A Model of the Unfolded Protein Response: Pancreatic β -Cell as a Case Study

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Key Words

Protein homeostasis • Mathematical model • Enzyme kinetics • Diabetes mellitus • Insulin production

We also present predictions for therapeutic strategies to ameliorate pancreatic β -cell death in diabetes patients.

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Abstract

Pancreatic β -cell failure is increasingly recognized as central to the progression of diabetes mellitus. Different causes are implicated in the onset of β -cell stress, dysfunction or death. Recent genetic and biochemical evidence both in humans and mice shows that failure in modulating the capacity and quality of the endoplasmic reticulum protein-folding machinery leads to β -cell death. The quality control of protein-folding is regulated by several signaling pathways, which are collectively termed the unfolded protein response. In β -cells proinsulin represents up to 50% of the total protein synthesis, and the rate of glucose-stimulated proinsulin translation is approximately 1 million molecules per minute per cell. When proinsulin folding is disrupted, the high burden imposed by proinsulin synthesis on the unfolded protein response is considered the leading cause of certain diabetes phenotypes. We have developed a model of the unfolded protein response to investigate the factors that can prevent misfolded protein accumulation.

Introduction

The endoplasmic reticulum (ER) is the largest intracellular compartment of eukaryotic cells, with an extensive array of interconnecting membranes, tubules, and flattened sacs that extend throughout the cell, including the nuclear envelope. ER membranes are physiologically active, and contain differentiated domains specialized for specific functions [1]. One of the main functions of the ER is to serve as the protein-folding factory [2]. The ER is responsible for the synthesis, folding, assembly and modification of one third of the eukaryotic proteome [3].

Proteins enter the ER as unfolded polypeptide chains with variable fluxes depending on the physiological state of the cell. A sudden increase in the demand for a protein or the disruption of a folding reaction causes an imbalance between protein-folding load and capacity of the ER, which can lead to the accumulation of unfolded or

misfolded proteins in the ER lumen [4]. These changes in the protein landscape of the ER lead to a condition known as ER stress [5]. If the protein homeostasis is not restored, apoptosis (programmed cell death) will be induced [6, 7].

Eukaryotic cells have evolved several signal transduction pathways collectively known as the unfolded protein response (UPR) to achieve the homeostatic control of protein production. The UPR ensures the fidelity of protein folding and prevents the accumulation of unfolded or misfolded proteins. The UPR accomplishes protein homeostasis through three pathways. The first pathway prevents the influx of nascent polypeptides into the saturated ER by inhibiting protein synthesis [8, 9]. The second, known as the ER-assisted folding (ERAF) pathway [10], increases the ER capacity to fold proteins through up-regulation of chaperones and folding catalysis [11, 12]. The third, known as the ER-associated degradation (ERAD) pathway [13], induces degradation of misfolded and aggregated unfolded proteins [14].

Recent genetic and biochemical evidence both in humans and mice shows that failure to modulate the UPR leads to a number of diseases [15], among them diabetes mellitus [16]. In β -cells the rate of glucose-stimulated proinsulin mRNA translation is approximately 1 million molecules of proinsulin per minute per cell. Proinsulin can represent up to 50% of the total protein synthesis in β -cell [17-19]. The high burden imposed by proinsulin on the unfolded protein response is considered the leading cause of certain diabetes phenotypes. Multiple studies have demonstrated that disruptions in the UPR pathways can cause accumulation of unfolded and misfolded proinsulin in the ER lumen, a condition that leads to death of β -cells and to the progression of diabetes mellitus [20]. As β -cell mass decreases, the surviving β -cells need to compensate and increase their insulin production. This condition increases protein folding demand and the risk of triggering ER stress in normal β -cells.

Reduced UPR signaling is sufficient to cause the onset of type-1 diabetes, type-2 diabetes, and other diabetic phenotypes. The protein kinase RNA(PKR)-like ER kinase gene (*PERK*), a transducer of the UPR, has been linked to type 1 diabetes in South Indian populations through analysis of polymorphisms at this gene [21]. Mutation in another UPR transducer, the activating transcription factor-6 (ATF6), is linked to type-2 diabetes in Dutch [22] and Pima Indian cohorts [23].

