Mathematical Modeling of the Effects of the Signal Recognition Particle on Translation and Translocation of Proteins Across the Endoplasmic Reticulum Membrane

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The kinetics of the signal recognition particle(SRP)-mediated process of protein translocation across the endoplasmic reticulum membrane was studied by mathematical modeling and complementary experiments. The following results were obtained.

(1) A model according to which SRP directs the ribosome, rather than the mRNA, to the membrane is supported by experiments designed to discriminate between the two alternatives.

(2) This model describes both steady-state and synchronized translation experiments and makes a number of predictions.

(3) The interaction between a nascent protein and SRP may be described by two parameters: (i) a binding constant which can be attributed to the structure of the signal peptide, and (ii) the size of the "SRP-window", i.e. the distance between the first and the last site on the polypeptide chain that can interact with SRP. For preprolactin a binding constant of 1 to $2 \cdot 5 \, \text{nmol}^{-1} \, l$ was estimated. Modeling of the synchronized synthesis of ovalbumin indicates that it has a much weaker binding constant than preprolactin ($\sim 0 \cdot 25 \, \text{nmol}^{-1} \, l$) although we cannot exclude the possibility that the SRP-window may be also smaller.

(4) A better understanding of the molecular effects of SRP on translation and translocation through the rough endoplasmic reticulum membrane has been achieved. Inhibition of the steady-state rate of translation by SRP requires a stoichiometric interaction of SRP with ribosomes carrying nascent polypeptide chains and will occur only when ribosomes are piled up back to the initiation site. Translocation, on the other hand, requires only the catalytic action of SRP and is determined by the local concentration of protein-synthesizing ribosomes accumulated at the site(s) of SRP interaction. As a consequence, translational inhibition by SRP may sometimes fail to occur, depending either on the type of protein or on experimental conditions, such as a high mRNA concentration, even if translocation can be demonstrated.

(5) A rough extrapolation to the conditions *in vivo* indicates that all synthesized polypeptide chains destined for translocation across or integration into the endoplasmic reticulum membrane are indeed quantitatively translocated and that no translational inhibition occurs.

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1. Introduction

The signal recognition particle (SRP)† is involved in the initiation of protein translocation across the RER membrane (Walter et al., 1981; Walter & Blobel, 1981a,b). In the case of amino-terminal, cleavable signal sequences, it exerts in the absence of microsomal membranes a site-specific translational arrest in a wheatgerm cell-free system. The size of the arrested nascent polypeptide fragment (~70 amino acid residues) suggests that chain elongation stops when the signal sequence has fully emerged from the ribosome (Walter & Blobel, 1981b). A direct interaction between the signal sequence and SRP has been demonstrated recently (Kurzchalia et al., 1986). The translational arrest is terminated by binding of the arrested complex by means of SRP to an SRP receptor (docking protein) present in the RER membrane (Meyer et al., 1982; Gilmore et al., 1982a; Tajima et al., 1987); concomitantly, initiation of polypeptide translocation across the membrane begins (for reviews, see Rapoport, 1986; Briggs & Gierasch, 1986; see also Fig. 1).

SRP has therefore two effects, which can be assayed separately: (1) inhibition of translation in the absence of microsomal membranes; and (2) reconstitution of the translocation competence of high salt-washed microsomes that are devoid of SRP and therefore inactive by themselves. It is still unclear how the two effects of SRP are interrelated and under what conditions they can be functionally dissociated.

It has not been decided whether SRP is required for each ribosome to become membrane-bound; an alternative model has been discussed according to which only the first ribosome on a mRNA molecule needs SRP (Briggs & Gierasch, 1986; Fig. 2). It is also not clear whether results *in vitro* can be applied directly to conditions *in vivo*.

Synchronized translation systems have been widely used for the experimental analysis of the translocation process (Walter & Blobel, 1981b; Rothman & Lodish, 1977). It would be desirable to define optimal conditions for such experiments.

The solution to these problems can be sought with profit by mathematical modeling of the translocation process, which is rather complex and can hardly be understood intuitively. Indeed, the kinetics and efficiency of protein translocation depend on a number of parameters, including the concentrations of mRNA, ribosomes, SRP and microsomal membranes, as well as on specific properties of the translocated protein, such as its length and the structure of the signal sequence. Further factors influencing the process may be the binding of SRP to monosomes and to its receptor in the absence of protein synthesis (Walter et al., 1981; Gilmore et al., 1982b). Finally, one should take into

account the fact that steady and transient states could be controlled in different ways. Mathematical modeling can help to make complex systems more transparent, to test alternative hypotheses, to suggest discriminating experiments and to extrapolate to conditions that are not easily accessible to experimentation (Heinrich et al., 1977).

2. Description of the Models

(a) General assumptions; treatment of the translation process

The models are based on the very general treatment described by MacDonald et al. (1968) and MacDonald & Gibbs (1969) and are extensions of our previous models of the translation process (Heinrich & Rapoport, 1980).

As in the previous model, ribosomes are regarded as hard bodies, occupying L codons on a mRNA and moving stepwise in an independent manner in only one direction. Dissociation of ribosomes into subunits and the role of initiation and elongation factors are neglected. Their influence is simply subsumed in a rate constant for the corresponding step.

The position j of a ribosome on the mRNA is denoted by the distance it has moved from the initiation codon. Denoting by R_j the concentration of ribosomes having their front at position j, one may write a differential equation for each codon:

$$dR_j/dt = v_{j-1} - v_j \quad j = 1, \ldots, M, \qquad (1)$$

where v_{j-1} and v_j are the fluxes for the movement of ribosomes from position $j-1$ to j and from j to $j+1$, respectively. M gives the total number of codons on the mRNA.

The fluxes may be written in the following form. For the last codons on the mRNA the following equations hold:

$$v_M = k_T R_M; \quad v_j = k_j R_j \text{ for } M - L < j < M, \qquad (2)$$

where v_M is the rate of termination, k_T is the rate constant for termination of protein synthesis and the k_j values are elongation rate constants.

The initiation step is assumed to be a bimolecular reaction between a free ribosome and a mRNA molecule having an unoccupied initiation region:

$$v_I = k_I r M_{tot} \left(1 - \sum_{s=1}^{L} (R_M/M_{tot}) \right), \qquad (3)$$

where k_I is the rate constant of initiation and r is the number of codons occupied by a ribosome. For r the following differential equation holds:

$$dr/dt = v_M - v_I. \qquad (4)$$

For the elongation steps, the fluxes may be written as follows (MacDonald et al., 1968; MacDonald & Gibbs, 1969; Heinrich & Rapoport, 1980):

$$v_j = k_j R_j P_{j+1}, \qquad (5)$$

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† Abbreviations used: SRP, signal recognition particle; RER, rough endoplasmic reticulum; SDS, sodium dodecyl sulfate.

with k_j being an elongation rate constant and P_{j+1} the conditional probability that codon $j+1$ is free given that codon j is occupied by a ribosome:

$$P_{j+1} = 1 - \left(R_{j+L} \bigg/ \left(M_{tot} - \sum_{s=1}^{L-1} R_{j+s} \right) \right)$$

$$= \left(1 - \sum_{s=1}^{L} (R_{j+s}/M_{tot}) \right) \bigg/ \left(1 - \sum_{s=1}^{L-1} (R_{j+s}/M_{tot}) \right),$$

$$\tag{6}$$

where M_{tot} is the total mRNA concentration.

R_j/M_{tot} represents the probability that a ribosome is located with its front at position j, which is equivalent to the density of nascent polypeptide chains of length j. The ribosome density at codon j will include all ribosomes that have part of their structures overlapping that codon:

$$\sum_{s=1}^{L} R_{j+s}/M_{tot}.$$

In all calculations a further simplification was made by setting all rate constants with the exception of k_I equal to each other ($k_j = k_T = k$).

(b) *Incorporation of the translocation process; the standard model*

Our present knowledge of the SRP-mediated process of protein translocation across the endoplasmic reticulum membrane is summarized in Figure 1(a) and, in a more formalized way, in Figure 1(b).

