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What drives the translocation of proteins?

(Brownian motion/heat shock proteins/chaperonins/model)

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ABSTRACT **We propose that protein translocation across membranes is driven by biased random thermal motion. This "Brownian ratchet" mechanism depends on chemical asymmetries between the cis and trans sides of the membrane. Several mechanisms could contribute to rectifying the thermal motion of the protein, such as binding and dissociation of chaperonins to the translocating chain, chain coiling induced by pH and/or ionic gradients, glycosylation, and disulfide bond formation. This helps explain the robustness and promiscuity of these transport systems.**

Many proteins and ribonucleoproteins, either during or after their synthesis, translocate into or across cellular membranes. A signal in the protein's sequence—the signal sequence—is both necessary and sufficient to target a protein for transport across a particular membrane and this targeting requires specific cytosolic factors. In contrast, little is known about how RNAs or complexes of RNAs and proteins are targeted to and transported across the nuclear envelope. Morphological and functional evidence indicate that both proteins and RNAs are transported through aqueous pores in the nuclear envelope (1–3). Recent evidence also suggests that proteins translocate across the endoplasmic reticulum (ER) through an aqueous translocation pore (TP) (4).

Translocated proteins may be hydrophobic or positively or negatively charged. Thus the translocation machinery must be quite promiscuous. There are no studies on the rates of protein translocation, but we can put some upper limits on the process. In yeast, the time between a protein's synthesis and its exocytosis can be <5 min. This suggests that translocation occurs in under a minute, perhaps much faster.

Translocation requires a driving force, but what could fulfill the apparently contradictory thermodynamic requirements of being powerful, fast enough, and nonspecific? One answer is Brownian motion. At room temperature, there is $\approx 0.5 k_B T = 4.1 \times 10^{-14}$ erg (1 erg = 0.1 μJ) per degree of freedom (where k_B is the Boltzmann constant and T is the absolute temperature), sufficient to keep a macromolecule in violent thermal motion. The second law of thermodynamics assures us that the energy in these random motions is unavailable to perform useful work, such as driving the protein through the TP. Feynman *et al.* (5) showed that a "Brownian ratchet" can use thermal fluctuations to perform directed work given a temperature gradient. There are no significant temperature gradients within a cell, but chemical reactions can play a similar role (6, 7). That is, different reactions on the cis or trans sides of a membrane (with respect to the direction of translocation) can bias the Brownian movements of the translocating chain. In this report, we demonstrate this by constructing a mathematical model of the translocation process. Thermodynamics sets limits on what is energetically possible but cannot address the mechanistic question of how to convert this energy into directed motion; for this we must turn to molecular mechanics.

The Model

We examine the post-translational translocation of a protein from the cis to trans side of a membrane addressing the process that begins after an initial tip (or loop) is threaded through the channel—a separate physical process we shall discuss elsewhere. To traverse the TP, a protein must be in an unfolded conformation. Brownian motion will cause the protein to fluctuate back and forth through the TP but with no net displacement. But if, upon emerging from the TP, a protein is modified in such a way that it cannot reenter the pore, then its random walk will be biased. For example, when a nascent chain is glycosylated, it cannot reenter the TP. Thus it will reptate—"move like a snake" (8)—until it fully translocates across the membrane. The more closely spaced the ratcheting sites are, the faster is the movement across the membrane. The model rests on two assumptions. (*i*) The protein is unfolded and free to reptate back and forth through the TP. (*ii*) Chemical asymmetries (specified below) rectify the protein's movements. Both assumptions are strongly supported by experimental data.

Several observations indicate a translocating polypeptide is free to reptate back and forth. (*i*) Upon release from cytosolic ribosomes, nascent polypeptides traverse the membrane and enter the lumen of the ER (9). Thus, without the input of additional energy, the 40 amino acids of the polypeptide in the ribosome and 20 amino acids spanning the membrane freely traverse the bilayer. (*ii*) Translocating polypeptides are extracted from the membrane with mild conditions that leave the membrane intact (10). (*iii*) Releasing translocating chains from the membrane reveals the presence of large aqueous pores, presumably protein-conducting channels (4).

