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A Semi-quantitative Treatment of Missense and Nonsense Suppression in the *str*A and *ram* Ribosomal Mutants of *Escherichia coli*

Evaluation of some Molecular Parameters of Translation *in vivo*

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The efficiency of missense and nonsense suppressors is affected in different ways in *Escherichia coli* strains carrying different kinds of ribosomal mutations: see Gorini's review (1970). This led Gorini (1971) to postulate a "ribosomal screen" able to distinguish between normal and mutant tRNAs. Here we examine the alternative hypothesis, that the results on suppression can be accounted for by minor changes in the kinetics of polypeptide chain elongation in mutant ribosomes.

A general kinetic scheme for the attachment of tRNA to the A site of ribosomes is described. It is postulated that the tRNA first makes a "loose" bond with the codon. A second event is required to stabilize binding and to allow transpeptidation. The probability that the second event occurs is related to the time that the tRNA sticks to the codon in the loose binding state. Ribosomal mutations would make the transition from loose to tight binding more probable (*ram*) or less probable (*str*A), per collision.

A method of analysis is developed that enables one to relate directly the experimental measurements on suppression to molecular parameters. By numerical fitting, it is shown that the following set of conditions can account for the behaviour of the various ribosomal mutants.

(a) In the wild-type cell, when a codon becomes associated with its cognate tRNA or release factor, the probability of ensuing chain elongation or termination is very close to unity. The probability of elongation decreases to about one-half in *str*A1 strains.

(b) Loaded nonsense suppressor tRNAs *su*1, *su*2 and *su*3 are present in such amounts that they collide with UAG about as often as the release factor does, but their binding is such that peptide bond formation is not very efficient.

(c) In contrast, the low efficiency of two missense suppressors appears related to a relatively low frequency of codon-loaded suppressor collisions, while the association itself may be either stronger (*su*58) or much weaker (*su*78) than that of the corresponding normal codon–tRNA association.

1. Introduction

The effect of streptomycin on translation ambiguity *in vivo* and *in vitro* has been studied extensively for the past ten years (see reviews by Pestka, 1971; Davies, 1969). A number of *Escherichia coli* ribosomal mutants have been isolated that differ

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from wild type in their behavior when cells are grown in the presence of the drug. They were first classified as streptomycin sensitive, resistant or dependent. More recent work has allowed a further characterization of these mutants in terms of two classes of mutations, namely *str*A and *ram*. The notations used here are those proposed by Gorini (1970).

The *str*A mutants appear to diminish the level of naturally occurring errors, as measured by the amount of reading through of nonsense codons. They also diminish the level of nonsense suppression by the tRNA suppressors *su*1, *su*2 and *su*3 (Gartner & Orias, 1966; Gorini *et al.*, 1966; Strigini & Gorini, 1970). In contrast, the *ram* mutants appear to increase the level of naturally occurring errors, and of nonsense suppression (Rosset & Gorini, 1969). The effects of both *ram* and *str*A on missense suppression were also studied (Biswas & Gorini, 1972).

Genes *str*A and *ram* code for two different proteins of the 30 S ribosome subunit (Ozaki *et al.*, 1969; Zimmerman *et al.*, 1971). Studies on cell-free protein synthesis showed that the *str*A and *ram* effects could be demonstrated *in vitro* and were mediated by the 30 S subunit (Rosset & Gorini, 1969). These experiments helped to establish the notion that the ribosome exerts a direct control on accuracy in protein synthesis. Thus, the influence of *strA* and *ram* is held to be exerted directly at the translational level rather than at the level of the error-correcting devices involving nucleases and proteases. However, the mechanism whereby the two loci control accuracy is unknown.

The change in efficiency of suppression brought about by the ribosomal mutations varies widely from case to case, with the occasional occurrence of reversed patterns. For instance, upon infection by bacteriophage T4 in a number of *str*A mutants, the larger the suppression by *su*1 and *su*2, the smaller it is with *su*3 (Rosset & Gorini, 1969). It appeared as though the ribosome was responding in a differentiated manner to the various suppressors. Gorini was led to put forward the hypothesis of a "ribosomal screen" that is able to distinguish between normal and suppressor tRNAs (Gorini, 1971).

Others held the view that the results obtained by Gorini and co-workers did not necessarily imply the ability of the ribosome to distinguish between wild-type and suppressor tRNA. The observed phenomena might instead be related to changes in the kinetics of polypeptide synthesis (Orgel, unpublished results; Ninio, 1973). An example was constructed and briefly described (Ninio, 1973), in which the capacity of the ribosome to discriminate between two competitors was dependent on a single kinetic parameter. This model is further examined in section 6. A model leading to simpler calculations will be considered in the next section.

I will attempt to show that there is a method for analyzing quantitatively the results obtained by Gorini and co-workers. The method is based on a kinetic scheme for the binding of tRNA to the A site of ribosomes and gives some insight into the molecular parameters of translation *in vivo*. The conclusions are not critically dependent on the precise kinetic model, provided that certain broad conditions are satisfied (see section 6).

