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KINETICS OF PROTEIN SYNTHESIS BY POLYRIBOSOMES

by

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Kinetics Of Protein Synthesis By Polyribosomes

Recently considerable evidence has been presented [1,2] that cytoplasmic protein synthesis is mediated by a group of ribosomes linked together by a strand of messenger RNA. Such aggregates are referred to as polyribosomes and may consist typically of 5-10 ribosomes per messenger (M),RNA strand. The present report investigates the kinetics of polyribosome mediated protein synthesis. A fairly general but still tractable deterministic linear model is presented which allows for messenger RNA sources of arbitrary functional form and also takes into account any deterioration of the messenger RNA which may occur during the course of protein synthesis. The ribosome (R) concentration will be considered constant.

1. FORMULATION

The physical model of the polyribosome mechanism is shown in figure 1. A messenger RNA strand possibly several thousand A^{o} units in length is shown occupied by ribosomes each of about 200 A^{o} in diameter. Amino acid residues in the form of aminoacyl-S-RNA compounds (AS) are delivered to the growing peptide chain. The sequencing of the amino acids in the peptide is determined by Watson-Crick base pairing between the trinucleotides of AS and those of the messenger RNA. Investigations, [3,4] of AS indicate that it retains a double helical configuration in solution with a bend near the middle. At the bend a minimum of three nucleotides must be present [5]. It is assumed that nucleotides pair with the messenger RNA, as shown in figure 1. An

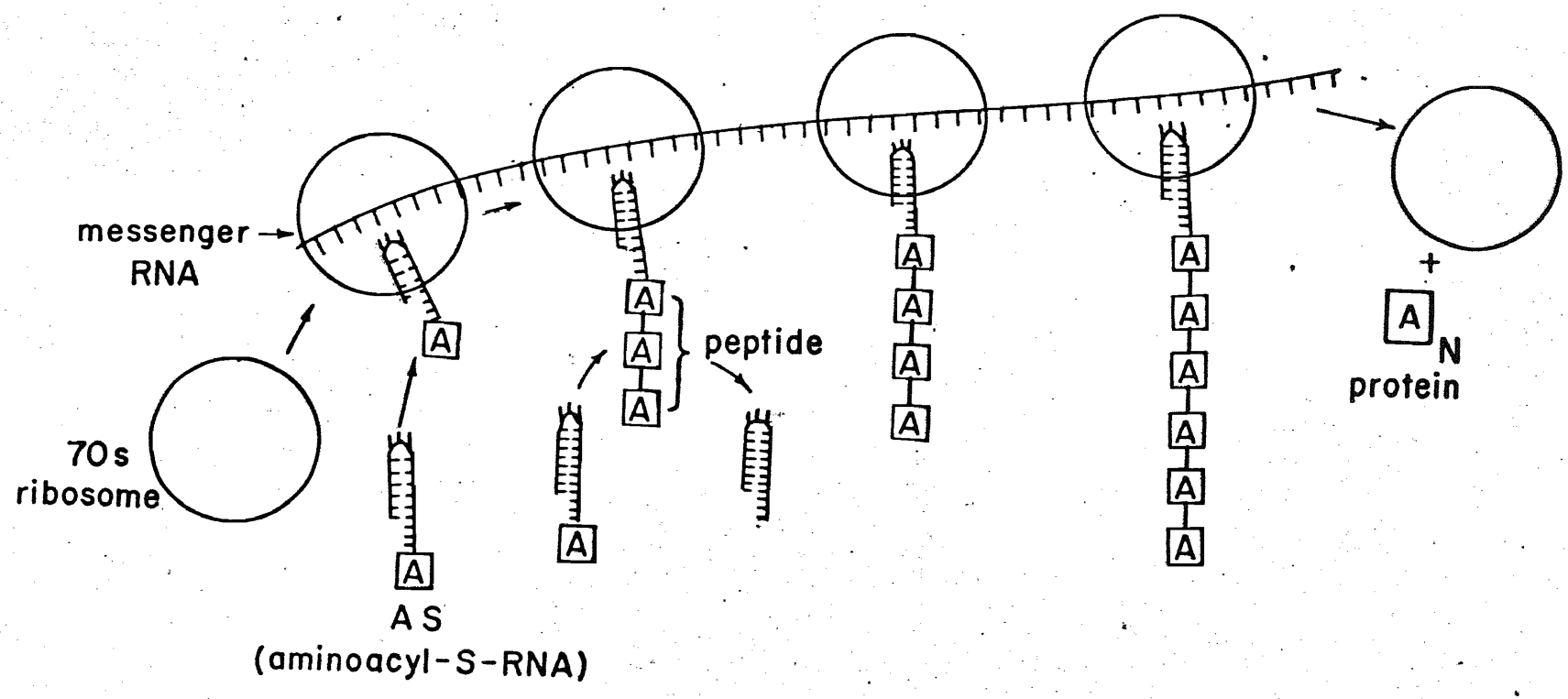
messenger
RNA
70s
ribosome
A S
(aminoacyl-S-RNA)
peptide
+
N
protein

FIG. 1

enzyme mediated reaction then transfers the amino acyl group from AS to the growing peptide. As the ribosome travels from left to right it "reads" the information on the messenger RNA strand while building up the polypeptide. It is evident that some sort of mechanism must be available to start the ribosome at the appropriate end of M and to prevent the reverse motion of the ribosome.

After the first ribosome has proceeded a certain distance along M corresponding to the addition of p amino acid residues, a second ribosome may now become attached to the messenger RNA. This process will continue until the full complement of ribosomes per messenger RNA is realized. When the synthesis of each peptide chain is complete the polypeptide and ribosome are liberated at the right hand end of the messenger RNA. The remaining bound ribosomes continue to move along the messenger RNA and eventually another ribosome becomes attached to the left end of the messenger RNA and the cyclic processes repeated until a vital constituent such as AS is depleted. It is well known [6] that messenger RNA may be short lived while the soluble RNA(S) shows no appreciable turn-over during protein synthesis. The stability of the messenger RNA is, however, enhanced when it is complexed with ribosomes [8]. The biochemical significance of this lability is evident since if the requirement for a messenger is ended then the production of new messenger RNA will cease and that already existing will be destroyed within a few minutes.

In the following we consider a model in which both the ribosome and AS concentrations are kept essentially constant during the synthesis. The case where the ribosomes and AS are variables results in a set of nonlinear differential equations which in general cannot be solved. However, M is considered to be added at a rate, g(t), while it deteriorates at a rate characterized by the rate constant k_d for the free M and at a rate characterized by k_m for the bound M.

