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TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS: A SIMULATION STUDY*

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SUMMARY

A model for the translation phenomenon is developed which allows computer simulations of control of protein synthesis at the translational level. The main parameters governing the model are: the rate of initiation, the rate of progression of the ribosomes along the mRNA, the rate of release of ribosomes from the mRNA, the amount of ribosomal subunits and the amount of mRNA in the system. Simulations are performed which evidence the influence of these parameters on experimentally available data such as: polyribosome profiles, rate of protein synthesis, mean transit time of ribosomes and distribution of ribosomes along the mRNA. The aim of this work is to lead to easier interpretation of experimental works dealing with translational control of protein synthesis.

INTRODUCTION

Understanding of the control mechanisms involved in protein synthesis is a very fruitful approach to many fundamental problems such as embryonic development, homeostasis and the mode of action of hormones. Since the classic paper of JACOB AND MONOD[1], attention was first principally paid to the transcriptional aspects of this control; many authors are now interested in studying both experimentally[2–7] and theoretically[8–11] its translational aspects. In many of these studies, polyribosome profiles (*i.e.* polysome distribution expressed as ultraviolet absorbance *vs.* sedimentation rate in ultracentrifugation) and rate of protein synthesis are the commonest means used to explore changes in the functioning of the translation machinery under various experimental conditions. A survey of the literature reveals that intuition alone often does not allow the interpretation of results in such experiments. It is therefore of great interest: (a) to define the parameters ruling polyribosome assembly and polypeptide elongation; (b) to predict the effect of alterations of these parameters on polysome profiles and rate of protein synthesis.

According to available data in the literature, we have defined a model for translation governed by the following parameters: amount of mRNA, 80-S ribosomes and ribosomal subunits, rate of binding of ribosomal subunits on mRNA, of progression of ribosomes along mRNA and of release of ribosomes from mRNA. Using

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the Monte Carlo method, we were able to animate such a model in "experiments" running on a digital computer under various experimental conditions, *i.e.* assigning various values to the defined parameters. These simulations allow: (a) generations of polyribosome profiles; (b) computation of the rate of protein synthesis; (c) evaluation of the statistical distribution of the ribosomes along the mRNA and (d) calculation of the mean time a ribosome remains on the mRNA (mean transit time). They will be of use in the analysis of results obtained on the regulation of protein synthesis under various conditions.

METHODS

(1) *The model*

In order to simulate the translation phenomenon, we need a model as accurate as possible. The general outline of the model we have chosen is that of the classical Warner–Rich model for protein synthesis[12], modified according to more recent discoveries, especially concerning the ribosome subunit cycle[13–16] (Fig. 1). Such a model requires two kinds of assumptions: structural assumptions and functional ones.

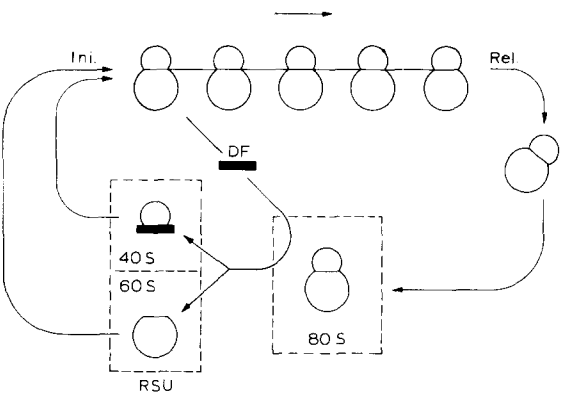

Fig. 1. Model for ribosome subunit cycle (according to SUBRAMANIAN *et al.*[16]). Abbreviations: RSU, ribosome subunits; 40 S, pool of 40-S subunits; 60 S, pool of 60-S subunits; 80 S, pool of 80-S ribosomes; DF, dissociation factor (it is supposed that DF remains fixed on the 40-S subunit after dissociation has occurred, to be released only during the formation of the initiation complex); INI., initiation; REL., release.

(a) *Structural assumptions*

The hemoglobin synthesizing system of the reticulocyte was chosen as a structural model because of its simplicity (2 mRNA of similar length: 141 and 146 codons for the mRNA of α and β chains, respectively). The system was further simplified: we consider that there is only one class of mRNA's of the same length and base sequence. Such a system possesses two structural parameters: the mRNA length and the ribosome diameter. The former was chosen equal to 150 codons. The ribosome diameter given by electron microscopy is about 220 Å (refs. 17, 12, 18). If we use the 6.5-Å measure given by EISENBERG AND FELSENFELD[19] for the interphosphate bridge of the "extended" mRNA, the ribosome diameter expressed in number of

codons is about 11. We assume in this work that the ribosome could mask 10 codons on the mRNA so that the maximum number of ribosomes on one mRNA is 15. This assumption is supported experimentally by the finding of KAZAZIAN AND FREEDMAN[20] in reticulocytes incubated in the presence of *O*-methylthreonine.

