PARTICIPATION OF INITIATION FACTOR IF-3 IN THE BINDING OF AcPhe-tRNA TO THE 30S RIBOSOMAL SUBUNIT

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SUMMARY

Initiation factor IF-3 is required in addition to IF-1 and IF-2 for maximal initial rate of poly(U)-directed binding of AcPhe-tRNA to 30S ribosomal subunits of E. coli. Incubation periods longer than 10 sec, by which time the reaction is virtually over, progressively obscure the requirement for IF-3 in AcPhe-tRNA binding. IF-3 also stimulates the poly(A, G, U)-directed binding of fMet-tRNA to the 30S ribosomal subunit, but in this case, significant stimulation can still be observed even with extended incubation. These results indicate that IF-3 functions similarly in the translation of synthetic mRNA, as it does with natural mRNA, participating in ribosome dissociation and in the formation of the initiation complex from the 30S ribosomal subunit.

The initiation factor IF-3 of bacteria is believed to facilitate the interaction of ribosomes with mRNA, in addition to functioning as a ribosome dissociating factor in the initiation of protein synthesis (1,2). According to some investigators, IF-3 enhances ribosome-mRNA interaction by conferring upon ribosomes the ability to recognize and bind specifically to the initiator sites of natural mRNA (3). Such a role for IF-3 is supported by the requirement reported for IF-3 in the translation of natural mRNA, but not of synthetic mRNA (4), and by the differential translation of various natural mRNAs observed in phage-infected E. coli extracts (5,6) and with purified IF-3 fractions (7,8). The observations that IF-3 is required in the translation of synthetic mRNAs initiated by fMet-tRNA and by AcPhe-tRNA (9-11), on the other hand, suggest a less precise role for IF-3 in the selection of the initiator sites of mRNA.

Studies carried out recently in two laboratories seemingly reconcile the observed requirement of IF-3 in the translation of synthetic mRNAs with its presumed role in recog-

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nizing specifically the initiator sites of natural mRNA (12, 13). On the basis of the observation that fMet-tRNA and AcPhe-tRNA binding to 70S ribosomes, but not to 30S ribosomal subunits, required IF-3, both groups concluded that IF-3 is necessary with synthetic mRNA merely to dissociate 70S ribosomes and that, unlike its dual role in the translation of natural mRNA, the factor is not involved in the formation of an initiation complex from the small ribosomal subunit. Since their observations and conclusion are at variance with those of this laboratory (14, 15), we present hereby further evidence for the participation of IF-3 in the formation of an initiation complex from the 30S ribosomal subunit with synthetic mRNA.

MATERIALS AND METHODS

Quarter-log E. coli B cells were purchased from Grain Processing Co., mid-log E. coli Q13 cells from General Biochemicals, poly(U) and poly(A, G, U) from Miles Laboratories, and dextran sulfate (MW 5×10^5) from Pharmacia. Phosphocellulose P-11 and DEAE cellulose DE-23 were from Reeve Angel. Purified $tRNA^{fMet}$ from E. coli K_{12} MO was a generous gift of Dr. G. D. Novelli.

The preparations of Ac[^{14}C]Phe-tRNA, f[^{3}H]Met-tRNA, and 30S ribosomal subunits were carried out as described previously (15). Initiation factors were purified from E. coli Q13 ribosomes, and were assayed by measuring the stimulation of AcPhe-tRNA binding to 30S ribosomal subunits (14).

The assay mixture contained in a final volume of 0.1 ml: 50 mM imidazole-HCl (pH 7.4), 8 mM $MgCl_2$, 80 mM NH_4Cl, 4 mM 2-mercaptoethanol, 0.2 mM GTP, about 10 µg of 30S ribosomal subunits preincubated 10 min at 37° in a buffer containing 0.5 M NH_4Cl and 10 mM $MgCl_2$ (16-18), and purified initiation factors. The mixture was equilibrated at 15°, and the binding reaction was initiated by addition of 20 pmoles of Ac[^{14}C]Phe-tRNA and 4 µg of poly(U). The reaction was stopped with 2.5 ml of buffer containing 10 mM imidazole-HCl (pH 7.4), 8 mM $MgCl_2$, 80 mM NH_4Cl, and 5×10^{-6} M dextran sulfate (freed of sulfate and phosphate by dialysis). The ribosomal

complexes were then adsorbed onto Millipore filters, washed with buffer, dried, and measured for radioactivity in a liquid scintillation system.

