The EMBO Journal vol.3 no.1 pp.91 – 94, 1984

Does codon composition influence ribosome function?

S.G.E. Andersson, R.H. Buckingham[1] and C.G. Kurland*

Department of Molecular Biology, Biomedical Center, Box 590, 751 24 Uppsala, Sweden, and [1]Institut de Biologie Physico-Chimique, 13 Rue Pierre et Marie Curie, 75005 Paris, France

*To whom reprint requests should be sent
Communicated by C.G. Kurland

Escherichia coli **ribosomes pre-initiated with N-acetyl-Val-tRNA^{Val} elongate strictly alternating poly(U-G) at a rate between eight and 12 peptide bonds per second per ribosome** *in vitro*. **Comparisons with poly(U)-primed poly(Phe) synthesis show that these systems function with the same rates which are close to those of protein synthesis** *in vivo*. **This indicates that, at least** *in vitro*, **codon composition has no marked influence on the speed of elongation when the concentration of ternary complex is saturating. Furthermore, the missense frequencies for the two polymers are within the same range: the missense substitution of Trp for Cys is 10^{-4} and that of Met for Val is 10^{-3} in the poly(U-G)-primed system. These data argue against models that explain the codon preference of certain gene families by postulating effects of high or low GC content of codons on the performance characteristics of ribosomes.**
Key words: ribosomes/codon composition/elongation rate

Introduction

Nucleotide sequences from a wide variety of sources indicate that the degenerate codons for any particular amino acid are not used at equal frequencies to specify proteins. Instead, there are characteristic codon preferences associated with different genomes and, within a given organism, characteristic preferences associated with highly or weakly expressed mRNAs (Fiers *et al.*, 1971; Grantham and Gautier, 1980; Grantham *et al.*, 1981; Wain-Hobson *et al.*, 1981; Grosjean and Fiers, 1982). Although the data are nowhere near as exhaustive, there is also evidence that the distribution of aminoacyl-tRNA isoacceptor species reflects to some degree the codon preference of the organism (Garel, 1974; Chavancy and Garel, 1981; Ikemura, 1981). It is clear that balance between the codon frequencies and the corresponding isoacceptor distributions is a prerequisite for optimal speed and accuracy of translation (von Heijne and Blomberg, 1979; Chavancy and Garel, 1981). A corollary notion is that rare codons translated by minor tRNA species can be used to regulate the rate of translation. Quite direct experimental support for the putative regulatory role of minor codons is found in the recent demonstration that the individual rates of translation of several proteins in *Escherichia coli* are significantly different from each other and are inversely related to their content of rare codons (Pedersen, 1983).

It has been suggested that the major codons of highly expressed genes are favoured because the energy of interaction between them and their cognate anticodons is optimal (Grosjean and Fiers, 1982). Thus, measurements with model systems in the absence of ribosomes are consistent with the idea that the stability of cognate codon-anticodon interactions can vary significantly depending on the base composition of the codon (Frieier and Tinoco, 1975; Grosjean *et al.*, 1978). Accordingly, Grosjean and Fiers (1982) have accounted for the tendency of the major codons to have an intermediate G-C content in terms of a model in which extremes of codon-anticodon stability of interaction are avoided. Thus, translation will 'proceed most smoothly if the energy between codons and corresponding anticodons were rather uniform' (Grosjean and Fiers, 1982).

Here, we attempt to test the supposition that the nucleotide composition of the codons influences the speed or accuracy of ribosome function. We employ an *in vitro* system derived from *E. coli* that was previously shown to translate the codon UUU at a rate and with an accuracy very similar to those for polypeptide synthesis *in vivo* (Wagner *et al.*, 1982; Andersson *et al.*, 1982). The present data indicate that poly(U-G), which contains the alternating codons UGU and GUG is translated at the same rate and with comparable missense errors as is poly(U). These data suggest that the performance characteristics of the ribosome may not be sensitive to the presence or absence of G nucleotides in the mRNA.