The mRNA is divided into three different regions (see Fig. 1). In the first part (up to position $N-1$), ribosomes travel along the mRNA as described above (unbound ribosomes). When a ribosome has reached position N, there is the first opportunity for an interaction of the signal peptide with SRP (S in the Figure). It is assumed that there is a region up to position $N+K$ in which SRP can be bound. Thereafter the signal peptide may be buried in a domain of the already synthesized part of the polypeptide chain or may be too far removed from the ribosome for SRP interaction. The region from position N to $N+K$ we call the "window". Once SRP is bound, the ribosome stops moving (arrested ribosome). Elongation arrest even late in translation has been demonstrated recently (Ainger & Meyer, 1986; Wiedmann *et al.*, 1987). The complex is bound to the SRP receptor (docking protein, D in the Figure) present in the microsomal membrane (docked ribosome). It is assumed that SRP-binding and the docking of the ribosome to the membrane by means of SRP are reversible steps. Reversibility of the SRP binding has been proved recently (Wiedmann *et al.*, 1987). Finally, the actual translocation complex is established by membrane-anchoring of the ribosome (bound ribosome). This step is assumed to be irreversible and coupled to an elongation step; both SRP and its receptor (docking protein) are released during this step. This assumption may be an oversimplification, since binding of the ribosome appears to take place in the
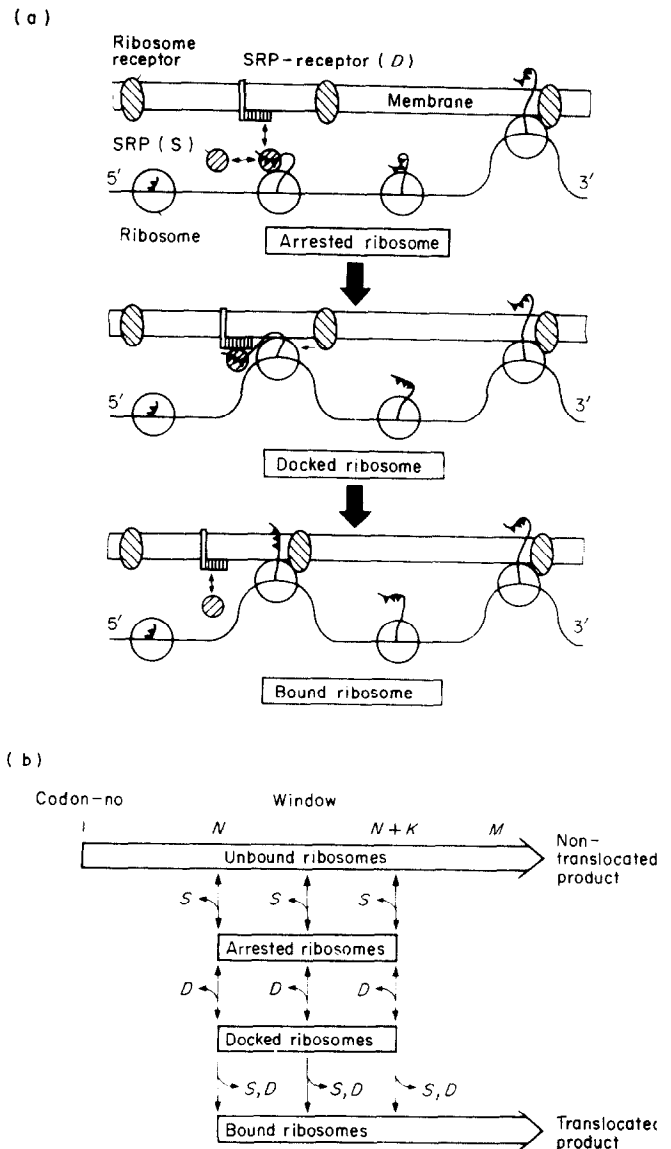

Figure 1. Schematic illustration of the SRP-mediated process of protein translocation as represented in the standard model. (a) The process of initiation of protein translocation across the RER membrane. (b) A more formalized version suitable for mathematical modeling. Ribosomes move along the mRNA from codon 1 to M. Within the window region (from codon N to $N+K$) there is the possibility for an interaction with SRP (S). Thereby the ribosomes are arrested. The ribosomes are directed to the endoplasmic reticulum membrane by interaction of SRP with its receptor (docking protein, D) yielding docked ribosomes. Finally, SRP and its receptor are released and the ribosomes become truly membrane-bound (bound ribosomes). This step, in contrast to the binding reactions, is assumed to be irreversible and coupled to an elongation step. Ribosomes are shown to be anchored to the RER membrane by presumed ribosome receptors (see (a)). Ribosomes beyond codon $N+K$ synthesize either non-translocated or translocated product. Note that a given mRNA molecule can carry both membrane bound and unbound ribosomes (a).

absence of protein synthesis (Gilmore & Blobel, 1983). However, incorporation of another irreversible reaction step would not change the main

results but merely introduce an additional (unknown) rate constant.

The third region of the mRNA (beyond position $N + K$) is characterized by the existence of two classes of ribosomes with no possibility of conversion between them: membrane-bound ribosomes synthesizing translocating protein chains and unbound ones synthesizing non-translocating protein chains. It should be noted in this scheme that a given mRNA molecule may carry both membrane-bound and unbound ribosomes (see Fig. 1(a)).

Mathematical treatment of the first and last region of the mRNA is analogous to that given before, except that for the last region two differential equations have to be written for each codon:

$$\mathrm{d}R_j^{nt}/\mathrm{d}t = kR_{j-1}^{nt}P_j - kR_j^{nt}P_{j+1}, \qquad (7)$$

$$\mathrm{d}R_j^{t}/\mathrm{d}t = kR_{j-1}^{t}P_j - kR_j^{t}P_{j+1} \qquad (8)$$

where the superscripts nt and t denote "non-translocating" and "translocating", respectively. The conditional probabilities are given by:

$$P_{j+1} = \frac{1 - \left(\sum_{s=1}^{L} (R_{j+s}^{nt} + R_{j+s}^{t})/M_{tot} \right)}{1 - \left(\sum_{s=1}^{L-1} (R_{j+s}^{nt} + R_{j+s}^{t})/M_{tot} \right)}. \qquad (9)$$

The differential equation for r has to be modified to take into account the liberation of free ribosomes from both classes:

$$\mathrm{d}r/\mathrm{d}t = -v_I + kR_M^{nt} + kR_M^{t}. \qquad (10)$$

For the window region (except for the boundary positions N and $N + K + 1$), we have four differential equations for each codon:

$$\mathrm{d}R_j^{nt}/\mathrm{d}t = kR_{j-1}^{nt}P_j - kR_j^{nt}P_{j+1} -$$
$$q_S R_j^{nt}S + q_{-S}[RS], \qquad (11)$$

$$\mathrm{d}[RS]_j/\mathrm{d}t = q_S R_j^{nt}S - q_{-S}[RS]_j -$$
$$q_D[RS]_j D + q_{-D}[RSD]_j, \qquad (12)$$

$$\mathrm{d}[RSD]_j/\mathrm{d}t = q_D[RS]_j D - q_{-D}[RSD]_j -$$
$$k[RSD]_j P_{j+1}, \qquad (13)$$

$$\mathrm{d}R_j^{t}/\mathrm{d}t = kR_{j-1}^{t}P_j - kR_j^{t}P_{j+1} + k[RSD]_{j-1}P_j. \qquad (14)$$

$[RS]_j$ gives the concentration of ribosomes at position j carrying SRP, $[RSD]_j$ gives the concentration of ribosomes docked to the SRP receptor, and S and D denote the free concentrations of SRP and its receptor, respectively. The q terms denote rate constants of the corresponding binding steps.

In order to simplify the system further, it is assumed that the binding processes are much faster than the elongation step. Indeed, an elongation step takes about a second or so, while moving the appropriate distance by diffusion both in solution (of SRP) or in the plane of the membrane (of the SRP receptor), is a matter of milliseconds or even

less. A quasi-steady-state approximation can then be used by eliminating all fast reactions from the differential equations (see Appendix). Instead, equilibrium relations are introduced:

$$Q_S = q_S/q_{-S} = [RS]_N/(R_N S)$$
$$= [RS]_{N+1}/(R_{N+1}S) = \ldots$$
$$= [RS]_{N+K}/(R_{N+K}S) \qquad (15)$$

$$Q_D = q_D/q_{-D} = [RSD]_N/([RS]_N D)$$
$$= [RSD]_{N+1}/([RS]_{n+1}D) = \ldots$$
$$= [RSD]_{N+K}/([RS]_{N+K}D). \qquad (16)$$

It is assumed here that the binding constants are equal within the window region. The extraction of slow steps involves tedious calculations but circumvents the known problems with stiff differential equations in which the step width of integration has to be adjusted according to the fastest reactions. The calculations are therefore speeded up considerably (Heinrich et al., 1977; Schauer & Heinrich, 1983).

The boundary positions of the window region N and $N + K + 1$ are treated in an analogous manner, taking into account the possible fluxes (not shown).

The system of differential equations was solved numerically using the following starting conditions:

$$R_j = 0 \quad \text{for } j < N$$
$$R_j^{nt} = R_j^{t} = 0 \quad \text{for } j > N + K \qquad (17)$$

and

$$R_j = \varepsilon/(1 + Q_S S_{tot} + Q_S Q_D S_{tot} D_{tot})$$
$$[RS]_j = Q_S S_{tot} R_j$$
$$[RSD]_j = Q_D D_{tot}[RS]_j$$

for $N < j < N + K$.

Here S_{tot} and D_{tot} are the total concentrations of SRP and its receptor, respectively, and ε is a small number (generally set equal to 10^{-6}). The starting values of R_j in the window region were set not equal to zero in order to comply with the quasi-steady-state approximation. The starting value for r was taken as the total ribosome concentration R_{tot}.

The program was written in FORTRAN for a BESM6 computer. A modified Runga–Kutta procedure was used for integration of the system of differential equations. In general, for $M = 200$ and realistic parameter values (see Table 1), it took about ten minutes computing time to reach a steady-state.

(c) Extension of the standard model by consideration of binding of free SRP to its receptor

The binding process:

$$S + D \rightleftharpoons SD,$$
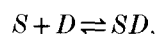
which takes place even in the absence of ribosomes (Gilmore et al., 1982b), can be taken into account without difficulties. Again, it is assumed that a quasi-steady-state approximation holds so that an

Table 1

Standard parameters of the models and their numerical values

Parameter	Meaning	Standard value	Reference
k	Rate constant of elongation and termination	20 min^{-1}	Heinrich & Rapoport (1980)
k_I	Rate constant of initiation	$0.012 \text{ min}^{-1} \text{ nmol}^{-1} \text{ l}$	Heinrich & Rapoport (1980)
M	Number of codons of mRNA	200	
N	Position of emergence of the signal peptide from the ribosome	70	Walter & Blobel (1981*b*)
K	Size of window of SRP interaction	10	
L	Number of codons occupied by a ribosome	12	Heinrich & Rapoport (1980)
Q_S	Binding constant for SRP binding to ribosome carrying an exposed signal peptide	$2.5 \text{ nmol}^{-1} \text{ l}$	
Q_D	Binding constant for docking of the SRP–ribosome–complex	$0.03 \text{ nmol}^{-1} \text{ l}$	
R_{tot}	Total ribosome concentration	$100 \text{ nmol}^{-1} \text{ l}$	
M_{tot}	Total mRNA concentration	$1 \text{ nmol}^{-1} \text{ l}$	
S_{tot}	Total SRP concentration	Variable	
D_{tot}	Total SRP receptor concentration	Variable	

equilibrium relation can be written:

$$Q_{SD} = [SD]/(SD). \qquad (18)$$

Changes in the calculations involve the quasi-steady-state approximation for the fast reactions (not shown).

