The Role of mRNA Competition in Regulating Translation

IV. KINETIC MODEL*

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A kinetic model of protein synthesis is presented, primarily designed to analyze the accompanying data (Brendler, T., Godefroy-Colburn, T., Carlill, R. D., and Thach, R. E. (1981) J. Biol. Chem. 256, 11747-11754; Walden, W. E., Godefroy-Colburn, T., and Thach, R. E. (1981) J. Biol. Chem. 256, 11739-11746). Our model treats initiation as a multistep process in which mRNA must bind to a "discriminatory factor" prior to its recognition by the native 40 S subunit. Interaction with the latter is followed by an irreversible rearrangement which yields the functional 40 S initiation complex capable of binding the 60 S ribosome with release of all the factors. Elongation is simply treated as a series of irreversible steps with a single rate constant. The model takes into account the recycling of ribosomal subunits, initiation factors, discriminatory factor, and message initiation site. We can thus mimic the simultaneous translation of several messages, each with its own concentration, size, binding constants, and rate constants. The only limit to the number of messages is the capacity of the computer (3 kilobytes of accessible memory is sufficient for 5 messages). Thus, we are able to evaluate quantitatively the effect of each parameter on the rate of synthesis of individual polypeptides, on polysome size, and on the repartition of message species between the untranslated and the polysomal pools.

Several applications are considered: (i) competitive translation of α- and β-globin in vitro (Kabat, D., and Chappel, M. R. (1978) J. Biol. Chem. 253, 2684-2690) to (ii) determination of the relative affinities of reoviral messages for the discriminatory factor in vitro (Brendler, T., Godefroy-Colburn, T., Carlill, R. D., and Thach, R. E. (1981) J. Biol. Chem. 256, 11747-11754); (iii) effect of elongation inhibitors on the translation of reoviral and cellular messages in SC-1 fibroblasts (Walden, W. E., Godefroy-Colburn, T., and Thach, R. E. (1981) J. Biol. Chem. 256, 11739-11746); and (iv) effect of the growth state on the initiation efficiency of Vero cell messages (Lee, G. T.-Y., and Engelhardt, D. L. (1979) J. Mol. Biol. 129, 221-233). In each case we find that the experimental data are consistent with the notion that mRNAs compete for a discriminatory factor independent of the ribosome. This factor has a high enough affinity for mRNAs to ensure nearly quantitative binding. When present in a limiting amount (with respect to the message pool but not necessarily with respect to the rest of the translation apparatus), the discriminatory factor selects against those messages for which its affinity is lowest, thereby modulating their initiation efficiency.

When the factor is present in excess, on the other hand, all the messages are translated at maximum efficiency. This form of translational control could be remarkably efficient as an on-off switch for the synthesis of a few key proteins.

A growing body of evidence points to the importance of mRNA competition for some limiting component of the initiation apparatus (referred to as "discriminatory factor") as a means of regulating the relative amounts of polypeptides synthesized from a complex mixture of messages. In vitro, the most clear-cut examples are those of α- and β-globin synthesis (1-3) and of the competition between EMC virus mRNA and cellular (or globin) mRNAs in a mouse ascites system (4). Similar observations were made in a wheat germ extract (5).

In the accompanying papers (6, 7), we describe a method which can be used to determine the relative affinities of several messages for the limiting discriminatory factor.

In vivo initiation rate differences between messages have been documented in a variety of systems. Evidence rests on two types of experiments. The first involves use of elongation and initiation inhibitors; as noted previously (8), fast-initiating messages are more sensitive to elongation inhibitors and less sensitive to initiation inhibitors than the slow initiators are. This approach was used in the reticulocyte (9), in virus-infected animal cells (10-12), and in T7-infected Escherichia coli (13). The second approach employs analysis of the distribution of specific messages in polysomes (9, 11-13). However, the exact mechanisms which control initiation rates in vivo are still open to speculation. A nonquantitative analysis of data presented in the accompanying paper (12) suggests that competition for a limiting component may largely determine the efficiency of translation of reovirus messages in the mouse SC-1 fibroblast. This may not be true of some other systems in which the specificity of the translation apparatus appears to change after infection (14). Messenger RNA competition also exists in T7-infected Escherichia coli (13).

Given the complexity of the initiation pathway and our present inability to dissect each step in vivo, we tried to extract the maximum amount of information from available kinetic data. It is well known that kinetic evidence alone cannot prove a reaction mechanism; curve-fitting attempts however can easily disprove some of the possible schemes. We therefore developed a rather versatile model primarily designed to quantitatively analyze data obtained by the "inhibitor" methods in vivo. The model was also applied to reconstituted systems in vitro. This analysis helped us interpret the accompanying data (6, 7, 12), as well as some other published

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1The abbreviations used are: EMC, encephalomyocarditis virus; m7GTP, 7-methylguanosine-5'-triphosphate; mILNP, messenger ribonucleoprotein; eIF, eukaryotic initiation factor; A60 unit, that amount of material which has an absorbance of 1.0 when dissolved in 1 ml of solvent with a light path of 1 cm at 260 nm.
proven useful in explaining the effects of specific elongation pathway protein synthesis rate and on polysome size. This model has the presence of initiation and elongation rate constants on the overall or initiation inhibitors on the relative synthesis rates of been proposed, each dealing with a particular aspect of the existence of the mechanism (2, 3) that M must bind to a discriminatory factor, F, as a prerequisite of its attachment to the native 40 S subunit, this feature was incorporated into Lodish's model (21, 23). This revised model, however, as it has been presented, implicitly assumes that all the components of the system are in large excess with the exception of mRNA initiation sites. It is therefore not possible to apply Lodish's analysis to the case of several mRNA species competing for a limiting factor for which their affinity is large enough to ensure quantitative binding. Moreover, the polysome density predictions made on the basis of this model may overlook the fact that ribosomes are in limited supply in the cell. For instance, if one assumes a minimum ribosome spacing of 12 codons (8), the maximum polysome packing density on a message coding for 350 amino acids (average cellular message) is achieved with 29 ribosomes per message. If the cell contains only 10–15 ribosomes for each mRNA molecule, a drastic reduction of the elongation rate will only increase the average packing density to one-half of its maximum value before free ribosomes become essentially exhausted. As a result, the initiation rate differences between messages may not be completely obliterated. A recent theoretical treatment analyzing GTP inhibition of the reticulocyte lysate does allow for ribosome limitation, in a different context however (22).

Our model takes into account the possibility of initiation factor and ribosome limitations, as well as the effect of poly- some density on initiation. The initiation process is treated as a multistep pathway in which the message-discriminatory step is the binding of factor F to mRNA. The critical assumptions, which enable us to reproduce the accompanying sets of data (6, 12) as well as some other published results (2, 15) are: (i) the molar ratio of F to total mRNA is slightly lower than unity and may vary with physiological conditions; (ii) the affinity of F for the mRNA initiation site, although variable with mRNA species, is high enough to ensure near-quantitative binding at the concentrations encountered in vivo.

