

# **Prosomes are Involved in the Repression of Viral mRNA**

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Prosomes, *in-vitro* Protein Synthesis,  
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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 parti-  
cles 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 gelectro-  
phoresis [1, 2] and unpublished results). Our ear-  
lier 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 experi-  
ments 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 pro-  
somal 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

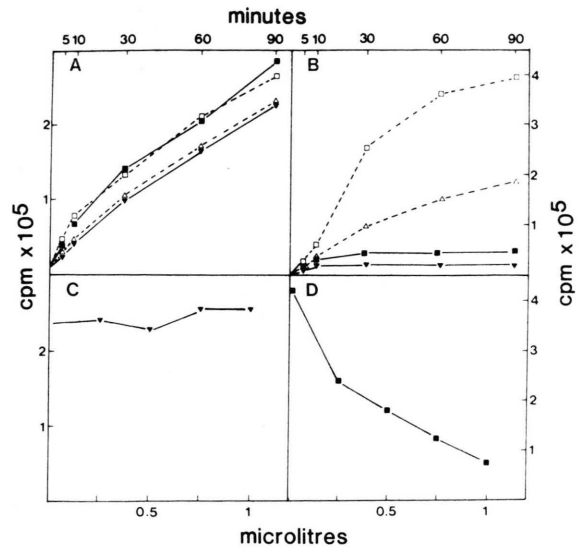


Fig. 1. Prosomes were isolated from mouse erythroblasts as reported earlier [1] and extracted with chloroform/phenol after digestion with proteinase k [4]. The purity of RNA was controlled by polyacrylamide gelelectrophoresis and the RNA was stored at  $-70^{\circ}\text{C}$  for the *in vitro* translation assays. Rabbit reticulocyte lysate was purchased from New England Nuclear and 1  $\mu\text{g}$  of mRNA or viral RNA/mRNA was tested in a 25  $\mu\text{l}$  *in vitro* translation assay with different amounts of prosomal RNA.

A. 1  $\mu\text{g}$  of globin mRNA or HeLa mRNA was incubated with 0.8–1  $\mu\text{g}$  of prosomal RNA of mouse erythroblasts. Incorporation of  $[^{35}\text{S}]$ methionine:

- $\Delta$ – $\Delta$ – $\Delta$  HeLa mRNA bound to polyribosomes, purified over oligo(dT)-cellulose,
- $\square$ – $\square$ – $\square$  rabbit globin mRNA purchased from Sigma,
- $\nabla$ – $\nabla$ – $\nabla$  HeLa mRNA + prosomal RNA,
- $\blacksquare$ – $\blacksquare$ – $\blacksquare$  rabbit globin mRNA + prosomal RNA.

B. 1  $\mu\text{g}$  of Adenovirus mRNA or TMV RNA was incubated with 0.8–1  $\mu\text{g}$  of prosomal RNA of mouse erythroblasts. Incorporation of  $[^{35}\text{S}]$ methionine:

- $\Delta$ – $\Delta$ – $\Delta$  Adenovirus mRNA bound to HeLa polyribosomes, purified over oligo(dT)-cellulose,
- $\square$ – $\square$ – $\square$  TMV RNA isolated from Tobacco mosaic virus,
- $\nabla$ – $\nabla$ – $\nabla$  Adenovirus mRNA + prosomal RNA,
- $\blacksquare$ – $\blacksquare$ – $\blacksquare$  TMV RNA + prosomal RNA,

C. 1  $\mu\text{g}$  HeLa mRNA was incubated with different amounts of prosomal RNA of mouse erythroblasts and tested for *in vitro* translation; 1  $\mu\text{l}$  contained approximately 0.8–1  $\mu\text{g}$  prosomal RNA;

- $\nabla$ – $\nabla$ – $\nabla$  incorporation of  $[^{35}\text{S}]$ methionine after 90 min;

D. 1  $\mu\text{g}$  of TMV RNA was incubated with different amounts of prosomal RNA of mouse erythroblasts and tested for *in vitro* translation; 1  $\mu\text{l}$  contained approximately 0.8–1  $\mu\text{g}$  prosomal RNA;

- $\blacksquare$ – $\blacksquare$ – $\blacksquare$  incorporation of  $[^{35}\text{S}]$ 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 specificity.

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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