Volume 233, number 1, 95–99 FEB 05913 June 1988

Is translation inhibited by noncognate ternary complexes?

Neşe Bilgin, Måns Ehrenberg and Charles Kurland

Dept of Molecular Biology, Uppsala University, The Biomedical Center, Box 590, S-751 24 Uppsala, Sweden

Received 30 March 1988

We studied the influence of an error-prone isoacceptor ($tRNA^{Leu}_4$), as well as an intermediate ($tRNA^{Leu}_2$) and a weak ($tRNA^{Val}$) competitor of $tRNA^{Phe}$ on the poly(Phe) synthesis rate. Even at very high excess concentrations of these noncognate ternary complexes there was no significant effect on the translation rate. Our result argues against the assertion that in vivo translation is slowed down by noncognate tRNA and favours the hypothesis that the incorrect ternary complex concentrations are too low to saturate the ribosomes in vivo.

Protein synthesis; Translational regulation; Ribosomal A-site; (*E.coli*)

1. INTRODUCTION

It has long been the view that because of its large mass the translation apparatus should operate close to its maximum rate [1,2]. Indeed, the semi-empirical calculations of Gouy and Grantham [3] seem to support this conjecture. In their model the time for tRNA association is an order of magnitude shorter than the time for a whole elongation cycle. It could be argued that given the large excess of noncognate tRNA species at each codon, the ribosome might spend more time sorting out the noncognate tRNA species than processing the cognate ones [3]. If this were the case, we might expect the ribosome to be saturated by noncognate tRNA species [4–6].

Our work on the optimal design of the translation apparatus suggests that it is only when cells grow at the very fastest rates that ribosomes should operate near their maximum rates [7]. Thus, we expect ribosomes to function in vivo under most growth conditions at rates that are determined by the concentrations of cognate ternary complexes and the kinetic efficiency of their interactions with codon-programmed ribosomes. This design strategy seems to be reflected in the growth characteristics of both wild type and mutant bacteria with altered translational accuracy phenotypes [8]. Here, we wish to explore the possibility of a related design strategy that is associated with the accuracy of ribosome function.

It is clear to the extent that noncognate tRNA species occupy the ribosome and block access of cognate tRNA species to the ribosome. In other words, noncognate tRNA species can function as competitive inhibitors of cognate translation, owing to the extent that they bind to the ribosome. The question we pose here is whether the time for sorting noncognate tRNA species on the codon-programmed ribosome can be made sufficiently short that this inhibitory effect is reduced to insignificance. Our data suggest that noncognate tRNA species have a vanishingly small inhibitory effect on cognate translation in vitro. We conclude that the evolutionary options for the translation apparatus include a strategy for minimizing the influence of noncognate tRNA species on the kinetics of cognate translation.

2. MATERIALS AND METHODS

2.1. *Chemicals*

^3H- and ^{14}C-labelled phenylalanine, leucine and valine were

Correspondence address: N. Bilgin, Dept of Molecular Biology, Uppsala University, The Biomedical Center, Box 590, S-751 24 Uppsala, Sweden

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

obtained from Amersham (Buckinghamshire). Non-labelled amino acids, phospho*enol*pyruvate, GTP, nutrescine, spermidine, myokinase (EC 2.7.4.3), pyruvate kinase (EC 2.7.3.40) were purchased from Sigma (St. Louis). Poly(U) and ATP were obtained from Pharmacia (Uppsala).

2.2. *Purifications and preparations*

E. coli MRE-600 cells for enzyme and tRNA preparations and 017 cells [9] for ribosome preparations were grown under good aeration at 37°C in TY2 medium supplemented with 0.2% glucose. All cells were harvested at the late exponential phase by centrifugation. 017 cells were in addition washed with polymix buffer [10]. Cells were stored at −80°C prior to use.

Ribosomes were purified as in [11]. EF-G was purified as in [12], EF-Tu as in [13,14], EF-Ts as in [15]. Phe-tRNA synthetase, Leu-tRNA synthetase [14] and Val-tRNA synthetase [16] were prepared as described before. One unit of tRNA synthetase is defined as the amount that aminoacylates 1 pmol of tRNA per second under the conditions of the assay. tRNA (bulk) was purified on DEAE-cellulose (Whatman DE-52) according to [17]. $tRNA^{Phe}$, $tRNA_2^{Leu}$, $tRNA_4^{Leu}$ and $tRNA^{Val}$ were partially purified on BD cellulose (Boehringer) according to [18]. $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$ were further purified on Sepharose 4B (Pharmacia) according to [19] and $tRNA^{Val}$ on DEAE-Sephadex A-50 (Pharmacia) according to [20]. ^3H- and ^{14}C-labelled *N*-acetyl-Phe-$tRNA^{Phe}$ was prepared as described earlier [14]. All components were dialyzed against polymix and stored at −80°C, except tRNA synthetases, which were kept at −20°C in polymix containing 50% glycerol.