The relevant reactions will now be considered. Synthesis is initiated by the binding of a ribosome (R) to a strand of free messenger RNA. According to Levinthal et. al. [8] this association may require the participation of AS so that the initial reaction will be represented as

(1)

$$M+R+AS \xrightarrow{k_o} MRAS \quad .$$

Binding of ribosomes requires magnesium ions and the complex is dissociated at low magnesium ion concentrations [2]. Growth of the peptide requires that the appropriate aminoacyl-S-RNA be adsorbed onto the MR complex at a position adjacent to the growing peptide site and that amino acid residue (A) then be added to the peptide by means of a nonspecific amino acid transferase. It is known that the amino acid is linked through the carboxyl group to the soluble RNA (S) and that the forma-

tion of the peptide bond is achieved by the transfer of the entire peptide chain to the amino group of AS [9]. Gilbert and others [2] have recently provided evidence that there is only one strong binding site for SA per ribosome so that presumably it is this site at which peptide bond formation occurs and which moves along with the ribosome. In view of these findings the growth step may be represented by binding at the active site followed by a transfer reaction:

(2) $$MR\ AS + AS \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MR\ AS \cdot AS \quad ,$$

(3) $$MR\ AS \cdot AS \xrightarrow{k} MRA_2S + S \quad .$$

In equation (2) the AS to the right of the dot represents the bound species. The above sequence of reactions is repeated during growth of the peptide so that in general we have

(4) $$MR\ A_{p-1}\ S + AS \rightleftharpoons MR\ A_{p-1}\ S \cdot AS \quad ,$$

(5) $$MR\ A_{p-1}\ S \cdot AS \longrightarrow MR\ A_p\ S + S \quad .$$

When the first ribosome has translated a distance d along M corresponding to say p amino acids in the peptide chain, another ribosome will be added and thereby initiate a second

growing peptide chain. This process will be repeated with a third ribosome and so on. Observations [1] indicate that the ribosomes are separated from each other by a gap of $100 - 50A^{\circ}$ so that d and p may be estimated. Allowing $3.5A^{\circ}$ for a nucleotide base in the messenger RNA then for a triplet coding scheme each amino acid residue corresponds to a distance of about $10A^{\circ}$ along the messenger RNA. Assuming an average gap of $100A^{\circ}$ and a ribosome with a diameter of about $200A^{\circ}$, then on the average $d \sim 300A^{\circ}$ and $p \sim 30$ amino acid residues.

The reactions subsequent to (5) may now be expressed by the relationship

(6)
$$MR\ A_p\ S + AS + R \longrightarrow M(RAS)\ (RA_pS) \quad ,$$

Iteration of reactions (1) - (6) gives rise in general to polyribosome which may be represented by

(7)
$$M(R\ A_{i_1}\ S)\ (R\ A_{i_2}\ S)\ \ldots\ldots\ (R\ A_{i_q}\ S) \quad ,$$

which indicates a polyribosome made up of q ribosomes in a state with a growing peptide chain of i_1 amino acid residues on the last added ribosome, i_2 amino acid residues on the adjacent ribosome, etc. The case $i_k = o$ corresponds to the absence of a ribosome located at the kth region of the messenger RNA.

A convenient notation is suggested by (7) since the state of the polyribosome is defined by the ordered set of subscripts which may be displayed as a vector $(i_1, i_2, i_3 \ldots i_q)$. As a slight extension of this notation we may designate an AS adsorbed to the binding site of a ribosome (i.e., $(i_1, i_2 \ldots i_q)$.AS) by means of the notation $(i_1, i_2 \ldots i_q/1)$.

It is evident that if we assume random growth of the peptides a very large number of intermediary states can occur. In order to simplify the algebraic manipulations arising in the analysis without however changing the essential character of the problem we shall assume that addition of the amino acid residues occurs in sequence starting with the ribosome farthest to the right. Under this sequential addition assumption a typical sequence of polyribosome states would be

$$(i_1, \ldots i_{q-1}, i_q) \longrightarrow (i_1, \ldots i_{q-1}, i_q/1) \longrightarrow (i_1, \ldots i_{q-1}, i_q+1) \longrightarrow$$

$$\ldots \quad (i_1, \ldots i_{q-1}+1, i_q+1) \longrightarrow \tag{8}$$

$$\ldots \quad (i_1+1, \ldots i_{q-1}+1, i_q+1) \quad .$$

Thus with the present model we assume in effect that the messenger RNA is divided into q regions. As the ribosome associated with any particular region of M moves along the

region p amino acid residues are added to the growing peptide chain so that p q peptide residues are added in all. Eventually a ribosome and its associated protein will arrive at the end of the messenger RNA strand where they will be released. While the details of the release remain to be elucidated, it is evident that after the last amino acid has been added the bond between the protein and AS must be severed. The free protein A_N is now free to assume its final tertiary structure, P_N. It is evident from the work on the reconstitution of denatured proteins that to a large extent the tertiary structure follows spontaneously from the amino acid sequence. In some cases it is known that the physiologically active form of the protein requires the association of several subunits. For the present purposes however the terminal reactions will be represented by

(9) $$(i_1, i_2 \cdots, pq) \xrightarrow{k_f} (i_1, i_2 \cdots, o) + A_N + S \quad ,$$

(10) $$A_N \xrightarrow{k_p} P_N \quad .$$

We shall also introduce a compact notation in order to express the various reactions. Thus the reactions

$$R + AS + (0,3,0) \xrightarrow{k} (1,3,0)$$

will be written as

$$(0,3,0) \xrightarrow{k,R,AS} (1,3,0) \quad ,$$

and similarly for other reactions.

2. THE MATHEMATICAL MODEL IN THE SPECIAL CASE p = 2, q = 3

In this section, we set up the mathematical model for the polyribosome process and solve it under certain conditions. In general, we can handle the case in which there are q ribosomes each of which occupies a space on the messenger RNA corresponding to the designation of p amino acids. However, this general case, while uncomplicated in principle, leads to some tedious algebra of little interest in itself, and accordingly we relegate it to an appendix. Instead, we consider here the special case of a peptide with six amino acid residues and for which p = 2, q = 3 which, although simplified, suffices to exhibit all the characteristics of our model from the mathematical point of view and is sufficiently simple to allow for a detailed treatment. At the end of the section, we supply the corresponding results for the general case.

(a) The Reaction Equations

The reaction equations are as follows:

$$g(t) \longrightarrow (o,o,o) \xrightarrow[\downarrow k_m]{k_o,R,AS} (1,o,o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,o,o/1) \xrightarrow[\downarrow k_d]{k} (2,o,o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}}$$

$$(2,o,o/1) \xrightarrow[\downarrow k_d]{k} (o,3,o) \xrightarrow[\downarrow k_d]{k_o,R_1AS} (1,3,o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,3,o/1) \xrightarrow[\downarrow k_d]{k}$$

$$(1,4,o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,4,o/1) \xrightarrow[\downarrow k_d]{k} (2,4,o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (2,4,o/1) \xrightarrow[\downarrow k_d]{k}$$

$$(2,o,5) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (2,o,5/1) \xrightarrow[\downarrow k_d]{k} (o,3,5) \xrightarrow[\downarrow k_d]{k_o,R,AS} (1,3,5) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}}$$

$$(1,3,5/1) \xrightarrow[\downarrow k_d]{k} (1,3,6) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,3,6/1) \xrightarrow[\downarrow k_d]{k} (1,4,6) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}}$$

$$(1,4,6/1) \xrightarrow[\downarrow k_d]{k} (2,4,6) \xrightarrow[\downarrow k_d]{k_f} (2,4,o) + A_6 \ ;$$

$$A_6 \xrightarrow{k_p} P_6 \quad .$$

In the terminology of control theory, this set of equations is open-loop until the compound (2,4,o) is reached at which point there is a feedback of (2,4,o) from the last of the chain reactions to close the loop.

