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Is there proofreading during polypeptide synthesis?

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The stoichiometric efficiency with which ternary complexes containing Phe-tRNA^{phe} and Leu-tRNA^{leu} support polypeptide synthesis has been compared in a poly(U)-directed, steady-state translation system. When unfractionated tRNA is used to support synthesis, the number of discharged ternary complexes per peptide bond formed is an average of 48 times greater for leucine than for phenylalanine. When three purified leucine isoacceptor species are tested, they each show a characteristic ratio of ternary complexes discharged per missense insertion, normalized to that for phenylalanine: these are 103, 76, and 45 for Leu-tRNA$_2^{leu}$, Leu-tRNA$_3^{leu}$, and Leu-tRNA$_4^{leu}$, respectively. The data are consistent with the functioning of a proofreading mechanism during translation.
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Introduction

Studies with model systems have suggested that at equilibrium codon-anticodon interactions, by themselves, are not sufficiently sequence-specific to account for the accuracy of protein synthesis (Grosjean *et al.*, 1978). However, it is conceivable that the ribosomes repeat such a limited selection step one or more times and, if the system is properly arranged, the overall accuracy of aminoacyl-tRNA selection can surpass that of the elementary steps. Such a proofreading mechanism requires a second free energy source to drive the additional editing steps far from equilibrium; this driving force has been identified in protein synthesis with the factor-dependent guanine nucleotide cycles (Hopfield, 1974; Ninio, 1975).

If aminoacyl-tRNA selection on ribosomes works this way, the discard of each incorrect aminoacyl-tRNA in the editing step will be accompanied by the hydrolysis of a GTP molecule. Therefore, the ratio of GTPs hydrolyzed to peptide bonds formed will be significantly greater for incorrect amino acids than for correct ones. This reasoning is the basis of the previous attempts to search for proofreading on ribosomes. For example, poly(U)-programmed ribosomes were confronted with ternary complex containing elongation factor Tu (EF-Tu), aminoacyl-tRNA, and GTP. Then, the ratio of GTPs hydrolyzed to aminoacyl-tRNAs accepted by the ribosome was measured with the correct aminoacyl-tRNA (Phe) and with the incorrect species (Leu). Ratios of between 5 and 50 GTPs per Leu-tRNA$_2^{leu}$ accepted on the ribosome have been observed and forwarded as evidence for the existence of a proofreading function in this system (Thompson and Stone, 1977; Yates, 1979; Thompson *et al.*, 1981a, 1981b).

Although these single-factor measurements appear to be convincing, they suffer from an ambiguity that is intrinsic to their experimental design. A proofreading mechanism is one in which the flow of incorrect substrate is preferentially diverted over the editing branches while the flow of correct substrate is preferentially forwarded to the final product. In other words, such a selection mechanism compares the rate of the discard flow with that of the forward flow for the different substrates. Such a comparison in polypeptide synthesis is not possible in the absence of elongation factor G (EF-G), because the normal forward flow of translocation is missing from the system. Accordingly, it is difficult to decide to what extent the excess GTP hydrolysis observed in single-factor measurements is relevant to the complete system operating in the steady state (Kurland, 1978).

The bias toward an ambiguous experimental design arises from the difficulty of measuring the EF-Tu-dependent GTP hydrolysis in a background of EF-G-catalyzed GTP hydrolysis. Nevertheless, we show that it is possible to circumvent this problem in a steady-state polypeptide elongation system. We take advantage of the fact that the rate of cycling of EF-Tu is dependent on the presence of EF-Ts, while the EF-G cycle is indifferent to EF-Ts (Lipmann, 1969). By adjusting the composition of our system so that the speed of EF-Tu cycling is rate-limiting for peptide bond formation, we can measure the relative number of times that a ternary complex containing Leu-tRNA^{leu} is discarded on the ribosome before forming a peptide compared with that for Phe-tRNA^{phe}; this ratio is the proofreading factor. Although our data superficially support the previous reports of proofreading on ribosomes (Thompson and Stone, 1977; Yates, 1979; Thompson *et al.*, 1981a, 1981b), there is very little quantitative agreement between the two sets of data.