RESULTS

The binding of AcPhe-tRNA to 30S ribosomal subunits can be used as a convenient assay in the purification of all three initiation factors. The assay for each factor is carried out by measuring the stimulation of AcPhe-tRNA binding to 30S ribosomal subunits in the presence of saturating amounts of the other two factors. Activity profiles of the three initiation factors obtained by assaying phosphocellulose fractions of a crude preparation of the factors are presented in Fig. 1. The detection of three distinct peaks of activity, each revealed maximally only when assayed in the presence of components from the other two peaks, provides further evidence for the participation of IF-3 in the

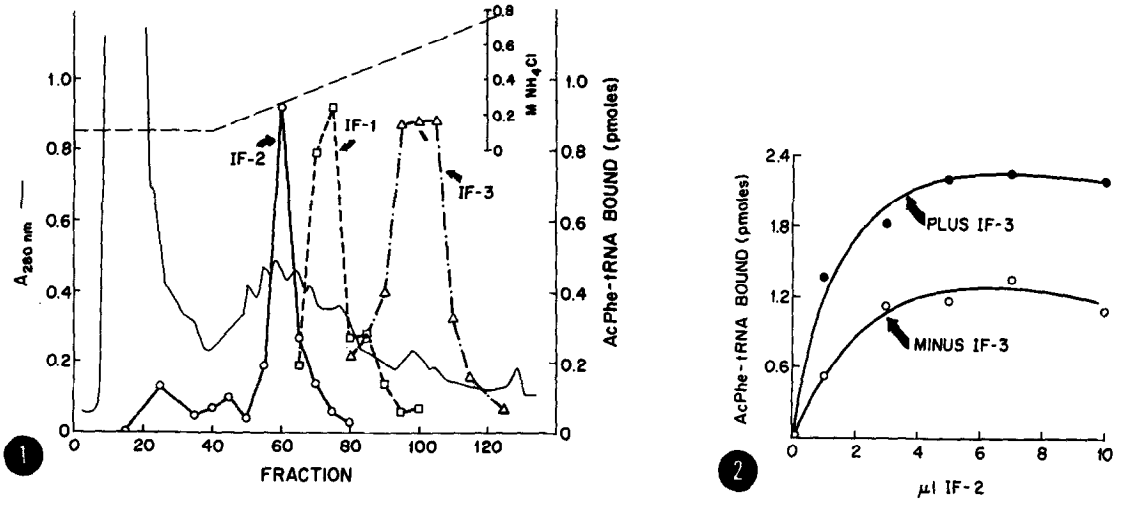


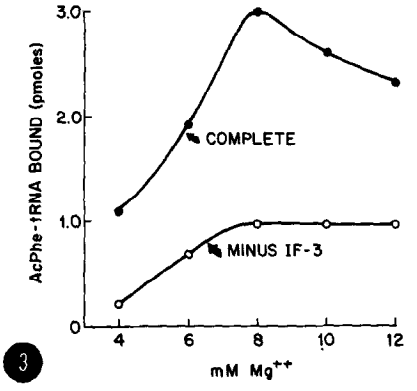
Fig. 1. Detection of initiation factors in phosphocellulose fractions by AcPhe-tRNA binding to the 30S ribosomal subunit. Chromatography of the factors on phosphocellulose was carried out as described previously (15). Each factor was assayed in a 10 sec incubation in the presence of saturating levels of the two complementary factors, as described in Materials and Methods. All values for binding have been corrected for background binding with complementary factors alone, which were: 0.5 pmoles, 0.1 pmoles, and 0.3 pmoles in the measurement of IF-1, IF-2, and IF-3 activity, respectively.

Fig. 2. Effect of increasing IF-2 concentration on the requirement of IF-3 in AcPhe-tRNA binding. Assay conditions were as described in Materials and Methods. Incubation was for 10 sec.

formation of an initiation complex from the 30S ribosomal subunit, poly(U), and AcPhe-tRNA. The peak fractions designated as IF-3 also exhibited ribosome dissociating activity (data not presented).

