Hypothesis

Uncharged tRNA error damping model

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Uncharged tRNA is known to bind to the ribosome in a codon-specific fashion. In this way, cognate uncharged tRNA competes with non-cognate aminoacyl-tRNA. If uncharged tRNA can be aminoacylated while on the ribosome, this will damp errors due to aminoacyl-tRNA imbalance. Kinetic analysis shows that this scheme reduces errors at 'hungry' codons considerably more effectively than J. Ninio's accuracy tuner model; for example, a 10-fold decrease in cognate aminoacyl-tRNA elicits only a 10% increase in the error frequency.

Translational accuracy *uncharged tRNA* *Ribosome*

Imbalances amongst the aminoacyl-tRNA pools, such as occur during amino acid limitation, ought to provoke corresponding increases in the frequency of translational error at the affected codons. For example, a 10-fold reduction in the concentration of one aminoacyl-tRNA species should result in a 10-fold increase in the relative frequency with which competing, noncognate aminoacyl-tRNAs bind to ribosomes which have the 'hungry' codons calling for the limiting species in their decoding sites. This passive response is observed in *relA*$^-$ mutants of *E. coli*, but *relA*$^+$ cells possess a mechanism to compensate for aminoacyl-tRNA imbalance so as to retain a normal or near-normal error frequency at hungry codons [1].

This adjustment of translational accuracy appears to apply *only* to hungry codons, and not to codons calling for fully aminoacylated tRNAs [2,3]. Ninio [4] has recently proposed the 'accuracy tuner' hypothesis to account for this unexpected selectivity. Briefly, the accuracy tuner model postulates that when ribosomes stall at a hungry codon, the increased time during which the A site remains empty permits a decrease in the intrinsic rate of action of peptidyl transferase. This has the effect of increasing accuracy at hungry codons through what Ninio has elsewhere [5] called 'kinetic amplification': a diminished rate of peptidyl transfer increases the time during which noncognate aminoacyl-tRNAs can dissociate from the ribosome (or can be ejected by a proofreading branch reaction) before the peptide bond is formed.

Here I will point out an experimental weakness of the accuracy tuner model, and propose a different way to increase accuracy selectively at hungry codons which does not suffer this difficulty.

If the increased time that ribosomes stall at hungry codons is what triggers the accuracy tuner, then one would expect nonsense codons to be translated with the increased accuracy characteristic of the accuracy tuner. This is because there are no tRNAs cognate to nonsense codons, and so ribosomes should stall with an empty A site even longer at nonsense codons than at hungry sense codons. It should be noted that the release factors are present at very low concentrations in *E. coli*, estimated at 100 [6] to 600 [7] molecules per cell. By contrast, iso-tRNAs are present at a range of concentrations with an average of about 5000 molecules per cell [8]. Therefore, it should take much more time for a ribosome to col-

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lide with a release factor than with a tRNA. This expected time lag at nonsense codons is probably the explanation of the specificity of phenotypic suppression by 5-fluorouracil incorporation into mRNA: the analogue suppresses many nonsense mutants strongly, but produces little or no suppression of missense mutants* [9,10].

The accuracy tuner model therefore predicts that mistranslation of nonsense mutations, i.e. readthrough, should occur much less frequently than mistranslation at non-starved sense codons. Experimental data contradict this prediction. The readthrough frequency of a large number of UAA and UAG nonsense mutations is broadly distributed about a mean of about 3×10^{-4} [11], while UGA mutations exhibit a higher readthrough frequency [12,13]. The mean frequency of mistranslation at unstarved sense codons, aggregated over a large number of positions, has been estimated as $2–4 \times 10^{-4}$ [14].

It follows that the accuracy tuner cannot be triggered simply by ribosome stalling, but by some other property of hungry sense codons which is *not* shared by nonsense codons. I suggest that this property is the binding of cognate *uncharged* tRNA to the ribosome. The codon-specific binding of deacylated tRNA has long been known [15,16], and in fact, it is the stimulus for ppGpp formation by the *relA*$^+$ gene product associated with the ribosome [17,18].

* Phenotypic suppression by 5-FU was discovered [9] during the bronze age of molecular biology, at a time when mutant classification was incomplete. By 1969, Rosen et al. were able to make some definitive assignments ([10], table 2). Of 32 mutants in the r_{II} and *Pho*A genes which were suppressed by 5-FU, 31 turned out to be nonsense (amber and ochre) and one remained unclassified. Of the many revertible mutants which were *not* amber or ochre and which did *not* respond to 5-FU, one can expect a reasonable number of missense mutants, and in fact 8 were so identified (most being unclassified). Putting this another way, 0 out of 8 known missense mutants, but 31 out of 51 known nonsense mutants, exhibited 5-FU phenotypic suppression, a highly significant difference ($\chi^2 = 7.3$ with Yates correction, $p < 0.01$). There were also highly significant differences in the frequency of 5-FU suppressible alleles amongst the three classes of nonsense mutants, which might reflect a difference in stalling time at terminators recognized by RF1 and those recognized by RF2.