Results

Figure 1 summarizes an experiment in which the tRNA^{Cys} and tRNA$_1^{Val}$ dependence of poly(Cys-Val) synthesis is measured with the poly(U-G)-primed system. The data show that the incorporation of either amino acid is dependent on the concentration of both tRNA species, but that the incorporation of each is always equal to the other, and that at amounts >20 pmol for both tRNA species the synthesis of poly(Cys-Val) is virtually at a maximum. Since we are interested in the effect of codon character on the rate of ribosome function and since we wish to avoid any influences of tRNA concentration on the kinetics of ribosome function, the experiments described below were usually done in the presence of 100 pmol of each tRNA species.

We have previously measured the elongation rate of poly(Phe) synthesis with poly(U)-programmed ribosomes by incubating the ribosomes with [^{3}H]NAc-Phe-tRNA and measuring the rate of [^{14}C]Phe incorporation per [^{3}H]NAc-Phe incorporated into polypeptide in an elongation burst; the elongation rates so obtained were close to 10 peptide bonds/s/active ribosome (Andersson *et al.*, 1982; Wagner *et al.*, 1982). We have performed the analogous measurement for poly(U-G)-programmed ribososomes by pre-incubation with [^{3}H]NAc-Val-tRNA and normalizing the incorporation of Cys and Val to the amount of [^{3}H]NAc-Val incorporated into polypeptide. Data summarized in Figure 2 show that a burst of elongation at 37°C could be maintained at a linear rate for 5 – 10 s, after which the rate decelerated. The amount of [^{3}H]NAc-Val incorporated was constant throughout. When the poly(U-G)-ribosome mixture had been pre-incubated at

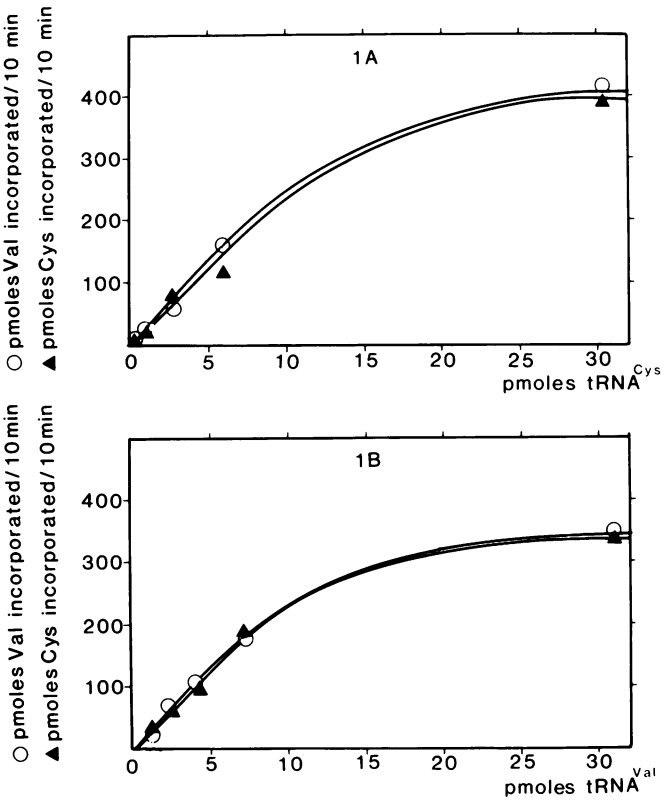

Fig. 1. Incorporation of radioactive Cys and Val was measured in the presence of different amounts of $tRNA^{Cys}$ and $tRNA_1^{Val}$ as described in Materials and methods. Mixture I contained 60 pmol of ribosomes in 50 μl. For the experiment in **A**, mixture II contained 100 pmol of $tRNA_1^{Val}$ and the indicated amounts of $tRNA^{Cys}$. For the experiment in **B**, mixture II contained 100 pmol of $tRNA^{Cys}$ and the indicated amounts of $tRNA_1^{Val}$. The reactions were carried out for 10 min at 37°C, and the incorporation of isotope into hot acid-precipitable material was followed for (○) Val and (▲) Cys.

