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Hypothesis

Maintenance of accuracy during amino acid starvation

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The kinetics of the tRNA cycle is in itself capable of keeping the translational error level almost unaffected by amino acid starvation. There is no need to assume any yet unknown mechanism or property. Kinetic analysis shows that the concentration of aminoacyl-tRNA can stay high even for large reductions in aminoacylation, since the pool of uncharged tRNA normally is very small. An enhanced binding of uncharged tRNA to the ribosome could increase the effect and produce an extremely efficient error damping. A similar result is obtained when EF-Tu is partially inhibited by ppGpp.

Translational accuracy; tRNA cycle; uncharged tRNA; Stringent response; ppGpp; Elongation rate

1. INTRODUCTION

The accuracy in protein synthesis is dependent on the relative concentrations of cognate and non-cognate aminoacyl-tRNA competing for the same codon. One would expect that a reduced aminoacylation of cognate tRNA relative to the aminoacylation of non-cognate tRNA would result in a corresponding increase in misinsertions at the 'hungry' codons. How, then, is it possible for the cell to keep the translational error level at a near normal value during amino acid starvation, as happens in stringent response? In the past few years, a great deal of attention has been paid to this question and recently Ninio [1] proposed the 'accuracy tuner', and Gallant [2] a model which includes acylation of uncharged tRNA on the ribosome, as suggested solutions to the problem.

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However, such hypothetical models are not needed to explain the absence of additional misinsertions at hungry codons. The paths of the tRNA cycle, and its relative pool sizes, are sufficient to account for the retained accuracy during amino acid starvation. In this context, one should clearly distinguish between the acylation step and the binding of ternary complex to the ribosome, something which is not made in, for example, Ninio's and Gallant's models. It is hence not the same to say that there is a 10-fold reduction in aminoacylation and that there is a 10-fold decrease in the concentration of the corresponding aminoacyl-tRNA. With the aid of a kinetic model of the tRNA cycle I show here how the relative concentration of cognate/noncognate ternary complex may vary as the acylation of the cognate tRNA is impeded.

It is important to note that the kinetics of the steps of protein synthesis is a complex interplay of all the different pools along the tRNA cycle. When aminoacylation of a particular species is reduced, the pool of the corresponding uncharged tRNA in-

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creases, which partially compensates for the rate decrease. The concentration of acylated tRNA will thus never be completely reduced proportionally to the aminoacylation level. This is consistent with the finding that a large portion (8–40%) of the 'starved' tRNA remains in the acylated form during amino acid starvation [3–5]. The pools of cognate aminoacyl-tRNA bound to the ribosomal A- and P-sites may stay almost unchanged for rather large reductions in aminoacylation. This is an effect of the kinetics of the system, which under normal conditions is keeping the largest portion of tRNA in the ternary complex pool. It has been estimated that about 65% of all tRNA is in the ternary complex pool and a very small part is uncharged [6]. A 10-fold reduction in aminoacylation may thus result in a 10-fold increase in the concentration of uncharged tRNA, but in just a slight decrease in the aminoacyl-tRNA pool. Hence, hungry codons may not be as hungry as it would seem.

As will be shown here, this kinetic effect, which is always present, can in itself explain why the error level will not necessarily increase dramatically during amino acid starvation. Thus, no extra error-correction mechanisms are needed. Still, as I shall discuss, certain effects will enhance the pure kinetic effect. With a 'shunt' in the tRNA cycle an extremely efficient error damping can be achieved, with a 10-fold reduction in aminoacylation resulting in a 2% increase in error frequency. If elongation factor Tu (EF-Tu) is partially blocked by ppGpp, as may happen during amino acid starvation in *relA*$^+$ (wild-type) cells of *E. coli*, this too will effectively contribute to the restriction of translational errors.

