Apolipoprotein B synthesis: a square lattice model

Lin Chen, Jie Zhang,* and Lawrence Chan[1]

Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030, and Department of Physics,* Rice University, Houston, TX

Abstract We have formulated a random walk model for emerging apolipoprotein B interaction with its translocation channel on the surface of the rough endoplasmic reticulum based on the assumption that this interaction is a diffusion-mediated process. From this model, we found that a substantial proportion of mRNA–ribosome complexes was not bound to their translocation channels at any given moment of quasi-steady state. The duration of polypeptide chain elongation was an important determinant of the number of mRNA–ribosome complexes bound to their channels, but it had little net effect on the integrated rate of protein synthesis and translocation. The rates of protein synthesis and translocation for different secretory proteins that were cotranslationally translocated were very similar provided that they had similar mRNA concentration. We conclude that one of the control mechanisms regulating apolipoprotein B production may rely on the properties of translocation channels to determine the fate of newly synthesized apolipoprotein B molecules.—**Chen, L., J. Zhang, and L. Chan.** Apolipoprotein B synthesis: a square lattice model. *J. Lipid Res.* 1994. **35:** 84–92.

Supplementary key words protein translocation • mRNA–ribosome complexes • random walk • translocation channel

Apolipoprotein B (apoB) is a major structural component of the triglyceride-rich lipoproteins. Two forms of apoB (apoB-100 and apoB-48) are synthesized in a tissue-specific manner (1, 2). In humans, apoB-100 is predominantly synthesized in liver and is required for the assembly of very low density lipoprotein (VLDL) (3, 4); apoB-48 is synthesized in the small intestine and is a requisite structural component of chylomicrons. ApoB-48 is also produced in the liver of rodents (5). The mechanism of apoB-48 production is unique. ApoB-48 mRNA is the product of apoB-100 mRNA editing, a post-transcriptional process by which a C is converted to a U at nucleotide 6666, changing the latter from CAA (encoding Gln-2153) to UAA (a stop codon). ApoB-48 thus comprises the N-terminal 48% of apoB-100 (6, 7). As a major protein component of VLDL and the only protein component of LDL (low density lipoprotein) (4, 8, 9), apoB plays a pivotal role in lipoprotein metabolism and its regulation has profound effects on cholesterol homeostasis (10). The rate of apoB secretion in vitro and in vivo is modulated by various metabolic, nutritional, and hormonal factors (11–13). Recent studies indicate that, despite wide fluctuations in apoB secretion under different conditions, its mRNA level varies little (14–16). This is especially true for studies performed on the cultured human hepatoma cell line (HepG2) (12–17). Thus, the control of apoB secretion appears to be mediated mainly at a post-transcriptional level through means such as translation, translocation, trafficking, intracellular degradation, and the re-uptake of the newly secreted VLDL particles (18). An important level of control may be on the surface of rough endoplasmic reticulum (rER) for the successful translocation of newly synthesized apoB molecules (19–21). In this study, we have confined our analysis to one of the possible control steps, namely, the interaction between apoB mRNA–ribosome complex and its hypothetical translocation channel on the surface of rER, with the hope that we may uncover some intrinsic properties of the process that modulates apoB production. We have constructed a model to study the conditions under which the search for apoB translocation channels could be an important determinant for the efficiency of translocation. Computer simulations were performed to assess the rate of protein production with respect to parameters that are potentially important for its control, including the number of apoB mRNA molecules, the quantity of translocation channels, the duration of polypeptide chain elongation, and the degree of apoB mRNA editing. Our findings may have important implications for the regulation of apoB production and our understanding of cholesterol homeostasis and for secretory protein production in general.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; rER, rough endoplasmic reticulum; SRP, signal peptide recognition particle.

[1]To whom correspondence should be addressed.

METHODS

Theory

ApoB translocation across the rER membrane is a prerequisite for its production and the assembly and secretion of VLDL (19–21). There is strong evidence to suggest that the translocation of apoB is a cotranslational process (19, 22, 23), and probably involves some specific proteins on the rER. The docking protein for the recognition of ribosome with rER (24–27) and an aquatic channel on the surface of rER may also be part of its translocation machinery (28). A schematic drawing of apoB translation and translocation on the surface of rER is presented in **Fig. 1** which may also serve as an example for the subset of secretory proteins that are cotranslationally translocated. Translation of apoB mRNA occurs on ribosomes, and apoB synthesis is initiated in the N-terminus producing the signal peptide that binds to the signal peptide recognition particle (SRP) in the cytoplasm. The whole complex (mRNA–ribosome–SRP) then touches down on the surface of rER and starts the search for pre-existing translocation channels. Once the complex "finds" a channel, translation is resumed (25, 27) coincident with translocation of the nascent apoB polypeptide. Upon completion of the polypeptide chain, mRNA and ribosomes dissociate from rER surface and the translocation channel is then available for another round of protein synthesis and translocation. One of the control steps in apoB translation and translocation may be the search for and/or the assembly of the translocation channel. This step contrasts with the post-translational events such as lipoprotein assembly or intracellular degradation which may be a regulated process in response to various physiological manipulations. The model we will be exploring deals exclusively with an initial part of apoB synthesis, i.e., the dynamic relationship of mRNA–ribosome complex with its hypothetical translocation channel; the steps subsequent to this interaction are not addressed in this study.

