EFFECT OF A DISCRIMINATING FACTOR ON α AND β GLOBIN mRNA TRANSLATION

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1. Introduction

In the last few years, several authors have demonstrated the translation of varous mRNAs in heterologous cell-free systems in the absence of homologous initiation factors [1–5]. The rate of translation of a given mRNA varies from one cell-free system to another. A differential effect of the initiation factors from various origins has been established and competition experiments between two mRNAs proved the preferential translation of one mRNA with regard to the factors [6].

Factors with specificity for particular mRNAs have been purified from muscle cells [7] and ascites cells [8]. The translation of myoglobin mRNA and EMC RNA seems to be dependent on a specific factor.

In 1973 Nudel et al. [9] described a factor from rabbit reticulocyte ribosomes, the discriminating factor which in ascites cell-free extract stimulates preferentially the translation of α globin mRNA. But this factor is not specific for globin mRNAs since it also stimulates the translation of some viral RNAs.

Recently Lodish [10] postulated that the differential rates of protein synthesis is due to changes in the level of non-specific components required for initiation rather than to the presence of some specific factors.

In this work we studied the activity of the discriminating factor from polysomal KCl wash of reticulocytes [9]. A similar factor was obtained from microsomal KCl wash of mouse liver and Krebs mouse ascites cells. The preferential increase of α globin synthesis was found with the factors isolated from the various cells. The synthesis rate of α globin was also investigated with regard to increasing amounts of the reticulocyte discriminating factor.

2. Materials and methods

The extracts of mouse ascites cells and mouse liver cells (S_{30}) were prepared and preincubated according to Mathews and Korner [11]. The discriminating factor was isolated from rabbit reticulocyte ribosomes [9], from mouse liver and mouse ascites microsomes and purified either to the DEAE-cellulose or to the phosphocellulose step.

The cell-free systems contained for 1 ml of incubation medium: 50 μM Tris–HCl pH 7.8, 3 μM Mg $(CH_3\ COOH)_2$, 60 μM KCl, 1 μM ATP, 0.25 μM GTP, 10 μM creatine phosphate, 150 μg creatine phosphokinase, 4.5 μM GSH, 600 μg of the 19 aminoacids minus leucine (according to Borsook [12]), 300 μCi [^{3}H] Leu (35 Ci/mM) or 1.5 μCi [^{14}C] Leu (300 mCi/mM), 165 μl of preincubated S_{30}, rabbit globin mRNA (Searle), EMC or Mengo RNAs and the various discriminating factors.

Total incorporation of radioactive leucine into protein was measured after incubation of 60 μl of cell-free system. 300 μl were used when α and β globin synthesis was studied. After 1 h at 37°C, 20 mg of rabbit hemoglobin were added. The globins were separated on CM 52 column [13] and the radioactivity of each fraction was measured.

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3. Results

The total incorporation of leucine into proteins was measured in ascites S_{30} with globin mRNA and with each fraction obtained by the DEAE-cellulose fractionation of the KCl wash. The result was identical with the three KCl washes ; the highest stimulation was observed with the fraction eluted with approximately 190 mM KCl.

The reticulocyte DEAE fractions were also incubated in the presence of EMC and Mengo RNAs. The fractions eluted with 190 mM KCl stimulated strongly EMC RNA translation and to a lower rate the translation of Mengo RNA (data not shown).

The 190 mM KCl fractions obtained from the three types of cells were incubated in ascites S_{30} in the presence of globin mRNA. Table 1 shows that these three different factors stimulate preferentially α globin synthesis.

The ascites factor was also investigated in liver S_{30}. The DEAE-cellulose fractions which stimulated the total aminoacid incorporation in ascites S_{30} in the presence of globin mRNA inhibited the total incorporation in liver S_{30} (data not shown).
The activity of 190 mM ascites fractions on α and β globin synthesis was investigated in liver S_{30} (table 2). We noticed as previously a stimulation of α globin mRNA translation, while β globin mRNA translation was inhibited. This effect on α and β globin synthesis was found with amounts of globin mRNA ranging from 5 μg to 30 μg per ml.

The DEAE-cellulose fractions from reticulocyte and ascites cells with stimulating activity were further purified on phosphocellulose column and eluted with a stepwise KCl molarity.

