REASSOCIATION OF EUKARYOTIC RIBOSOMAL SUBUNITS BY A FACTOR FROM MOUSE FIBROBLAST RIBOSOMES

David Kyner and Daniel H. Levin

Institute for Muscle Disease, Inc.
New York, New York 10021

Received October 5, 1972

SUMMARY

A factor contained in a 0.8 M KCl extract of L-cell ribosomes promotes the reassociation of highly purified L-cell 40S and 60S ribosomal subunits. No amino acyl-tRNA or mRNA is required. Only subunits stripped of initiation factors by differential treatment with 1 M KCl are responsive to the factor. Factor-dependent reassociation is optimal at 1.5 mM $MgCl_2$; above that concentration, there is increasing spontaneous reassociation in the absence of factor. During chromatography on DEAE-cellulose, the reassociation factor elutes at 50 mM KCl. Factor-dependent reassociation is considerably enhanced by GDPCP, and partially stimulated by GTP. Subunit reassociation is completely inhibited by 0.05 mM aurin tricarboxylic acid.

INTRODUCTION

During the course of studies on the function of eukaryotic initiation factors, we have partially purified a ribosomal factor from mouse fibroblasts (L-cells) which promotes the reassociation of highly purified L-cell 40S and 60S subunits. The eukaryotic reassociation factor (RF) is contained in a 0.8 M KCl extract of L-cell ribosomes and elutes at 50 mM KCl during chromatography on DEAE-cellulose. As previously reported, L-cell ribosomal subunits prepared in 0.5 M KCl retain their initiation factors and are not responsive to exogenous factors (1). However, differential treatment of these subunits with 1 M KCl (2) yields particles which respond to the reassociation factor at low $MgCl_2$ concentrations. The reassociation activity of the eukaryotic RF reported in these studies is similar to that of the prokaryotic association factor described by Garcia-Patrone et al (3, 4).

METHODS

The isolation and separation of L-cell subunits in 0.5 M KCl and the differential treatment of these subunits with 1 M KCl have been described (1, 2). All of the experiments in this report were carried out with the 1 M KCl-treated subunits. A 0.8 M KCl extract of L-cell ribosomes was

prepared as previously described (1). Chromatography on DEAE-cellulose of the ribosomal extract by a discontinuous KCl gradient yielded 4 protein fractions which eluted at 0.05 M KCl (fraction A), 0.1 M KCl (fraction B), 0.2 M KCl (fraction C), and 0.3 M KCl (fraction D). The reassociation factor (RF) was contained in fraction A. All fractions were concentrated by ammonium sulfate precipitation as described for the unfractionated factors (2).

RESULTS

A high salt wash of L-cell ribosomes containing initiation factors (IF) induced reassociation of 1 M KCl-treated 40S and 60S subunits of L-cell ribosomes (Fig. 1). Complete dependence on IF was demonstrated at 1.5 mM $MgCl_2$. Above this concentration, there was increasing spontaneous reassociation in the absence of factors reaching a maximum at 5 mM $MgCl_2$ (Fig. 1). Chromatography of the IF on DEAE-cellulose yielded 4 protein fractions A, B, C, and D (see Methods). The subunit reassociation factor (RF) was contained in fraction A which eluted at 50 mM KCl. None of the other protein fractions were effective in promoting reassociation (Fig. 2). Formation of the 80S monomer from the subunits was enhanced with increasing concentrations of RF (Fig. 3).

In the absence of added GTP, the efficiency of factor-dependent subunit reassociation was about 25%; this value increased to a level of 45% following GTP addition (Fig. 4). However, replacement of GTP with GDPCP produced a reassociation efficiency of 70% (Fig. 4). These data suggest that GTP is required for the association, but that no GTP hydrolysis occurs. The reassociation observed in the absence of GTP suggests the possibility that some GTP may be present in the factor preparation. The limited stimulation obtained with GTP cannot be explained at present. However, some GTPase activity is present in the factor preparation and it is possible that GTP hydrolytic products inhibit reassociation; this assumption is supported in part by the efficient reassociation obtained with GDPCP.