Results

In all of these experiments we employ a system for the translation of poly(U) that, when complete, supports a rate of polypeptide elongation close to 10/s and has a missense error ratio for leucine of 4×10^{-4} (Wagner *et al.*, 1982a). Since this system is made up of purified elongation factors, aminoacyl-tRNA synthetases, etc., it is possible to adjust the concentration of the individual components so that the rates of phenylalanine and leucine incorporation are determined by one or other of the components. For example, in order to favor the flows associated with leucine we can limit the rate of tRNA^{phe} charging by adding appropriately small amounts of the corresponding synthetase (PRS) and by adding saturating amounts of Leu-tRNA synthetase (LRS). The ternary complex pool will then be biased in favor of Leu-tRNA^{leu} and variations of the PRS concentration will lead to a proportional variation of phenylalanine incorporation into polypeptide in the steady state (Wagner *et al.*, 1982b).

Our objective is to measure how often a ternary complex is bound to the poly(U)-ribosome complex with the result that a GTP is hydrolyzed but the aminoacyl-tRNA is discarded from the system. One way to do this is to arrange the system so that the rate of EF-Tu.GDP regeneration into ternary

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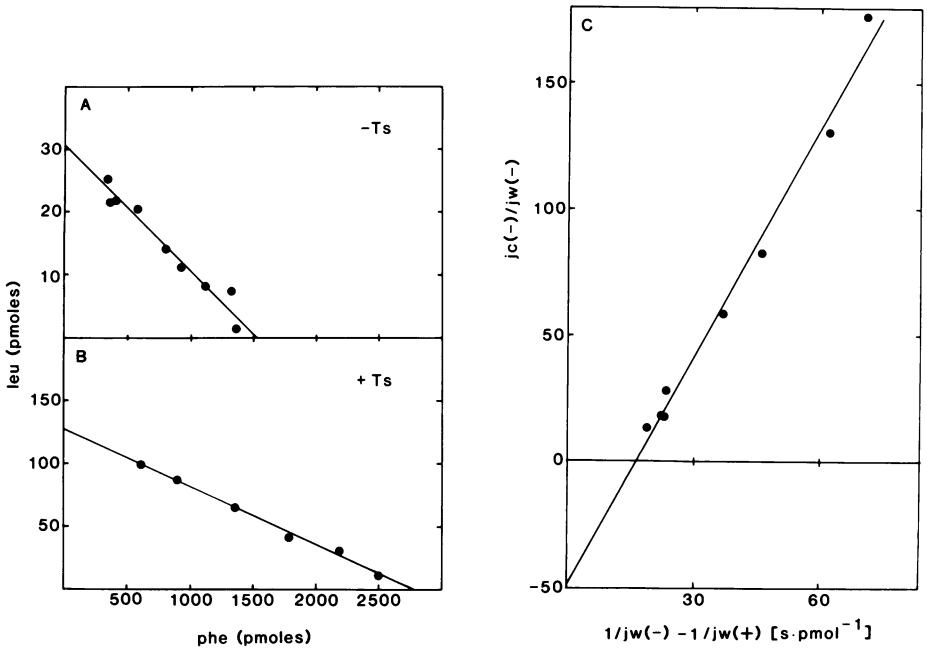

Fig. 1. A. Incorporation of phenylalanine and leucine with unfractionated tRNA in the absence of EF-Ts at different concentrations of PRS. The slope of the line relates the number of EF-Tu cycles per peptide bond for leucine to that for phenylalanine. **B.** Incorporation of phenylalanine and leucine as in **A** but in the presence of EF-Ts. The slope of the line reflects the decrease in the effective ribosome concentration when the phenylalanine flow increases (Materials and methods). A comparison of the intercepts at the ordinate in **A** and **B** shows the extent to which the EF-Tu cycle in **A** is saturated by the discharge of ternary complexes with Leu-tRNA^{leu}. **C.** Determination of the proofreading factor *F*. The data from **A** and **B** are combined according to equation (5) to give a linear plot. The intercept of the line with the ordinate is $-F$. The line is estimated by linear regression analysis.

complex is fixed and is the slowest step in the process. Under this constraint, the number of peptide bonds formed plus the number of proofread ternary complexes will be limited by the rate with which ternary complex can be regenerated from EF-Tu.GDP. This means that variation of the ratio of ternary complexes containing Phe-tRNA^{phe} to those containing Leu-tRNA^{leu} will lead to a change in the total number of peptide bonds formed per dissipated ternary complex if proofreading of Leu-tRNA^{leu} takes place at a significant rate. Alternatively, if there is no proofreading of Leu-tRNA^{leu}, a variation of the composition of the ternary complex pool will have no influence on the stoichiometric efficiency of peptide bond formation; it will only influence the error frequency.

