COMPUTATIONAL MODELING OF CHAPERONE INTERACTIONS IN THE ENDOPLASMIC RETICULUM OF *SACCHAROMYCES CEREVISIAE*

Marc Griesemer [*] **Carissa Young** [***]
David Raden [***] **Francis Doyle III** [**]
Anne Robinson [***] **Linda Petzold** [*]

[*] *Department of Computer Science, University of California, Santa Barbara*
[**] *Department of Chemical Engineering, University of California, Santa Barbara*
[***] *Department of Chemical Engineering, University of Delaware*

Abstract:
In eukaryotes, the endoplasmic reticulum (ER) acts as a protein gatekeeper for protein folding, maturation, and transport. Molecular chaperones, of the Hsp70 family of proteins, participate in assisting these processes and are essential to cellular function and survival. BiP is the resident chaperone in the ER of *Sacchromyces cerevisiae*. In this study we have created two deterministic models to examine how BiP interacts with the membrane-bound co-chaperone Sec63 in translocation, a process in which BiP assists in guiding a nascent protein into the ER lumen.

Keywords: chaperones, endoplasmic reticulum, computational modeling, *S. cerevisiae*

1. INTRODUCTION

Molecular chaperones, of the Hsp70 family of proteins, participate in a wide range of processes essential to cellular function and survival. Found in all organisms and major compartments of eukaryotic cells, many are expressed in response to cellular stress (Georgopoulos and Welch, 1993). These highly conserved proteins assist in protein folding and maturation, assembly or disassembly of complexes, translocation of newly synthesized proteins, suppression of aggregation, and protein degradation. Such versatility is intriguing because molecular chaperones have a single activity: peptide binding. The phenomenon of a protein having multiple, sometime competing, functions indicates a high level of systems control. Co-chaperones stimulate the binding of chaperone to substrate and play a critical role in regulating chaperone activities (Hennessy *et al.*, 2005).

The formation of a trimeric complex (consisting of chaperone, co-chaperone, and substrate) has been studied extensively by *in vitro* experimentation. However, experimental evidence indicates that co-chaperones are localized on a sub-organelle level through interactions with other proteins or lipid membranes. *In vitro* experimental methods cannot capture these cellular spatial effects. Therefore it is plausible that the involvement of a molecular chaperone in diverse cellular functions is regulated by spatial distribution or the formation of molec-

ular gradients by co-chaperones. In this work we test this hypothesis via iterations between computation and experiments.

Our first step was to develop an ordinary differential equation (ODE) model to describe the kinetic characteristics of chaperone regulation in yeast, *Sacchromyces cerevisiae*. The model and parameters are based on a kinetic model developed for simulating chaperone protein refolding in the bacterium *Escherichia coli* (Hu *et al.*, 2006). Experiments to determine the parameters specific to the yeast system are in progress, however most of those results are not yet available. Thus our ODE model currently uses the reaction rate constants resulting from *in vitro* experimentation of the well characterized DnaK and DnaJ chaperone system in *E. coli*.

Our next step was to incorporate spatial effects, creating a time-dependent partial differential equation (PDE) model. The PDE model was then used to simulate a freely diffusing chaperone regulated by a co-chaperone with limited mobility. This models a chaperone interacting with a co-chaperone that is bound to a lipid membrane. Diffusion coefficients were estimated from the literature and initial species concentrations were based on experimental work on *S. cerevisiae*.