Several chemical asymmetries could bias the Brownian walk of a chain. As a polypeptide emerges from the translocation apparatus, often before much of the protein has been synthesized, the chain is subjected to glycosylation (11, 12), formation of disulfide bonds, binding of chaperonins, and cleavage of the signal sequence (which affects folding of the chain). Any, or all, can induce the asymmetry required for the Brownian ratchet.

Consider a protein in the process of translocating with one node ratcheted on the trans side (Fig. 1). The polymer's thermally driven random walk will eventually translate the free segment through the pore to the trans side of the membrane. Is this process fast enough to account for the observed rates of translocation? If a chain 100 nm long, with a diffusion coefficient of $D = 10^{-8}$ cm^2/sec, is allowed to diffuse freely, it will take a time $\tau \approx L^2/2D = 0.005$ sec to diffuse its own length (L). This assumes the chain is a structureless point mass; a more accurate estimate must take

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Abbreviations: TP, translocation pore; ER, endoplasmic reticulum.

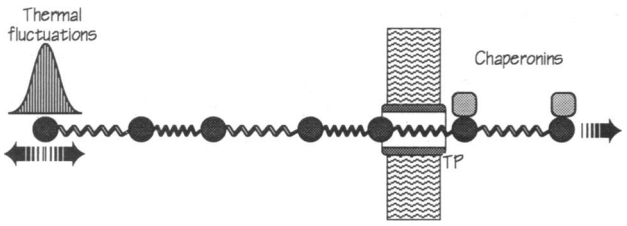

FIG. 1. One-dimensional model of a flexible protein chain diffusing through the TP. Each node experiences elastic forces from the neighboring nodes, random and viscous drag forces from the fluid surroundings, and, if a chaperonin is bound to the node, a repulsive force from the TP. The protein fluctuates back and forth until a chaperonin comes close enough to the pore-bound enzyme to be stripped from the chain. This allows a segment of the protein to diffuse through the pore. When the segment exits from the pore, it is bound by a lumenal chaperonin, preventing the segment from fluctuating backward.

into account the extended geometry of the chain, its accompanying elastic flexibility, the constraint of the pore on its entropic configurations, and the effects of the asymmetric factors enumerated above. To answer this we have constructed a computational model of the process.

We first treat the case where the ratchet is implemented by the binding of chaperonins (Fig. 1). We adopt a standard model for polymer dynamics by representing the protein by a chain of elastically linked subunits (13) that may be individual amino acids or larger segments. Each node of the model chain experiences four forces: viscous drag forces from the surrounding fluid, elastic forces from the neighboring nodes, repulsive forces of the membrane and the TP, and random forces due to the thermal environment. The equations describing the motion of the chain are derived by balancing the various forces that act on each node:

$$f_k(dX_k/dt) = F_k^{\text{elastic}}(X_{k-1}, X_k, X_{k+1}) + F_k^{\text{pore}}(X_k) + R_k \quad (k = 1, \ldots, N), \quad \textbf{[1a]}$$

where $f_k(dX_k/dt)$ is the frictional drag on kth node, $F_k^{\text{elastic}}(X_{k-1}, X_k, X_{k+1})$ are elastic forces on segment k from adjacent segments, $F_k^{\text{pore}}(X_k)$ is the pore force on segment k, R_k is the random force, N is the number of subunits in the protein, $X_k(t)$ is the position of the kth subunit at time t, and f_k is the friction coefficient of the kth subunit.

In Eq. **1a**, the pore force depends on whether a particular node is ratcheted-bound by a chaperonin on the trans side of the membrane. But as we discuss below, other modifications can effect a Brownian ratchet as well. These are incorporated into the equations by allowing each segment to have a bound chaperonin or not:

$$X_k^{\text{bound}} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} X_k^{\text{free}}. \quad \textbf{[1b]}$$

Transition rates are the binding and dissociation rate constants, k_+ and k_-, respectively (if chaperonins are abundant, rate constants are pseudo-first-order and $1/k_+ \propto$ mean time for a free segment to bind a chaperonin). Segments with a bound chaperonin have a higher friction coefficient, f_k, than free segments; more importantly, they interact with the translocation pore differently. The lipid bilayer is impermeable to the chain, and thus we can model the membrane as a very high energy barrier. The translocation pore allows free subunits—those with no chaperonin bound—to pass through the membrane but a subunit binding a chaperonin will see an energy barrier preventing its entry into the pore.

