INITIATION AND PROTEIN SYNTHESIS: TRANSLATION OF DI- AND TRI-CODON MESSENGERS

Richard W. Erbe and Philip Leder

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health Education, and Welfare, Bethesda, Maryland 20014

Received May 6, 1968

Translation of mRNA[1] involves a complex array of enzymes, factors and cofactors first identified in the laboratories of Lipmann (Nakamoto et al., 1963), Moldave (Fessenden and Moldave, 1962) and Schweet (Hardesty et al., 1963). In order to correlate these elements with protein synthesis we have synthesized two small RNA messengers initiated with an F-Met codon, $AUGU_3$ and $AUGU_6$. These direct binding of F-Met- and Phe-tRNA's to ribosomes, and synthesis of F-Met initiated Phe peptides. A simple assay specifically measures synthesis of the latter. We have found that translation of the first codon of these small messengers requires purified initiation factors and GTP. Translation of the second codon depends upon translation of the first and requires one of two complementary transfer factors. The product formed by binding in response to the first two codons is converted to dipeptide without further additions. The second transfer factor stabilizes the peptidyl-tRNA-ribosome complex and makes the third codon available for translation, apparently by translocation of both peptidyl-tRNA and mRNA.

---

[1] Abbreviations: mRNA, messenger RNA; F-Met, formyl-methionine; Met, methionine; Phe, phenylalanine; tRNA, transfer RNA; $AUGU_3$ and $AUGU_6$, trinucleoside diphosphate, ApUpG, followed by three and six, 5'-uridylic acid residues.

MATERIALS AND METHODS

Ribosomes, initiation and transfer factors, AA-tRNA, and oligonucleotides:

1 M NH_4Cl-washed E. coli MRE-600 ribosomes were prepared as described previously (Leder and Nau, 1967); initiation factors free of transfer factors by a modification of the method of Stanley et al., (1966); and transfer factors T and G of Nishizuka and Lipmann (1966) by a modification of Lucas-Lenard and Lipmann (1966). The preparation of mixed F-^{3}H-Met- and ^{14}C-Phe-tRNA's, and ^{14}C-Phe-tRNA from unfractionated E. coli B tRNA has been described (Leder and Bursztyn, 1966). $AUGU_3$ and $AUGU_6$ were prepared, purified and characterized by a modification (Leder, 1968) of the method of Leder, Singer and Brimacombe (1966).

Assays: Reaction components and conditions are given in the legend to each table and figure. Binding: mRNA directed ribosomal binding was measured by the nitrocellulose filter assay (Nirenberg and Leder, 1964). Synthesis of blocked oligopeptides: The reaction, in conical 12 ml centrifuge tubes, was stopped by addition of 1.2 ml 0.1 N KOH, and incubation resumed for 30 min at 37^o to hydrolyze aminoacyl- and peptidyl-tRNA's. Following acidification with 0.1 ml 12 N HCl, F-Met-^{14}C-Phe and/or F-Met-^{14}C-Phe-^{14}C-Phe were extracted with 1.5 ml ethyl acetate. Tubes were centrifuged at 2000 rpm for one min and 1.0 ml of the upper ethyl acetate phase was transferred to 10 ml Bray's solution for liquid scintillation counting. Product analysis: The products extracted in ethyl acetate were electrophoresed on Whatman No. 1 paper in pyridine acetate, pH 3.5, 0.05 M, at 53 volts/cm for 75 min. One by three cm strips were taken for liquid scintillation counting. Migration of the peptides containing ^{14}C-Phe was compared to that of authentic samples of F-Met-Phe, F-Met, Met and

Phe. All peptides were characterized further by cyanogen bromide degradation and paper chromatographic comparison to authentic Phe and Phe-Phe.

RESULTS AND DISCUSSION

At 5 mM Mg^{2+}, Table I, recognition of the initial AUG codon, reflected by ribosomal binding of F-Met, depends upon initiation factors and GTP (Salas et al., 1967; Leder and Nau, 1967; Anderson et al., 1967). Recognition of the second codon, reflected by ribosomal binding of Phe, also requires initiation factors and GTP, and depends stringently upon T. In the absence of transfer factor G, binding of both F-Met- and Phe-tRNA's is moderately reduced compared to the complete system, an observation related to the stabilizing effect of G noted below.