2. BACKGROUND

The first organelle of the secretory pathway, the ER is frequently referred to as the protein gatekeeper within the cell, allowing only properly folded proteins to continue to their final destination (Figure 1). It is a major site of protein synthesis and responds to cellular stress conditions in order to maintain homeostasis of the cell. The molecular chaperone BiP resides within the ER lumen of yeast. Through biochemical and genetic experiments, BiP has been identified in critical cellular processes including protein translocation, folding and maturation, karyogamy (nuclear fission), and ERAD (ER Associated Degradation) where unfolded or abnormally folded proteins are sent back to the cytosol for degradation (Brodsky and Schekman, 1993; Latterich and Schekman, 1994; McCracken and Brodsky, 2003; Nishikawa and Endo, 1997; Schlendstedt *et al.*, 1995). The aforementioned processes are associated with selective co-chaperones (Figure 2). Proteomic studies have verified the location of associated proteins to the ER and identified absolute levels of protein expression (Ghaemmaghami *et al.*, 2003; Huh *et al.*, 2003). Results indicate that the concentration of BiP exceeds the level of co-chaperones at normal growth conditions by at least an order of magnitude. Additional experiments have verified that co-chaperones must be localized at a sub-organelle level in order for BiP-required cellular activities to occur (Corsi and Schekman, 1997). Based upon these three factors a) BiP's involvement in various cellular functions and interaction with multiple co-chaperones, b) concentration differences between specified proteins, and c) validation that protein interactions within this system are dependent upon localization, we seek to address the following hypothesis: Spatial effects upon BiP are regulated by co-chaperones in order to control cellular functions and behavior within the ER of *S. cerevisiae*.

The focus of our work is to evaluate the interaction between BiP and unfolded protein regulated by the co-chaperone, Sec63. This protein complex is involved in the process of translocation, defined as nascent protein transit into the ER. Sec63 is associated with the ER membrane, localizing its interaction with BiP to a thin zone adjacent to the membrane. Several experiments with protein variants of Sec63 have studied the molecular nature of this interaction (Corsi and Schekman, 1997). Results indicate that a loss of co-chaperone localization at the membrane inhibits efficient transport of nascent protein to the lumen. Our computational model of BiP localization in *S. cerevisiae* attempts to capture the observed experimental results. We are equating the amount of BiP at the membrane with translocation efficiency. In addition, spatial effects of co-chaperone Sec63 are accounted for in our PDE model.

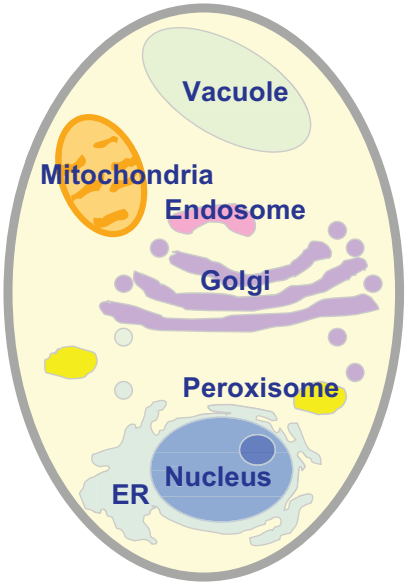

Fig. 1. Schematic of an eukaryotic cell. The Endoplasmic Reticulum (ER) is the first organelle in the secretory pathway.

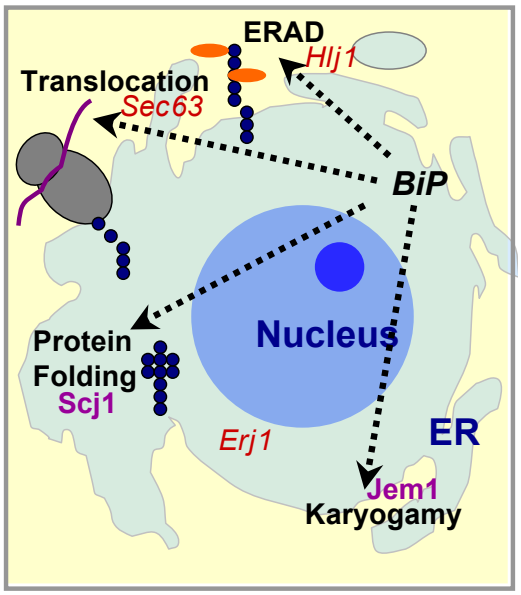

Fig. 2. Depiction of chaperone BiP and its involvement in multiple cellular processes e.g. translocation, protein folding and maturation, karyogamy, and ERAD. Respective proteins for each process are depicted in italics (membrane proteins) and boldface (freely diffusing).