To arrange our system so that EF-Tu is rate limiting for polypeptide synthesis and is present primarily as EF-Tu.GDP binary complex, we take advantage of our earlier studies of the EF-Tu cycle (Ruusala *et al.*, 1982). There we found that the rate of release of GDP from the binary complex is reduced to ~ 0.01/s when EF-Ts is absent from the system. Accordingly, we prepare our incubation mixtures with saturating concentrations of all components, including bulk tRNA, except that we omit EF-Ts and we limit the phenylalanine incorporation to rates dependent on the amounts of PRS added.

If the rate of ternary complex regeneration from EF-Tu.GDP is truly rate limiting and there is no proofreading of Leu-tRNA^{leu}, a plot of the rate of leucine incorporation (J_w^-) against phenylalanine incorporation (J_c^-) will yield a negative slope of one as we vary the degree of Phe-tRNA^{phe} charging, i.e., in the absence of proofreading the stoichiometric efficiency of polypeptide synthesis per ternary complex will be constant. The other extreme is the situation where the whole selectivity of the system depends on proofreading. Here, we would expect the slope of the plot to correspond directly to the error of the selection. As can be seen from Figure 1A, the results of our PRS titration experiment in the absence of EF-Ts are intermediate between these two extremes. The slope of this plot indicates that the number of EF-Tu.GDP molecules generated for every leucine incorporated is >50 times the number for phenylalanine.

The validity of this estimate of the proofreading factor (*F*) depends on the degree to which the system meets two underlying preconditions. One of these is that the rate of polypeptide synthesis, even at low PRS concentrations, is strictly limited by the speed of ternary complex regeneration from EF-Tu.GDP and is not limited by the slow rate with which ribosomes discharge Leu-tRNA^{leu} ternary complex. The degree to which the EF-Tu cycle limits polypeptide synthesis in the absence of EF-Ts can be estimated by comparing this activity with that obtained in the presence of EF-Ts (Figure 1B). The greater the stimulation of leucine incorporation by EF-Ts, the closer the system is to saturating the EF-Tu cycle in the absence of EF-Ts. The data in Figure 1A and 1B show close to a 5-fold stimulation of the leucine incorporation by EF-Ts, which suggests that the EF-Tu cycle in the absence of EF-Ts was 80% saturated (see Materials and methods).

A second aspect of our measurement that needs attention is an estimate of the degree to which the variation of the PRS concentration influences the number of active ribosomes in the system. This variation of effective ribosome concentration is evident in Figure 1B. Thus, all of our experiments are carried out with limiting PRS and the overwhelmingly dominant species of the ternary complex pool is Leu-tRNA^{leu}. Therefore, in the presence of EF-Ts the concentration of Leu-tRNA^{leu} ternary complex will be virtually unchanged when the PRS varies. This means that the observed decrease of the leucine incorporation associated with increased PRS concen-

tration could be caused by a decrease in the effective ribosome concentration (see Materials and methods). Indeed, this effect has been seen previously (Wagner *et al.*, 1982a) and it has been explained as an accumulation of stuck ribosomes at the 3′ end of the poly(U) messengers. Since the magnitude of this effect for a given amount of polypeptide synthesis is independent of the presence or absence of EF-Ts, the data obtained in the presence of EF-Ts can be used to correct for this effect in the absence of EF-Ts (see Materials and methods).

As shown in Materials and methods, we can combine the incorporation rates observed in the absence of EF-Ts (J_w^-, J_c^-) with those obtained in the presence of EF-Ts (J_w^+, J_c^+) in a single plot that gives a reliable estimate of the proofreading factor (F). Here we plot J_c^-/J_w^- as a function of the difference: $1/J_w^- - 1/J_w^+$, and the intercept is $-F$. As shown in Figure 1C, the proofreading factor (F) is 48 for the leucine incorporation supported by bulk tRNA from *Escherichia coli*.

The proofreading factor of 48 obtained with unfractionated tRNA for leucine incorporation is only an average value for a mixture of tRNAs. We have also studied five leucine isoacceptor species that we purified from *E. coli* bulk tRNA. First, we attempted to characterize each of these with respect to their ability to support leucine incorporation by a poly(U)-programmed ribosome. Here, we measure the leucine missense error obtained when the concentration of the leucine isoacceptor is equal to that of the Phe-tRNA^{phe} with which it is competing; we refer to this error level as the characteristic error frequency (E).