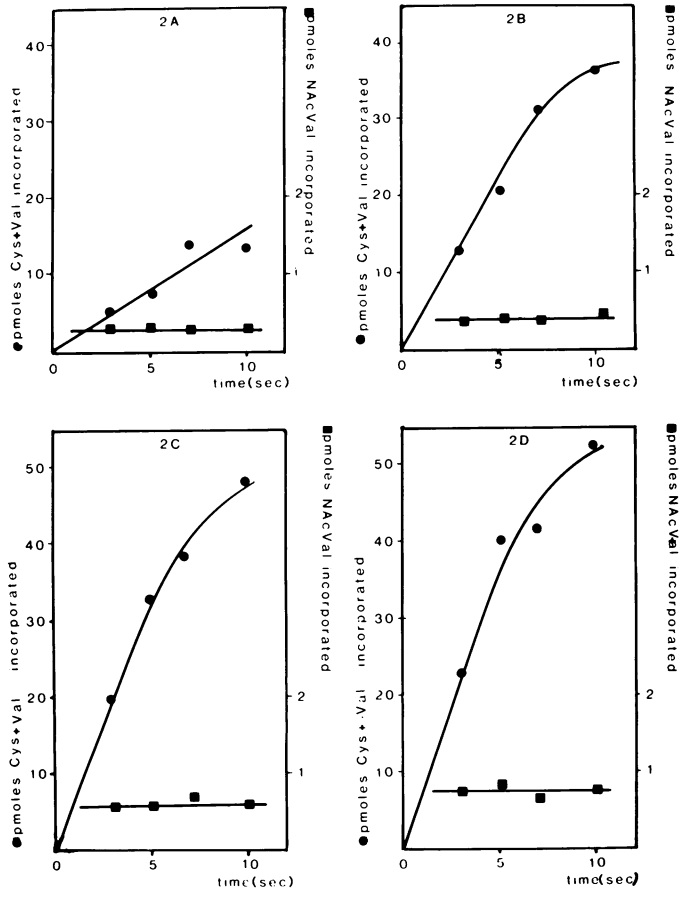

Fig. 2. The time course of (●) Cys plus Val and (■) [^{3}H]-NAc-Val incorporation into acid-precipitable material was studied as described in Materials and methods. Each sample contained 20 pmol of ribosomes and 50 pmol [^{3}H]NAc-Val-tRNA in the 50 μl mixture I which was pre-incubated at 37°C **(A)**, 43°C **(B)**, 47°C **(C)** and 49°C **(D)**. After the pre-incubation mixture was chilled, 50 μl of mixture II were added and the incubation was carried out for the indicated times at 37°C.

37°C, the amount of [^{3}H]NAc-Val incorporated corresponds to 1% active ribosomes, and the incorporation of Cys plus Val per active ribosome corresponds to eight peptide bonds per second.

The fraction of active ribosomes calculated in the poly(U) assay is routinely 10–20 times greater than that which we obtained with the poly(U-G)-programmed system (Figure 2A). Furthermore, we could not improve the activity with poly(U-G) by changing the concentrations of different critical components such as the NAc-Val-tRNA. It has been suggested that poly(U-G) has a stable secondary structure (Gray and Ratliff, 1977). If this putative structure lowers the affinity of the polymer for ribosomes, pre-incubation at higher temperatures might disrupt the structure and favour ribosome association.

The data summarized in Figure 2 are consistent with this guess. Ribosomes were pre-incubated with poly(U-G) at 37°, 44°, 47° and 49°C, and then the activated ribosomes were assayed at 37°C. As the pre-incubation temperature was increased, the percentage of active ribosome rose from 1.0%, to 2.25%, to 2.9% and finally to 3.5%, respectively. Nevertheless, the elongation rate per active ribosome was relatively constant at 8.2, 9.5, 10.3 and 10.0 peptide bonds per second, at the respective temperatures. When the pre-incubation was carried out at higher temperatures, the ribosomes were inactivated.