3. MODELS

Modelers have attempted to discern BiP's role in assisting translocation of proteins in the ER. Previous work has focused on the mechanisms of transport, with the most popular being the Brownian ratchet model (Elston, 2000; Elston, 2002). Our work is not specifically concerned with the mechanism of translocation, but rather with how the BiP-Sec63 interaction enhances translocation. Experiments have posited that Sec63 recruits BiP to the membrane surface to perform translocation (Corsi and Schekman, 1997). As previously mentioned, the distribution of BiP in the ER is believed to be inhomogeneous. Given that the populations of BiP and other proteins in the system range from tens to hundreds of thousands of each species, a deterministic model of molecular concentrations seems to be justified. To this end, we first constructed an ODE model, and then extended it to a PDE model to capture the spatiotemporal dynamics.

3.1 Model Descriptions

3.1.1. ODE model Our core model is described by a system of ordinary differential equations. It is a 7 state, 13 parameter model that represents the interactions of BiP with the co-chaperone Sec63 and unfolded proteins (U), and is shown as a schematic in Figure 3. It is derived from a larger kinetic model (Hu *et al.*, 2006) that examined the protein folding activity of chaperones DnaK and DnaJ. These chaperones are involved in heat-shock remediation in *E. coli*, and have been extensively studied *in vitro*. In these studies, it has been found that DnaK associates loosely with the unfolded protein until DnaJ causes tighter binding through the stimulation of ATPase activity of the chaperone. In our model, BiP and Sec63 play the roles of DnaK and DnaJ, respectively.

Using the states of the model in Figure 3, we constructed a largely unidirectional cycle which describes the system. Free BiP associated with ATP (state $X1$) can bind to the unfolded protein and can then associate with Sec63 to form a trimeric complex ([BiP-ATP-U-Sec63], state $X3$). ATP hydrolysis occurs either on Sec63-dependent (X3→X4) or independent (X6→X7) pathways. Therefore, BiP exists in two conformational domains: ATP-bound states represented in the upper triangular portion of the diagram; and ADP-bound states in the lower triangular portion. The former has high association rates and low affinity to unfolded protein, while the ADP-bound states have low association rates and high affinity to unfolded protein. The cycle completes with the dissociation of Sec63 and the nucleotide exchange enhanced by the protein GrpE. BiP then releases the unfolded protein and returns to its free ATP-bound state, and the cycle can repeat.

We conducted simulations using this model configuration and collected concentration data for each state. The simulation produced results in which states $X1$ ([BiP-ATP]) and $X6$ ([BiP-U-ATP]) dominated the concentration levels. This confirmed an important point: that BiP strongly interacts with unfolded protein, although it should be mentioned that the association rate is on the higher end of the range of experimental data (Hu *et al.*, 2006). This initial model served as a description of reaction kinetics between BiP, Sec63, and unfolded protein, and was a building block for constructing a spatially-dependent model describing chaperone interactions in the ER.

3.1.2. PDE Model Proceeding from the ODE model, our next objective was to describe the distribution of BiP in the ER due to spatial effects. The partial differential equation model describes translocation. The model incorporates: (1) chemical reactions representing transitions between states in the ODE system that take place on the inner membrane, and (2) diffusion into the lumen of the ER. This spatially dependent system