**MODEL AND METHOD OF SOLUTION**

**Main Features of the Model**

**Initiation**—Our scheme (Fig. 1) tries to incorporate what is known of the mechanism of initiation (24–26) and follows the pathway given by Benne and Hershey (26). Initiation is assumed to consist of five steps:

1. Upon release of ribosomal subunits from an mRNA molecule after completion of a polypeptide chain, the individual initiation factors, Met-tRNA, and GTP bind to the 40 S ribosome to yield the "native 40 S subunit," $R^*$. Even though these events are not simultaneous, they are treated as a single binding equilibrium between the "derived 40 S subunit," $R$, and the initiation factor which happens to be present in limiting quantity, $I$. We are allowing for the possibility that binding of the initiation factors to the 40 S ribosome would be influenced by the competitive binding of a 60 S subunit, $R'$, to produce a vacant 80 S ribosome, $RR'$.

2. The mRNA initiation site, $M$, on the other hand, is activated for subsequent initiation steps by binding of the discriminatory factor, $F$; the strength of this interaction is characteristic of the mRNA species.

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results concerning the competitive synthesis of $\alpha$- and $\beta$-globin in a reticulocyte cell-free system (2), and the repartition of the different species of mRNA between a polysomal and a nonpolysomal fraction in growing and stationary Vero cells (15).

A number of theoretical models for protein synthesis have been proposed, each dealing with a particular aspect of the pathway (8, 16–22). Lodish's model (8, 21) describes the influence of initiation and elongation rate constants on the overall protein synthesis rate and on polysome size. This model has proven useful in explaining the effects of specific elongation or initiation inhibitors on the relative synthesis rates of $\alpha$-globin (slow initiating message) and $\beta$-globin (fast initiating message) in the reticulocyte. Lodish's original model (8) represents the initiation pathway in terms of one overall bimolecular reaction between the message initiation site, $M$, and the native 40 S subunit, $R^*$. The elongation rate is assumed to be independent of the polypeptide sequence. The overall protein synthesis rate is shown to be governed by both the initiation rate and the spacing of ribosomes on the message ("polysome density," which is itself dependent on the ratio of elongation to initiation rates). Polysome density determines the proportion of the message initiation sites which are free to initiate at any given time, i.e. which are unbound by elongating ribosomes still in the vicinity of the initiation site. After the notion was introduced (2, 3) that $M$ must bind to a discriminatory factor, $F$, as a prerequisite of its attachment to the native 40 S subunit, this feature was incorporated into Lodish's model (21, 23). This revised model, however, as it has been presented, implicitly assumes that all the components of the system are in large excess with the exception of mRNA initiation sites. It is therefore not possible to apply Lodish's analysis to the case of several mRNA species competing for a limiting factor for which their affinity is large enough to ensure quantitative binding. Moreover, the polysome density predictions made on the basis of this model may overlook the fact that ribosomes are in limited supply in the cell. For instance, if one assumes a minimum ribosome spacing of 12 codons (8), the maximum polysome packing density on a message coding for 350 amino acids (average cellular message) is achieved with 29 ribosomes per message. If the cell contains only 10–15 ribosomes for each mRNA molecule, a drastic reduction of the elongation rate will only increase the average packing density to one-half of its maximum value before free ribosomes become essentially exhausted. As a result, the initiation rate differences between messages may not be completely obliterated. A recent theoretical treatment analyzing GTP inhibition of the reticulocyte lysate does allow for ribosome limitation, in a different context however (22).

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3. The native 40 S subunit binds to the activated initiation site. This step is generally assumed not to be mRNA-discriminatory in the present treatment, although there is no a priori reason to exclude this possibility (8, 21, and Fig. 6C).

4. An irreversible reaction, possibly involving ATP hydrolysis (20–27), leads to the formation of a functional 40 S initiation complex. Our model allows for a possible variation of the rate of this reaction with the message species.

5. The rest of the initiation pathway (binding of the 60 S subunit, release of the initiation factors) is treated as a single irreversible reaction (28). Available evidence indicates that the "junction step" is not rate-limiting under normal circumstances in the reticulocyte (21); however, it is obvious that under conditions of limiting ribosome concentration (in vitro, or in cycloheximide-treated SC-1 cells, Ref. 12), the junction rate could have an influence on the overall rate of protein synthesis. Indeed, some published profiles (28) indicate the presence of "half-polysomes," bearing a 40 S subunit at the initiation site, in a normal reticulocyte lysate. In addition, introducing junction in our scheme as a separate step enables us to consider the case of cells treated with specific inhibitors of this reaction, in the absence of specific information, we assume that the discriminatory factor cycles at the junction step as well as the other initiation factors.

Elongation—As done by Ledish (6), we consider each elongation step as a first order reaction with rate constant $k_1$, which is independent of the amino acid being added. The distribution of ribosomes is assumed to be uniform along any given mRNA molecule; this approximation is only valid at low polysome packing density (less than one-half the maximum density) (21), but we have seen that the packing density is likely to rise above that level in our cells since the number of ribosomes is simply not large enough. The termination rate is thought to be larger than the initiation rate in vitro and should not have any significant effect on the overall protein synthesis rate (21).

Principle of the Derivation

RESULTS

Four applications of this model will be considered here: (i) simulation of in vitro experiments such as those reported by Kabat and Chappell (2) concerning the simultaneous translation of α- and β-globin in a reticulocyte-derived system; (ii) analysis of in vitro experiments devised to measure the relative affinity of several messages for the discriminatory factor (6); (iii) simulation of the effect of elongation inhibitors in reovirus-infected mouse fibroblasts (12); (iv) modeling of the distribution of mRNA between polysomes and "mRNP" in fast-growing and stationary Vero cells (15).

Message Discrimination in Vitro and Its Relief by Initiation Factors—The characteristic findings of Kabat and Chappell (2), also obtained in another in vitro system by Golini et al. (4), are the following.

1. When the natural mixture of α- and β-globin mRNAs (molar ratio $\alpha/\beta = 1.5$) is added in increasing concentrations to a reticulocyte protein synthesis system, the quantity of α-chains synthesized reaches a maximum and then declines, while the quantity of β-chains synthesized keeps increasing to a saturation level. The maximum quantity of α-chains produced at a nonsaturating mRNA concentration is roughly $\frac{1}{2}$ the maximum quantity of β-chains produced at message saturation (Fig. 3 of Ref. 2).

2. When α-globin message is used alone, its translation is as efficient as that of β-globin message.

3. The ratio of α- over β-chains synthesized can be restored to a value closer to 1.5 by adding eIF-4B or eIF-4A, the former being more effective. The data presented also suggest that the larger mRNA concentration, the more eIF-4B is needed to relieve competition.