2. Simplified Kinetic Model

The productive binding of tRNA to the acceptor site of the ribosome is considered here to require two events. The first event is the collision of a tRNA with a codon, leading to a transitory association. Then a second event sets up the process that leads

ultimately to peptide bond formation. The second event may be, for instance, the formation of a second contact with the ribosome, or the interaction with a "supernatant" protein. Our crucial assumption is that the probability that the second event occurs subsequent to a primary collision is related to the time that the tRNA sticks to the codon. If the sticking time is short, the tRNA is likely to fall off before the amino acid incorporation can occur. We further make the reasonable simplifying assumption that for very short "sticking times" the probability of incorporation (p) is proportional to the sticking time (θ) and for long enough sticking times, the probability of incorporation is close to unity. In this simple treatment, we shall use the Laplace distribution for p, namely $p = 1 - e^{(\theta/\tau)}$, where τ is the characteristic time-constant of the process.

Now, there is a way to refine the picture of the codon–anticodon transitory association, which at the end will bring a simplification. When two molecules are associated, after a collision, their sticking time is not uniform, but follows a probability distribution. A very reasonable probability distribution is the "exponential decay". Such a distribution simply means that the molecules have no memory of anterior states; if two molecules are associated at time t, their probability of getting dissociated between times t and $t + dt$ is independent of the time at which the association started. An exponential decay can be characterized by a time-constant θ. The probability of remaining associated at time t for one hybrid takes the form: $p(t) = e^{-(t/\theta)}$.

Combining the exponential decay for the codon–anticodon transitory association, and the Laplace distribution for the occurrence of the second event, and integrating over t yields the extremely simple formula:

$$p(\theta,\tau) = \frac{\theta}{\theta + \tau}. \tag{1}$$

The principle of our explanation for the restriction in the efficiencies of various suppressors stems from the consideration of quantitative relations of the type expressed in equation (1). If there is a ribosomal mutation that changes the value of

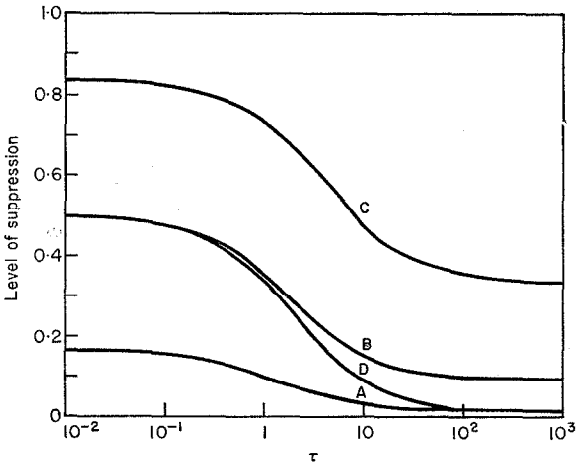

FIG. 1. Theoretical cases of competition. In A, B and C we assume that a suppressor tRNA with $\theta = 1$ is competing against a wild-type tRNA with $\theta = 10$, the relative multiplicity (m) being 0·2 for curve A, 1 for curve B and 5 for curve C. In D, a suppressor tRNA with $\theta = 1$ competes against a wild-type tRNA with $\theta = 100$, the multiplicity being 1.

τ, it affects the probability of amino acid incorporation subsequent to codon–anticodon collision. However, the change in the probability differs from one tRNA to the other, depending upon the time-constant of the loose association. A decrease in the value of τ has a large effect on the probability of incorporation if $\theta < \tau$, but has little effect if $\theta \gg \tau$ since in the latter case, p is already close to unity. It would be equally possible and equally plausible to propose that ribosomal mutations have the effect of increasing (*ram*) or decreasing (*str*A) all, or many, of the sticking times θ.

In order to reduce the number of disposable parameters when fitting the data, we make two simplifying assumptions (more general treatments will be discussed later).

(a) The value of τ depends only on the ribosome. To each genotype, for instance *str*A2 *ram*$^+$ is associated one, and only one, value of τ.

(b) The value of θ depends only on the tRNA species, and the site on mRNA to which it binds, irrespective of the τ characteristic of the ribosome.

Then, equation (1) can be read this way: when a tRNA–codon association of characteristic sticking time θ is formed on a ribosome of characteristic transition time τ, there is a probability $\theta/(\theta + \tau)$ of transferring the amino acid to the growing polypeptide chain.

TABLE 1

Ribosomal mutants

Designation	Genotype		τ
A	*str*A$^+$	*ram*1	<1
B	*str*A$^+$	*ram*$^+$	1
C	*str*A60	*ram*$^+$	3·2
D	*str*A1	*ram*1	7
E	*str*A40	*ram*$^+$	9
F	*str*A2	*ram*$^+$	30
G	*str*A1	*ram*$^+$	100

The parameter τ indicates the ease with which the amino acid is transferred to the polypeptide chain once the codon–anticodon association is formed. The proposed values for τ were obtained by numerical fitting of the data provided by Gorini (1971) and Biswas & Gorini (1972) (see text). In the *ram*1 mutants, the level of errors in translation is very high. As a result, amino acid substitutions occur not only at the considered missense or nonsense site, but at other sites as well. This feature was elegantly demonstrated by Biswas & Gorini (1972), using a missense suppressor and a nonsense suppressor in combination. The incidence of this effect on the measured suppression levels makes a quantitative treatment involving state A pointless. The τ value for state D should also be taken with caution. With the exception of state D, all the ribosomal mutants considered here can be ordered on a unique scale with respect to their ability to perform phenotypic suppression.