(b) The System of Differential Equations

Let (i_1, i_2, i_3), $(i_1, i_2, i_3/1)$, A_6, P_6 now also denote the concentration of each of these species as a function of time, while r and u denote the corresponding concentrations of R and AS respectively. When kinetic analysis is applied to the system of reaction equations, we get the following system of ordinary differential equations connecting these concentrations.

$$\frac{d(o,o,o)}{dt} = g(t) - (k_o ru + k_m)\ (o,o,o)$$

$$\frac{d(1,o,o)}{dt} = k_o ru\ (o,o,o) - (k_1 u + k_d)\ (1,o,o) + k_{-1}\ (1,o,o/1)$$

$$\frac{d(1,o,o/1)}{dt} = k_1 u\ (1,o,o) - (k_{-1} + k + k_d)\ (1,o,o/1)$$

$$\frac{d(2,o,o)}{dt} = k\ (1,o,o/1) - (k_1 u + k_d)\ (2,o,o) + k_{-1}\ (2,o,o/1)$$

$$\frac{d(2,o,o/1)}{dt} = k_1 u\ (2,o,o) - (k_{-1} + k + k_d)\ (2,o,o/1)$$

$$\frac{d(o,3,o)}{dt} = k\ (2,o,o/1) - (k_o ru + k_d)\ (o,3,o)$$

$$\frac{d(1,3,o)}{dt} = k_o ru\ (o,3,o) - (k_1 u + k_d)\ (1,3,o) + k_{-1}\ (1,3,o/1)$$

$$\frac{d(1,3,o/1)}{dt} = k_1 u\ (1,3,o) - (k_{-1} + k + k_d)\ (1,3,o/1)$$

$$\begin{cases} \dfrac{d(1,4,o)}{dt} = k\,(1,3,o/1) - (k_1 u + k_d)\,(1,4,o) + k_{-1}\,(1,4,o/1) \\ \dfrac{d(1,4,o/1)}{dt} = k_1 u\,(1,4,o) - (k_{-1} + k + k_d)\,(1,4,o/1) \end{cases}$$

$$\frac{d(2,4,o)}{dt} = k\,(1,4,o/1) - (k_1 u + k_d)\,(2,4,o) + k_{-1}\,(2,4,o/1) + k_f\,(2,4,6)$$

$$\frac{d(2,4,o/1)}{dt} = k_1 u\,(2,4,o) - (k_{-1} + k + k_d)\,(2,4,o/1)$$

$$\frac{d(2,o,5)}{dt} = k\,(2,4,o/1) - (k_1 u + k_d)\,(2,o,5) + k_{-1}\,(2,o,5/1)$$

$$\frac{d(2,o,5/1)}{dt} = k_1 u\,(2,o,5) - (k_{-1} + k + k_d)\,(2,o,5/1)$$

$$\frac{d(o,3,5)}{dt} = k\,(2,o,5/1) - (k_o ru + k_d)\,(o,3,5)$$

$$\begin{cases} \dfrac{d(1,3,5)}{dt} = k_o ru\,(o,3,5) - (k_1 u + k_d)\,(1,3,5) + k_{-1}\,(1,3,5/1) \\ \dfrac{d(1,3,5/1)}{dt} = k_1 u\,(1,3,5) - k_{-1} + k + k_d)\,(1,3,5/1) \end{cases}$$

(ж)
$$\begin{cases} \ldots \end{cases}$$

$$\frac{d(2,4,6)}{dt} = k\,(1,4,6/1) - (k_f + k_d)\,(2,4,6)$$

$$\frac{dA_6}{dt} = k_f\,(2,4,6) - k_p\,A_6$$

$$\frac{dP_6}{dt} = k_p\,A_6 \quad .$$

Here the notation $(\ast)\{\ldots$ indicates that the preceding two equations are iterated for the next two pairs of species (1, 3, 6), (1, 3, 6/1) and (1, 4, 6), (1, 4, 6/1).

(c) The Transform Solution

As already indicated, the system of differential equations developed in the preceding paragraph is non-linear if r and u vary with time, and no exact solution for them is apparent. We shall linearize them by making the assumption that r and u are each constant. Physically, this will be justified if the reservoir of free ribosome and aminoacid is large compared to the concentration of the other species so that the depletion of these substances during the reaction process is negligible.

When r and u are constant, we have a non-homogeneous system of linear differential equation with constant coefficients. These will be handled by the Laplace transform technique under the assumption that, initially, all species have zero concentration except free messenger RNA, (o,o,o). Let its initial value be m_o. We use s for the transform variable and denote the transforms of $g(t)$, (i_1,i_2,i_3), $(i_1,i_2,i_3/1)$, A_6, and P_6 by $G(s)$, $[i_1,i_2,i_3]$, $[i_1,i_2,i_3/1]$, $\overline{A}_6$ and $\overline{P}_6$ respectively. Then, after some simplification, the first few equations in the transformed set read as follows:

$$(s + k_o ru + k_m)\,[o,o,o] = G(s) + m_o$$

$$A:\begin{cases} (s + k_1 u + k_d)\,[1,o,o] - k_{-1}\,[1,o,o/1] = k_o ru\,[o,o,o] \\ -k_1 u\,[1,o,o] + (s + k_{-1} + k_d)\,[1,o,o/1] = o \end{cases}$$

$$B:\begin{cases} (s + k_1 u + k_d)\,[2,o,o] - k_{-1}\,[2,o,o/1] = k\,[1,o,o/1] \\ -k_1 u\,[2,o,o] + (s + k_{-1} + k + k_d)\,[2,o,o/1] = o \end{cases}$$

$$C:\quad (s + k_o ru + k_d)\,[o,3,o] = k\,[2,o,o/1]$$

The pairs of equations A and B and the equation C occur again but with different species; and we can abreviate the remainder of the set of equations in easily understandable form as:

$$A\{[1,3,o],\ [1,3,o/1],\ [o,3,o]\}$$

$$B\{[1,4,o],\ [1,4,o/1],\ [1,3,o/1]\}$$

$$\begin{cases} (s + k_1 u + k_d)\,[2,4,o] = k\,[1,4,o/1] + k_{-1}\,[2,4,o/1] + k_f\,[2,4,6] \\ (s + k_{-1} + k + k_d)\,[2,4,o/1] = k_1 u\,[2,4,o] \end{cases}$$

$$B\{[2,o,5],\ [2,o,5/1],\ [2,4,o/1]\}$$

$$C\{[o,3,5],\ [2,o,5/1]\}$$

$$A\{[1,3,5],\ [1,3,5/1],\ [o,3,5]\}$$

$$B\{[1,3,6],\ [1,3,6/1],\ [1,3,5/1]\}$$

$$B\{[1,4,6],\ [1,4,6/1],\ [1,3,6/1]\}$$

$$(s + k_f + k_d)\,[2,4,6] = k\,[1,4,6/1]$$

$$(s + k_p)\,\overline{A}_6 = k_f\,[2,4,6]$$

$$s\overline{P} = k_p\,\overline{A}_6$$

We shall now set up simple recursion relationships between transforms of adjacent species. This will enable us to determine [1,4,o/1] in terms of [o,o,o] and [2,4,o/1] in terms of [2,4,6]. Then equations (11) will determine [2,4,6] in terms of [o,o,o]. This result, used in the next to the last equation above will yield $\overline{I}_6$; and then $\overline{P}_6$ is given by the last equation.