(*b*) *Functional assumptions*

We assume that a population of free 80-S ribosomes is exposed to the action of a dissociation factor acting stoichiometrically[16,20], resulting in the formation of a constant amount of 40-S and 60-S ribosomal subunits[15,22]. These subunits interfere with the 5′ ends of a homogenous population of free, monocistronic mRNA to make initiation complexes[23], the rate of this reaction depending upon the presence of the initiation factors[24], GTP[25,26], formylable methionyl-tRNA[27–29], the availability of the initiator codon AUG (the initiation site being possibly masked by a ribosome at a given time) and the amount of ribosomal subunits[13]. The ribosome on the mRNA will now progress in a 5′,3′ direction, moving from one codon to the next one while decoding the genetic information. The rate of this progression is a function of the activity of translation factors[30,31], the concentration of GTP[32–34], of aminoacyl-tRNA's corresponding to the codons being decoded and of the relative crowding on the mRNA. When the ribosomes reach the 3′ end of a mRNA, we assume (which is still controversial in eukaryotes)[14,35,36] that they are released as 80-S ribosomes while releasing a completed polypeptide chain. This reaction depends upon the presence of releasing factors[37,38] and the arrival of the ribosome at a termination codon[39]. When released from the mRNA, we assume, although some authors do not agree with this hypothesis[35], that the 80-S ribosomes become ready to reenter the cycle being dissociated into ribosomal subunits by the dissociation factor[14].

The simulation of this model requires some extra assumptions:

(1) Unless specified otherwise, the amount or activity of ribosomes, ribosomal subunits, dissociation factor, mRNA and all other factors involved in the system remains constant during one simulation.

(2) The rate at which the ribosomes on the mRNA move from one codon to the next one is the same for any codon throughout one simulation (*i.e.* we exclude possible slowdowns).

(3) The dissociation factor acts in a stoichiometric way as described by SUBRAMANIAN *et al.*[16] in prokaryotes and PÊTRE[21] in eukaryotes. This implies that the amount of ribosomal subunits remains constant whâtever the amount of 80-S ribosomes.

(4) The action of the dissociation factor is quasi instantaneous.

Such a model possesses three important functional parameters governing the state of the system: the rates of initiation, progression and release of the ribosomes.

(2) *The simulation*

In order to enable a computer to perform simulations of the model, we have to provide the structural parameters and to animate these structures according to the functional properties of the system. A simulation program proposed by GORDON[10] did not take into account ribosome cycling and dealt only with one mRNA; it could therefore not generate data similar to experimental ones. We have elaborated another program to answer these criticisms.

A population of polysomes is represented by a matrix; each horizontal row of this matrix stands for one mRNA (Fig. 2). One row possesses 15 elements corres-

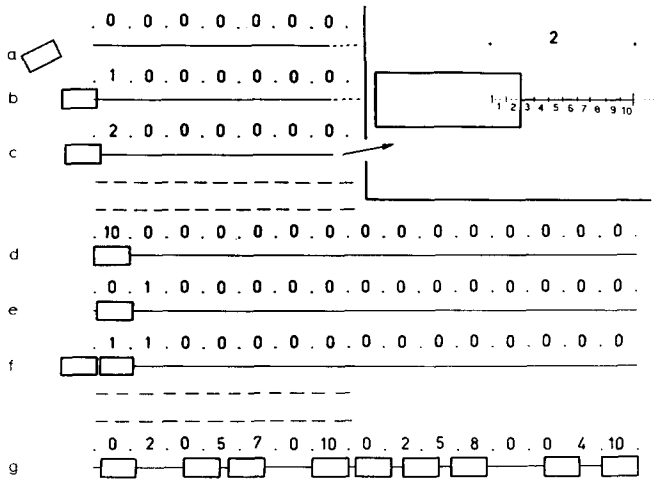

Fig. 2. Simulation of ribosome progression along the mRNA. Correspondance between one horizontal row of the matrix and the state of the polysome it represents. a, empty mRNA; b, one ribosome at the initiation site; c, the ribosome is at the second codon, . . .; e, the ribosome is now at the 11th codon; the initiation site is accessible; f, a second ribosome attaches on the mRNA; g, example of a polysome with 9 ribosomes and its representation by one row of the matrix. The ninth ribosome is ready to be released. The box shows a detail of c: the ribosome is at the second codon and the value of the first element of the matrix is set to the value 2.

ponding to 15 regions of 10 codons on the mRNA. The value of each element ranges from 0 to 10, accounting for the location of a possible ribosome on the region corresponding to that element. For instance, if the mRNA is empty, all the elements of the row will be zero; if there is one ribosome at the initiation site of the mRNA, the first element of the row is set to the value 1, the others remaining at zero. If the ribosome now progresses one codon forward, the first element of the row is set to the value 2, and so on; if the ribosome, after 9 progressions passes from the 10th codon to the 11th, the first element of the row is set to zero and the second one to 1. A new ribosome can now eventually be adsorbed at the initiation site, resulting in the formation of a disome which is simulated by setting the first element of the row to the value 1, the second one remaining at its anterior value. The number of non zero elements of the row gives thus the number of ribosomes of the polysome, and the value of the matrix elements indicates the location of the ribosomes on the mRNA (the value 15 for the number of elements and 10 for the maximal value of each element comes evidently from the fact that we assume that each ribosome covers 10 codons and that there is a maximum of 15 ribosomes on 1 mRNA).