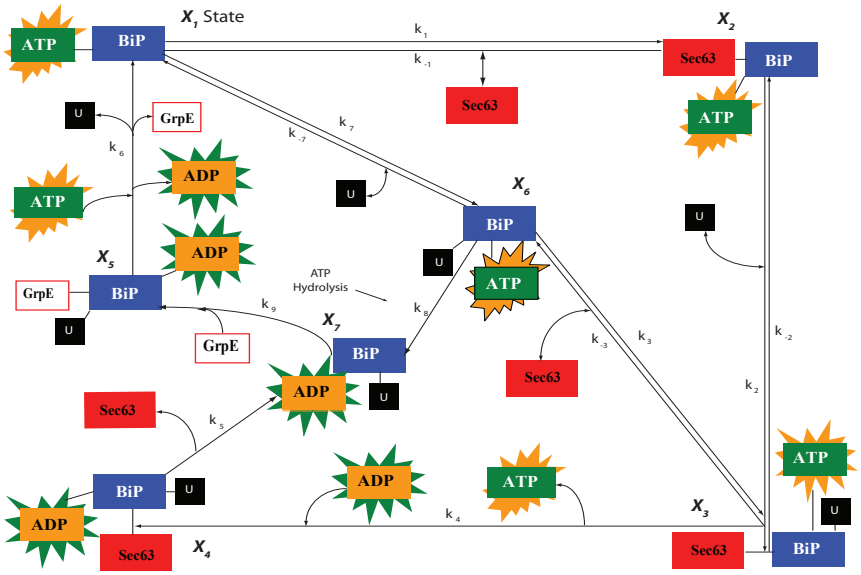

Fig. 3. The ODE model consisting of 7 states, representing the interaction of BiP with the other proteins in the system.

of equations was approximated by the method of finite differences (Figure 4). The irregular geometry of the ER was simplified to a sphere (surrounding a spherical nucleus) and assumed to be symmetric. With these assumptions, the (time-dependent) system can be modeled in one spatial dimension.

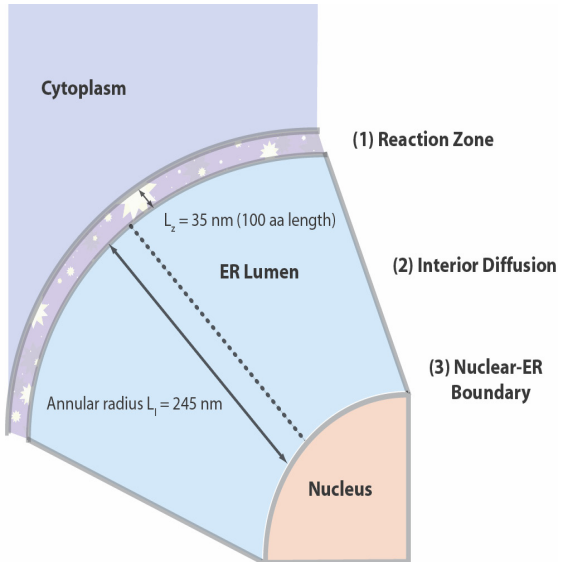

Fig. 4. The PDE model consists of a reaction zone and lumen represented by reaction-diffusion equations. The length of the reaction zone, L_z, was taken to be 35 nm, while the annular radius of the lumen, L_l, is 245 nm.

We define the reaction zone as the membrane portion of the ER where protein-protein interactions take place between BiP and the other proteins in the system. The rate of change of the concentration of species k is the sum total of the concentrations of the free and bound species plus the diffusion in the interior.

$$L_z \frac{\partial C_{z,k}}{\partial t} = R_k + D \frac{\partial C_{l,k}}{\partial x}\Big|_{x=0} \tag{1}$$

Pure (one-dimensional) diffusion occurs in the lumen

$$\frac{\partial C_{l,k}}{\partial t} = D \frac{\partial^2 C_{l,k}}{\partial x^2} \tag{2}$$

with the nuclear-ER boundary condition given by

$$\frac{\partial C_{l,k}}{\partial x}\Big|_{x=L_l} = 0. \tag{3}$$

Here, $C_{z,k}$ is the reaction zone concentration and $C_{l,k}$ represents the concentration in the lumen for diffusing species k. (If this species exists only in the reaction zone, then this term is absent.) D is the diffusion coefficient (assumed to be the same for all species), and R_k is the reaction term for species k. L_z is the length of the reaction zone (in one dimension), and L_l is the annular radius of the lumen. The variable x represents the distance from the reaction zone for a particular point on the grid.

