Hypothesis

Fine tuning of ribosomal accuracy

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If the rate constant for peptide bond formation were high just after an amino acid incorporation and occasionally switched to a lower value afterwards, then the ribosome could compensate for tRNA imbalance specifically at hungry codons. A rigorous analysis of the scheme proves its effectiveness. For instance, a 10-fold reduction in cognate tRNA concentration may increase the error rate by only a factor of two.

Translational accuracy *Stringent control* *tRNA imbalance* *Mnemonic enzyme*

1. INTRODUCTION

The translation machinery has the potential to be more accurate than it normally is. High fidelity ribosome mutants are easily found both in procaryotes [1] and eucaryotes [2,3]. A single cut in *E. coli* ribosomal RNA suffices to make translation more accurate [4]. Under conditions of amino acid starvation, there is a deficit in tRNA acylated with the missing amino acid. One normally expects missense errors at 'hungry' codons to increase in proportion to tRNA imbalance. If, for instance, starvation reduces the concentration of cognate tRNA by a factor of 10 without changing the concentrations of non-cognate tRNAs, missense errors should be multiplied by a factor of 10. Now, it seems that stringent *E. coli* cells, when starved for an amino acid can compensate somewhat for the tRNA imbalance and recover their normal accuracy [5]. Apparently, the kinetics of protein synthesis are altered in many aspects [6], but there is no redress of the tRNA imbalance so the change presumably occurs at the ribosome level. There is no lack of theoretical models in which a metabolite, say ppGpp, by changing a kinetic constant of the protein synthesis machinery could increase the accuracy of translation at all codons [5,6]. However, it seems that the stringent response, although it restores accurate readings at hungry codons, does not increase the accuracy at other codons [7]. We need a model to account for such paradoxical observations. I will now show that there is indeed a way ribosomes can overcome problems of tRNA imbalance at hungry codons without changing their accuracy at other codons.

2. THE ACCURACY TUNER

To simplify the argument, we reduce ribosome kinetics to a Michaelis scheme, as in [8], although we know that things are far more complex:

$$\text{Ribosome} + \text{tRNA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Rib}\cdot\text{tRNA} \overset{h}{\rightleftharpoons} \text{peptide}$$

For such a simplified scheme, the lower the value of h, the higher the accuracy of translation [8]. For more complex schemes, a decrease in a rate constant for a given forward step may either increase or decrease accuracy, depending upon the exact location of the step within the reaction pathways [9]. Both direct and inverse correlations between speed and accuracy have been observed [10].

Let us imagine that peptidyltransferase can switch, in our simplified scheme, between 2 states of high (rate constant h) or low (rate constant l) efficiency of peptide bond formation. Suppose that

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the rate is set to the high value just after a peptide bond is formed, and that it may switch afterwards to the low value. If cognate tRNAs are abundant, the peptide bond will usually be formed without leaving time for the $h \rightarrow l$ switch to occur, and ribosomes will go on translating at their normal (low) accuracy. When a hungry codon is encountered, the deficit in cognate tRNA will give time for the $h \rightarrow l$ transition to occur, hence the increased accuracy will compenate somewhat for tRNA imbalance.

A mathematical treatment of the scheme is given in the appendix. The effectiveness of the scheme is demonstrated in fig.1 where it can be seen that, within a certain range, a 10-fold decrease in cognate tRNA concentration provokes a mere 2-fold increase in error rate.

3. DISCUSSION

The model predicts that all ribosomes have the potential to be in 2 states of high or low peptidyltransferase activity, corresponding to states of low and high accuracy. There is a large body of in vitro and in vivo evidence that under superficial examination may sustain or disprove the model. Fig.1 illustrates the difficulty. According to the range of the observations, and to the scatter of the experimental points, the log-log plot may appear concave, convex or linear. Furthermore, at very low cognate tRNA concentrations the sequestration effect [11] will bias the results. Shpaer [12] suggested that small hairpins occurring after rare codons in *E. coli* mRNAs contribute to slowing down translation at these codons. If correct this feature would add complexity to the scheme.

