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Ratcheting in Post-translational Protein Translocation: A Mathematical Model

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We have developed a non-steady-state mathematical model describing post-translational protein translocation across the endoplasmic reticulum membrane. Movement of the polypeptide chain through the channel in the endoplasmic reticulum membrane is considered to be a stochastic process which is biased at the lumenal side of the channel by the binding of BiP (Kar2p), a member of the Hsp70 family of ATPases (ratcheting model). Assuming that movement of the chain through the channel is caused by passive diffusion (Brownian ratchet), the model describes all available experimental data. The optimum set of model parameters indicates that the ratcheting mechanism functions at near-maximum rate, being relatively insensitive to variations of the association or dissociation rate constants of BiP or its concentration. The estimated rate constant for diffusion of a polypeptide inside the channel indicates that the chain makes contact with the walls of the channel. Since fitting of the model to the data required that the backward rate constant be larger than the forward constant during early diffusion steps, translocation must occur against a force. The latter may arise, for example, from the unfolding of the polypeptide chain in the cytosol. Our results indicate that the ratchet can transport polypeptides against a free energy of about 25 kJ/mol without significant retardation of translocation. The modeling also suggests that the BiP ratchet is optimized, allowing fast translocation to be coupled with minimum consumption of ATP and rapid dissociation of BiP in the lumen of the ER. Finally, we have estimated the maximum hydrophobicity of a polypeptide segment up to which lateral partitioning from the channel into the lipid phase does not result in significant retardation of translocation.

Keywords: protein translocation; mathematical model; endoplasmic reticulum; Brownian ratchet; BiP

Introduction

Many proteins are transported post-translationally across the endoplasmic reticulum (ER) membrane. Studies in yeast have shown that post-translational translocation requires a seven-component membrane-protein complex, the Sec complex (Deshaies *et al.*, 1991; Panzner *et al.*, 1995). This complex consists of a heterotrimeric Sec61p complex, which is the central component of a protein-conducting channel, and a tetrameric Sec62/63p complex. The Sec complex binds the signal sequence of the translocation substrate (Plath *et al.*, 1998), and the polypeptide chain is inserted into the channel as a loop, exposing a small segment to the ER lumen. The subsequent movement of the polypeptide through the channel requires an additional component, the lumenal protein BiP (called Kar2p in yeast; Vogel *et al.*, 1990). BiP is a member of the Hsp70 family of ATPases and interacts with the Sec complex *via* a lumenal domain of Sec63p, the J-domain, a segment of about 70 residues that defines the Hsp70-interacting J-protein family (Sadler *et al.*, 1989; Sanders *et al.*, 1992; Brodsky & Schekman, 1993; Scidmore *et al.*, 1993; Lyman & Schekman, 1995; Corsi & Schekman, 1997; Matlack *et al.*, 1997, 1999). The final step in translocation is the cleavage of the signal sequence, resulting in the mature polypeptide chain being localized in the ER lumen. Signal sequence cleavage is not essential for translocation, however.

Abbreviations used: ER, endoplasmic reticulum; mtHsp70, mitochondrial Hsp70.

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The protein-conducting channel has the potential to open sideways towards the lipid because it is used in the cotranslational translocation pathway to integrate hydrophobic segments of membrane proteins. BiP may provide the driving force for post-translational translocation by acting as a molecular ratchet (Simon *et al.*, 1992 Schneider *et al.*, 1994). This mechanism is supported by recent experiments (Matlack *et al.*, 1999). Using purified components, it was shown that multiple BiP molecules associate with the translocation substrate prepro-α-factor during its translocation through the channel. Binding required the prior interaction of BiP with the J-domain of Sec63p. Once bound to the substrate, BiP minimized passive backwards movement of the polypeptide through the channel. A Brownian ratchet, in which forward movement is caused by passive diffusion, is sufficient to achieve translocation, as shown by the fact that antibodies against the substrate could replace BiP. These data did not exclude, however, the possibility that BiP also actively promotes forward movements, for example by ''pulling'' on the incoming polypeptide chain, in addition to serving as a molecular ratchet. A ratcheting model was also proposed for mitochondrial protein import; here, mitochondrial Hsp70 (mtHsp70) in the matrix of mitochondria serves an analogous function to BiP in the ER lumen, and Tim44 on the inner mitochondrial membrane is believed to replace the J-domain of Sec63p (Schneider *et al.*, 1994). More recent data suggest that in mitochondrial protein import both ratcheting and pulling mechanisms may operate together (Voisine *et al.*, 1999).

A specific model for how BiP may act as a ratchet is as follows (Matlack *et al.*, 1999; see also Figure 1). BiP binding to the substrate is initiated by a transient interaction of BiP in its ATP form with the J-domain of Sec63p. The J-domain activates BiP and induces rapid hydrolysis of the nucleotide, resulting in the ADP form bound to the translocating polypeptide chain. J-activated BiP binds peptides with a wide range of amino acid sequences (Misselwitz *et al.*, 1998). Attachment of BiP prevents the bound segment of the substrate from re-entering the channel, but does not hinder forward movements. Once enough of the polypeptide chain has moved into the lumen, the next BiP molecule binds by the same mechanism, and this process is repeated until the polypeptide is entirely translocated across the membrane. BiP dissociates from the substrate following conversion of BiP-ADP to BiP-ATP, although a slower dissociation may occur without nucleotide exchange (Misselwitz *et al.*, 1998). Substrate molecules that are fully released from the channel still carry bound BiP molecules but these eventually dissociate, resulting in a free polypeptide chain that can continue with folding and modification reactions in the ER lumen.

Testing and understanding the ratcheting process requires mathematical modeling. Modeling can test whether all experimental data are in quantitative agreement with BiP functioning solely as a Brownian ratchet. In addition, it can identify the key parameters that determine the efficiency of the ratchet. For example, one can ask whether the rate constant for association of BiP with the substrate is a decisive parameter. Or, is the dissociation constant or perhaps even the rate constant for polypeptide sliding within the channel more important? Finally, modeling can determine the limits of the ratcheting mechanism. For example, it is intuitively clear that too tightly folded a polypeptide chain cannot be transported by a Brownian ratcheting mechanism, but how folded can the polypeptide be? Similarly, if hydrophobic segments of a polypeptide chain can laterally exit the channel into the lipid phase, what is the maximum hydrophobicity up to which translocation of a secretory protein would not be perturbed?

Several attempts have been made to describe the ratcheting process in mathematical terms. The first model assumed that a steady-state situation prevails, in which a polypeptide chain moves with a constant rate through the channel (Peskin *et al.*, 1993). It was also assumed that any lumenal binding partner can act as a ratcheting molecule. This model was recently extended for mitochondrial protein import. The steady-state assumption was maintained, but the binding and dissociation of mtHsp70 were taken into account (Chauwin *et al.*, 1998). Since the parameters of the ATPase cycle of the mtHsp70-Tim44 system have not yet been determined experimentally, they were taken from the *Escherichia coli* DnaK-DnaJ system, assuming that Tim44 has a similar effect on mtHsp70 as a J-protein, even though it shares only marginal sequence homology. The model took into account only mtHsp70 molecules bound next to the channel and neglected the effect of molecules that may remain bound to more distant portions of the chain. A modification of the model was used to analyze the pulling mechanism (Chauwin *et al.*, 1998). In this case the assumption was made that mtHsp70 is always bound to its membrane partner Tim44, rather than being transferred to the substrate; a similar assumption would not be appropriate for the ER system in which BiP is eventually associated only with the substrate (Matlack *et al.*, 1999).

The recent progress on the mechanism of post-translational translocation into the ER offers new possibilities for mathematical modeling. Several parameters of the ATPase cycle of BiP have been determined experimentally (Misselwitz *et al.*, 1998), and actual translocation rates, without the complicating effect of the preceding binding step, have been measured in a soluble system that contains only purified membrane components (Matlack *et al.*, 1999). In addition, the number of BiP molecules associated with fully translocated chains and the rate of backsliding of a polypeptide chain through the channel have been determined (Matlack *et al.*, 1999). All these data correspond to non-steady-state conditions and the previous math-

ematical models are therefore not applicable. Here, we have therefore developed models that describe the new experimental results.

Modeling

Model assumptions

Our model treats protein translocation as a stochastic process in which both the position of the polypeptide substrate with respect to the plane of the membrane and the positions of bound BiP molecules on the substrate are taken into account. The elementary steps include forward and backward diffusion of the protein substrate through the channel, the J-mediated association of BiP molecules with the substrate at the lumenal side of the channel, the spontaneous dissociation of BiP molecules from the substrate, and the release of the translocated protein from the channel into the lumen of the ER.