Equation pairs A and B are of the form

$$(s + k_1 u + k_d)\, X - k_{-1} Y = aZ$$

$$-k_1 u\, X + (s + k_{-1} + k + k_d)\, Y = o \quad ,$$

where X, Y and Z are concentrations of certain consecutive species and $a = k_o ru$ or k respectively in cases A or B. Solving this pair of equations for X and Y in terms of Z, we find

$$(12) \qquad \begin{cases} X = \dfrac{an(s)}{f(s)}\, Z \ , \\ Y = \dfrac{ak_1 u\, Z}{f(s)} \ , \end{cases}$$

where we have used the abreviations

$$f(s) = (s + k_{-1} + k + k_d)\,(s + k_1 u + k_d) - k_1 k_{-1} u \quad ,$$

$$n(s) = (s + k_{-1} + k + k_d).$$

The single equations like C are, of course, immediately convertible to a simple recursion relation of this kind.

We start by writing down the recursion relation for [1,4,o/1] and in succession the recursion relations for the species appearing in the right members of these relations using equations (12). With the notation $m(s) = s + k_o ru + k_d$, we get

$$[1,4,o/1] = k\, k_1 u\, [1,3,o/1] \,/\, f(s)$$

$$[1,3,o/1] = k_o k_1 ru^2\, [o,3,o] \,/\, f(s)$$

$$[o,3,o] = k\, [2,o,o/1] \,/\, m(s)$$

$$[2,o,o/1] = k\, k_1 u\, [1,o,o/1] \,/\, f(s)$$

$$[1,o,o/1] = k_o k_1 ru^2\, [o,o,o] \,/\, f(s)$$

$$[o,o,o] = (G(s) + m_o) \,/\, (s + k_o ru + k_m) \,.$$

Eliminating intermediate species from these equations we obtain

$$(13) \qquad [1,4,o/1] = \frac{k_o^2\, k^2\, k_1^4\, r^2\, u^6 [G(s) + m_o]}{(s + k_o\, ru + k_m)\, m(s)\, [f(s)]^4} \,.$$

Similarly, writing down the recursion relation for [2,4,6] and working backwards we get the following sequence of equations

$$[2,4,6] = \frac{k[1,4,6/1]}{(s+k_f+k_d)}$$

$$[1,4,6/1] = k\, k_1 u\, [1,3,6/1] \,/\, f(s)$$

$$[1,3,6/1] = k\, k_1 u\, [1,3,5/1] \,/\, f(s)$$

$$[1,3,5/1] = k_o k_1 ru^2\, [o,3,5] \,/\, f(s)$$

$$[o,3,5] = k[2,o,5/1] \,/\, m(s)$$

$$[2,o,5/1] = k\, k_1 u[2,4,o/1] \,/\, f(s) \,.$$

Eliminating, we find

$$(14) \qquad [2,4,6] = \frac{k_o k^5 k_1^4 \, r \, u^5 \, [2,4,o/1]}{(s+k_f+k_d)\, m(s)\, [f(s)]^4} \,.$$

In the pair of equations (11), use the second equation to eliminate [2,4,o] in the first, and then replacing [1,4,o/1] by its value from eq. (13) and [2,4,o/1] from its value in eq. (14), solve for [2,4,6]. The result is

$$[2,4,6] = \frac{r^3 k_o^3 k^9 k_1^9 u^{12} \, [G(s) + m_o]}{(s+k_o ru+k_m)\, m(s)\, h(s)\, [f(s)]^4} \,,$$

where

$$h(s) = (s+k_f+k_d)\, m(s)\, [f(s)]^5 - r\, k_f k_o k_1^5 k^5 u^6 \,.$$

Finally, from the last two transform equations of the original set, we have

$$(15) \quad \begin{cases} \overline{A}_6 = \dfrac{k_f r^3 k_o^3 k^9 k_1^9 \, u^{12} \, [G(s) + m_o]}{(s + k_p)\,(s + k_o ru + k_m)\, m(s)\, h(s)\, [f(s)]^4} \,, \\ \overline{P}_6 = \dfrac{k_p}{s} \cdot \overline{A}_6 \,. \end{cases}$$

In the appendix, we carry out this same line of reasoning for the general case of q ribosomes. Here $pq = N$, the total number of aminoacids in the protein chain. The result is

$$\overline{A}_N = \frac{k_f \, (k_o ru)^q \, (k\, k_1 u)^{\frac{(q+1)N}{2} - 3} \, [G(s) + m_o]}{(s+k_p)\,(s+k_o ru+k_m)\, h(s)\, [m(s)]^{q-2} [f(s)]^{\frac{(q-1)N}{2} - 2}} \,,$$

$$\overline{P}_N = k_p \, \overline{A}_6 / s \,,$$

where

$$h(s) = (s+k_d+k_f)\,(s+k_o ru+k_d)\, [f(s)]^{N-1} - r k_o k_f \, (k k_1)^{N-1} u^N \,.$$

(d) The Time Domain Solution

Equation (15) must be inverted to obtain the solution for the protein concentration in the time domain. Before proceeding to carry out this inversion, we note that the feedback system for which eq. (15) is the system function, is semi-stable in the sense that the zeros of the denominator of $\overline{P}_6$, aside from s = o, are all in the left half-plane. This follows first for both zeros of f(s) (which are in fact negative real), since f(s) is of the form $f(s) = s^2 + (a+b)s + ab-K$ with a, b, K all > o, where f(o) = ab-K may easily be shown to be positive for $k_d > o$. Next, by applying the Nyquist criterion, a similar conclusion is obtained for h(s). For, writing

$$h(s) = (s+k_f+k_d)\ (s+k_o ru+k_d)\ [f(s)]^5 \left\{1 - \frac{c}{h_1(s)}\right\} ,$$

where $h_1(s) = (s+k_f+k_d)\ (s+k_o ru+k_d)\ [f(s)]^5$ and $c = rk_f k_o k_1{}^5 u^6$, we easily calculate that c/h(o) = 1 when k_d = o and therefore c/h(o) < 1 when $k_d > o$. Since the zeros of $h_1(s)$ are all negative real, $\left|c/h_1(i\omega)\right|$ is a monotonic decreasing function for $o \leq \omega < \infty$, which approaches zero as $\omega \longrightarrow \infty$. Thus the point + 1 can never be encircled by the map of $c/h_1(s)$ along the Nyquist contour.

We now return to the consideration of the inverse of eq. (15). The situation we shall consider here is the case where the initial concentration of messenger RNA is zero, i.e. m_o = o. We shall discuss the synthesis of protein associated with a buildup in messenger RNA concentration. An example of the present model is provided by enzyme induction where initially the messenger RNA associated with the induced enzyme is absent and subsequent buildup in the messenger occurs with the addition of inducer.