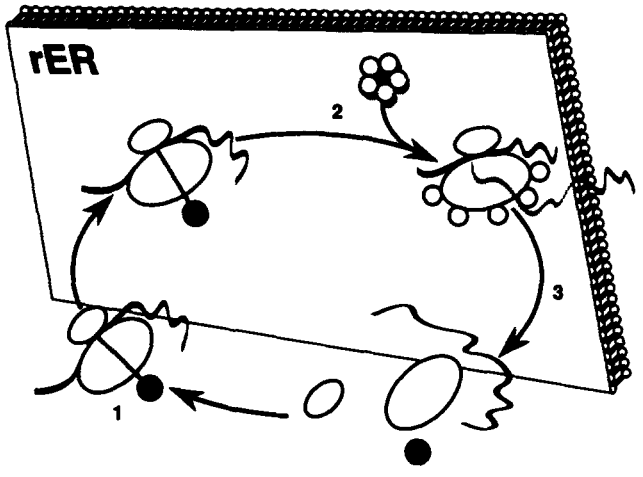

Fig. 1. Schematic diagram of apoB synthesis and translocation. The process of apoB synthesis/translocation is divided arbitrarily into three steps. Step 1 represents the binding of ribosome to mature apoB mRNA and the initiation of the synthesis of signal peptide, which is recognized by the SRP particle, and translation is on hold until the beginning of translocation; this complex then rapidly diffuses towards the surface of rER. Step 2 represents the random walk of mRNA–ribosome complex in search of translocation channel on the surface of rER. Step 3 represents apoB chain elongation and translocation and the subsequent dissociation of ribosome and mRNA from the rER surface, thus to start a new cycle of the whole process (flower, translocation channel; shaded circle, SRP).

Model design

The basic assumption is that the process by which an mRNA–ribosome complex searches for its translocation channel is diffusion-mediated. The surface area (A) of rER has been simplified as an N×N square (where N×N = A) with a periodic boundary (a torus) to mimic the continuity of the total cellular rER and to simplify our mathematical treatment. The channels and the complexes are randomly distributed on the entire surface of N×N lattice with each occupying a lattice point. The diffusion pattern of mRNA–ribosome complexes on the surface of rER and the collision of the complex with its channel are further simplified as a random walk on the N×N square lattice with every step the complex walks to one of its four nearest neighboring points with equal probability. The channels are held stationary (which is equivalent to assuming that both the complex and the channel are mobile, but with lower relative diffusion coefficients (29, 30)). When an mRNA–ribosome complex walks into the position of a channel, a successful binding of the complex to this channel occurs. The complex then occupies this channel for the period of time that is required for completion of the entire polypeptide chain while it is being translocated. Then, the channel will be available again for binding to new mRNA–ribosome complexes. In choosing the approach described, we have effectively eliminated some unknown parameters such as the effective collision area of the complex and channel, the direction of collision, and the binding interaction of the complex with its channels. This approach serves as the basis for the simulation programming. An analytical solution of the number of mRNA–ribosome complexes bound to translocation channels at n steps is established, provided that n is smaller than the number of steps required to finish the polypeptide chain elongation.

Simulation procedure

Programs were written in Fortran 77 and are available upon request. Most of the simulations were performed on 100 × 100 lattices. From our analytical results, however, it is clear that there is little gain in using larger lattice sizes, because the number of complexes bound to their translocation channels at n steps is directly related to the ratio of the average number of distinctive positions visited

by a walker after n steps over the lattice size (see Appendix for detail).

RESULTS

Analytical solution

We only solved the problem of the number of successful binding of walkers (mRNA-ribosome complexes) to their channels at n steps with the initial condition of uniformly distributed walkers and the channels on the surface of rER, and provided that n is smaller than the number of steps required to complete the protein chain elongation and the translocation process. B(n) (the number of walkers that are bound to their translocation channels at n steps) is determined by the following functions:

$$B(n) = \sum_{i=1}^{w} P_i H_i \qquad \text{Eq. 1)}$$

$$P_i = \frac{\binom{i}{w}\left(\frac{S_n}{N^2}\right)^i \left(1 - \frac{S_n}{N^2}\right)^{w-i}}{1 - \left(1 - \frac{S_n}{N^2}\right)^w} \qquad \text{Eq. 2)}$$

$$H_i = \begin{cases} W(iF_i + \sum_{j=1}^{i-1} jF_j') & (i < R) \\ R & (i \geq R) \end{cases} \qquad \text{Eq. 3)}$$

Where P_i is the probability of the lattice point visited by i number of different walkers; H_i is the average number of channels bound with walkers on the lattice points that have been visited by i number of different walkers; F_i is the probability that at least i number of channels are at the lattice points that have been visited by i different walkers; F_j' is the probability that j number of channels are at the lattice points that have been visited by j number of different walkers; W is the number of walkers; R is the number of translocation channels that are within the lattice points visited by W number of different walkers at n steps; and S_n is the average of distinctive lattice points visited by a walker after n steps (see detail in Appendix).

Therefore, B(n) is solely determined by the number of channels and the complexes, the size of the lattice, the duration of translation, and the number of steps the complexes have walked. If the number of channels and complexes are to remain constant, then B(n) is determined by $S_n/N \times N$. This is the basis for our scaled-down simulations to avoid the use of huge numbers of $N \times N$ and n and the realistic condition to save computing time.