2. THE tRNA CYCLE

The model for the tRNA cycle is essentially the same as described previously [7]. Here, however, the model has been extended to include the possibility that uncharged (free, deacylated) tRNA can bind to the ribosome, thus creating a shunt in the tRNA cycle (see fig.1). Also, the possibility of errors due to competition by non-cognate aminoacyl-tRNA is now included. For simplicity, I use only one kind of codon and two tRNA species competing for that codon: the cognate tRNA (1)

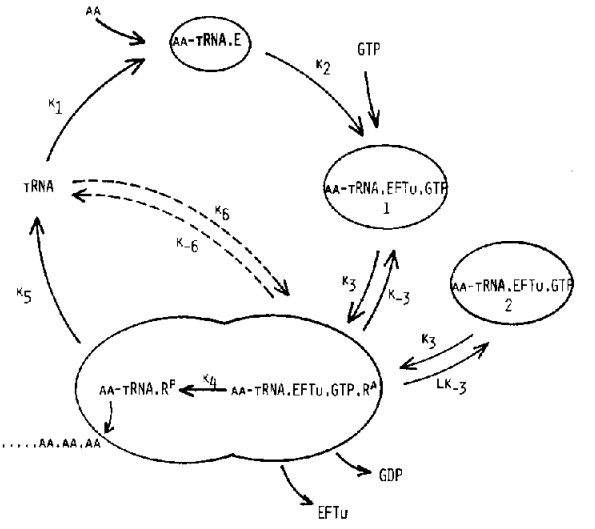

Fig.1. The tRNA cycle with a shunted pathway from the uncharged tRNA pool to the ribosomal A-site, and with competing non-cognate aminoacyl-tRNA.

for which different pools are calculated, and the non-cognate tRNA (2), which is kept at a constant aminoacyl-tRNA concentration (in ternary complex, aa-tRNA·EF-Tu·GTP). Besides, in the constant ternary complex pool, non-cognate tRNA also appears in the ribosomal A- and P-sites in this model.

The kinetic equations for the tRNA cycle of fig.1 are solved numerically for the steady-state case. The various tRNA pools are expressed in terms of the rate constants, k_1, k_2, k_3, k_{-3}, k_4, k_6, k_{-6}, and l, and the total amounts of ribosomes (loaded with the specific codon), synthetases, and tRNA of each species, which all are externally determined constants. The rate constant k_5, which determines the elongation rate, can be expressed as $k_5 = k_4(T_1^A + T_2^A)/R_1$, where T_1^A and T_2^A designate cognate and non-cognate aminoacyl-tRNA in ribosomal A-sites (aa-tRNA·EF-Tu·GTP·R^A), and R_1 is the total amount of active ribosomes loaded with codons of type 1 (see [7] for further details).

The choice of the constants above was based on experimental and calculated data on relative pool sizes [6,8], as well as on the values used by Ninio [1] and Gallant [2], for the sake of comparison (here only the approximate relative numbers are of importance).

Selection of substrate comes here only from the

constants l and k_{-3}/k_3, and the error frequency is calculated as,

$$e = P_2/P_1 = \bar{T}_2^P/T_1^P = T_2^A/T_1^A = (k_{-3} + k_4)/(lk_{-3} + k_4)T_2^T/T_1^T,$$

where T_1^P and T_2^P are the tRNA in P-sites (aa-tRNA·R^P), and T_1^T and T_2^T are the tRNA in ternary complex.

3. RESULTS

Simulation of the tRNA cycle model, with and without a shunt ($k_6 = 1000$ and $k_6 = 0$, respectively), is shown in fig.2, together with the models by Ninio and Gallant. In order to facilitate a comparison between the models, the error frequency ($e = T_2^P/T_1^P = P_2/P_1$) is plotted relative to the error frequency obtained for $k_1 = 1000$ for each model.

The error frequency is related to the ratio of cognate to non-cognate Tu-bound aminoacyl-tRNA, and this ratio varies in a complex way when acylation of uncharged tRNA is impeded, as discussed in section 1. This is shown in fig.3 for two different values of the tRNA/ribosome ratio (and hence also of the relative size of the ternary complex pool).

From fig.2 it is obvious that both the shunted and the unshunted tRNA cycle model may keep the error frequency lower than, or as low as, the models proposed by Ninio and Gallant for a large range of k_1. For the shunted cycle, where uncharged cognate tRNA may block the ribosomal A-site, the error frequency can always be kept close to the experimentally observed value (around 3×10^{-4}), even for a 1000-fold reduction in k_1. The shunted tRNA cycle is extremely stable to variations in pool sizes and rate constants. For instance, a 2-fold reduction in total amount of cognate (and non-cognate) tRNA, and thereby of Tu-bound tRNA, only slightly increases the error level at very low k_1 values. Also, if the binding of uncharged tRNA is weakened, so that the ratio k_6/k_{-6} decreases from, say, 10 to 2, the error frequency is only doubled for $k_1 = 1$. Simulations with unsaturated ribosomes or synthetases and with inhibition of EF-Tu gave similar results.