If the accuracy tuner does indeed operate in ribosomes, there are many ways it can be regulated. Some organisms may have it turned on permanently, others may turn it on or off according to circumstances or to developmental stage. In *E. coli rel+*, the switch would be under control of ppGpp, while in *rel−* mutants, the ribosomes would always be in the low accuracy state. The *rel−* and *rel+* error rates are given, under comparable conditions, by the quantities e_1 and e_0 in the appendix. Ways in which the scheme may be amended to fit best the *E. coli* data will be discussed elsewhere (Gallant and Ninio, in preparation).

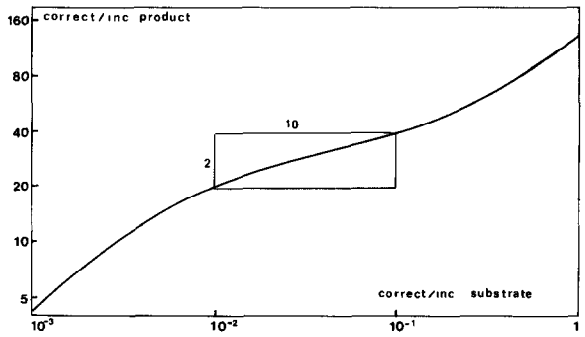

Fig.1. Efficiency of the accuracy tuner. The ratio of correct to incorrect product was computed for the following values of the kinetic parameters of the scheme in fig.2a: k_1 varying from 1 to 1000, $k_2 = 1000$, $k_{-1} = 10$, $k_{-2} = 100\,000$, $h = 1000$, $l = 10$, $r = R = 300$. k_1 and k_2 are the frequencies of associations between codons and cognate or non-cognate tRNAs, k_{-1} and k_{-2} are the dissociation rates, h and l are the rates of peptide bond formation in the low and high accuracy states respectively, r or R is the rate of ribosome switching from the low to the high accuracy state. As indicated by the rectangle, a 10-fold decrease in tRNA concentration brings about a 2-fold increase in error rate. Without accuracy buffering, the curve would be a straight line of slope 45°. The simulation does not take into account the effect of binding of deacylated tRNA to ribosomes. Such binding, increasing with amino acid starvation, would make the scheme even more effective.

The existence of the proposed device may have bearings on several domains of research, including codon usage, context effects, variability in translation rates and the maintenance of translational accuracy in perturbed environments.

APPENDIX: KINETIC ANALYSIS

A scheme for accuracy tuning is shown in fig.2a. The high and low accuracy ribosomal states are designated by H and L. Subscripts 1 and 2 stand for cognate and non-cognate tRNAs. The scheme is of the 'mnemonic' (hysteretic) type [13–15]. Note that *E. coli* DNA polymerase I is a mnemonic enzyme [16]. The calculation of error rates for a given kinetic scheme can be simplified, without loss of rigour, by considering the probabilities of various paths in the reaction scheme. The method, inspired from [17], was outlined in [18] and further developed in [9]. The sceptical reader can check the