Simulation

a) The analytical solution shows a good match with the results of simulation up to about 500 steps but deviates slightly thereafter (**Fig. 2**). The reason for the deviation may be that, with the analytical approach, we have assumed that channels are uniformly (instead of randomly, as in simulation) distributed on the entire surface of rER, thus maximizing the probability of collision with walkers in the long term.

b) The total number of translocation channels on the surface of rER correlates well with the efficiency of binding. For a constant number of mRNA-ribosome complexes, the total number of translocation channels determines the time the system takes to achieve its full capacity (**Fig. 3**).

c) Only a fraction of the total complexes is bound to their translocation channels at quasi-steady state. Simulations were performed at equal numbers of walkers and channels with respect to different lengths of polypeptide chain. The number of ribosome-mRNA complexes bound with their translocation channels at quasi-steady state with different lengths of hypothetical polypeptide chain is shown in **Fig. 4A.** When the simulation steps reached the time that was required for the duration of chain elongation, an expected sharp drop in the bound complexes was observed which represents the start of the re-opening of translocation channels to other complexes. Note that under these conditions, for duration of chain elongation that varies from 1000 to 3000 steps, the number of bound complexes is almost indistinguishable.

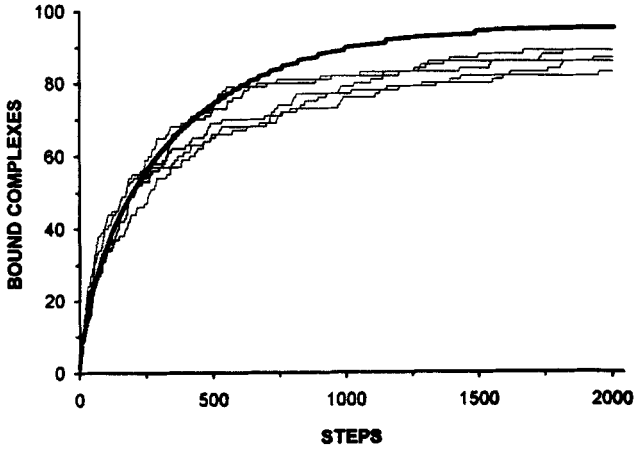

Fig. 2. Comparison of analytical and simulation results. The parameters used are: 100 mRNA-ribosome complexes, 100 translocation channels, with the surface area of rER as 100×100. The X axis represents the number of steps taken by the complexes, and the Y axis represents the number of mRNA-ribosome complexes bound to translocation channels. The heavy line is the analytical solution of the number of bound complexes at each step, while the thin lines represent the results of five different simulations.

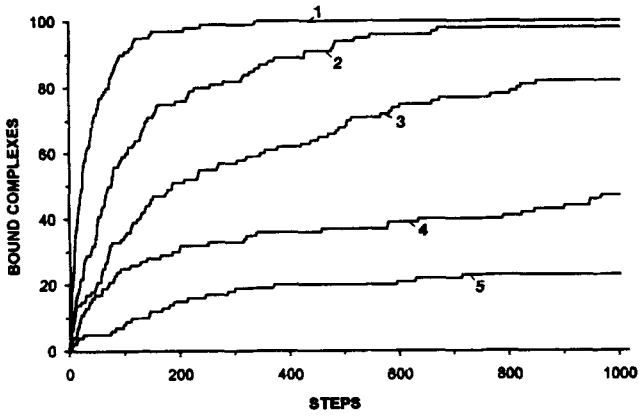

Fig. 3. Effect of total number of channels on the efficiency of binding of complexes to the channels. Simulations were performed under the conditions of 100 mRNA-ribosome complexes, 100×100 lattice size of rER equivalent area, and the number of translocation channels from top to bottom at 500 (line 1); 250 (line 2); 100 (line 3); 50 (line 4); and 25 (line 5). The X axis is the number of steps taken by the complexes, and the Y axis is the number of bound mRNA-ribosome complexes.

It is clear that for proteins with 100 to 1000 amino acid residues (the range of most secretory proteins), the number of bound complexes at quasi-steady state is proportional to the length of polypeptide chain (Fig. 4B). As the time (or number of steps taken) that is required to finish the individual polypeptide chain is also proportional to its length (assuming a constant rate of polypeptide chain elongation), then the overall number of molecules synthesized per unit time will be very similar for most secretory proteins that are cotranslationally translocated. Furthermore, most of the mRNA-ribosome complexes are not bound to their channels at any given moment of protein synthesis (Fig. 4B).

d) Simulation of apoB synthesis and translocation of a single human hepatocyte is shown in **Fig. 5.** Parameters for simulation are based on currently available experimental data (16–18) (see figure legend for detail). When the system achieves its quasi-steady state, only 40–60% of apoB mRNA-ribosome complexes have found their translocation channels. On average, for a single liver cell, there are about 58 apoB molecules being synthesized at any moment.

e) Scaled up simulations of apoB synthesis from 100×100 lattice size are shown in **Table 1.** At quasi-steady state, use of a 1:1 ratio of the mRNA-ribosome complexes versus translocation channels only slightly underestimates the bound complexes using a ratio of 1:500. Use of the 1:1 ratio saves considerable computing time. Another feature evident in this table is that the number of bound apoB mRNA-ribosome complexes with translocation channels at quasi-steady states is not very sensitive to several-fold variation of the number of total translocation channels (see the 316×316 groups in Table 1).

f) The degree of apoB mRNA editing has some interesting effects on the amount of apoB-100 and apoB-48 synthesized in a single rodent hepatocyte. In our simulation, two species of mRNAs (apoB-100 and apoB-48) are competing for the same translocation channels. The only difference is that the time of completion of an apoB-48 polypeptide chain is assumed to be 48% that of apoB-100, i.e., it converts to the equivalent of 875 steps for apoB-100 and 380 steps for apoB-48 in our 100×100 lattice system during the completion of one apoB-100 or one apoB-48 molecule, respectively. **Table 2** summarizes the collective effects of degree of editing (i.e., % of apoB mRNA that is in the apoB-48 form) on the number of apoB-100 and