If $m_o = o$ then, as is well known, the general time-domain solution for $P_6(t)$ may be written out in the form of a convolution integral in terms of the impulse response, i.e. the solution when $G(s) \equiv 1$ or $g(t) = \delta(t)$ where $\delta(t)$ is the Dirac delta function. If $P_o(t)$ is the impulse response we have, in general,

$$(16) \qquad P_6(t) \equiv \int_o^t P_o(\tau) g(t-\tau)\,d\tau \equiv \int_o^t P_o(t-\tau) g(\tau)\,d\tau \quad .$$

The form for $P_o(t)$ may be determined easily by inverting eq. (15), taking $G(s) \equiv 1$, $m_o = o$. Let $-\rho_1$ and $-\rho_2$ be the zeros of $f(s)$, and $-\gamma_1, -\gamma_2, \ldots, -\gamma_{12}$ the zeros of $h(s)$, and suppose, for simplicity, that these are all distinct and different from the remaining three zeros of the denominator of $\overline{P}_6$, which we also assume to be distinct. Then we may write

$$(17) \qquad P_o(t) = A_o + A_1 e^{-k_p t} + A_2 e^{-(k_o ru + k_m)t} + A_3 e^{-(k_o ru + k_d)t}$$

$$+ e^{-\rho_1 t} \sum_{i=o}^{3} B_i t^i + e^{-\rho_2 t} \sum_{i=o}^{3} C_i t^i + \sum_{i=1}^{12} D_i e^{-\gamma_i t} \quad .$$

Here the coefficients A_i, B_i, C_i, D_i, may be calculated by expanding $\overline{P}_6$ with $G(s) \equiv 1$, $m_o = o$, into partial fractions and inverting termwise, (or alternatively by means of residues). As the reaction constants are not yet known numerically, this procedure will yield literal expressions for the coefficients. We do not carry out this development explicitly except for A_o which represents the steady-state term of the impulse response. Since A_o is the residue of $\overline{P}_o(s)$ at $s = o$, we find from eq. (15) that

$$(18) \qquad A_o = \frac{k_f r^3 k_o^3 k^9 k_1^9 u^{12}}{(k_o ru + k_m)(k_o ru + k_d)[f(o)]^4 h(o)} \quad .$$

where

(19) $$f(o) = (k_{-1}+k+k_d)\,(k_1 u+k_d) - k_1 k_{-1} u\ ,$$

$$h(o) = (k_f+k_d)\,(k_o ru+k_d)\,[f(o)]^5 - rk_o h_f k_1^5 k^5 u^6 .$$

Equation (16) is now specialized for two cases which are considered to be of prime interest for the theory: g(t) a step function, and g(t) a ramp pulse.

Case (i) $g(t) = au(t)$ where u(t) is the unit step function. Here eq. (16) gives

$$P_6(t) = a\int_o^t P_o(\tau)\,d\tau\ .$$

Carrying through the integration, we obtain

(20) $$P_6(t)/a = A_o t + E - \frac{A_1}{k_p} e^{-k_p t} - \frac{A_2}{k_o ru+k_m} e^{-(k_o ru+k_m)t}$$

$$- \frac{A_3}{k_o ru+k_d} e^{-(k_o ru+k_d)t} + e^{-\rho_1 t}\sum_{i=o}^{3} B_i' t^i + e^{-\rho_2 t}\sum_{i=o}^{3} C_i' t^i$$

$$- \sum_{i=1}^{12} \frac{D_i}{\gamma_i} e^{-\gamma_i t}\ .$$

Here the A_i and D_i are as in eq. (17). The B_i' may be found from the B_i by means of the recursion formulas

$$-\rho_1 B_3' = B_3\ ,$$

$$-\rho_1 B_i' + (i+1)B_{i+1}' = B_i \quad (i = o, 1, 2)\ .$$

Similar formulas relate C_i' and C_i with ρ_2 replacing ρ_1.

Finally, E may be expressed in terms of the coefficients of eq. (17) or may be found directly by means of the relation

$$E = \frac{d}{ds}\left\{\frac{k_f k_p k_c^3 k^7 k_1^9 r^3 u^{12}}{(s+k_p)(s+k_o ru+k_m)\, m(s)h(s)[f(s)]^4}\right\}_{s=o} . \tag{21}$$

While the steady state behavior of this solution is evident from eq. (20), any discussion of the transient behavior will require a more complete and accurate specification of the constants than is presently available. It is conjectured that all except the first few exponential terms will be negligible thus leading to an essentially monotonic increasing behavior of the output. For very small values of time, the initial value theorem leads to the representation

$$p_6(t)/a = k_f k_p k_o^3 k^7 k_1^9 r^3 u^{12} t^{24} + \text{higher powers of } t .$$

Case ii

$$g(t) = at \quad o \le t \le \alpha, \; a > o ,$$

$$= o, \; t > \alpha .$$

In this case, eq. (16) becomes

$$P_6(t) = a \int_o^t \tau P_o(t-\tau)\, d\tau , \; o \le t \le \alpha ,$$

$$= a \int_o^{\alpha} \tau P_o(t-\tau)\, d\tau , \; t \ge \alpha .$$

Let $p_o(t) = \int_o^t P_o(\tau) d\tau$ so that $p_o(t)$ is given explicitly by eq. (20). Then, using integration by parts, we have for $o \le t \le \alpha$,

$$P_6(t)/a = \int_o^t (t - \tau)\, P_o(\tau)\, d\tau = \int_o^t p_o(\tau)\, d\tau \quad .$$

Similarly, for $t \geq \alpha$,

$$P_6(t)/a = \int_{t-\alpha}^t (t - \tau)\, P_o(\tau) d\tau = -\alpha p_o(t - \alpha) + \int_{t-\alpha}^t p_o(\tau) d\tau .$$

The integrals in the right members of these equations may be evaluated using eq.(20), and thus we arrive at the representations

$$P_6(t)/a = A_o \frac{t^2}{2} + Et + F + \frac{A_1}{(k_p)^2} e^{-k_p t} + \frac{A_2}{(k_o ru + k_m)^2} e^{-(k_o ru + k_m)t}$$

$$+ \frac{A_3}{(k_o ru + k_d)^2} e^{-(k_o ru + k_d)t} + e^{-\rho_1 t^3} \sum_{i=o}^{3} B_i'' t^i + e^{-\rho_2 t} \sum_{i=o}^{3} C_i'' t^i$$

$$+ \sum_{i=1}^{12} \frac{D_i}{(\gamma_i)^2} e^{-\gamma_i t} \quad , \quad (o \leq t \leq \alpha) \; ;$$

$$P_6(t)/a = \frac{A_o \alpha^2}{2} + \frac{A_1}{(k_p)^2} [e^{\alpha k_p}(\alpha k_p - 1) + 1] e^{-k_p t} + \frac{A_2}{\beta_1^2} [e^{\alpha \beta_1}(\alpha \beta_1 - 1) + 1] e^{-\beta_1 t}$$

$$+ \frac{A_3}{\beta_2^2} [e^{\alpha \beta_2}(\alpha \beta_2 - 1) + 1] e^{-\beta_2 t} + e^{-\rho_1 t} \sum_{i=o}^{3} B_i''' t^i + e^{-\rho_2 t} \sum_{i=o}^{3} C_i''' t^i$$

$$+ \sum_{i=1}^{12} \frac{D_i}{(\alpha_i)^2} [e^{\alpha \gamma_i}(\alpha \gamma_i - 1) + 1] e^{-\gamma_i t} \quad , \quad t \geq \alpha .$$

Here we have written $\beta_1 = k_o ru + k_m$, $\beta_2 = k_o ru + k_d$. The new coefficients F, B_i'', C_i'', B''', C''' can be expressed in terms of the previous ones but we do not carry this out here.