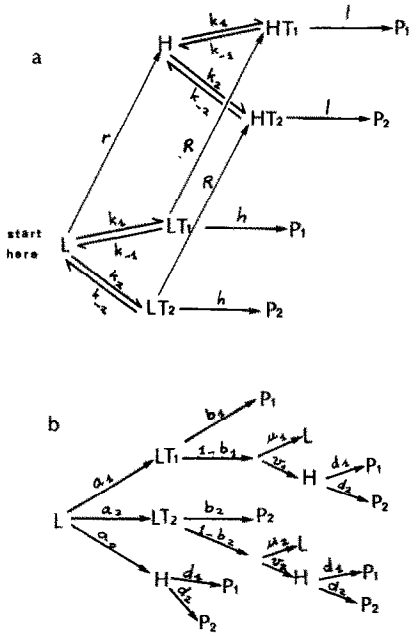

Fig.2. Kinetic scheme for buffering accuracy. (a) Ribosomes in a state of low (L) or high (H) accuracy may bind cognate or non-cognate tRNAs (T_1,T_2) and thus incorporate correct or incorrect amino acids. After a peptide bond is formed, the ribosome always starts in state L, but may thereafter switch to state H. Here, k_1 and k_2 represent collision frequencies and stand for the more traditional $k_1(T_1)$ and $k_2(T_2)$ terms. When cognate tRNAs are rare, the ribosome has higher chances to switch to the H state, and thus become more discriminative in its choice between correct and incorrect substrates. (b) The scheme can be rewritten as a graph, showing the various reaction pathways. For the purpose of error rate calculations, pathways that involve a return to the initial state need not be taken into account [7].

result by applying the standard steady-state treatment. The probabilities shown in fig.2b obey the following constraints:

$$a_0+a_1+a_2=1;\ d_1+d_2=1;\ u_1+v_1=u_2+v_2=1$$

Their expression, in terms of the kinetic parameters in the scheme, are as follows:

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

$$b_1=\frac{h}{h+k_{-1}+R}+\frac{R}{h+k_{-1}+R}\times\frac{l}{l+k_{-1}}$$

$$d_1=\frac{k_1(l+k_{-2})}{k_1(l+k_{-2})+k_2(l+k_{-1})}$$

$$v_1=\frac{R}{R+k_{-1}+l}$$

For the probabilities of the paths involving the second substrate, invert subscripts 1 and 2 in the corresponding expressions.

The error rate (incorrect/correct product) is given by:

$$e_0=\frac{a_2b_2+\epsilon d_2}{a_1b_1+\epsilon d_1}$$

with:

$$\epsilon=a_0+a_1(1-b_1)v_1+a_2(1-b_2)v_2$$

The intuitive meaning of the expression for e_0 is as follows: d_2/d_1 is the error rate that would be obtained if the ribosome were always in the high accuracy state. ϵ is the probability that the ribosome switches from the L to the H state before a peptide bond can be formed. a_2b_2/a_1b_1 is the error rate that would be obtained if binding of tRNA were allowed only in the L state.

ϵ contains 2 terms: a_0, which corresponds to the L→H transition in the absence of tRNA, and the rest to the same transition in its presence. In most reasonable situations, and in particular, in the numerical example of fig.1, the second part is negligible so that a_0 is preponderent. This amounts to say that one can make $R=0$ in the scheme. By doing so, the scheme would be simplified on paper, but be more complex in reality, for it would imply that tRNA binding raises a barrier against a possible transition.

By and large, there are several error rates to consider: the error rates e_1 $(=k_2(h+k_{-1})/[k_1(h+k_{-2})])$ and e_3 $(=d_2/d_1)$ of the pure low and high accuracy pathways of the scheme, the blended error rate e_2 $(=a_2b_2/a_1b_1)$ and the true error rate of the scheme, e_0. Contrary to intuition, e_2 can be larger than e_1. On the other hand, e_0 is necessarily between e_1 and e_3. This puts limitations on the effectiveness of the scheme. If for some set of conditions $e_3/e_1 = 100$, then the maximum compensation that can be obtained for a reduction in cognate tRNA concentration is 100. As shown in the practical example of fig.1, the scheme can be quite

effective in a 10-fold range of concentration changes.

The kinetic tuner can be imbedded into a more complex and realistic scheme of ribosome kinetics [19,20]. Whereas in the energy-relay scheme [21] the free energy of an enzymatic reaction is used to amplify the accuracy of the next reaction, in the present scheme, part of the free energy excess of protein synthesis [22] may be diverted to switch the ribosome to the low accuracy state. The proposed scheme for accuracy buffering need not be restricted to ribosomes and may of course apply to other enzymatic systems.

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