(d) *An alternative model; SRP mediates binding of mRNA to the RER membrane*

Whereas in the standard model SRP is required for each ribosome to become membrane-bound, in the alternative model ribosomes can be transferred to the membrane before having reached codon N (see Fig. 2). Mathematical treatment of the alternative model of SRP function can be done by extension of the standard model. For simplicity, it is assumed that SRP binding is only possible at a single codon N (the size of the window is assumed to be only one residue).

Two classes of mRNA-bound ribosomes are distinguished from the very first position on: ribosomes traveling along a free mRNA or along a membrane-bound mRNA. Thus, for position 1 we have:

$$dR_1^f/dt = k_I \left(1 - \sum_{s=1}^{L} (R_s^f/M^f) \right) rM^f - kR_1^f P_2^f - w_1, \qquad (19)$$

$$dR_1^b/dt = k_I \left(1 - \sum_{s=1}^{L} (R_s^b/M^b) \right) rM^b - kR_1^b P_2^b + w_1. \qquad (20)$$

The superscripts f and b refer to free and membrane-bound mRNA, respectively. M^f and M^b are the total concentrations of free and membrane-bound mRNA. w_1 is a flux characterizing the transfer of ribosomes at position 1 to the membrane. P_2 is the conditional probability that codon 2 is free, given that codon 1 is occupied by a ribosome.

Analogous equations hold for all positions up to N. The region beyond position N is treated
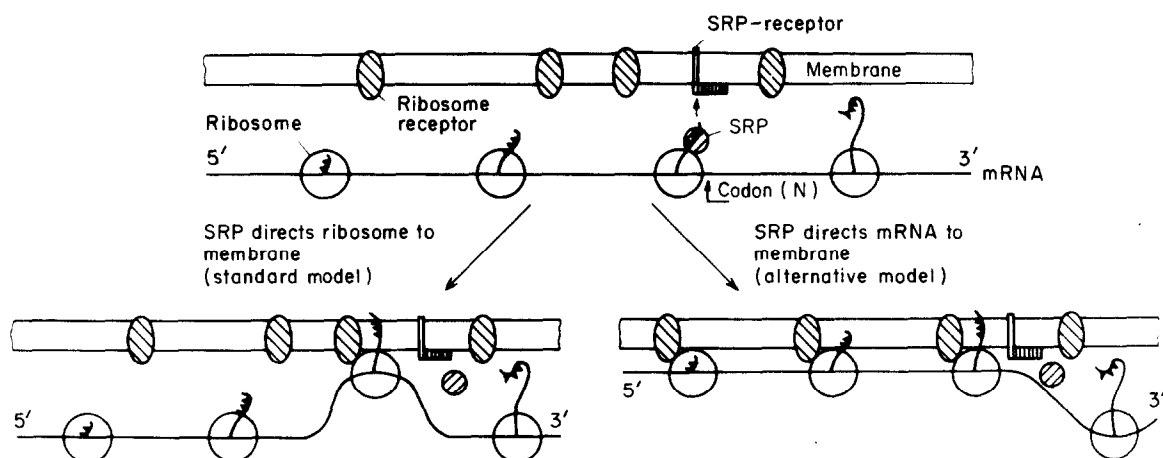

Figure 2. Schematic illustration of the 2 models of SRP functioning. According to the standard model, SRP is required for each ribosome to become membrane-bound. By contrast, according to the alternative model, if SRP directs a ribosome at codon N to the membrane, ribosomes following it on the same mRNA molecule would also become membrane-bound. Ribosomes can, therefore, be transferred to the membrane even before reaching codon N. They too will synthesize translocated product. Ribosomes beyond codon N that are transferred to the membrane are nevertheless assumed to continue synthesis of non-translocated product. In this model SRP is assigned a role in directing the mRNA, rather than a ribosome, to the membrane.

similarly except that it is assumed that ribosomes transferred to the membrane at these positions continue to produce non-translocated protein chains.

The transfer fluxes w_j are given by the expression:

$$w_j = k[RSD]_N P^f_{N+1}(R^f_j/M^f).   (21)$$

Finally, it has to be taken into account that the conditional probabilities P^f_j and P^b_j have to be calculated separately for the membrane-bound and free mRNA species. A new differential equation also holds for the free and membrane-bound mRNA concentration:

$$dM^f/dt = -dM^b/dt = -k[RSD]_N P^f_{N+1};$$

$$(M_{tot} = M^f + M^b).   (22)$$

All other calculations are similar to those for the standard model.

(e) Parameter values

The system is determined by the parameters given in Table 1. Most standard values are based on experimental data; in particular, those for k_1, k and L have been justified by consideration of the translation process alone (Heinrich & Rapoport, 1980). The standard values of Q_S and Q_D have been obtained by a fit to the experimental data (see Results) on the basis of $K = 10$ (K is the size of the window). Most parameters have been varied systematically in order to test their influence on the behavior of the system (see Results).

3. Experimental Methods

Cell-free translation of human placental RNA was carried out in a wheatgerm system in the presence of [^{3}H]leucine, as described (Bassüner et al., 1984). Dog pancreatic rough microsomes were added to the system in different subsaturating amounts. After different times of translation, samples were taken, precipitated by trichloroacetic acid and processed for SDS/polyacrylamide gel electrophoresis (Wiedmann et al., 1984). The gel was fluorographed for different times and the amounts of prelactogen and lactogen synthesized were quantified by densitometry of the X-ray films. A correction was made for the different number of leucine residues present in the 2 proteins.

Cell-free translation of oviduct mRNA was carried out in a synchronized system. After 30 s, 4 mM-^{7}mGp and 10 μM-edeine were added to inhibit further initiation of translation. Both drugs were required to inhibit initiation completely (not shown). Translation was carried out at 22°C. Portions were removed after different times and processed for SDS/polyacrylamide gel electrophoresis. The amount of completed ovalbumin chains was determined by densitometry of the preflashed X-ray films used for the autoradiography.

4. Results

(a) Does SRP direct a ribosome or a mRNA molecule to the membrane?

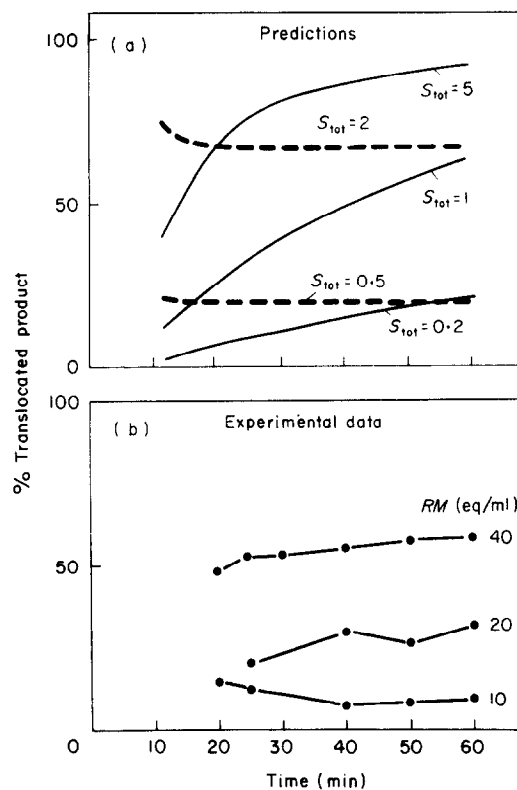We have tried to discriminate between two models of SRP function (see Fig. 2). According to

Figure 3. Predicted behavior of the 2 models of SRP functioning and comparison with experimental data. (a) The percentage of translocated product predicted to be synthesized at different times during cell-free synthesis was calculated for the 2 models of SRP functioning: broken curves, SRP is required for each ribosome (standard model); continuous curves, SRP directs the mRNA to the membrane. The curves were calculated for $D_{tot} = 1$ nmol 1^{-1} and standard parameters, for different values of S_{tot}. (b) Experimental data obtained by translation of human placental lactogen in a wheatgerm cell-free system in the presence of suboptimal amounts of rough microsomes (RM) from dog pancreas (equivalents (eq), as defined by Walter et al. (1981)).

one model (standard model), SRP is required for each ribosome individually to become membrane-bound. According to the alternative model, if a ribosome is transferred by SRP to the membrane, other ribosomes following on the same mRNA molecule would also be directed to the membrane without the need for SRP, possibly because a spatial neighborhood already exists. This model would assign to SRP the function of directing a mRNA molecule, rather than a ribosome, to the membrane.

For the standard model, once the first ribosomes reach the termination codon, a constant average number of membrane-bound and of unbound ribosomes per mRNA molecule is soon attained, and therefore a linear time-course of product formation is expected for both classes of proteins. Consequently, the percentage of translocated protein synthesized for a given concentration of rough microsomes (or SRP) should be constant with time (Fig. 3(a)). In contrast, according to the

alternative model, the rate of formation of translocated protein increases with time until all ribosomes synthesize only translocated products (Fig. 3(a)). In fact, the only steady state of this system is one where all the mRNA is membrane-bound (100% synthesis of translocated product). If the SRP concentration is sufficiently high, the conversion of free to membrane-bound mRNA is fast. The rate of transfer of the mRNA to the membrane would be even faster if the window were larger than just one codon (as assumed for the calculations shown in Fig. 3(a)).

The different time-courses of product formation predicted according to the two models permit an easy discriminating experiment to be done (Fig. 3(b)). It may be seen that the percentage of translocated protein synthesized in a cell-free system remains constant within the error of the measure. One may therefore dismiss the model according to which SRP is required for mRNA binding; the data are in agreement with the standard model. It should be noted that the result does not depend on the particular parameter values chosen for the calculations.