Let us now define our functional parameters in the following way:

(*a*) *Initiation*

Let P_1 be the probability for each accessible initiation site of any mRNA of our population to adsorb a pair of ribosomal subunits during one time interval Δt if there was only one pair of subunits available. The maximum number of initiations occurring in our system during Δt is thus $P_1 \times n \times R_s$, where n is the number of mRNA in the system and R_s the number of ribosomal subunit pairs.

(*b*) *Progression of the ribosomes*

P_p is defined as the probability for any unhindered ribosome on the mRNA

to move one codon forward during one time interval Δt. The maximum number of progressions in the system during Δt is thus $P_p \times R_0$, where R_0 is the number of ribosomes engaged in protein synthesis at that time.

(c) *Release of the ribosomes*

P_r is the probability for the 3′ terminal end of any mRNA, if covered by a ribosome to release it during Δt. $P_r \times n$ being the maximum number of releases during that time interval.

The P_i, P_p and P_r are evidently homologous of the initiation, progression and release "rates" described in the model. One important difference however is that these probabilities are conditional probabilities, *i.e.* they apply to events which would occur with a probability P only if the actual situation of the system was ideal (initiation site unmasked; ribosome unhindered; ribosome ready to be released). This is to say that these probabilities and hence, the maximum number of events of each kind calculated above, are an expression of potential initiation, progression or release and that they do not take any account of the state of the system which is precisely, a function of the relative ratio between these probabilities. For instance, if we imagine a theoretical situation in which the release of ribosomes becomes almost zero ($P_r = 0$; lack of releasing factors, poisoning of the releasing system, *etc.*), the mRNA will quickly be overcrowded and, although the system will still possess the same initiation and progression potential, these two events will soon become rare. The conditional character of the probabilities involved in such a system makes plain probability calculus cumbersome. Therefore, another means was used to animate the model: the Monte Carlo method[40].

By means of a Fortran IV program running on a CDC 6400 computer, we offer to our system (the matrix) the maximum number of events of each kind which could occur during Δt, according to given values of P_i, P_p, P_r, n R_s and R_0 (the value of R_0 is continuously computed from the state of the matrix). The program is planned so that: (1) only the possible events are really made to occur; (2) that they occur with the given probabilities and (3) at random as far as the sequence and the place are concerned. By making these operations several ten thousand times, we are able, for any values of P_i, P_p, P_r, n and R_s to obtain the polysome distribution, and the rate of protein synthesis (from the number of released ribosomes/Δt). By measuring the ratio between the maximum number of events of each kind and the actual number of events occurring during a given time interval, we are able to objectivate which kind of events is rate limiting in a given situation. The steady state is obtained when the number of actual initiations equals that of releases.

RESULTS

We empirically choose such values for the parameters P_i, P_p, P_r, R_s and n, so that we were able to generate a typical polyribosome profile of reticulocyte, showing a peak in the class of the pentamers[18] (Fig. 3a). Since this will be our starting situation for further experiments, it needs some words of comment:

(a) In order to minimize slight variations in the shape of the profiles at steady state due to the fact that we are dealing with a limited number of polysomes, the profiles presented on Fig. 3 are obtained by averaging five profiles from five different states of the system.