ER stress caused by proinsulin misfolding has been implicated as a proximal event in the etiology of young adult onset [24, 25] and neonatal [26] diabetes. Studies

of *Akita* and *Munich* mouse models show mutations at cysteine residues that impair proinsulin disulfide bond formation [27] and activate ER stress. *Akita* mice have pancreatic insulin deficiency [28, 29] and suffer loss of β -cell mass within weeks after birth [30]. *Akita* mice β -cells are highly susceptible to proteotoxicity [31] even when the ER stress response is fully activated [32] and the ERAD pathway is up-regulated to clear misfolded proteins [33]. In humans, proinsulin with the analogous *Akita* mutation shows that expression of such proinsulin is proteotoxic in comparison with non-mutants. Recently, neonatal onset of dominantly inherited diabetes in 16 families had been associated with a missense mutation in the human *Ins* gene [26]. This mutation impairs proinsulin disulfide bond formation leading to proinsulin misfolding. In summary, disruption of disulfide bond pairing in proinsulin is sufficient to induce diabetes in both humans and mice [20].

Can the accumulation of misfolded protein be managed to prevent β -cell failure? The mechanisms that cause β -cells to fail upon ER stress are not well understood yet. The UPR is a complex system which adapts to fluctuations in cell physiology and demand for protein synthesis. Potential therapeutic interventions will require the identification of molecular targets for both the upstream activators of the UPR and the downstream responses to UPR. At the moment, this is not an easy task as 20% of known genes go through transcriptional changes when the UPR is activated [20]. Efforts are being made to provide an accounting of proteins in the endoplasmic reticulum of rat liver parenchyma [34]. The only way we can accomplish this task is through a complete analysis of the physiological function of each UPR subpathway.

In recent years, the use of mathematical and computational modeling has proved to be essential for the analysis of complex biochemical and signaling pathways [35-37]. Models serve as a tool, comparable to any experimental laboratory tool, for hypothesis testing and making experimentally testable predictions [38]. Recently a mathematical model of the UPR provided an integrative view of the ER function in protein export [39]. In this paper we explore a different aspect of the UPR: we develop a mathematical model of UPR kinetics to investigate the factors driving the accumulation of newly synthesized protein as misfolded protein. We employ this model to investigate potential therapeutic strategies to prevent newly synthesized protein from accumulating as misfolded protein. The primary objective of therapies for diseases related to ER stress is preventing the

accumulation of misfolded proteins, which leads to proteotoxicity and cell death [31]. Presently there are two management strategies proposed to prevent accumulation of misfolded proteins. For mutant misfolded proteins causing recessive loss-of-function diseases, the improvement of protein folding efficiency [40, 41] is considered beneficial for patients. For genetically dominant protein defects causing ER stress and cell death, such as proinsulin mutations [26], the proposed strategies are reducing ER stress or apoptosis signals [20]. Our model shows neither strategy alone would effectively prevent accumulation of protein misfolding. Our model predicts that improvement in both protein folding efficiency and degradation of misfolding-prone proteins is the best treatment for protein misfolding diseases.

Current model for activation of the unfolded protein response

In the mammalian cell, the main UPR signaling cascade is initiated by three ER stress transducers: inositol-requiring protein-1 α (IRE1 α), ATF6 and PERK. Each transducer defines a distinct subpathway of the UPR. Currently there are three models explaining how these transducers sense the unfolded protein load [6, 42]. The model consistent with most experimental evidence suggests that all three sensors are maintained in an inactive state through the association with the abundant ER chaperone immunoglobulin-binding protein (BiP) in resting cells. In ER stress, BiP is sequestered by unfolded or misfolded proteins, thereby leading to release and activation of the ER transducers [43].

The most immediate response to ER stress is the activation of the UPR subpathway initiated by the PERK transducer. PERK is an ER-localized type I transmembrane protein with a cytoplasmic portion containing a protein kinase domain. This domain undergoes activating trans-autophosphorylation following the release of BiP from PERK. PERK phosphorylates the α -subunit of eukaryotic translation initiation factor-2 (eIF2 α). eIF2 α phosphorylation inhibits the assembly of the 80S ribosome and consequently the synthesis of proteins. This subpathway mediates protein homeostasis by reducing load of newly synthesized proteins entering the ER lumen [44, 45].