The solution of the model equations lets us compute the translocation rate (e.g., residues per sec) as a function of chain flexibility, differential coiling potentials, pore characteristics, and the kinetics of chain modification (chaperonin binding/dissociation, glycosylation, etc.)—a formible task for three-dimensional polymers of significant length. We detail these calculations elsewhere; below we describe a one-dimensional version of the model.

Conditions maximizing translocation rates are as follows: (*i*) ratcheting each node on the trans side, (*ii*) instantaneous chaperonin binding on the trans side, and (*iii*) instantaneous dissociation of chaperonins from the cis side upon reaching the TP. The computation begins with the chain just threaded through the pore and continues until the left-most node just clears the membrane (Fig. 1); this is the translocation time τ. We model the polypeptide as an elastic chain between 5 and 75 nm, with potential ratcheting sites at 5-nm intervals. Since the pattern of random forces always differs, the chain follows a different trajectory for each simulation. Repeated calculations yield a distribution of translocation times whose mean velocity and mean transit time, $\langle\tau\rangle$, we can compute by averaging (Fig. 2).

It is not known how many ratcheting sites are in each translocating chain. Translocation is slowest when sites are only at each end of the chain (Fig. 2*A*). Still, a 100-nm chain translocates in <3 msec. So, even if one site is ratcheted on the trans side, diffusion is sufficient to move proteins across

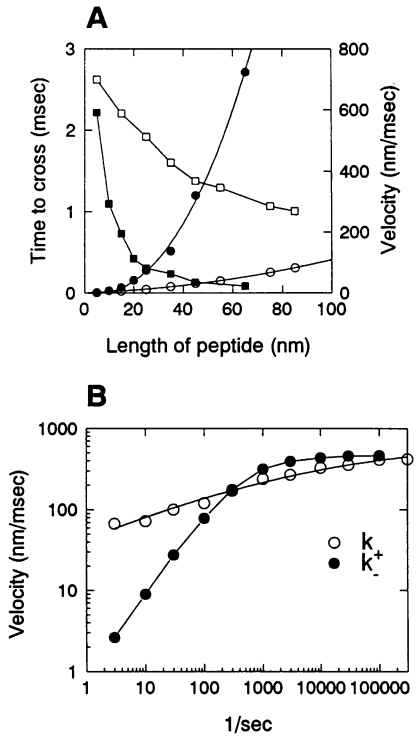

FIG. 2. Numerical solutions of the model shown in Fig. 1. (*A*) The mean translocation time $\langle\tau\rangle$ (circles) and mean velocity $\langle v\rangle \equiv L/\langle\tau\rangle$ (squares) are shown as a function of chain length. The results are plotted for simulations that assumed that either every node could be ratcheted (open symbols) or only the node farthest on the trans side was ratcheted (solid symbols). The open circles are fit with a quadratic, $\langle\tau\rangle \propto L^2$, and the solid circles are fit by a cubic, $\langle\tau\rangle \propto L^3$ (see discussion of Eq. 2). (*B*) The effects of chaperonin kinetics on translocation velocity were shown by varying the trans binding rate (k_+) or the cis dissociation rate (k_-). When $k_+ > 1000$ bindings per sec, the velocity is diffusion limited. In the simulations the following parameters were used: membrane thickness, 5 nm; pore repulsion range, 0.75 nm; chain elastic constant $\approx k_BT/\delta^2$, 1/25; friction coefficient, 1×10^{-7} dyne·sec/cm (1 dyne = 100 mN); Brownian force, $(fkT/\Delta t)^{1/2}$ dyne; integration step size, $\Delta t < 1 \times 10^{-7}$ sec.

the membrane faster than the minimum limit set by experimental observations. Ratcheting significantly speeds translocation (Fig. 2*A*) by a factor of about L/δ (where δ is the distance between ratcheting sites), as discussed below.