Even the unshunted tRNA cycle is able to keep the error level low, through the bulking of

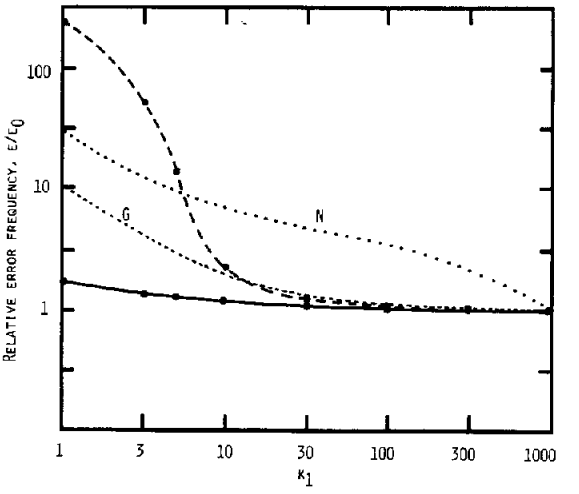

Fig.2. Simulation of the tRNA cycle model, together with curves from Ninio's and Gallant's models (N and G, respectively; parameter values as in fig.3 of [2]). The relative change in error frequency, e/e_0, where $e = P_2/P_1 = T_2^P/T_1^P$, and e_0 is the calculated error frequency for $k_1 = 1000$ in each model, is plotted vs the aminoacylation rate, k_1, as it varies from 1000 down to 1. Other parameters in the tRNA cycle model: $k_2 = 100$, $k_3 = 1000$, $k_{-3} = 100$, $k_4 = 120$, $k_5 = k_4(T_1^A + T_2^A)/R$ (as explained in the text), $k_6 = 1000$ in the shunted case (——) and $k_6 = 0$ in the unshunted case (– – –), $k_{-6} = 100$ and $l = 10000$. The relative amounts of cognate tRNA : synthetase : codon-loaded ribosomes, $T_1 : E_1 : R_1$, is 100:15:10. With these values $e_0 = 2.3 \times 10^{-4}$.

aminoacyl-tRNA in the ternary complex pool. The tRNA cycle without a shunt is however more sensitive to variations in pool sizes and rate constants. With the values used here (see legend to fig.2), assuming saturated ribosomes, the unshunted tRNA cycle can restrict errors almost as well as the shunted cycle, even for a large reduction of aminoacylation. It is only for extremely low k_1 values that the binding of uncharged tRNA to the ribosome becomes important for error damping. Inhibition of EF-Tu (by ppGpp) can also reduce the errors effectively at low k_1 values, by a reduction in the competing aminoacyl-tRNA pool as well. For example, a 1000-fold reduction of aminoacylation yields only a 10-fold increase in error frequency, if 25% of the EF-Tu molecules are blocked.

The variation in elongation rate when aminoacylation is reduced is shown in fig.4 for the shunted and unshunted tRNA cycle models. The higher accuracy of the shunted tRNA cycle is ac-