In our model the binding of a substrate to the channel is assumed to be fast and its dissociation into the cytosol is neglected. Experimental data indicate that dissociation of bound substrate into the cytosol does not occur even after extended incubation periods (Matlack *et al.*, 1997), and that the half-time of association of substrate with the channel is relatively short (about three minutes; Plath & Rapoport, 2000). In addition, many of the experiments modeled have been performed under conditions in which the binding phase is completed before translocation is initiated.

For the actual translocation process the following specific assumptions were made: (a) the substrate polypeptide is described as a chain of L discrete and equal segments which move stepwise through the channel (the chain is assumed to emerge in the ER lumen with its N terminus first (Figure 1), although in reality the presence of the signal sequence would likely result in the emergence of a loop); (b) the thickness of the channel is neglected; (c) within the channel, the polypeptide can diffuse in either direction, but the rate of forward movement may be slower than that of backward movement (see below); (d) each segment that has moved into the lumen can be occupied by a BiP molecule; (e) BiP association can occur only at an unoccupied segment next to the lumenal end of the channel; (f) BiP dissociation can occur from any segment with the same rate constant; (g) substrate molecules released into the lumen cannot rebind to the channel (irreversibility of the release reaction is caused *in vivo* by the removal of the signal sequence and *in vitro*, in a soluble translocation system, by the choice of conditions preventing the rebinding of substrate molecules; Matlack *et al.*, 1997, 1999); and (h) there is no interaction between different membrane channels or between different BiP molecules on a substrate molecule.

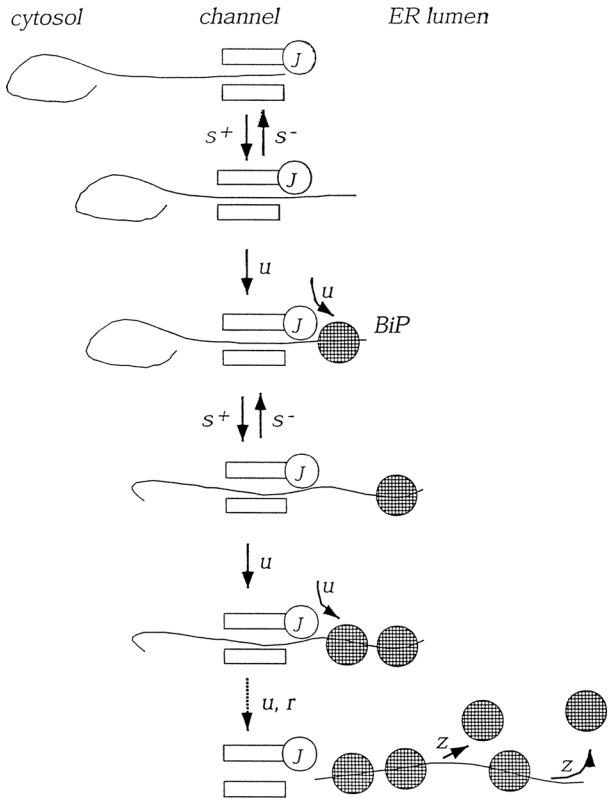

Figure 1. Scheme of the Brownian ratcheting mechanism of translocation. A part of the channel, the J-domain, catalyzes binding of BiP molecules to the substrate protein. BiP prevents the substrate from sliding backwards through the channel, leading to a biased diffusion towards the ER. After release into the ER lumen and dissociation of BiP, the substrate protein can undergo folding and further processing.

Model equations

During translocation, a polypeptide chain may attain different states σ_i which are specified by the number l of segments that are translocated into the lumen, and by the number n of segments occupied by BiP molecules. For example, for $L = 10$ the state:

$$\sigma^B : \underline{0}\,\underline{0}\,\underline{0} \mid \underline{1}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}\,\underline{0}\,\underline{1}$$

indicates that seven segments are located in the lumen (the vertical line indicates the position of the channel), and that the segments indicated by a 1 are occupied by a BiP molecule (three in total). Similarly, the state:

$$\sigma^R : \underline{1}\,\underline{1}\,\underline{1}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}$$

indicates that the chain has been released into the lumen and is occupied at the indicated positions with BiP molecules. Of course, segments on the left side of the membrane are always empty. The total number S of states is $3 \times 2^L - 1$ with $2^{L+1} - 1$

states in the subclass of ''bound'' states and 2^L states in the subclass of ''released'' states.

We describe the time-dependent change of the probability $P_i(t)$ of finding at time t the protein in state σ_i by a master equation:

$$\frac{dP_i(t)}{dt} = \sum_{j=1}^{S} w_{ij}P_j(t) - \sum_{j=1}^{S} w_{ij}P_i(t) \qquad (1)$$

where w_{ij} denotes the transition probability per time from state σ_j to σ_i. The two sums on the right side of the equation correspond to the overall rates of increase and decrease, respectively, of the occupancy of state i. For specifying w_{ij} we take into account that there are five different types of elementary processes: (1) s_+ for inward chain movement; (2) s_- for outward chain movement; (3) u for binding of BiP to the translocation substrate (the concentration of free BiP is assumed to be constant and is incorporated into the pseudo-first-order rate constant u, i.e. $u = u'$[BiP], where u' denotes a second-order rate constant); (4) z for dissociation of BiP from the substrate; and (5) r for the release of the chain from the channel into the ER lumen.

Despite the large total number of states for even small values of L, there exist only a small number of states σ_k which can be reached from a given state σ_i by one of these transitions. Furthermore, a state has to fulfil the following conditions to undergo certain transitions: inward diffusion can occur only as long as the chain is not yet fully translocated ($l < L$); outward movement and BiP binding can occur only if there is a free segment next to the lumenal end of the channel; and release of the chain into the lumen can occur only if $l = L$.

In our calculations we have considered the possibility that the sliding rate constants for the forward and backward directions may not be equal. For example, if a post-translational translocation substrate is not completely unfolded in the cytosolic compartment, sliding in the forward direction would require energy of unfolding. On the other hand, folding on the lumenal side may not play a role because this would be prevented by bound BiP molecules. To take into account unfolding of the polypeptide chain on the cytoplasmic side of the channel, we used two different simplified models. In both of them, the ratio s_{+i}/s_{-i} for a given diffusion step i may vary between the steps. These ratios are related to the free energy difference $\Delta G = \Sigma_{i=1}^{L}\Delta G_i$, where $\Delta G_i = -RT\ln(s_{+i}/s_{-i})$. In the first model, the average value $s = (s_+ + s_-)/2$ of the diffusion parameters s_+ and s_- is assumed to be constant for all steps. This is equivalent to assuming that for each step there is an unfolding-refolding equilibrium for the segment proximal to the cytoplasmic end of the channel before diffusion of this segment through the channel can occur (''stepwise-unfolding model''; derivations not shown). The folding equilibrium would decrease the rate of forward sliding and increase that of backward sliding. In the alternative model, s_- (instead of s) is assumed to be constant for all steps, while s_+ may vary. This is equivalent to assuming that all segments on the cytoplasmic side (not just the one proximal to the channel) are in an unfolding-refolding equilibrium (''domain-unfolding model''; derivations not shown). All calculations are shown for the first model, but the fit to the data with the second model is only slightly less satistfactory (not shown). It should be noted that the ΔG term does not necessarily include only unfolding energy, but also other energy terms counteracting forward movement of the polypeptide chain, including conformational entropy contributions (Sung & Park, 1996).

Summation over all states with a given value of l yields the probability $p_L(l,t)$ for finding chains with l segments in the lumen. Similarly, summation over all states with a given number n yields the probability $p_N(n,t)$ for finding a chain with n bound BiP molecules. Under the given model assumptions the maximal number N of bound BiP equals the total number of segments L. Using these probabilities, one may define a mean translocation length $\bar{l}$ and a mean number of bound BiP molecules $\bar{n}$ as follows:

$$\bar{l}(t) = \sum_{l=0}^{L} lp_L(l,t), \quad \bar{n}(t) = \sum_{n=0}^{N} np_N(n,t) \qquad (2a, b)$$

With the initial condition that the chains have all their segments on the cytoplasmic side, one derives from $\bar{n}$ an expression for the average number of BiP binding events per translocated chain, which is equivalent to the average number of hydrolyzed ATP molecules c_{ATP} per translocated chain. This quantity is simply given by the time integral of the average BiP dissociation rate:

$$c_{ATP} = z\int_0^\infty \bar{n}(t)dt \qquad (3)$$

The fraction $p_R(t)$ of released chains can be used to calculate an average time for the appearence of released substrate molecules in the ER lumen:

$$\tau_R = \int_0^\infty (1 - p_R(t))dt \qquad (4)$$

where $p_R = 0$ for $t = 0$ and $p_R \to 1$ for $t \to \infty$. Similarly, from the fraction $p_F(t)$ of chain that are released into the ER lumen and no longer carry bound BiP one can calculate the average time τ_F for the appearence of such molecules (for the definition of average transition times, see Heinrich & Rapoport (1975); Lloréns *et al.* (1999)).

