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Rate and accuracy of bacterial protein synthesis revisited

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Our understanding of the accuracy of tRNA selection on the messenger RNA programmed ribosome has recently increased dramatically because of high-resolution crystal structures of the ribosome, cryo-electron microscopy reconstructions of its functional complexes, and fast kinetics experiments. Application of single-molecule spectroscopy with fluorescence resonance energy transfer to studies of tRNA selection by the ribosome has also provided new, albeit controversial, insights. Interestingly, when the fundamental trade-off between rate and accuracy in substrate-selective biosynthetic reactions is taken into account, some aspects of the current models of ribosome function appear strikingly suboptimal in the context of growing bacterial cells.

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Introduction

In this brief review, we first outline the physical–chemical basis for the existence of a fundamental trade-off between speed and accuracy of tRNA selection in protein synthesis (Box 1). This type of trade-off is general and also applies to other substrate discriminating biosynthetic activities, like aminoacylation of tRNA, transcription, reverse transcription, and chromosome replication. We discuss how its deleterious effects can be attenuated by energy-driven proofreading of substrates, and then set the speed–accuracy dilemma in evolutionary context to suggest optimal kinetics solutions based on a criterion of fitness maximization (Box 2). These novel considerations, showing for the first time that rapid protein synthesis in the living cell actually *requires* high accuracy, are used to discuss recent advances in the current understanding of the accuracy of tRNA selection, based on crystal structures and new kinetics experiments, including single-molecule spectroscopy.

Trade-off between speed and accuracy in protein synthesis

The fundamental trade-off between the speed and accuracy by which the messenger RNA (mRNA) programmed ribosome selects aminoacyl-tRNAs in ternary complex with EF-Tu·GTP can be understood from elementary Michaelis–Menten kinetics (Box 1). The translation accuracy depends not only on how well the ribosome discriminates a cognate from a near-cognate ternary complex in terms of different binding standard free energies ($\Delta\Delta G$) but also on how well the ribosome utilizes this difference to repress amino acid substitution errors. Most probably, the ribosome has evolved to maximize $\Delta\Delta G$, and thereby the *intrinsic selectivity*, by an optimized design of the substrate-binding pocket in the transition state for product formation, while the degree of utilization, determined by the *discard parameters*, has evolved to maximize the growth rate of bacteria (Box 2). Interestingly, as described in the example below, the trade-off between speed and accuracy becomes significant *only* when the accuracy (A) is tuned close to its upper limit given by the $\Delta\Delta G$ (Box 1, Figure 1a).

The actual accuracy (A) is defined as the ratio between the effective rate constants (k_{cat}/K_m) for the association of cognate and near-cognate substrates, telling us how much more often a cognate substrate is chosen compared to a near-cognate substrate at the same concentration. Assume, for example, that the standard free-energy difference $\Delta\Delta G$ corresponds to a factor of 1000 larger binding affinity for the cognate than for a near-cognate substrate, then the actual accuracy A can only approach the intrinsic selectivity 1000 at the cost of a greatly decreased rate of cognate product formation ($k_{cat}/K_m \rightarrow 0$) (Box 1, Figure 1a (black line)). The reason is that full utilization of the intrinsic selectivity requires the concentrations of cognate ternary complex on and off the ribosome must be equilibrated. This trade-off was clarified by Ninio [1] in the analysis of experimental data on ribosomal mutants with hyper-accurate as well as error-prone phenotypes [2].

According to the analysis in the previous paragraph, there exists an upper limit to the accuracy of protein synthesis which depends on $\Delta\Delta G$. Already in 1957, before the advent of molecular biology, Linus Pauling postulated that this intrinsic (thermodynamic) limitation to the selection of similar amino acids would give rise to very large amino acid substitution errors in intracellular proteins [3]. However, both his own and others' experimental data showed much smaller frequency of amino