The discriminating effect was essentially found in the fraction eluted with 300 mM KCl (P_{300}) (table 3). The reticulocyte and ascites P_{300} stimulated α globin

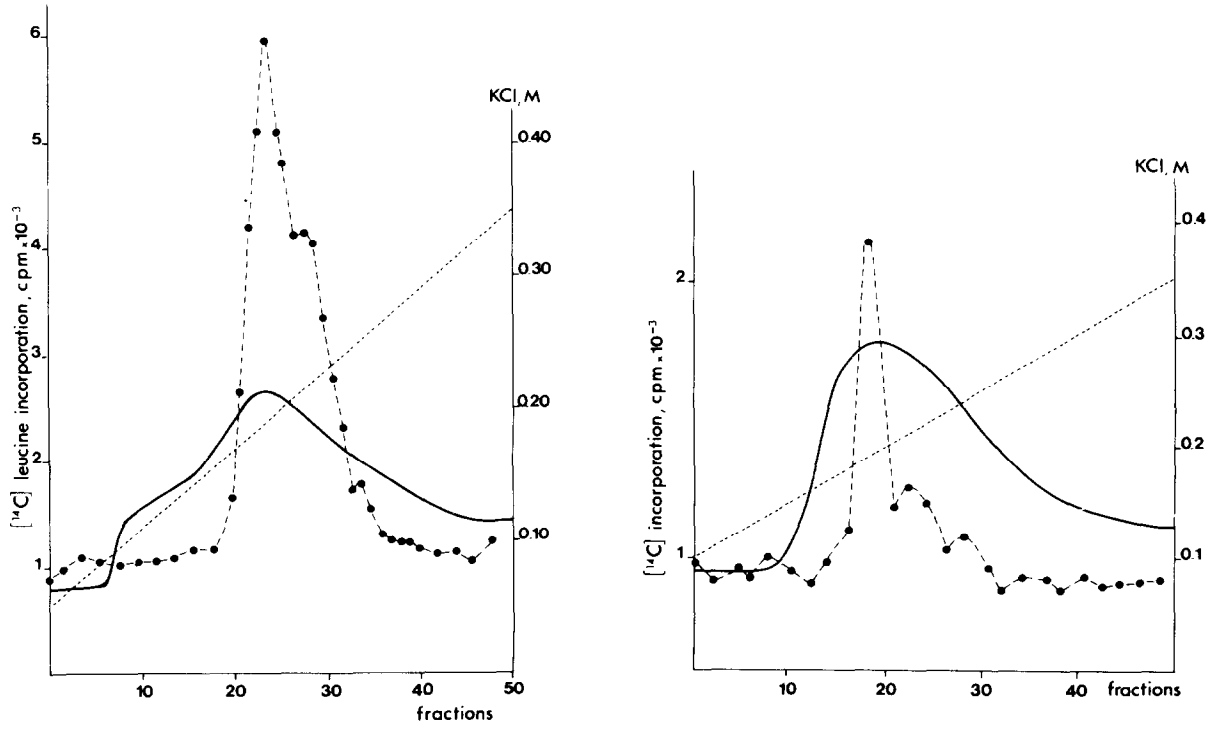

Fig.1. Effect of DEAE fractions on total ^{14}C incorporation. (a) Ribosomal 0.5 M KCl wash from rabbit reticulocytes was eluted from DEAE column by 50–350 mM KCl gradient. (b) Microsomal 0.5 M KCl wash from mouse liver was eluted from DEAE column by 100–350 mM KCl gradient. The fractions were incubated in ascites S_{30} in the presence of 2.5 μg globin mRNA/ml: (——) optical density; (– – – –) KCl molarity; (●–●) ^{14}C incorporation.

Table 1
Effect on α and β globin synthesis of DEAE fractions isolated from different cells

DEAE factor	[^{3}H]Leu incorporation (cpm) α globin	β globin	α/β ratio	Stimulation percent α globin	β globin
None	510	740	0.69		
Reticulocyte	2800	1910	1.46	450	160
None	2610	3230	0.81		
Liver	6710	4790	1.40	160	50
None	8170	25 400	0.32		
Ascites	52 930	59 560	0.89	550	130

The fractions eluted at 190 mM KCl on DEAE cellulose were incubated in ascites S_{30} in the presence of 2.5 μg of globin mRNA as indicated in Materials and methods. The globin chains were separated on CM 52 according to Dintzis and the incorporation for each chain was calculated by adding up the radioactivity of the fraction corresponding to optical density peaks.

synthesis with little or no effect on β globin (fig.3).

Increasing amounts of the P_{300} factor from reticulocytes were incubated in ascites S_{30} with globin mRNA. Stimulations of α and β globin synthesis with regard to the addition of increasing of P_{300} factor are shown in fig.4. A much greater effect is found on α globin synthesis.

4. Discussion

In this work we describe the isolation of a factor from rabbit reticulocytes, mouse liver and mouse ascites cells which stimulate preferentially α globin synthesis in an ascites cell-free system in the presence of globin mRNA. It is likely that this factor is not highly specific since it also stimulates viral mRNA

Table 2
Effect on α and β globin synthesis of DEAE fractions isolated from mouse ascites microsomes and incubated in liver S_{30}

Ascites Factor	Total incorporation (cpm) α globin	β globin	α/β ratio	Stimulation percent α globin	β globin
None	4390	4390	1.00		
Plus	7900	2915	2.71	79	− 34

The chain incorporation was established as in table 1.

