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A low-M_r factor isolated from *Escherichia coli* inhibits eukaryotic in vitro protein synthesis

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The effect of a low-M_r factor, partially purified from *E. coli* B, was investigated in *E. coli*, reticulocyte, and wheat germ lysate in vitro protein synthesis systems. Equal concentrations of factor were needed to inhibit protein synthesis in the eukaryotic system as compared to the prokaryotic system. Experiments suggested that the factor inhibits the initiation step in the eukaryotic systems.

Translation *Initiation* Escherichia coli *Protein synthesis inhibitor* *Protein synthesis inhibition* *Cell-free system*

1. INTRODUCTION

The isolation from *E. coli* K-12 of a low-M_r substance that inhibits protein synthesis in vitro has been reported in [1]. Using an in vitro translation system containing 'S-100' supernatant fraction, washed ribosomes from *E. coli*, R-17 RNA as a template and crude initiation factors, it was demonstrated that this factor inhibits formation of the 70 S initiation complex [2]. Although the chemical identity of the factor is not known, we have shown that it has $M_r < 500$, it is weakly cationic, it is highly polar, and it is stable to periodate oxidation, alkaline hydrolysis (1 M NaOH, 100°C, 0.5 h), and acid hydrolysis (6 M HCl, 110°C, 96 h) [1,2].

We describe here the purification procedure for the factor and its effect in vitro in wheat germ and reticulocyte lysate protein synthesis systems. The factor was able to inhibit eukaryotic protein synthesis completely, most probably at the initiation level.

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2. MATERIALS AND METHODS

2.1. *Partial purification of factor*

Escherichia coli B cells were grown in 0.4% glucose, minimal salts and 1 μg thiamine/ml [1] to 2×10^8 cells/ml and harvested by centrifugation. The pellet was resuspended in 1/10th vol. of the above media, colicin A at a multiplicity of ~10 was added, and the culture was incubated at 37°C for 5 min with vigorous shaking; 0.25 μCi [^{14}C]glucose/ml was then added and incubation continued for 35 min. The incubation was stopped when trichloroacetic acid was added to 10% final conc. After centrifugation the supernatant fluid was extracted with ether up to pH > 5. The supernatant fluid was then concentrated by flash evaporation, absolute ethanol was added to 90% final conc, and the precipitate was discarded. A second ethanol precipitation was done and the supernatant fluid was applied to an activated alumina column (Matheson, Coleman and Bell type F-20). Further details of the purification procedure are described under fig.1–4. One unit of factor was defined as the amount necessary to inhibit β-galactosidase induction in vivo by 50% in 20 min with 1 mg cellular protein as in [1].

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2.2. *In vitro protein synthesis*

The rabbit reticulocyte and wheat germ lysates were purchased from Bethesda Research Laboratories and 10 μg globin message/ml was used in a 0.01 ml reaction volume to direct translation. The *E. coli* cell-free extract was prepared as in [3] and 0.5 mg QB RNA/ml was used in a 0.01 ml reaction volume to direct translation [2]. After incubation at 37°C for the indicated times, a sample was removed, spotted on Whatman 3MM paper and boiled in 10% trichloroacetic acid for 10 min. The filters were washed once in H_2O, twice in ethanol, once in acetone, dried and 3 ml scintillation fluid was added to determine the amount of [^{35}S]methionine incorporated into acid-insoluble material.

3. RESULTS AND DISCUSSION

The purification procedure for factor described here yields material that is homogeneous on cation-exchange chromatography (fig.1–4). It is not pure, however, as determined by mass spectrometry. This lack of purity probably does not contribute artifactual inhibition of protein synthesis, since the effect of this factor preparation is a very specific inhibition of formation and destabilization of the *E. coli* 70 S initiation complex [2].