Box 1 Calculating the trade-off between speed and accuracy in protein synthesis

Most ribosomes in growing bacteria participate in protein elongation as polysomes [23], and their mode of action is therefore similar to that of enzymes working in the steady state. Accordingly, the Michaelis–Menten formalism and, in particular, the parameter k_{cat}/K_m, is essential for discussions about the accuracy of tRNA selection by mRNA-programmed ribosomes in the living cell. k_{cat}/K_m is defined as the second-order association rate constant k_a for substrate binding times the probability that a bound substrate forms a product:

$$\left(\frac{k_{cat}}{K_m}\right) = k_a P(\text{product}) \tag{1}$$

The k_{cat}/K_m parameters define the relative rate by which an enzyme can form product from one substrate compared to that from other substrates and a comparison of k_{cat}/K_m values for different substrates can therefore be used to calculate the accuracy of an enzyme. First, we apply a simple textbook version of a Michaelis–Menten scheme to the initial selection mechanism of ternary complexes (T_3) on the ribosome (R):

$$R + T_3 \underset{k_d}{\overset{k_a}{\rightleftarrows}} R_1 \xrightarrow{k_1} \tag{2}$$

Here k_a is the association rate constant for a ternary complex to the ribosomal A-site, while k_d is the corresponding dissociation rate constant and k_1 is the forward rate constant for ternary complex, associated with the GTPase activation of EF-Tu, so that $P(\text{product}) = k_1/(k_1 + k_d)$. With equal concentrations of cognate and near-cognate ternary complex, the (normalized) accuracy, A, of ternary complex selection, is the ratio between k_{cat}/K_m for formation of cognate (superscript c) and near-cognate (superscript nc) products:

$$A = \frac{(k_{cat}/K_m)^c}{(k_{cat}/K_m)^{nc}} = \frac{k_a k_1^c}{k_1^c + k_d^c}\frac{k_1^{nc} + k_d^{nc}}{k_a k_1^{nc}} = \frac{1 + d_I a_I}{1 + a_I} \tag{3}$$

The accuracy A is determined by the intrinsic selectivity, d_I, and the discard parameter, a_I, for a cognate ternary complex, which for Scheme (2) can be written:

$$d_I = \frac{k_1^c}{k_1^{nc}}\frac{k_d^{nc}}{k_d^c} = e^{-\Delta\Delta G_I/RT} \quad \text{and} \quad a_I = \frac{k_d^c}{k_1^c} \tag{4}$$

Here $\Delta\Delta G_I$ is the difference between the cognate and near-cognate standard free energies in the GTPase activation step, R is the molar gas constant, and T is the absolute temperature. The coupling between intrinsic selectivity d_I and $\Delta\Delta G_I$ reflects that the intrinsic selectivity is reached when the system is equilibrated, that is, when the rate $(k_{cat}/K_m)^c$ goes to zero. Rearranging Eq. (3) gives the expression for the trade-off between rate for cognate substrate, $(k_{cat}/K_m)^c$, and accuracy (A):

$$\left(\frac{k_{cat}}{K_m}\right)^c = k_a\left(\frac{d_I - A}{d_I - 1}\right) \tag{5}$$

Although d_I confers an upper limit to the accuracy of ternary complex selection, it is possible to use the same d_I parameter for aminoacyl-tRNA selection in subsequent proofreading steps. The reason is the near-irreversible GTP-hydrolysis step, which allows for kinetic proofreading step à la Hopfield [5]:

$$\begin{array}{l} R + T_3 \underset{k_d}{\overset{k_a}{\rightleftarrows}} R_1 \xrightarrow{k_1} R_2 \xrightarrow{k_{pep}} \\ \qquad\qquad \downarrow q_d \\ \qquad EF\text{-}Tu \cdot GDP + aa\text{-}tRNA \end{array} \tag{6}$$

The intrinsic selectivity, d_F, and a cognate discard parameter, a_F, can be defined also for the proofreading step according to

$$d_F = \frac{k_{pep}^c}{k_{pep}^{nc}}\frac{q_d^{nc}}{q_d^c} = e^{-\Delta\Delta G_F/RT} \quad \text{and} \quad a_F = \frac{q_d^c}{k_{pep}^c} \tag{7}$$

Here k_{cat}/K_m is determined by two probabilities:

$$\frac{k_{cat}}{K_m} = k_a\frac{k_1}{k_1 + k_d}\frac{k_{pep}}{k_{pep} + q_d}$$

The overall accuracy, A, is now factorized into an initial selection, I, and a proofreading selection, F:

$$A = \frac{k_{cat}^c/K_m^c}{k_{cat}^{nc}/K_m^{nc}} = IF = \frac{1 + d_I a_I}{1 + a_I}\frac{1 + d_F a_F}{1 + a_F} \tag{8}$$