The calculations assumed chaperonin binding on the trans side was fast. But chaperonins are large (60–90 kDa) and may not bind very quickly to the nascent chain. We next investigate the effect of finite rates of attachment and detachment on translocation time by varying the binding rate constant k_+ over several orders of magnitude for a chain of $L = 45$ nm with ratcheting sites every $\delta = 5$ nm (Fig. 2*B*). At high binding rates, velocity is independent of k_+ since motion is limited by the polypeptide's diffusion. As the binding rate decreases, the ratchet has a smaller effect on accelerating translocation.

Since chaperonins cannot enter the TP, translocation velocity is limited by the rate they are stripped from the chain on the cis side. If chaperonin binding affinity was small enough to frequently free up a segment for diffusion into the TP, the chain could not be held in a linear configuration. Thus, we postulate a pore-associated enzyme (perhaps an ATP or GTP dissociating enzyme) that detaches the chaperonin from the chain. The maximum velocity corresponds to instantaneous cis dissociation and trans binding, so translocation is diffusion-limited. When the rate of cis dissociation falls below about 500 removals per sec, the velocity varies approximately linearly with k_-^{cis} (Fig. 2*B*).

The following formula is an approximate analytical solution describing the average translocation velocity, $\langle v \rangle$ (the derivation will be presented elsewhere):

$$\langle v \rangle = \left(\frac{2D}{\delta}\right)\left(\frac{k_+}{k_+ + 2k_-}\right) = \left(\frac{2D}{\delta}\right)\left(\frac{1}{1 + 2K_d}\right), \quad [2]$$

where $K_d = k_-/k_+$. The assumptions behind Eq. 2 are as follows: the rod is rigid;§ chaperonins exist on the trans side only (or are enzymatically removed rapidly at the cis side of the TP); and reaction rates k_+ and k_- are fast. (The approximation in Eq. 2 improves as the rates increase.) Note the velocity of translocation increases as δ decreases. When $k_+ >> k_-$, the velocity becomes simply $\langle v \rangle = 2D/\delta$, so average translocation time is given by $\langle \tau \rangle \approx L/\langle v \rangle = L\delta/2D$. Generally, D varies inversely with size, so we expect $\langle \tau \rangle \sim L^2\delta$. If chaperonins bind only at the ends of the chains ($\delta = L$), then $\langle \tau \rangle \sim L^3$. Numerical simulations in Fig. 2*A* show this dependence.

These results put quantitative limits on translocation times. The slowest time is when chaperonins bind only at the ends of the chain. By taking $\delta \approx 100$ nm and $D \approx 10^{-8}$ cm^2/sec, the translocation time is 5 msec; but if $\delta = 5$ nm, the transit time is 0.25 msec—faster by a factor of 20. This estimate of τ is probably too short, since our one-dimensional calculation cannot account for chain coiling. Nevertheless, numerical and analytical calculations show the ratchet mechanism is more than sufficient to account for the observed rates of translocation.

Other Brownian Ratchet Mechanisms. This thermal ratchet model depends on the asymmetry of chaperonin binding to the translocating chain. But a number of chain modifications occur cotranslationally that can bias thermal reptation. Any ligand that binds differently on the two sides of the membrane will bias reptation. For example, if chaperonin concentration differs on the cis and trans sides of the membrane, differential binding equilibria bias reptation toward the side with the higher concentration. Moreover, the lumenal space of the ER contains enzymes that glycosylate many proteins inhibiting their backward fluctuations and rectifying their Brownian movements.

