# REQUIREMENT OF A DIALYZABLE COMPONENT FROM CRUDE INITIATION FACTORS FOR THE TRANSLATION OF VIRAL AND EUKARYOTIC MESSENGER RNA

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SUMMARY

A dialyzable factor has been isolated both from the crude 0.5 M KCl wash of reticulocytes and Krebs II Ascites tumor polyribosomes. This component is not only required for the in vitro translation of different eukaryotic messengers like globin and lens crystallin mRNA, but also for the translation of polio and adeno viral RNA.

## INTRODUCTION

Recently Bogdanovsky et al (1) isolated a dialyzable factor from the 0.5 M KCl wash of reticulocyte ribosomes, which restores the activity of dialyzed crude initiation factors. The assays were performed in a reticulocyte cell-free system. The authors suggested that the dialyzable component was a new RNA species of very small size.

We have recently shown (2) that, whatever the nature of this factor might be, its activity is rather unspecific in that it stimulates the translation of globin and lens crystallin messenger RNA in a variety of cell-free heterologous systems.

In the present paper we demonstrate that also viral messengers require the factor for translation in vitro. Moreover, we isolated a dialyzable component with similar activity as the reticulocyte factor from the 0.5 M KCl wash derived from polysomes of Krebs II Ascites tumor cells.

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## MATERIALS AND METHODS

The isolation of the dialyzable factor from reticulocytes, the preparation of RNAse ribosomes and Krebs II pH 5 enzymes were performed as described previously (2). The dialyzable factor from Krebs II Ascites cells was isolated in a similar way as reported for the reticulocyte factor. An amount corresponding to 450 $A_{260}$ units of polyribosomes was treated with 0.5 M KCl at $0^{o}$ for 2 h. The ribosomes were spun by centrifugation at 105,000 x g for 3 h and the crude initiation factors (26 mg/ml protein) were dialyzed for 5 h against 10 volumes of distilled water. The dialysate was lyophilized and resuspended in one quarter of the original volume of the crude initiation factor preparation.

Cell-free protein synthesis was performed at $30^{o}$ for 1 h. The reaction mixture contained per ml: 10 $A_{260}$ units of RNAse ribosomes from reticulocytes, 0.3 mg of pH 5 enzymes, 40 µg mRNA, 100 mM KCl, 2 mM $MgAc_2$, 1 mM DTT, 10 mM Tris-HCl pH 7.4, either 3 mg of crude initiation factors or 2 mg of dialyzed initiation factors and 40 µl of the dialyzable factor. The ATP-generating system and amino acid mixture were as described earlier (2). The final volume was 25 µl.

Electrophoresis was performed on SDS slab gels according to Laemmli (3). However, a linear gel gradient from 6-18% polyacrylamide was used and the autoradiographic analysis was performed as described elsewhere (4).

Polio virus (Strain Mahoney type I) was grown in primary monkey kidney cells on microcarriers (5) and purified as described by Van Wezel (6). RNA was extracted from purified polio virus with SDS-acetate buffer pH 3.5 (7). This RNA was further purified by centrifugation through a 15-30% glycerol density gradient, containing 10 mM Tris-HCl pH 7.4, 100 mM NaCl, 1 mM EDTA, for 4.5 h at 180,000 x g.

Adeno-RNA was extracted from KB-cells 35 h after infection with adenovirus type 5 (Strain Ad 75) (8). Crystallin mRNA was isolated as described earlier (4).