By setting $a_F = a_I = a$ and $d_F = d_I = d$, in line with early experimental estimates suggesting equal contribution of initial selection and proofreading to A in tRNA selection by ribosomes [9], we obtain:

$$A = \frac{k_{cat}^c/K_m^c}{k_{cat}^{nc}/K_m^{nc}} = \left(\frac{1 + da}{1 + a}\right)^2 \tag{9}$$

Thus, assuming two equal selection steps separated by an almost irreversible reaction, the expression for the trade-off between rate and accuracy in the selection of tRNA on the ribosome becomes:

$$\left(\frac{k_{cat}}{K_m}\right)^c = k_a\left(\frac{d - \sqrt{A}}{d - 1}\right)^2 \tag{10}$$

acid substitution errors than he thought physically possible [3,4]. This apparent paradox led to a search for ways by which enzymes could transcend the intrinsic accuracy limit.

Here, the concept of kinetic proofreading, or kinetic amplification, enters the story. The idea of proofreading, independently clarified by Hopfield [5] and Ninio [6], is that the initial selection of substrates is followed by one or more additional selection steps, in which the substrates can be irreversibly discarded from the enzyme. The irreversibility requires coupling to a thermodynamic driving force, which in the case of protein synthesis is provided by GTP-hydrolysis on EF-Tu [7]. The stoichiometry between GTP-hydrolysis and peptide bond formation was used by Thompson and Stone to identify the proofreading of aminoacyl-tRNAs by the translating ribosome [8]. In the presence of proofreading, the overall accuracy can be factorized into an initial selection and a proofreading selection, and, in line with early experimental estimates suggesting equal contribution of initial selection and proofreading to the overall accuracy in tRNA selection by ribosomes [9], we obtain a maximal accuracy of $1000^2 = 1\,000\,000$ for our hypothetical case. Again, this value is not reached unless the rate of elongation goes to zero (Figure 1a (red line)). However, as illustrated by Figure 1a, it is possible with only minor effects on the product formation rate, to utilize up to 10 and 1% of the maximal (intrinsic) accuracy for one-step

Box 2 Optimal rate and accuracy of protein synthesis in an evolutionary context

For bacteria growing under stationary or varying conditions, their fitness will be related to their constant or time averaged growth rate, respectively. The growth rate, μ, in turn, is determined by the concentration, [R], of elongating ribosomes in the cell, the average protein elongation rate, v_e, on the ribosome and the quality, q, of the synthesized proteins [24]:

$$\mu = \frac{[R]v_e q(A)}{\rho_0}$$

Here ρ_0 is the total concentration of amino acids that are used in the proteins of a cell, and the quality q increases monotonically with the normalized accuracy, A, of tRNA selection on the ribosome. There will be an optimal accuracy, A, that maximizes the growth rate, μ, and the fitness of the population because a large amino acid substitution (missense) error frequency reduces q and a large accuracy, A, reduces the rate of protein elongation (Figure 1). In line with this prediction, it was found that bacterial mutants with error-prone or hyper-accurate ribosomes both have growth rates below that of wild-type [25].

Assuming tRNA selection in two equivalent steps as in Scheme (6) of Box 1, an intrinsic discrimination parameter d of 30 000, a rough estimate of how amino acid substitution errors affect the average quality, q, of proteins and taking the trade-off between rate and accuracy (Figure 2) into account, the optimal missense error level has been estimated as about 10^{-5} substitutions per amino acid residue ($A \approx 10^5$) [15]. This value is substantially higher than older estimates of the *in vivo* accuracy [26], but in the same range as recent estimates on error frequencies in living cells [27•].

It should also be noted that these predictions were all made on the assumption that neither near-cognate nor noncognate tRNAs inhibit protein elongation in the living cell. This is in line with early biochemical experiments suggesting that near-cognate tRNAs are very poor inhibitors of binding and processing of cognate tRNAs by the *E. coli* ribosome [28], but in contrast to results obtained by others (as discussed in the main text).

(black line) and two-step (red line) selection mechanisms, respectively. When the rate–accuracy relation is brought into the context of the living cell, where cognate tRNAs always compete with a large number of near-cognate and noncognate tRNAs, it is seen that the speed of protein synthesis as a function of the accuracy of tRNA selection has a well-defined maximum, before the onset of the trade-off close to near-maximal accuracy (Figure 1b).