In closing this discussion of cases (i) and (ii) we remark that for the case of q ribosomes considered in the appendix, the form of the steady state solution is the same as that obtained above. However, the constants A_o and E which figure in the solutions are now defined as follows:

$$A_o = \frac{k_f (k_o ru)^q (k\, k_1 u)^{\frac{(q+1)N}{2} - 3}}{(k_o ru + k_m)(k_o ru + k_d)^{q-2} [f(o)]^{\frac{(q-1)N}{2} - 2} h(o)} \quad ,$$

where $f(o)$ is given by eq. (19) and

$$h(o) = (k_d + k_f)(k_o ru + k_d)[f(o)]^{N-1} - rk_o k_f (k\, k_1)^{N-1} u^N \; ;$$

$$E = \frac{d}{ds}\left[\frac{k_f k_p (k_o ru)^q (k\, k_1 u)^{\frac{(q+1)N}{2} - 3}}{(s + k_o ru + k_m)(s + k_o ru + k_d)^{(q-2)} [f(s)]^{\frac{(q-1)N}{2} - 2} h(s)}\right] s = o \quad ,$$

where

$$h(s) = (s + k_d + k_f)(s + k_o ru + k_d)[f(s)]^{N-1} - rk_o k_f (k\, k_1)^{N-1} u^N \; .$$

3. SUMMARY AND DISCUSSION

A detailed model of protein synthesis by polyribosomes has been developed and the resulting equations solved on the assumption that the kinetics of the system depends linearly on messenger RNA concentration. Specifically, the assumption is made that such components as the amino acid-adapter RNA and ribosome concentrates are constant. However, the messenger RNA input is taken to be time dependent and the analysis is given for both step and ramp inputs.

It is interesting to compare the above results with the available experimental data. Pardee and Prestige have made some very careful studies of the kinetics of enzyme induction in E. coli [10]. These workers have shown that with each induced enzyme investigated a lag of about 3 minutes occurred, at 37°C between the addition of inducer and the appearance of the enzyme. It was also shown that the lag is not associated with penetration of the inducer but rather with the synthesis of messenger RNA. It is significant that once started the initial buildup in enzyme concentration consists of a nonlinear portion followed by a linear increase in concentration vs. time, the general form of the enzyme buildup is shown in figure 2. In terms of the present analysis the nonlinear phase corresponds to case (ii) above when the concentration of messenger RNA is increasing over the interval $\beta \leq t \leq \alpha$, where β is the time of the induction period. The solution in this case is given by (22). If the transient terms are such that the

exponentials damp out within a minute or so at some time following the induction period then subsequent steady state increase will be a quadratic polynomial of the form

$$P_6(t) = \frac{A_o}{2} t^2 + Eat + Fa \quad , \qquad \beta \leq t \leq \alpha \ .$$

When the messenger RNA concentration reaches the steady value due to the balance between synthesis and degradation then the solution corresponding to case (i) above applies and we have a linear dependency given by

$$P_6(t) = A_o at + Ea \quad , \qquad t > \alpha \ .$$

Unfortunately, it is evident from (18) and (21) that no simple relationship is apparent between A and E and the basic kinetic constants. However, it is interesting to note that other things being equal the slope of the linear portion is proportional to the rate of messenger RNA production:

$$\frac{dP_6(t)}{dt} = A_o a \quad ,$$

It is interesting to note that experimental studies [10] indicate a dependency of the role of enzyme synthesis on inducer concentration and hence presumably on the rate of messenger RNA synthesis. The reported dependency on inducer was, however, not linear. The exact significance of the latter is not clear since the relationship between inducer and RNA synthesis is not established.

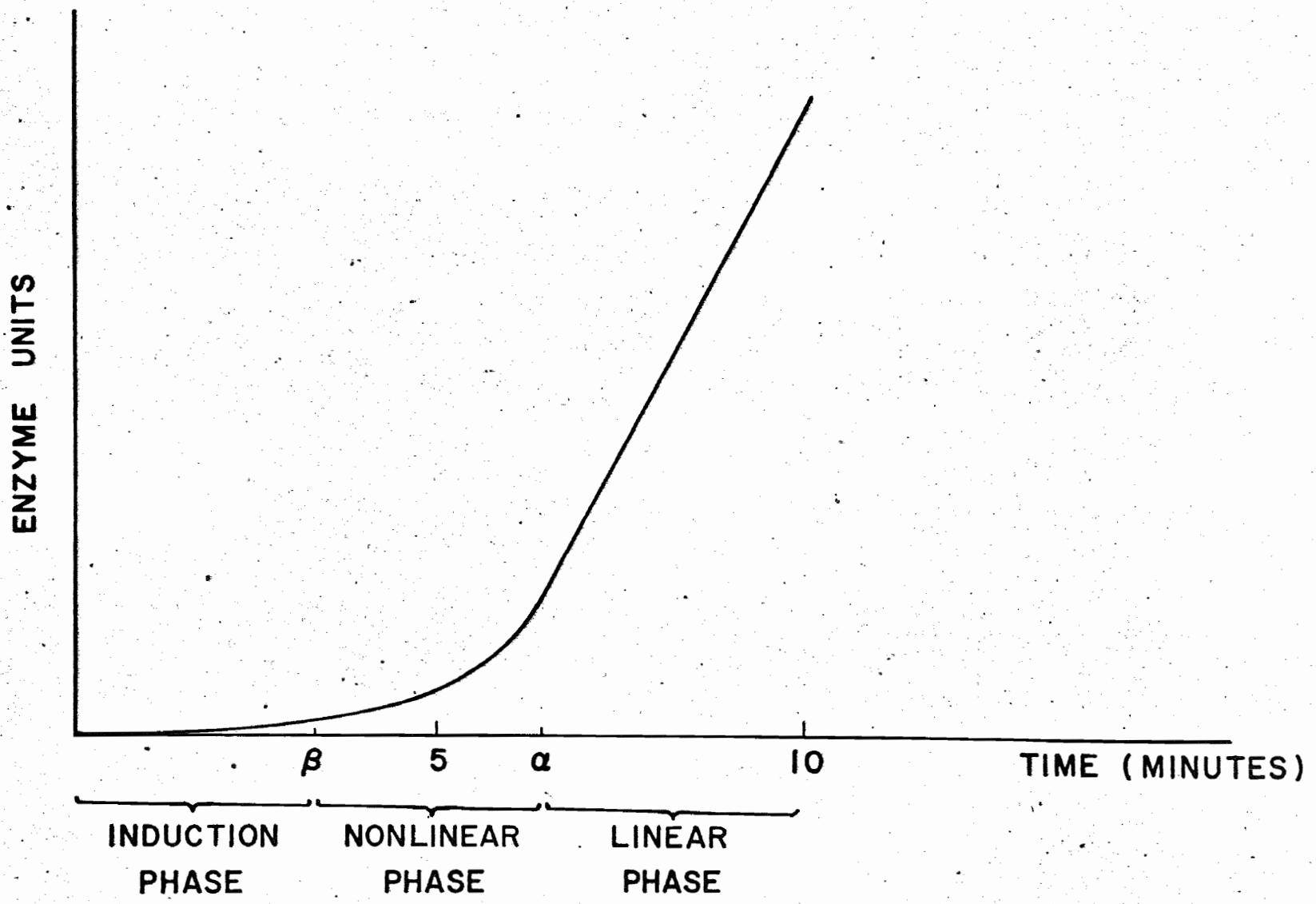
ENZYME UNITS
β
5
α
10
TIME (MINUTES)
INDUCTION PHASE
NONLINEAR PHASE
LINEAR PHASE

FIG. 2

Appendix

In this section, we consider the general case of q ribosomes, each having a "coverage" of p amino acids on the messenger RNA. Use will be made of the notions and notations developed in Section for the special case $p = 2$, $q = 3$. Our species are now designated by q vectors, $(i_1, i_2 \ldots, i_q)$ where each i_k may have the value zero, and otherwise $(n - 1)\, p + 1 \leq i_n \leq n_p$, $(n = 1, 2, \ldots, q)$. The complex formed after absorption of an AS molecule will be denoted by $(i_1, i_2 \ldots, i_q/1)$. The proteins in initial and native form are represented by A_N and P_N respectively. All other notation is the same as given above.