If the chain coils more tightly in the cisternal space than in the cytoplasm, this ratchets thermal motions, independent of ligand binding. Virtually all biological polymers carry fixed charges—usually negative—that affect their degree of coiling (14). If the ionic strength on the trans side is higher than on the cis side, counter ions will shield these charge interactions allowing the protein to coil more tightly. Similarly, a reduced pH in the compartment will titrate charge groups on the chain; if this shifts the protein toward its isoelectric point, it will coil more compactly. Kagan *et al.* (15) suggested a pH-dependent folding as a way to translocate diphtheria toxin across lysosomes. A higher-than-cytosolic concentration of calcium may do the same in the lumen of the ER, in the matrix of the mitochondria, or in the periplasm of *Escherichia coli*. But, since no systematic charge configuration characterizes all translocated proteins, folding may not be a general mechanism for rectifying protein diffusion, though it may assist in specific instances. There is evidence that the signal sequence confers differential coiling potentials on a protein, since many proteins, such as bacterial alkaline phosphatase, are sensitive to protease prior to translocation; after signal peptide cleavage, they fold into a tighter protease-resistant form (16).

Many chain modifications may occur cotranslocationally (e.g., glycosylation or signal peptide cleavage), because it would be difficult for the modifying enzyme to access its site once the protein has folded. But these modifications could also ratchet the nascent chain and speed translocation. So, the modifying enzymes could be intimately involved in the translocation process. Indeed, *in situ* many of these mechanisms may work in parallel. Binding of chaperonins, glycosylation of the translocated chain, and cleavage of the signal sequence may all ensure vectorial transport.

The Brownian ratchet hypothesis assumes that the diffusion of a protein back and forth through the pore is unbiased but that once a step of a certain size is made the ratchet locks the protein in place. Alternatively, diffusion itself could be biased. A voltage across the mitochondrial membrane is required for protein import (17). Mitochondrial proteins have, on average, a pI value that is 1.5 units more basic than that of cytosolic proteins (18). Thus, the membrane potential of 50 mV (corresponding to 10^5 V/cm) would bias mitochondrial protein reptation into the matrix. Unfortunately, such a mechanism would retard the import of regions with local negative charges. But a membrane potential may facilitate the initial threading of the protein into the TP.

Thermodynamic Considerations. Potential thermodynamic driving forces for translocation include transmembrane differences in pH, ionic strength, membrane potential, or other electrochemical gradients. By examining the translocation process at a more detailed level, we can see that all these forces can contribute to biasing the random diffusion of the translocating protein. Fig. 3 summarizes the role of different factors in promoting translocation.

The energy for translocation ultimately derives from free energy of the kinetic processes associated with translocation, for the second law of thermodynamics prohibits extracting work from an isothermal reservoir. For example, in the chaperonin model the free energy sources are the concentration of chaperonin across the membrane and the binding energy of chaperonins to the chain. A site emerging from the pore has no bound chaperonin and so is not in equilibrium with the trans compartment. When the emerging site binds a chaperonin, the free energy of trans binding, ΔG_+, must be large enough to ensure a chaperonin is bound to the site often

§Although the center of mass of a flexible chain will diffuse more slowly than that of a rigid chain, a flexible chain will translocate faster than a rigid chain so long as $\delta << L$. This is because each node in an elastic chain can fluctuate somewhat independently of the chain as a whole, which allows a node to fluctuate out the right side of the TP, even though the rest of the chain may be moving to the left. Further, the tension in the internodal spring of each ratcheted node helps pull the rest of the chain through the TP.

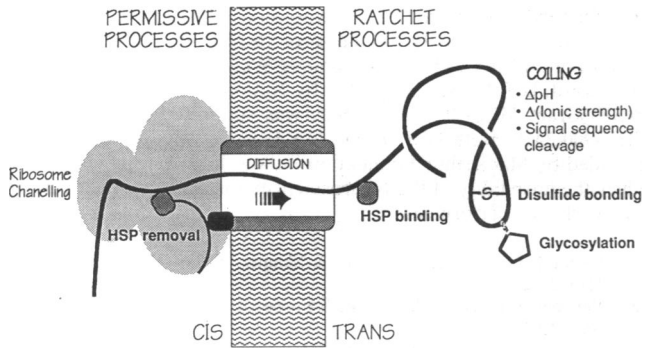

FIG. 3. Summary of the factors that could ratchet protein translocation. On the cis side there are at least three permissive processes: keeping the chain unfolded by chaperonin binding, containment with the ribosome, and removal of blocking chaperonins adjacent to the TP. On the trans side forces include binding of chaperonins, glycosylation, disulfide bonding, and chain coiling (the latter process being affected by pH, ionic strength near the protein's pI value, or cleavage of the signal sequence). HSP, heat shock protein.