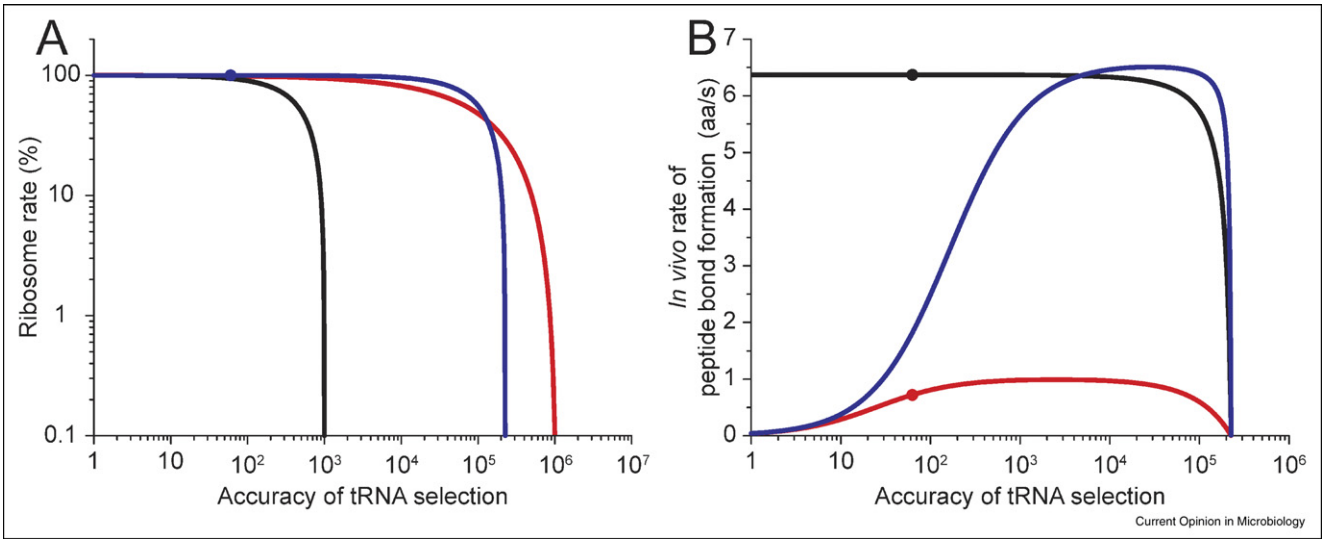
Recent advances in molecular level understanding of accuracy in protein synthesis from structures and kinetics

Kinetic studies of the *Escherichia coli* ribosome have in the past decade been dominated by Rodnina, Wintermeyer and collaborators at the University of Witten-Herdecke. An important part of their work on tRNA recognition and peptidyl-transfer is schematically summarized in Figure 2a [10••]. The kinetic constants they measure propose a very high intrinsic discrimination in the initial selection of tRNAs, that is, 226 000 instead of 1000 as in the example discussed above. This intrinsic selectivity is partitioned between a binding selectivity of 347 (i.e. the ratio between k_{-2} for the near-cognate and cognate cases in Figure 2a) and a GTPase activation selectivity of 650 (i.e. the ratio between k_3 for the cognate and near-cognate cases in Figure 2a) [10••].

It has been a long-standing mystery how the ribosome allows a tRNA, when in ternary complex with EF-Tu and GTP, to form the codon–anticodon interactions required for a large intrinsic accuracy. This enigma was recently resolved by cryo-EM, revealing the anticodon stem loop region of a cognate, EF-Tu-bound tRNA to be bent and twisted such that the anticodon is optimally oriented for precise codon recognition [11,12]. The discovery of a large GTPase activation selectivity [10••] is nicely in line with an important hypothesis by Ramakrishnan and collaborators, based on the crystal structure of the 30S ribosome in various complexes with error-inducing antibiotics and cognate or near-cognate analogs of tRNA bound to the ribosomal A site [13,14]. These structures reveal that upon cognate, but not near-cognate, codon–anticodon interaction, there is a domain closure of the 16S rRNA structure, concomitant with a 'flipping out' of its bases 1492 and 1493, allowing for stereo-chemical recognition of correct pairing between bases one and two of a codon and the corresponding bases of the tRNA anticodon. Accordingly, the partial intrinsic binding selectivity of 347 in the scheme in Figure 2a would correspond to the free-energy difference between cognate and near-cognate tRNA provided by Watson–Crick hydrogen bonding alone, and the GTPase activation selectivity of 650 could be provided by the stereo-selectivity because of interaction of the codon–anticodon helix with bases 1492 and 1493 of 16S rRNA.