It is possible to illustrate each of these points with our model system, using the proper sets of constants. The discriminatory step must be reaction 1 of our scheme since it is not rate-limiting and must therefore precede interaction with the limiting component of the system. (One could postulate discriminatory binding of mRNA to the native 40 S subunit followed by a rate-limiting enzyme-catalyzed reaction. However, a counterargument against this possibility is the finding that competition between EMC (uncapped) and globin mRNA (capped) in the ascites system is absolutely independent of the 40 S ribosome concentration (4). Moreover, as will be shown below, discrimination at this step is not necessary to describe certain types of in vivo data obtained in fibroblasts, whereas discrimination in reaction 1 is. Of course, it could be argued that these observations may not apply to the special case of competition between α- and β-globin messages in the reticulocyte. In any case both schemes are kinetically equivalent.)

The constants used are listed in the figure legends. The total ribosome concentration, $r_s$, was calculated from Ref. 2 by using 22 pmol/μg unit as the absorbance of 80 S ribosomes (30). The elongation rate, $k_e = 45$ amino acids/min/active ribosome, is typical of a reticulocyte lysate (9, 30) and was also obtained in a reconstituted Krebs ascites system. The dissociation constant of the 80 S vacant ribosomes, $K_{1s} = 0.04$ μM, is that measured at 2 mM Mg$^{2+}$ by Trachsel and Staehelin (31). The dissociation constant of the 40 S initiation factor complex, $K_t = 0.04$ μM, was calculated at 2 μM Mg$^{2+}$ from Fig. 6 of Ref. 31. The initiation factor concentration, $e_i = 0.015$ μM, was evaluated from the typical eIF-3 content of a crude initiation factor preparation reported by Thomas et al. (32). We also calculate this value to be optimum for the given ribosome concentration (data not shown). In the absence of specific data for the reticulocyte system, $k_1$ was obtained from the maximum initiation frequency of the mouse ascites reconstituted system, i.e. 0.15 initiation/min/message for both EMC virus and globin messages. The dissociation constant of the (40 S initiation factor) complex, $K_{1s}$, was determined at 2.5 μM Mg$^{2+}$ from Fig. 3 of Ref. 2. However, certain constraints on the range of choices exist. For example, the ratio of $K_{1s}$ for α- and β-globin mRNAs is close to 50 (2). In addition, the product $K_{1s}$ determines the overall stability of message-ribosome interaction (complex C) and can be evaluated very roughly for α-globin mRNA from the height of the synthesis peak in Fig. 3 of Ref. 2. In Fig. 2 we used the following parameters: $K_{1s} = 0.001$ μM, $K_{1s}(\alpha) = 0.015$ μM, and $K_{1s}(\beta) = 0.0003$ μM. However, it is possible to model the experimental results just as adequately with a higher value of $K_s$ and lower values of $K_{1s}(\alpha)$ and $K_{1s}(\beta)$ (not shown). The total discriminatory factor concentration, $e_i$, was treated as an adjustable parameter. Two values, 0.025 and 0.050 μM, were employed in Fig. 2A. The curves obtained using these values compare favorably to the actual experimental data (Fig. 3 of Ref. 2). It is of interest that the total mRNA concentrations which give equivalent rates of α- and β-globin synthesis are less than twice the corresponding $e_i$ values, in each case.

Increasing the concentration of factor $F$ results in an increase in the α/β-globin synthesis ratio, i.e. "competition relief" (Fig. 2B). This occurs without significant increase of the overall protein synthesis rate (not shown). Moreover, the level of $F$ needed to obtain a given α/β synthesis ratio in-2

Kinetic Model of Messenger RNA Competition

**FIG. 2.** Competition between α- and β-globin mRNAs in vitro and its relief by the discriminatory factor and other initiation factors. The parameters are chosen as follows (see Table I for notations): \( L = 12, K_f(\alpha\text{-globin}) = 0.015 \mu\text{M}, K_f(\beta\text{-globin}) = 0.0003 \mu\text{M}, K_i = 0.001 \mu\text{M} \) for both messages, \( K_f(\alpha\text{-globin}) = 0.040 \mu\text{M}, K_i = 0.040 \mu\text{M}, k_3 = 0.3 \text{ min}^{-1} \) for both messages, \( k_2 = 2 \text{ min}^{-1} \mu\text{M}^{-1}, k_s = 45 \text{ min}^{-1} \), \( r_l = 0.130 \mu\text{M}, f_i = 0.010 \mu\text{M}, e_i = 0.015 \mu\text{M}, S(\alpha) = 480, S(\beta) = 720 \). The maximum protein synthesis rate obtained with the unmethylated mixture of messages is 37% of that obtained with the methylated mixture (for the individual messages in the mixture this number varies slightly, e.g., 35% for α and 38% for β).

Under certain conditions, increasing the concentration of \( R^* \), which is assumed net to discriminate between messages, may result in partial competition relief. This will occur if two conditions are realized: (i) binding of \( M \) to \( F \) (reaction 1) is weak, i.e., \( R^* \) contributes significantly to the stability of complex C and (ii) the concentration of both message and factor \( F \) are low. This effect is shown in Fig. 2C, where the \( R^* \) concentration is varied by virtue of changes in concentration, \( e_i \), of the nondiscriminatory factor, I. According to our model, competition relief obtained in this case should correlate with an increase of the protein synthesis rate (not shown). However, this increase might not necessarily be observed if a rate-limiting step (enzyme working at saturation) occurred downstream from reaction 2. In short, a factor which relieves competition between messages is not necessarily the discriminatory factor itself, but may act by contributing to the stability of the mRNA/ribosome complex in a nondiscriminatory way. A factor of this type, however, will have very little effect at high message levels, even when added in stoichiometric amounts. A steady state analysis of our pathway in the

**FIG. 3.** Saturation of a fractionated translation system by reovirus mRNAs. The reaction scheme of Fig. 1 is used to model experiments presented in Fig. 2A of Ref. 6 and Fig. 2A of Ref. 7, i.e., titration of the in vitro system by; the capped methylated messages for \( \mu_{\text{NS}} (- - -) \) or \( \alpha \) (---), an equimolar mixture of the methylated messages for \( \mu_{\text{NS}} \) and \( \alpha \) (---), an equimolar mixture of the unmethylated messages for \( \mu_{\text{NS}} \) and \( \alpha \) (---). The overall rate of protein synthesis is normalized to that obtained with 0.8 pmol of mRNA in a 25-μl assay. The parameters are chosen as follows (see Table I for notations): \( L = 12, K_f = 0.005 \mu\text{M} \) for \( \sigma \) messages, \( K_f = 0.0008 \mu\text{M} \) for \( \mu_{\text{NS}} \) messages, \( K_i = 0.001 \mu\text{M} \) for methylated messages, \( K_i = 0.015 \mu\text{M} \) for unmethylated messages, \( K_i = 0.040 \mu\text{M}, K_e = 0.040 \mu\text{M}, k_3 = 0.3 \text{ min}^{-1} \), \( k_2 = 2 \text{ min}^{-1} \mu\text{M}^{-1}, k_s = 45 \text{ min}^{-1} \), \( r_l = 0.130 \mu\text{M}, f_i = 0.010 \mu\text{M}, e_i = 0.015 \mu\text{M}, S(\alpha) = 480, S(\mu_{\text{NS}}) = 720 \). The maximum protein synthesis rate obtained with the unmethylated mixture of messages is 37% of that obtained with the methylated mixture (for the individual messages in the mixture this number varies slightly, e.g., 35% for \( \sigma \) and 38% for \( \mu_{\text{NS}} \)).