(b) *Tests of the standard model and its extensions by experimental data and estimation of parameter values*

(i) *Tests for steady-state conditions*

First, the model was tested by steady-state data obtained from the literature for the translation *in vitro* of preprolactin mRNA in a wheatgerm cell-free system (Walter *et al.*, 1981; Walter & Blobel, 1981a; Gilmore *et al.*, 1982a).

Figure 4(a) shows that the inhibition by SRP of translation of preprolactin mRNA in the absence of microsomes (D_{tot} = 0) is well described by the model assuming a value of $Q_S = 2.5 \, \text{nmol}^{-1} \, \text{l}$ for the binding constant of SRP to ribosomes carrying exposed signal peptides. Owing to uncertainties in the conversion of units/volume to $\text{nmol} \, \text{l}^{-1}$, this value may be actually somewhat lower ($\sim 1 \, \text{nmol}^{-1} \, \text{l}$). Such differences have no effect on the conclusions or on the quality of describing the data.

A lower estimate of the binding constant of SRP has been obtained experimentally by determining the amount of iodinated SRP bound to polysomes synthesizing preprolactin (Q_S (experimental) $> 0.16 \, \text{nmol}^{-1} \, \text{l}$: Walter *et al.*, 1981). With our model we can calculate the amount of SRP bound to polysomes and compare it with the experimental data. For $Q_S = 2.5 \, \text{nmol}^{-1} \, \text{l}$ and $S_{tot} = 1 \, \text{nmol}^{-1} \, \text{l}$ (corresponding roughly to the conditions used by Walter *et al.*, 1981) we find about 45% of the SRP bound (28% for $Q_S = 1 \, \text{nmol}^{-1} \, \text{l}$), which is in fair agreement with the experimental observation†.

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† Between 30% and 43% were found to be bound, depending on the class to which SRP bound to monosomes is assigned (Walter *et al.*, 1981).

$K\text{-}RM \, (\text{eq}/25 \, \mu\text{l}) = D_{tot} \, (\text{nmol}/\text{l})$
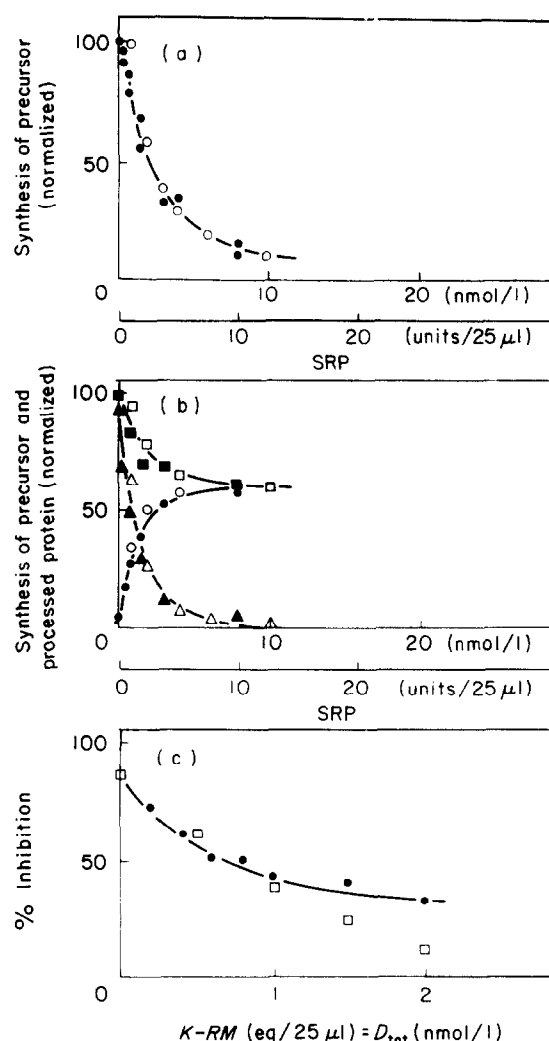
Figure 4. Comparison of steady-state data from the literature with predictions according to the standard model. (a) Inhibition by SRP of translation of preprolactin in the wheatgerm system. Predicted rates of synthesis of preprolactin (open circles) were calculated according to the standard model. D_{tot} was set equal to zero and standard parameters were used. The system of differential equations was integrated, for different values of S_{tot}, until a steady-state was obtained (less than 60 min required). Experimental data (filled circles) were taken from Walter *et al.* (1981). (b) Dependence on SRP of the synthesis of preprolactin and prolactin in the presence of dog pancreatic microsomes. The predicted rates of synthesis (open symbols) were calculated for $D_{tot} = 1 \, \text{nmol} \, \text{l}^{-1}$ (corresponding to 1 eq $K - RM/25 \, \mu\text{l}$ assay) and standard parameter values. The experimental data (filled symbols) were taken from Walter & Blobel (1981a). Triangles, synthesis of precursor; circles, synthesis of translocated product; squares, total synthesis of translocated plus non-translocated product. (c) Dependence on salt-washed microsomes ($K - RM$) of the translational inhibition exerted by SRP. The predicted translational inhibition by SRP (open squares) was calculated for $S_{tot} = 8 \, \text{nmol} \, \text{l}^{-1}$ (corresponding to 10 units/25 μl assay) and standard parameters. The experimental data (filled circles) were taken from Gilmore *et al.* (1982a).

The interaction of a polypeptide with SRP depends not only on the parameter Q_S but also on the size (K) of the SRP-window. It will be shown in a later section, however, that for a large range of K values (from about 8 to about 80) the estimates for Q_S change very little. Therefore, one may obtain a rough estimate for Q_S from experimental data, such as those shown in Figure 4(a), by simply taking the concentration of SRP for which half-maximum inhibition is observed.

The standard model also describes the data reasonably well if microsomal membranes are present during cell-free translation (Fig. 4(b) and (c). For modeling it was assumed that 1 equivalent $K - RM_S/25 \, \mu l$ corresponds roughly to 1 nmol^{-1} 1 of active SRP receptor protein†. The best fit to the data was obtained with a binding constant for "docking" of $Q_D = 0.03$ nmol^{-1} 1. Experimental estimates for comparison are not available.

SRP is known to bind weakly to 80 S ribosomes that are not engaged in translation, with a binding constant about four orders of magnitude lower than Q_S (Walter *et al.*, 1981). If the standard model was extended to include this binding process, the calculated behavior of the system did not change significantly, particularly if it was assumed that ribosomes carrying SRP initiate translation as efficiently as "naked" monosomes (not shown).

SRP also binds to its membrane receptor in the absence of protein synthesis and independently of the presence of ribosomes (Gilmore *et al.*, 1982*b*). Incorporation of this binding reaction into the standard model leads to an inhibition of translation by SRP even in the presence of microsomal membranes (Fig. 5): at high concentrations, free SRP can compete with ribosome-bound SRP for receptor sites. However, even if it is assumed that the binding constants Q_D and Q_{SD} are equal (Fig. 5), the predicted inhibition of translocated protein synthesis is moderate unless very high SRP concentrations are used. The weak competition is due to the fact that only a small percentage of receptor molecules is normally occupied. For example, at concentrations of $S_{tot} = 16$ nmol l^{-1} and $D_{tot} = 1$ nmol l^{-1} about 98% of the receptor is still unoccupied. If the binding constant Q_{SD} were smaller than Q_D. corresponding to an allosteric effect of the signal peptide binding site of SRP on its membrane attachment site, the competition phenomenon would be even weaker.

(ii) *Modeling of synchronized translation experiments*

Synchronized translation experiments have been used in order to analyze the consecutive molecular events occurring in protein translocation (Walter & Blobel, 1981*b*; Rothman & Lodish, 1977). Ideally,

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† It was estimated that 1 equivalent (eq), RM contains about 100 fmol of receptor (Tajima *et al.*, 1987). Consequently, 1 eq. $K - RM/25 \, \mu l$ corresponds to 4 nmol l^{-1}. We have rounded off this value since it is likely that only a fraction of the receptor is active.
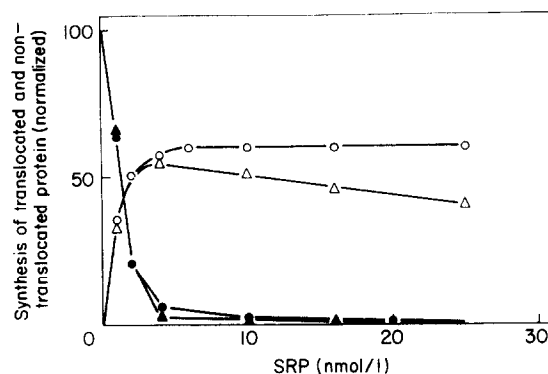

Figure 5. Influence of binding of free SRP to its receptor on the synthesis of translocated and non-translocated product. For the calculations, the standard model was extended by taking into account that free SRP can interact with its membrane receptor even in the absence of protein synthesis (characterized by the binding constant Q_{SD}, see the text). Integration of the system of differential equations was done for different values of S_{tot} and standard parameters until a steady-state was obtained. Open symbols, synthesis of translocated product; filled symbols, synthesis of non-translocated product; triangles $Q_{SD} = 0.03$ nmol^{-1} 1 (i.e. identical with Q_D); circles, $Q_{SD} = 1 \times 10^{-4}$ nmol^{-1} 1.

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only one ribosome should travel along a given mRNA molecule and all ribosomes should have moved the same distance from the initiation codon. Such a situation is approximated experimentally by inhibiting the initiation of translation after a short time. In practice, the time-window during which initiation of translation is allowed, is chosen to be a compromise between the extent of synchronization on the one hand (the shorter the better) and the number of translating ribosomes on the other (the more the easier the detection of the synthesized polypeptide chains).