## RESULTS AND DISCUSSION

In an earlier paper we demonstrated the unspecificity of a dialyzable factor from the 0.5 M KCl wash, prepared from reticulocyte polyribosomes, in stimulating messenger RNA translation in vitro (2).
However, since exclusively globin and crystallin messengers were utilized in our assay systems, the possibility that the factor exhibits its activity only towards a limited number of rather small messengers could not be excluded. Therefore we investigated the effect of the factor on the translation of two messengers derived from virus: one an RNA virus (polio), the other a DNA virus (adeno).
Cell-free protein synthesis was performed under optimal ionic conditions, namely 100 mM KCl and 2 mM $MgAc_2$. These optima deviate slightly from the optima for the translation of globin and crystallin mRNA (120 mM KCl and 2.4 mM $MgAc_2$, respectively) (2).
In Fig. 1 it is clearly shown that the translation of the two viral messenger species tested is dependent upon the addition of the dialyzable factor (1E and 1H). As far as the synthesis of the high molecular weight proteins is concerned (above 80,000 daltons), the stimulation is considerably higher with the crude KCl wash than with the reconstituted dialyzed KCl wash (compare Fig. 1F with 1H). This holds true for both polio and adeno viral messengers. It cannot be excluded that this is due to a loss of some component(s) upon dialysis of the crude KCl wash. We observed that dialysis of the initiation factors always causes a small precipitate of protein which cannot be redissolved. The translation of the globin and crystallin messengers, however, (Fig. 1L) is virtually unaffected upon recombination of dialyzed initiation factors and the dialyzable component. An active dialyzable factor could also be obtained from Krebs II Ascites KCl wash. Its stimulating activity on the translation of viral and crystallin mRNA was identical to that derived from

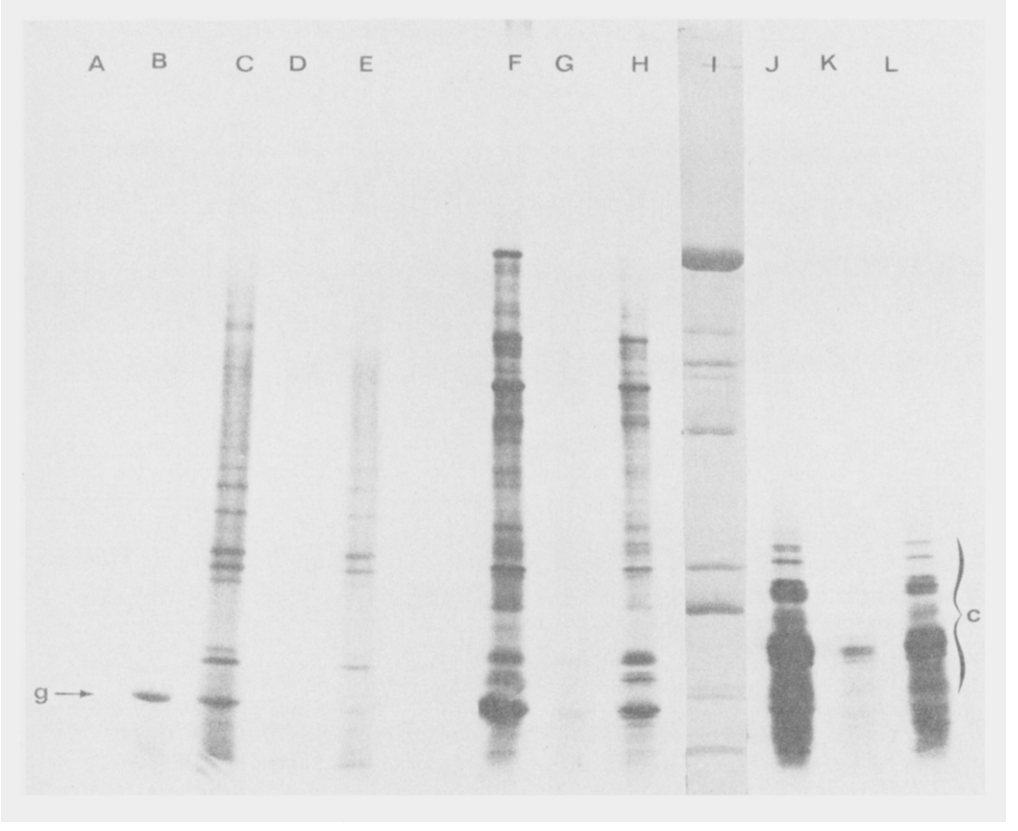


Fig. 1. Autoradiography of the SDS gel pattern of products synthesized in vitro under direction of viral and crystallin messengers in a heterologous system. Incubations were performed as described in the Methods section.