These results correspond to what was observed with eIF-4B by Kabat and Chappell (2) and by Golini et al. (3), or with eIF-2 by Di Segni et al. (4). Under certain conditions, increasing the concentration of \( R^* \), which is assumed net to discriminate between messages, may result in partial competition relief. This will occur if two conditions are realized: (i) binding of \( M \) to \( F \) (reaction 1) is weak, i.e., \( R^* \) contributes significantly to the stability of complex C and (ii) the concentration of both message and factor \( F \) are low. This effect is shown in Fig. 2C, where the \( R^* \) concentration is varied by virtue of changes in concentration, \( e_i \), of the nondiscriminatory factor, I. According to our model, competition relief obtained in this case should correlate with an increase of the protein synthesis rate (not shown). However, this increase might not necessarily be observed if a rate-limiting step (enzyme working at saturation) occurred downstream from reaction 2. In short, a factor which relieves competition between messages is not necessarily the discriminatory factor itself, but may act by contributing to the stability of the mRNA/ribosome complex in a nondiscriminatory way. A factor of this type, however, will have very little effect at high message levels, even when added in stoichiometric amounts. A steady state analysis of our pathway in the
nonequilibrium situation (not shown) reveals additional possibilities (see also Ref. 21). Thus, short of independent evidence, one could have used a complete kinetic study of the system (especially at high ribosome concentration) in order to establish the exact role of any putative "competition relief" factor.

**Measurement of the Relative Affinities of mRNAs for the Factor in Vitro**—It is customary to evaluate the efficiency of a message from the dependence of the protein synthesis rate upon mRNA concentration. Apart from the fact that it is difficult to determine what proportion of the message molecules is active, these curves are very insensitive to the overall affinity of the message for ribosomes or factors, since binding is nearly quantitative. Thus, the theoretical titration curves obtained with two messages differing by a factor of 6 in their affinity for F are almost indistinguishable within normal experimental error (Fig. 3). This result corresponds closely with actual observations (2, 6, 7). Furthermore, the midpoint of the titration curve varies with the concentration of the limiting component of the system, which may be F, I, or R in our model (not shown). Message length is another variable which may influence the titration curve if the message is large enough to sequester a significant proportion of the ribosomes (Table II in miniprint).

To circumvent these difficulties in obtaining useful data from mRNA titration curves, a competition method was devised (6). In this method, a nonsaturating mixture of "test" messages is translated in the presence of increasing amounts of a competing message (a convenient competitor is rabbit globin mRNA). After quantitation of the individual translation products, one observes that the translation of each test message is affected by competition in a characteristic way. One can show by a straightforward extension of previous models (2, 4) that these "competition curves" should possess certain useful characteristics. First, translation of test messages with the lowest affinities for discriminatory factor (i.e., highest values for $K_f$) will be most rapidly inhibited by increasing globin mRNA concentrations; conversely, those of greatest affinity will be least affected. Second, each individual mRNA in a mixture of test mRNAs will exhibit its characteristic competition curve irrespective of its own concentration, or the concentration of the other mRNAs in the mixture (within certain well-defined limits of overall test mRNA concentration). Third, if Q is defined as the rate of translation of a test message in the presence of globin mRNA and $Q_0$ that without globin mRNA, it can be shown that, under certain conditions,$^4$ the quantity $Q' = Q/(Q_0 - Q)$ is proportional to the free factor concentration, $f$, and inversely proportional to $K_f$. Hence, a plot of $Q'$ for one of the test messages versus $Q'$ for a reference test message should be linear, with slope $K_f/2K_i$. That all these theoretical predictions are in fact observed in the mouse ascites system has been documented (6). We present here a more detailed analysis of these competition experiments which takes into account the complete pathway, in order to define the conditions of validity of the plots.

Since elongation is nonlimiting in *in vitro* and since we assume that junction is not the limiting step of initiation, Equation 6 can be rewritten (omitting superscripts for simplicity):

$$m_c = c(1 + K_f/r^* + K_i/r_f)$$  \hspace{1cm} (18)

Several cases may now be considered.

1. At very low concentration of test message and if $K_f$ and $K_i$ are, respectively, much smaller than $f$ and $r^*$, the message is almost entirely ribosome-bound, i.e. $c_0 \approx m_c$. In this case, addition of competing mRNA serves to reduce $r^*$ and $f$, thus

\hspace{1cm} $^4$ R. E. Thach, unpublished results.

\[ Q' = \frac{Q}{Q_0 - Q} = \frac{c}{m_c - c} = \frac{r^*f}{K_f(f + K_f)} \]  \hspace{1cm} (19)

(i) When the concentration of competing message is sufficient to completely saturate F, the level of free $F$ falls below $K_f$. Under these conditions:

$$Q' = \frac{r^*}{K_f}$$  \hspace{1cm} (20)

This is also true in large excess of $r^*$ over $F$ (see Equation 18). In either of these cases, if two messages (1 and 2) are translated simultaneously in the presence of a competing message, $r^*$ and $f$ are common to both and one gets:

$$Q^{(1)}/Q^{(2)} = K_i^{(1)}/K_i^{(2)}$$  \hspace{1cm} (21)

We refer to this quantity as the "discrimination ratio." Obviously, this argument can be extended to a system containing any number of test messages and the discrimination ratio for any pair of test mRNAs can be measured independently of this absolute concentration, or of the presence of other test mRNAs. (As noted above, this approximates very closely the actual experimental results, as shown in Ref. 6.)

(ii) When $F$ is in large excess over $r^*$, the method is very sensitive to any discrimination at step 1 since addition of enough competing message to reduce $f$ significantly would drastically inhibit translation of the test message and make measurements unreliable. Equation 19 then becomes:

$$Q' \approx \frac{r^*}{K_f}$$  \hspace{1cm} (22)

2. When the affinity of $M^*$ for $R^*$ is low ($K_f > r^*$ and $f$), but $K_i < f_i$, the $Q'$ plots obtained at a low concentration of test message essentially measure discrimination at reaction 1 since in this case:

$$Q' \approx \frac{f}{K_i}$$  \hspace{1cm} (23)

This is an equivalent expression to that derivable$^4$ from earlier studies (2, 4), as noted above. Clearly, the consequences are the same. The "discrimination ratio" in this case is simply $K_i^{(1)}/K_i^{(2)}$.