2.3. *Assays*

A poly(U) directed poly(Phe) synthesizing system optimized

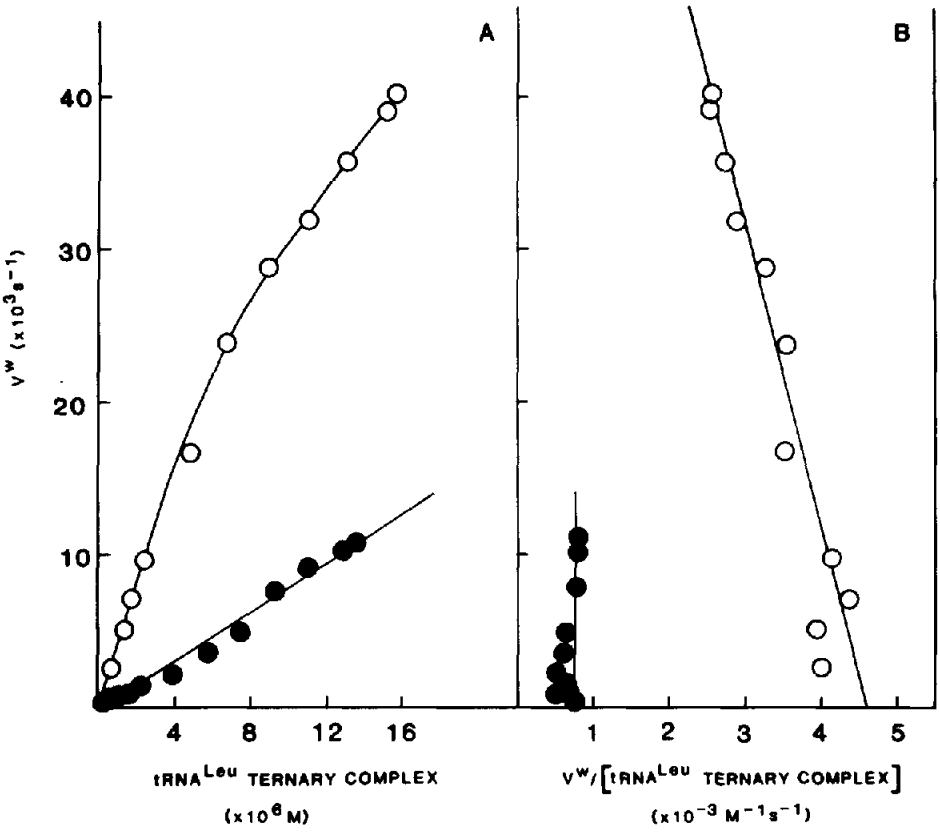

Fig.1. (A) Rate of Leu incorporation (v^w) in the absence of the Phe ternary complex as a function of $tRNA_2^{Leu}$ (●—●) or $tRNA_4^{Leu}$ (○—○) ternary complex concentrations. In the standard burst assay conditions [14,21] 10 pmol active ribosomes were used in the ribosome mix (25 μl). A primer mix (25 μl) was made containing 160 pmol cold Phe, 160 pmol $tRNA^{Phe}$ and 150 units of Phe-tRNA synthetase, in addition to the other components of the standard factor mix. In the factor mix (50 μl) either $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$ were varied with equimolar amounts of EF-Tu from 0 to 1600 pmol in the presence of [^{14}C]Leu (450 cpm/pmol) and 10 units of Leu-tRNA synthetase. All mixes were incubated for 10 min at 37°C, then the ribosome mix was added into the primer mix and poly(Phe) chains of 10–15 amino acids per ribosome were allowed to form in 3–5 min. The primers were then added into the factor mix and assayed for Leu incorporation in 2 min. The amount of ternary complex in each point was separately determined by cold trichloroacetic acid precipitation of the factor mix following 10 min preincubation. (B) Eadie-Hofstee plots of the data in A to determine K_M values (from the intercepts at the *x*-axis) for $tRNA_2^{Leu}$ and $tRNA_4^{Leu}$.

for rate and accuracy [10,14] was used throughout the work. Modifications of the standard burst and error assays [14,21] are described in the figure legends.