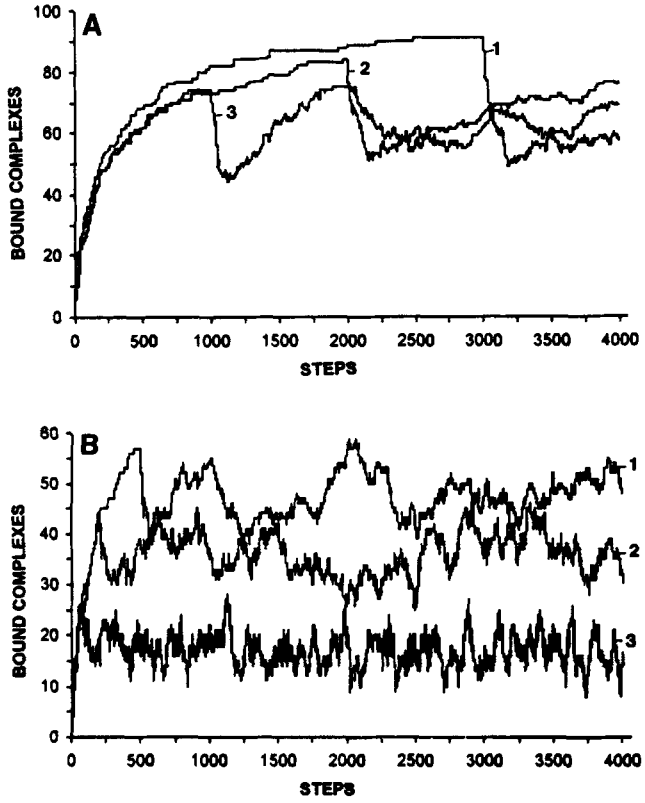

Fig. 4. Effect of the equivalent length of polypeptide chain on the number of bound complexes. The X axis represents the number of steps taken by the complexes, and the Y axis is the number of bound mRNA-ribosome complexes. Simulations up to 4000 steps were performed under the following conditions: 100×100 lattice size of rER equivalent area, 100 mRNA-ribosome complexes, 100 translocation channels, and different steps of a bound complex that will occupy the channels during the polypeptide chain elongation. Assuming that the rate of chain elongation is relatively constant for different proteins at 5 residues per second, then, as detailed in the legend of Fig. 5, 3000 steps (A, line 1) of a bound complex that will occupy the channels during the polypeptide chain elongation is equivalent to a polypeptide of 20,000 hypothetical amino acid residues. And so, iteratively, 2000 steps (A, line 2) is equivalent to a polypeptide of 10,000 amino acid residues, 1000 steps (A, line 3) is equivalent to 5000 amino acid residues, 500 steps (B, line 1) is equivalent to 3000 amino acid residues, 200 steps (B, line 2) is equivalent to 2500 amino acid residues, and finally 100 steps (B, line 3) is equivalent to 500 amino acid residues.

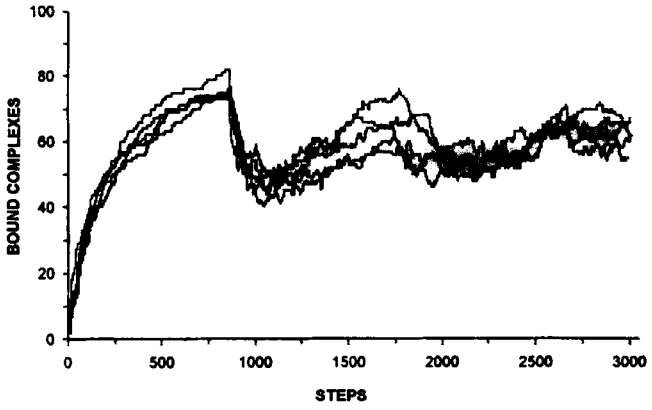

Fig. 5. Simulation of apoB-100 synthesis in a single human hepatocyte. The average kinetic energy of each molecule is determined by $mv^2/2 = kT/2$ (where m is the mass of the molecule, v the velocity, k the Boltzman constant, and T the absolute temperature). With a combined molecular weight of 9.7×10^6, the average velocity of apoB mRNA-ribosome complex is about 50 cm/sec. Taking the diffusion coefficient ($D = 1 \times 10^{-9} cm^2/sec$) of a typical membrane-bound protein as the D of the mRNA-ribosome complex (41), the average of the free distance (d) the complex can diffuse and the time for each step (t) are then defined by $d = 2D/v$ and $t = 2D/v^2$ respectively (42). Then, for the duration of 900 seconds of apoB-100 chain elongation (2), it takes the equivalent of about 1.13×10^{15} steps. Taking $2.5 \times 10^{-3} cm^2$ as the total area of liver rER (35, 36) and assuming the ratio of available translocation channels and apoB mRNA-ribosome is about 500 to 1 (26), then an area of $5 \times 10^{-6} cm^2$ rER will have about 100 translocation channels for apoB mRNA-ribosome complexes. From here we scale down to 100×100 lattice, and the value of $S_n/N \times N$ at 900 seconds for apoB-100 in a hepatocyte is equivalent to the value of $S_n/N \times N$ 857 steps simulation in 100×100 lattice. The simulation conditions are chosen for 100 mRNA-ribosome complexes, 100 translocation channels, lattice size of 100×100, and the steps that required to finish one round of chain elongation is 857. Five simulations were performed up to 3000 steps. The X axis represents the number of steps taken by the complexes, and the Y axis represents the number of mRNA-ribosome complexes bound to translocation channels.

apoB-48 molecules being synthesized on the surface of the rER. Note that the percentage of apoB-48 molecules being synthesized is significantly lower than expected from the percentage of editing, and the total number of apoB molecules (apoB-100 plus apoB-48) being synthesized decreases with increasing proportion of edited mRNA.