Hereafter we shall designate the ribosomal states by roman capital letters, according to Table 1. Thus, *str*A$^+$*ram*$^+$ is state B, while *str*A1*ram*$^+$ is state G. Arabic numerals will be allocated to tRNAs and release factors. Then equation (1) can be rewritten in the case, for instance, of an association relative to a tRNA1 and a ribosome in state A:

$$p_1^A = \frac{\theta_1}{\theta_1 + \tau_A}. \qquad (2)$$

Let us consider a tRNA1 and a tRNA2 competing for the same codon. If Z_1 and Z_2 are the frequencies of collisions that lead to an effective codon–tRNA transitory association, one can define a relative multiplicity factor $m_{1,2} = Z_1/Z_2$. Z_1 and Z_2 should be roughly proportional to the concentrations of loaded tRNA1 and tRNA2. The relative rates of incorporation of the amino acids carried by the two tRNAs will depend upon p_1^A, p_2^A and $m_{1,2}$. In practice, one of the competitors is a nonsense or a missense suppressor, the other is a wild-type tRNA or a release factor. The experimentally observed quantity, the "level of suppression", is given by

$$s = \frac{mp_1}{mp_1 + p_2}, \tag{3}$$

or to be more precise:

$$s_{1,2}^A = \frac{m_{1,2}p_1^A}{m_{1,2}p_1^A + p_2^A}. \tag{4}$$

This equation, which relates the level of suppression to the relative efficiencies of collisions and to the probabilities of transition from loose binding to tight binding, is independent of the precise kinetic model used. The model is involved when one tries to assign precise values to the p terms, for example through equation (1).

Equation (3) can be rewritten introducing a quantity μ:

$$\mu \equiv \frac{s}{1-s} = \frac{mp_1}{p_2}. \tag{5}$$

In the case of nonsense suppression, μ is the transmission/termination ratio.

Introducing the μ values allows us to get rid of the multiplicity parameters when analyzing the data. For instance, one can write

$$\frac{\mu_{1,2}^A}{\mu_{1,2}^B} = \frac{p_1^A}{p_2^A} \times \frac{p_2^B}{p_1^B}. \tag{6}$$

The ratio on the left is directly related to the experimental data, and the expression on the right is devoid of the m values. In Gorini's terminology, the "restriction index" for tRNA2, when switching from mutant A to mutant B, is $s_{1,2}^A/s_{1,2}^B$. Using the μ values instead of the s values makes the mathematics simpler.

It is easy to see that if $\tau_B > \tau_A$,

$$\frac{\mu_{1,2}^A}{\mu_{1,2}^B} < 1 \qquad \text{implies } \theta_2 < \theta_1.$$

If we have in two separate experiments, two competitors 2 and 3 against the same "normal" tRNA1, and if again we take $\tau_B > \tau_A$, then

$$\frac{\mu_{1,2}^A}{\mu_{1,2}^B} \times \frac{\mu_{1,3}^B}{\mu_{1,3}^A} > 1 \qquad \text{implies } \theta_2 < \theta_3.$$

Therefore, if the experiments give a number of μ values, a rapid comparison enables one to rank some of the various θ values involved in increasing order, irrespective of the multiplicity factors.

It is easy to demonstrate that for $\tau_B > \tau_A$ and $\theta_1 < \theta_2$,

$$\frac{\mu^B_{1,2}}{\mu^A_{1,2}} > \frac{\tau_A}{\tau_B}. \tag{7}$$

This inequality corresponds to the expected property that an increase in transition time τ cannot produce a higher relative increase in discrimination between two competitors. However, if θ_1 is small compared to the τ values, and θ_2 is large compared to them, the two ratios of inequality (7) are close.

To make the situation intuitive, several theoretical cases of competition are shown in Figure 1.

Equation (1) is rather demanding, and it is generally impossible to choose τ values to fit sets of "suppression" values chosen at random. Consider the figures presented in Table 2.

TABLE 2

An example of the type of data susceptible to quantitative analysis

Ribosomal state	Suppressors 1	2
A	0·40	0·40
B	0·30	0·26
C	0·10	0·18

The figures are levels of suppression by tRNA1 or tRNA2 acting at the same locus, in the 3 ribosomal mutants A, B and C.

We assume that we have two cases of suppression by tRNA1 or tRNA2 competing against the normal tRNA3.

We can fix arbitrarily $\tau_A = 1$, and we then have seven parameters to fit the data: τ_B, τ_C, θ_1, θ_2, θ_3, m_1 and m_2.

From

$$\frac{\mu^A_{1,3}}{\mu^B_{1,3}} \times \frac{\mu^B_{2,3}}{\mu^A_{2,3}} = 0{\cdot}82,$$

we deduce that $\theta_2 < \theta_1$.

From

$$\frac{\mu^A_{1,3}}{\mu^C_{1,3}} \times \frac{\mu^C_{2,3}}{\mu^A_{2,3}} = 2,$$

we deduce that $\theta_1 < \theta_2$.

Therefore, in that case, although we have seven disposable parameters we cannot fit the six observations. On the other hand, if we allow for the possibility of experimental error, we may consider that the two columns of Table 2 are the results of a duplicate experiment with tRNA1. Fixing arbitrarily $\tau_A = 1$, we have five parameters to fit the data, and this can be done with some flexibility.