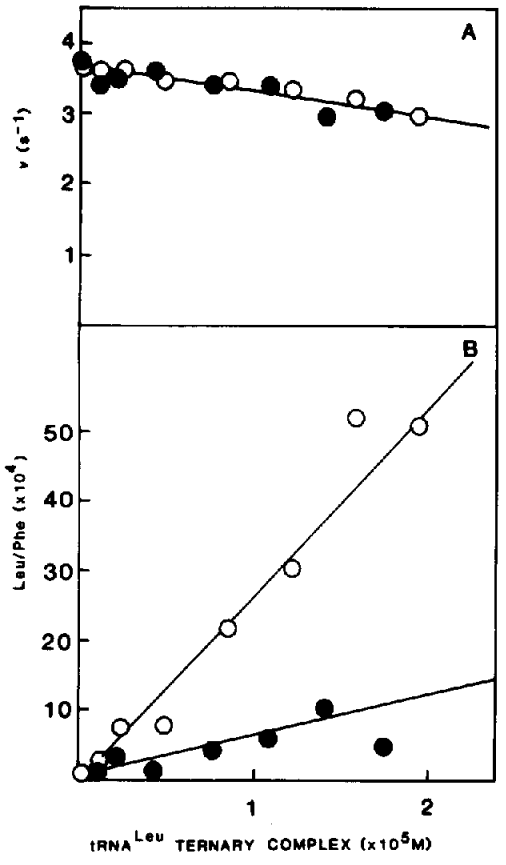

Fig.2. Effects of $tRNA_2^{Leu}$ (●—●) and $tRNA_4^{Leu}$ (○—○) ternary complexes on the rate of poly(Phe) synthesis. (A) Phe incorporation/s per ribosome (ν) is plotted against $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$ ternary complex concentrations in the presence of 1.3×10^{-6} M Phe ternary complex. In the standard burst assay [14,21], 130 pmol $tRNA^{Phe}$ and 300 pmol EF-Tu, 1 mM cold Leu and 10 units of Leu-tRNA synthetase were originally present in the factor mix. $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$, together with equimolar amounts of EF-Tu, were varied from 0 to 1600 pmol. [^{3}H]NAc-Phe-$tRNA^{Phe}$ (180 cpm/pmol) and 0.3 mM [^{14}C]Phe (6 cpm/pmol) were used to determine the rates of elongation in 7 s. (B) Leu missense error levels as a function of the Leu ternary complex concentrations. The experiment was performed as in A. [^{3}H]NAc-Phe-$tRNA^{Phe}$ was replaced with [^{14}C]NAc-Phe-$tRNA^{Phe}$ (1 cpm/pmol) in the ribosome mix and [^{3}H]Leu (1350 cpm/pmol) was included in the factor mix. The amount of Phe and Leu ternary complexes in each point was determined by measuring the charge levels of isoacceptors by cold trichloroacetic acid precipitation of the factor mixes in B following 10 min preincubation. Errors were calculated as in [14].

3. RESULTS

We have explored the influence of $tRNA_2^{Leu}$, $tRNA_4^{Leu}$ and $tRNA^{Val}$ on the kinetics of Phe incorporation into polypeptide by ribosomes coded by poly(U). The noncognate tRNA species can be ranked according to the error generated when the cognate species and noncognate competitor are at