Two features of the scheme in Figure 2a are surprising. Firstly, the actual initial selectivity is 60 [10••], which is but a tiny fraction of the maximally possible, intrinsic selectivity, of 226 000. As discussed in the previous section, an increase of the accuracy by orders of magnitude with virtually no kinetic losses can be achieved without changing the intrinsic selectivity of the ribosome but merely tweaking its utilization (Figure 1a, blue line). Secondly, in the proposed scheme (Figure 2a) [10••] there is a tRNA binding site of high and uniform affinity for all types of tRNAs before they contact the mRNA codon. This step leads to strong inhibition of protein elongation *in vivo* [15], as illustrated in Figure 1b. Here we use the scheme in Figure 2a to predict the average rate of cognate protein elongation in the *E. coli* cell when the accuracy of ternary complex selection is varied by changing the rate constant k_{-2} by the *same* factor for both cognate and near-cognate substrates (Figure 1b, red line) (this kind of universal change can easily be probed in nature, e.g. by mutations affecting the ternary complex binding site). The prediction from this analysis is that an accuracy increase will, in fact, *increase* rather than decrease the

Figure 1

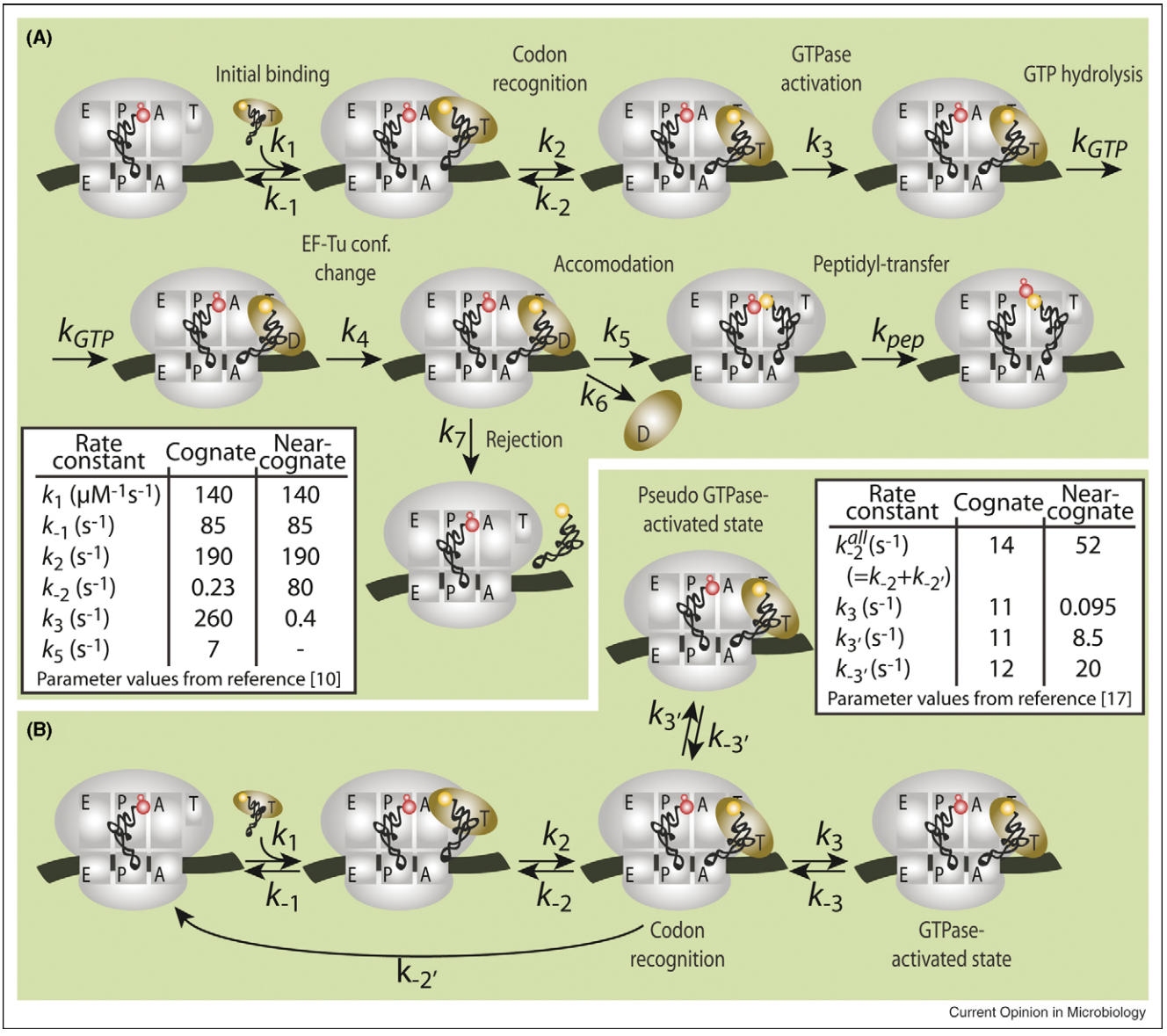


The trade-off between rate and accuracy. Panel A: the rate constant for cognate peptide bond formation $(k_{cat}/K_m)^c$ (normalized to $(k_{cat}/K_m)^c$ without any selectivity, $A = 1$) is plotted versus the normalized accuracy of ternary complex selection on the ribosome (see Box 1 for details). The black line illustrates a one-step selection scheme (Scheme (2) in Box 1) with a maximal ('intrinsic') selectivity of 1000. The red line illustrates a hypothetical two-step selection mechanism (Scheme (6) in Box 1) where each step has the intrinsic selectivity of 1000, giving an overall intrinsic selectivity of 10^6. The blue line represents the rate–accuracy trade-off in the initial selection of ternary complex on the ribosome, based on the scheme and rate constants from [10••] which is presented in Figure 2a. The parameters are here adjusted by scaling the rate constant k_{-2} for both cognate and near-cognate substrate by the same factor. The intrinsic selectivity of the initial selection of this scheme is 226 000, while the actual selectivity utilized, 60, is shown as a blue dot. Panel B: the black line shows how the *in vivo* rate of peptide bond formation depends on the initial selection of ternary complexes on the ribosome, according to the scheme and rate constants from [10••] illustrated in Figure 2a (see Box 1 for details). The accuracy is varied by changing the rate constants k_{-2} for both cognate and near-cognate ternary complex by the same factor. The *in vivo* concentration of cognate tRNA is here assumed to be 2 μM (2% of 100 μM total tRNA) in accordance with [22] and no binding