Figure 6(a) shows the density distribution of ribosome-bound nascent polypeptide chains along the mRNA for different times after the start of translation in the absence of SRP. It may be seen that even for a time-window of 0·5 minute, which is possibly the shortest attainable experimentally, the synchronization becomes poor after a short incubation period. For example, after six minutes the mean distance of the ribosomes from the initiation codon will be about 115 codons and the half-width of the peak is about 30 codons. It is therefore expected (and found) that even under the most favorable conditions the nascent polypeptide chains constitute a heterogeneous population. It is important to note that such synchronization experiments are not accurate enough to follow the defined growth of a polypeptide chain. The extent of synchronization can be improved by choosing a shorter time-window of initiation. However, besides problems of detection of the synthesized polypeptide chains, there may also be a limit to the extent of inhibition of translation that can be achieved: the shorter the time-window, the more severe the
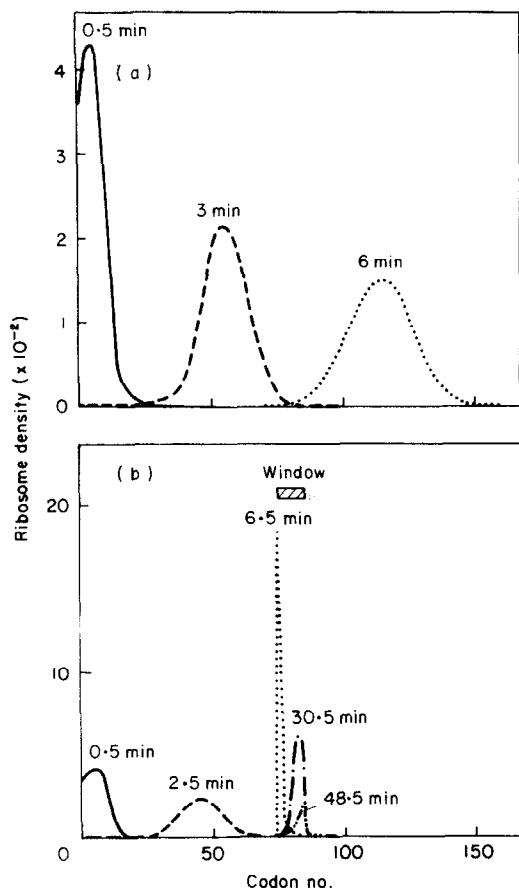
Figure 6. Predicted distribution of ribosome-bound nascent polypeptide chains for different time points in synchronized translation experiments. The probability that a ribosome is located with its front at position j, R_j/M_{tot} was plotted for each codon j. This corresponds to the Q density of nascent polypeptide chains having a length of j amino acid residues. (a) Translation in the absence of SRP. After 0·5 min of initiation, the value of k_I was changed from its normal value of 0·012 to 1×10^{-6} and integration of the system was continued. (b) Translation in the presence of 25 nmol l^{-1} of SRP. The time-window was 0·5 min as for (a).

Figure 7. Appearance of completed polypeptide chains with time in simulated synchronized translation systems for different SRP concentrations and values of Q_S and K. The time-window of initiation was taken as 0·5 min and k_I was reduced thereafter to 1×10^{-6}. The cumulated amount of completed polypeptide chains at different time-points was calculated for different values of S_{tot}. The data in (a) were obtained for $Q_S = 2·5$ nmol^{-1} l and $K = 10$, and correspond roughly to the case of preprolactin. Reduction of either the size of the SRP-window ((b), $K = 2$) or of the binding constant for SRP ((c), $Q_S = 0·25$ nmol^{-1} l) increases the concentration of SRP required to block the ribosomes for a given period of time. Panel (c) may correspond to the case of ovalbumin (compare with Fig. 8).

inhibition must be in order to minimize the effects of "leakiness". For example, if the time-window is 0·5 minute, the initiation rate has to be inhibited by a factor of at least 10^4 to get less than 2% leakage through the initiation. Such a strong inhibition can only be achieved by the combined action of two inhibitors (Braell & Lodish, 1982; see also Fig. 8).

If SRP is present during translation of a protein to be translocated (at $D_{tot} = 0$), the elongation arrest leads to a high density of ribosome-bound nascent polypeptide chains at position N, where there is the first opportunity for an interaction of the signal sequence with SRP (Fig. 6(b)). Indeed, experimentally an arrested polypeptide fragment of about 70 amino acid residues in length has been observed for several proteins under such conditions (Walter & Blobel, 1981b; Kurzchalia *et al.*, 1986; Meyer *et al.*, 1982). Collection of all the ribosomes at this site, i.e. maximum appearance of the arrested
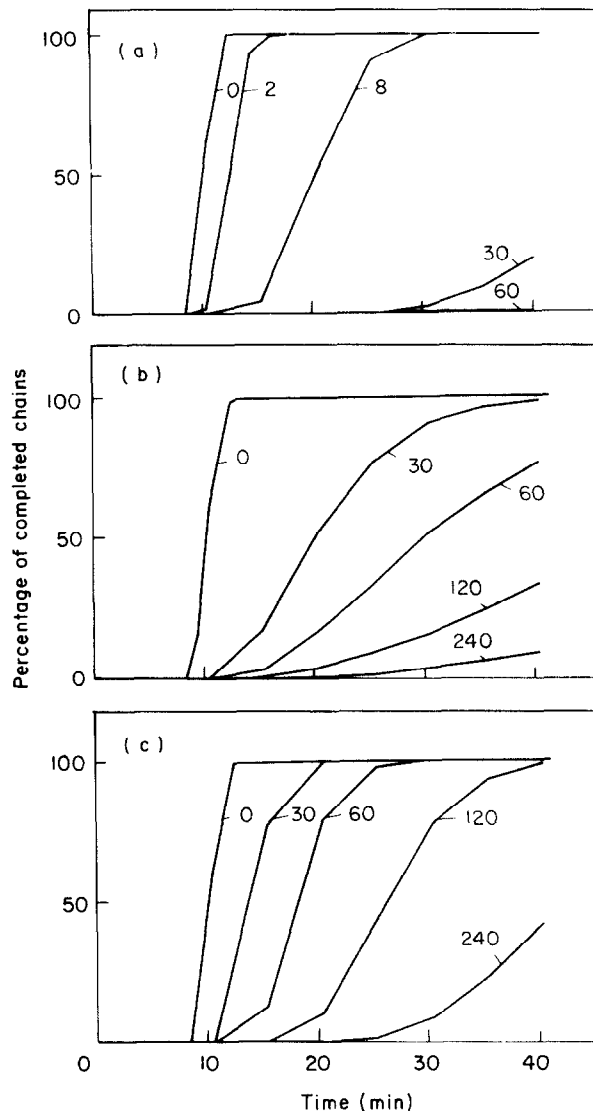
fragment, should take a little longer than expected from the size of the arrested polypeptide fragment (about 5 min *versus* 3·5 min for the chosen parameter values) in agreement with the experimental observation (Gilmore & Blobel, 1983). With time the arrested ribosomes move slowly "downstream" leading to a broadening of the density distribution (Fig. 6(b)). Eventually, all ribosomes manage to reach the termination codon of the mRNA. The
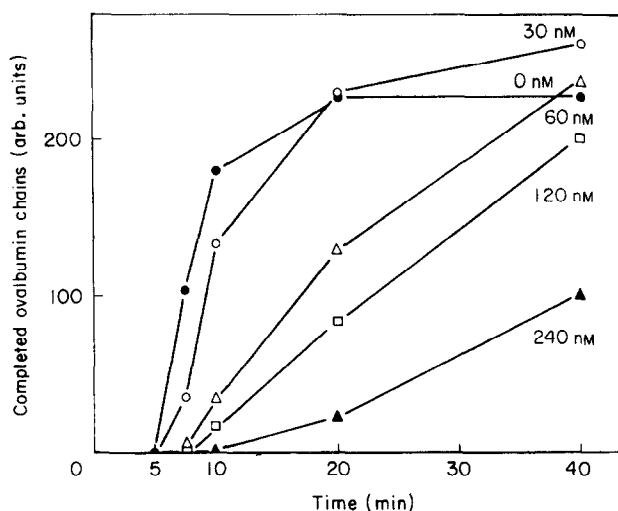
Figure 8. Synchronized cell-free synthesis of ovalbumin dependent on the SRP concentration. Translation of oviduct mRNA was carried out in a wheatgerm cell-free system at 22 °C in the presence of different concentrations of SRP. Initiation of translation was stopped after 0·5 min by the addition of ^{7}mGp and edeine. Quantification of completed ovalbumin chains found at different time points was carried out after SDS/polyacrylamide gel electrophoresis. Note the similarity of the curves with those shown in Fig. 7(b) and (c).

time it takes for the disappearance of the arrested polypeptide fragment depends on the SRP concentration and on the parameter values for Q_S and K (compare Fig. 7(a) with (b) and (c)). Indeed, it has been verified experimentally that the arrested fragment of preprolactin disappears with a slow kinetics depending on the SRP concentration (Wiedmann *et al.*, 1987, and unpublished results). If the SRP binding constant Q_S is small or the SRP-window comprises few codons, the translational arrest exerted by SRP is predicted to be manifest only for a short time.

Figure 8 shows our experimental data for the synchronized translation of ovalbumin mRNA in the presence of different concentrations of SRP (at $D_{tot} = 0$). The curves correspond closely to the theoretical ones obtained for small K or Q_S values (cf. Fig. 7(b) and (c)). Since $K < 2$ appears unlikely, one may conclude that the ovalbumin signal sequence has a low SRP binding constant (~ 0.25 nmol^{-1} l), much lower than that of preprolactin (modeled in Fig. 7(a)). We cannot exclude the possibility, however, that *both* Q_S and K are reduced.

It was of interest to determine in a general manner the conditions under which an arrested polypeptide fragment can be expected in synchronized translations.