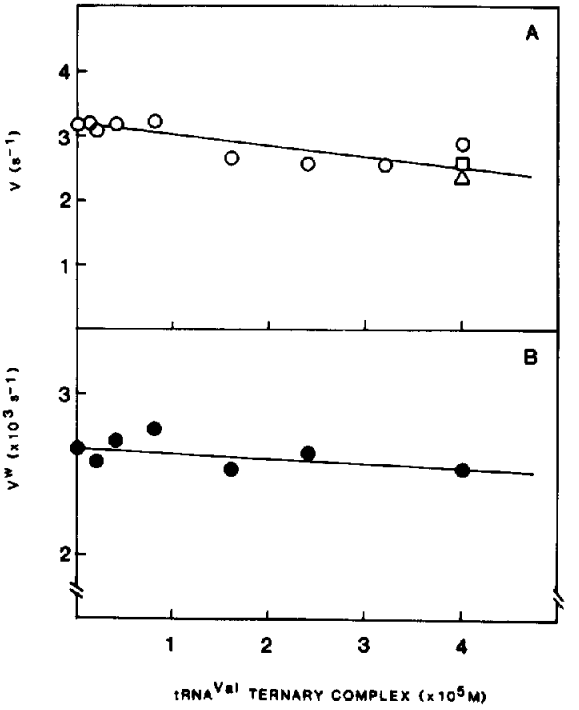

Fig.3. (A) Effect of $tRNA^{Val}$ ternary complex on the rate of poly(Phe) synthesis (ν) with 10^{-7} M Phe ternary complex (○—○). 4×10^{-5} M EF-Tu without $tRNA^{Val}$ (□). 4×10^{-5} M $tRNA^{Val}$ without EF-Tu (△). In the burst assay [14,21], 10 pmol $tRNA^{Phe}$, 300 pmol EF-Tu, 250 pmol EF-Ts, 150 units Phe-tRNA synthetase, 10 units of Val-tRNA synthetase and 1 mM cold Val were present in the factor mix (70 μl). Ribosome mix (30 μl) contained 5 pmol total (0.6 pmol active) ribosomes. $tRNA^{Val}$, together with EF-Tu were varied from 0 to 4100 pmol. The amount of Val ternary complex present at each point was determined by cold trichloroacetic acid precipitation of the factor mix replacing cold Val with [^{3}H]Val (136 cpm/pmol). Rates were monitored by [^{3}H]NAc-Phe-$tRNA^{Phe}$ (900 cpm/pmol) and [^{14}C]Phe (27 cpm/pmol) in ribosome mix and in factor mix respectively. (B) Effect of $tRNA^{Val}$ ternary complex on the rate of Leu misincorporation (ν^w) in the absence of Phe ternary complex. Leu misincorporation was measured on primer poly(Phe) chains of 10–15 amino acids/ribosome as described in the legend of fig.1A. In the presence of 240 pmol $tRNA_4^{Leu}$ in the factor mix, Val ternary complex was varied and measured as described above.

equal concentrations (P_e). The P_e values for the Leu_4, Leu_2 and Val isoacceptor species are 6×10^{-4}, 1×10^{-4} and less than 10^{-6}, respectively.

First we measured the Leu incorporation in the absence of the Phe ternary complex in titrations with Leu_4 and Leu_2 ternary complexes (fig.1A). Eadie-Hofstee plots of these titrations are shown in fig.1B, from which we calculate a K_M value of 2×10^{-5} M for $tRNA_4^{Leu}$ ternary complex and estimate that the K_M value for $tRNA_2^{Leu}$ ternary complex must be larger than 10^{-4} M.

The outcome of direct competition for the ribosome between $tRNA^{Phe}$ ternary complex, on the one hand, and $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$, on the other, is shown in fig.2A. A small decrease in the poly(Phe) synthesis rate is observed when either one of the Leu ternary complexes is titrated to about 2×10^{-5} M. At the same time the Leu errors respond linearly with the concentration of the Leu ternary complex, as expected (fig.2B). The same small decrease in the poly(Phe) synthesis rate can be obtained simply by increasing the concentration of EF-Tu (fig.3) so this decrease is presumably not related to the competition between Phe and Leu ternary complexes.

The effects of the Val ternary complex on the poly(Phe) synthesis rate or on the rate of Leu incorporation in the absence of the Phe ternary complex are shown in fig.3A and B, respectively. The experiment demonstrates that there is no inhibitory effect of the Val ternary complex at concentrations up to 5×10^{-4} M either on the rate of Phe (fig.3A) or on the rate of Leu (fig.3B) elongation.

4. DISCUSSION

Our in vitro study has demonstrated that the cognate ternary complex search for open A-sites is uninhibited by huge excess concentrations of incorrect ones. This shows that the time that ribosomes are blocked while they reject or process incorrect tRNAs is negligible. In accordance with this we have found extremely high K_M values for noncognate ternary complexes. These K_M values can be ranked according to the missense error levels associated with the different tRNAs so that the lower the error level is the higher is the K_M value. Our study addresses the question, implicit in the study of Gouy and Grantham [3], concerning the time ribosomes spend sorting out incorrect tRNAs. The data suggest that this time is negligible compared to all other times in the elongation cycle. It furthermore removes a misconception, introduced earlier [4–6], that ribosomes are slow because they are blocked by incorrect ternary complexes.