In parallel, ER stress activates the UPR subpathways initiated by the IRE1 α and ATF6 transducers. IRE1 α is transmembrane protein that undergoes autophosphorylation in response to unfolded

Fig. 1. The unfolded protein response is mediated by three stress transducers resident in the endoplasmic reticulum (ER). This diagram illustrates the main events leading to the activation of the unfolded protein response (UPR). The protein chaperone BiP is presently considered the master regulator of the UPR. Under nonstressed conditions BiP binds to the stress transducers PERK, ATF6 and IRE1. With the accumulation of unfolded proteins (U), BiP dissociates from the stress transducers and binds to the unfolded proteins. This leads to the activation of stress transducers. The arrows show the progression of events after the stress transducers activation. For instance, activation of the PERK pathway requires the autophosphorylation of PERK. Later PERK phosphorylates the α -subunit of eukaryotic translation initiation factor-2 (eIF2 α). eIF2 α phosphorylation inhibits the assembly of the 80S ribosome and consequently synthesis of proteins.

proteins. IRE1 α then cuts the precursor x-box binding protein 1 (XBP1) mRNA. This results in a XBP1 isoform, known as active XBP1, a potent transcription factor [6, 46]. On the other hand, ATF6 is transported from the ER to the Golgi apparatus, where it is cleaved by site 1 protease and site 2 protease. This process results in release of the cytosolic DNA-binding portion of ATF6, known as ATF6f [47]. From there ATF6f migrates to the nucleus to specifically induce along with active XBP1 the activation of genes encoding for the ERAF and ERAD pathways [48, 49]. We illustrate schematically the activation of the unfolded protein response in Figure 1.

The ERAF pathway uses many chaperones and folding catalysts to direct folding [10]. In the ER lumen,

folding is aided by chaperones like BiP and glucose-regulated protein 94 (GRP94); the calnexin/calreticulin cycle also facilitates N-linked glycoprotein folding chaperones. The folding catalysts include protein disulfide isomerases (PDI) and *cis/trans* peptidyl-prolyl isomerases [50-52]. The ERAF and ERAD pathways are versatile and complex biochemical pathways, whose complete mechanisms remain to be established. If these mechanisms fail to restore the protein homeostasis in the ER, the UPR will initiate apoptosis to protect the cells from producing misfolded or malfunctioning proteins [6, 46].

A mathematical formulation of the model for the unfolded protein response

The UPR is a complex and integrated system of pathways. To assess and understand the physiological functions of each UPR subpathway, we need to develop an integrated mathematical formation of UPR. Our mathematical formulation models the rates of the populations of unfolded, misfolded and folded protein. The concentration levels of the unfolded (u), misfolded (m) and (f) folded proteins are dictated by the ERAF pathway, ERAD pathway as well as the rate of protein misfolding. To simplify the model construction we investigate the UPR after the three transducers have been activated, which makes our model independent of the activation mechanism of the UPR transducers. Under this simplification, time is initiated ($t=0$) in our model immediately after the transducers have been activated. We introduce the following assumptions to develop the model:

- In ER stress, the UPR subpathway initiated by the PERK transducer completely halts the influx of unfolded protein in the ER lumen. The unfolded protein concentration triggering the UPR defines the initial unfolded protein concentration, which we denote as u_0 . u_0 in our model is also equivalent to the concentration of newly synthesized protein. The assumption that the newly synthesized protein is constant during the UPR is reasonable under the observation that the PERK transducer subpathway promotes cell survival by preventing the influx of nascent polypeptides under conditions of ER stress [9, 53].

- The rate of protein misfolding is first-order kinetics [54], and is proportional to its reactant concentration: the unfolded protein concentration. The misfolding rate constant is defined as k_0 . The rate of transition of

misfolded protein to unfolded or folded protein is very slow and does not occur within a relevant physiological timescale. In the literature, the transition between folding states of proteins *in vivo* has been reported to follow first-order kinetics [55-57].