and tightly enough to prevent the chain from diffusing back through the TP. If $\Delta G_+ < k_BT$, the binding site will likely be empty when a reverse fluctuation occurs, carrying the site back into the TP—or if the site is occupied, the force of its collision with the TP will likely dislodge it. If the ratchet mechanism is chain coiling, an emerging site is not in entropic equilibrium and this free energy difference implements the ratchet. A close inspection of each ratchet mechanism reveals the process needs a free energy source that ultimately derives from intermolecular bond energies and/or entropic conformations (for example, ATP hydrolysis taps into 8.3×10^{-13} erg per molecule $\approx 20\ k_BT$ of chemical bond energy). This model provides a mechanistic look at how these free energies are transduced into vectorial translocation.

Membrane Proteins. Not all proteins translocate entirely through the channel. Intermittent hydrophobic stretches are intercalcated into the lipid bilayer to integrate membrane proteins. A model of translocation must explain how some stretches of amino acids translocate across and others integrate into the membrane. To see how this could be accomplished by a ratchet mechanism we make the following assumptions. The TP is composed of subunits (of the same or separate proteins) (4, 19–22). These subunits can thermally fluctuate radially (Fig. 4)—this "breathing" fluctuation opens a path between the pore and lipid bilayer (23). Upon synthesis of a latent transmembrane domain, there is a pause in protein translation/translocation. The chain is displaced into the bilayer when there are coincident fluctuations: an opening in the gap between the subunits and a transverse fluctuation in the chain. Once such a fluctuation has occurred, it is unlikely to be reversed because of the considerable entropy gain of the chain as it mixes with the bilayer.

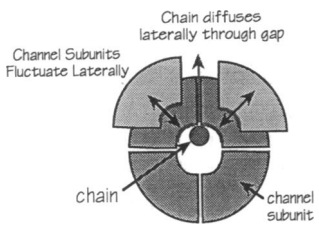

FIG. 4. Subunits of the TP fluctuate radially opening a path for the chain to fluctuate laterally into the membrane. If pore fluctuation coincides with lateral chain fluctuation, the segment enters the bilayer. A stop transfer signal that causes translocation to pause in this configuration enhances the probability of such a coincident pair of fluctuations.

If a nonhydrophobic segment is in the channel during a "breathing" fluctuation, it remains there for it is energetically unfavorable for the nonhydrophobic segment to partition into the bilayer. Hydrophobic segments can partition into the bilayer. For latent transmembrane domains, this is the desired result though it could lead to inappropriate integration of segments into the bilayer. This problem can be minimized if the time scale of a "breathing" fluctuation is rapid relative to the rate the protein moves across the membrane. The probability of integrating a polypeptide into the bilayer is substantially increased if translocation and/or translation are slowed during synthesis of latent transmembrane domains. Consistent with this assumption is the observation that ribosomes slow when translating signal sequences (24).

By studying the time scales and dynamics of membrane insertion, we can ascertain if this model is plausible. It does not violate the laws of physics but can it take place on the observed time scale? How long must the chain remain in the pore until a sufficiently large lateral chain fluctuation and a "breathing" fluctuation of the channel occur simultaneously? Simulation of the model can provide some answers.

Chaperonins. Chaperonins are needed for *in vitro* translocation across the ER (24, 25) and for *in vitro* import across mitochondrial (27–30), chloroplast (31), and bacterial membranes (32–35). They have been cross-linked to translocation intermediaries (28), and their deletion in yeast blocks ER translocation (36). Despite these clues, their functional significance is unknown. They may be required on the cis side to keep proteins unfolded, a state permissive for translocation (25). Trans chaperonins may be required for facilitating proper folding of the translocated chain (29). It has been suggested that differences in energies of folding, perhaps affected by the chaperonins, provide energetics for protein movement (37). While these may be important in particular cases, we propose a specific mechanism by which chaperonins are provocateurs of chain movement. Consistent with this is the observation that by solubilizing mitochondria precursors with urea the requirements for both heat shock proteins and ATP are bypassed (38). This implies ATP is required only for the chaperonins. In contrast, even after ER-targeted translocation precursors are solubilized in urea, ATP is still needed (39). These observations are in accord with our model and suggest two roles for chaperonins in the translocation process. On the cis side of the membrane, they keep the protein unfolded. On the trans side, they keep it from diffusing back through the TP. Since slowing their dissociation rate on the cis side slows translocation, this may regulate how much of the nascent chain is folded on the trans side prior to glycosylation, disulfide bond formation, or other post-translational modifications.