The addition of excess IF-2 does not eliminate the requirement of IF-3 for maximal rate of AcPhe-tRNA binding to 30S ribosomal subunits (Fig. 2). Each assay mixture contained IF-1 in saturating amounts. Factor IF-1 had been previously separated and was distinguished in our system from IF-2 and IF-3 by chromatography on DEAE cellulose, since IF-1 is not retained by the resin (19). The results presented in Fig. 2, therefore, in addition to distinguishing IF-3 from IF-2, show that IF-3 participates in the formation of the initiation complex from the 30S ribosomal subunit with AcPhe-tRNA as initiator tRNA.

Stimulation of the binding of AcPhe-tRNA to 30S ribosomal subunits by IF-3 is optimal at about 8 mM Mg^{++} with saturating levels of IF-1 and IF-2 (Fig. 3). This concentration of Mg^{++} is lower than that observed for polyphenylalanine synthesis initiated by Phe-tRNA; it corresponds to the optimum concentration of Mg^{++} observed for polyphenylalanine synthesis initiated with AcPhe-tRNA (17,20). The binding of AcPhe-tRNA to 30S



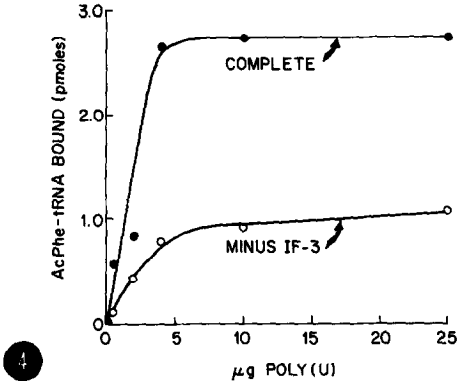


Fig. 3. Stimulation of AcPhe-tRNA binding by IF-3 at different concentrations of Mg^{++}. Assay conditions were as in Fig. 2.

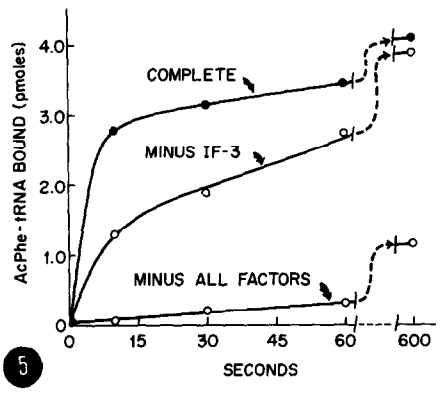
Fig. 4. Stimulation of AcPhe-tRNA binding by IF-3 at different concentrations of poly(U). Assay conditions were as in Fig. 2, except for the variation in poly(U) concentration.

ribosomal subunits is stimulated by IF-3 over a wide range of poly(U) concentrations (Fig. 4), not only at low concentrations as suggested for synthetic mRNAs (21).

The stimulation of AcPhe-tRNA binding to 30S ribosomal subunits by IF-3 is greatest with short periods of incubation (Fig. 5). Stimulation is strong at 10 seconds, but there is almost no stimulation by IF-3 after 10 minutes of incubation. The necessity of brief incubations in order to demonstrate dependence of AcPhe-tRNA binding on IF-3 is obvious from these results. The binding of fMet-tRNA to 30S ribosomal subunits directed by poly(A, G, U) is also stimulated by IF-3 in the presence of saturating amounts of IF-1 and IF-2 (Fig. 6). The stimulation by IF-3 after a 10-minute incubation in this case, however, is still significant.

DISCUSSION

This study has provided additional evidence for the participation of IF-3 in the formation of an initiation complex from the 30S ribosomal subunit with synthetic RNA as messenger. Thus, IF-3 appears to have a dual role in the translation of synthetic mRNA, as it has in the translation of natural mRNA. This suggests that IF-3 does not facilitate spe-



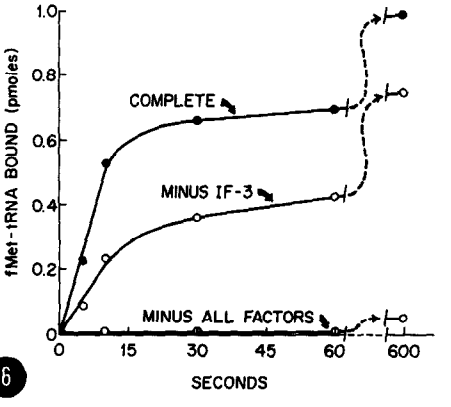


Fig. 5. Stimulation of AcPhe-tRNA binding by IF-3 as a function of time. Assay conditions were as described in Materials and Methods.