These conclusions are of course valid only provided that our in vitro results can be extrapolated to living cells. We think that this is the case and for two reasons. First, our in vitro system has missense error levels as well as elongation rates approximating those in vivo for wild type as well as for a whole set of ribosomal mutants [8]. Our second argument is based on the assertion, extensively corroborated by experiments [22,23], that bacterial populations with impaired translation rates are at a severe selective disadvantage in relation to strains with optimal ribosomes. This means, put in another way, that there is a tremendous selection pressure to avoid inhibition of protein synthesis in bacterial populations. One strategy to achieve this is obviously to design ribosomes in such a way that inhibitory effects of incorrect ternary complexes on the translation rate are minimized. That it is possible to successfully accomplish such strategies is indeed proven by the performance of our in vitro system and there is no reason to expect *E. coli* to be less efficient than we are.

REFERENCES

[1] Maaløe, O. (1979) in: Biological Regulation and Development (Goldberger, R.F. ed.) pp.487–542, Plenum, New York.
[2] Ingraham, J.L., Maaløe, O. and Neidhardt, F.C. (1983) in: Growth of the Bacterial Cell, Sinauer Associates Publishers, Sunderland, MA.
[3] Gouy, M. and Grantham, R. (1980) FEBS Lett. 115, 151–155.
[4] Chavancy, G. and Garel, J.P. (1981) Biochimie 63, 187.
[5] Garel, J.P. (1974) J. Theor. Biol. 43, 211–225.
[6] Varenne, S., Buc, J., Lloubes, R. and Lazdunski, C. (1984) J. Mol. Biol. 180, 549–576.
[7] Ehrenberg, M. and Kurland, C.G. (1984) Q. Rev. Biophys. 17, 45–82.
[8] Kurland, C.G. and Ehrenberg, M. (1987) Annu. Rev. Biophys. Biophys. Chem. 16, 291–317.
[9] Olsson, M.O. and Isaksson, L.A. (1979) Mol. Gen. Genet. 169, 251–257.
[10] Jelenc, P.C. and Kurland, C.G. (1979) Proc. Natl. Acad. Sci. USA 76, 3174–3178.
[11] Jelenc, P.C. (1980) Anal. Biochem. 105, 369–374.
[12] Wagner, E.G.H. and Kurland, C.G. (1980) Biochemistry 19, 1234–1240.

[13] Lebermann, R., Antonsson, B., Giovanelli, R., Guariguata, R., Schumann, R. and Wittinghofer, A. (1980) Anal. Biochem. 104, 29–36.
[14] Wagner, E.G.H., Jelenc, P.C., Ehrenberg, M. and Kurland, C.G. (1982) Eur. J. Biochem. 122, 193–197.
[15] Arai, K.J., Kawakito, M. and Kaziro, Y. (1972) J. Biol. Chem. 247, 7029–7037.
[16] Andersson, S.E.G., Buckingham, R.H. and Kurland, C.G. (1984) EMBO J. 3, 91–94.
[17] Kelmers, A.D., Hancher, C.W., Phares, E.F. and Novelli, G.D. (1971) Methods Enzymol. 20, 3–9.
[18] Gillam, J.C., Millward, S., Blew, D., Von Tigerstrom, M., Wimmer, E. and Tener, G.M. (1967) Biochemistry 6, 3043–3056.
[19] Holmes, N.M., Hurd, R.E., Reid, B.R., Rimmerman, R.A. and Hatfield, G.W. (1975) Proc. Natl. Acad. Sci. USA 72, 1068–1071.
[20] Seno, T., Kobayashi, M. and Nishimura, S. (1968) Biochim. Biophys. Acta 169, 80–94.
[21] Ehrenberg, M. and Kurland, C.G. (1987) Methods Enzymol., in press.
[22] Ruusala, T., Andersson, D., Ehrenberg, M. and Kurland, C.G. (1984) EMBO J. 3, 2575–2580.
[23] Andersson, D.I., Bohman, K., Isaksson, L.A. and Kurland, C.G. (1982) Mol. Gen. Genet. 187, 467–472.