Discussion

Translocation of macromolecules across membranes is robust and promiscuous: almost any molecule can be translocated when given the proper signal sequence. Cytosolic proteins have been targeted to the ER (40), ER proteins have been targeted to the chloroplast (41), double-stranded DNA has been translocated into mitochondria (42), and gold particles have been targeted into nuclei (1). This suggests, with few exceptions, that nothing special about macromolecules makes them translocation-competent; hence, the mechanism that drives translocation must be equally nonspecific. We have shown that a Brownian ratchet mechanism is both indiscriminate and fast enough to explain the observed rates of translocation. Moreover, our model predicts specific functional dependencies on molecular size and kinetic rate constants, so that its predictions can be experimentally addressed.

Several other mechanisms have been proposed for driving translocation. (*i*) The ribosome pushes the nascent chain through the membrane using the energy associated with chain elongation. (*ii*) A pump in the membrane mechanically grabs the chain and pulls it across (46). (*iii*) Electrochemical gradients across the membrane (e.g., pH or other ionic gradients) drive translocation. (*iv*) Energy associated with post-translocational folding might "pull" the chain through the membrane (15, 29). Randall (44) has summarized arguments against the first proposal. The second proposal requires a pump that binds tightly enough to move the translocating peptide but has little specificity, since translocated segments vary considerably in their polarity and charge. There are no universally present transmembrane gradients that could affect all proteins, and in any event the diversity of charge in macromolecules precludes electrophoretic forces as a general mechanism. The effect of chain folding on translocation rates can only be computed from a full three-dimensional simulation; we will report on this elsewhere.

A Brownian ratchet mechanism has several advantages for translocating proteins. (*i*) Specificity is not programmed into the translocating protein—consistent with the observation that many cytosolic proteins are translocated if expressed with a signal sequence. (*ii*) A ratchet provides specific physical mechanisms for transducing the chemical energy of ATP to mechanical movement (6). (*iii*) The translocating segment is not bound to specific proteins, freeing latent transmembrane domains to partition laterally into the lipid bilayer. (*iv*) Since several independent processes promote biased diffusion (Fig. 3), this gives a reliable, fast, and nonspecific translocation mechanism. By defining and quantifying the model's parameters, it should be possible to predict the effects of ionic strength and changes in pH, temperature, and protein flexibility on translocation rates.

Macromolecules cross many different intracellular membranes. ATP-binding cassette (ABC) proteins mediate peptide transport across the ER membrane for antigen presentation and toxin transport across bacterial membranes. Both proteins and RNAs move in both directions across the nuclear envelope. In all cases hydrophilic molecules cross membranes, sometimes against a concentration gradient. In no instance has a mechanism for moving the macromolecules been implicated. The ratchet mechanism, being nonspecific, may drive these translocations. For example, mRNAs are associated with one set of proteins in the nucleus, but after transport into the cytosol, they affiliate with a second set (48). This asymmetry in the nuclear pore could bias the thermal reptation of the molecules out of the nucleus. Similarly, disposition of ATP binding regions in the ABC transporters may provide the asymmetry needed for vectorial movement across the membrane. Brownian ratchets may be a ubiquitous mechanism for moving macromolecules across biological membranes.

This model predicts translocation can be driven by several thermodynamic energy sources and that the relative role of each varies among translocated proteins. It should be possible to experimentally distinguish between different ratchet mechanisms by measuring translocation rates. Further, it may explain why different laboratories have observed results that implicate so many different energy sources for translocation.

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