Table I

$AUGU_3$ Directed Ribosomal Binding of F-Met-, Phe- and F-Met-Phe-tRNA's, and Synthesis of F-Met-Phe at 5 mM Mg^{2+}

Reaction System	F-^{3}H-Met in Ribosome	^{14}C-Phe Bound Product	F-Met-^{14}C-Phe Synthesized
		μμ moles	
Complete	3.20	2.08	2.48
minus initiation factors	0.31	0.35	0.72
minus T factor	2.94	0.35	0.11
minus G factor	2.20	1.61	2.75
minus GTP	1.03	0.69	0.59

Each complete 0.05 ml reaction mixture contained Tris-Ac, pH 7.2, 0.05 M; $MgAc_2$, 5 mM; NH_4Cl, 0.05 M; ribosomes, 0.3 A^{260} unit; $AUGU_3$ or $AUGU_6$ as oligonucleotide, 0.04 mM; ^{14}C-Phe-tRNA, 7 μμmoles (single label), or F-^{3}H-Met-tRNA, 20 μμmoles, with ^{14}C-Phe-tRNA, 9 μμmoles (double label); GTP, 1 mM; initiation factors, 5.2 μg; T factor, 12 μg; G factor, 11 μg. Incubation was at 23° for 30 min.

Synthesis of the dipeptide F-Met-Phe depends upon all components required for binding in response to both first and second codons. G, however, is not required for dipeptide synthesis. In experiments not shown (Erbe, Nau and Leder, in preparation) at 10 mM Mg^{2+} binding of Phe-tRNA requires only T and GTP, and is independent of both recognition of the first codon and peptide bond formation. The effect of T on binding agrees with previous observations of Ravel (1967), Lucas-Lenard and Haenni (1968) and Ertel et al., (1968). It is clear from Table I that dipeptide formation occurs readily when F-Met- and Phe-tRNA's are bound to ribosomes in response to adjacent codons. The peptide bond forming activity is associated with the 50S ribosomal subunit (Monro, 1967).

Although G is not required for F-Met-Phe synthesis, the binding of F-Met- and Phe-containing product to ribosomes is reduced by one-third when G is omitted (Table I). Under these conditions, over 90% of the bound product is in the form of the dipeptidyl-tRNA (Erbe, Nau and Leder, in preparation). G stabilizes the F-Met-Phe-tRNA-ribosome complex as shown in Table II. In the absence of G the amount of Phe-containing product bound to ribosomes is reduced. This is accompanied by the appearance of an approximately equimolar amount of F-Met-Phe-tRNA in the ribosome-free filtrate. The product is apparently released as peptidyl-tRNA, for it is not extractable in ethyl acetate prior to alkaline hydrolysis. The stabilizing effect of G is compatible, among other possibilities, with translocation of of the peptidyl-tRNA to a site of greater affinity on the ribosome.

The role of G in the translocation process was examined more directly by comparing its effect on the translation of di- and tri-codon messengers. Fig 1 shows an electrophoretic analysis of the peptide products extracted in ethyl acetate. With the dicodon messenger, F-Met-Phe is synthesized in

Table II

Effect of G Factor on the Stability of the F-Met-Phe-tRNA-Ribosome Complex

Reaction System	^{14}C-Phe in Ribosome Bound Product	F-Met-^{14}C-Phe-tRNA Released
Complete	2.21	1.06
minus G factor	1.24	1.88

Components of reaction mixtures and conditions of incubation are as noted in Table I. F-Met-^{14}C-Phe-tRNA release was measured in the nitrocellulose filtrate by the blocked oligopeptide assay technique.