The differential equation system (1) was solved numerically by applying a fourth-order Runge-Kutta algorithm. The initial states were chosen according to the experimental conditions to be modeled. Since we assume no interactions between different translocation channels, the concentration of substrate chains in a certain state σ_i can be

derived from the single-chain model simply by multiplying the state probability P_i with the total concentration of substrate chains. Calculated probabilities can be equated with experimentally measured fractions of the total chains population.

Our model assumes that each segment can bind a ratcheting molecule. In reality, however, a diffusion segment may correspond to a single amino acid residue of the polypeptide chain, while each BiP molecule would cover several residues on the substrate. In the Supplementary Material we present a mathematical treatment of this case with some simplifying assumptions. The analysis shows that assuming that diffusion and ratcheting steps are the same introduces only a small error.

We have also performed a simplified steady-state treatment of the translocation process in which the BiP occupancy of the sites is extrapolated from that of the first two sites behind the membrane (Supplementary Material).

Determination of standard parameter values

All modeling was performed for prepro-α-factor as the translocation substrate, although, of course, with appropriate modification of the parameters the model is applicable to any protein. The prepro-α-factor chain comprises 165 amino acid residues. In our calculations we have generally used $L = 10$, resulting in a system of 3071 differential equations. Assuming that the first ~25 amino acid residues of prepro-α-factor, containing the signal sequence, are located within the channel and do not participate in BiP binding, this would correspond to BiP covering about 14 residues ($(165 - 25)/10 = 14$).

Constraints for the parameter values s_+ and s_-, describing the forward and backward sliding of a polypeptide in the channel, were derived from theoretical considerations and from a comparison with experimental data. If $s_+ = s_-$ for all steps, the maximum stimulation of translocation by a ratcheting molecule can be calculated. For a perfect ratchet, i.e. one with $u \to \infty$ and $z = 0$, the average translocation time is $\tau_R = L/s$, while without a ratcheting molecule the translocation time is $\tau_R = L(L + 1)/(2s)$. The maximum stimulation is therefore $(L + 1)/2$, or 11/2 for the chosen chain length. The experimental data show, however, that the stimulation is much higher: in the presence of BiP, essentially 100 % of all substrate molecules are transported within ten minutes, whereas in the absence of BiP, only 2 % are translocated within 60 minutes. The discrepancy indicates that s_+ must be smaller than s_-, i.e. there must be an energy barrier for at least some of the steps. One extreme would be that $s_+ < s_-$ for only the first step, and that for all subsequent steps $s_+ = s_-$. However, this also does not allow a description of the data: for translocation in the absence of BiP, s_{+1} has to be chosen very much smaller than s_{-1}, and therefore translocation in the presence of BiP is much too slow. Another extreme would be that the energy barrier is distributed equally among all steps, but again, a fit with the data cannot be obtained: the binding of a single ratcheting molecule would not be expected to have a significant effect on translocation, whereas the experimental finding is that an antibody to the amino terminus of the polypeptide chain causes a significant stimulation of translocation compared with the level seen in its absence (15 % *versus* 2 % translocation in 60 minutes). In fact, a compromise between these two extreme assumptions is the only possibility that describes the data. For our modeling, we have assumed that $s_+ < s_-$ for the first four steps and that the energy barrier is equally distributed among them, and that $s_+ = s_-$ for all subsequent steps. The numerical values were derived from a comparison with the data. Specifically, the amount of substrate translocated without BiP and translocation with antibodies lead to constraints of the parameters. Using the stepwise-unfolding model, we determined as optimal values $s_+ = 2.85\ \text{min}^{-1}$ and $s_- = 21.15\ \text{min}^{-1}$ for the first four steps, and $s_+ = s_- = 12\ \text{min}^{-1}$ for the other steps. These values would correspond to a total energy barrier of $\Delta G = 20$ kJ/mol. For the domain-unfolding model the optimal values were $s_+ = 1.62\ \text{min}^{-1}$ for the first four steps and $s_+ = 12\ \text{min}^{-1}$ for the remaining ones ($s_- = 12\ \text{min}^{-1}$ for all steps).

We have tested whether the exact choice of L and of the number of steps during which sequential unfolding occurs is critical for the fit to the data. Calculations based on the definition (4) for the average translocation time show that for $L = 15$ the data can be described with $s_+ = 5.27\ \text{min}^{-1}$ and $s_- = 18.27\ \text{min}^{-1}$ for the first six steps, and for $s_+ = s_- = 11.77\ \text{min}^{-1}$ for the remaining nine steps. The derived ΔG value of 18.6 kJ/mole is not much different from that used for the lower L-value. Similarly, changing the number of slow unfolding steps by one or two has only a small effect, in particular on ΔG.

The parameter u, describing the association of BiP with the substrate, was set to zero under conditions of ATP depletion, and in the presence of ATP to $50\ \text{min}^{-1}$ for the stepwise-unfolding model and to $60\ \text{min}^{-1}$ for the domain-unfolding model. The latter values were derived by fitting the model to the experimental data on the time-course of translocation and on the dependence on the BiP concentration. With a standard BiP concentration of 1 μM, a u-value of $50\ \text{min}^{-1}$ corresponds to a bimolecular rate constant $u' = 50 \times 10^6\ \text{min}^{-1}\ \text{M}^{-1}$, in reasonable agreement with the rate constant determined for the association of DnaK and peptides ($27 \times 10^6\ \text{min}^{-1}\ \text{M}^{-1}$; Schmid *et al.*, 1994).

The parameter z, describing the dissociation of BiP from the substrate, is highly constrained by the data obtained in backsliding experiments with prepro-α-factor (see Results). In addition, z was independently determined in peptide-dissociation experiments using a BIAcore machine (Misselwitz *et al.*, 1998). These data also indicated that the dissociation is faster in ATP than in ADP. We used a z-value of $1\ \text{min}^{-1}$ in the presence of ATP, and a

z-value of $0.2\ \text{min}^{-1}$ for conditions of ATP depletion. For the modeling of experiments with a BiP mutant lacking the lid domain, we used $z = 0.7\ \text{min}^{-1}$ under ATP-depletion conditions, consistent with its three- to fourfold higher dissociation rate from peptide substrates (Misselwitz *et a1.*, 1998).

Results

Description of experimental data

We first tested whether the model can describe data in which the translocation of a substrate through the channel is followed in a soluble system. In these experiments, prepro-α-factor is bound to the channel in proteoliposomes, the complex is solubilized in detergent, and translocation is started by the addition of BiP and ATP. The experiments show that the polypeptide chain is moved through the soluble channel and released at its lumenal side (Matlack *et al.*, 1997, 1999). Molecules that are no longer associated with the channel are scored as being translocated.

For modeling, we used as the starting condition that all substrate molecules have no segment on the lumenal side of the channel before addition of BiP and ATP. Using the standard parameter set for the stepwise-unfolding model, the fraction of substrate molecules that are fully translocated through the channel was calculated as a function of time (Figure 2, continuous curve). A fair agreement with the experimental data points was obtained (diamonds). The calculation indicates the existence of a lag time before released substrate molecules appear, consistent with the expectation that it takes time for the first polypeptide chains to be released from the channel. A lag phase was not obvious from the data (Matlack *et al.*, 1999), but it may have been missed. Not only is the predicted lag phase relatively short (one to two minutes), but it is also difficult to measure the small amount of substrate released at early time points (released substrate is measured as the decrease of channel-associated substrate).