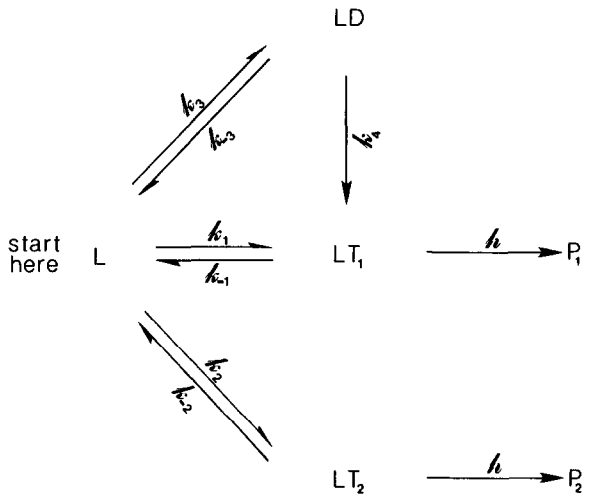

Fig.1. Kinetic scheme for error-damping by deacylated tRNA. The terminology follows that of [4]. A ribosome (L) can associate with a cognate aminoacyl-tRNA at rate k_1 to form complex LT_1, which dissociates with rate k_{-1}; it can associate with a noncognate aminoacyl-tRNA with rate k_2 to form complex LT_2, which dissociates with rate k_{-2}; or it can associate with cognate *uncharged* tRNA at rate k_3 to form complex LD, which dissociates with rate k_{-3}. Aminoacylation of the tRNA in complex LD can occur with rate k_4. Peptide bond formation converts complexes LT_1 or LT_2 to correct product P_1 or incorrect product P_2, both occurring at rate h.

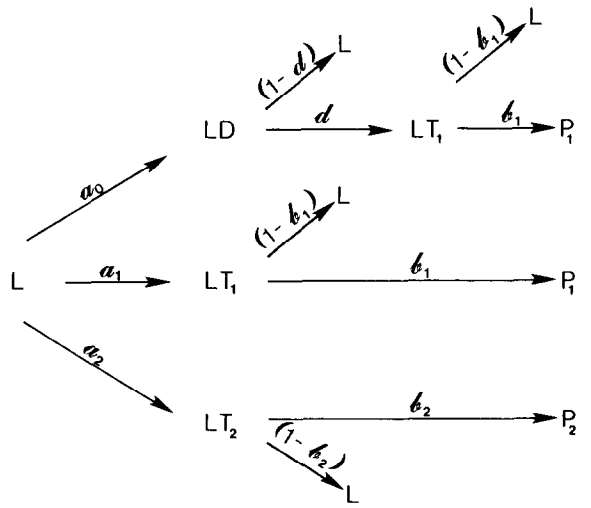

Fig.2. Path probability diagram of the kinetic scheme. Relative probabilities of transitions are shown on the arrows, and defined in terms of the rate parameters of fig.1 in the text.

One possibility, therefore, is that the binding of deacylated tRNA triggers the accuracy tuner, i.e. a decrease in peptidyl transferase activity. An accuracy tuner triggered in this way will damp errors at hungry codons even more effectively than one triggered by simple ribosome stalling (Ninio, personal communication).

The binding of deacylated tRNA at hungry codons could damp errors in a different way, without the need to postulate a switch in peptidyl transferase activity. The presence of a cognate deacylated tRNA in the ribosome's A site will block association with a noncognate aminoacyl-tRNA. If the cognate deacylated tRNA can be charged while on the ribosome, even at a greatly reduced rate, then very effective error damping is achieved.

Fig.1 presents this kinetic scheme, using the same terminology as that of Ninio's accuracy tuner with the following modifications: (A) there is no accuracy tuner, i.e. peptidyl transferase occur at a single rate h in all ribosomes; (B) cognate deacylated tRNA associates with ribosomes to form complex LD and dissociates at rate k_{-3}; (C) deacylated tRNA can be charged whilst on the ribosome at rate k_4.