3. Finally, it is obvious that the slope of the $Q'$ plots would be 1.0 in large excess of test mRNA, or if the overall affinity of the message for the system were very low.

Employing these considerations, we have mimicked the translation of two reovirus messages, $\sigma$ and $\mu$ (nomenclature as in Ref. 33), either separately or together, in the presence of increasing amounts of competing globin mRNA (that is, the system described in Fig. 4 of Ref. 6). The constants used are listed in the legends of Figs. 3 and 4. Except as required by systematic investigation of the parameters, the characteristics of the mouse ascites in *in vitro* system are generally assumed to be the same as those of the reticulocyte with the exception of factor and ribosome concentrations. As discussed further below, $R^*$ seems to be in excess over $F$ in our present system, justifying the choice: $f_f = 0.01 \mu M$ and $r_f = 0.015 \mu M$; it was measured by UV absorption. Values of S correspond to the average length of rabbit $\alpha$- and $\beta$-globin (34) and to the approximate size of reovirus proteins (35). We used the ratio of 6.2 between $K_f$ values for the two reovirus messages which results from experiments done at 100 mM KCl and 3 mM Mg(OAc)$_2$ (6). The absolute values of these constants were chosen in the same range as for the preceding section; however, the constant $K_i$ for globin (not necessarily the same in two *in vitro* systems of different biological origins) was adjusted to reproduce experimental results.

Fig. 4, similar to Fig. 4 of Ref. 6, shows that with the constants chosen the concentration of test mRNA (up to 80% saturation) has little influence on the shape of the inhibition curves. Moreover, reovirus messages behave essentially the same whether translated separately or together. This result agrees closely with the experimental observation (6). More-
over, the $Q'$ plots of data for capped mRNAs shown in Fig. 4 (C and D) are more or less linear, passing through the origin with a slope approaching 6.2 (Fig. 5, curves 1 to 3). They closely approximate a straight line at high concentrations of globin mRNA, where $F$ is saturated. Deviation from linearity is however significant at lower concentrations of globin mRNA. As pointed out earlier, the curvature is much more reduced when $R^*$ is in excess (curve 4). Inasmuch as the experimentally observed $Q'$ plots do not deviate detectably from linearity (6), we assume that the ratio of $f_i$ to $e_i$ is lower in the mouse ascites system than in the reticulocyte system, in which case the linear Equations 20 and 21 would apply to the ascites system.

It is important to emphasize that the slope of the $Q'$ plots actually measures the overall discrimination, whether it occurs at reaction 1, at reaction 2, or at both. This is illustrated by curve 5 of Fig. 5, in which it is assumed that all the discrimination occurs at the level of binding to $R^*$ (the ratio of the $K_v$ values is set at 6.2). Curvature is undetectable in this case (see Equation 19). However, as shown below in the discussion of the in vivo case, variation in $K_v$ values is not necessary to fit the experimental data. This suggests that the discrimination ratios measured in vitro are in fact simply ratios of $K_v$ values for the mRNAs under study. Nevertheless, it is entirely possible that at least some variation in $K_v$ will be found to occur for at least some natural messages (8, 21). This seems especially likely for uncapped mRNAs, as will be discussed below.

The choice of a competing mRNA is theoretically indifferent. However, it will obviously be more practical to use a message with an affinity for the system in the same range as that of the test messages. If the competing mRNA is too weak, it will not be able to outcompete the test mRNAs when added at reasonable concentrations. If the competing mRNA is much stronger than the test messages, inhibition will be very sharp and difficult to reproduce. The length of the competing message is an important consideration if ribosomes are not in large excess; a large message such as EMC RNA may indeed sequester a significant number of ribosomes, making $R^*$ the limiting component of the system. We have seen that $Q'$ plots are nonlinear under these conditions (compare curves 2 and 4 of Fig. 5).

**Role of the Cap-binding Protein**—The accompanying paper (7) shows that prevention of cap methylation of reovirus messages synthesized in vitro decreases their affinity for the protein synthesizing system (globin mRNA outcompetes them more efficiently) and reduces the apparent discrimination ratio measured from the $Q'$ plots. It is also observed (Fig. 2 of Ref. 7) that cap removal reduces by 60% or more the maximum protein synthesis obtained at message saturation, without modifying the normalized titration curves. The reduction of maximum protein synthesis may vary with the batch of initiation factors, the ionic conditions, etc., but is independent of the message, at least in the reovirus series.

We have considered the possibility that the cap-binding protein itself might be the message discriminatory factor. However, this hypothesis does not explain the titration curve data nor the markedly different behavior of EMC virus and unmethylated reovirus mRNAs (7). We tend to favor another interpretation which accounts for all the observations presented in Refs. 6 and 7. In this interpretation we assume the following.

(i) The cap-binding protein has the same affinity for all capped messages.

(ii) The cap-binding protein is a part of $R^*$ in vitro. This is in accord with its strong affinity for eIF-3 (36).
Kinetic Model of Messenger RNA Competition

Cycloheximide Inhibition of Protein Synthesis in Neonatally Infected Mouse Fibroblasts—Inhibition by low doses of cycloheximide (up to 2 μM) was used as a tool to study the relative initiation efficiency in vivo of the different reovirus messages and of the host messages. The data (presented in Ref. 12) show some interesting features.

(i) Reovirus translation is less sensitive to cycloheximide than is host translation. This indicates that initiation is more efficient on host than on reovirus messages in the SC-1 cells. Among the viral polypeptides studied, the order of sensitivity to cycloheximide is as follows: αs < μss < λi.

(ii) The pattern of inhibition changes with the time post-infection; although the order of sensitivity to cycloheximide stays the same, the differences between individual responses increase as infection progresses. As will be shown elsewhere,5 this cannot be attributed to a change in the extent of “capping” of the viral messages nor to a qualitative modification of the protein synthetic machinery.

(iii) Most strikingly, addition of minute amounts of cycloheximide significantly increases the synthesis of αs and to a lesser extent μss, whereas the overall protein synthesis rate decreases. The extent of the increase depends on the time postinfection and may reach 40 to 50%.

We now show that these results are quite consistent with the hypothesis of message competition for a discriminatory factor. The parameters of the model were chosen as follows.

(i) Ribosome concentration was evaluated from the rRNA content of L-cells (41), assuming a cytoplasmic volume of 4 pl. We thus find \( r_i \approx 1 - 2 \mu m \).