We also calculated the fraction of free substrate molecules that are not only fully released from the channel but also no longer associated with BiP (Figure 2, broken curve). Experimentally, this fraction was determined by subtracting from the population of released prepro-α-factor molecules those that can be immunoprecipitated with BiP antibodies (Matlack *et al.*, 1999). The calculated time dependence is clearly steeper than the experimental points (re-plotted from Matlack *et al.*, 1999; Figure 2). In addition, in the calculations all substrate molecules are eventually free, whereas in the experiments, about 15% remain associated with BiP, even after long incubation times. The discrepancy may be due to our simplifying assumption that BiP does not bind at all in the absence of J-activation, or it could be caused by some unspecific binding of prepro-α-factor to BiP, to the antibodies,

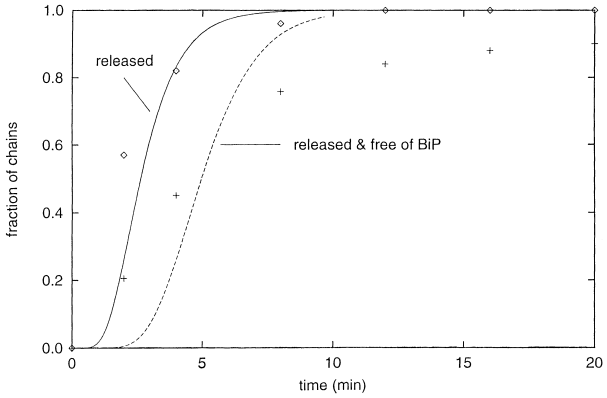

Figure 2. Modeling of translocation in solution. A soluble complex of prepro-α-factor and channel was generated, and translocation started by the addition of BiP and ATP. Molecules that are no longer associated with the channel are scored as being translocated. The continuous line represents the calculated time-dependent fraction of substrate molecules released from the channel as derived from the model. The experimental data from Matlack *et al.* (1999) are shown as diamonds. Also shown is the calculated fraction of molecules that are not only released from the channel, but also no longer associated with BiP (dotted line). The data points (crosses) were replotted from Matlack *et al.* (1999). The theoretical curves correspond to $\tau_R = 2.87$ and $\tau_F = 5.19$.

or to the protein A beads used to collect the antibodies. For the domain-unfolding model the best parameter set gave a slightly less satisfactory fit with the data (not shown).

Next, we simulated experiments in which translocation in solution was performed at different BiP concentrations (Figure 3). In our model, this corresponds to varying the parameter u describing the association of BiP with the substrate, which is proportional to the BiP concentration. The u-value at 1 μM BiP corresponds to the one used in the calculations for Figure 2. Using the standard parameters of the stepwise-unfolding model the observed fit with the data is good (compare the broken curve and the experimental data (diamonds) in Figure 3). A similarly good fit was obtained with the domain-unfolding model (not shown).

We also compared with experimental data the size distribution of substrate-BiP complexes released from the channel. In the experiments the release reaction in a soluble system was stopped at different time points, and the released prepro-α-factor-BiP complexes were separated by sucrose-gradient centrifugation according to their size (Matlack *et al.*, 1999). In the calculations, summation over all released states with a given value of n was performed for different time points (Figure 4). In agreement with the experimental data (Matlack *et al.*, 1999), at early time points (two minutes after addition of BiP and ATP), a heterogeneous population was seen. The predictions are consistent with the experimental average of about

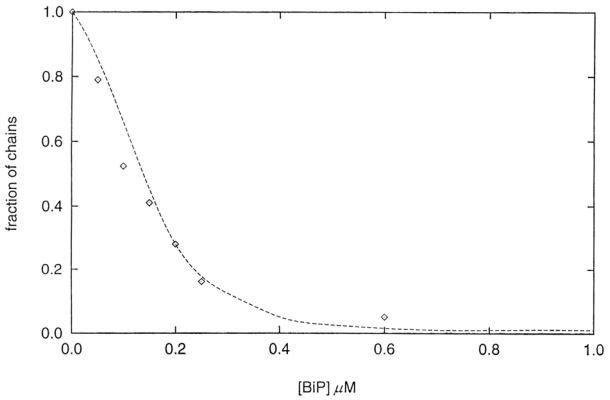

Figure 3. Effect of BiP concentration on translocation efficiency. Translocation in a soluble system was performed as for Figure 2 with different BiP concentrations. The fraction of substrate molecules associated with the channel after ten minutes of incubation is plotted *versus* the BiP concentration. The BiP binding rate constant u is assumed to be proportional to the BiP concentration, with the standard value $u = 50\ \text{min}^{-1}$ corresponding to a concentration of 1 μM. Experimental data from Matlack *et al.* (1999) are shown as diamonds.

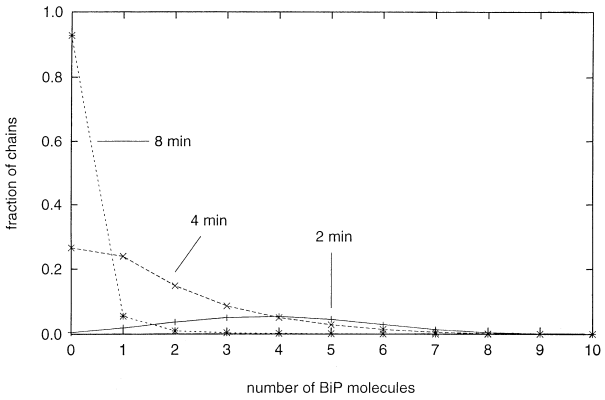

Figure 4. Size distribution of released substrate-BiP complexes as a function of time. Translocation in a soluble system was performed as for Figure 2 and the fraction of chains associated with 0,1,2,...,10 BiP molecules calculated at different time points. The data are in qualitative agreement with the size distribution determined experimentally by sucrose-gradient centrifugation (Matlack *et al.*, 1999; data not shown).

four molecules of BiP per polypeptide chain. At four minutes the distribution was shifted to lower numbers (an average of one or two molecules), and at eight minutes essentially all substrates had lost their BiP molecules, as observed experimentally (Matlack *et al.*, 1999).

Another test of the model is provided by backsliding experiments (Matlack *et al.*, 1999). Here, prepro-α-factor with a bulky chemical group at its C terminus is first imported into proteoliposomes containing lumenal BiP and ATP, resulting in a stalled molecule with the bulky group abutting the cytoplasmic end of the channel. When ATP is depleted, BiP can no longer bind to the substrate and the polypeptide chain slides backwards. In the experiments, after incubation for a certain time period, the samples are put on ice and treated with protease. Chains with no segment exposed to the cytosol give a specific protected fragment, whereas this fragment is lost when even a single polypeptide segment has appeared in the cytosol. The loss of the specific fragment with time is thus a measure of the rate of backsliding.

To model backsliding, we used as an initial state the situation in which all ratcheting sites are in the lumen and occupied by BiP. To simulate a steady-state situation in the presence of ATP, prior to the start of the backsliding experiment, a one minute pre-incubation period was used with association and dissociation rate constants of $u = 50\ \text{min}^{-1}$ and $z = 1\ \text{min}^{-1}$, respectively. The fraction of non-translocated chains after the pre-incubation period was set to 1 ($t = 0$ in Figure 5). As expected from a steady state, when ATP conditions were maintained no backsliding occurred (Figure 5, continuous curve), in agreement with the experimental data (squares). To model conditions of ATP depletion, the parameters were set to $u = 0$ and $z = 0.2\ \text{min}^{-1}$ after the pre-incubation period (Figure 5, broken curve). Again, a good fit with the data (diamonds) was obtained.

Backsliding data are also available for a BiP mutant that lacks the lid domain (Matlack *et al.*, 1999). This mutant has about the same rate of association with a peptide as the wild-type protein, but a three- to fourfold higher dissociation rate (Misselwitz *et al.*, 1998). When we used $z = 0.7\ \text{min}^{-1}$ instead of $z = 0.2\ \text{min}^{-1}$, backsliding following ATP depletion could be well described (Figure 5, compare dotted curve with experimental data (crosses)).

Finally, we tested whether the model can describe translocation driven by antibodies to the substrate (Matlack *et al.*, 1999). In these experiments, peptide-specific antibodies to prepro-α-factor were included in proteoliposomes containing the Sec complex, and import of the substrate was assessed after 60 minutes by a protease-protection assay. Out of three antibodies directed against distinct regions of prepro-α-factor, only one against the segment immediately following the signal sequence caused significant translocation (15 %). In the absence of antibody, only 2 % translocation was seen. We modeled these experiments by assuming that the antibody binds to only one segment with kinetic parameters $u = 0.5\ \text{min}^{-1}$ and $z = 0$, i.e. it binds relatively slowly, but does not dissociate. With these values we get significant antibody-driven translocation if the antibody binds to sites 1 or 2, some stimulation if it binds to site 3, and no stimulation if it binds to one of the following sites (Figure 6). These results are thus in agreement with the experimental data that antibodies binding to the C terminus have little effect (sites 4

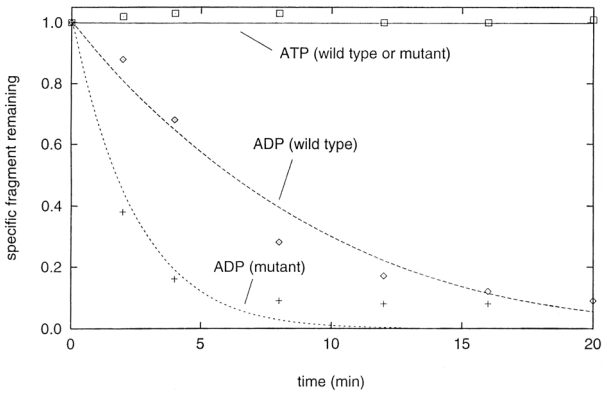

Figure 5. Simulation of backsliding experiments. Prepro-α-factor with a bulky chemical group at its C terminus was first imported into proteoliposomes containing lumenal BiP and ATP, resulting in a stalled molecule with the bulky group abutting the cytoplasmic end of the channel. Following protease treatment, it gives rise to a characteristic fragment. Backsliding of the chain into the cytosol was determined by the loss of this fragment. The continuous line shows the simulation under ATP conditions where no backsliding occurs. The broken line shows the calculations after ATP depletion at $t = 0$. These conditions were modeled by setting $u = 0$ and $z = 0.2\ \text{min}^{-1}$. The dotted line shows calculations for a BiP mutant lacking the lid domain, which has a higher dissociation rate ($z = 0.7\ \text{min}^{-1}$). The points are experimental data taken from Matlack *et al.* (1999).