3. Fitting the Data

Let us consider first the data provided on missense suppression by Biswas & Gorini (1972), and on nonsense suppression by Gorini (1971).† Both concern cells grown at 37°C. A connection between the two sets of experiments is provided by the *su*3 data (Gorini, 1971), which involve all the ribosomal states (see Table 3). The data indicate the percentage of enzymatic activity per mg of total proteins found in the various mutants, as compared to wild type. The nonsense suppression data (*su*1, *su*2 and *su*3) describe the suppression of one amber mutation in the ornithine transcarbamylase gene. The missense suppression data (*su*58 and *su*78) refer to two different loci in the tryptophan synthetase *A* gene. There are 27 experimental observations and 21 disposable parameters, including 5 m, 8 θ and 8 τ values, one of which can be chosen arbitrarily (here, $\tau_B = 1$).

Unfortunately, the experimental levels of suppression cannot be equated unequivocally to the s terms defined in equations (3) or (4). (a) The three nonsense suppressors *su*1, *su*2 and *su*3 insert three different amino acids in response to UAG. These are serine, glutamine and tyrosine, respectively. The way these replacements affect the activity of the enzyme and its susceptibility to proteases is unknown. Furthermore, the messenger RNA carrying the nonsense codon may be more labile than the corresponding wild-type messenger. (b) Another source of uncertainty stems from the possibility that, through indirect consequences, the error-correcting devices are not working with the same efficiency in the various mutants.

In order to deal with *both effects* described in (a) above, I have considered arbitrary corrections for enzymatic activity. Thus, there are two columns for *su*2 in Table 3, one corresponding to no correction, and the other to the situation where the experimental data underestimate the level of suppression by a factor of four. Similarly, the *su*3 data are analyzed both uncorrected, and with a twofold correction. The multiplicative coefficients were chosen so as to bring the efficiencies of suppression by *su*1, *su*2 and *su*3 more in line with the previous evaluations obtained in other situations, using rather direct methods (Kaplan *et al.*, 1965; Garen *et al.*, 1965).

There is no way to deal here with effect (b). Our treatment is valid if the experimental data reflect authentic ribosomal effects, and invalid if the differential performance of error-correcting devices in the various mutants is the source of the difference between them.

Equations (1) or (2) relate to tRNA binding. At this stage, we have no idea of how *str*A and *ram* may change the probability of termination subsequent to the association of a nonsense codon with a release factor. We will make use of a computation artifact, that equations (1) or (2) apply to release factors as well.

The various assumptions for fitting the data are now entirely explicit. One can attempt to find the best values of the parameters through a trial and error procedure, using a computer. However, I have preferred to take advantage of the mathematical simplicity of the equations. The calculations were done with a slide rule (or, when an additional decimal place was required, with a desk calculator). The reader can check very easily that the proposed values for the τ terms (Table 1) combined with the proposed values for the θ and m terms (Table 5) do indeed fit the data, by

† The latter are mainly from Dr G. A. Jacoby's unpublished work and reproduced with his permission in Breckenridge (1969) and Gorini (1969). The work on *su*3 suppression was completed by Biswas & Gorini (1972).

TABLE 3

Nonsense and missense suppression data

Ribosomal state	*su*1		*su*2			*su*3			*su*58		*su*78	
	Obs.	Calc.	Obs.	Calc.	Calc.†	Obs.	Calc.	Calc.†	Obs.	Calc.	Obs.	Calc.
B	41·8	42·1	3·4	3·5	3·3	26·5	26·5	26·8	7·75	7·60	7·50	7·57
D‡									7·75	7·65	6·75	6·71
C	30·1	31·3	1·6	1·5	1·5	21·5	21·0	21·3				
D						14·8	15·7	15·9	7·50	7·73	5·60	5·50
E	19·1	19·0	0·55	0·58	0·66	14·2	13·8	14·0				
F	9·1	8·4	0·15	0·21	0·23	6·5	6·6	6·7				
G‡						4·0	5·0	5·1	8·50	8·37	3·00	2·14
G	5·7	3·7	0·10	0·08	0·09	2·7	3·0	3·0	8·75	8·89	0·75	1·25

The observed values indicate the percentage of specific enzymatic activity found in various strains as compared to wild type. The nonsense suppression data (*su*1, *su*2 and *su*3) correspond to the suppression of the same amber mutation in the ornithine transcarbamylase gene (Gorini, 1971). The missense suppression data (*su*58 and *su*78) correspond to two different loci in the tryptophan synthetase *A* gene (Biswas & Gorini, 1972).

The ribosomal states were defined in Table 1. The θ and the m values used in the computation of the predicted suppression levels are given in Table 5.

† The case where one takes into account possible corrections for loss of enzymatic activity following the insertion of a wrong amino acid, according to Table 5.

‡ Cells grown in the presence of streptomycin. For this case, we used $\tau_D = 3$ and $\tau_G = 45$.

following the instructions given in the legend of Table 5. This may be the best exercise for understanding the truly intuitive simplicity of the treatment.

In practice, the strategy for fitting the data makes use of the following equation:

$$\frac{\mu^{B}_{1,0}}{\mu^{C}_{1,0}} = \frac{(\theta_0 + \tau_B)(\theta_1 + \tau_C)}{(\theta_1 + \tau_B)(\theta_0 + \tau_C)}, \tag{8}$$

which is obtained by combining equations (2) and (6).