Generally speaking, the better the ribosomes are blocked at the first arrest site (position N), the greater will be the amount of the arrested fragment. Therefore, high SRP concentrations are favorable, ideally much higher than those used for efficient inhibition of the steady-state rate. For example,

more than 75 nmol l^{-1} of SRP are required to prevent movement by the ribosome peak of more than five codons within 60 minutes (for standard parameter values). By contrast, 10 nmol SRP l^{-1} lead to an almost complete inhibition of the steady-state translation (see Fig. 4). A second factor is obviously the time of incubation. For an N-terminally located signal sequence and for a normal rate of translation it will take about five minutes for maximum appearance of the arrested fragment. Longer incubation periods will lead to a gradual elongation of the polypeptide fragment and simultaneous broadening of the nascent chain distribution. Finally, the time-window during which initiation of translation is allowed plays an important role. If it is too long (>1 min for the chosen parameter values), on average more than one ribosome will be found on a given mRNA molecule; blockade of the first ribosome will lead to a high ribosome density at position N-L giving rise to a second shorter apparent arrested fragment. With time both ribosome peaks move forward and broaden until a heterogeneous population is obtained. It is clear that such a situation may obscure the observation of the actual SRP-arrested fragment. It should also be noted that for some proteins with small Q_S values the arrested fragment may be visible only for a very short time even for high SRP concentrations.

(c) *Analysis of the molecular effects of SRP on steady-state translation and translocation*

In order to get a better understanding of the molecular effects of SRP on translation and translocation, the influence of various conditions, depending either on the type of protein (intrinsic factors) or on experimental circumstances (extrinsic factors), were analyzed. Fundamental differences between the effect of SRP on translation and on translocation were discovered and are discussed in this section.

(i) *Consequences of the spatial separation on the mRNA between initiation of translation and of translocation*

Blockade of ribosomes by SRP binding within the window region does not automatically inhibit the steady-state rate of translation. This is illustrated in Figure 9, where the steady-state ribosome density along the mRNA is plotted for two SRP concentrations. For a low SRP concentration, piling up of ribosomes at the arrest site ($N = 70$) is only a local event, whereas for higher concentrations a "traffic jam" occurs reaching all the way back to the initiation region at the mRNA (compare the two panels of the Figure). Only in the latter case is the steady-state rate of translation inhibited. Transition between the two cases occurs abruptly at a certain SRP concentration ($S_{tot} \sim 1.5$ nmol l^{-1}). A threshold is therefore predicted for the translational inhibition by SRP (e.g. see Figs 4 and 10).
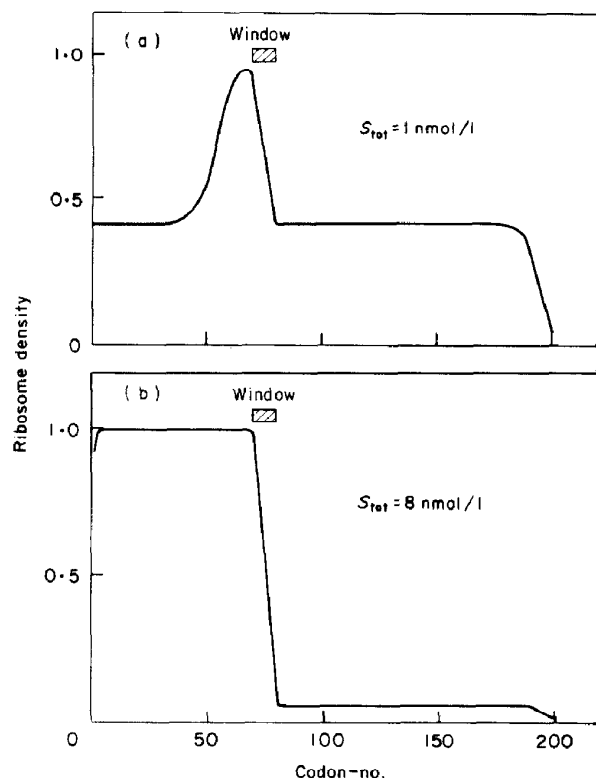
Figure 9. Steady-state distribution of ribosomes along the mRNA for low and high SRP concentrations. The steady-state density of ribosomes at a given codon is plotted *versus* the codons of the mRNA. The density of ribosomes at codon j is given by the expression:

$$\left(\sum_{s=1}^{L} R_{j+s} \right) \Big/ M_{tot}.$$

Standard parameter values were used and D_{tot} was set equal to zero. S_{tot} was either 1 nmol l^{-1} (upper part), corresponding to an only local accumulation of ribosomes, or 8 nmol l^{-1} (lower part), giving a traffic jam situation with the initiation region of the mRNA being partially blocked.

In contrast to the inhibition of translation, the efficiency of translocation depends only on the situation at the arrest site. Since this condition is less stringent, one can expect that in a variety of circumstances translational inhibition by SRP would fail to occur even if translocation can be demonstrated. For example, if the binding constant Q_S is reduced to 0·1 of its standard value (Fig. 10), the translational inhibition is greatly diminished by a shift of the threshold concentration of SRP to higher values, whereas translocation is much less reduced.

Not only the SRP binding constant but also the size (K) of the window may differ among proteins. Figure 11(a) shows the extent of inhibition as a function of SRP for different K values. It is apparent that differences in K exert their greatest effect at low SRP concentrations. The inhibition of translation increases with K up to values in the range of L (L is the number of codons occupied by a ribosome) to decrease again at very large K values.
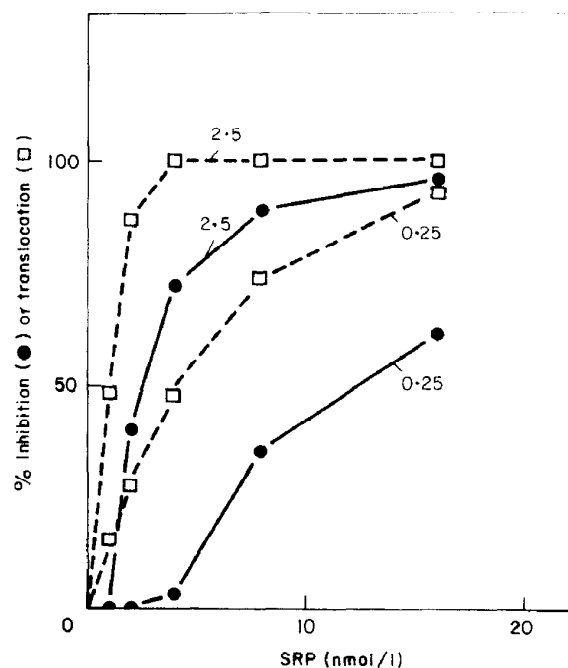

Figure 10. Theoretical dependence of translational inhibition and translocation on the SRP binding constant (Q_S). The system of differential equations describing the standard model was integrated for different values of Q_S (numbers in the Figure) and for different values of S_{tot} until a steady-state was obtained. Standard parameters were used with the exception of $K = 25$. The calculations for translocation were performed for $D_{tot} = 1 \text{ nmol l}^{-1}$. ($\bullet$) Inhibition; ($\square$) translocation.

These results are understood by consideration of two opposing effects. The longer the window, the more opportunity for SRP to bind to a ribosome and to stop its movement. Consequently, the translational inhibition is increased. If the window is very long, however, the limited amount of SRP is distributed among many binding sites and less piling up of ribosomes in front of the arrest zone occurs.

The efficiency of translocation in the presence of microsomal membranes shows a monotonic dependence on K (Fig. 11(b)). Here, the opportunity of ribosomes to become membrane bound is increased with a longer window.

As a consequence of the different dependence of the two processes on the K values, there is a range where the translational inhibition by SRP can be weak (small or very large K values), but translocation occurs. If a polypeptide chain is short, both the translational arrest and the efficiency of translocation are reduced, but the former to a greater extent (not shown). The effect is essentially a reduction of the size (K) of the window for SRP interaction. It is therefore predicted that polypeptides comprising less than 80 residues show a weak SRP-induced translational inhibition and are relatively poorly translocated. It is interesting that small polypeptides like the procoat protein of the f1 phage are indeed poorly translocated *in vitro*, possibly by a different pathway (Watts *et al.*, 1983). According to
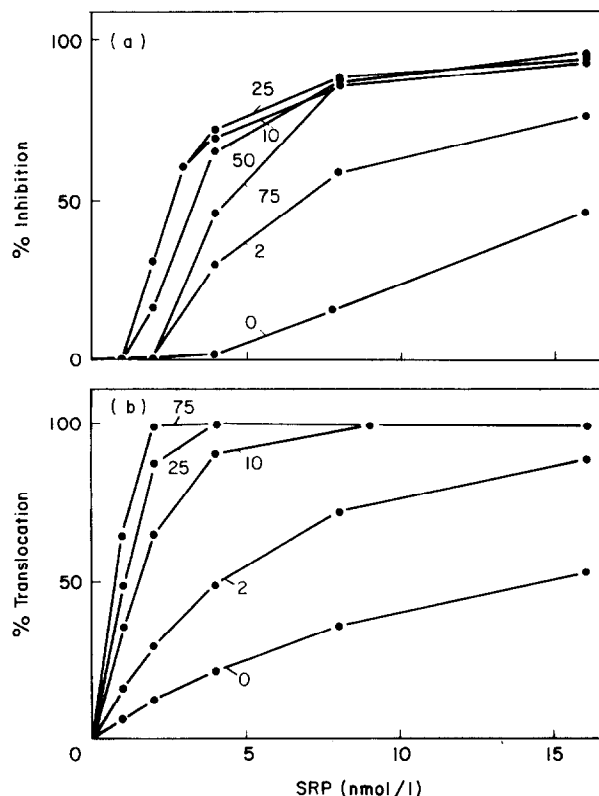
Figure 11. Theoretical dependence of translational inhibition and translocation on the size of the SRP-window (K). The system of differential equations describing the standard model was integrated for different values of K (numbers in the Figure) and for different values of S_{tot} until a steady-state was obtained. Otherwise, standard parameters were used. (a) Translational inhibition by SRP in the absence of microsomal membranes ($D_{tot} = 0$); (b) translocation in the presence of microsomal membranes ($D_{tot} = 1$ nmol l^{-1}). $K = 0$ means that SRP can only interact with ribosomes at position N.