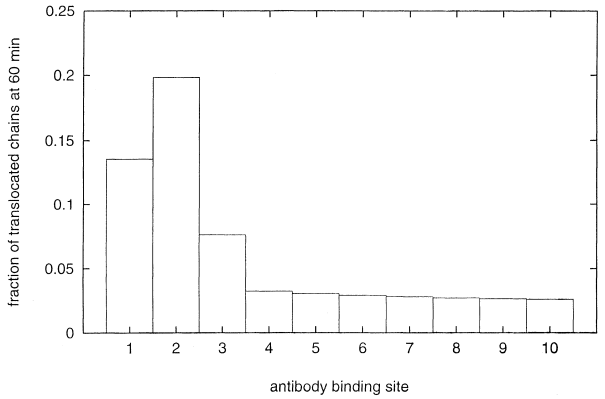

Figure 6. Modeling of antibody-driven translocation. Peptide-specific antibodies to prepro-α-factor were included into proteoliposomes containing the Sec complex, and import of the substrate was assessed after 60 minutes by a protease-protection assay. These experiments were modeled by assuming that the antibodies bind to a single site on the chain with $u = 0.5\ \text{min}^{-1}$ and $z = 0$. The fraction of substrate molecules translocated after 60 minutes was calculated. Experimentally, an antibody binding to site 1 gave 15% translocation, and antibodies binding to regions corresponding to sites 4 or 6 gave 2% translocation (Matlack *et al.*, 1999).

and 6 would be the approximate binding sites of the two antibodies that did not stimulate translocation). In summary, we conclude that the model describes all available experimental data reasonably well.

Properties of the system under standard conditions

The model allows us to calculate variables for a normal translocation reaction that cannot be easily determined experimentally. In Figure 7(a) we have calculated with standard parameter values the fraction of substrate molecules that have $l = 0,1,2,\ldots,10$ ratcheting sites in the lumen, or are fully released into the lumen as a function of time. As expected, the number of molecules with no segment in the lumen rapidly decreases monotonically, and that released into the lumen increases with a lag phase. Molecules with four to ten segments in the lumen are present in lower numbers than those with fewer segments, because we have assumed energy barriers for the first four steps and none thereafter. Thus, once the substrate has moved beyond step 4, it rapidly slides through and leaves the channel.

Figure 7(b) gives the number of substrate molecules that carry $n = 0,1,2,\ldots,10$ BiP molecules as a function of time, as well as the corresponding mean value $\overline{n}(t)$. As expected, molecules with no BiP bound are the only species at the beginning and end of the translocation reaction. Similarly, molecules with one BiP bound show two maxima in a time course, whereas those with more BiP molecules bound show only one maximum. The results indicate that a maximum mean number of two or three BiP molecules are bound to the substrate at about 2.5 minutes into the translocation reaction, roughly at the time when 50% of all molecules are translocated (Figure 2).

Figure 7(c) shows the BiP occupancy of the different ratcheting sites in unreleased substrates as a function of the distance from the lumenal end of the channel. These data were calculated for 2.5 minutes because in a time interval around this time point the distribution was almost constant (not shown). It may be seen that for 86% of all substrate molecules the site relevant for the ratcheting mechanism, i.e. the one next to the channel, is occupied by BiP. Thus, with our standard parameters the ratchet is functioning close to its maximum efficiency. A similar conclusion was derived with a simplified steady-state model presented in the Supplementary Material: with realistic parameters the site next to the channel was occupied to 88%.

The effect of model parameters on the performance of the system

The model can be used to determine which parameter has the most pronounced effect on the efficiency of the ratcheting mechanism. As expected, when the association rate constant u is

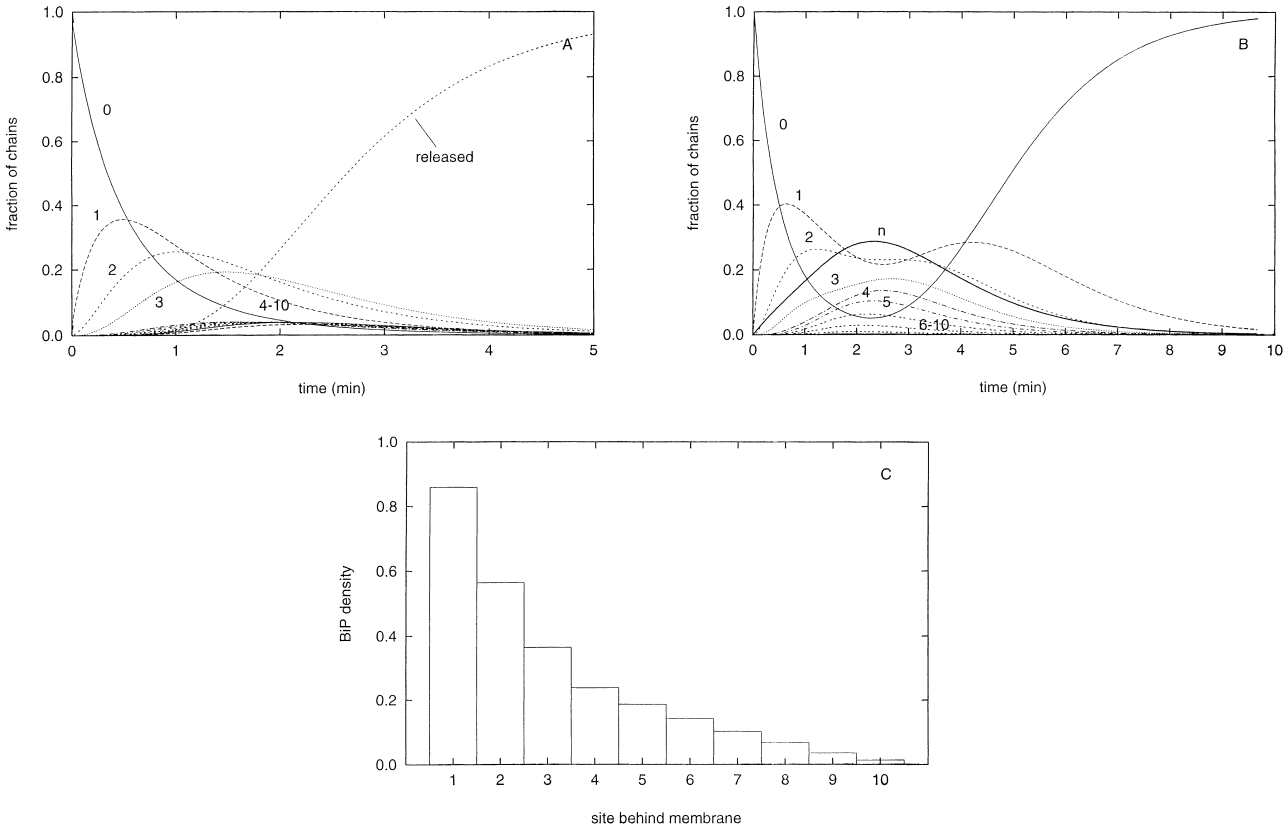

Figure 7. Predicted behavior of the system. (a) A normal translocation reaction was simulated with the initial condition that all substrate molecules are bound to the channel and all polypeptide segments are located on its cytosolic side. Using standard parameter values, the fraction of substrate molecules that have $l = 0,1,2,\ldots,10$ ratcheting sites in the lumen, or are fully released into the lumen, was calculated as a function of time. (b) The fraction of substrate molecules that carry $n = 0,1,2,\ldots,10$ BiP molecules was calculated as a function of time. Also shown is the calculated mean value $\overline{n}(t)$. (c) The density of BiP at different ratcheting sites was calculated as a function of the distance from the lumenal end of the channel at $t = 2.5$ minutes. The profile deviates only slightly from a decaying exponential.

increased, the average translocation time τ_R becomes shorter (Figure 8(a), continuous curve). However, with the chosen standard value of $u = 50\ \text{min}^{-1}$, variation of u has, around its standard value, relatively little effect. We conclude that the rate constant for association of BiP with the substrate, and thus the BiP concentration, do not significantly determine the efficiency of the ratcheting mechanism. Also plotted in Figure 8(a) is the average time τ_F for the appearence of released substrate molecules that no longer carry BiP (broken curve). The difference between the two curves indicates that at standard conditions it takes on average about 2.5 minutes for a released substrate molecule to lose all BiP molecules. Again, as may be expected, the parameter u has only little influence on the appearance of free substrate molecules. Finally, we have calculated in Figure 8(a) the average number c_{ATP} of ATP molecules hydrolyzed per translocated polypeptide chain (dotted curve). This number is always only slightly higher than ten, i.e. close to the chosen L-value.