of near-cognate or noncognate tRNAs is accounted for. The red line shows the same calculation as for the black line, but now taking into account 15 μM of near-cognate tRNAs and 83 μM of noncognate tRNAs [22] interacting with the ribosome according to the scheme and rate constants from [10••] which is shown in Figure 2a. The blue line illustrates what happens when the affinity of the mRNA-independent binding step in Figure 2a is reduced by increasing the rate constants k_2 and k_{-1} by the same factor of 10 000. The black and red dots show the values of the protein synthesis rate and the actual accuracy utilized for the scheme and rate constants as presented in [10••].

rate of protein elongation *in vivo*, because of reduced inhibition by near-cognate ternary complex. It also shows the deleterious effects of the mRNA-independent binding of all tRNAs, which brings the maximal rate at optimal accuracy down from seven to about one per second. This means that the parameter values in [10••] for the scheme in Figure 2a are far off from those in the living cell, and strikingly suboptimal with respect to *both* rate and accuracy [15].

A new and exciting approach to study the rate and accuracy of protein synthesis has recently been taken by Puglisi, Chu and collaborators [16•,17•]. They used single-molecule spectroscopy and fluorescence energy transfer to explore how a ternary complex, containing Phe-tRNA^{Phe} and EF-Tu bound to the noncleavable GTP analog GDPNP, interacts with ribosomes programmed either with a cognate UUU (Phe) or with a near-cognate CUU (Leu) codon. One advantage of the single-molecule approach is that different ribosomal states have distinct and identifiable FRET levels, and based on these experiments they suggested how initial selection of ternary complex occurs (Figure 2b) [17•]. However, this scheme, along with a similar scheme in the previous paper [16•], has a dissociation rate constant (k_{-2} in Figure 2b) directly from the codon recognition to the free state of ternary complex without a matching association rate constant, thereby violating the detailed balance constraint [6,18] and, ultimately, the second law of thermodynamics. Their proposed scheme partitions the total intrinsic selectivity of ternary complex recognition in a binding selectivity (i.e. the ratio between k_{-2}^{all} for the near-cognate and the cognate case in Figure 2b) of about 4 and a GTPase activation selectivity (i.e. the ratio between k_3 for the cognate and the near-cognate case in Figure 2b) of about 120 [17•], leading to an overall intrinsic selectivity for ternary complex recognition of 450. We note that the binding selectivity of 4 in Figure 2b is much smaller than the corresponding selectivity of 370 estimated by Gromadski and Rodnina [10••], and that also the overall intrinsic selectivity is much smaller (450 versus 226 000). According to Lee *et al.* [17•], therefore, AU-base pairing in