- The rate of protein folding follows Michaelis-Menten kinetics. We propose that the ERAF pathway can be modeled as a single enzyme, which recognizes the unfolded protein as its substrate. Complex biochemical pathways have been simplified with Michaelis-Menten kinetics while still capturing the overall dynamics of the pathway [35, 36, 39]. In our case, the folding rate exhibits maximum velocity V_f with Michaelis-Menten constant K_f . K_f may be considered as the overall dissociation constant of all folding enzymes bound to unfolded proteins in the ERAF pathway [58]. The fraction of unfolded protein converted into folded protein per unit time by the ERAF is determined by $r_f = V_f/K_f$ [59].

- The rate of unfolded and misfolded protein degradation follows a linear competitive substrate Michaelis-Menten kinetics. We propose that proteins recruiting substrates to the ERAD pathway act like a single enzyme competing for their substrates (unfolded and misfolded proteins) [60]. Recently it has been shown that this assumption captures the overall dynamics of the ERAD pathway in a general model of protein export from the ER [39]. In our model, the unfolded protein degradation exhibits a maximum velocity V_u with Michaelis-Menten constant K_u , while the misfolded protein degradation has maximum velocity V_m , with Michaelis-Menten constant K_m . K_u may be considered as the overall dissociation constant of all protein degradation enzymes bound to unfolded proteins in the ERAD pathway, while K_m may be treated as the overall dissociation constant of all protein degradation enzymes bound to misfolded proteins in the ERAD pathway. The fraction of unfolded protein that is degraded per unit time by the ERAD pathway is $r_u = V_u/K_u$, while the fraction of misfolded protein that is degraded per unit time by the ERAD pathway is $r_m = V_m/K_m$ [59].

Our mathematical formulation of the UPR is based on protein misfolding kinetics and a Michaelis-Menten treatment of the ERAF and ERAD pathways, where the pathways are represented as single enzyme reactions. We do not anticipate changes in the model behavior if the pathways are modeled with Hill functions instead of Michaelis-Menten equations. However, the selection of different competitive mechanisms for the rate of unfolded and misfolded protein degradation can have substantial effects on the model dynamics.

Fig. 2. The unfolded protein response in the ER lumen is influenced by the interaction of the ER-assisted folding (ERAF, solid line) and ER-assisted degradation (ERAD, broken line) pathways. The complex reactions in each of these pathways are assumed to have a lumped behavior following Michaelis-Menten kinetics. Each pathway is considered like an enzyme. The folding protein (F) is synthesized from the unfolded protein (U) via the ERAF pathway represented as the enzyme A. The folding rate exhibits maximum velocity V_f with Michaelis-Menten constant K_f . The unfolded protein and misfolded protein (M) are degraded (D) via the ERAD pathway, which is represented by the enzyme B. The unfolded protein degradation exhibits a maximum velocity V_u with Michaelis-Menten constant K_u , while the misfolded protein degradation has maximum velocity V_m with Michaelis-Menten constant K_m . The enzyme-substrate complex intermediate for each pathway is represented by C. The unfolded protein is misfolded through a first-order reaction with constant k_0 .

We illustrate our model schematically in Figure 2. The unfolded protein is released into the ER lumen, where it can be folded in the presence of chaperones and folding catalysts through the ERAF pathway. The unfolded proteins can be transformed irreversibly into misfolded proteins under the influence of mutations or changes in the energy landscape of unfolded protein. Unfolded and misfolded proteins are subject to protein degradation through recruiting chaperones of the ERAD pathway. Our model does not consider the case where the UPR initiates apoptosis, because it occurs in a longer timescale.