Factor-dependent reassociation of the 40S and 60S subunits was completely inhibited by the presence of 0.05 mM aurin tricarboxylic acid (ATA) in the reaction mixture (Fig. 5a). When reassociation was allowed to proceed normally (Fig. 5b), the delayed addition of ATA induced dissociation of the 80S couple (Fig. 5c). Inhibitors of protein synthesis such as

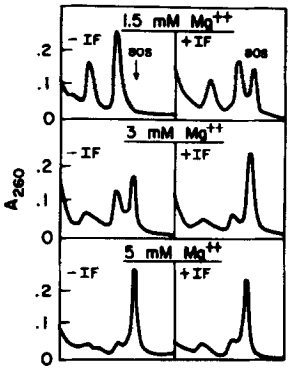

Fig. 1.

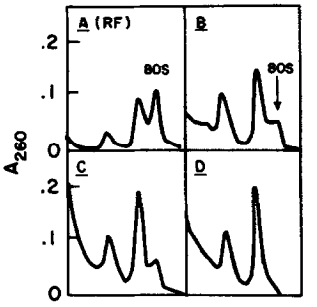

Fig. 2.

Fig. 1. Effect of unfractionated L-cell ribosomal initiation factors (IF) on reassociation of purified L-cell 40S and 60S subunits. Incubations contained the following components in a final volume of 50 µl: 40 mM Tris-HCl (pH 7.5), 50 mM KCl, $MgCl_2$ as indicated, 0.4 mM GTP, 1 mM DTT, 0.16 A_{260} units of 1 M KCl-treated 40S subunits (2), 0.32 A_{260} units of 1 M KCl-treated 60S subunits (2), and 25 µg of unfractionated initiation factors as indicated. After 10 min. incubation at 37°C, the reaction mixture was layered on a 5-20% sucrose density gradient containing 30 mM Tris-HCl (pH 7.5), 50 mM KCl, and the corresponding concentration of $MgCl_2$, and centrifuged in the SW-50.1 Spinco rotor at 40,000 rpm for 50 min. at 12°C. Gradients were monitored at A_{254} in an Isco Fractionator. Direction of sedimentation is left to right.

Fig. 2. Partial purification of reassociation factor (RF). Fractions A (RF), B, C, and D were obtained by chromatography on DEAE-cellulose of the unfractionated factors as described (see Methods). RF eluted at 50 mM KCl. Assays were as described in Fig. 1 except that 1.5 mM $MgCl_2$ was used throughout, and 12 µg of each protein fraction was added where indicated.

---

pactamycin (0.1 mM), fusidic acid (0.2 mM), puromycin (0.2 mM), and cycloheximide (0.2 mM) had no effect on reassociation; however, 0.2 mM streptomycin enhanced factor-dependent reassociation. This phenomenon is still under investigation.

DISCUSSION

In the present studies, it was observed that the reassociation of highly purified L-cell ribosomal subunits, which occurs spontaneously at 3-5 mM $MgCl_2$, becomes factor-dependent at 1.5 mM $MgCl_2$. The eukaryotic reassociation factor (RF) appears to be derived from the 40S subunits since the stripped but not the unstripped 40S subunits respond to factor-dependent

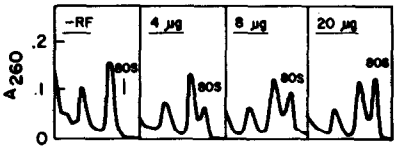

Fig. 3.

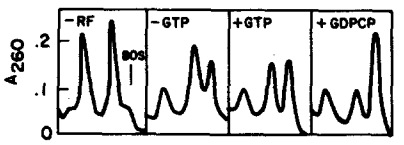

Fig. 4.

