PRESENCE OF A NEW RNA SPECIES AMONG THE INITIATION PROTEIN FACTORS ACTIVE IN EUKARYOTES TRANSLATION

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SUMMARY : We have isolated a RNA of very small size from 0.5 M KCl wash prepared from rabbit reticulocytes in absence of magnesium. Some physico-chemical properties have been studied : it is single stranded, its base composition in p. 100 is 33 U, 7 G, 46 A and 14 C. The M.W. is approximately 11,000. This RNA is necessary for the biological activity of the dialyzed KCl-wash. It is probably a component either of an initiation factor (IF E_3) or of an interference factor. This RNA is required for the hemoglobin chain synthesis in a reticulocyte cell-free system.

Since MILLER et al. (1) and GODIN et al. (2) first described the effect of the 0.5 M KCl ribosomal wash fraction on in vitro hemoglobin synthesis in a cell-free system, there have been many data reported by different authors demonstrating that the active components of the ribosomal wash are protein factors required for the initiation of mRNA translation in eukaryotes (3, 4, 5). As reported by SCHAPIRA et al. (6), exogenous mRNA can be translated in a haterologous cell-free system, a fact which supposes a non specificity of initiation factors. Some recent studies (7, 8, 9), however, suggest the existence of factors able to select and bind specifically to mRNA.

In 1972, FUHR and NATTA (10) reported in the ultrafiltrate of a ribosomal wash from β-thalassaemia reticulocytes a RNA stimulating specifically β chain production in the complete system.

The present studies started from our observation that the 0.5 M KCl wash fraction of rabbit reticulocyte ribosomes (3, 4) lost much of its activity when dialyzed. We are able to reconstitute in a great part the activity of the dialyzed 0.5 M

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TABLE I

Activity of the KCl wash-RNA after phenol extraction

FACTORS	Activity p. Moles of ^{14}C leucine
0.5 M KCl wash	326
0.5 M KCl wash dialyzed	39
0.5 M KCl wash dialyzed + RNA	187
0.5 M KCl wash dialyzed + RNA extracted with phenol	214

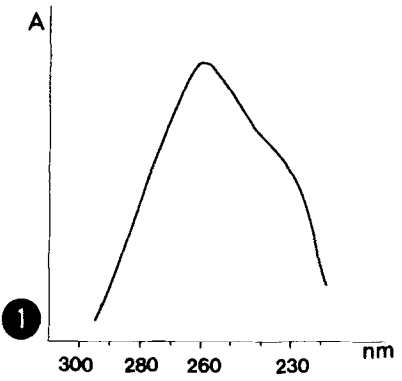
Activity was expressed in p. Moles of ^{14}C leucine incorporated in the mixture of 130 µl containing 0.1 mg of washed ribosomes, 500 µg of protein for the 0.5 M KCl wash dialyzed or not, and 0.5 µg of RNA, and 0.5 mg of pH 5 enzyme.

KCl wash fraction by adding the lyophilized ultrafiltrate, having first excluded the possibility that this was due to a mere ionic effect. We concentrated on isolating the active part of the ultrafiltrate, which turned out to be a RNA and which under the described conditions of preparation is free from associated proteins. This RNA was subsequently characterized by some physico-chemical properties as well as by its biological activity.

MATERIAL AND METHODS

Rabbit reticulocytes and ribosomes were prepared as indicated previously by MILLER and SCHWEET (11). For the extraction of the KCl factor, the ribosomes were resuspended in 0.25 M sacharose, 0.1 M Tris, adjusted at 0.5 M KCl and stirred for 2 hours. The final centrifugation (3 hrs at 130,000 g) separated washed ribosomes and 0.5 M KCl wash fraction containing initiation factors :

The 0.5 M KCl wash, diluted to 50 A_{260} units per ml, was dialyzed for 5 hours at 4° C in ten volumes of water. The