(ii) Translatable cellular mRNA concentration was evaluated relative to the ribosome concentration from the polysome profile in the presence of 1 μM cycloheximide. With uninfected growing SC-1 cells we find that the polysome profile varies somewhat from one experiment to another and we calculate a ratio of 6 to 12 ribosomes per message molecule (Ref. 12 and Footnote 6). From data presented in Ref. 42, the number average size of polypeptide synthesized by uninfected mouse fibroblasts is evaluated at 350 to 500 amino acids. Since the method used would tend to underestimate the contribution of low molecular weight protein, we chose the lower value for our calculations. Cellular messages do not seem to be significantly degraded during the first 10 h of infection by reovirus (12).

(iii) Reovirus mRNA represents over 30% of the total message mass after 8 h of infection (12). Since the average coding capacity of the viral messages is approximately 550 (on the basis of a typical size distribution, Ref. 43, and of the individual molecular weights of reoproteins, Ref. 35), we estimate that the molar ratio of viral to cellular messages is at least 0.2. At earlier times postinfection, this ratio is obviously lower and the pattern of proteins synthesized after 5 h suggests a value of 0.05 (12).

(iv) Quantitation of eIF-2 (44) and evaluation of the relative concentration of the different initiation factors in the reticulocyte lysate (32) suggest that there is nearly 1 molecule of each initiation factor for 10 ribosomes. From the amount of Met-tRNA bound to 40 S subunits in the presence of edeine, we find that the growing SC-1 cells must contain at least 1 active molecule of each initiation factor for 20 to 40 ribosomes. A ratio of 1:36 was thus chosen.

(v) The elongation rate was measured in L-cells both in the absence and in the presence of cycloheximide (42). Constant \( k_e \) was thus found to be 600 to 700 amino acids per min in normal growth conditions and the concentration of cycloheximide which inhibits elongation by 50% is 0.05 μg/ml. We

\[ \text{Footnote 6: } \text{B. M. Detjen, W. E. Walden, and R. E. Thach, manuscript in preparation.} \]

\[ \text{Footnote 7: } \text{R. Wyatt, T. Godefroy-Colburn, and R. E. Thach, unpublished results.} \]
assume that inhibition by cycloheximide follows classical kinetics.

(vi) Rate constant $k_1$ was adjusted to give an overall initiation rate compatible with observed polysome size. Junction rate was assumed to be nonlimiting.

(vii) Dissociation constants $K_r$ and $K_t$ are not known in vivo. We rather arbitrarily used values determined at 1.5-2 mM in vitro (31). However, the choice of these values is not critical for our conclusions.

(viii) Constants $K_t$ for the various messages were chosen in the same range as for in vitro modeling and adjusted to obtain a reasonable fit to experimental data.

(ix) Constant $K_r$ (assumed to be the same for all messages unless otherwise mentioned) was evaluated from the typical content of a reticulocyte lysate in free native subunits carrying Met-tRNAs, i.e. 2 to 4% of the total ribosomal content, or 20 to 40% of the initiation factors (44). We thus arrive at $K_r = 0.1 \mu M$. This value is significantly higher than the value of 0.091 \mu M used for the in vitro calculation. Apart from the differences in ionic environment, viscosity, subcellular structure (45), etc., it may be relevant to point out that the overall initiation rates are 2 orders of magnitude higher in the cell than in the reconstituted system, possibly creating a nonequilibrium situation. Thus, if dissociation of complex C were much slower.
than its transformation into $C^*$ in vivo, $K_v$ would be a steady state, "Michaelis-Menten" type constant, nearly proportional to $k_v$. In this case, its numerical value would be greatly increased ($k_v$ is 170 times higher in vivo than in vitro; Figs. 5 and 6), thereby justifying the value $K_v = 0.1 \mu m$.

Fig. 6 enables one to distinguish between three possible discrimination mechanisms. In A, discrimination is the result of competition between host and viral mRNAs for factor $F$. $B$ assumes competition for $R^*$, and $C$ models the case in which rate constant $k_v$ (assumed to govern the overall pathway as in refs. 8 and 21) is different for the two messages. The three sets of conditions yield nearly the same protein synthesis rates and polysome sizes in the uninhibited case, but they lead to three different responses to cycloheximide. In situations A and B, the viral protein synthesis rate increases initially, whereas host protein synthesis is inhibited. The difference between the two responses appears in the range of cycloheximide concentrations at which the pool of free 40 S subunits becomes depleted: at cycloheximide levels in excess of 0.1 pg/ml, viral protein synthesis decreases at the same rate as host in situation A (the polysome size levels off on both messages), whereas in situation B the size of viral polysomes decreases drastically, causing a precipitous drop of the protein synthesis rate. In situation $C$, unlike the other two, the rate decreases monotonically on both viral and host messages. A comparison of these theoretical behaviors with experiment (Figs. 2-4 of Ref. 15) supports the view that messages compete for a factor which occurs independently of the ribosome, in accord with previous observations (4).

Fig. 7 illustrates the effect of ribosome concentration on the response to cycloheximide. It appears that a large excess of ribosome (as in $C$) is necessary for cycloheximide to induce a significant increase of reoviral protein synthesis.

Curves $6A$ and $7B$ mimic the experiments done after 5 h and 8 h of infection (Figs. 2 and 3 of Ref. 12), in which we assume that the molar concentration of viral message increases from 5% to 20% of host messages, everything else being constant. Agreement with the experimental results is as good as can be expected from the number of unknown parameters. It is evident that the behavior of reovirus-infected SC-1 cells can be explained without invoking any specific effect of reovirus infection on the translational apparatus, such as the change of specificity toward capped messages reported in reovirus-infected L-cells (14). Indeed, no evidence either for a decrease in viral mRNA capping or for a change in initiation factor activity has been detected in the SC-1 cell system.

**Effect of the Growth State on Protein Synthesis in Vero Cells**—The data of Lee and Engelhardt (15) show that the growth state of Vero cells has a marked effect on the proportion of the translatable message found in polysomes. In the exponential state, 75% of the poly(A)-containing RNA is polysomal, whereas the proportion drops to 35% in stationary cells. In addition, the concentration of poly(A)-containing RNA is reduced in stationary cells to 25% of that in exponen-