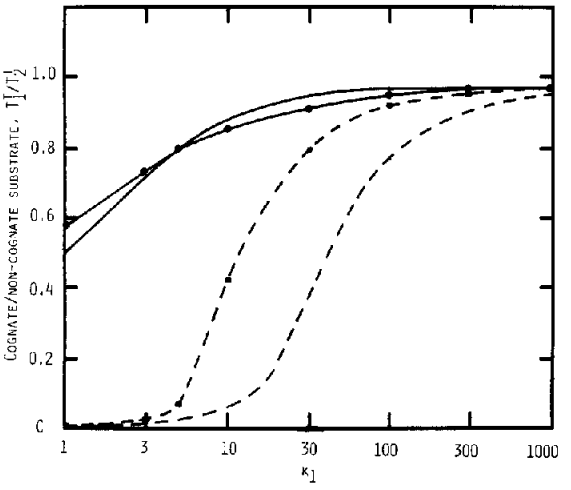

Fig.3. The variation of cognate/non-cognate aminoacyl-tRNA in ternary complex, T_1^T/T_2^T vs k_1. The solid lines show the variation for the shunted tRNA cycle, when $T_1 = 100$ and $T_2^T = 70$ (circles), and when $T_1 = 50$ and $T_2^T = 19$ (no circles). The dashed lines show the corresponding variation for the unshunted tRNA cycle (squares: $T_1 = 100$, $T_2^T = 70$; no squares: $T_1 = 50$, $T_2^T = 19$). All other parameter values as in fig.2.

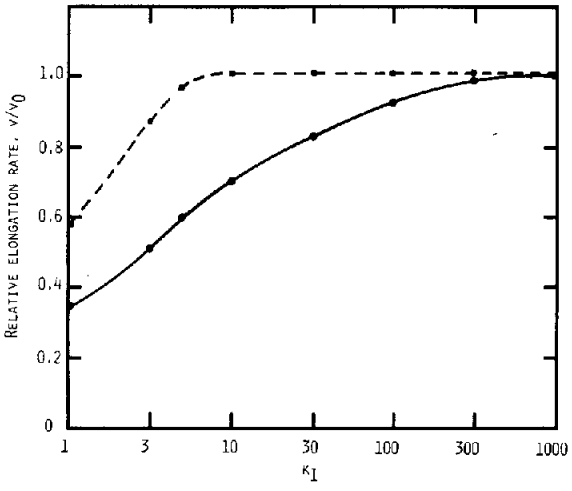

Fig.4. The normalized variation in elongation rate, v/v_0 vs k_1. $v = k_5$ and v_0 is the calculated rate for $k_1 = 1000$. The solid line is plotted for the shunted tRNA cycle and the dashed line for the unshunted cycle.

complished at the cost of a lower elongation rate, since in this model uncharged tRNA is blocking the ribosomal A-site and there is no acylation on the ribosome. It should be noted, though, that the small effect on elongation rate in fig.4 is due to the assumed saturation level of the ribosomes. For less saturated ribosomes, as when EF-Tu is partially blocked, the effect is much greater. Blocking of EF-Tu by ppGpp would reduce the elongation rate at all codons during starvation, and the overall protein production would decrease.

If non-cognate aminoacyl-tRNA is discarded, and translocated, at a lower rate than for cognate aminoacyl-tRNA, then the non-cognate tRNA could block the ribosomal A-site, through its longer binding time, in the same way as cognate uncharged tRNA. This was simulated by decreasing the constants l and k_4 for the non-cognate species. A considerable reduction in error frequency could be achieved, without the concomitant drop in elongation rate as for the binding of uncharged tRNA. However, at very large reductions of aminoacylation ($k_1 < 10$) the blocking by non-cognate aminoacyl-tRNA is unable to reduce the errors as effectively as that by cognate uncharged tRNA, simply because the concentration of the latter increases with decreasing acylation.

4. DISCUSSION

Considering the kinetics of the tRNA cycle, there is no need for any yet unknown mechanism to explain the maintenance of accuracy during amino acid starvation. In fact, recent experiments could not reveal any trace of the mechanisms proposed by Ninio and Gallant (Ehrenberg, M., personal communication). Since a major portion of the tRNA molecules is likely to be acylated and in ternary complexes, and a much smaller part is uncharged, a large reduction in aminoacylation is not followed by a corresponding reduction in ternary complexes. Hence, cognate tRNA may be able to compete with non-cognate tRNA to a greater extent than is normally thought. However, as the concentration of cognate acylated tRNA decreases and the pool of uncharged tRNA increases, there will also be a growing competition by the uncharged tRNA.