Since the *E. coli* factor inhibits prokaryotic in vitro protein synthesis, it was of interest to determine if it could also inhibit eukaryotic in vitro protein synthesis. The ability of various concentra-

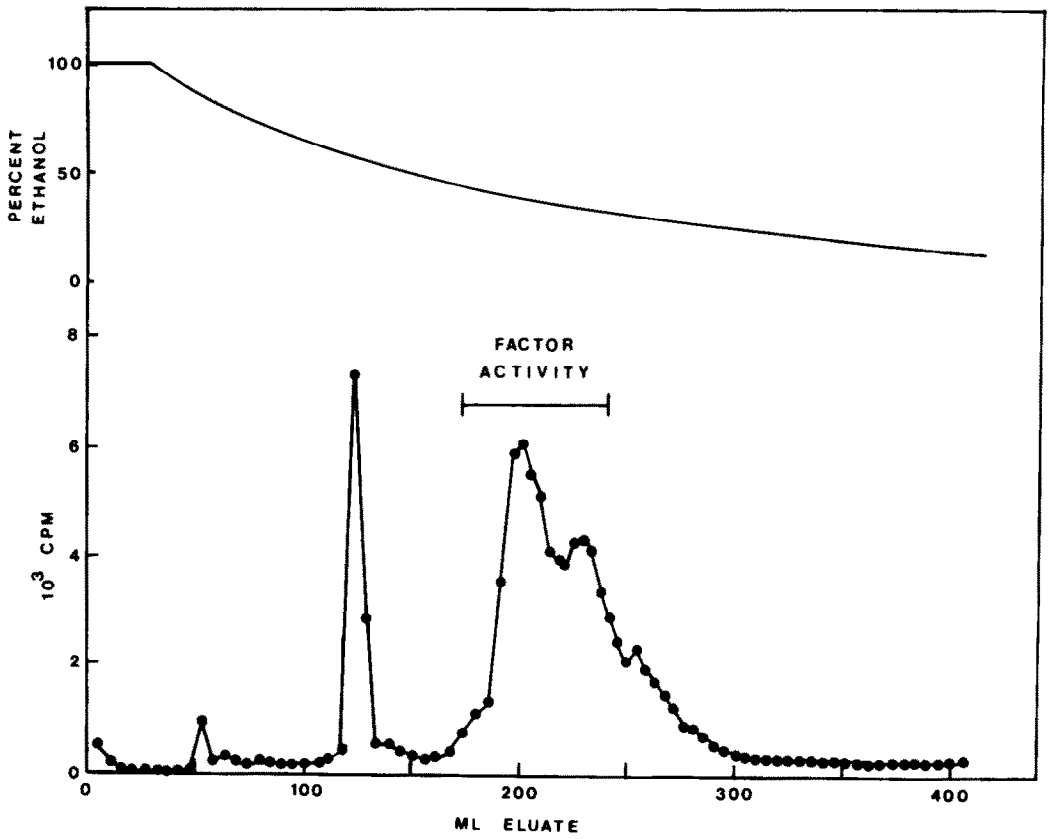

Fig.1. Alumina column chromatography: The supernatant fluid, prepared as in section 2, was applied to a dry column of alumina (2.5 cm × 41 cm). The column was eluted with a non-linear ethanol–H_2O gradient as indicated.

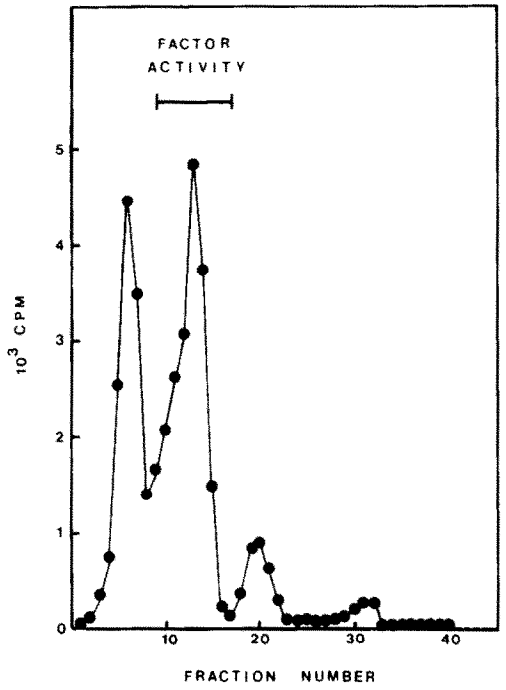

Fig.2. Paper chromatography: The fractions from the alumina column containing factor activity were pooled and applied to Whatman 3MM paper (1.5 cm × 60 cm). After descending chromatography overnight with *N*-butanol:ethanol:water (40:11:19, by vol.), the strip was dried, cut into 1 cm fractions, and water was used to elute the factor.