DISCUSSION

The mechanism that controls protein translocation is a complex process that involves multiple steps and the interaction of numerous protein components of the rER (27); however, the translocation machinery could be universal for all secretory proteins (31). Some specific features of protein translocation such as that of "pause transfer" phenomenon in apoB (32, 33) indicate a unique interaction between the elongating polypeptide chain and its translocation channels. If the translocation machinery or the translocation channels were unique for a certain subset of proteins (e.g., apoB), the ratio of translocation channels versus mRNA-ribosome complexes should remain high; otherwise, the protein production or translocation could be severely restricted. As shown in Fig. 5, even at 1:500 ratio, which probably is an overestimate (26, 28, 34–36), one surprising prediction from this model is that the majority of the mRNA-ribosome complexes of secretory proteins that are being cotranslationally translocated on the surface of rER are not bound to their translocation channels, but just "idling" around. This does not mean that the idling complexes do not contribute to protein synthesis and translocation. In fact, every single complex contributes equally to enhancing the probability of finding their channels collectively. On the other hand, if the translocation channels are universal to most proteins, then, as shown in Fig. 3, five- to ten-times more channels versus mRNA-ribosome complexes could easily saturate the system.

The assumption that the mRNA-ribosome complexes that are idling on the surface of rER are not synthesizing apoB protein until they successfully interact with their translocation channels is a logical consequence of the action of SRP, which will slow down or stop translation until the complexes touch down on the surface of rER (25–27) and successfully interact with translocation machinery, thus minimizing the possibility of premature synthesis of the protein that may become non-translocatable. It is clear that this model is only suitable for secretory proteins that are cotranslationally translocated. Although translocation has been shown to be independent of, or uncoupled from, the process of translation for certain secretory proteins or membrane-bound proteins (36), these proteins seem to represent a small subset of secretory proteins that either have short peptide chain length or do not require the participation of membrane for its folding or assembly (36, 37). It is also possible that some apoB mRNA-ribosome complexes escape the translocation constraint and

TABLE 1. Effect of simulation scale on the bound apoB-100 mRNA-ribosome complexes at quasi-steady state

Lattice Size	Total Number of Complexes	Total Number of Translocation Channels	C/T[a]	Bound Complexes[b]
100^2	100	100	1:1	61
142^2	100	200	1:2	65
224^2	100	500	1:5	70
316^2	100	200	1:2	47
316^2	100	500	1:5	65
316^2	100	1,000	1:10	73
1000^2	100	10,000	1:100	74
2236^2	100	50,000	1:500	75

[a]C/T is the ratio of total number of ribosome-mRNA complexes versus total translocation channels in simulation.

[b]The value of bound complexes at quasi-steady state is the average over the value from step 1000 to step 3000 of a single simulation.

TABLE 2. Effects of the degree of apoB mRNA editing on the synthesis of apoB-100 and apoB-48 in a single rodent hepatocyte, assuming that regulation is exerted solely at the level of mRNA–ribosome complex interaction with its translocation channel[a]

Editing (%)	ApoB-100 Bound Complexes	ApoB-48 Bound Complexes	Total Bound Complexes	% ApoB-48 Bound Complexes	Rate of ApoB-100 (# per h)	Rate of ApoB-48 (# per h)	Total Rate of ApoB (# per h)	% ApoB-48 Molecules Produced
0	61	0	61	0	244	0	244	0
10	54	3	57	5.3	216	25	241	10.3
20	49	6	55	11	196	50	246	20.3
30	45	9	54	16.7	180	75	255	29.4
40	36	16	52	31	144	133	277	48
50	32	18	50	36	128	150	278	54
60	26	23	49	47	104	192	296	65
70	22	26	48	54	88	217	305	71
80	14	33	47	70	56	275	331	83
90	7	39	46	84	28	325	353	92
100	0	44	44	100	0	367	367	100

Five simulations were performed for each % of editing. The average number of apoB-100 and apoB-48 mRNA–ribosome complexes that are engaged in polypeptide chain elongation on the surface of rER at quasi-steady state and the integrated rate of apoB-100 and apoB-48 production are presented. The parameters used are as follows: 100 total (apoB-48 and apoB-100) number of apoB mRNA–ribosome complexes, 100 translocation channels, and lattice size of 100 × 100. Under these conditions, a total of 857 steps is required for the duration needed to complete chain elongation for apoB-100 and 380 steps for apoB-48 (see legend of Fig. 5 for the derivation of these numbers).

[a]Undoubtedly other sites of regulation exist, but are not addressed in this computation.

start chain elongation on the surface of rER without interaction with translocation channel itself, or some translocating apoB molecules through local vibration may escape the constraint of the translocation channels (31), rendering them only partially translocated. The apoB molecules that are only partially translocated without further interaction with translocation channels may represent an intrinsic property of the system and may contribute to be a part of the pool of apoB molecules that are degraded intracellularly (21, 22).