(a) The Reaction Equations

As already pointed out in Section 2 , there are essentially three basic reactions involved in the process. Two of them, the absorption and the polymerization of an amino acid molecule by each ribosome in turn, are repeated over and over until there is space available on the messenger RNA for the addition of a new ribosome; the latter process constitutes the third basic reaction. In the following abbreviated set of reactions, the missing equations can be supplied by following along with this basic sequence of operations.

We have

$$\underline{g(t)} \rightarrow (o,o,\underset{\downarrow k_m}{\ldots},o) \xrightarrow{k_o,R,AS} (1,o,\underset{\downarrow k_d}{\ldots},o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,o,\underset{\downarrow k_d}{\ldots},o/1) \xrightarrow{k}$$

$$(2,o,\underset{\downarrow k_d}{\ldots},o) \rightleftharpoons \cdots \xrightarrow{k} (p,o,\underset{\downarrow k_d}{\ldots},o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (p,o,\underset{\downarrow k_d}{\ldots},o/1) \xrightarrow{k} (o,p+1,\underset{\downarrow k_d}{o},\ldots,o) \xrightarrow{k_o,R,AS}$$

$$(1,p+1,o,\underset{\downarrow k_d}{\ldots},o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (1,p+1,o,\underset{\downarrow k_d}{\ldots},o/1) \xrightarrow{k} (1,p+2,o,\underset{\downarrow k_d}{\ldots}o) \rightleftharpoons \cdots \xrightarrow{k}$$

$$(p,2p,o,\underset{\downarrow k_d}{\ldots},o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}} (p,2p,o,\underset{\downarrow k_d}{\ldots},o/1) \xrightarrow{k} (p,o,2p+1,o,\underset{\downarrow k_d}{\ldots},o) \underset{k_{-1}}{\overset{k_1,AS}{\rightleftharpoons}}$$

$$(p,o,2p+1,o,\underset{\downarrow k_d}{\ldots}o/1) \xrightarrow{k} (o,p+1,2p+1,o,\underset{\downarrow k_d}{\ldots},o) \xrightarrow{k_o,R,AS}$$

$$(1,p+1,2p+1,o,\ldots,o) \rightleftharpoons \cdots (p,2p,\underset{\downarrow k_d}{\ldots},qp) \xrightarrow{k_f} A_N + (p,2p,3p,\ldots,(q-1)p,o) \; ;$$

$$A_N \xrightarrow{k_p} P_N \; .$$

(b) The System of Differential Equations

Setting up the reaction kinetics corresponding to the reaction equations, and using a notation corresponding to that of Section we arrive at the following system of differential equations:

$$\frac{d(o,o,\ldots,o)}{dt} = g(t) - (k_o ru + k_m)\ (o,o,\ldots,o)$$

$$\frac{d(1,o,\ldots,o)}{dt} = k_o ru\ (o,o,\ldots,o) - (k_1 u + k_d)\ (1,o,\ldots,o) + k_{-1}(1,o,\ldots,o/1)$$

$$\frac{d(1,o,\ldots,o/1)}{dt} = k_1 u(1,o,\ldots,o) - (k_{-1} + k + k_d)\ (1,o,\ldots,o/1)$$

$$\frac{d(2,o,\ldots,o)}{dt} = k\ (1,o,\ldots,o/1) - (k_1 u + k_d)\ (2,o,\ldots,o) + k_{-1}(2,o,\ldots,o/1)$$

. . . . . .

$$\frac{d(p,o,\ldots,o)}{dt} = k(p-1,o,\ldots,o/1) - (k_1 u + k_d)\ (p,o,\ldots,o) + k_{-1}(p,o,\ldots,o/1)$$

$$\frac{d(o,p+1,o,\ldots,o)}{dt} = k\ (p,o,\ldots,o/1) - (k_o ru + k_d)\ (o,p+1,\ldots,o)$$

$$\frac{d(1,p+1,o,\ldots,o)}{dt} = k_o ru(o,p+1,o,\ldots,o) - (k_1 u + k_d)\ (1,p+1,o,\ldots,o)$$

$$+ k_{-1}\ (1,p+1,o,\ldots,o)$$

$$\frac{d(1,p+1,o,\ldots,o/1)}{dt} = k_1 u(1,p+1,o,\ldots,o) - (k_{-1} + k + k_d)\ (1,p+1,o,\ldots,o/1)$$

. . . . . .

$$\frac{d(o,p+1,2p+1,o,\ldots,o)}{dt} = k\ (p,o,2p+1,o,\ldots,o/1) - (k_o ru + k_d)$$

$$(o,p+1,2p+1,o,\ldots,o)$$

$$\frac{d(1,p+1,2p+1,o,\ldots,o)}{dt} = k_o ru(o,p+1,2p+1,o,\ldots,o/1) - (k_1 u + k_d)$$

$$(1,p+1,2p+1,o,\ldots,o) + k_{-1}(1,p+1,2p+1,o,\ldots,o/1)$$

. . . . . .

$$(23)\begin{cases} \dfrac{d(p,2p,\ldots,(q-1)p,o)}{dt} = k(p-1,2p,\ldots,(q-1)p,o/1)+k_f(p,2p,\ldots,qp) \\ \qquad -(k_1u+k_d)\ (p,2p,\ldots,(q-1)\ p,o/1) \\ \dfrac{d(p,2p,\ldots,(q-1)\ p,o/1)}{dt} = k_1u(p,2p,\ldots,(q-1)\ p,o)-(k_{-1}+k+k_d) \\ \qquad [p,2p,\ldots,(q-1)\ p,o/1] \end{cases}$$

. . . . . .

$$\frac{d(p,2p,\ldots,qp)}{dt} = k(p-1,2p,\ldots,qp/1) - (k_d+k_f)\ (p,2p,\ldots,qp)\ ;$$

$$\frac{dA_N}{dt} = k_f\ (p,2p,\ldots,qp) - k_p\ A_N\ ;$$

$$\frac{dP_N}{dt} = k_p\ A_N\ .$$

(c) The Transform Solution

We employ the notation of Section 2 for Laplace transforms. As in that section, we assume r and u to be constant and all initial conditions to be zero except that for the free messenger RNA which is taken to be m_o .

