and polyribosomes appear.[1] We have compared active polyribosomal mRNP particles from developing embryos with latent, membrane-associated mRNP particles from dormant cysts, with particular emphasis on their protein composition. A slight but distinct difference in protein complement has previously been observed between free and polyribosomal mRNP particles in normal mammalian cells.[2]

Dormant cysts were homogenized, and a fraction of cytoplasmic membranes was isolated and purified.[3] Latent mRNP particles were detached by treatment with zwitterionic detergent and purified by centrifugation in sucrose and Cs_2SO_4 gradients.[4,5] Polyribosomes were isolated from embryos developed for 18 h (50 % emergence). Polyribosomal mRNP particles were released by EDTA treatment and purified by sucrose gradient and Cs_2SO_4 density gradient centrifugation, or by affinity chromatography on oligo(dT) cellulose. The mRNA distribution was determined by poly(A)$^+$RNA analysis.

Latent mRNP particles from the membranes of dormant cysts sedimented at about 40S, and contained poly(A)$^+$RNA with a sedimentation peak around 14S. The purified particles banded in Cs_2SO_4 at 1.27 g/cm^3 (Fig. 1A). The low density was accounted for by a complex protein pattern, indicating a high protein:RNA ratio (Table 1), and the presence of some lipid, including phosphatidylcholine. The data suggest that lipoprotein(s) may be included in the structure.[5]

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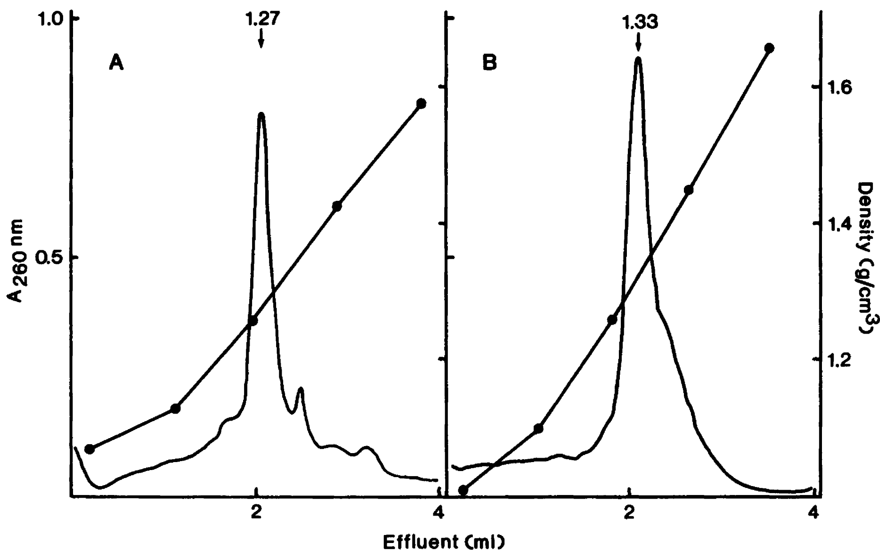

Fig. 1. Cs_2SO_4 density gradient centrifugation of mRNP particles. Poly(A)$^+$RNA-containing fractions from sucrose gradients were precipitated with ethanol, dissolved in buffer and fixed with 4 % glutaraldehyde. After dialysis overnight, the particles were centrifuged for 17 h (4 °C) at 110 000 g_{av} in a preformed Cs_2SO_4 gradient, using a Beckman SW60 rotor. (A) Membrane-bound particles. (B) Polyribosomal particles.

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Table 1. Protein composition of mRNP particles. Values in italics represent protein bands common to both types of particles.

Protein ($M_r \times 10^{-3}$)
Membrane-bound particles
95, *82*, *72*, 67, *62*, *58*, *56*, 53, *47*, 43, 40, *37*, 35, *32*, 30, 24, *20*, 19, 16
Polyribosomal particles[a]
135, 126, 98, *82*, *72*, *63*, *59*, *55*, *46*, *38*, *33*, *20*

[a] Purified by affinity chromatography on oligo(dT) cellulose.

The polyribosomal poly(A)$^+$RNP particles sedimented around 30S in sucrose gradients (Fig. 2). The extracted poly(A)$^+$RNA formed a broad sedimentation peak within the range of 12–20S. The density of the isolated particles in Cs_2SO_4 was 1.33 g/cm^3 (Fig. 1B), *i.e.* appreciably higher than that of the latent, membrane-associated mRNP particles from dormant cysts. The protein pattern was also less complex (Table 1). Some proteins with the same electrophoretic mobility in SDS polyacrylamide gels may be identical in the two kinds of particle.

The more complex structure of the latent, membrane-bound particles may represent an adaptation to long-term storage during the cryptobiotic phase. Lipoprotein(s) may serve to anchor the particles to the surface of the membranes and may thereby facilitate the transportation of messenger to morphogenetically active regions within the embryonic syncytium. After the resumption of development the stored mRNP particles apparently have to be extensively modified in order to become available for translation.

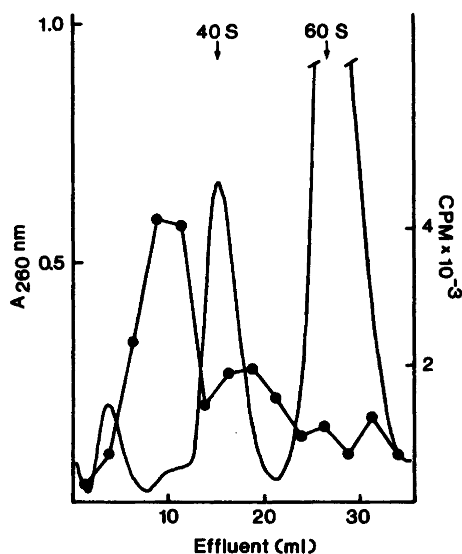

Fig. 2. Polyribosomal mRNP particles prepared by EDTA (10 mM) dissociation of polyribosomes from developed cysts. Sucrose gradients (10–30 %) were centrifuged for 17 h (0 °C) in a Beckman SW27 rotor. RNA (●) was extracted and analyzed by hybridization to [^{3}H]poly(U).[5]

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