This model also predicts that under the same conditions (same relative concentration of mRNA, and sharing the same translocation channels), the number of polypeptide chains synthesized per unit time for most secretory proteins should be very similar irrespective of the size of the proteins, although more time is needed to complete the synthesis of the larger ones. This is because of the diffusion nature of the interaction between the complexes and the translocation channels (30), and because the longer the polypeptide chain, the longer the ribosome complexes that will occupy the translocation channels during polypeptide chain elongation, and more of the mRNA–ribosome complexes are bound to their channels.

ApoB-100 and apoB-48 provide a unique system to examine some of the possibilities stated above. As the mRNAs of these two proteins differ only in a single base, it is reasonable to expect that their translation and translocation properties are very similar, and that they share the same translocation channels. Thus, a specific prediction of this model as to the relative amount of apoB-100 and apoB-48 on the surface of rER under different degrees of editing could be obtained (Table 2). The disparity between apoB-100 and apoB-48 in the amount of membrane-associated apoB protein (38) probably represents a later, perhaps post-translocational event of the regulation of lipoprotein assembly and secretion.

The mechanism for the control of apoB synthesis and secretion is poorly understood (1, 2, 12, 15). It has been suggested that the control mechanism depends on the sorting of the newly synthesized apoB molecule and its assembly in lipoprotein particles (19). It is apparent that a substantial amount of apoB synthesized is degraded intracellularly (20, 21). It is, however, unclear whether the intracellular degradation serves as a regulating mechanism of apoB secretion or as a "quality control" mechanism that removes molecules that are not properly folded or assembled (19, 20, 22). It is possible that the control of apoB synthesis and secretion may also be partly regulated on the surface of rER; our simulations on the early event of the interaction of apoB mRNA–ribosome complexes with its translocation showed that the rate of synthesis could be affected by the property and/or the quantity of translocation channels.

There are some unique features in apoB that require special consideration in our model. In the case of apoB, translocation may be a discontinuous process that involves "pause-transfer" sequences that represent specific interaction of apoB polypeptide chain with its translocation machinery (33). However, as long as the "pauses" are transitory compared with the time required for elongation of the entire apoB polypeptide, the binding profile of translocation channels and apoB mRNA–ribosome complexes and the integrated rate of apoB production will not change significantly. If, indeed, the pauses during translocation for apoB-100 and apoB-48 are very long, then from Fig. 4, it is apparent that the number of bound apoB-100

mRNA–ribosome complexes at steady state will be almost identical to that in the absence of any pause, but the number of bound complexes of apoB-48 will almost double. Contrary to the effect of long pauses on the number of bound complexes, the consequences for the overall rate of apoB production for apoB-100 and apoB-48 are in the opposite direction in that apoB-100 production will be reduced to half and apoB-48 production will remain the same compared to the situation where there is no pause. The different effects of "very long" pauses for apoB-100 and apoB-48 or other shorter apoBs (produced in vitro) on their synthesis profile and their binding to translocation channels on the surface of rER predicted from this model can be tested by mutating the "pause-transfer" sequences within the same experimental system.

The possibility of apoB secretion being regulated at the level of translation may minimize the apparent futile cycle of synthesis and degradation. However, subsequent steps in apoB assembly may also be important, as in the case of abetalipoproteinemia, where the impairment of secretion may result from the malfunctioning of the assembly and secretory machinery (39).

In summary, we have presented a square lattice model for an early event in apoB synthesis. This model is applicable to secretory proteins in general, but is especially interesting in terms of apoB biosynthesis, not only because of the unusually large size of apoB, but also because of the unique phenomenon of apoB mRNA editing that allows the production of two proteins of vastly different sizes from two almost identical mRNAs. Some of the predictions from this model can be explored in future experiments on the regulation of apoB synthesis in various experimental systems.

APPENDIX

From Montroll's original study of random walk on lattices (40), we can deduce the following iterative formulas to calculate the average distinctive lattice points visited by a walker on a N×N square lattice with periodic boundary condition (S_n):

$$S_n = 1 + \Delta_1 + \Delta_2 + \cdots + \Delta_n \qquad \textit{Eq. A-1)}$$

Where

$$\Delta_n = 1 - \frac{1}{N^2}\left(\sum_{i=1}^{n-1} \Delta_{n-1} b_i\right) \qquad (\Delta_1 = 1) \qquad \textit{Eq. A-2)}$$

And

$$b_i = \left(\sum_{k=0}^{N-1}\left(\cos\frac{2k\pi}{N}\right)^i\right)^2 \qquad \textit{Eq. A-3)}$$

Thus, we have S_t, the total average number of distinctive lattice points visited collectively by W, number of walkers, at n steps.

$$S_t = N^2\left(1 - \left(1 - \frac{S_n}{N^2}\right)^w\right) \qquad \textit{Eq. A-4)}$$

Then, the average number of channels that will be included in those lattice points is

$$R = R_t\left(1 - \left(1 - \frac{S_n}{N^2}\right)^w\right) \qquad \textit{Eq. A-5)}$$

Where R_t is the total number of channels on the N×N lattice.