Discretizing in space, we obtain

$$L_z \frac{\partial (C_{z,k})}{\partial t} = R_k + D \frac{C^1_{l,k} - C_{z,k}}{\Delta x} \tag{4}$$

$$\frac{\partial C^1_{l,k}}{\partial t} = D\frac{C_{z,k} - 2C^1_{l,k} + C^2_{l,k}}{\Delta x^2} \tag{5}$$

$$\vdots$$

$$\frac{\partial C^N_{l,k}}{\partial t} = D\frac{C^{N-1}_{l,k} - C^N_{l,k}}{\Delta x^2}, \tag{6}$$

where $C^i_{l,k}$ represents the concentration of species k at grid point i in the lumen, and Δx is the spatial separation between two consecutive grid points. The boundary at $x = 0$ is the ER membrane, and the boundary at $x = L_l$ is the nucleus. Given this formulation, the concentration of each species is tracked spatially and temporally.

3.2 Model Assumptions

Several assumptions were made to simplify the model and approximate the dynamics of the system. In the reaction zone, the reactions are not assumed to be localized to any particular region of the zone. The focus was on the ER-lumen boundary, setting the flux to zero from the cytoplasm. We also do not have any substrate in the lumen; it remains in the reaction zone throughout our simulations. Physiologically, the nascent protein chains would be released from the translocation channel and diffuse into the lumen. Furthermore, we are not modeling reactions occuring in the lumen. BiP normally interacts with proteins or other co-chaperones which would be in competition for free BiP molecules, but this is not a focus of our present work.

3.3 PDE Model Scenarios

Four scenarios were defined to simulate different conditions in the ER. In each scenario, different species were allowed to diffuse in the lumen. The scenarios are

(1) The wild-type case which assumes that only free BiP is allowed to diffuse into the lumen.
(2) Sec63 and BiP are both allowed to diffuse into the lumen. This occurs with an experimental variant of Sec63p lacking its membrane tether, Sec63v.
(3) Sec63 is removed from the system and free BiP is allowed to diffuse.
(4) Unfolded protein (U) is removed from the system. As a result, translocation is inhibited (free BiP diffusing). This gives a baseline interaction of BiP's distribution in the ER.

It should be noted that scenario 1 is the only normal condition. Scenarios 2-4 are special conditions that can be experimentally obtained using either genetic manipulation or chemical treatment of the yeast cell.

3.4 Ratio Metric

From the ER PDE model, we determine the ratio of reaction zone concentration of BiP to the concentration in the interior. This gives an indication of spatial localization and homogeneity of BiP, and can be verified experimentally. The default scenario is to allow free BiP to diffuse while bound BiP and the other players (namely Sec63 and unfolded protein) remain in the reaction zone. From these scenarios, one can make predictions of the importance of these processes on translocation. This is described by the equation:

$$r = \frac{\left[BiP\right]_{z+} + \left[BiP^b\right]_z}{[BiP]_L}, \tag{7}$$

At steady-state, the concentrations of free BiP in the reaction zone and in the lumen are the same,

$$\left[BiP\right]_z = \left[BiP\right]_L, \tag{8}$$

however the total BiP concentration is much higher in the reaction zone.

3.5 Preliminary Model Results

Using the DASSL(DASPK) ODE/DAE solver (Brenan *et al.*, 1996), we ran simulations for each of the scenarios until all species reached steady-state, at t=5 s. The system starts from conditions where all the BiP is free and present in the reaction zone. Diffusion is fast, equalizing gradients of free BiP across the ER. Reactions then take place on a slower timescale, locking up BiP in the surface zone. The output was the concentration of each species in the reaction zone and at interior grid points. We then calculated the ratio of total BiP (free + bound) concentration in the reaction zone over the free BiP in the lumen.

Table 1. BiP ratio by scenario.

Scenario		BiP ratio
1	BiP	7.06
2	Sec63	7.02
3	Sec63=0	6.35
4	U=0	1.44

The results (Table 1) show that BiP preferentially remains in the reaction zone, giving an inhomogeneous distribution throughout the ER. This is expected since BiP is reacting on the surface and forming non-diffusing species. When Sec63 is removed from the system, however, the BiP ratio essentially remains unchanged. This presents an

interesting result which contrasts with the understanding that BiP is recruited to the surface by Sec63 in the process of translocation. Finally, when translocation is inhibited (U=0), the BiP ratio (1.44) is much lower. This calculation was initially done with 11 spatial grid points, and was repeated with 91 spatial grid points, yielding identical results for the ratios.