Both the major species Leu-tRNA$_1^{leu}$ and the minor species Leu-tRNA$_5^{leu}$ are so inefficient at supporting missense errors in this system that we have been unable to obtain reliable estimates of their Es, which are $< 10^{-5}$ (see Table I). The remaining isoacceptor species yield measurable missense error frequencies and, of these, Leu-tRNA$_4^{leu}$ is the most error-prone (see Table I). The proofreading factors (F) for each of these three isoacceptors have also been estimated (Figure 2 and Table I). We note that all three species appear to be proofread to different extents, with the most error-prone (Leu-tRNA$_4^{leu}$) having the smallest F factor and the least error-prone (Leu-tRNA$_2^{leu}$) having the largest F factor.

Table I. Error and proofreading with tRNA^{leu}: characteristics of the tRNA^{leu} isoacceptors

tRNA	E[a]	F[a]
Leu 1	$< 10^{-5}$	–
Leu 2	1.1×10^{-4}	103
Leu 3	1.3×10^{-4}	76
Leu 4	6×10^{-4}	45
Leu 5	$< 10^{-5}$	–
Bulk	4×10^{-4}	48

[a]For details of determination of factors E and F, see Results.

Discussion

The present experiments show that when the rate of polypeptide synthesis is limited strictly by the availability of ternary complex, the stoichiometric efficiency of peptide bond formation can be shown to be much lower for incorrect ternary complexes than for correct ones. Furthermore, when three different leucine isoacceptors are tested in our poly(U)-primed system, each species displays a characteristic number of dissipated ternary complexes per missense insertion (see Table I). These are precisely the kinds of results that would be expected if proofreading is part of the mechanism of aminoacyl-tRNA selection by ribosomes (Hopfield, 1974; Ninio, 1975).

Although we have studied too small a sample of tRNA species to permit any strong generalizations to be drawn, the data obtained with three leucine isoacceptors are consistent with a simple proofreading scheme. Thus, if some allowance is made for the errors of our measurements, it appears that the proofreading factor (F) is approximately the square root

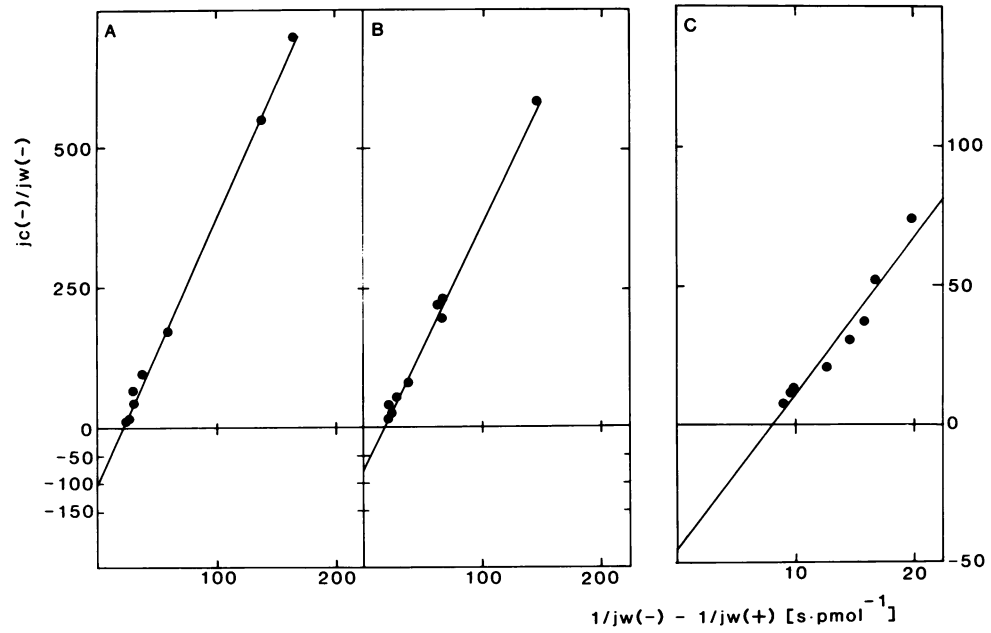

Fig. 2. Determination of the proofreading factor F for Leu-tRNA$_2^{leu}$ (**A**), Leu-tRNA$_3^{leu}$ (**B**), and Leu-tRNA$_4^{leu}$ (**C**) as in Figure 1C. The lines have been estimated by linear regression analysis.

of the reciprocal of the characteristic error frequency (E) for all three isoacceptors. This result is compatible with a mechanism in which the accuracy of the process is equipartitioned between an initial selection and a proofreading selection.