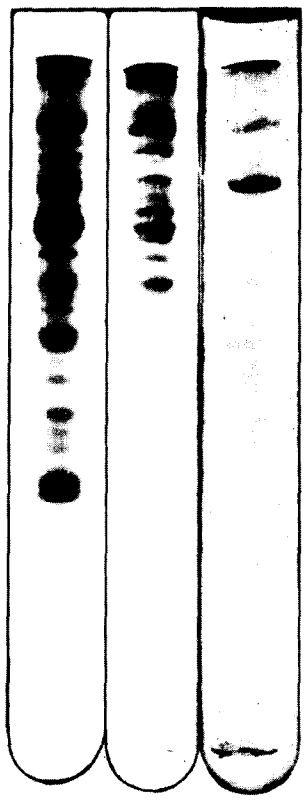

Fig.2. SDS polyacrylamide gel of: (a) crude reticulocyte KCl wash, (b) ammonium sulfate precipitate, (70%) (c) reticulocyte P_{300} fraction.

Table 3
Effect on α and β globin synthesis of the factors purified on phosphocellulose column

Factor	Total incorporation (cpm) α globin	β globin	α/β ratio	Stimulation percent α globin	β globin
None	25 510	35 750	0.71		
Reticulocyte	49 490	34 980	1.41	94	0
None	37 080	48 130	0.77		
Ascites	52 180	51 245	1.02	41	6

The factors eluted by 300 mM KCl (P_{300}) were incubated in ascites S_{30} with globin mRNA. Radioactivity was calculated as previously indicated.

translation. But its major characteristic is a differential translational effect on the different mRNAs. It translates EMC RNA with more efficiency than Mengo RNA, and α globin mRNA rather than β globin mRNA. This effect could be related to the secondary structure of these RNAs.

The question arises of the 'broad specificity' of the discriminating factor. A very similar factor is distributed among different cells even in non-hematopoietic cells. We cannot tell yet whether it is universally present in the cells. According to Lodish [10] a differential effect on the rate of synthesis does not require specific factors. Nevertheless in our experiments in liver cell-free systems, it is difficult to explain opposite effects on α and β globin synthesis by only non specific components. The relation of the discriminating factor with the various initiation factors described by Anderson [14] and by Staehelin [15,16] has not yet been determined.

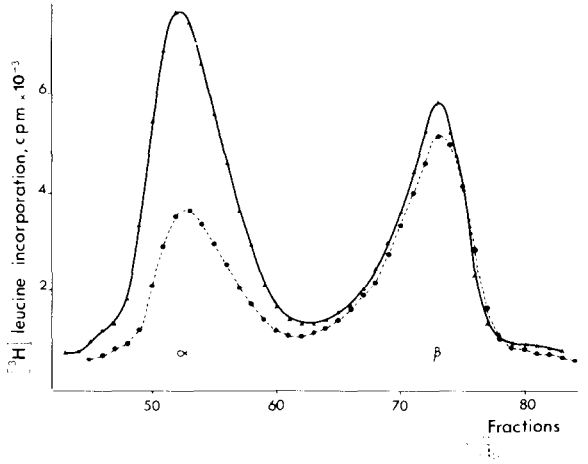

Fig.3. Effect of reticulocyte P_{300} factor on α and β globin synthesis. The globin synthesis was performed as described in Materials and methods. The product of the synthesis was separated on CM 52 column [13]. (●---●) incubation without reticulocyte factor; (▲——▲) incubation in the presence of P_{300} reticulocyte factor.

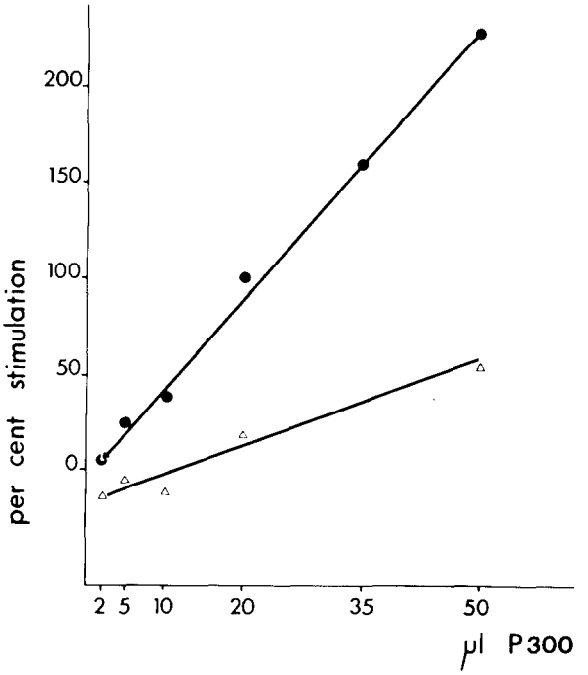

Fig.4. Effect of increasing amounts of reticulocyte P_{300} factor on the α and β globin synthesis in ascites S_{30}. For incubation conditions, see Materials and methods. (●——●) ^{3}H incorporation in α globin; (△——△) ^{3}H incorporation in β globin.

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