In accordance with the assumptions, u and m are governed in our model by the following system of ordinary differential equations:

$$\frac{du}{dt} = \underbrace{-k_0 u}_{\text{Unfolded protein rate}} - \underbrace{\frac{r_f u}{1 + \frac{u}{K_f}}}_{\text{First-order depletion due to protein misfolding}} - \underbrace{\frac{r_u u}{1 + \frac{m}{K_m} + \frac{u}{K_u}}}_{\text{Depletion due to unfolded protein degradation}} \quad (1)$$

$$\frac{dm}{dt} = \underbrace{k_0 u}_{\text{Misfolded protein rate}} - \underbrace{\frac{r_m m}{1 + \frac{u}{K_u} + \frac{m}{K_m}}}_{\text{First-order production from unfolded protein}} - \underbrace{\frac{r_m m}{1 + \frac{u}{K_u} + \frac{m}{K_m}}}_{\text{Depletion due to misfolded protein degradation}} \quad (2)$$

We can also write expressions for f and degradation product (d), but these are not essential for our preliminary analysis, because they are dependent on u and m . The initial condition for Equations (1)-(2) is $(u, m)(t = 0) = (u_0, 0)$. Note that we are taking the concentration of misfolded protein equal to zero when the UPR is initiated: $m(t = 0) = 0$. This assumption does not affect the

derivation of our model, or the response of the model to variations in the parameter values.

Dynamical behavior of the unfolded and misfolded protein populations in the model of the unfolded protein response

We will first examine the time course of the unfolded and misfolded populations. We can accomplish this by inspecting the signs in our model equations (1)-(2). The rate constant k_0 and kinetics parameters V_u , K_u , V_f , K_f and V_m , K_m , are always positive; otherwise, the reaction kinetics of the model would lead to negative concentrations. As a consequence, the rate of u , Equation (1), is always negative $du/dt < 0$ while the unfolded protein is depleted. Therefore, u decreases monotonically from its initial concentration u_0 towards zero during the time-course of UPR.

The situation is different for the time course of m . Equation (2) has two terms: the first-order production rate, which is greater than or equal to zero, and a depletion rate which is less than or equal to zero. At the beginning of UPR, m is zero or very small. This implies that the rate of depletion due to unfolded protein degradation is negligible in comparison with the rate of production of misfolded protein from unfolded protein, which is positive. Therefore, $dm/dt > 0$ during the initial transient of the UPR. As u decreases and m increases, the rate of depletion of misfolded protein will increase above the rate of

Fig. 3. The time course of the unfolded protein (u) and misfolded protein (m) concentrations in our mathematical model of the unfolded protein response. After the unfolded protein response (UPR) is activated, the unfolded protein is depleted monotonically by the UPR machinery. In contrast, the misfolded protein reaches a maximum value (50% of protein) before being depleted by the UPR machinery. This general behavior is characteristic for our model. The equations were solved numerically with the Runge-Kutta-Rosenbrock algorithm for stiff ordinary differential equations in MAPLE 12. The initial unfolded protein concentration is $1 \mu\text{M}$. The parameter values used in this figure are: $k_0 = 0.25 \text{ s}^{-1}$, $V_f = V_u = V_m = 0.1 \mu\text{M s}^{-1}$ and $K_f = K_u = K_m = 1.1 \mu\text{M}$. The rate constants employed to determine these Michaelis-Menten constants are discussed in the text.

production of misfolded protein. This will make $dm/dt < 0$ after the initial transient of the UPR. The change of the misfolding protein rate from positive to negative implies that m increases from zero to a maximum value, and then decreases again back to zero.

The dynamical behavior of u and m is illustrated in Figure 3. The unfolded protein starts at its initial concentration $1 \mu\text{M}$ and is depleted monotonically by the UPR machinery. In contrast, the misfolded protein reaches a maximum value before being depleted by the UPR machinery. To calculate the numerical solution to Equations (1)-(2), we used the Runge-Kutta-Rosenbrock method for stiff ordinary differential equations implemented in MAPLE 12 and assigned the kinetics parameters that described each pathway in our model. The values selected for the kinetics parameters are based on rate constants for similar processes or models in the literature. The enzyme-substrate association constants were set equal to $10^6 \text{ M}^{-1} \text{ s}^{-1}$, which is within the same order of magnitude for typical protein-protein association

reactions [61]. The enzyme-substrate complex dissociation constants were set to 0.1 s^{-1} , while the catalytic rate constants were set to 1 s^{-1} . These values have been assumed to be reasonable in a model for folding for export from the ER [39], because they reflect the high affinity and faster processing of the ERAF and ERAD machineries. These rate constants give Michaelis-Menten constant values of $1.1 \mu\text{M}$. Using the above catalytic rate constant, we have set the maximum velocities to $0.1 \mu\text{M s}^{-1}$. The first order dissociation constant k_0 is assumed to be 0.25 s^{-1} .