When the dissociation rate constant z was increased beyond its standard value of $1\ \text{min}^{-1}$, the average translocation time τ_R increased as expected, but the increase was only moderate (Figure 8(b), continuous curve). Thus, the efficiency of the ratchet is also relatively insensitive to changes of the dissociation rate constant z. The average time τ_F for the appearance of released substrate molecules that no longer were associated with BiP showed a minimum (Figure 8(b), broken curve). Such a minimum is expected, because with slow BiP dissociation (low z-values) translocation is fast, but BiP remains bound for a long time. The standard conditions ($z = 1\ \text{min}^{-1}$) are not exactly at this minimum ($z = 2.5\ \text{min}^{-1}$), but are not far from it. Thus, the system is close to an optimal compromise between an efficient ratchet on the one hand, and rapid release of the ratcheting molecules, which *in vivo* could otherwise block subsequent folding and modification reactions, on the other. Interestingly, a more important optimization may have occurred with respect to ATP consumption. As can be seen from Figure 8(b) (dotted curve), the number of ATP molecules hydrolyzed per translocated chain increases drastically with higher z-values. The standard conditions may therefore represent a compromise between the

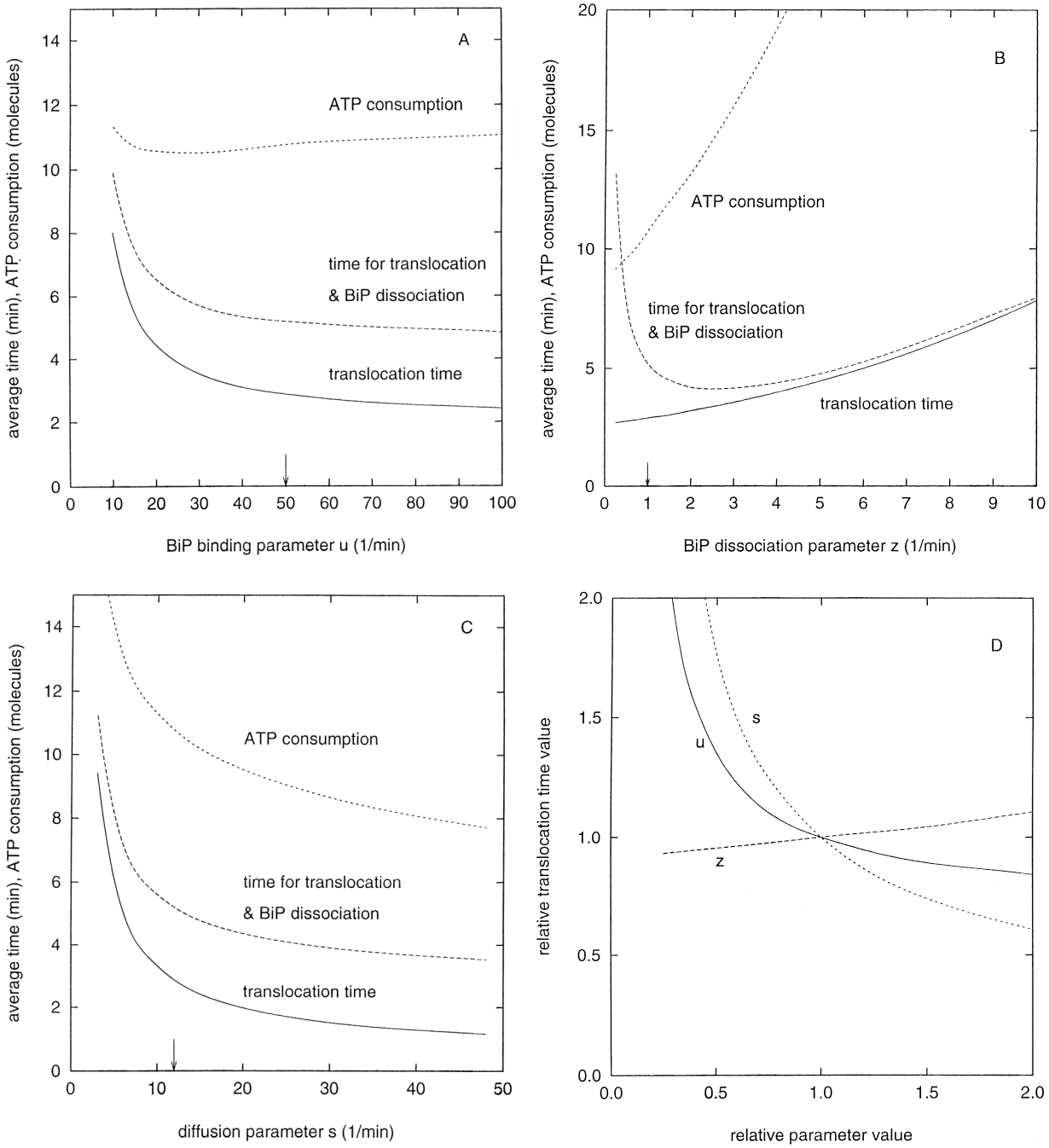

Figure 8. Influence of model parameters u, z, and s on translocation times and ATP consumption. The average time of translocation τ_R, the average time τ_F for the appearance of translocated substrate molecules that no longer are associated with BiP molecules, and the number c_{ATP} of ATP molecules consumed per translocated substrate molecule were calculated for different values of the BiP binding rate constant $u = u'$ [BiP] (standard value $u = 50$ min^{-1}). All other parameters were kept constant at their standard values. The position of the reference state is indicated by an arrow. (b) As in (a), but with variation of the dissociation rate constant z (standard value $z = 1$ min^{-1}). (c) As in (a), but with variation of the diffusion parameter s (standard value $s = 12$ min^{-1}). (d) The curves in (a) to (c) were replotted to allow a direct comparison of the importance of parameters in controlling the average translocation time. The point (1,1) corresponds to the reference state obtained with the standard parameter values.

rapid release of ratcheting molecules on the one hand, and high ATP consumption on the other.

When the average value of the diffusion parameter s was increased, the average time τ_R of translocation and the average time τ_F of the appearance of free substrate molecules in the lumen both decreased (Figure 8(c), continuous and broken curves, respectively). Around the standard value of $s = 12$ min^{-1}, an increase of s had relatively little effect, whereas a decrease led to a sig-

nificant inhibition of translocation. In addition, low s-values led to an increase of ATP consumption (dotted curve). The system may thus be at the borderline where contact of a polypeptide chain with the walls of the channel could have a significant effect on the translocation rate.

In Figure 8(d) the theoretical results depicted in Figure 8(a) to (c) are replotted to allow a direct comparison of the importance of the parameters for the average translocation time τ_R. The curves show relative variations of τ_R as a function of relative variations of the parameters u, s, and z around the reference state. It may be seen that the translocation time is most sensitive to variations of s, followed by those of u, particularly at low values (Figure 8(d)); z has almost no influence. It should be noted that the slopes of the curves at the reference state represent the control coefficients of the various parameters with respect to τ_R (see Heinrich & Rapoport, 1974; Fell, 1996).