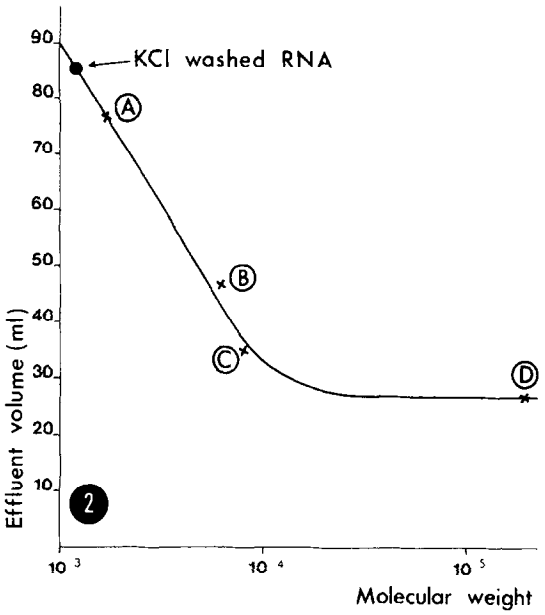


Figure 1 : U.V. Spectrum of KCl wash-RNA

Figure 2 : The Molecular Weight of the KCl wash-RNA determined according to (13) takes place in a curve of reference : A-myoglobin, B-hemoglobin, C-creatine kinase, D-blue dextran.

dialysis tubing had previously been boiled for 15 min. The dialysate was lyophilized and desalted on "Sephadex G 25" (column 48 x 1.5 cm), absorbance of the eluate was automatically recorded at 260 nm.

Biological tests were performed in a reticulocyte cell-free system containing : 30 mM Tris pH 7.8, 1.35 mM $MgCl_2$, 80 mM KCl, 0.8 mM ATP, 0.1 mM GTP, 10 mM creatine phosphate, 150 µg of creatine-kinase, 4.6 mM glutatione, 0.82 mg of an amino-acid mixture without Leucine, 2.5 µ Ci of ^{14}C Leucine (297 mCi/mM), 1 mg of washed ribosomes, 5 mg of pH 5 fraction and 0.1 ml of supernatant obtained after precipitation of the pH 5 enzyme in a total volume of 1.3 ml.

Hemoglobin chains were separated on CM cellulose columns as described by DINTZIS (12) with a linear gradient, between 0.02 N pyridine plus 0.2 N formic acid, and 0.2 N pyridine plus 2 N formic acid.

RESULTS

When desalting the 0.5 M KCl wash prepared according

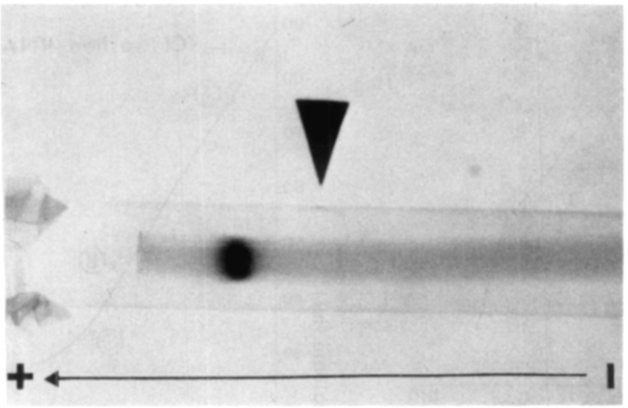


Figure 3 : Electrophoresis in polyacrylamide gel of KCl wash-RNA. The electrophoresis was carried out on 5 p. 100 polyacrylamide gel at 5 mA/tube for 90 min. The big arrow localizes the place of 4 S which is not present in this electrophoresis, and the single band is the KCl wash-RNA.

to (3) by simple dialysis against distilled water before polyacrylamide electrophoresis, we noticed that the dialysate contained a product which absorbed in U.V. ; dialysis against various buffers also leads to extraction of the KCl wash-RNA. On the average, we obtained 0.250 A_{260} units of RNA for 50 A_{260} units of the 0.5 M KCl wash fraction. However when the KCl wash was prepared in the presence of 4 mM $MgCl_2$, as indicated by SCHREIER and STAEHELIN (5), the ultrafiltrate contained almost no RNA.

Physico-chemical properties :

The U.V. absorbance spectrum of the RNA (fig. 1) showed a maximum at 255 nm and the A_{260}/A_{280} ratio was 1.9.

The molecular weight determined on "Sephadex G 25" superfine (13) was approximately 11,000 (Fig. 2).

Electrophoretic analysis of the KCl wash-RNA in 5 p. 100 polyacrylamide gels as indicated by LABRIE (14) revealed one single band, migrating ahead of bromophenol blue and ahead of 4 S RNA (Fig. 3).

For the determination of the base composition of the RNA, we hydrolyzed the fraction in 0.3 N KOH for 18 hours at 37° C. The ribonucleotides were separated on "Dowex 50" ion exchange resins according to KATZ and COMB (15). The base composition, as calculated from standard extinction coefficients, is in p. 100 : 33 U, 7 G, 46 A, 14 C.