In another experiment, we compared the elongation rates at 37°C obtained as in Figure 2 with poly(U) and poly(U-G) after pre-incubation at 37°C; they are 10.2 and 9.3 peptide bonds per active ribosome per second. When the systems are pre-incubated at 49°C the corresponding rates are 8.6 and 11.2, respectively. In other words, there is no significant difference between any of these rates if an uncertainty of ±15% is allowed for these measurements.

We have also studied the missense errors generated at the UGU and GUG codons. Here, we measure the ratio of two competing amino acids incorporated into polypeptide when the ratio of the competing tRNA species is varied (Ruusala *et al.*, 1982). It is possible to calculate from the slope of this plot (Figures 3 and 4) a characteristic error frequency (E), which corresponds to the missense substitution rate when the two species are at equal concentrations.

The Trp missense substitution for Cys at the UGU codon is analyzed in Figure 3. Here, the Trp incorporation was measured at a fixed $tRNA^{Cys}$ concentration and the amounts of $tRNA^{Trp}$ were increased. The characteristic error frequency calculated by linear regression from this titration is 1.0×10^{-4}; a comparable figure was obtained when the $tRNA^{Trp}$ concentration was fixed and $tRNA^{Cys}$ concentration was changed (data not shown). When the same titration was performed with streptomycin-resistant ribosomes, the

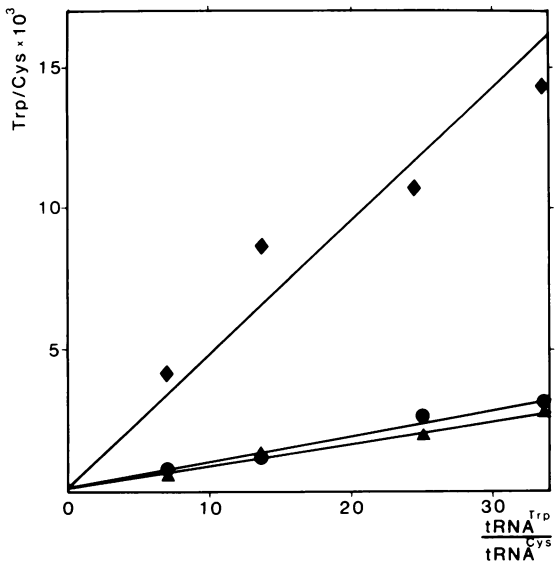

Fig. 3. The determination of the characteristic error frequency for tryptophan misincorporation with the UGU codon was carried out as described in Materials and methods. Mixture I contained 60 pmol of ribosomes. Mixture II contained 100 pmol of $tRNA^{Cys}$ and 40–160 pmol of $tRNA^{Trp}$. Assays were incubated for 15 min. Calculated ratio of Trp incorporation over cysteine incorporation for D14 *Ram* ribosomes (♦), O17 wild-type ribosomes (●) and UK235 str-R ribosomes (▲). E was calculated from the slopes as described by Ruusala *et al.* (1982).