It is known that codon-specific binding of uncharged tRNA to the ribosome does occur [9], and that it results in the formation of ppGpp in *relA*$^+$ (normal) *E. coli* cells [8,10]. In these cells the translational accuracy is almost unaffected by amino acid starvation, whereas the error frequency

increases dramatically in *relA*$^-$ mutants, where no ppGpp is formed [4]. The exact action of ppGpp is not known, but it seems to affect the accuracy specifically at hungry codons [11]. The rate of gene expression in *relA*$^+$ cells may also be governed by ppGpp, through its inhibiting effects on RNA and protein synthesis (rather than by the limitation of aminoacyl-tRNA) [4,8]. Several theoretical models (e.g. [4,12,13]) have tried to explain its action, but so far none has been wholly successful. It has been suggested that ppGpp can bind to EF-Tu and then block the binding of aminoacyl-tRNA to EF-Tu in a non-specific way [12,14]. In the model described here this can enhance the error damping, even if EF-Tu is not saturated by ppGpp. The EF-Tu·ppGpp complex may possibly also facilitate the binding of uncharged tRNA to the ribosome, perhaps by forming a ternary complex with the uncharged tRNA. (In fact, certain uncharged tRNAs have been observed to carry an unidentified molecular group which protects them from degradation during amino acid starvation [3].) This action of ppGpp would reduce the elongation rate generally *and* specifically at the starved codon, and together with the reduced levels of rRNA, tRNA, and mRNA, this could explain the observed inhibition of protein synthesis during severe amino acid starvation.

During mild starvation in both animal cells [5,15] and *relA*$^-$ cells [3], or when the action of ppGpp is excluded for *relA*$^+$ cells [4], the rate of protein synthesis is surprisingly little affected. Even if there is a reduction of the elongation rate at hungry codons, it does not need to affect the overall rate of protein synthesis, as has been pointed out in previous work [15,16]. It depends on the degree of starvation and on the location of the hungry codons in the mRNA molecule. If the hungry codons appear far from the initiation region, and not in clusters, the risk is small that a local delay of a ribosome will affect initiation, and by that the rate of protein production.

The restriction of translational errors described here is solely an effect of the kinetics of the system. In addition to this effect, error-correction mechanisms, such as proofreading [12,14] and peptidyl-tRNA drop-off [17], may contribute to the maintenance of accuracy during amino acid starvation.

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REFERENCES

[1] Ninio, J. (1986) FEBS Lett. 196, 1–4.
[2] Gallant, J.A. (1986) FEBS Lett. 206, 185–188.
[3] Yegian, C.D. and Stent, G.S. (1969) J. Mol. Biol. 39, 45–58.
[4] O'Farrell, P.H. (1978) Cell 14, 545–557.
[5] Lofgren, D.J. and Thompson, L.H. (1979) J. Cell. Physiol. 99, 303–313.
[6] Gouy, M. and Grantham, R. (1980) FEBS Lett. 115, 151–155.
[7] Liljenström, H., Von Heijne, G., Blomberg, C. and Johansson, J. (1985) Eur. Biophys. J. 13, 115–119.
[8] Ingraham, J., Maaloe, O. and Neidhardt, F. (1983) Growth of the Bacterial Cell, Sinauer Associates, Sunderland, MA.
[9] Levin, J. and Nirenberg, M. (1968) J. Mol. Biol. 34, 467–480.
[10] Haseltine, W. and Block, R. (1973) Proc. Natl. Acad. Sci. USA 70, 1564–1568.
[11] Parker, J. and Holtz, G. (1984) Biochem. Biophys. Res. Commun. 131, 487–492.
[12] Rojas, A.-M., Ehrenberg, M., Andersson, S. and Kurland, C.G. (1984) Mol. Gen. Genet. 197, 36–45.
[13] Gallant, J., Weiss, R., Murphy, J. and Brown, M. (1985) in: The Molecular Biology of Bacterial Growth (Schaechter, M. et al. eds) pp.92–107, Jones and Bartlett, Boston.
[14] Dix, D.B. and Thompson, R.C. (1986) Proc. Natl. Acad. Sci. USA 83, 2027–2031.
[15] Harley, C.B., Pollard, J.W., Stanners, C.P. and Goldstein, S. (1981) J. Biol. Chem. 256, 10786–10794.
[16] Liljenström, H. and Von Heijne, G. (1987) J. Theor. Biol. 134, 43–55.
[17] Menninger, J.J. (1983) J. Mol. Biol. 171, 383–399.