A rather broad range of proofreading numbers have been reported from single factor experiments (Thompson and Stone, 1977; Yates, 1979; Thompson *et al.*, 1981a, 1981b). In these studies the largest stoichiometric ratio for Leu-tRNA$_2^{leu}$ (f_w) was 50 and that for Phe-tRNA^{phe} (f_c) in the same study was 2 (Thompson *et al.*, 1981a). Such values lead to a proofreading factor (F) close to 25, while our estimate of F for Leu-tRNA$_2^{leu}$ is close to 100. Since the smaller value reported by Thompson *et al.* (1981a) was obtained in an incomplete system, at 0°C, in an ionic mixture that has been shown to be suboptimal for translation of poly(U) (Jelenc and Kurland, 1979; Pettersson and Kurland, 1980), the discrepancy between their estimate and ours is not surprising.

According to Ikemura (1981), *E. coli* contains close to three times as much tRNA^{Phe} as tRNA$_4^{leu}$. Since the characteristic error rate for Leu-tRNA$_4^{leu}$ is 6 x 10^{-4} (Table I) and the leucine error frequency is 4 x 10^{-4} with unfractionated tRNA, our data suggest that approximately half of the leucine error of poly(U) translation with bulk tRNA is due to Leu-tRNA$_4^{leu}$. This conclusion is also consistent with the proofreading factors obtained with the different tRNAs. The identity of the isoacceptor species responsible for the remaining leucine incorporation cannot be decided until we have characterized the major Leu-tRNA$_1^{leu}$ species and compared the affinities of the different leucine isoacceptors for EF-Tu.

The proofreading factor (F) that we have estimated for different tRNA species is only a ratio that compares the relative stoichiometry for competing ternary complexes (f_w/f_c). Clearly, it would be useful to be able to evaluate the absolute stoichiometries. We note in this connection that the slope of the plots in Figures 1C and 2A, B, C is determined by the dissociation rate constant for the EF-Tu.GDP complex, which we have determined previously (Ruusala *et al.*, 1982), as well as by the concentration of EF-Tu and f_c. If we could devise an independent assay to measure accurately the concentration of active EF-Tu molecules in our assays, it would be possible to obtain a direct measure of f_c. At present we know only the total concentration of EF-Tu molecules (without regard to their activity) that we add to the incubation mixtures, and this yields estimates of f_c close to 1.

The experiments described here show that it is possible to study the stoichiometry of the flows for different components of a complete steady-state translation system. Although the results obtained in this study are consistent with the idea that the flows over the ribosome are arranged in such a way as to support a proofreading selection mechanism, there is at least one more problem that must be solved before we can be fully confident that ribosomes proofread. We lack direct evidence that the same catalytic centers which mediate the formation of peptide bonds with ternary complex are also responsible for all the dissipative discharge of the ternary complexes. In other words, it is not inconceivable that what appears to be a proofreading flow, in our experiments as well as in the single factor ones, is only a dissipative artifact supported by defective ribosomes.

We have performed a large number of experiments to identify the sources of ternary complex dissipation. In these we have found that the consumption of ternary complexes apparently depends on the charging level of the Leu-tRNA^{leu}, on the amounts of EF-G that are present as well as on the mutant phenotype of the ribosomes. All of these observations are consistent with the interpretation that the normal mechanism of translation involves proofreading. We will return to this problem in a later communication.

Materials and methods

Kinetic calculations

The flow into peptide bonds (J_i) from ternary complex (T^3_i) when the concentration of ribosomes with an open A site is given by [R] can be described simply as:

$$J_i = \frac{[T^3_i][R]R_i}{f_i} \quad (1)$$

where R_i is a characteristic rate factor (Ehrenberg and Blomberg, 1980) and f_i is the number of ternary complexes dissipated per peptide bond formed. Here, the subscript (i) can refer to the cognate species (c) or a noncognate one (w). When the EF-Tu cycle is rate limited by the absence of EF-Ts the flow of ternary complexes (J_t^-) is described by:

$$J_t^- = k_d\,[\text{Tu.GDP}] = f_c J_c^- + f_w J_w^- \quad (2)$$

Here, k_d is the rate constant for the dissociation of the EF-Tu-GDP complex, and the superscript (−) refers to the absence of EF-Ts.