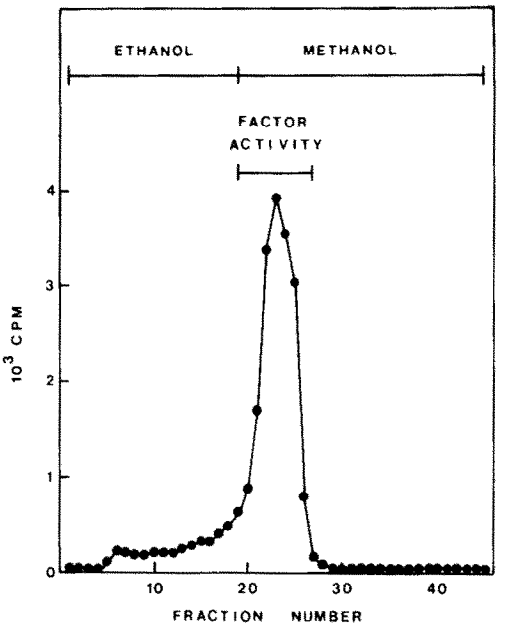

Fig.3. Silicic acid column chromatography: The pooled factor peak from the paper chromatography was dried, dissolved in absolute ethanol, and applied to a silicic acid column (1.2 cm × 20 cm). The column was eluted successively by absolute ethanol and methanol.

tions of factor to inhibit two different eukaryotic systems was compared with its inhibition of the *E. coli* in vitro system (fig.5). As can be seen, essentially complete inhibition by factor is achieved in all three systems. A *Tetrahymena thermophila* in vitro synthesis system using poly(U) as template was also completely inhibited (not shown).

Since the factor inhibits the initiation of translation in prokaryotes [2], attempts were made to determine if initiation is also the site of inhibition

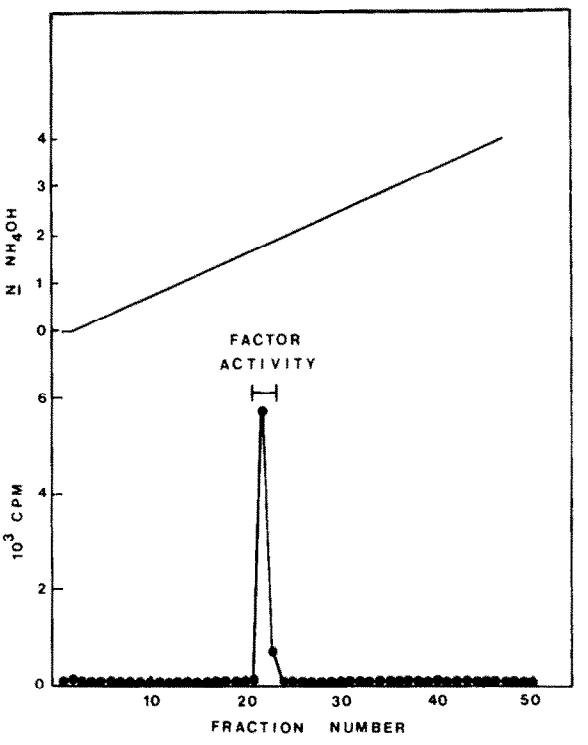

Fig.4. Dowex 50 (H^+) column chromatography: The pooled factor peak from the silicic acid column was dried, dissolved in H_2O, and applied to a Dowex 50 (H^+) column (1.2 cm × 20 cm). The column was eluted with a linear gradient of 0–4 N NH_4OH, the NH_4OH was removed by evaporation, and the residue, dissolved in H_2O, was used in the protein synthesis inhibition studies.