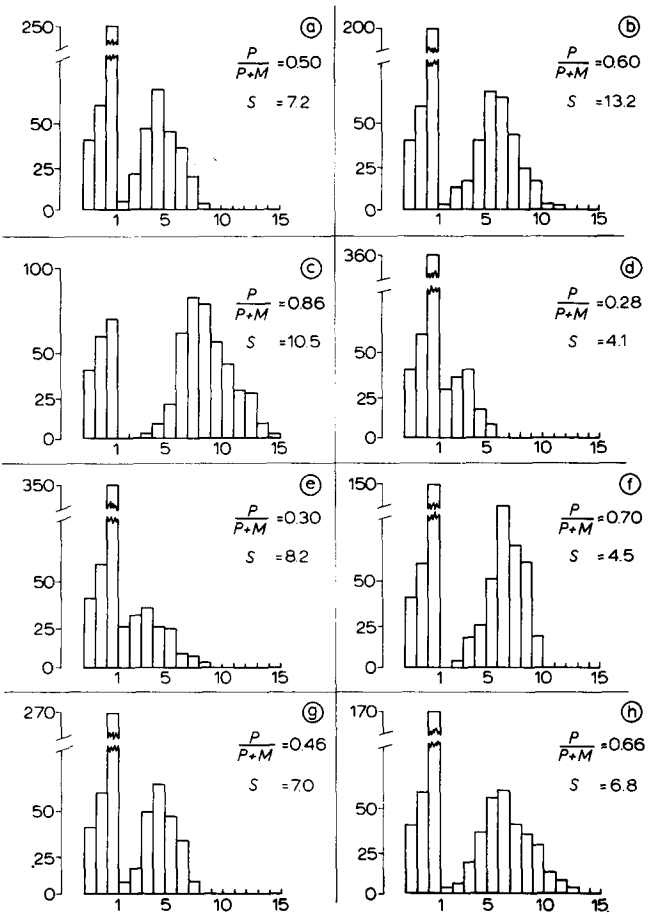

Fig. 3. Polysome profiles and protein synthesis as a function of initiation, progression and release. P, number of ribosomes in the polysomes; M, number of free ribosomes; S, rate of protein synthesis expressed as number of released ribosomes during one arbitrary time interval. The profiles are expressed as following: abscissae indicates the polysome classes (from 1 to 15); the two classes at the left of the monosomes stands for 40-S and 60-S subunits. Ordinate: number of ribosomes in each class. (a) Starting situation. $P_1 = 4 \cdot 10^6$; $P_p = 1 \cdot 10^{-2}$; $P_r = 1 \cdot 10^{-3}$ (for definition of P_1, P_p, P_r, see text). (b) 2-fold increase in P_1 and P_p. (c) 2-fold increase in P_1. (d) 2-fold decrease in P_1. (e) 2-fold increase in P_p. (f) 2-fold decrease in P_p. (g) 2-fold increase in P_r. (h) 2-fold decrease in P_r. Profiles are obtained from a population of 50 polysomes by averaging 5 profiles corresponding to 5 states of the system distant from each other of about 10 000 events.

(b) The amount of 80-S ribosomes and ribosome subunits relative to the amount of ribosomes in polysomes was chosen following HOGAN AND KORNER[15], and R. LECOCQ (personal communication). The ratio $P/(P+M)$ was set to 0.5 (P = number of ribosomes in polysomes; M = number of ribosomes in the monomer peak), and $R_s/(P+M)$ to 0.2 (R_s = number of subunit pairs) in our starting situation.

(c) The values of the initiation, progression and release probabilities corresponding to the starting situation are $P_1 = 4 \cdot 10^{-6}$; $P_p = 1 \cdot 10^{-2}$; $P_r = 1 \cdot 10^{-3}$. To one set of the three probabilities corresponds only one polysome profile and one

rate of protein synthesis. On the contrary, to one polysome profile may correspond an infinite number of different values for the three parameters. This results from the fact that in a given situation, modifying the probabilities which are not rate limiting does not alter the state of the system.

(d) Analysis of the ratio of actual events occurring in our starting situation at steady state to possible events shows that in this situation release of ribosomes from mRNA is not rate limiting, while initiation and progression of ribosomes indeed are.

Fig. 3 shows how 2-fold alterations of initiation, progression and release probabilities affect the polysome profiles, $P/(P+M)$ ratios and rate of protein synthesis expressed as the number of released ribosomes during one constant arbitrary time interval Δt. Except in Fig. 3b, only one probability is modified in each experiment, the others remaining at their initial value. It can be seen that alteration in the initiation probability is the most effective in modifying both the polysome profile and the rate of protein synthesis (Figs. 3c and 3d). 2-fold increase of P_I results in an increase of $P/(P+M)$ from 0.5 to 0.86 accounting for the shift of the polysome profile from monomers towards heavier aggregates and in a 50 % stimulation of protein synthesis. Modification of the progression probability affects the polysome profile in the opposite direction (Figs. 3e and 3f). Increasing P_p results in a shift of the profile towards lighter polysomes. The protein synthesis is stimulated but this stimulation is limited because in this situation, initiation becomes still more rate limiting. An increase in the release probability (Fig. 3g) does not affect both the polysome profile and the rate of protein synthesis since this parameter was seen not to be rate limiting. On the contrary, a 2-fold decrease of P_r (Fig. 3h) induces a slight shift in the polysome profile towards heavier aggregates and slightly reduces protein synthesis. Fig. 3b shows the effect of increasing simultaneously P_I and P_p. Protein synthesis is greatly enhanced while the polysome profile only shows a slight modification, demonstrating that a polysome profile alone gives no information about the functional state of the system.

On Fig. 4, we have plotted the rate of protein synthesis as a function of the probability of initiation, P_p and P_r remaining constant. Protein synthesis follows an almost linear increase for small values of P_I and reaches a plateau for higher values of this parameter. It is interesting to note that the normal polysome profile of the reticulocyte (our starting situation) corresponds to values of P_I where the function is linear. This would allow easy and efficient regulation of protein synthesis at the level of initiation in such a system. The extreme value of P_I corresponding to 5-fold stimulation of initiation corresponds to a quite unrealistic situation where the polysome shift is important enough to exhaust the 80-S ribosome population.

The mean transit time of ribosomes (*i.e.* the mean time a ribosome remains on a mRNA) is plotted as a function of P_I and P_p, respectively, on Figs. 5 and 6. The mean transit time decreases hyperbolically when P_p increases, the horizontal asymptote being the mean time a ribosome must wait at the 3′ end of the mRNA before release occurs (P_r^{-1}). More surprising is the mean transit time increase associated with increasing of P_I (Fig. 5). It can be seen that the mean transit time given by simulations is always greater than the ideal computed one which appears on the ordinate axis ($149 \times P_p^{-1} + P_r^{-1}$). This is interpreted as a consequence of the relative crowding of the polysomes.