The basic idea of our experiment is to vary the charging level of the Phe-tRNA^{phe} while holding the charging level of the Leu-tRNA^{leu} at 100% so that the relative amounts of leucine and phenylalanine incorporated into peptide change significantly. If there is proofreading (i.e., $f_w/f_c = F >> 1$), it will be detectable as a decrease in the stoichiometry of peptide bond formation per discharged ternary complex as the mole fraction of leucine incorporated into peptide increases.

Under the conditions of our experiments, the flow of leucine into polypeptide can be described as:

$$J_w^- = \frac{T\mathring{u} - J_c^- f_c/k_d}{f_w\left(\frac{1}{k_d} + \frac{1}{[R]\,R_w}\right)} \quad (3)$$

This expression, where $T\mathring{u}$ is the total concentration of EF-Tu, has the virtue of describing the ternary complex flows even when the EF-Tu cycle is not being driven at its maximum rate, which is defined by the condition: $[R]R_w >> k_d$. A way still has to be found to account for the variation of active ribosomes (R) as the PRS concentration varies.

We recall that even when we restrict the flow of phenylalanine into peptide by keeping the PRS concentration low, this flow is much larger than the leucine incorporation under all of our conditions. Thus, to a good first approximation the phenylalanine incorporation will determine the extent to which ribosomes run to the end of the poly(U) and become stuck. Furthermore, for a given rate of phenylalanine incorporation, the extent to which the ribosomes are inactivated will be independent of whether or not EF-Ts is present. We have maintained the charging level of Leu-tRNA^{leu} close to saturation, and that for Phe-tRNA is greatly restricted. Therefore, in the presence of EF-Ts almost all of the EF-Tu will be in the form of the Leu-tRNA^{leu} ternary complex. Therefore, we can write to a good approximation:

$$J_w^+ = T\mathring{u}\,[R]R_w \quad (4)$$

By substituting (4) into (3) we obtain the correction for the variation in active ribosome concentration:

$$\frac{J_c^-}{J_w^-} = \frac{T\mathring{u}k_d}{f_c}\left(\frac{1}{J_w^-} - \frac{1}{J_w^+}\right) - F \quad (5)$$

Thus, the data from the incorporation measurements in the presence and absence of EF-Ts can be plotted according to (5), and the intercept yields the value of the proofreading factor F, while the slope is given by $T\mathring{u}k_d/f_c$.

Chemicals

All chemicals were of the highest purity available. Poly(U), L-phenylalanine, L-leucine, phosphoenolpyruvate (PEP), GTP, ATP, putrescine, spermidine, myokinase (EC 2.7.4.3) and pyruvate kinase (EC 2.7.1.40) were purchased from Sigma (St. Louis, MO). *E. coli* tRNA was obtained from Public Health Laboratory Service (Porton, UK). Radioactive amino acids were purchased from Amersham International (Amersham, UK). BD-cellulose was from Boehringer Mannheim (Mannheim, FRG). Plaskon was a

kind gift from G. Dirheimer and Adogen 464 was purchased from Aldrich.

Buffer

Polymix (Jelenc, 1980; Jelenc and Kurland, 1979): 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, 1 mM dithioerythritol, pH 7.5. Working strength buffer was prepared by mixing in the correct proportions, 100 mM potassium phosphate (pH 7.5) and a 10-fold concentrate without phosphate (pH 7.5) according to the procedure described by Jelenc (1980).

Purifications

Ribosomes were prepared from frozen MRE 600 cells (Public Health Laboratory Service) and stored in buffer containing 30% methanol at −20°C as described by Jelenc (1980).

The purification of EF-Tu followed the method of Lebermann *et al.* (1980) with modifications described in Wagner *et al.* (1981). The factor was stored at −80°C in polymix containing 10 μM GDP. EF-Ts was obtained through the procedure of Arai *et al.* (1972) and kept in polymix at −80°C. EF-G was purified according to Wagner and Kurland (1980) and stored in polymix buffer at −80°C.

The purification of PRS followed the scheme described in Wagner *et al.* (1982a). LRS was purified as described before (Wagner *et al.* 1982a). Both enzymes were stored at −20°C in polymix with 25% glycerol.

Isoacceptor tRNAs

Phenylalanine-specific tRNA was purified with the aid of BD-cellulose (Gillam *et al.*, 1967) and RPC-5 (Pearson *et al.*, 1971) column procedures.