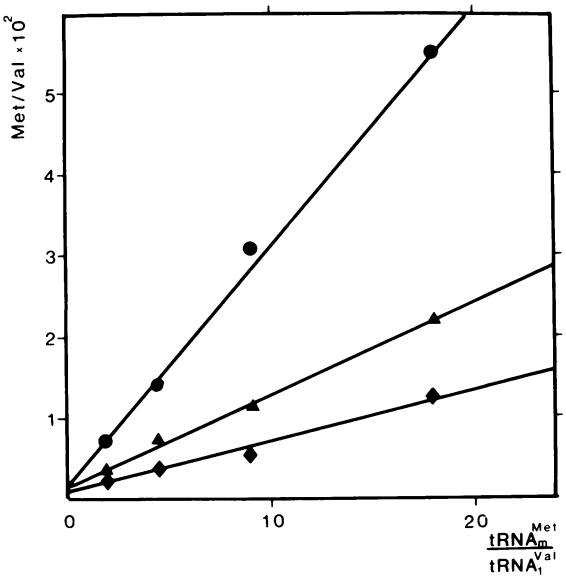

Fig. 4. Determination of the characteristic error frequency for methionine misincorporation with the GUG codon was carried out as described in Materials and methods. Mixture I contained 100 pmol of ribosomes. Mixture II contained 100 pmol of $tRNA^{Cys}$. 10 pmol of $tRNA_1^{Val}$ and 20–180 pmol of $tRNA_m^{Met}$. Assays were incubated for 15 min. The ratio of Met incorporation over valine incorporation for D14 *Ram* ribosomes (●), O17 ribosomes (▲) and UK235 str-R ribosomes (♦). E was calculated from the slopes as described in Ruusala *et al.* (1982).

characteristic error frequency for $tRNA^{Trp}$ was only slightly reduced (0.8×10^{-4}), but when ribosomes from a *Ram* mutant were present the characteristic error frequency increased to 4×10^{-4}.

We have also measured a missense substitution frequency at the GUG codon by studying the competition between $tRNA_m^{Met}$ and $tRNA_1^{Val}$ (Figure 4). When wild-type ribosomes are present the characteristic error rate for $tRNA_m^{Met}$ is 1×10^{-3}. This frequency is reduced to 6×10^{-4} by streptomycin-resistant ribosomes and it is raised to 3×10^{-3} by ribosomes from a *Ram* mutant.

Discussion

The experiments described here were carried out with an *in vitro* system that had been optimized for the speed and accuracy of poly(U) translation into poly(Phe) (Jelenc and Kurland, 1979; Pettersson and Kurland, 1980; Wagner *et al.*, 1982). Nevertheless, the rate of elongation of poly(Cys-Val) programmed by poly(U-G) was indistinguishable from that for poly(Phe). Likewise, the missense substitutions of Trp for Cys and Met for Val respond in the expected way to changes in the ribosome phenotype (Gorini, 1971; Bouadloun *et al.*, 1983). Furthermore, these missense frequencies are within the same range as the Leu missense frequencies expressed by wild-type and mutant ribosomes translating poly(U) in the same *in vitro* system (Ruusala *et al.*, 1982; Andersson and Kurland, 1983). In other words, the data suggest that the performance characteristics of the ribosomes during elongation are unaffected by the presence or absence of guanine nucleotides in mRNA, at least *in vitro*. Unfortunately, an extrapolation from these data to the situation *in vivo* is not without its uncertainties.

Estimates of polypeptide elongation rates for *E. coli* at 37°C tend to cluster around 17 peptide bonds per second per active ribosome (Kjeldgaard and Gausing, 1974; Kennell and Riezman, 1977; Andersson *et al.*, 1982). More detailed studies of six different proteins in *E. coli* suggest that the average rates at which they are elongated can vary by as much as 60%, i.e., from 0.18 to 0.29 seconds per peptide bond at 24.5°C (Pedersen, 1983). In addition, Talkad *et al.* (1976) have suggested that conventional estimates of β-galactosidase synthesis rates are biased towards the very fastest ribosomes and that the rate of elongation varies from ribosome to ribosome at values between eight and 15 peptide bonds per second. Indeed, our estimate of 10 peptide bonds per second is close to the average that would be predicted from the data of Talkad *et al.* (1976).

According to the calculations of Gouy and Grantham (1980) the concentration *in vivo* of an average ternary complex and that of an average codon-programmed ribosome are such that the elongation rate should be limited primarily by the maximum rate of ribosome function. Accordingly, the high frequency codons found in highly expressed mRNA species, that are translated by major tRNA isoacceptor species, should be processed at the maximum rate supported by ribosomes. It is for this reason that we have focused our attention on the influence of guanine nucleotides on the maximum rates of elongation *in vitro*. Our data suggest that there may be no influence of codon composition on these rates. Although more data will be required to substantiate this tendency, there are at least two conclusions that are consistent with our observations.