Figure 2



Rate constant	Cognate	Near-cognate
k_1 ($\mu M^{-1}s^{-1}$)	140	140
k_{-1} (s^{-1})	85	85
k_2 (s^{-1})	190	190
k_{-2} (s^{-1})	0.23	80
k_3 (s^{-1})	260	0.4
k_5 (s^{-1})	7	-

Rate constant	Cognate	Near-cognate
k_{-2}^{all} (s^{-1}) ($=k_{-2}+k_{-2'}$)	14	52
k_3 (s^{-1})	11	0.095
$k_{3'}$ (s^{-1})	11	8.5
$k_{-3'}$ (s^{-1})	12	20

Recently suggested schemes for ribosome catalyzed peptide bond formation. Panel A represents the model proposed by Gromadski and Rodnina in [10••]. Panel B shows the proposed model of initial selection from Lee *et al.* [17•].

the first codon position of a ternary complex in ground state only results in an intrinsic selection advantage of a factor of 4, that is, about two orders of magnitude less than suggested by other estimates [10••,13]. The single-molecule experiments also suggest that the cognate GDPNP-containing ternary complex attains stable binding to the ribosome in the GTPase-activated state [17•], while Gromadski and Rodnina suggest the GDPNP-containing ternary complex to be in the ground state [10••]. If Lee *et al.* are right, it would mean that the value of the cognate rate constant k_{-2} in Figure 2a is greatly underestimated by Gromadski and Rodnina and that their estimate of the intrinsic selectivity of ternary complex selection is greatly overestimated. The answer to the question, if the GDPNP-containing ternary complex is in the ground state [10••] or in the GTPase-activated state [17•], may be resolved by a comparison of the cryo-EM structure of a GDP-containing cognate ternary complex stabilized on the ribosome by the antibiotic kirromycin [11,12] with a very recent cryo-EM structure of the ribosome bound to a GDPNP-containing cognate ternary complex (J Frank, submitted).

Conclusions

The detailed scheme for tRNA selection and peptidyl-transfer provided by the Witten–Herdecke groups

(reviewed in [19]) has provided us with unprecedented knowledge of important details of mechanism of ribosome function, and the fruitful integration between these results and intelligently interpreted information from crystallography (reviewed in [14,20••]) is a scientific break-through. However, when the widely accepted kinetic model by Gromadski and Rodnina [10••] is brought into the context of the living cell, it completely fails to reproduce *in vivo* data on protein elongation [15,21]. One reason for this failure is the very small utilization (60) of the extremely large (226 000) intrinsic ternary complex selectivity, meaning that an increased utilization would not only reduce the frequency of amino acid substitution errors, but also *increase* the rate of peptide-elongation in the living cell (Figure 1b). So, if the model and its parameter values are basically correct, why did evolution not bring it from a suboptimal performance with low accuracy and small elongation rate to an optimal performance with much higher accuracy and faster protein synthesis? Another peculiar feature relates to the high-affinity, mRNA-independent binding state of the model, which greatly reduces the protein elongation rate through strong inhibition by noncognate and near-cognate tRNAs alike (Figure 1b). So, if this feature of the model also is basically correct, what has prevented Mother Nature from reducing this affinity and removing the inhibition? Is there, for instance, a physical constraint implying that the high-affinity state is necessary to preserve a high association rate constant for cognate ternary complex?

When the biochemistry of ribosome function has attained full *in vivo* compatibility, an important step toward its integration with bacterial physiology has been taken. Such an integration will, we suggest, greatly improve the relevance of *in vitro* experiments as well as the interpretability of *in vivo* experiments, and eventually tell us whether the parameter values associated with the Gromadski–Rodnina model are unrealistic or if they reflect physical constraints on the evolution of ribosome function that are unknown at the present time.

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