As an example, consider that the level of suppression by *su*1 is 41·8% in state B and 30·1% in state C. From there, the μ values of the left side of equation (8) are computed according to equation (5). τ_B is set as equal to 1, and we shall attempt to see if it is possible to fit the data for various values of τ_C. From equation (8) one can deduce the sticking time of *su*1 (θ_1) as a function of that of the release factor (θ_0). The results are shown in Table 4 for $\tau_C = 3$, 5 and 10. As soon as θ_1 is computed, m_1 can be deduced from equation (5).

TABLE 4

Mutual dependency of the kinetic parameters

Assumed	Computed					
	($\tau_c = 3$)		($\tau_c = 5$)		($\tau_c = 10$)	
θ_0	θ_1	m_1	θ_1	m_1	θ_1	m_1
∞	1·98	1·08	5·00	0·860	12·5	0·775
200	1·92	1·09	4·71	0·865	11·2	0·777
100	1·85	1·10	4·46	0·869	10·0	0·781
50	1·71	1·12	4·01	0·878	8·36	0·788
25	1·51	1·16	3·33	0·896	6·24	0·800
15	1·28	1·20	2·40	0·952	4·26	0·828

A typical stage in fitting the data. The computation makes use of only 2 experimental values: the levels of nonsense suppression by *su*1 in states B and C (see Table 3). Then, for every value of τ_c, θ_1, the sticking time of *su*1, is computed as a function of θ_0, the sticking time of its competing release factor, using eqn (8).

The value of τ_C cannot be chosen arbitrarily. If it is taken as $\leq 2{\cdot}1$, there will be no way to fit the *su*2 data, according to the inequality (7). If it is taken as too large a value (above 10 for instance), we shall get into another kind of trouble. A large value for τ implies a large value for θ_1 and, therefore, a p value close to unity. Then one could not expect *ram*1 to be much less discriminative than *str*A$^+$*ram*$^+$ with respect to suppression by *su*1 (or *su*3), which appears contrary to the evidence, although the data are somewhat ambiguous (see the legend of Table 1). There are also some limitations on θ_0. Given θ_0, θ_1 and m_1, one can compute a "minimum level of suppression" that would be reached for $\tau = \infty$. This level is very simply given by

$$s_\infty = \frac{m\theta_1}{m\theta_1 + \theta_0}. \tag{9}$$

Now, if we take $\theta_0 \leq 25$ (for $\tau_C = 3$) or $\theta_0 \leq 50$ (for $\tau_C = 5$) and compute s_∞, it will be seen that it is impossible to go down to the suppression level of 5·7%

observed for state G. Therefore, the study can be restricted to the large values for the parameter θ_0. However, θ_0 cannot be taken as equal to infinity, for then it will not be possible to interpret the missense *su*78 data. The argument is a bit involved. For both *su*1 and *su*78, there is roughly a tenfold decrease in the value of μ when going from state B to state G. Calling θ_{78} and θ_{10} the sticking times of *su*78 and of its wild-type competitor (a $tRNA^{Cys}$), one can write that:

$$\text{sign of } (\theta_0 - \theta_{10}) = \text{sign of } (\theta_1 - \theta_{78}). \tag{10}$$

This relation can be derived by equating the right side expressions of equation (8) for *su*1 and *su*78. In general, when the suppression level is plotted as a function of τ, a rapid variation occurs around the value of τ that is equal to the shorter of the sticking times (see Fig. 1). It is perceptible from the data that the major decline in suppression levels occurs for *su*78 later (i.e. for larger τ values) than for *su*1 or *su*3. This says that *su*78 has a longer sticking time than *su*1, and from equation (10) one then deduces that the θ value for $tRNA^{Cys}$ must be taken as $>\theta_0$, which in turn cannot be taken as equal to infinity.

The preceding discussion should make one thing clear. Although the proposed solution is not unique, any other numerical fitting of the same data must lead to a very similar pattern of results, as expressed in the coarse-grained statements (a) to (f) of the next section.

4. Results

If our treatment is valid, the following conclusions are reached (Table 5).

(a) When wild-type $tRNA^{Asp}$ or $tRNA^{Cys}$ bind to their respective codons, on a wild-type ribosome, the sticking time is long enough to let the amino acid be incorporated in the growing polypeptide chain with a probability close to unity. Less than 2% of the associations are abortive. In the most restrictive strain, *str*A1, the probability decreases to about one-half, thus producing a twofold slowing in one of the steps involved in amino acid incorporation. It also appears that the association of the release factor R1 with UAG is rarely abortive.

(b) When nonsense suppressors *su*1, *su*2 and *su*3 bind to UAG, the sticking time is relatively short (0·3 to 5 times the transition time τ_B). The probability of having the amino acid transferred to the polypeptide chain is in the range 0·3 to 0·85.

(c) The concentration of acylated nonsense suppressors *su*1 and *su*3 is such that they hit the UAG codons about as often as the release factor does (the multiplicity is in the range 0·4 to 1·6). The concentration of *su*2 may be slightly lower.

(d) The variation in efficiency of nonsense suppression as a function of the location of the nonsense mutation appears related then to small variations in suppressor tRNA binding, rather than to variations in release factor binding.