It is possible that the differences between the stabilities of interaction seen with GC-rich and GC-poor oligonucleotides (Freier and Tinoco, 1975) are not characteristic of codon-anticodon interactions at the ribosomes A-site. Indeed, a tendency in this direction has already been noted in model system studies of tRNA-tRNA interactions in the absence of ribosomes (Grosjean *et al.*, 1978). Alternatively, the rate-limiting step in elongation, which has not as yet been identified, may be insensitive to the character of the codons.

Whatever the explanation turns out to be, and bearing in

mind the caveat mentioned above, we have begun to explore explanations of codon preferences that do not involve directly the physical characteristics of codon-anticodon interactions. As we show elsewhere, it is possible to view the development of codon preferences as a growth optimization strategy and to account for the major codon preference as a statistical reflection of the nucleotide composition of the DNA, rather than *vice versa* (Kurland and Ehrenberg, 1983).

Materials and methods

Chemicals

ATP, GTP, phosphoenolpyruvate (PEP) trisodium salt, putrescine, spermidine, L-cysteine, L-valine, L-methionine and L-tryptophan were purchased from Sigma, [^{3}H]cystine, [^{3}H]tryptophan, [^{3}H]methionine, [^{3}H]- and [^{14}C]-valine were obtained from Amersham International, Bucks, UK and [^{14}C]-cystine from New England Nuclear. *E. coli* tRNA, myokinase (EC 2.7.4.3), pyruvate kinase (EC 2.7.1.40), and DNA polymerase I were obtained from Boehringer Mannheim, FRG and $d(A\text{-}C)_5$, $d(T\text{-}G)_5$ from Collaborative Research Inc., Waltham, MA.

Purifications and preparations

Poly(U-G) was prepared by transcription of poly d(A-C):d(T-G): this was synthesized according to Wells *et al.* (1965). The DNA-polymer was purified on a Sephadex G-50 column. The pooled polymer fractions were precipitated in EtOH, resuspended in H_2O and stored at −20°C.

RNA polymerase was prepared by a procedure described by Burgess and Jendrisach (1975).

Using purified poly d(A-C):d(T-G) as a template for DNA-dependent RNA polymerase, poly(U-G) was prepared according to Nishimura *et al.* (1965). The RNA-polymer was passed through a G-50 column. The ethanol-precipitated polymer was resuspended in H_2O and stored at −80°C.

Ribosomes were prepared from *E. coli* strains O17, D14 (Olsson and Isaksson, 1979) and UK235 (Bohman, unpublished) as described by Jelenc (1980) except that the ribosomes were stored at −80°C in polymix buffer. The purification of elongation factor (EF)-G (Wagner and Kurland, 1980), EF-Tu (Lebermann *et al.*, 1980; Wagner *et al.*, 1982) and EF-Ts (Arai *et al.*, 1972) has been described. Cysteine-tRNA synthetase (Cys-S), tryptophan-tRNA synthetase (Trp-S), methionine-tRNA synthetase (Met-S) and valine-tRNA synthetase (Val-S) were partially purified to remove cross contaminations as well as Ts. The synthetases were obtained as a by-product of the standard EF-Tu preparation from a DEAE-Sepharose CL-6B column followed by an Ultrogel Aca 44 column as described in Leberman *et al.* (1980). They were then applied to a DEAE-52 column according to Kern and Lapointe (1979). The synthetases were stored in polymix containing 25% v/v of glycerol at −20°C.

Cysteine-specific tRNA as well as valine-specific tRNA were purified with the aid of BD-cellulose (Gillam *et al.*, 1967). Tryptophan-specific tRNA was charged with tryptophan prior to chromatography on BD-cellulose. The Trp-$tRNA^{Trp}$ was discharged in Bicine buffer as described by Joseph and Muench (1971). The pure $tRNA^{Trp}$ was free of cross-contamination by $tRNA^{Cys}$ and $tRNA_1^{Val}$. Purification of $tRNA_m^{Met}$ was performed by a procedure described by Seno *et al.* (1968).