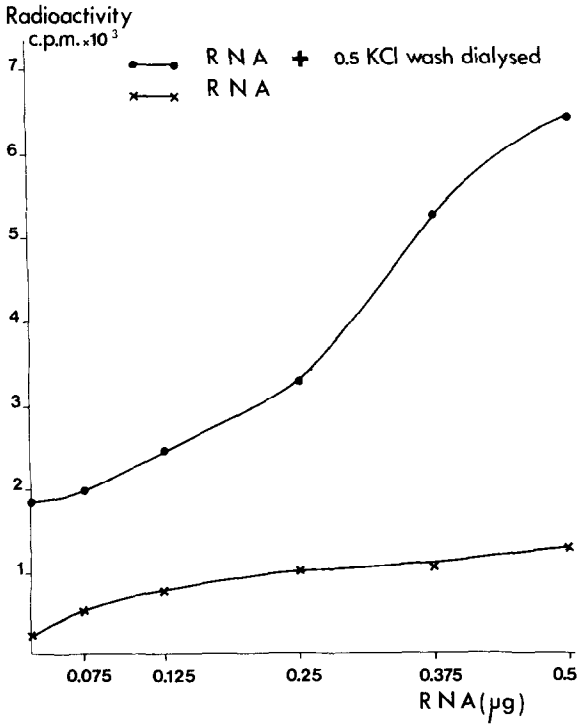


Figure 4 : Study of biological activity of the KCl wash-RNA, as measured by the biosynthesis of hemoglobins.

During heating from 20 to 95° C, the RNA showed constant optical absorbance at 260 nm.

It is concluded that the RNA is single stranded, a result which coincides with the base composition, and its molecular weight is very small.

Biological activity :

Without complementation, the dialyzed 0.5 M KCl wash fraction which still contains active initiation factors, showed only 5-15 p. 100 of the activity of crude initiation factors (Fig. 4). We could prove that this activation of the dialyzed initiation factors was really due to RNA and not to protein contaminant by submitting the RNA to repeated phenol extractions, which had no influence on its biological activity (Table I).

The addition of KCl wash-RNA is required for the synthesis of hemoglobin. Its effect is higher on the synthesis of α than of β globin chains (Table II).

DISCUSSION

We have been able to isolate a RNA of very small mole-

TABLE II

Effect of the KCl wash-RNA on the synthesis of α and β chains from globin

FACTORS	Specific activity cpm/A 280 nm	
	α chains	β chains
0.5 M KCl wash dialyzed (5 mg)	0.625	0.630
0.5 M KCl wash dialyzed (5 mg) + RNA (5 μg)	7.75	4.55

cular weight from the 0.5 M KCl wash of rabbit reticulocyte ribosomes, and to demonstrate its capacity to reactivate dialyzed initiation factors. FUHR and NATTA (10) described the existence of RNA both in the pH 5 fraction and in the KCl wash from β-thalassaemia reticulocytes, and obtained different activities for α and β globin chain synthesis according to the origin of the factor. As for rabbit reticulocyte initiation factors, the KCl wash stimulated mainly α chain synthesis, but the RNA was not isolated.

Recent studies on the specificity of some of the initiation factors may provide a possibility of interpreting our results. As reported by SCHREIER and STAEHELIN (9), a subcomponent of factor IF-E_3 seems to account for the binding of natural messenger after formation of the initiation complex. The authors suppose that this subcomponent, which could contain RNA, might determine specificity. Therefore, it may be possible that we have isolated an RNA molecule, or a family of RNA molecules or a fragment of RNA, which normally binds to an initiation factor.

Moreover it is now established that interference factors

intervene in eukaryotic protein synthesis (NUDEL et al.)(16). These factors are able to modify the α/β ratio in globin chain synthesis. The results, obtained with the KCl wash-RNA, correspond, as far as the biological activity is concerned, to those reported for interference factors.

We do not know yet to which initiation factor the newly described RNA is bound. The following hypothesis can be made : the RNA described here might be specifically complementary to a non coding sequence of mRNA.

In conclusion, the KCl wash-RNA has some of the characteristics of an initiation factor ($IF\text{-}E_3$), or of an interference factor ; it is absolutely necessary for initiation, as dialyzed factors are ineffective. We do not know if this RNA is intact or a fragment of a larger molecule ; even if it is a fragment, it retains its main biological activity.

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