(e) The missense suppressor *su*58 sticks better to its codon than the corresponding wild-type tRNA. The low efficiency of suppression is related to the small amount of suppressor tRNA that reaches the codon (8 to 10% compared to $tRNA^{Asp}$).

(f) In another case (*su*78) the sticking time is short, but larger than those obtained with nonsense suppressors. The low level of suppression is again a result of a low multiplicity (m).

TABLE 5

Molecular parameters of suppression

Suppressor	θ_1	p_1^B	$m_{1,2}$	Competitor	θ_2	p_2^B
*su*58	130	0·99	0·082	$tRNA^{Asp}$	90	0·989
*su*78	13	0·93	0·088	$tRNA^{Cys}$	400	0·998
*su*1	2·6	0·72	1·0	Release factor R_1	200	0·995
*su*2	0·56	0·36	0·10	—	—	—
*su*2†	0·62	0·38	0·40	—	—	—
*su*3	5·0	0·83	0·43	—	—	—
*su*3†	2·86	0·74	1·55	—	—	—

The parameter θ_1 characterizes the binding efficiency of the suppressor; θ_2, characterizes the binding efficiency of its competitor (normal tRNA or release factor); m, the frequency of suppressor–codon bond formation, relative to the frequency of non-suppressive association. The levels of suppression are computed as follows. First, the θ values from this Table and the τ values from Table 1 are combined according to eqns (1) or (2) to yield the probabilities of amino acid insertion or termination after the association is formed. We have displayed here the resulting values for the wild-type strain (*str*A$^+$ *ram*$^+$). In that case, one has by convention $p = \theta/(\theta + 1)$. Then, the m values are combined with the p values according to eqns (3) or (4) to yield the computed suppression levels, which can be matched against the observed suppression levels observed in Table 3. When a correction for enzyme activity is considered, the final result should be divided by 2 or by 4, according to the case.

† Assuming a 2-fold correction of enzymatic activity for *su*3 and a 4-fold correction for *su*2.

Because of the many simplifications that were used in the treatment, and because of the basic limitations of the experimental data, the numbers given in Tables 1 and 5 are to be taken as illustrative. What we have tried to do is to show that a kinetic interpretation of the available data is consistent with realistic values of the parameters involved.

(a) *Discussion of other relevant data*

We have tried to apply the same treatment to similar published data by Strigini & Gorini (1970). This cannot be done unless one assumes that some of the figures are in error by 15 to 20%, a level compatible with the author's estimate of the accuracy of their data. The figures they give for nonsense suppression by *su*1, *su*2 and *su*3 are different from those we have used (displayed in Table 3). This may be related to differences in the conditions of cell culture (growth in this case was at 30°C) and enzyme assay. The θ values of the release factors appear smaller (30 to 50) in the case of suppression in the *lac zyl* gene, while the θ values of the suppressors may be larger (10 to 15), the multiplicities remaining in the range 0·5 to 2. Thus, the general conclusions of the Results section can be maintained for the 30°C data.

It is difficult to fit quantitatively, the data on suppression in T4-infected cells (Rosset & Gorini, 1969; Strigini & Gorini, 1970). This can be attributed in part to the fact that the experimentally determined quantity, the burst size, is a very poor reflection of the useful quantity, the s term of equation (4). Furthermore, under the conditions of phage infection, a suppressor tRNA may become deficient in a modified base that contributes to its binding properties. The evidence for such a possibility is discussed by Strigini & Gorini (1970) and in Gorini's review (1970).

Strigini & Gorini (1970) observed that the efficiencies of suppression of the suppressors *su*Y and *su*G were reduced by a factor of 1·4 to 4·5 when going from *str*A$^+$ to *str*A1 at 30°C. The two nonsense codons suppressed were in the *lac* operon. Now, a very recent report by Strigini & Brickman (1973) indicates that the efficiencies of the same suppressors are reduced by a factor of 21 to 25 when going from *str*A$^+$ to *str*A2 at 30°C. This time, the suppressed codons have sequences such that the base pairs involved in suppression include non-complementary associations in the first or second position of the codon–anticodon association. Thus, as expected, the levels of suppression are very low. The result is in very good agreement with our quantitative treatment, which predicts that the smaller the θ values, the larger the effect of the ribosomal mutations. Note that *str*A2 is a state intermediate between *str*A$^+$ and *str*A1. Furthermore, the background of suppression or "amount of leakiness" was determined, and found to be, in the same experiments, reduced by a factor of 36 to 61 from *str*A$^+$ to *str*A2.

5. Consistency of the Results with Current Knowledge on Protein Synthesis

The conclusions described in the preceding section could not be derived by previous theoretical treatments of the experimental data. The picture that emerges can be tested against the available experimental data on some aspects of protein biosynthesis.

(a) *Properties of nonsense suppressors*

A single *E. coli* cell, of the strain MRE600, contains about 30,000 ribosomes and 500 molecules of the release factor R1 (Klein & Capecchi, 1971). In exponentially growing cells there may be 15 molecules of tRNA per ribosome (Maaløe & Kjeldgaard, 1966; data given for *Salmonella typhimurium*). Approximately 2% of the tRNA is tRNA^{Tyr} (Yegian *et al.*, 1966) and 20% of tRNA^{Tyr} is *su*3 (Goodman *et al.*, 1970). The chargeability of *su*3 appears to be normal (Gefter & Russell, 1969). If 50% of the *su*3 molecules were acylated, there would be a ratio of one molecule of R1 for 1·7 molecules of competitive *su*3. We estimated, from the numerical fitting of the data, that *su*3 hits its codon 1·5 times more frequently than R1 (Table 3, in the case where an enzyme activity correction is allowed). Therefore, the collision numbers are in ratios not very different from the concentration ratios.