Preparation of N-acetyl-Val-tRNA was done according to Ruusala *et al.* (1982) except that [^{3}H]valine and $tRNA_1^{Val}$ were used instead of [^{3}H]Phe and $tRNA^{Phe}$.

Translation assay

The standard protocol was as follows: two mixtures, initiation mix (I) and factor mix (II) were prepared on ice. Both of them contain the polymix buffer components, 5 mM Mg_2^+, 0.5 mM Ca_2^+, 8 mM putrescine, 1 mM spermidine, 5 mM phosphate, 5 mM NH_4^+, 95 mM K^+ and 1 mM dithioerythritol (DTE) at pH 7.5. Mixture I in addition contains in 50 μl: 0.005 OD of poly(U-G) as well as ribosomes and NAc-Val-$tRNA^{Val}$ as indicated in the figure legends. Mixture II contains in 50 μl; 2 mM ATP, 2 mM GTP, 12 mM PEP, 5 μg pyruvate kinase, 0.3 μg myokinase, 50 pmol EF-G, 300 pmol EF-Tu, 30 pmol EF-Ts, 15 units of Cys-tRNA synthetase and Val-tRNA synthetase, 100 pmol $tRNA^{Cys}$ and $tRNA^{Val}$ (unless otherwise stated), 100 μM cysteine and valine (5–30 c.p.m./pmol). One unit of synthetase can produce 1 pmol of aminoacyl-tRNA from free amino acid and tRNA in 1 s at the substrate concentration used here. When misincorporation is assayed, 10 μM [^{3}H]tryptophan or 10 μM [^{3}H]methionine (500 c.p.m./pmol) and 2 units of Met-S and Trp-S are included. The amounts of $tRNA^{Trp}$ and $tRNA^{Met}$ are indicated in the figure legends. [^{3}H]- or [^{14}C]cystine is reduced to cysteine prior to addition to the factor mix, by incubating it with DTE for 10 min at 37°C. Mixture I is pre-incubated for 10–15 min at 49°C unless otherwise stated in the figure legends. Mixture II is pre-incubated for 15 min at 37°C. The elongation reaction is started by transferring 50 μl of mixture I (using a pipette tip pre-warmed in water at 37°C) to an aliquot of mixture II. The whole mixture is incubated for the indicated times at 37°C. The reaction was stopped by the addition of 5 ml trichloroacetic acid containing 0.5% w/v of Val, Cys, Trp and Met.

Filtrations and calculations were done as described by Jelenc and Kurland (1979). Backgrounds for cysteine, valine and methionine were measured by omission of poly(U-G) in control samples and were 20–30 c.p.m. in the ^{14}C channel. Backgrounds for tryptophan and methionine were obtained by the omission of $tRNA^{Trp}$ or $tRNA_m^{Met}$ in control samples and were 200–400 c.p.m.

Acknowledgements

We wish to thank our colleagues at the Department for their critical comments. This work was supported by grants from the Swedish Natural Sciences Research Council, The Swedish Cancer Society, The EMBO short-term fellowship program, and by grants from CNRS (GR18) as well as INSERM to Dr. Marianne Grunberg-Manago, to whom we are grateful for support and interest.