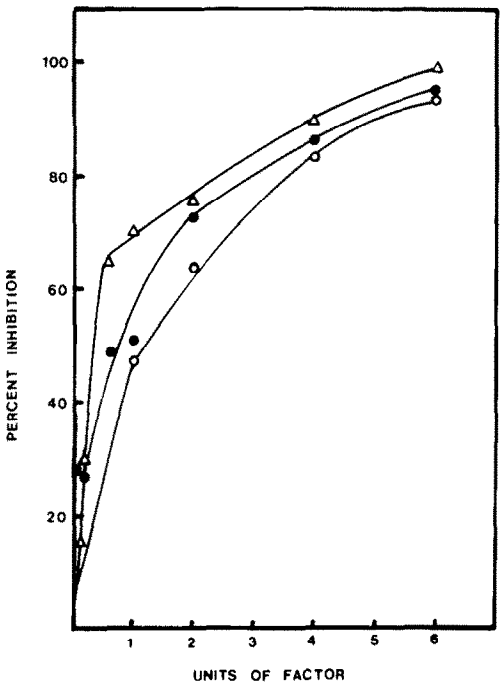

Fig.5. Inhibition of translation by the factor using the S-30 (●), rabbit reticulocyte (○) and wheat germ (△) in vitro systems. Concentrations of factor as shown were added prior to the lysate and the reaction continued for 20 min.

in the eukaryotic systems (table 1). When the factor was added to any of the 3 systems before the initiation of translation, essentially complete inhibition of synthesis was observed. No detectable high-M_r proteins were observed by SDS–polyacrylamide gel electrophoresis when the factor was added before protein synthesis was initiated (not shown). To differentiate between effects on initiation and elongation, the factor was added 12 min after initiation. The reaction was allowed to proceed for 15 min, and the inhibition of protein synthesis during the latter 15 min was determined (table 1). In contrast to addition of factor at 0 min, the addition of factor at 12 min did not completely inhibit further protein synthesis. The control experiments adding inhibitors of elongation, chloramphenicol and cycloheximide, resulted in complete inhibition of protein synthesis during this time period. The control experiment adding kasugamycin, a specific inhibitor of initiation in prokaryotes [4,5] gave results similar to those obtained with factor. These results suggest

Table 1

Inhibition of protein synthesis using eukaryotic and prokaryotic in vitro translational systems

In vitro system	% Inhibition		
	Inhibitor[a]	0 min[b]	12 min[c]
E. coli S-30 lysate	None	0	0
	CAP	–	100
	KSG	–	52
	Factor	95	63
Rabbit reticulocyte lysate	None	0	0
	CH	–	100
	Factor	94	67
Wheat germ lysate	None	0	0
	CH	–	96
	Factor	99	74

[a] Chloramphenicol (CAP), kasugamycin (KSG), cycloheximide (CH) and the *E. coli* factor were added to final conc. 200 μg/ml, 400 μg/ml, 50 μM and 600 units/ml, respectively

[b] Inhibitors were added before the addition of the designated lysate; the reaction was allowed to continue for 20 min

[c] Inhibitors were added to the in vitro assay 12 min after the initiation of translation and the reaction was allowed to continue for a further 15 min. Incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble material that occurred only in the latter 15 min interval was determined as in section 2

that once peptidyl chains have been initiated, the factor does not inhibit their completion, and that, therefore, the factor acts at the level of initiation of protein synthesis in eukaryotes as well as in prokaryotes.

It is extremely intriguing that a low-M_r compound isolated from a prokaryote is capable of affecting eukaryotic protein synthesis. The results presented here suggest that there is some step, probably in initiation, that is common to both prokaryotes and eukaryotes, and that this step is the target of action of the *E. coli* factor.

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REFERENCES

[1] Clark, V.L. (1979) J. Bacteriol. 137, 340–349.
[2] Clark, V.L. (1980) Proc. Natl. Acad. Sci. USA 77,1181–1184.
[3] Wilhelm, J.M. and Haselkorn, R. (1971) Methods Enzymol. 20, 527–536.
[4] Tanaka, N., Nishimura, T., Yamaguchi, H., Yamamoto, C., Yoshida, Y., Sashikata, K. and Umezawa, H. (1965) J. Antibiot. 18, 139–144.
[5] Okuyama, H., Machiyama, N., Kinoshita, T. and Tanaka, N. (1971) Biochem. Biophys. Res. Commun. 43, 196–199.