It appears that R1 is a better collider (for UAG) than *su*3 by a factor of $1{\cdot}7/1{\cdot}5 = 1{\cdot}1$. A completely independent estimate can be made from the analysis of *in vitro* competition between *su*3 and R1 in the translation of a phage T4 messenger (Beaudet & Caskey, 1970). From their data, we deduce† a relative collision efficiency of 0·9. Neither of these values is significantly different from unity in view of the uncertainties of the analysis.

We have estimated that *su*1 and *su*3 are synthesized in comparable amounts. This

† Beaudet & Caskey (1970) show that the probability of termination is equal to that of propagation when the reaction mixture contains 1 A_{260} unit of unfractionated tRNA and 0·0745 microunit of R_1. One unit of enzyme corresponds to 2·08 mg of pure R_1 (Caskey *et al.*, 1969). The molecular weight of R_1 is around 44,000 (Klein & Capecchi, 1971). One mg of tRNA corresponds approximately to 23 A_{260} unit. From there the concentration of "competitive" tRNA^{Tyr} is computed as above. Since the discrimination in *in vitro* systems is not as good as *in vivo*, we take equal p values for *su*3 and R_1. Hence our estimate of collision efficiencies.

is consistent with the observation that *su*1 is a minor component of $tRNA^{Ser}$, in the same way as *su*3 is a minor component of $tRNA^{Tyr}$ (Andoh & Garen, 1967; Söll, 1968).

(b) *Missense suppressors*

The rate of acylation of various missense suppressor $tRNA^{Gly}$ species by the glycyl-tRNA ligase is considerably reduced compared with the rate for wild-type $tRNA^{Gly}$ (Carbon *et al.*, 1966; Squires & Carbon, 1972). This property was exploited for the isolation of the missense suppressors. Thus the conclusion that the low efficiency of these suppressors is attributable to the low frequency with which loaded missense $tRNA^{Gly}$ species get to the ribosome, is supported by observations on their decreased chargeability.

(c) *Elongation factors*

A question arises, when trying to compare the collision frequencies of tRNAs and release factors. Is it loaded tRNA, or a complex of loaded tRNA with an elongation factor that recognizes the codon? An interesting possibility is the following. Most of the loaded tRNA goes uncomplexed to codon recognition, with subsequent addition of elongation factors. A small portion of the loaded tRNA would travel complexed with elongation factors, and on binding to a triplet, there would be a high probability of transition to the tight binding state. Thus, one may think of a kinetic treatment qualitatively different from the one we have considered. A factor affecting the availability of elongation factors for complexing tRNA before the codon recognition step would alter the discriminative abilities of the ribosome. These speculations are based on the following knowledge. The binding of $tRNA^{Phe}$ to the A site of the ribosomes *in vitro*, at low magnesium concentration, is speeded up when the tRNA is preincubated with elongation factors and GTP for a prokaryote system (Lucas-Lenard & Haenni, 1968). This is not so for a eukaryote system (Ravel *et al.*, 1973). In an *in vitro* system studied by Kreuzer *et al.* (1972), errors in amino acid incorporation increase with the concentration of elongation factors. It is thought that the binding of unloaded tRNA (therefore uncomplexed with elongation factors) to the A site of ribosomes is of physiological significance in the stringent/relaxed response (Haseltine & Block, 1973).

6. Other Kinetic Treatments

Equation (1) is, in fact, much more general than it would appear at first sight. $p(\theta,\tau)$ is really a function of a single parameter, θ/τ. Therefore, we can make the τ terms tRNA dependent. The only requirement would be that the ribosomal mutations should affect all the τ terms in some multiplicative, uniform fashion. Such would be the case, for instance, if the transition from loose to tight binding is made across an energy barrier, the upper level u_R being determined by the ribosomal state, and the lower level u_T being dependent on the tRNA. Then one could write that, for very short times, the probability of transition is proportional to

$$e^{-(u_R - u_T)/k_T},$$

and one would end up again with equation (1), where

$$\tau = \alpha \, e^{u_R/k_T}. \tag{11}$$

In equation (11), α is characteristic of the tRNA. A change in u_R of 1·4 kcal/mo would produce a tenfold increase in the value of τ.

In the oscillating ribosome scheme (Ninio, 1973), transpeptidation can occur only at certain times, separated by a period t. Clearly the period here plays the same role as the transition time. If we consider that the period is random and follows a Laplace distribution, we end up again with equation (1). If, on the other hand, the probability distribution of the period is very sharp around its mean value, one gets:

$$p(\theta,t) = \frac{\theta}{t}(1 - e^{-t/\theta}). \tag{12}$$

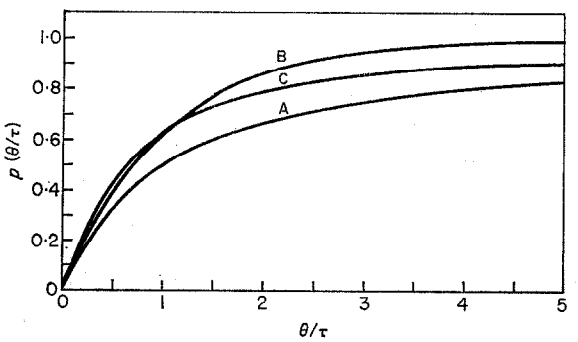

FIG. 2. Probability distributions associated to various kinetic cases discussed in the text. p, in ordinate is plotted as a function of θ/τ. Curve A, $p = \theta/(\theta + \tau)$; curve B $p = 1 - e^{-\theta/\tau}$; curve C, $p = \theta/\tau$ $(1 = e^{-\tau/\theta})$.