Finally, we investigated the effect of the energy barrier. We maintained the assumption that the energy barrier ΔG is distributed equally among the first four steps and varied its magnitude (Figure 9). Since we assumed that substrates cannot be released into the cytosol but can always be released into the ER lumen, there is no finite stalling force that would prevent translocation. However, as ΔG is increased, translocation eventually becomes so slow that for all practical purposes it can be considered stalled. Interestingly, when ΔG is increased from its standard value of 20 kJ/mol to 25 kJ/mol or even somewhat higher, there is only little effect on the average time of translocation (Figure 9, continuous curve). However, when increased to 35 kJ/mol translocation is about six times slower, and at $\Delta G = 40$ kJ/mol it comes essentially to a halt (more than 30-fold slower). As may be expected, both the time for the appearance of free substrate molecules in the lumen and the number of ATP molecules hydrolyzed per translocated polypeptide also increase at high ΔG values. Again, around the standard conditions, ΔG variations have little effect. We conclude that up to an energy barrier of about 25 kJ/mol the Brownian ratchet can transport polypeptides without significant retardation. Similar results were obtained with the domain-unfolding model (data not shown).

To calculate a finite stalling force, we used the simplified steady-state model (see Supplementary Material). This model allows one to calculate the steady-state translocation velocity as a function of a force opposing the inward movement of the chain. With parameter values that are derived from the more complete model, translocation comes to a halt at a ΔG value of 90 kJ/mol (Figure B2 in Supplementary Material). This value is an upper limit for the stalling force because a uniform distribution of ΔG was assumed.

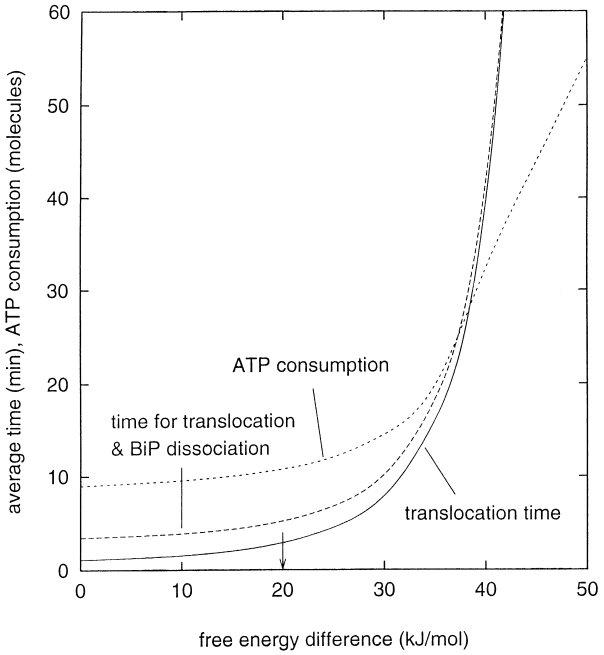

Figure 9. Influence of the energy barrier on translocation times and ATP consumption. The average time of translocation τ_R, the average time τ_F for the appearance of translocated substrate molecules that no longer are associated with BiP molecules, and the number c_{ATP} of ATP molecules consumed per translocated substrate molecule were calculated as for Figure 8 for different values of the energy barrier ΔG (standard value $\Delta G = 20$ kJ/mol).

Hydrophobicity of polypeptide segments sets limits for ratcheting mechanism

The protein-conducting membrane channel not only transports secretory proteins across the membrane, but also allows hydrophobic segments of membrane proteins to partition laterally into the lipid phase. Conversely, if a segment in a secretory protein were too hydrophobic, its transport across the membrane would be retarded because it could transiently leave the channel laterally. We have made a very approximate calculation of how hydrophobic a ratcheting segment can be without causing a significant reduction of the translocation rate.

We assume that the ratchet is perfect, with L irreversible sliding steps of equal rate, and that one polypeptide segment is hydrophobic, allowing it to rapidly partition laterally from the channel into the lipid phase (equilibrium constant K_{eq}). The translocation time is then given by:

$$\tau_R^* = \frac{1}{s}(L + K_{eq}) \qquad (5)$$

Thus, the ratio between the translocation times in the absence and presence of a partitioning equilibrium is:

$$\frac{\tau_R^*}{\tau_R} = \frac{L + K_{eq}}{L} \quad (6)$$

The formula indicates that for $L = 10$ and $K_{eq} = 10$ the translocation time would be doubled, and that for $K_{eq} = 100$, it would be increased about tenfold. The K_{eq} values translate into ΔG values of about -5 kJ/mol and -11 kJ/mol, respectively. The conclusion is that a segment of about 14 amino acid residues (the size of a ratcheting site for $L = 10$) cannot have a higher lipid-partitioning energy of about 5-10 kJ/mol without causing significant retardation of translocation of the polypeptide. Indeed, when the prepro-α-factor sequence was analyzed with the program MPEx, which calculates the free energy of lipid partitioning for segments of 19 amino acid residues in length (White & Wimley, 1999), none had a free energy level above 3 kJ/mol (disregarding the signal sequence; S. White, personal communication).

Discussion

Our mathematical model contains a minimum number of parameters and yet can describe all available experimental data. The experimental data were obtained under different conditions, and it is therefore by no means trivial that they could all be described with the same numerical parameter values. In addition, some parameters were independently confirmed in totally unrelated experiments. The fit demonstrates that all available data are consistent with BiP acting as a Brownian ratchet in post-translational protein translocation across the ER membrane. Our results do not exclude the possibility that BiP could also actively promote forward movement of the substrate, in addition to acting as a ratchet. Indeed, a motor mechanism could also describe the data. We have modeled a motor by replacing s_+ by s_+m (with $m > 1$) for cases where the first ratcheting site next to the channel is occupied. The data can be fitted with ΔG concentrated on one early diffusion step, and the quality of the fit is slightly better than with the pure ratcheting mechanism due to the additional free parameter (data not shown). However, our results show that there is no necessity to assume such a mechanism. Although it has been postulated that mitochondrial protein import can only be explained by assuming the simultaneous action of both ratcheting and motor mechanisms (Voisine *et al.*, 1999), experimental data are relatively scarce so that modeling becomes difficult, and theoretical considerations alone cannot decide between a Brownian ratchet and a motor mechanism (Chauwin *et al.*, 1998).

The model parameters include rate constants for the association of BiP with the translocation substrate (u), for the dissociation of BiP from the substrate (z), and for the sliding of the polypeptide chain within the channel (s). While the standard values for u and z are similar to those obtained in experiments in which the binding of BiP (or the related DnaK) to peptides was measured, values for s cannot be easily verified experimentally. Our estimate for s (about 12 min^{-1}) indicates that sliding is much slower than calculated for the diffusion of a polypeptide chain through a wide pore with which it makes no contact (this would be in the millisecond range; Peskin *et al.*, 1993). We therefore conclude that the polypeptide chain makes contact with the walls of the channel when it slides through it. A similar statement was made before, based on backsliding experiments with mitochondrial import substrates (Chauwin *et al.*, 1998). However, that conclusion was based on the erroneous assumption that the rate of backsliding was solely determined by the sliding parameter, and that the dissociation of mtHsp70 from the substrate can be neglected. Our modeling of the backsliding reaction in the ER system demonstrates that the z-parameter for dissociation of BiP from a substrate is much more important than the sliding rate constant s, in agreement with the experimental data, which show that a BiP mutant with an increased dissociation rate gives much higher backsliding rates (Figure 5). In fact, the slowness of sliding inside the channel cannot be deduced from backsliding experiments alone.

We were unable to describe the experimental data with a Brownian ratcheting mechanism unless we made the assumption that there is a free energy barrier for the first several diffusion steps. Without this assumption, translocation of prepro-α-factor was either too fast in the absence of added BiP or too slow in the presence of BiP or of an antibody to the substrate. Interestingly, neither the assumption that the energy barrier was present only in the first diffusion step nor that it was distributed equally among all steps, was appropriate to obtain a fit with the data. Only a model in which several of the initial diffusion steps encounter an energy barrier could describe the data. The total free energy barrier calculated for prepro-α-factor was 20 kJ/mol. Although we have assumed that the entire energy is spent on the unfolding of the polypeptide chain at the cytosolic side of the channel, the magnitude of the calculated energy seems somewhat high in view of the fact that native proteins have a ΔG of about 25-60 kJ/mol for their unfolding (Pfeil, 1981). Prepro-α-factor cannot be correctly folded in the cytosol but it could adopt secondary structure or other loosely folded conformations. If unfolding was the major contributor to the energy barrier, our results suggest that it would occur gradually during the first several diffusion steps of the translocation process. Although the *in vitro* unfolding of native proteins is generally a cooperative process, it seems possible that the sum of unfolding of local conformations may not be negligible and could be of particular importance as long as a sizable portion of the chain is still located in the cytosol. However, given the size of the energy barrier, it seems possible that factors other than the unfolding of the polypeptide chain may contribute to the energy barrier encountered

during early ratcheting steps. For example, energy could be required to strip the polypeptide of cytosolic chaperones. However, most cytosolic proteins are released from the substrate when it is bound to the channel, i.e. before it begins its actual translocation (Plath & Rapoport, 2000). Other reasons why early ratcheting steps may be particularly slow could be related to the small lumenal loop formed by the polypeptide chain after its insertion into the channel; the high curvature may, for example, hinder efficient binding of the first BiP molecules. Regardless of which process contributes the most to the energy barrier, our results put an upper limit on the unfolding energy compatible with translocation by a Brownian ratcheting mechanism. We have estimated that up to ΔG values of about 25 kJ/mol, the average translocation time is only slightly increased. However, polypeptides which require more than 40 kJ/mol unfolding energy cannot be transported with reasonable rates, supporting the idea that post-translational translocation requires that a substrate maintains a loosely folded structure after its completion by translation.