Taking the Laplace transform in each of the differential equations of the system above and collecting like terms, we find that the set of transform equations may be characterized as follows. Aside from the first equation, the equations corresponding to (23), and the last three equations, the system is made up by repetition of certain pairs of equations and certain single equations. The single equations are of the form

$$(s+k_oru+k_d)\ [o,p+1,2p+1,\ldots,mp+1,o,\ldots,o]=k[p,o,2p+1,\ldots,mp+1,o,\ldots,o/1]\ ,$$

where $m = 1,2,\ldots,q-1$, and for $m = 1$ the species appearing in the right member of this equation is to be interpreted as $[p,o,o,\ldots,o/1]$. Thus there are altogether $q-1$ of these single equations. The pairs of equations are of two types, both of which may be encompassed in the single form

$$\begin{cases} (s + k_1 u + k_d)\, X - k_{-1}\, Y = aZ \\ -k_1 u\, X + (s + k_{-1} + k + k_d)\, Y = o \end{cases}$$

Here $a = k_o ru$ when $X = [1,\ p+1,\ 2p+1,\ldots,mp+1,\ o,\ldots,o]$

$Y = [1,\ p+1,\ 2p+1,\ldots,mp+1,\ o,\ldots,o/1]$, $Z = [o,\ p+1,\ 2p+1,\ldots,$

$mp+1,o,\ldots,o]$,

where $m = o,\ 1,\ldots,q-1$.

And $a = k$ for all cases other than those specified up to this point. When $a = k$, then $X = [i_1,\ i_2,\ldots,i_q]$, $Y = [i_1,\ i_2,\ldots,i_q/1]$ and Z is the species immediately preceding X in the process. If we denote by A and B the two pairs of equations when $a = k_o ru$ and $a = k$ respectively, and by C the single equation, then it may be verified that the system of transform equations can be written out symbolically in the following fashion:

$$(s + k_o ru + k_m)\,[o,o,\ldots,o] = G(s) + m_o\ ,$$

$$A,\ \underbrace{B,\ B\ldots,}_{p-1\text{ times}}\ ,\quad C,\ A,\ \underbrace{B,\ B,\ldots,}_{2p-1\text{ times}}\ ,\quad C,\ A,\ \underbrace{B,\ B,\ldots,}_{3p-1\text{ times}}\ldots,$$

$$
\left\{
\begin{aligned}
(s+k_1u+k_d)\,[p,2p,\ldots,(q-1)p,o] &= k[p-1,2p,3p,\ldots,(q-1)p,o/1] \\
&\quad +k_{-1}[p,2p,\ldots,(q-1)p,o/1] \\
&\quad +k_f[p,2p,\ldots,qp] \\
(s+k_{-1}+k+k_d)\,[p,2p,\ldots,(q-1)p,o/1] &= k_1u[p,2p,\ldots,(q-1)p,o]\ ,
\end{aligned}
\right.
$$

B, ...,

$$(s + k_d + k_f)\,[p,2p,\ldots,qp] = k[p-1,2p,\ldots,qp/1]\ ,$$

$$(s + k_p)\,\overline{A}_N = k_f\,[p,2p,\ldots,qp]\ ,$$

$$s\overline{P}_N = k_p\,\overline{A}_N\ .$$

The single equations in this set can immediately be interpreted as simple recursion relations between transforms of consecutive species. Recursion relations of similar simple form are obtained by solving the equation pairs A and B for X and Y in terms of Z. We find

$$X = \frac{a\,(s + k_{-1} + k + k_d)}{f(s)}\ .\ Z\ ,\quad Y = \frac{ak_1u}{f(s)}\ .\ Z\ ,$$

where f(s), as in Section 2 , is given by

$$f(s) = (s + k_{-1} + k + k_d)\,(s + k_1u + k_d) - k_1k_{-1}u.$$

It will turn out that in our subsequent development we require only the second of these relations, i.e. the one between Y and Z.

We now sketch the remainder of the argument leaving the verification of details to the reader. It is evident from the set of transform equations that a knowledge of [p, 2p,...,qp] will determine $\overline{A}_N$ and then $\overline{P}_N$. Now [p, 2p,...,qp] occurs in the feedback equation,

which is the first of the equation pair (24,. We can use the second equation in (24) to eliminate [p, 2p,...,(q-1)p,o] from the feedback equation and this yields

5) $$\frac{f(s)}{k_1 u}\,[p,2p,\ldots,(q-1)p,o/1]=k[p-1,2p,3p,\ldots,(q-1)p,o/1] + k_f[p,2p,\ldots,qp]\,.$$

But [p,2p,...,qp] also appears in the equation which is second from the last in the system of equations, where the right member involves [p-1,2p,...,qp/1]. By using our recursion relations, we can successively eliminate [p-1,2p,...,qp/1], [p-1,2p-1,3p,...,qp/1] etc. until we work our way down to [p,2p,...,(q-1)p,o/1].

The result is

6) $$[p,2p,\ldots,qp]=\frac{k_o k^{qp-1} k_1^{qp-2} r u^{qp-1}[p,2p,\ldots,(q-1)p,o/1]}{(s+k_d+k_f)\,(s+k_o r u+k_d)\,[f(s)]^{qp-2}}\,.$$

This formula can be used to eliminate [p,2p,...(q-1)p,o/1] in equation (25).

We also require [p-1, 2p, 3p,...,(q-1/p),o/1] in eq. (25) and this can be obtained once again by using the recursion relations starting from [p-1, 2p, 3p,...,(q-1)p,o/1]. Useful in this process is the relation (used for i = p, n = q-2 to start)

7) $$[i-1,p+i,2p+i,\ldots,np+i,o\ldots o/1]=\frac{(kk_1u)^{(n+1)i-n+2}[1,p+1,\ldots,np+1,o/1]}{[f(s)]^{(n+1)i-n+2}}\,,$$

which is easily proved by induction. The transform [1,p+1,...,np+1,o/1] can be expressed in terms of [o,p+1,2p+1,...,np+1,o] by using

$Y = ak_1uZ/f(s)$ with $a = k_o ru$. Next, the single equation C expresses $[o,p+1,2p+1,\ldots,np+1,o]$ in terms of $[p,o,2p+1,\ldots np+1,o,\ldots,o/1]$ and we can then use formula (27) to continue the elimination. The end result is

$$[p-1,2p,\ldots(q-1)p,o/1] = \frac{k_o^{q-1}k^{\frac{q(q-1)p}{2}-3}k_1^{\frac{q(q-1)p}{2}-2}r^{q-1}u^{\frac{q(q-1)p}{2}+q-3}.[G(s)+m.]}{(s+k_o ru+k_m)\,(s+k_o ru+k_d)^{q-2}\,[f(s)]^{\frac{q(q-1)p}{2}-2}}.$$

Finally, we use (26) and (28) in (25) and solve for $[p,2p,\ldots,qp]$ to obtain

$$[p,2p,\ldots,qp] = \frac{k_o ru)^q kk_1 u)^{\frac{(q+1)N}{2}-3}\,[G(s)+m_o]}{(s+k_o ru+k_m)\,h(s)\,(s+k_o ru+k_d)^{q-2}\,[f(s)]^{\frac{(q-1)N}{2}-2}},$$

where

$$h(s) = (s+k_d+k_f)\,(s+k_o ru+k_d)\,[f(s)]^{N-1} - rk_o k_f (kk_1)^{N-1} u^N .$$

It follows immediately that

$$\overline{A}_N = \frac{k_f\,[p,2p,\ldots,qp]}{(s+k_p)},$$

$$\overline{P}_n = \frac{k_f k_p [p,2p,\ldots,qp]}{s(s+k_p)},$$

with $[p,2p,\ldots,qp]$ as in (29).

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