Figure 12. Theoretical dependence on the mRNA concentration (M_{tot}) of translational inhibition and translocation. The calculations were performed with standard parameters. The mRNA concentration (M_{tot}) was varied as indicated in the Figure. The calculations for translocation were performed for $D_{tot} = 1$ nmol l^{-1}.

the assumptions made in our model, polypeptides less than 70 residues in length should not react with SRP at all.

It should be noted that SRP produces a lag phase in the formation of completed polypeptide chains. Its length depends both on the SRP concentration and on K (not shown).

It is of interest to consider whether the location of a signal peptide in a polypeptide influences the effects of SRP. It has been speculated that the absence of a translational arrest observed for two membrane proteins is due to the internal location of the signal sequence (Anderson *et al.*, 1983). Perhaps, somewhat surprisingly, our calculations indicate the absence of such an effect as long as the signal peptide is sufficiently far away from the termination codon (>80 amino acid residues) (results not shown). Transition from the low density case to the traffic jam situation is so abrupt that the site of blocking is unimportant. Indeed, we have recently shown that a normally N-terminal signal sequence triggers the SRP arrest of translation even if placed at an internal location in a polypeptide chain (Wiedmann *et al.*, 1986).
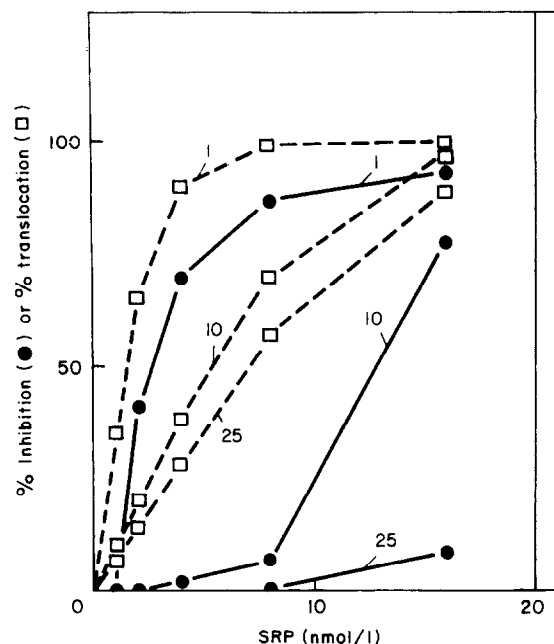
(ii) *Stoichiometric* versus *catalytic action of the SRP*

Translational inhibition involves a stoichiometric interaction of SRP and ribosomes carrying exposed signal peptides. On the other hand, translocation only requires a catalytic function of SRP, since it is released immediately after membrane binding of the complex. Therefore, more SRP is needed for the former effect.

This difference is clearly born out by modeling the effects of varying the mRNA concentration (Fig. 12). With an increase in the mRNA concentration the translational inhibition by SRP is predicted to be much more reduced than the efficiency of translocation. This example shows that the failure to observe translational arrest may not necessarily indicate a peculiarity of the polypeptide studied and may instead be the result of experimental conditions. It should also be noted that other mRNA species present in the assay may compete with the mRNA studied for the SRP.

Conditions of ribosome limitation also affect the translational inhibition more strongly than the translocation process (not shown).

(d) *Extrapolation to the conditions* in vivo

It has been suggested that the physiological meaning of the translational arrest is to prevent misdirection of proteins destined for export (Walter & Blobel, 1981b). Ribosomes that do not find a membrane binding site immediately would wait in an arrested state until docking becomes possible.
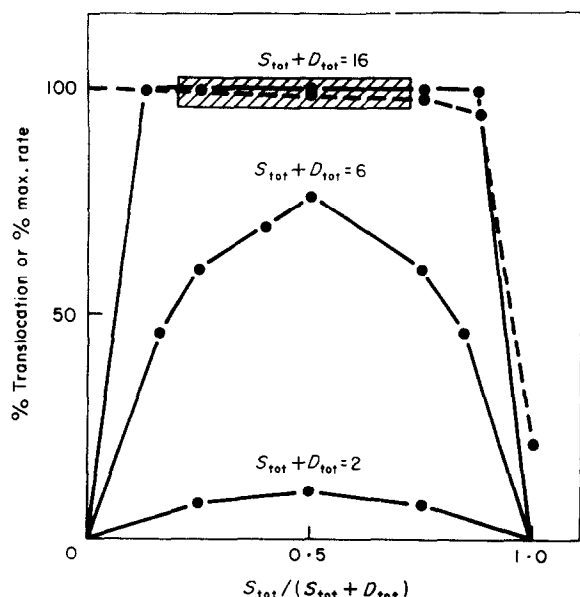
Figure 13. Extrapolation to the conditions *in vivo*. The system of differential equations describing the standard model was integrated until a steady-state was obtained. Continuous lines give the percentage of translocation, the broken line gives the translational rate at $S_{tot} + D_{tot} = 16\,\mathrm{nmol\,l^{-1}}$ in relation to the maximum rate of translation obtained for $S_{tot} = 0$. All curves were calculated for $S_{tot} + D_{tot} = \mathrm{constant}$. Standard parameters were used except for $M_{tot} = 10\,\mathrm{nmol\,l^{-1}}$. The box indicates the range for which favorable *in vivo* conditions are expected: essentially complete translocation and no inhibition of translation.

We have tried to test this hypothesis by extrapolation to conditions *in vivo*.

Plots were constructed giving the translocation efficiency and percentage of inhibition of translation for various values of the ratio $S_{tot}/(S_{tot} + D_{tot})$. The total sum of SRP and its receptor was also varied (Fig. 13). It may be seen that the optimum of translocation is achieved if SRP and its receptor are present in equimolar concentrations. If the sum of their concentrations is equal to or higher than the mRNA concentration, the translocation efficiency approaches 100% for a wide range of the ratio of S_{tot}/D_{tot}. It is also apparent that inhibition of translation by SRP is insignificant unless $S_{tot} \gg D_{tot}$. Similar results were obtained if all concentrations were scaled up to comply with the much higher concentrations in a cell (not shown).

The absolute concentrations of SRP and its receptor in a cell are uncertain but their ratio in a pancreatic exocrine cell has been estimated to be about 1 to 0·5 (Gilmore *et al.*, 1982*b*). Rough estimates indicate that there are about equal numbers of molecules of SRP and mRNA molecules[†].

[†] Calculated from the fact that there is about 1 SRP and 1 mRNA molecule for every 10 ribosomes (Gilmore *et al.*, 1982*b*; Heinrich & Rapoport, 1980).

We therefore conclude that in a living cell translocation of all the synthesized polypeptide chains occurs with no "miscompartmentalization" of exported proteins in the cytoplasm or translational inhibition. Owing to the spatial separation on the mRNA of initiation of translation and of translocation, a local accumulation of ribosomes occurs at the arrest site, as suggested previously (Walter & Blobel, 1981*b*), which leads to efficient protein translocation without piling up of ribosomes back to the initiation site of translation. Consequently, the rate of synthesis of translocated proteins is predicted to be as fast as that of cytoplasmic ones.

5. Discussion

Although the mathematical models proposed may appear to be rather complicated, they are probably the simplest ones compatible with reality. The complexity is due to the fact that a non-homogeneous ribosome distribution along the mRNA is an essential feature of the system.

The models are general, in that no assumptions are made concerning the actual mode of protein translocation, i.e. coupled to elongation (Blobel & Dobberstein, 1975) or in the form of polypeptide domains (Randall, 1983), and that they are applicable to both secretory and membrane proteins.

Of course, a number of simplifications had to be made to retain both generality and manageability of the model. For example, it is known that ribosomes actually do not move with equal rates at all codons of the mRNA (Bergmann & Lodish, 1979). Pauses may occur due to limitation of certain tRNA species. Consideration of differences in ribosome density would not change the qualitative results obtained. One would expect the natural "stop-sites" of the ribosome to be the major SRP interaction sites as well, since the higher ribosome density would favor SRP binding. The addition of SRP would thus augment the pausing in translation by slowing down the movement of ribosomes even further at the critical codons. One would also expect the nascent chains corresponding to these "stops" to be the major translocated species.

Despite such subtle modifications, which the model might need for certain cases, the introduction of only three parameters, i.e. two binding constants (Q_S and Q_D) and the size of the window (K) during which SRP can interact with the nascent chain, was found to be sufficient to describe translocation. The fact that the standard model accounts for a variety of experimental data indicates that the essential features of the translation/translocation system are reproduced by the model.

The interaction between a protein and SRP may be described by only two parameters: a binding constant (Q_S), which can be attributed to the structure of the signal peptide, and the size of the SRP-window (K), which is influenced additionally by the structure of the succeeding part of the

polypeptide chain. For example, it is expected that the window may be small for proteins with internally located signal peptides, since these may be buried rapidly in a domain of the already synthesized portion of the polypeptide chain. On the other hand, translocation of entire polypeptide domains (Randall, 1983) or post-translational transport across the RER membrane may be assumed to proceed through a long window.

We have provided evidence that SRP binding is significantly weaker for ovalbumin than for preprolactin. In keeping with this result is the observation that translocation is also inefficient for ovalbumin (Palmiter et al., 1980). It may be significant in this regard that the hydrophobic core of the signal sequence of ovalbumin (Tabe et al., 1984) is interrupted by Ser and Tyr residues (Rapoport, 1986).

It would be desirable to estimate both parameters describing the interaction with SRP (Q_S and K) independently. Unfortunately, however, a general and easy method for doing that is not yet available. Estimation of Q_S, therefore, relies on K being in the range between 8 and 80 within which our model predicts its effect to be insignificant. We have recently provided evidence that SRP can indeed interact with nascent preprolactin chains exceeding 70 residues in length (Wiedmann et al., 1987). The data indicate that the binding constant may be unaltered within a certain range (perhaps up to ~120 residues) and decreases with chain length thereafter. It may be concluded that the assumption of a SRP-window is a simplification; the actually observed gradual decrease of SRP binding with chain length is approximated in the model by an abrupt drop of the binding constant.