Additionally, we calculated the ratio of Sec63 for scenario 2 where it exists in its variant (diffusing) form, and for scenario 4 when unfolded protein is removed from the system. The result is given in Table 2.

Table 2. Sec63 ratio

Scenario	Sec63 ratio
Sec63	3497
U=0	$2.2*10^6$

In these cases, Sec63 remains overwhelmingly on the membrane. Further cases would give insight into other parts of the system.

4. CONCLUSION

We have constructed two deterministic models of chaperone interactions: (1) a preliminary ODE model with most of its parameters taken from the literature for *E. coli* while we wait for experimental results; and (2) a spatial PDE model describing the chaperone activity in the ER of *S. cerevisiae* through reaction-diffusion equations. From the simulations, we found that the concentration of BiP and other proteins in the system was inhomogeneous, with the concentration being greater at the membrane. Our scenarios showed, however, that BiP had the same distribution whether Sec63 was present at the membrane or not. This result runs counter to the idea that Sec63 specifically recruits BiP in transporting nascent protein into the ER lumen. We plan to add reactions to the lumen and let the substrates freely diffuse there as a next step.

In creating these models, we have taken a step in making predictions to determine the relative importance of BiP in the translocation of proteins. The model can be extended, if necessary, to more complicated geometries, or to incorporate stochasticity.

REFERENCES

Brenan, K E, S L Campbell and L R Petzold (1996). *The Numerical Solution of Initial Value Problems and Differential-Algebraic Equations*. second ed.. SIAM Classics in Applied Mathematics Series. New York, NY.

Brodsky, J L and R Schekman (1993). A sec63p-bip complex from yeast is required for protein translocation in a reconstituted proteoliposome. *J. Cell Biol.* **123**(6 Pt 1), 1355–63.

Corsi, A K and R Schekman (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.* **137**(7), 1483–93.

Elston, T (2000). Models of posttranslational protein translocation. *Biophys. J.* **79**(5), 2235–2251.

Elston, T (2002). The brownian ratchet and power stroke models for posttranslational protein translocation into the endoplasmic reticulum. *Biophys. J.* **82**(3), 1239–1253.

Georgopoulos, C and WJ Welch (1993). Role of the major heat shock proteins as molecular chaperones. *Annu. Rev. Cell Biol.* **9**, 601–634.

Ghaemmaghami, S, W K Huh, K Bower, R W Howson, A Belle, N Dephoure, E K O'Shea and J S Weissman (2003). Global analysis of protein expression in yeast. *Nature* **425**(6959), 737–41.

Hennessy, F, W Nicoll, R Zimmermann, M Cheetham and G Blatch (2005). Not all j domains are created equal: Implications for the specificity of hsp40-70 interactions. *Prot. Sci.* **14**, 1697–1709.

Hu, B, M P Mayer and M Tomita (2006). Modeling hsp70-mediated protein folding. *Biophys. J.* **91**(2), 496–507.

Huh, W K, J V Falvo, L C Gerke, A S Carroll, R W Howson, J S Weissman and E K O'Shea (2003). Global analysis of protein localization in budding yeast. *Nature* **425**(6959), 686–91.

Latterich, M and R Schekman (1994). The karyogamy gene kar2 and novel proteins are required for er-membrane fusion. *Cell* **78**(1), 87–98.

McCracken, A A and J L Brodsky (2003). Evolving questions and paradigm shifts in endoplasmic-reticulum-associated degradation (erad). *BioEssays* **25**(9), 868–77.

Nishikawa, S and T Endo (1997). The yeast jem1p is a dnaj-like protein of the endoplasmic reticulum membrane required for nuclear fusion. *J. Bio. Chem.* **272**(20), 12889–92.

Schlendstedt, G, S Harris, B Risse, R Lill and PA Silver (1995). A yeast dnaj homologue, scj1p, can function in the endoplasmic reticulum with bip/kar2 via a conserved domain that specifies interactions with hsp70s. *J. Cell Biol.* **129**, 979–988.