Fig. 6. Stimulation of poly(A, G, U)-directed binding of fMet-tRNA by IF-3 as a function of time. Reaction conditions were exactly as described in Materials and Methods for AcPhe-tRNA, except that poly(A, G, U) was added in place of poly(U) and f[^{3}H] Met-tRNA in place of Ac[^{14}C]Phe-tRNA.

cific ribosome recognition of initiation signals that are present only in natural mRNA, since it is very probable that the factor has the same dual role in the translation of synthetic mRNA (14).

The absence of an effect of IF-3 in the formation of an initiation complex from the 30S ribosomal subunit with synthetic mRNAs observed by Meier et al. (12) and Suttle et al. (13) may be due to the long incubation periods of 5-15 minutes utilized by these investigators. Our results have shown that the reaction is extremely vigorous, and that incubation periods of 5 to 10 seconds are sufficient. Indeed, prolonged incubation obscures the effect of IF-3.

It may well be that IF-3 facilitates the interaction of ribosomes with mRNA, but the precise role of the factor in this interaction and in the selection of the initiator site remains to be elucidated by further study. Such studies must involve the role of initiator tRNA, since recent evidence from this laboratory (15) and elsewhere (22) suggests that selection of initiation signals by ribosomes is accomplished via a complex of 30S ribosomal subunit and fMet-tRNA.

REFERENCES

1. Lucas-Lenard, J., and Lipmann, F. (1971) Annu. Rev. Biochem. 40, 409-448.
2. Haselkorn, R., and Rothman-Denes, L. (1973) Annu. Rev. Biochem. 42, 397-438.
3. Revel, M., Herzberg, M., and Greenspan, H. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 261-275.
4. Iwasaki, K., Sabol, S., Wahba, A., and Ochoa, S. (1968) Arch. Biochem. Biophys. 125, 542-547.
5. Hsu, W.-T., and Weiss, S. (1970) Proc. Nat. Acad. Sci. USA 64, 345-351.
6. Revel, M., Brawerman, G., Lelong, J., and Gros, F. (1968) Nature 219, 1016-1019.
7. Lee-Huang, S., and Ochoa, S. (1971) Nature New Biol. 234, 236-239.
8. Revel, M., Greenspan, H., Aviv, H., Groner, Y., and Pollack, Y. (1970) FEBS Lett. 9, 213-217.
9. Dubnoff, J., and Maitra, N. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 301-306.
10. Grunberg-Manago, M., Rabinowitz, J., Dondon, J., Lelong, J., and Gros, F. (1971) FEBS Lett. 19, 193-200.
11. Vermeer, C., Talens, J., Bloemsma-Jonkman, F., and Bosch, L. (1971) FEBS Lett. 19, 201-206.
12. Meier, D., Lee-Huang, S., and Ochoa, S. (1973) J. Biol. Chem. 248, 8613-8615.

13. Suttle, D., and Ravel, J. (1974) Biochem. Biophys. Res. Comm. 57, 386-393.
14. Bernal, S., Blumberg, B., and Nakamoto, T. (1974) Proc. Nat. Acad. Sci. USA 71, 774-778.
15. Blumberg, B., Bernal, S., and Nakamoto, T. (1974) Biochemistry 13, 3307-3311.
16. Nakamoto, T., and Hamel, E. (1968) Proc. Nat. Acad. Sci. USA 59, 238-245.
17. Kiem, E., and Nakamoto, T. (1968) Proc. Nat. Acad. Sci. USA 61, 1349-1355.
18. Zamir, A., Miskin, R., and Elson, D. (1969) FEBS Lett. 3, 85-88.
19. Dubnoff, J., and Maitra, U. (1971) Methods Enzymol. 20, 248-261.
20. Economou, A., and Nakamoto, T. (1967) Proc. Nat. Acad. Sci. USA 58, 1033-1039.
21. Yoshida, M., and Rudland, P. (1972) J. Mol. Biol. 68, 465-481.
22. Gilbert, J., and Kaempfer, R. (1974) Proc. Nat. Acad. Sci. USA, in press.