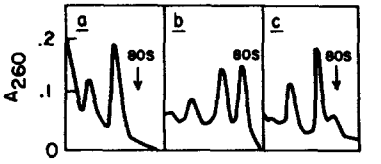

Fig. 5.

Fig. 3. Dependence of subunit reassociation on RF concentration. Assays were as described in Fig. 1 except that 1.5 mM $MgCl_2$ was used throughout. Amounts of RF protein are as indicated.

Fig. 4. Effect of GTP and GDPCP on subunit reassociation. Assays were as described in Fig. 1 except that 1.5 mM $MgCl_2$ was used throughout and 0.4 mM GTP or 0.4 mM GDPCP was added where indicated. In addition, 12 µg of RF was present in all experiments except the control (first panel).

Fig. 5. Effect of ATA on subunit reassociation. Assays were as described in Fig. 1 except that 12 µg of RF and 1.5 mM $MgCl_2$ were present in all experiments: (a) 0.05 mM ATA added at 0 min; (b) minus ATA; (c) 0.05 mM ATA added after 10 min. incubation followed by an additional 5 min. incubation at 37°C.

---

association; on the other hand, both the stripped and unstripped 60S subunit are responsive, which suggests that factor specificity resides in the 40S subunit. This finding is in agreement with Garcia-Patrone et al (4) who have demonstrated that the prokaryotic factor binds functionally to the 30S subunit. In addition, the prokaryotic and eukaryotic ribosomal factors are independent of amino acyl-tRNA and mRNA (3) in contrast to the reassociation factor from rat liver cytosol described by Wettenhall et al (5). It was of interest to find that the L-cell factor was inactivated by heating at 60°C for one minute whereas the prokaryotic factor, derived from Bacillus stearothermophilus (3), remained active after heating at 80°C for ten minutes (4).

It should also be noted that the partial requirement for GTP or GDPCP displayed by the eukaryotic factor is in contrast to the prokaryotic factor which requires no GTP (3).

The data reported here suggest that RF may play a role in the eukaryotic ribosome cycle in vivo by forming a reservoir of 40S-60S couples from subunits released from polyribosomes during protein synthesis. The presence of a reassociation activity implies the need for a dissociation mechanism to supply subunits for protein initiation. At present, it is not clear if this is accomplished by a RF which is reversible or by a mechanism similar to those described for the prokaryotic system (6, 7).

Studies still in progress indicate that the protein fraction A, which contains RF, also has a function in the final step of the protein initiation sequence which involves the formation of the 80S initiation complex from its precursors, the [40S, mRNA, Met-tRNA] complex and the 60S subunit (unpublished data). Whether this function is related to the reassociation activity or to some other factor(s) present in the same protein fraction is not yet resolved.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. George Acs for helpful discussions during the course of these studies and to Miss Gilda Castillo and Mr. Cornelius Whelan for excellent technical assistance. These studies were supported by the National Institutes of Health (CA-08751), the American Cancer Society (NP-36I), and the Muscular Dystrophy Associations of America.

REFERENCES

1. Levin, D.H., Kyner, D. and Acs, G., Proc. Natl. Acad. Sci. U.S. 69, 1234 (1972).
2. Levin, D.H., Kyner, D. and Acs, G., FEBS Lett. 25, 258 (1972)
3. Garcia-Patrone, M., Gonzalez, N.S. and Algranati, I.D., Proc. Natl. Acad. Sci. U.S. 68, 2822 (1971).
4. Garcia-Patrone, M., Gonzalez, N.S. and Algranati, I.D., FEBS Lett. 24, 126 (1972).
5. Wettenhall, R.E.H., Leader, D.P. and Wool, I.G., Biochem. Biophys. Res. Commun. 43, 994 (1971).
6. Subramanian, A.R. and Davis, B.D., Nature 228, 1273 (1970).
7. Kaempfer, R., Proc. Natl. Acad. Sci. U.S. 68, 2458 (1971).