Prosomes are Involved in the Repression of Viral mRNA

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Prosomes are small cytoplasmic RNP complexes. We present evidence that their RNA is a potential and selective inhibitor of viral mRNA translation while translation of normal cellular mRNA e.g. rabbit globin mRNA or HeLa cell mRNA is not affected.

Prosomes, novel and ubiquitous small RNP particles were recently found associated with repressed free mRNP complexes in the cytoplasm of duck, mouse and HeLa cells [1]. They consist of a specific set of proteins; some of them are similar to the small heat shock proteins as reported for the prosomes of *Drosophila* cells [2]. Their RNA content seems to be cell type specific, since prosomes of erythroblasts, *Drosophila* and HeLa cells revealed different RNA patterns in one and two dimensional RNA gelelectrophoresis [1, 2] and unpublished results). Our earlier investigations implicated, that prosomes play an important role as control factors of cytoplasmic gene expression.

Prosomes of mouse erythroblasts partially repress the protein synthesis of endogenous mRNA of an Krebs II Ascites lysate [3], and hybridization experiments suggested that prosomal RNA mediates the binding of prosomes to mRNA. Therefore, we tested the influence of prosomal RNA of highly purified prosomes on the translation of different mRNA species more carefully. When prosomal RNA was added to a lysate containing HeLa mRNA or globin mRNA the efficiency of the protein synthesis was not affected (Fig. 1A). Also increasing amounts of prosomal RNA had no influence on the translation of normal cellular mRNA (Fig. 1C).

However the translation efficiency of viral mRNA was remarkably reduced. When equal amounts of prosomal RNA (in correlation to HeLa mRNA in

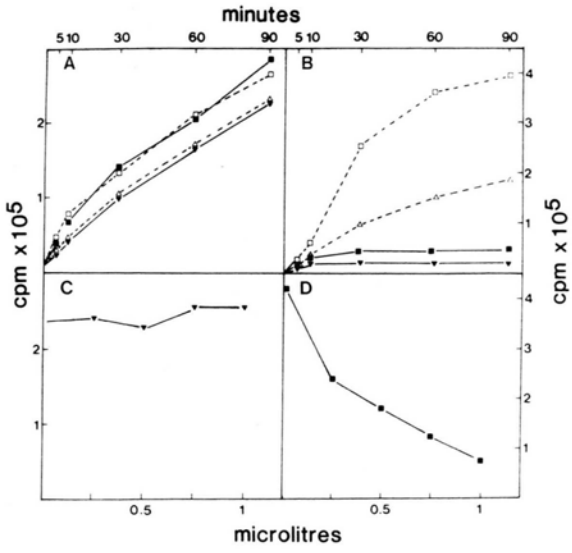

Fig. 1. Prosomes were isolated from mouse erythroblasts as reported earlier [1] and extracted with chloroform/phenol after digestion with proteinase k [4]. The purity of RNA was controlled by polyacrylamide gelelectrophoresis and the RNA was stored at −70 °C for the *in vitro* translation essays. Rabbit reticulocyte lysate was purchased from New England Nuclear and 1 µg of mRNA or viral RNA/mRNA was tested in a 25 µl *in vitro* translation essay with different amounts of prosomal RNA.

A. 1 µg of globin mRNA or HeLa mRNA was incubated with 0.8–1 µg of prosomal RNA of mouse erythroblasts. Incorporation of [^{35}S]methionine:
−△−△−△ HeLa mRNA bound to polyribosomes, purified over oligo(dT)-cellulose,
−□−□−□ rabbit globin mRNA purchased from Sigma,
−▼−▼−▼ HeLa mRNA + prosomal RNA,
−■−■−■ rabbit globin mRNA + prosomal RNA.

B. 1 µg of Adenovirus mRNA or TMV RNA was incubated with 0.8–1 µg of prosomal RNA of mouse erythroblasts.
Incorporation of [^{35}S]methionine:
−△−△−△ Adenovirus mRNA bound to HeLa polyribosomes, purified over oligo(dT)-cellulose,
−□−□−□ TMV RNA isolated from Tobacco mosaic virus,
−▼−▼−▼ Adenovirus mRNA + prosomal RNA,
−■−■−■ TMV RNA + prosomal RNA,

C. 1 µg HeLa mRNA was incubated with different amounts of prosomal RNA of mouse erythroblasts and tested for *in vitro* translation; 1 µl contained approximately 0.8–1 µg prosomal RNA;
−▼−▼−▼ incorporation of [^{35}S]methionine after 90 min;

D. 1 µg of TMV RNA was incubated with different amounts of prosomal RNA of mouse erythroblasts and tested for *in vitro* translation; 1 µl contained approximately 0.8–1 µg prosomal RNA;
−■−■−■ incorporation of [^{35}S]methionine after 90 min.

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μg) were added to Adenovirus mRNA or Tobacco mosaic virus RNA the protein synthesis was inhibited (Fig. 1B). The inhibition was proportional to the quantity of prosomal RNA:viral mRNA/RNA added (Fig. 1D).

Similar results were obtained with cow pea mosaic virus mRNA (data not shown). These experiments suggested strongly, that prosomal RNA as essential constituents of prosomes repress the protein synthesis of viral mRNA selectively. In conclusion prosomal RNA should contain sequences which recognize viral mRNA with a certain specifity.

Preliminary hybridization experiments of viral mRNA:prosomal RNA or prosomes :viral mRNA and globin mRNA:prosomal RNA revealed indeed a higher affinity of prosomal RNA for viral RNA sequences as for example to globin mRNA (results not shown). We are currently investigating these interactions more closely.

Summarizing we postulate that prosomes are involved in protein synthesis as selective repressors of viral mRNA.

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[1] H. P. Schmid, O. Akhayat, C. Martins de Sa, F. Puvion, K. Köhler, and K. Scherrer, EMBO J. **3,** 29−34 (1984).

[2] A. P. Arrigo, J. L. Darlix, E. W. Khandjian, M. Simon, and P. F. Spahr, EMBO J. **4,** 399−406 (1985).

[3] H. P. Schmid, Thesis, University of Stuttgart, F. R. G. (1982).

[4] H. P. Schmid, K. Köhler, and B. Setyono, J. Cell. Biol. **93,** 893−898 (1982).