Isoaccepting $tRNA^{leu}$ species were first separated on BD-cellulose (Gillam *et al.*, 1967). Three of the isoacceptor tRNAs were eluted from the column by a salt gradient and the remaining two by 10% ethanol in the buffer with 1 M salt. The two first eluting acceptor activities ($tRNA_1^{leu}$ and $tRNA_2^{leu}$), were free of cross contamination by each other and by $tRNA^{phe}$ as judged by the elution pattern. These tRNAs were not further purified. The third isoacceptor peak overlapped with the $tRNA^{phe}$ peak. Separation was achieved by RPC-5 chromatography (Pearson *et al.*, 1971). The leucine isoacceptors in the ethanol wash were divided into two activity peaks by Sepharose 4B chromatography (Holmes *et al.*, 1975). The purified tRNAs were numbered according to their elution order from BD-cellulose (1, 2, and 3) and according to Holmes *et al.* (1975) for 4 and 5.

Preparation of N-acetyl-Phe-$tRNA^{phe}$

N-acetyl-Phe-$tRNA^{phe}$ was prepared according to Rappoport and Lapidot (1974). After the acylation reaction, non-acetylated amino acids were stripped by Cu^{2+} treatment (Schofield and Zamecnik, 1968) and the remaining peptidyl-tRNA was finally purified on a BD-cellulose column in 50 mM NaOAc (pH 5) buffer containing NaCl. The column was loaded and washed in buffer with 0.2 M NaCl, washed at 1 M NaCl and the peptidyl-tRNA finally released with the buffer containing 1 M NaCl and 10% ethanol. The product was tested in a "burst" translation assay for maximal acceleration of poly(U)-directed peptide synthesis. Saturation of the activation of ribosomes was practically complete (~90%) at 1:1 ratio of NAc-Phe-$tRNA^{phe}$ to 70S.

Translation assays

PRS dependence of amino acid incorporation with bulk tRNA. The assay consists of two mixtures that are prepared on ice. Both of them contain the polymix buffer components. Mixture I additionally contains in 40 μl: 52 pmol of ribosomes, 27 μg poly(U), 80 pmol of N-acetyl-Phe-$tRNA^{phe}$ and 30 nmol [^{14}C]phenylalanine (4 c.p.m./pmol). Mixture II contains in 60 μl: 1.7 mM ATP, 1.7 mM GTP, 10 mM PEP, 550 μg of total *E. coli* tRNA, 160 pmol EF-G, 200 pmol EF-Tu, 0 or 150 pmol EF-Ts, 2.5 nmol [^{3}H]leucine (500 c.p.m./pmol), 5 units of LRS and 0.5–100 units of PRS. One unit of synthetase can produce 1 pmol of aminoacyl-tRNA from free amino acid and tRNA in 1 s at the substrate concentrations used here.

Mixtures I and II are preincubated for 15 min at 37°C. The elongation reaction is started by pipetting 40 μl of mixture I into reaction tubes containing 60 μl of mixture II. The reaction is stopped at 10 min by addition of 3 ml trichloroacetic acid containing 0.5% w/v of both phenylalanine and leucine. The background value (20 c.p.m. ^{14}C, 80 c.p.m. ^{3}H) was obtained from an assay performed in the absence of poly(U).

The samples were processed, counted and calculated as described by Jelenc and Kurland (1979).

PRS dependence of amino acid incorporation with purified tRNAs. The experiment was conducted similarly to the one above with three exceptions. Purified $tRNA^{phe}$ and $tRNA^{leu}$ were used instead of total tRNA. Mixture II contained 200 pmol of $tRNA^{phe}$ and 300 pmol of one of the $tRNA^{leu}$ isoacceptors. The EF-Tu content in mixture II was 300 pmol. The ribosome content in mixture I was 130 pmol (methanol-free ribosomes).

Determination of errors. Error for the total tRNA was determined as described in Wagner *et al.* (1982a). The error frequencies of the leucine isoacceptors were measured in a similar assay with a fixed input of $tRNA^{phe}$ (50 pmol) and different amounts of $tRNA^{leu}$ isoacceptor (0–200 pmol). The amount of available EF-Tu was kept higher (300 pmol) than the sum of all charged tRNA. The error was calculated by dividing the increase in leucine incorporation caused by 50 pmol of charged $tRNA^{leu}$ by the total phenylalanine incorporation.

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