Following the same argument of equation A-4, the average total number of lattice points that have been visited by i number of walkers S_i is then determined by

$$S_i = N^2\binom{i}{w}\left(\frac{S_n}{N^2}\right)^i\left(1 - \frac{S_n}{N^2}\right)^{w-i} \qquad \textit{Eq. A-6)}$$

Thus, P_i, the probability of the distinctive lattice sites visited by i number of different walkers is

$$P_i = \frac{\binom{i}{w}\left(\frac{S_n}{N^2}\right)^i\left(1 - \frac{S_n}{N^2}\right)^{w-i}}{1 = \left(1 = \frac{S_n}{N^2}\right)^w} \qquad \textit{Eq. A-7)}$$

For a lattice domain that has been visited by i different walkers, the average number of channels bound to the walkers is determined by the probability of at least i number of channels is within this domain F_i (with a weight of i), and the probability of j (j<i) number of channels is within this domain F_j' (carrying a weight of j), then the total average number of channels being occupied by walkers in the lattice points that have been visited by i number of different walkers is

$$H_i = \begin{cases} W(iF_i + \sum_{j=1}^{i-1} jF_j') & (i < R) \\ R & (i \geq R) \end{cases} \qquad \textit{Eq. A-8)}$$

Where

$$F_i = \sum_{j=i}^{R}\binom{j}{R}\left(\frac{1}{W}\right)^j\left(1 - \frac{1}{w}\right)^{R-j} \qquad (i = 1,2,\ldots.R) \qquad \textit{Eq. A-9}$$

$$F_j' = \binom{j}{R}\left(\frac{1}{W}\right)^j\left(1 - \frac{1}{w}\right)^{R-j} \qquad (i = 1,2,\ldots.R) \qquad \textit{Eq. A-10}$$

Thus, when we combine equations A-7 and A-8, the average number of walkers (mRNA–ribosome complexes) that are bound to their translocation channels at n steps B(n) is

$$B(n) = \sum_{i=1}^{w} P_i H_i \qquad \textit{Eq. A-11)}$$

Then we know, from these serial equations that the only determinant of B(n) is the ratio of S_n and N×N for a fixed number of W, R_t, and n. This provided us with a great convenience for simulating very large values of n and N×N as long as we kept tracking the value of S_n/N×N.

This work was supported in part by NIH grant HL27341. We thank Sally Tobola for assistance with tables and one of us (L. Chen) is grateful for the mathematical and programming advice provided by Kaining Wang and Medea Q. Meng.

Manuscript received 5 May 1993 and in revised form 27 July 1993.