Our calculations also put limits on the hydrophobicity of a polypeptide segment in a translocating polypeptide. If too hydrophobic, a segment would partition laterally from the channel into the lipid phase and would thus cause a significant decrease in the rate of translocation. This inhibitory effect turned out to be much stronger than that caused by the unfolding of a polypeptide chain, and it can occur even when the BiP ratchet functions at its optimum. One would therefore predict that secretory proteins do not contain hydrophobic segments with partitioning energies exceeding 5-11 kJ/mol. Indeed, our model substrate prepro-α-factor does not contain such a segment in the sequence following its signal peptide.

Under standard conditions, it seems that the Brownian ratchet functions close to its maximum efficiency: in about 86 % of all molecules, the crucial ratcheting site next to the channel was occupied. Interestingly, the average translocation time of a polypeptide was quite insensitive to individual changes of the parameters u, z, and s. The only exception may be a decrease in the average sliding rate constant s, suggesting that in cases where polypeptide segments make particularly strong contacts with the walls of the channel translocation could be slowed. However, it is remarkable that the properties of BiP, i.e. its association or dissociation rate constants or its concentration, do not appear to be major factors determining the rate of translocation. Rather, the properties of the translocation substrate itself, i.e. its length L and its folding properties (ΔG) are more important.

Our results suggest that *in vivo* the system is close to the state of optimal performance: BiP remains bound to the substrate long enough to serve as an efficient ratcheting molecule, but dissociates fast enough to prevent blockade of subsequent folding and modification reactions of the substrate. Under standard conditions, it takes on average about 2.5 minutes to move prepro-α-factor through the channel and an additional 2.5 minutes to release all BiP molecules, a time period that is not insignificant. In cotranslational translocation, in which BiP binding to the substrate probably does not occur, such a long delay in folding and modification is not expected, providing an advantage of this pathway over the post-translational one. The BiP dissociation parameter z had the most profound effect on the time difference between the release of substrate molecules into the lumen and the point where they were no longer associated with BiP. A decrease in the dissociation rate constant z slightly increased the translocation rate, but led to a significant delay in the generation of free substrate molecules (Figure 8(b)). An increase of z, on the other hand, allowed the more rapid generation of free polypeptide chains and increased the translocation time only moderately, but it led to excessive ATP consumption (Figure 8(b)). The BiP molecule itself seems to be optimized with respect to its z-value and, unlike its bacterial homolog DnaK, does not appear to require a cofactor that changes its intrinsic nucleotide exchange rate. Our data also show that about ten molecules of ATP are hydrolyzed for each prepro-α-factor molecule translocated. Again, this appears to be a compromise between the rate of dissociation of BiP from the substrate and the number of ATP molecules hydrolyzed. In conclusion, the system appears to be designed to be a compromise between different requirements: fast translocation, rapid release of BiP molecules from translocated molecules, and a small number of ATP molecules hydrolyzed per translocated polypeptide chain.

Acknowledgments

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References

Brodsky, J. L. & Schekman, R. (1993). A sec63p-BiP complex from yeast is required for protein translocation in a reconstituted proteoliposome. *J. Cell Biol.* **123**, 1355-1363.

Chauwin, J.-F., Oster, G. & Glick, B. S. (1998). Strong precursor-pore interactions constrain models for mitochondrial protein import. *Biophys. J.* **74**, 1732-1743.

Corsi, A. K. & Schekman, R. (1997). The lumenal domain of Sec63p stimulates the ATPase activity of BiP and mediates BiP recruitment to the translocon

in *Saccharomyces cerevisiae*. *J. Cell Biol.* **173**, 1483-1493.
Deshaies, R. J., Sanders, S. L., Feldheim, D. A. & Schekman, R. (1991). Assembly of yeast SEC proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex. *Nature,* **349**, 806-808.
Fell, D. A. (1996). *Understanding the Control of Metabolism*, Portland Press, London.
Heinrich, R. & Rapoport, T. A. (1974). A linear steady-state treatment of enzymatic chains; general properties, control and effector strength. *Eur. J. Biochem.* **42**, 89-95.
Heinrich, R. & Rapoport, T. A. (1975). Mathematical analysis of multienzyme systems. II. Steady state and transient control. *BioSystems*, **7**, 130-136.
Lloréns, M., Nuño, J. C., Rodríguez, Y., Meléndez-Hevia, E. & Montero, F. (1999). Generalization of the theory of transition times in metabolic pathways: a geometrical approach. *Biophys. J.* **77**, 23-36.
Lyman, S. K. & Schekman, R. (1995). Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of *Saccharomyces cerevisiae*. *J. Cell Biol.* **131**, 1163-1171.
Matlack, K. E. S., Plath, K., Misselwitz, B. & Rapoport, T. A. (1997). Protein transport by purified yeast Sec complex and Kar2p without membranes. *Science,* **277**, 938-941.
Matlack, K. E. S., Misselwitz, B., Plath, K. & Rapoport, T. A. (1999). BiP acts as a molecular ratchet during posttranslational transport of prepro-α factor across the ER membrane. *Cell,* **97**, 553-564.
Misselwitz, B., Staeck, O. & Rapoport, T. A. (1998). J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. *Mol. Cell.* **2**, 593-603.
Panzner, S., Dreier, L., Hartmann, E., Kostka, S. & Rapoport, T. A. (1995). Posttranslational protein transport in yeast reconstituted with a purified complex of Sec proteins and Kar2p. *Cell,* **81**, 561-570.
Peskin, C. S., Odell, G. M. & Oster, G. F. (1993). Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys. J.* **65**, 316-324.
Pfeil, W. (1981). The problem of stability of globular proteins. *Mol. Cell Biochem.* **40**, 3-28.
Plath, K. & Rapoport, T. A. (2000). Spontaneous release of cytosolic proteins from posttranslational substrates before their transport into the endoplasmic reticulum. *J. Cell Biol.* **151**, 167-178.
Plath, K., Mothes, W., Wikinson, B. M., Stirling, C. J. & Rapoport, T. A. (1998). Signal sequence recognition in posttranslational protein transport across the yeast ER membrane. *Cell,* **94**, 795-807.
Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J. & Silver, P. (1989). A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an *Escherichia coli* heat shock protein. *J. Cell Biol.* **109**, 2665-2675.
Sanders, S. L., Whitfield, K. M., Vogel, J. P., Rose, M. D. & Schekman, R. W. (1992). Sec61p and BiP directly facilitate polypeptide translocation into the ER. *Cell,* **69**, 353-365.
Schmid, D., Baici, A., Gehring, H. & Christen, P. (1994). Kinetics of molecular chaperone action. *Science,* **263**, 971-973.
Schneider, H. C., Berthold, J., Bauer, M. F., Dietmeier, K., Guiard, B., Brunner, M. & Neupert, W. (1994). Mitochondrial Hsp70/MIM44 complex facilitates protein import. *Nature,* **371**, 768-774.
Scidmore, M. A., Okamura, H. H. & Rose, M. D. (1993). Genetic interactions between KAR2 and SEC63, encoding eukaryotic homologues of DnaK and DnaJ in the endoplasmic reticulum. *Mol. Biol. Cell,* **4**, 1145-1159.
Simon, S. M., Peskin, C. S. & Oster, G. F. (1992). What drives the translocation of proteins? *Proc. Natl Acad. Sci. USA,* **89**, 3770-3774.
Sung, W. & Park, P. J. (1996). Polymer translocation through a pore in a membrane. *Phys. Rev. Letters,* **77**, 783-786.
Vogel, J. P., Misra, L. M. & Rose, M. D. (1990). Loss of Bip/Grp78 function blocks translocation of secretory proteins in yeast. *J. Cell Biol.* **110**, 1885-1895.
Voisine, C., Craig, E. A., Zufall, N., von Ahsen, O., Pfanner, N. & Voos, W. (1999). The preprotein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70. *Cell,* **97**, 565-574.
White, S. H. & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annu. Rev. Biophys. Biomol. Struct.* **28**, 319-365.

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