![Fig. 8. Effect of growth state on the polysomal distribution of mRNAs. A, results of Lee and Engelhardt (15). From data presented in Table I of Ref. 15, we calculated the polysomal fraction of each message in exponentially growing and in stationary cells. This calculation takes into account the overall proportion of mRNA found in polysomes (75% in growing cells and 35% in nongrowing cells). Concentric circles are used wherever several data points are superimposed. B, simulation of the results of Lee and Engelhardt. The simultaneous translation of several mRNAs is modeled in two sets of conditions representative of the growing and of the nongrowing state. In both cases, we calculate the proportion of each mRNA translated on polysomes carrying at least 3 ribosomes. The parameters are the same as in Fig. 6a except for the following changes: the system now translates 5 messages present in equimolar amounts, $m = 0.024 \mu m$ for each message in growing cells, and $m = 0.006 \mu m$ in nongrowing cells; the size of all the messages is 350 codons except for $A$ and $B$ where the size of one of the messages is 700 codons, respectively, message 1 and message 5. The discriminatory binding constants are $K_{f1} = 0.003 \mu m, K_{f2} = 0.001 \mu m, K_{f3} = 0.003 \mu m, K_{f4} = 0.01 \mu m,$ and $K_{f5} = 0.03 \mu m.$ The factor concentrations are adjusted to reproduce the experimental data for overall synthesis and polysomal mRNA fraction, in 3 hypotheses: $f_i$ and $e_i$ decrease proportionally as the cells become stationary (curve 1), $f_i$ decreases and $e_i$ remains the same (curve 2), and $f_i$ increases and $e_i$ remains the same (curve 3). These three curves correspond to messages coding for 350 amino acids. In the first hypothesis, data points corresponding to messages coding for 250 to 700 amino acids are expected to fall into the shaded area below curve 1. In growing cells, $f_i = 0.1 \mu m$ and $e_i = 0.04 \mu m.$ In stationary cells, $f_i = 0.013 \mu m$ and $e_i = 0.013 \mu m.$ In all cases, the total protein synthesis in stationary cells is 12% of that in growing cells; the fraction of total message found in polysomes in growing cells is 0.75 (C, $\Delta$, $\Box$), or 0.77 (C); in nongrowing cells this fraction drops to 0.38 (C), 0.45 (A and $\Box$), and 0.42 (C).
tially growing cells, whereas the ribosome content does not change significantly. These changes fully account for the overall decrease of the protein synthetic activity (15, 46). Individual messages vary drastically in their efficiency of recruitment into polysomes. From Table I of Ref. 15, we have calculated the proportion of mRNA found in polysomes for all the polypeptides listed. The proportion in stationary cells was then plotted as a function of the proportion in growing cells (Fig. 8A). It is obvious that, on the average, reduction of the growth rate has a larger effect on the translation of weak messages than of the strong ones. The data points appear to be clustered along a curve (not drawn, in order to avoid any bias in the presentation of the data) located below the first diagonal of the figure. This is not what one would expect if a limited number of messages were specifically "masked" and prevented from being translated when cells enter the stationary state.

Modeling of the experimental data was done with 5 messages of equal size (350 codons) mixed in equimolar proportion. Their affinities for the discriminatory factor were assumed to vary within 2 orders of magnitude. The discriminatory factor level in growing cells was chosen as 57% of the total mRNA concentration, to account for the observed proportion of mRNA in polysomes. The other parameters are similar to those of the previous section. We calculated the proportion of each message in polysomes carrying at least three ribosomes, since this was the criterion for separating the "polysomal" from the "nonpolysomal" fraction (15). The stationary cells were assumed to contain 25% as much mRNA as the growing cells, the same number of ribosomes, 33% as much discriminatory factor, and other initiation factors. Curve 1 of Fig. 8B was thus obtained, and it is seen to fit the experimental results in Fig. 8A reasonably well.

It is also possible to account for the data by assuming that the concentration of initiation factor I stays the same, whereas that of F decreases, as cells become stationary, to 7.3% of the growing state value (curve 2). This hypothesis however appears unlikely since it would require proteins I and F to turn over at vastly different rates. Moreover, the decrease of the eIF-2 level (probably limiting for the formation of $R^*$) has been documented in starved Ehrlich ascites cells (47).

The converse possibility, namely that the concentration of I would decrease and that of F would stay unchanged, leads to curve 3 of Fig. 8B. These results do not fit the experimental results, although this hypothesis does account for the overall decrease of the translation rate and of the overall proportion of mRNA found in polysomes.

We have assumed thus far that F effects discrimination in Vero cells as it does in SC-1 cells. However, data of Lee and Engelhardt (46) and Lodish and Porter (49) of a bimodal distribution of mRNA found in polysomes, since for a given ribosome spacing (which only depends on initiation and elongation rates) the number of ribosomes per message is proportional to its length. However when the theoretical points are plotted as in Fig. 8B, they fall near the original curve regardless of message size as long as the molar ratio of F to total mRNA is kept constant (shaded area in Fig. 8B). This result was obtained by doubling the size of individual messages one by one without changing their molar concentration. From this and other considerations, it is evident that the position of experimental points on the graph (Fig. 8A) was not a trivial effect of message size unrelated to initiation efficiency.

In summary, our model can account for the growth state-related variations of message translational efficiency. However, this does not necessarily imply that the rate of synthesis of the corresponding proteins is controlled at the translational level. On the contrary, by examining Table I of Ref. 15 one finds the absolute amount of a given mRNA in the polysomal fraction to be fairly independent of the growth state and in many cases unrelated to its translational efficiency. Thus, it seems that for many weak messages the sharp reduction of their translational efficiency upon reaching the stationary state is counteracted by an increase of the proportion of these messages in the translatable pool. Whether this results from differential mRNA stability, or from a feedback mechanism which would affect transcription or a post-transcriptional event, is open to speculation. Even more interesting is the fact that the counteraction or "compensation" mechanism does not function for all the messages. Some of the weak mRNAs (coding for polypeptides number 35, 39, 40, 48, and 88) are in fact under-represented in the stationary cells. This might indicate that the synthesis of the corresponding proteins is indeed regulated at the translational level, and it would be interesting to know to which extent these proteins are involved in the control of cell growth rate.

**DISCUSSION**

Protein synthesis is such a complex set of reactions that one cannot hope to describe it kinetically without making a number of simplifying assumptions. Our model was designed to study the effects of a few parameters related to initiation on the simultaneous translation of several mRNA species. It does not include provisions for varying selectively the rates of the different steps of elongation or termination. It is assumed that termination is not limiting and that the elongation rate does not vary unless one inhibits it specifically. In other words, the system is assumed to be saturated with elongation factors, aminoacyl-tRNA synthetases, and tRNAs. Moreover, we have analyzed a linear pathway, that is, one in which the order of binding of the different components is strictly determined. This may lead to oversimplification. There is indeed no solid evidence that the initiation pathway is exactly the same in the cell as in the reconstituted system. The choice of rapid equilibrium analysis may also lead to oversimplification. For example, this analysis would be inadequate to describe the "recruitment factor" of oocytes which is thought to be quasi-permanently bound to active message, as postulated by Lingrel and Woodland (48). The observation by Lee and Engelhardt (46) and Lodish and Porter (49) of a bimodal distribution of mRNA in polysomal gradients further supports the idea that the translation pathway may involve some slow relaxation phenomena. It has been repeatedly suggested, for instance, that the mRNA molecules which are being translated undergo a partial melting which makes their initiation site more apt to bind additional ribosomes. These relaxation phenomena are, however, reversible within minutes since it takes long for cycloheximide to displace the polysomal distribution of Vero cells (46). We thus think that our rapid equilibrium treatment of the pathway is valid in interpreting qualitatively the overall behavior of each message species.