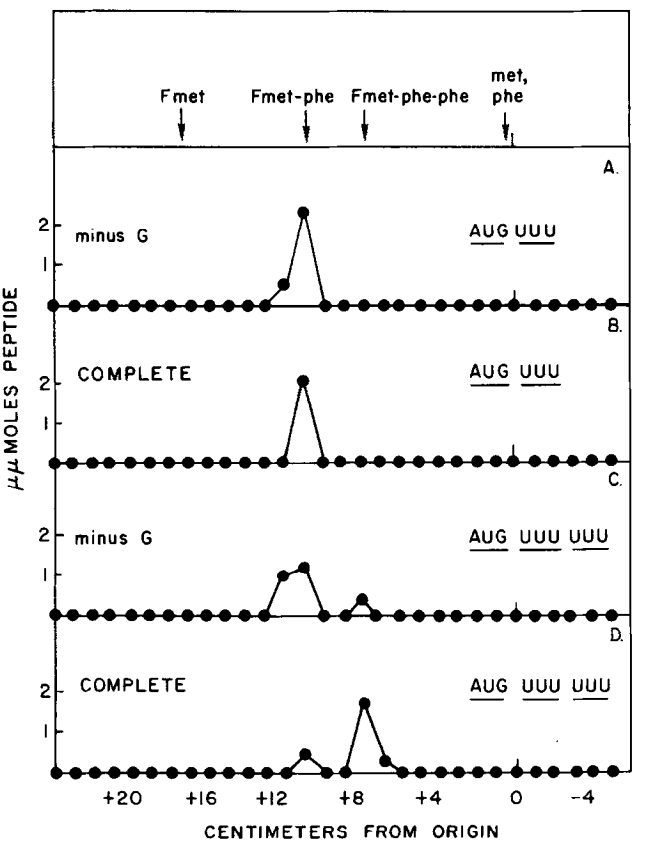

Fig. 1. Electrophoretic analysis of peptide products. Components of two-fold reaction mixtures and conditions of incubation are as noted in Table I and in the figure. Analytic procedure is given under Materials and Methods.

nearly equal amounts in the absence and presence of G (Fig 1A and 1B). In the absence of G translation of the tricodon messenger is incomplete, for only the first two codons are translated and the major product is the dipeptide, F-Met-Phe (Fig 1C). Translation of the third codon depends upon transfer factor G and the product of this reaction is the tripeptide, F-Met-Phe-Phe (Fig 1D). Thus only in the presence of G is the third of three adjacent codons translated. This is consistent with translocation of mRNA, exposing the next available codon for translation.

REFERENCES

Anderson, J. S., Dahlberg, J. E., Bretscher, M. S., Revel, M., and Clark, B. F. C., Nature, 216, 1072 (1967).

Erbe, R. W., Nau, M. M., and Leder, P., Manuscript in preparation.

Ertel, R., Brot, N., Redfield, B., Allende, J. E., and Weissbach, H., Proc. Nat. Acad. Sci., 59, 861 (1968).

Fessenden, J. M., and Moldave, K., Biochemistry, 1, 485 (1962).

Hardesty, B., Arlinghaus, R., Shaffer, J., and Schweet, R., Cold Spring Harbor Symp. Quant. Biol., 28, 215 (1963).

Leder, P., Methods in Enzymology, XII (1968). In the press.

Leder, P., and Bursztyn, H., Proc. Nat. Acad. Sci., 56, 1579 (1966).

Leder, P., and Nau, M. M., Proc. Nat. Acad. Sci., 58, 774 (1967).

Leder, P., Singer, M. F., and Brimacombe, R. L. C., Biochemistry, 4, 1561 (1965).

Lucas-Lenard, J., and Lipmann, F., Proc. Nat. Acad. Sci., 55, 1562 (1966).

Lucas-Lenard, J., and Haenni, A.-L., Proc. Nat. Acad. Sci., 59, 554 (1968).

Monro, R. E., J. Mol. Biol., 26, 147 (1967).

Nakamoto, T., Conway, T. W., Allende, J. E., Spyrides, G. J., and Lipmann, F., Cold Spring Harbor Symp. Quant. Biol., 28, 227 (1963).

Nirenberg, M. W., and Leder, P., Sci., 145, 1399 (1964).

Nishizuka, Y., and Lipmann, F., Proc. Nat. Acad. Sci., 55, 212 (1966).

Ravel, J. M., Proc. Nat. Acad. Sci., 57, 1811 (1967).

Salas, M., Miller, M. J., Wahba, A. J., and Ochoa, S., Proc. Nat. Acad. Sci., 57, 1865 (1967).

Stanley, W. M., Jr., Salas, M., Wahba, A. J., and Ochoa, S., Proc. Nat. Acad. Sci., 56, 290 (1966).