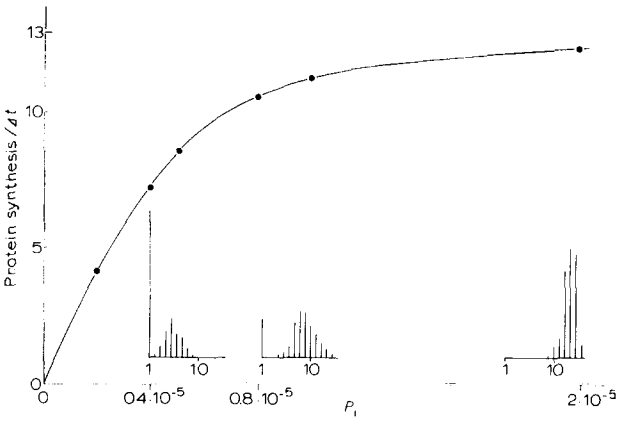

Fig. 4. Rate of protein synthesis as a function of probability of initiation. The rate of protein synthesis is expressed (ordinate) as a number of released ribosomes at steady state during one arbitrary time interval Δt. We show the polysome profiles corresponding to three particular values of P_i: $0.4 \cdot 10^{-5}$, $0.8 \cdot 10^{-5}$ and $2 \cdot 10^{-5}$ below the curve. P_p and P_r are those of the starting situation (Fig. 3a).

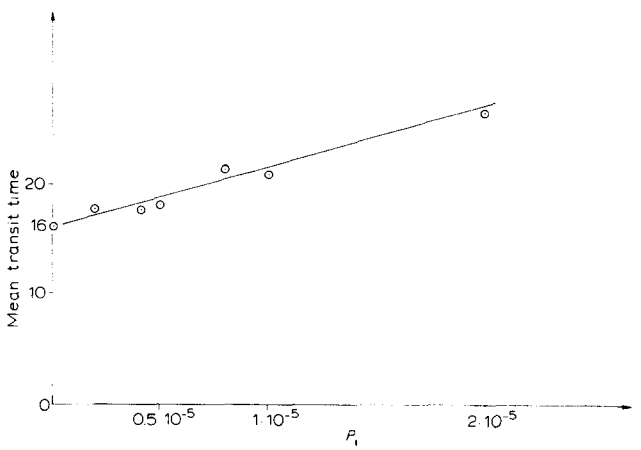

Fig. 5. Mean transit time as a function of probability initiation. The mean transit time (mean time a ribosome spends attached to a mRNA) is computed from the observed number of progressions and releases occurring at steady state and expressed in arbitrary units. The value 16 on the ordinate axis is the minimal observable value $149 \times P_p^{-1} + P_r^{-1}$. P_p and P_r are those of the starting situation (Fig. 3a).

The kinetics of alterations in protein synthesis under different conditions have been explored in the following way (Fig. 7). We continuously plotted the amount of protein synthesized as a function of time during simulations in which the probabilities of initiation (P_i) and progression (P_p) were modified. Fig. 7 shows the differences between the kinetics of the phenomenon respectively in the case of modifications of P_i (2-fold increase and decrease) and P_p (idem). Our starting situation was that of Fig. 3a. It appears that in the case of modification of P_i, the rate of protein synthesis remains unchanged during a time interval corresponding to the mean transit time, after which it reaches rather abruptly its new value. On the contrary, in the case of P_p alterations, the rate of protein synthesis changes immedia-

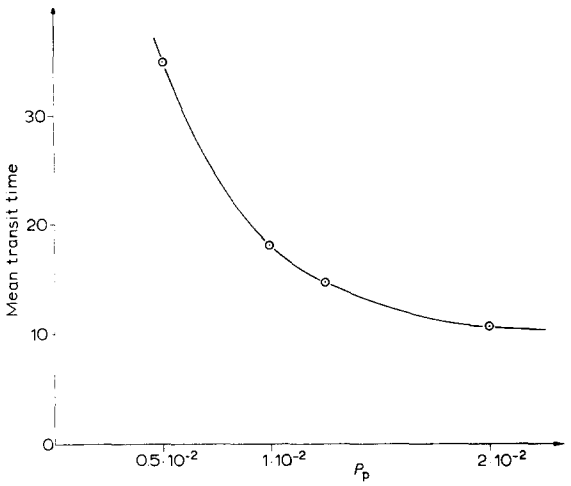

Fig. 6. Mean transit time as a function of probability of progression. The mean transit time is computed as in Fig. 5. The horizontal asymptote of the curve has the equation: mean transit time $= P_r^{-1}$. P_i and P_r are those of the starting situation (Fig. 3a).

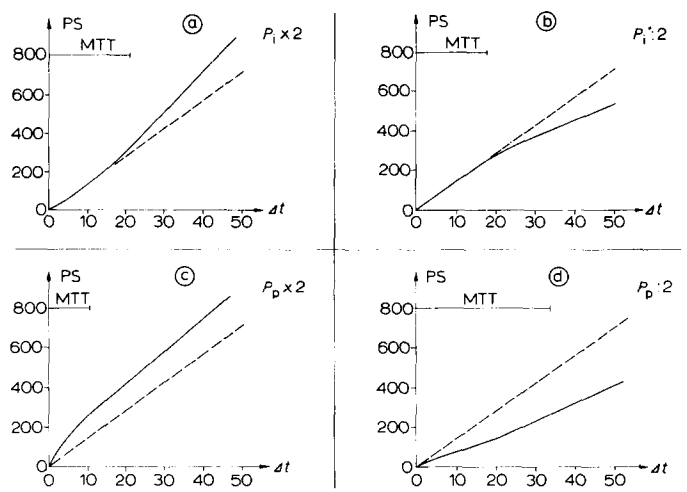

Fig. 7. Kinetics of protein synthesis. Dotted line, kinetics of protein synthesis by our starting situation. (a) 2-fold increase in P_i. (b) 2-fold decrease in P_i. (c) 2-fold increase in P_p. (d) 2-fold decrease in P_p. MTT = mean transit time. PS = protein synthesis.

tely and is already stabilized at its new value after a time interval of the mean transit time. The modification of the slope of the curves is much greater in the case of alteration of P_i, indicating the greater efficiency of initiation in the control of the system.