More generally, one can make a purely phenomenological analysis of the data, based on equations (4) and (6). One would try to see if the data can be analyzed in terms of m and p, where the p values behave like probabilities. Then, from the properties of the p values one would get information on the general character of the functions p (tRNA, ribosomal state), and one would deduce the correct kinetic model. Such an approach would require much more data than is available.

In a conventional kinetic treatment of polypeptide chain elongation, one would write

$$R + T \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RT_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} RT_2 \rightarrow \text{etc.} \tag{13}$$

R stands for the ribosome, T for tRNA, RT_1 for the loose complex and RT_2 for the tight complex. Then one can say: when the loose complex RT_1 is formed, it can either decay with a rate k_2, or lead to peptide bond formation with a rate k_3. Thus, the probability of success is $k_3/(k_2 + k_3)$. In a convenient system of units, one can write $\tau = 1/k_3$ and $\theta = 1/k_2$.

However, as soon as the events that take place on the ribosome are a little more complicated than the description given in equation (13), the use of the k values is too

restrictive, and one gets a much easier access to the physics of the competition problem through the probability distributions of sticking times and transition times. For instance, non-linear features and threshold devices can be directly built in the function $p(\theta,\tau)$.

7. Discussion

The proposed kinetic description of polypeptide elongation accounts in a simple manner for the complex behaviour of the *str*A and *ram* mutants. The scheme is consistent with the following features, which were repeatedly emphasized by Gorini and co-workers.

(a) The ribosomal mutants do not introduce new types of misreading but amplify or reduce misreadings that are detectable in wild-type cells. This feature invites considering explanations other than those involving a distorted geometry of the codon–anticodon binding site. While Gorini rationalized the results of his group by postulating a screen that operates before the codon–anticodon recognition step or simultaneously with it, the proposed kinetic treatment involves changes in the probability of the elongation step after the recognition step.

(b) All the *str*A alleles can be ordered with respect to increasing ability for phenotypic suppression. This order relation is expressed in our simplified treatment by the *single* kinetic parameter τ. This is to be contrasted with Gorini's interpretation, which involves a "restriction coefficient" that varies, not only with the ribosomal mutant, but also with the tRNA and the codon being translated.

(c) There is a generalization considered by Gorini (1970), that weak suppressors are more restricted than strong suppressors. There are a few exceptions, notably with some ochre suppressors. Our interpretation is, that a weak suppressor is strongly affected by the ribosomal mutants only when its weakness involves low sticking times, and not when it results from a low multiplicity.

There are two conclusions to this paper. The first is that in order to understand ribosomal discrimination, it is not needed to invoke mysterious properties of the ribosome. Simple kinetic models account for previously unrecognized effects. Two problems are encountered that bear some resemblance to that of ribosomal discrimination, namely how the DNA polymerase participates in base selection, and what is the basis for the specificity of the aminoacylation reaction. Work in progress, in collaboration with L. E. Orgel, shows again that a number of puzzling effects can be interpreted using very simple kinetic considerations.

The second conclusion is that the set of ribosomal mutants constructed by Gorini and co-workers may constitute an extremely important tool for acquiring knowledge on the details of protein synthesis *in vivo*, far beyond the expectations. The estimates of some kinetic and concentration data *in vivo* that were made from numerical fitting of the data are by no means trivial. The fact that they appear extremely reasonable suggests that we may be on the right track.

In this paper, the effects of streptomycin addition and *str*A or *ram* mutations were treated in a uniform manner as acting on the τ terms rather than the θ terms. This constraint was chosen, not because of its likelihood, but because it reduced the number of disposable parameters, thus making the treatment much less accommodating than it could be. My personal bias is that *str*A acts on the τ terms, while *ram*

acts on the θ terms. A satisfactory quantitative treatment of the effect of streptomycin will probably require more elaboration. τ and θ effects can be clearly separated. Suppose we have one set of authentic τ mutants, say the *str*A mutants, and we would like to know the class to which the *ram* mutants belong. Let us consider a determination of the level of suppression in the strain *su*1*str*A40*ram*1. Formally, if *ram* is a θ mutant, we should report the result in Table 3 as that of suppression by (*su*1*ram*1) on a *str*A40 ribosome, whereas if *ram* is a τ mutant, we should report the result at the intersection of the *su*1 column and the *str*A40*ram*1 line. It it interesting to note in that context that in the early publications of Gorini's group, experiments with streptomycin were reported in a separate column. This was at a time when the conceptions of codon–anticodon recognition were essentially geometrical. In the more recent publications, the streptomycin effects lead to the opening of additional rows. That there are more complications ahead is suggested by the recent findings of Garvin *et al.* (1973).

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