References

Andersson,D.I., Bohman,K., Isaksson,L.A. and Kurland,C.G. (1982) *Mol. Gen. Genet.*, **187**, 467-472.
Andersson,D.I. and Kurland,C.G. (1983) *Mol. Gen. Genet.*, **191**, 378-381.
Arai,K.I,. Kawakita,M. and Kaziro,Y. (1972) *J. Biol. Chem.*, **247**, 7029-7037.
Bouadloun,F., Donner,D. and Kurland,C.G. (1983) *EMBO J.*, **2**, 1351-1356.
Burgess,R.R. and Jendrisach,J.J. (1975) *Biochemistry (Wash.)*, **14**, 4634-4638.
Chavancy,G. and Garel,J.P. (1981) *Biochimie*, **63**, 187-195.
Fiers,W., Contreras,R., De Wachter,R., Haegeman,G., Merregaert,J., Min Jou,W. and Vandenberghe,A. (1971) *Biochimie*, **53**, 495-506.
Freier,S.M. and Tinoco,I.Jr. (1975) *Biochemistry (Wash.)*, **14**, 3310-3311.
Garel,J.P. (1974) *J. Theor. Biol.*, **43**, 211-225.
Gillam,J.C., Millward,S., Blew,D., von Tigerstrom,M., Wimmer,E. and Tener,G.M. (1967) *Biochemistry (Wash.)*, **6**, 3043-3056.
Gorini,L. (1971) *Nature New Biol.*, **234**, 262-264.
Gouy,M. and Grantham,R. (1980) *FEBS Lett.*, **115**, 151-155.
Grantham,R. and Gautier,C. (1980) *Naturwissenschaften*, **67**, 93-94.
Grantham,R., Gautier,C., Gouy,M,. Jacobzone,M. and Mercier,R. (1981) *Nucleic Acids Res.*, **9**, r43-r47.
Gray,D.M. and Ratliff,R.L. (1977) *Biopolymers*, **16**, 1331-1342.
Grosjean,H., de Henaue,S. and Crothers,D (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 610-614.
Grosjean,H. and Fiers,W. (1982) *Gene*, **18**, 199-209.
Ikemura,T. (1981) *J. Mol. Biol.*, **146**, 1-21.
Jelenc,P.C. and Kurland,C.G. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3174-3178.
Jelenc,P.C. (1980) *Anal. Biochem.*, **105**, 369-374.
Joseph,D.R. and Muench,K. (1971) *J. Mol. Biol.*, **246**, 7602-7609.
Kern,D. and Lapointe,J. (1979) *Biochimie*, **61**, 1257-1272.
Kennell,D. and Riezman,H. (1977) *J. Mol. Biol.*, **114**, 1-21.
Kjeldgaard,N.O. and Gausing,K. (1974) in Nomura,M., Tissières,A. and Lengyel,P. (eds.), *Ribosomes*, Cold Spring Harbor Laboratory Press, NY, pp. 369-392.
Kurland,C.G. and Ehrenbeg,M. (1983) *Prog. Nucleic Acids Res. Mol. Biol.*, in press.
Lebermann,R., Antonsson,B., Giovanelli,R., Guariguata,R., Schumann,R. and Wittinghofer,A. (1980) *Anal. Biochem.*, **104**, 29-36.
Nishimura,S., Jones,D.S. and Khorana,H.G. (1965) *J. Mol. Biol.*, **13**, 302-304.
Olsson,M.O. and Isaksson,L.A. (1979) *Mol. Gen. Genet.*, **169**, 251-257.
Pedersen,S. (1983) *Alfred Benzon Symp.*, **19**, in press.
Pettersson,I. and Kurland,C.G. (1980) *Proc.Natl. Acad. Sci. USA*, **180**, 275-281.
Ruusala,T., Ehrenberg,M. and Kurland,C.G. (1982) *EMBO J.*, **1**, 741-745.
Seno,T., Kobayashi,M. and Nishimura,S. (1968) *Biochim. Biophys. Acta*, **169**, 80-94.
Talkad,V., Schneider,E. and Kennell,D. (1976) *J. Mol. Biol.*, **104**, 299-303.
von Heijne,G. and Blomberg,C. (1979) *J. Theor. Biol.*, **78**, 113-120.
Wagner,E.G.H. and Kurland,C.G. (1980) *Biochemistry (Wash.)*, **19**, 1234-1240.
Wagner,E.G.H., Jelenc,P.C., Ehrenberg,M. and Kurland,C.G. (1982) *Eur. J. Biochem.*, **122**, 193-197.
Wain-Hobson,S., Nussinov,R., Brown,R.J. and Sussman,J.L. (1981) *Gene*, **13**, 355-364.
Wells,R.D., Ohtsuka,E. and Khorana,H.G. (1965) *J. Mol. Biol.*, **14**, 221-240.

Received on 12 September 1983