Kinetic analysis was done by the path probability method described by Ninio [4]. The path diagram is shown in fig.2, where the relative path probabilities are as follows.

$$a_0 = \frac{k_3}{k_1 + k_2 + k_3};\ a_1 = \frac{k_1}{k_1 + k_2 + k_3};$$

$$a_2 = \frac{k_2}{k_1 + k_2 + k_3}$$

$$b_1 = \frac{h}{h + k_{-1}};\ b_2 = \frac{h}{h + k_{-2}}$$

$$d = \frac{k_4}{k_4 + k_{-3}}$$

$$\text{The error rate } \left(\frac{p_2}{p_1}\right) = \frac{a_2 b_2}{(a_1 b_1 + a_0 d b_1)}$$

Simulation was done as follows. I assume that the binding of charged cognate and noncognate tRNAs occurs at the same rate ($k_1 = k_2$) when both species are fully charged, and there is no deacylated tRNA present ($k_3 = 0$). I simulate limitation for the cognate amino acid by reducing k_1 and replacing each cognate aminoacyl tRNA by a cognate deacylated tRNA ($k_3 = k_2 - k_1$). For simplicity, I assume that cognate aminoacyl-tRNA and deacylated tRNA dissociate from the ribosome at the same rate ($k_{-3} = k_{-1}$). I assume that amino acid limitation reduces the charging of free tRNA and ribosome-bound deacylated tRNA identically ($k_1 = k_4$).

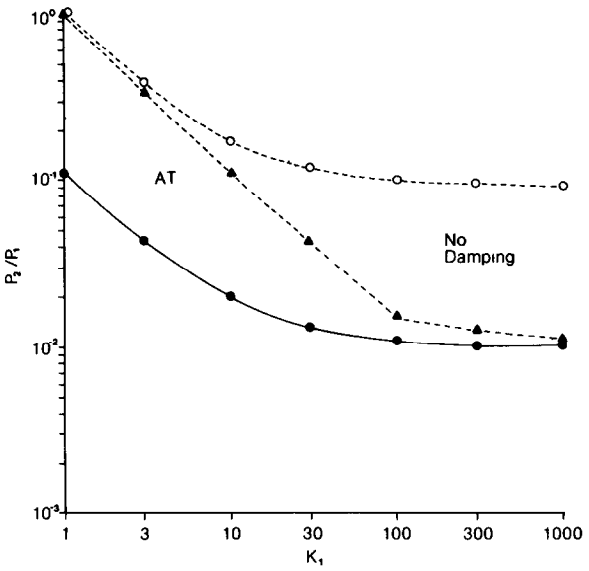

Fig.3. Simulation of the model. Error frequencies (P_2/P_1) are shown vs k_1. The dotted lines without points show the variation in error frequency with no error damping, or with Ninio's accuracy tuner (marked AT). The latter uses the same parameter values modelled in [4]: k_1 varies from 1000 down to 1; $k_2 = 1000$; $k_{-1} = 10$; $k_{-2} = 100000$; $h = 1000$; $\ell = 10$; $R = 300$. The solid line with filled circles shows the behaviour of the present model for the same parameter values of k_1, k_2, k_{-1}, k_{-2}, and h. R and ℓ do not exist in the present model. As described in the text, $k_3 = k_2 - k_1$, $k_4 = k_1$, and $k_{-3} = k_{-1}$. The dashed lines show the model's behaviour with different choices of parameter values. The filled triangles use the same values as above, except that k_{-1} is 100, i.e. cognate aminoacyl-tRNA dissociates from the ribosome 10-times faster. The open circles use the same values as the filled circles except that $k_{-2} = 10000$, i.e. noncognate aminoacyl-tRNA dissociates 10-times slower.

Simulation of the model is shown in fig.3, where the error frequency (P_2/P_1) is plotted vs the rate of

cognate tRNA binding to the ribosome (k_1) on a log-log scale. It can be seen that error damping is very effective: a decrease of k_1 from 1000 to 100 generates only a 10% increase in the error frequency, and a decrease of k_1 from 1000 to 10 generates only a 2-fold increase in the error frequency. The accuracy tuner model, shown for comparison, is considerably less effective.

Neither model damps errors effectively for very large decrease in k_1. This leads us to consider what range of variation in k_1 obtains in vivo. Virtually all measurements of tRNA charging in cells subjected to amino acid starvation or aminoacyl-tRNA synthetase inhibition indicate residual charging levels of 4–20%. If tRNAs are nearly fully charged during normal growth, then a roughly 10-fold decrease in k_1 is a realistic estimate for hungry codons. Through this range, the present model achieves excellent error damping, whereas the accuracy tuner model does not (see fig.3).

The present model is also robust in that it works over a large range of parameter values, illustrated for two examples in fig.3. The error damper's crucial requirement is only that k_4 be considerably greater than k_{-3}, i.e. that deacylated tRNA on the ribosome experiences a considerably greater chance of being aminoacylated than of dissociating from the ribosome.

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