These limitations and reservations aside, we have been able to mimic quite well the simultaneous translation of different mRNAs in several systems, both in *vivo* and in *vitro*. Some strikingly unusual findings are readily explained by assuming that prior to incorporation into a functional 40 S initiation complex, mRNA must bind to a separate entity, a "discriminatory factor," which selects the mRNA species for which its affinity is highest provided that the factor concentration is...
limiting. Thus, Kabat and Chappell's findings (2) concerning competition between $\alpha$- and $\beta$-globin messages in vitro can be precisely reproduced. So is the observation of Walden et al. (12) that, upon addition of very low doses of cycloheximide, the amount of certain reovirus proteins made in infected SC-1 cells increases significantly, whereas overall protein synthesis is inhibited. Similarly, data presented by Lee and Engelhardt (15), which deal with the proportion of the different mRNA species present in polysomes of Vero cells under different growth conditions, can be mimicked by assuming that there is a shortage of discriminatory factor in those cells and that the initiation and discriminatory factor levels drop together with mRNA concentration as the cells become stationary.

It therefore appears that the discriminatory factor level (and, to a lesser extent, the level of the other initiation factors) may play an important role in the regulation of translation. Indeed, many of the differences observed between cell lines (sensitivity to viral infection, behavior of the cell line at saturating density) could well result from differences in the relative concentrations of factors, mRNA, and ribosomes. It is easy to imagine situations in which the cell requires a rapid response to some changes in its environment. A small variation of the discriminatory factor activity might be an efficient way of achieving the desired result, by switching on or off the synthesis of a few similarly kinetic schemes.

Furthermore, the fact that the same kinetic scheme applies to different virus types suggests that there is a shortage of discriminatory factor in those cells. Indeed, many of the differences observed between cell lines (sensitivity to viral infection, behavior of the cell line at saturating density) could well result from differences in the relative concentrations of factors, mRNA, and ribosomes. It is easy to imagine situations in which the cell requires a rapid response to some changes in its environment. A small variation of the discriminatory factor activity might be an efficient way of achieving the desired result, by switching on or off the synthesis of a few similarly kinetic schemes.

In spite of the fact that most obvious peculiarities of mRNA competition may be accounted for with a single discriminatory binding step, a strong case can be made for the existence of the $\text{S}^\ast$ site of competition. On the other hand, a few eukaryotic messages have been shown to contain strong “Shine and Dalgarno” sequences (50) which are expected to play a role in initiation. A possible function of these sequences, quite compatible with the kinetic results, would be to stabilize the final form of the 40 S initiation complex ($C^\ast$ of Fig. 1) without being involved in primary message recognition.

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The rate of initiation on each message (1) of a mixture of messages is
\[ \frac{dN}{dt} = k_i (1) \]
and the rate of amino acid incorporation is
\[ \frac{dC}{dt} = k_i (1) \]
The next derivation is therefore intended to evaluate the individual \( k_i \)’s in the steady-state. Calculations presented in this paper are the “quasi-equilibrium” approximations since we have to reason on the basis that the overall rates of initiation would displace timing equivalences to any significant extent. Complete steady-state treatment was also done, but only for a single message. It was shown that the detailed results obtained by supposing that the elongating ribosome away from the initiation site, that is, reacting with (1) to (5) of Fig. 1, Part II represents elongation, termination and recycling of ribosomal subunits. The total concentration of free activated 40S subunits is considered an adjustable parameter which is evaluated by cyclic iteration through parts I and II. Under normal conditions, the value of \( r \) is determined by the total concentration of the limiting initiation factor necessary in the formation of the native 60S ribosome. Since this may not be true of cycloheximide-treated cells or in cells in which the message concentration is very large, in these cases the amount of free 40S ribosomes may become limiting.

Part I: For a given value of the parameter \( r \), the equilibrium concentrations of intermediate complexes \( c_{ij} \) are calculated from the following set of equations which represent the flow of message molecules through the different steps of initiation:
\[ \frac{dN}{dt} = k_i (1) \]
\[ \frac{dC}{dt} = k_i (1) \]
\[ \frac{dN}{dt} = k_i (1) \]
\[ \frac{dC}{dt} = k_i (1) \]
\[ \frac{dC}{dt} = k_i (1) \]

In addition, conservation of \( N \) requires:
\[ N = a_{11} (1) + a_{12} (2) + a_{13} (3) + a_{14} (4) + \ldots \]

which allows for the fact that the initiation site is blocked until the ribosome has translated 1 codons. Conservation of \( N \) requires:
\[ f_r = r \frac{dN}{dt} = f_{11} (1) \]

By combining equations (1), (2) and (3) with (4) and (5), one gets:
\[ a_{ij} (1) = a_{ij} (2) \]
\[ a_{ij} (2) = a_{ij} (3) \]
\[ a_{ij} (3) = a_{ij} (4) \]

and
\[ f_r = r \frac{dN}{dt} = f_{11} (1) + f_{12} (2) + f_{13} (3) + \ldots \]

Since the set of equations (4) and (7) does not yield \( c_{ij} \) as a function of \( r \), \( f_r \) and \( f_{11} \) in a simple analytical form, our approach is to use the intermediate parameter \( f \). Adjustment of \( f \) is aimed at satisfying the function:
\[ A(f) = c_{ij} = \ldots \]

The parameter is first made to vary stepwise until \( f \) changes stop, then adjusted within 0.1% by linear interpolation. The whole process usually requires less than 10 iterations.

The initial value of \( r \) is chosen as the smaller of \( r_{max} \) or \( r_{min} \). Since \( r \) is unknown at the beginning of the calculation, it is assumed that \( r = r_{max} \). Subsequent runs through the algorithm make use of the value of \( r \) resulting from the previous step.

Part II: Reversing the ribosomal subunits and of initiation factor 1 is described by the following equations:
\[ K_{11} = k_e + k_i \]
\[ k_{12} = k_r \]
\[ r = r + r_{max} \]
\[ q_{11} = q_{12} + q_{13} \]
\[ c_{11} = c_{12} + c_{13} \]
\[ q_{11} = q_{12} + q_{13} \]
\[ r_{11} = r_{12} + r_{13} \]

Where \( n_{ij} \) is the probability of finding a ribosome in any given state of messages (2), calculated from equation (5) as follows:
\[ n_{ij} = p_i (1) q_{ij} = p_i (1) q_{ij} \]

Variables \( r \) and \( c \) are evaluated as a function of \( r \) from equations (8) through (10). Then, parameter \( f \) is adjusted by an iteration method analogous to the one used in Part I, aimed at solving equation (12). Each run through Part II involves adjustment of \( f \) with the current value of 0 in Part I. The calculation takes 2 to 10 seconds on a small 700 desk computer.