We have not excluded the possibility that the third parameter, the binding constant describing the docking process (Q_D), is also dependent on the type of protein. An allosteric effect of the signal binding on the membrane docking of SRP is conceivable. We have shown that such an effect, even if it exists, would not be manifest since only a small percentage of the SRP receptor is normally occupied, resulting in only a weak competition between free and ribosome-bound SRP.

One of the main results obtained from our calculations is the rejection of a model according to which SRP would direct the mRNA, rather than each ribosome separately, to the membrane. One may conclude that it is not the proximity of a ribosome to the membrane *per se* that triggers binding.

The analysis has revealed fundamental differences between the effects of SRP on translation and translocation. It has been shown that, because of the spatial separation between initiation of translation and that of translocation, intrinsic factors specific for a given protein, notably its binding constant for SRP and the size of the SRP-window, may lead to dissociation of the two effects of SRP; translational inhibition may fail to occur even though translocation is demonstrable. The

same effect may be caused by the fact, subject to manipulation by experimental conditions (extrinsic factors), that SRP is required in stoichiometric amounts for a translational arrest but only in catalytic quantities for translocation. The prediction that the translational arrest exerted by SRP should be dependent on the mRNA concentration provides a test for the model.

It has been shown that a defective SRP lacking the 9000 and 14,000 M_r polypeptides loses its arrest activity but retains its function in protein translocation (Siegel & Walter, 1985). It is, therefore, obvious that the two functions of SRP can be separated physically. We have shown here that even for intact SRP, which contains the portion of SRP responsible for translational arrest *in vitro*, the two functions can be dissociated.

The inhibition of translation by SRP has only been found in cell-free systems derived from wheatgerm (Meyer, 1985). Although we cannot exclude the possibility of a peculiarity in the interaction of dog pancreatic SRP with wheatgerm ribosomes, it is also possible that the binding of SRP is simply weaker in other systems. Such an effect would explain why SRP does not inhibit translation even though it is required for translocation (see Fig. 10). This hypothesis should be testable by measuring the binding of SRP to polysomes, for example in the reticulocyte lysate system.

For our extrapolation to conditions *in vivo* it was assumed that the data obtained in the wheatgerm system are applicable. The results show that even if a translational arrest occurred *in vitro* it would not be expected *in vivo*. On the contrary, the translational rate is predicted to be comparable to that of cytoplasmic proteins, uninhibited by SRP. Translocation of the polypeptide is expected to be close to 100%. Such conditions are expected even if the concentrations of SRP, its receptor and the mRNA are in the same range.

Our theoretical treatment allows one to analyze transient kinetics for the conditions of synchronized translations. Although it is shown that such experiments are not sufficiently accurate to follow the defined growth of a polypeptide chain, the presence of SRP leads to resynchronization since all ribosomes are stopped at the same site. Conditions were determined under which the occurrence of an arrested polypeptide fragment can be optimized. In general, the use of our mathematical model may serve to pretest the behavior of the system before experiments are performed.

Appendix

We shall sketch in the following the application of the quasi-steady-state approximation for the standard model for elimination of the fast binding reactions.

For S and D the following differential equations hold:

$$\mathrm{d}S/\mathrm{d}t = -q_S S \sum_{j=N}^{N+K} R_j^{\mathrm{nt}} +$$
$$q_{-S} \sum_{j=N}^{N+K} [RS] + k \sum_{j=N}^{N+K} [RSD]_j P_{j+1}, \quad (A1)$$

$$\mathrm{d}D/\mathrm{d}t = -q_D D \sum_{j=N}^{N+K} [RS]_j +$$
$$q_{-D} \sum_{j=N}^{N+K} [RSD]_j + k \sum_{j=N}^{N+K} [RSD]_j P_{j+1}. \quad (A2)$$

By addition of the differential equations (11), (12) and (13) one gets:

$$\mathrm{d}R_j^{\mathrm{nt}}/\mathrm{d}t + \mathrm{d}[RS]_j/\mathrm{d}t + \mathrm{d}[RSD]_j/\mathrm{d}t$$
$$= kR_{j-1}^{\mathrm{nt}} P_j - kR_j^{\mathrm{nt}} P_{j+1} - k[RSD]_j P_{j+1}$$
$$(j = N, \ldots, N+K). \quad (A3)$$

Summation of equations (11), (12) or (13) over j and taking into account equations (A1) and (A2) yields:

$$\left(\sum_{j=N}^{N+K} \mathrm{d}R_j^{\mathrm{nt}}/\mathrm{d}t \right) - \mathrm{d}S/\mathrm{d}t$$
$$= kR_{N-1}^{\mathrm{nt}} P_N - kR_{N+K}^{\mathrm{nt}} P_{N+K+1} -$$
$$k \sum_{j=N}^{N+K} [RSD]_j P_{j+1}, \quad (A4)$$

$$\left(\sum_{j=N}^{N+K} \mathrm{d}[RS]_j/\mathrm{d}t \right) + \mathrm{d}S/\mathrm{d}t - \mathrm{d}D/\mathrm{d}t = 0, \quad (A5)$$

$$\left(\sum_{j=N}^{N+K} \mathrm{d}[RSD]_j/\mathrm{d}t \right) + \mathrm{d}D/\mathrm{d}t = 0. \quad (A6)$$

Note that now the right-hand sides of the equations do not contain the fast reactions. Now $\mathrm{d}S/\mathrm{d}t$ and $\mathrm{d}D/\mathrm{d}t$ are eliminated. From:

$$S = [RS]_N/(R_N Q_S)$$

one obtains

$$\mathrm{d}S/\mathrm{d}t = \mathrm{d}[RS]_N/\mathrm{d}t(1/(R_N Q_S)) -$$
$$\mathrm{d}R_N/\mathrm{d}t([RS]_N/(Q_S R_N^2)). \quad (A7)$$

Similarly, one gets:

$$\mathrm{d}D/\mathrm{d}t = \mathrm{d}[RSD]_N/\mathrm{d}t(1/(Q_D[RS]_N)) -$$
$$\mathrm{d}[RS]_N/\mathrm{d}t([RSD]_N/(Q_D[RS]_N^2)). \quad (A8)$$

Finally, we use the equilibrium relations (15) and (16) to eliminate the sums

$$\sum_{j=N}^{N+K} \mathrm{d}R_j^{\mathrm{nt}}/\mathrm{d}t$$

and

$$\sum_{j=N}^{N+K} \mathrm{d}[RS]_j/\mathrm{d}t.$$

For example, one obtains:

$$\mathrm{d}R_{N+1}^{\mathrm{nt}}/\mathrm{d}t = (\mathrm{d}[RS]_{N+1}/\mathrm{d}t)(R_N^{\mathrm{nt}}/[RS]_N) +$$
$$(\mathrm{d}R_N/\mathrm{d}t)([RS]_{N+1}/[RS]_N) -$$
$$(\mathrm{d}[RS]_N/\mathrm{d}t)(R_{N+1}^{\mathrm{nt}}/[RS]_N), \quad (A9)$$

and similar expressions for $\mathrm{d}R_{N+j}^{\mathrm{nt}}/\mathrm{d}t$ for $j > 1$.

By addition of these equations one gets:

$$\sum_{j=N}^{N+K} \mathrm{d}R_j^{\mathrm{nt}}/\mathrm{d}t = \mathrm{d}R_N/\mathrm{d}t \left(\sum_{j=N}^{N+K} [RS]_j \right) \bigg/ [RS]_N -$$
$$\mathrm{d}[RS]_N/\mathrm{d}t \left(\sum_{j=N+1}^{N+K} R_j^{\mathrm{nt}} \right) \bigg/ [RS]_N +$$
$$\left(\sum_{j=N+1}^{N+K} \mathrm{d}[RS]_j/\mathrm{d}t \right) R_N/[RS]_N. \quad (A10)$$

In a similar way one obtains

$$\sum_{j=N}^{N+K} \mathrm{d}[RSD]_j/\mathrm{d}t = \mathrm{d}[RSD]_N/\mathrm{d}t \left(\sum_{j=N}^{N+K} [RS]_j \right) \bigg/ [RS]_N -$$
$$\mathrm{d}[RS]_N/\mathrm{d}t \left(\sum_{j=N+1}^{N+K} [RSD]_j \right) \bigg/ [RS]_N +$$
$$\left(\sum_{j=N+1}^{N+K} \mathrm{d}[RS]_j/\mathrm{d}t \right) [RSD]_N/[RS]_N. \quad (A11)$$

Solving equation (A5) for

$$\sum_{j=N}^{N+K} \mathrm{d}[RS]_j/\mathrm{d}t$$

and inserting it into equations (A10) and (A11) yields expressions for

$$\sum_{j=N}^{N+K} \mathrm{d}R_j^{\mathrm{nt}}/\mathrm{d}t$$

and

$$\sum_{j=N}^{N+K} \mathrm{d}[RSD]_j/\mathrm{d}t.$$

These are now inserted into equations (A4) and (A6), which then contain only the derivations $\mathrm{d}R_N/\mathrm{d}t$, $\mathrm{d}[RS]_N/\mathrm{d}t$ and $\mathrm{d}[RSD]_N/\mathrm{d}t$. Together with equation (A3) for $j = N$ this yields a system of linear equations that can be easily solved for the individual derivatives. Finally, relations such as equation (A9) and equation (A3) can be used to obtain $\mathrm{d}R_{N+j}/\mathrm{d}t$, $\mathrm{d}[RS]_{N+j}/\mathrm{d}t$ and $\mathrm{d}[RSD]_{N+j}/\mathrm{d}t$ $(j = 1, \ldots, K)$.

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