On Fig. 8, we have represented the distribution of the ribosomes along the mRNA in the same experimental conditions as in Fig. 3. It appears that although we have seen that the release probability does not affect both the shape of the polyribosome profile and the rate of protein synthesis in our starting situation, P_r indeed affects the distribution of the ribosomes along the mRNA in that situation, being

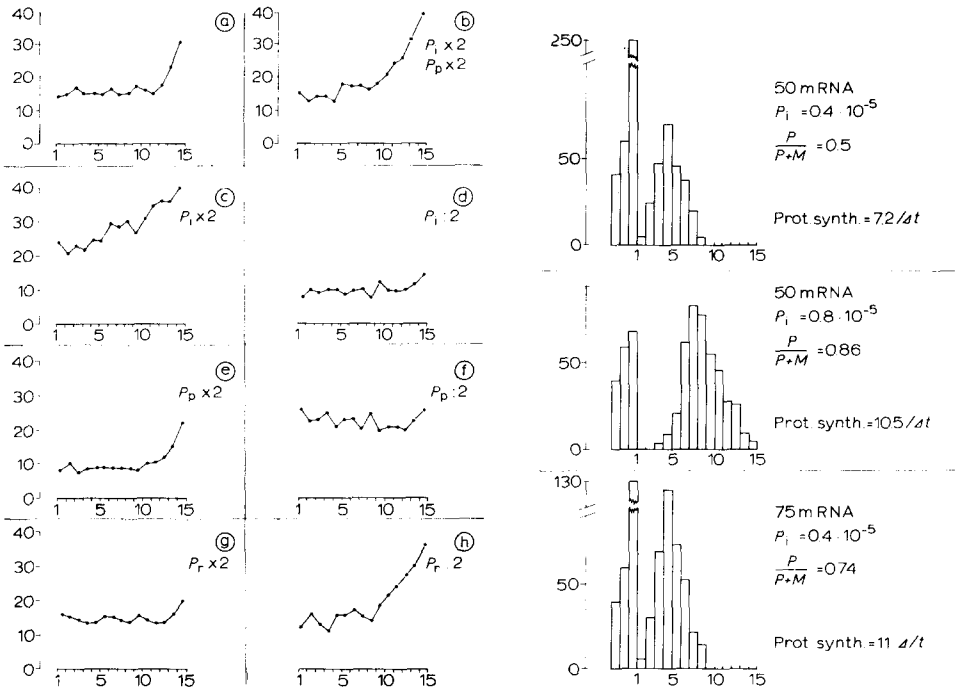

Fig. 8. Distribution of the ribosomes along the mRNA. In abscissae: 15 regions of 10 codons of the mRNA. In ordinates: the number of times a ribosome is found on the region throughout the polysome population at a given time at steady state. Since we deal with 50 polysomes, the maximum is 50. (a) Starting situation (as in Fig. 3a). (b) $P_i \times 2$; $P_p \times 2$. (c) $P_i \times 2$. (d) $P_i : 2$. (e) $P_p \times 2$. (f) $P_p : 2$. (g) $P_r \times 2$. (h) $P_r : 2$.

Fig. 9. Comparison between stimulation of initiation and increase in the amount of mRNA. The $P/(P+M)$ ratio, polysome profiles and rate of protein synthesis are expressed as in Fig. 3.

responsible for the accumulation of ribosomes at the 3′ end of the mRNA. The real pile of ribosomes which can be seen near the 3′ end of the mRNA in the case of 2-fold stimulation of initiation (Fig. 8c) accounts for the increasing of the mean transit time observed under this condition and must be interpreted in the same way as the slackening of motor cars on an overcrowded highway.

Fig. 9 shows how a 50 % increase in the number of mRNA alters the polysome profile and protein synthesis of our starting situation as compared with the experiment of stimulated initiation (Fig. 9b). The polysome distribution is not affected by this modification since none of the values of P_i, P_p, P_r and R_s is a function of the number of mRNA. However, the $P/(P+M)$ is enhanced, accounting for the participation of more 80-S ribosomes. This points out that a shift from monosomes to polysomes must be interpreted very carefully. In the case of stimulated initiation, the distribution of the polysomes is modified by lateral shift; in this case, only the number of polysomes is altered by an upward shift.