REFERENCES

1. Kane, J. P. 1983. Apolipoprotein B: structure and metabolic heterogeneity. *Annu. Rev. Physiol.* **45:** 637–650.
2. Olofsson, S-O., G. Bjursell, K. Boström, P. Carlsson, J. Elovson, A. A. Protter, M. A. Reuben, and G. Bondjers. 1987. Apolipoprotein B: structure, biosynthesis and role in the lipoprotein assembly process. *Atherosclerosis.* **68:** 1–17.
3. Yang, C-Y., S-H. Chen, S. H. Gianturco, W. A. Bradley, D. A. Sparrow, H. Deloof, M. Rosseneu, F-S. Lee, Z-W. Gu, A. M. Gotto, Jr., and L. Chan. 1986. Sequence, structure, receptor-binding domain and internal repeats of human apolipoprotein B-100. *Nature.* **323:** 738–742.
4. Sparks, J. D., and C. E. Sparks. 1985. Apolipoprotein B and lipoprotein metabolism. *Adv. Lipid Res.* **21:** 1–46.
5. Elovson, J., Y. O. Huang, N. Baker, and R. Kaman. 1981. Apolipoprotein B is structurally and metabolically heterogenous in rat. *Proc. Natl. Acad. Sci. USA.* **78:** 157–161.
6. Chen, S-H., G. Habib, C-Y. Yang, Z-W. Gu, B. R. Lee, S-A. Weng, S. R. Silberman, S-J. Cai, J. P. Deslypere, M. Rosseneu, A. M. Gotto, Jr., W-H. Li, and L. Chan. 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science.* **238:** 363–366.
7. Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein B-48 in intestine. *Cell.* **50:** 831–840.
8. Hamilton, R. L., A. Moorehouse, and R. J. Havel. 1991. Isolation and properties of nascent lipoproteins from highly purified rat hypatocytic Golgi fractions. *J. Lipid Res.* **32:** 529–543.
9. Cladaras, C., M. Hadzopoulou-Cladaras, R. T. Nolte, D. Atkinson, and V. L. Zannis. 1986. The complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48 forms. *EMBO J.* **5:** 3495–3507.
10. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
11. Fuki, I. V., S. N. Preobrazhensky, A. Y. Misharin, N. G. Bushmakina, G. B. Menschikov, V. S. Repin, and R. S. Karpov. 1989. Effect of cell cholesterol content on apolipoprotein B secretion and LDL receptor activity in human hepatoma cell line, HepG2. *Biochim. Biophys. Acta.* **1001:** 235–238.
12. Pullinger, C. R., J. D. North, B-B. Teng, V. A. Rifici, A. E. Ronhild de Brito, and J. Scott. 1989. The apolipoprotein B gene is constitutively expressed in HepG2 cells: regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J. Lipid Res.* **30:** 1065–1077.
13. Sparks, C. E., J. D. Sparks, M. Bolognino, A. Salhanick, P. S. Strumph, and J. M. Amatruda. 1986. Insulin effects on apolipoprotein B lipoprotein synthesis and secretion by primary cultures of rat hepatocytes. *Metabolism.* **35:** 1128–1136.
14. Gibbons, G. F. 1990. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* **268:** 1–13.
15. White, A. L., D. L. Graham, J. LeGros, R. J. Pease, and J. Scott. 1992. Oleate-mediated stimulation of apolipoprotein B secretion from rat hepatoma cells. *J. Biol. Chem.* **267:** 15657–15664.
16. Srivastava, R. A. K., B. A. Pfleger, and G. Schonfeld. 1990. Expression of LDL receptor, apolipoprotein B, apolipoprotein A-I and apolipoprotein A-IV mRNA in various mouse organs as determined by a novel RNA-excess solution hybridization assay. *Biochim. Biophys. Acta.* **1090:** 95–101.
17. Dashti, N. 1992. The effect of low density lipoproteins, cholesterol, and 25-hydroxycholesterol on apolipoprotein B gene expression in HepG2 cells. *J. Biol. Chem.* **267:** 7160–7169.
18. Williams, K. J., R. W. Brocia, and E. A. Fisher. 1990. The unstirred water layer as a site of control of apolipoprotein B secretion. *J. Biol. Chem.* **265:** 16741–16744.
19. Borén, J., M. Wettesten, A. Sjöberg, T. Thorlin, G. Bondjers, O. Wiklund, and S-O. Olofsson. 1990. The assembly and secretion of apoB-100-containing lipoproteins in HepG2 cells. Evidence for different sites for protein synthesis and lipoprotein assembly. *J. Biol. Chem.* **265:** 10556–10564.
20. Davis, R. A., R. N. Thrift, C. C. Wu, and K. E. Howell. 1990. Apolipoprotein B is both integrated into and translocated across the endoplasmic reticulum membrane: evidence for two functionally distinct pools. *J. Biol. Chem.* **265:** 10005–10011.
21. Furukawa, S., N. Sakata, H. N. Ginsberg, and J. L. Dixon. 1992. Studies of the sites of intracellular degradation of apolipoprotein B in HepG2 cells. *J. Biol. Chem.* **267:** 22630–22638.
22. Borén, J., L. Graham, J. Wettesten, J. Scott, A. White, and S-O. Olofsson. 1992. The assembly and secretion of apoB-100-containing lipoproteins in HepG2 cells. ApoB-100 is cotranslationally integrated into lipoproteins. *J. Biol. Chem.* **267:** 9858–9867.
23. Pease, R. J., G. B. Harrison, and J. Scott. 1991. Cotranslational insertion of apolipoprotein B into the inner leaflet of the endoplasmic reticulum. *Nature.* **353:** 448–450.
24. Gilmore, R., P. Walter, and G. Blobel. 1982. Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the Signal Recognition Particle receptor. *J. Cell Biol.* **95:** 470–477.
25. Walter, P., and G. Blobel. 1981. Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes. *J. Cell Biol.* **91:** 557–561.
26. Gorkich, D., E. Hartmann, S. Prehn, and T. A. Rapoport. 1992. A protein of the endoplasmic reticulum involved early in polypeptide translocation. *Nature.* **357:** 47–52.
27. Sanders, S. L., and R. Schekman. 1992. Polypeptide translocation across the endoplasmic reticulum membrane. *J. Biol. Chem.* **267:** 13791–13794.
28. Simon, S. M., and G. Blobel. 1991. A protein-conducting channel in the endoplasmic reticulum. *Cell.* **65:** 371–380.
29. Keizer, J. 1981. Effect of diffusion on reaction rates in solution and in membranes. *J. Phys. Chem.* **85:** 940–941.
30. Torney, D. C., and H. M. McConnell. 1983. Diffusion-limited reaction rate theory for two-dimensional systems. *Proc. R. Soc. (London)* **A387:** 147–170.
31. Simon, S. M., C. S. Peskin, and G. F. Oster. 1992. What drives the translocation of proteins? *Proc. Natl. Acad. Sci. USA.* **89:** 3770–3774.
32. Chuck, S. L., Z. Yao, B. D. Blackhart, B. J. McCarthy, and V. R. Lingappa. 1990. New variation on the translocation of proteins during early biogenesis of apolipoprotein B. *Nature.* **346:** 382–385.
33. Chuck, S. L., and V. R. Lingappa. 1992. Pause transfer: a topogenic sequence in apolipoprotein B mediates stopping and restarting of translocation. *Cell.* **68:** 9–21.

34. Loud, A. V. 1968. A quantitative stereological description of the ultrastructural of normal rat liver parenchymal cells. *J. Cell Biol.* **37:** 27–46.
35. Paumgartner, D., G. Losa, and E. R. Weibel. 1981. Resolution effect on the stereological estimation of surface and volume and its interpretation in terms of fractal dimensions. *J. Microsc.* **121:** 51–63.
36. Müller, G., and R. Zimmermann. 1987. Import of honeybee prepromelittin into the endoplasmic reticulum: structural basis for independence of SRP and docking protein. *EMBO J.* **6:** 2099–2107.
37. Perara, E., R. Rothman, and V. R. Lingappa. 1986. Uncoupling translocation from translation: implications for transport of protein across membranes. *Science.* **232:** 348–352.
38. Sparks, J. D., and C. E. Sparks. 1990. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *J. Biol. Chem.* **265:** 8854–8862.
39. Wetterau, J. R., L. P. Aggerbeck, M-E. Bouma, C. Eisenberg, A. Munck, M. Hermier, J. Schmitz, G. Gay, D. J. Rader, and R. E. Gregg. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science.* **258:** 999–1001.
40. Montroll, E. W. 1964. Random walk on lattices. *Proc. Symp. Appl. Math. Am. Math. Soc.* **16:** 193–220.
41. McCloskey, M., and M-M. Poo. 1984. Protein diffusion in cell membranes: some biological implications. *Int. Rev. Cytol.* **87:** 19–81.
42. Berg, H. C. 1983. Random Walks in Biology. Princeton University Press, Princeton, NJ. 11–16.