Incubation

A. minus initiation factors
B. with crude initiation factors, without added messenger
C. with crude initiation factors and polio mRNA
D. with dialyzed initiation factors and polio mRNA
E. with dialyzed initiation factors, polio mRNA and the dialyzable factor
F. with crude initiation factors and adeno mRNA
G. with dialyzed initiation factors and adeno mRNA
H. with dialyzed initiation factors, adeno mRNA and the dialyzable factor
I. stained control gel (adeno proteins)
J. with crude initiation factors and crystallin mRNA
K. with dialyzed initiation factors and crystallin mRNA
L. with dialyzed initiation factors, crystallin mRNA and the dialyzable factor.

On gel A and D the amount of protein synthesized is below detection level.
g = globin
c = crystallin

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reticulocytes (Fig. 2)(only shown for polio mRNA translation).

We stressed earlier that the RNA nature of the factor proposed by

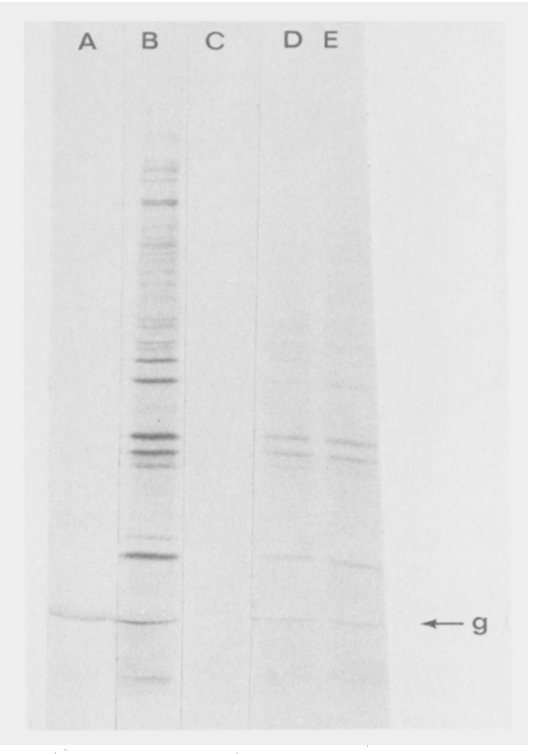


Fig. 2. Autoradiography of the SDS gel analysis of products synthesized in vitro in the presence of either crude reticulocyte KCl wash or dialyzed wash and the dialyzable factor from Krebs II Ascites tumor cells.

A. control minus mRNA, with crude initiation factors
B. incubation with crude initiation factors and polio mRNA
C. incubation with dialyzed initiation factors and polio mRNA
D. incubation with dialyzed initiation factors, polio mRNA and the dialyzable factor from reticulocytes
E. incubation with dialyzed initiation factors, polio mRNA and the dialyzable factor from Krebs II Ascites tumor cells.

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Bogdanovsky et al (1) is at least questionable (2). We observed that the activity of the factor was affected neither by alkali (0.3 N KOH at 37° for 15 h), nor by pancreatic RNAse treatment nor by heating for 5 min at 100°. In this connexion it has to be mentioned that Fuhr and Natta (9) could not detect any loss of activity of a presumptive small RNA species isolated from reticulocytes from patients with β-thalassemia upon treatment with RNAse A.

We separated the factor from KCl by gel filtration on a Sephadex G25

fine column. Furthermore we observed that the factor was not retained on a polystyrene column, and that it appeared to be soluble in ethanol but could not be extracted with ether. This excludes the possibility that the dialyzable factor is a lipid.

In all of our experiments only stimulation but no inhibition of heterologous messenger translation has been found. Therefore it is not very likely that the factor is identical to the so-called tcRNA (translation control RNA) described by Heywood et al (10). The latter authors isolated the activity from a purified $IF_3$ preparation and found only a negative control of translation of heterologous mRNA. The elucidation of the real nature of the respective factors awaits further investigation.

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