The effects of inhibition of 80-S ribosome dissociation into subunits on protein synthesis and polyribosome profiles are detailed on Figs. 10 and 11. It can be seen from comparison between Fig. 10 and Fig. 11 that protein synthesis goes on rather linearly for a long time while polysome profiles show a typical shift towards lighter

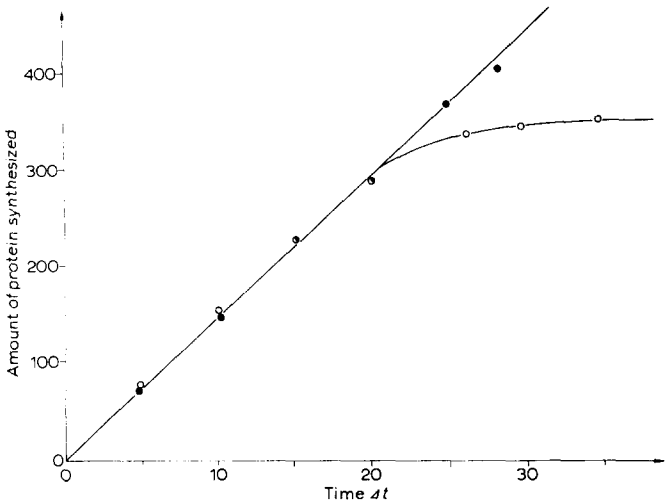

Fig. 10. Simulation of inhibition of 80-S ribosome dissociation. Effect on protein synthesis. ●, amount of protein synthesized at steady state by the system at the starting situation as a function of time; ○, amount of protein synthesized by the system as a function of time after dissociation of 80-S ribosomes has been inhibited at time 0. The amount of protein synthesized is given by the number of released ribosomes. The time scale is arbitrary.

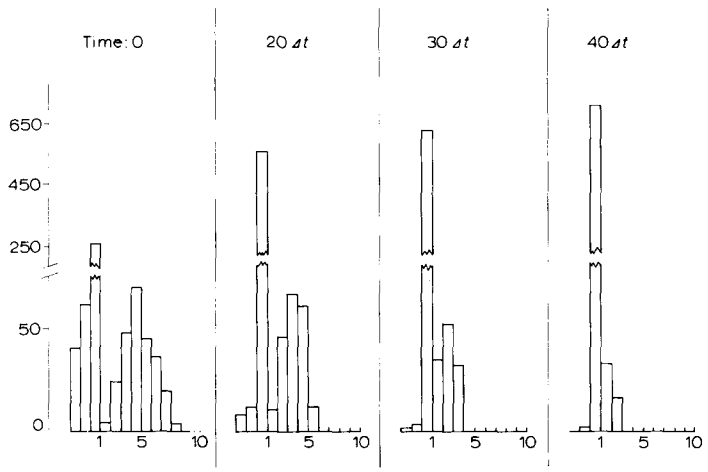

Fig. 11. Simulation of inhibition of 80-S ribosome dissociation. Effect on polysome profile. The profiles are expressed as in Fig. 3. The four profiles correspond to time 0, 20 Δt, 30 Δt and 40 Δt of Fig. 9.

aggregates and monosomes associated with reduced initiation, and ribosome subunits decrease dramatically. It is necessary to wait until the polysomes are almost empty to observe the inhibition of protein synthesis. This would correspond to the case of Fig. 7, where P_1 would have been progressively set to zero.

DISCUSSION

A model for protein synthesis by polyribosomes has been developed which allows computer simulations of translational control. Polyribosomes profiles, mean transit time of ribosomes on the mRNA, rate of protein synthesis, and distribution of ribosomes along the mRNA have been studied as a function of the following main parameters of the model: probability of initiation (P_I), of progression (P_p), of release (P_r), amount of mRNA and ribosomal subunits. Since the results presented have no value except within the limits of the model, some assumptions relative to this model need to be discussed.

We deal only with one class of free mRNA excluding investigations about possible specific translational control of one type of mRNA among a population of heterogenous mRNA[23,41] The model applies only to free polysomes. It is very likely that translation on endoplasmic reticulum-bound polysomes involves some extra parameters which we have not considered here (*e.g.* the smallest distance between ribosomes may be determined by the location of hypothetical ribosome-accepting sites on the reticulum[42].

According to JOKLIK AND BECKER[22] and HOGAN AND KORNER[15] we have postulated that the amount of ribosomal subunit pairs was constant, which is in agreement with the observation of SUBRAMANIAN *et al.*[16], and PÊTRE[21] concerning the stoichiometric mode of action of the dissociation factor. This implies that in physiological conditions the rate of initiation is independent of the sum, subunits *plus* 80-S ribosomes, which can be interpreted from a teleological point of view as a convenient means for allowing translational control of the synthesis of one kind of protein without affecting the synthesis of the others. Otherwise, indeed, the general initiation probability would decrease in any situation where the amount of ribosomes engaged in mRNA reading (*i.e.* on polysomes) would increase.

Since recent works by HUNT *et al.*[43] failed to reveal the existence of slackening points in the translation of hemoglobin mRNA, we have not considered here this hypothesis, which had been proposed by WINSLOW AND INGRAM[44]. ITANO[11] has shown that such slowdowns, if located near the 5′ end of the messenger, would have the same effect as a decrease in the initiation rate. On the contrary, if it is located near the termination codon, it would mimic a reduction in the rate of release of ribosomes.

It seems difficult at present to be completely definite about some particularities of the ribosome cycle. Are 80-S ribosomes released as such, or does dissociation occur simultaneously with the termination step[36]? Are the 80-S ribosomes an obligatory step in the ribosome cycle, or does the great majority of them not participate in the cycle[35]? There is evidence from isotopic tracer studies that subparticles recycle through polyribosomes without equilibrating with 80-S ribosomes[35]. This could suggest that dissociation occurs simultaneously with the termination step, an alternative explanation being that topographical parameters favor local recycling of subunits on particular mRNA's[22,14,45]. We chose a model in which the 80-S ribosomes would behave like a kind of store furnished with ribosomes falling off the mRNA and submitted to the random action of the dissociation factor[14].

We think that the results presented here would allow easier interpretation of experiments dealing with translational control of protein synthesis. From the results

of Fig. 3, it appears that experiments showing a shift in polysome profile from light aggregates and monosomes towards heavier polysomes can be interpreted in two ways when no new mRNA is synthesized. If it is associated with enhanced protein synthesis, it would indicate stimulation of initiation. Many experimental or physiological circumstances present such characteristics, *e.g.* Landscütz ascites cells grown on a medium enriched with amino acids[15]; liver cells from fasted mice receiving tryptophan[46]; thyroid cells exposed to thyrotropin action[47]; liver cells from rats fed a threonine-devoid diet[48]. On the contrary, if concomitant decrease of protein synthesis is observed, it is likely that a decrease in the rate of progression—as in cycloheximide-treated cells[41]—or release of ribosomes is responsible. From Fig. 7, it appears that it would be theoretically possible to differentiate between these two possibilities by electron microscope study of the distribution of ribosomes along the mRNA. A queue of ribosomes is more likely to be formed in the case of limited release of ribosomes. An interesting technique of demonstration of such queues have been presented by HUNT *et al.*[43].

An increase in protein synthesis associated with a shift of the polysome profile towards light aggregates and monomers could be seen in situations where increase of the progression rate of ribosomes takes place. We would propose this interpretation of the results of WITTMAN AND MILLER[49], who observed a disaggregation of polysomes concomitantly with an increased protein synthesis in rat liver after injection of fructose *in vivo*. The same shift with concomitant decrease in protein synthesis would be indicative of a limited initiation rate[50]. Several experimental systems present such characteristics: hamster ovary cells during mitosis[41]; diabetic muscle cells[3]; rat liver cells treated with ethionine[51]. It would be tempting to explain the action of ethionine in terms of competition with methionine in the reaction leading to methionyl-$tRNA_f^{Met}$ (where $tRNA^{Met}$ is formylable $tRNA_f^{Met}$) at the level of initiation. A particular case is that of NaF-treated cells[14], where decrease of initiation seems secondary to inhibition of dissociation of 80-S ribosomes. We have tested this situation on our model, and results appearing on Figs. 10 and 11 are in close agreement with those of VESCO AND COLOMBO[14].

From Fig. 7, giving the kinetics of protein synthesis under various circumstances, it is possible to differentiate between modifications of initiation rate or progression rate of ribosomes without looking at polysome profiles. For instance, the delay in manifestation of inhibition of protein synthesis is typical of a decrease in the initiation rate. The delay equals the mean transit time of ribosomes, as shown by the simulation reported on Fig. 7b. Such a delay is found experimentally in NaF[14]- and pactamycin[52]-treated cells; it agrees with the value calculated by HUNT *et al.*[53] for the mean translation time of hemoglobin mRNA. A kinetic of the progression inhibition type (Fig. 7d) is found in sparsomycin-treated cells[52], showing that diffusion of the antibiotics is probably not involved in the delay.

Analysis of mean ribosomal transit time (Figs. 5 and 6) provides one more criterion for analyzing regulation of translation. Modification in polysome profiles and protein synthesis with slight if any alterations of the mean transit time of ribosomes in a system where mRNA synthesis does not occur would indicate modifications in the initiation rate.

If our starting situation (where termination is not rate limiting) possesses some physiological value, it appears from Figs. 3, 7a, and 4 that the most effective para-

meter for linear regulation of protein synthesis at the translational level is the rate of initiation. It would therefore be tempting to consider initiation as one of the most likely parameters to be involved where regulation of translation occurs. Looking back, in retrospect, to the literature, we found a number of experiments confirming this assertion. While, in some cases, this hypothesis has been proposed at first[54,55] in other cases, either it has not been considered explicitly[56–58], or it required a lot of additional experimental work to demonstrate what the model would have suggested from the early works[52,41,3].

As a conclusion, we shall try to give a summary of what may theoretically be expected by the experimentator under specific circumstances of translational control:

(*a*) *Enhanced initiation*: shift of polysome profiles towards heavy polysomes; delay of about one transit time before increase in protein synthesis occurs; minute if any increase in mean transit time of ribosomes; uphill gradient of ribosomes along the mRNA.

(*b*) *Reduced initiation*: shift in the opposite direction; delay before decrease in protein synthesis occurs; slight reduction of the mean transit time; distribution of ribosomes along the mRNA almost homogenous.

(*c*) *Enhanced progression rate of ribosomes*: shift of profiles towards light aggregates; no delay in alteration of protein synthesis; dramatic decrease in mean transit time; possible queue at the 3′ end of the mRNA.

(*d*) *Reduced progression rate*: shift in the opposite direction; no delay in decrease of protein synthesis; great increase of mean transit time; distribution of ribosomes along the mRNA homogenous. It must be emphasized that in all our simulations modifying the intiation, progression or release probability, the maximum time required to reach the new steady state from the preceeding one equals the mean transit of ribosomes along the mRNA.

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