Our objective is now to determine the parameters that increase or reduce the maximum value of newly synthesized protein accumulated as misfolded protein.

Parameters affecting the accumulation of newly synthesized protein as misfolded protein

To investigate the parameters affecting the accumulation of newly synthesized protein (u_0) as misfolded protein (m), we examined the influence of our model parameters on the ratio of misfolded protein concentration to the initial unfolded protein concentration (m/u_0). We systematically varied each individual parameter while we maintained the other parameters fixed to the unit. We assigned the parameters to Equations (1)-(2), and calculated numerically the solution for m using the Runge-Kutta-Rosenbrock algorithm for stiff ordinary differential equations implemented in MAPLE 12. If the model operates in a parametrically sensitive region, its behavior can change sharply with small variations in the parameters. We expect that different parameters will affect the model to different extents, and the effect for each parameter may depend on the range over which is varied. It will be of great value to physiologists to understand how the model behavior is affected by the parameter values.

The accumulation of misfolded protein depends on the misfolding rate

We first investigate the influence of the misfolding rate, k_0 , on the accumulation of newly synthesized protein as misfolded protein. We expect the maximum value of the ratio m/u_0 to increase with k_0 as it is reported for loss-of-function diseases. We plot the time course of the ratio m/u_0 for different values of k_0 in Figure 4(a). We set the values of $r_f = r_u = r_m = 1 \text{ s}^{-1}$ and $K_f = K_u = K_m = 1 \mu\text{M}$. The curves show that the maximum value of m/u_0

Fig. 4. The effect of model parameters on the accumulation of newly synthesized protein (u_0) as misfolded protein (m) in our model of the unfolded protein response. In (a), the accumulation of misfolded protein increases with the higher misfolding rates, k_0 . In (b)–(c), protein misfolding is inhibited by increasing the catalytic efficiency of pathways operating in the unfolded state. r_f increases the catalytic efficiency of folding the unfolded ensemble, while r_u increases the activity for the degradation of unfolded protein. Note changes in the y-axis scale. In (d), misfolding protein accumulates at higher concentration for a longer timescale when the fraction of misfolded protein degraded per unit time r_m diminishes. The equations were solved numerically with the Runge-Kutta-Rosenbrock algorithm for stiff ordinary differential equations in MAPLE 12. The parameter values used are: $k_0 = 1\text{ s}^{-1}$, $r_f = r_u = r_m = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$, unless otherwise indicated in the figure.

increases with higher values of k_0 . Interestingly the maximum value is reached within a smaller timescale for higher values of k_0 . This means that increasing the misfolding rate has the potential of triggering proteotoxicity and apoptosis faster. It is possible that this is one of the factors that makes fast folding mutants, such as the *Akita* mice, highly susceptible to proteotoxicity [31] even when the UPR response to ER stress is fully activated [32].

In our model we are assuming that the misfolded state is irreversible. Misfolded proteins can revert to the unfolded state with the assistance of chaperones [50, 52], providing an opportunity to decrease the accumulation of misfolded proteins and give another chance to misfolded proteins to fold. Although this is potentially an interesting feature, which we could incorporate into our model, misfolded proteins generally form protein aggregates that cannot be reverted to the unfolded state [62].

Fast protein folding (or fast unfolded protein degradation) inhibits protein misfolding accumulation

The fraction of unfolded protein converted into folded protein per unit time, r_f , is increased in our model by introducing folding chaperones in the system. The effect of increasing r_f on the accumulation of

newly synthesized protein into misfolded protein is plotted in Figure 4(b). The parameters were set to $k_0 = 1\text{ s}^{-1}$, $r_u = r_m = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$. The curves show that fast folding inhibits protein misfolding. This effect results from a decrease in the unfolded protein pool available for misfolding. Our model also predicts the reverse effect: a decrease in the folding activity induces higher concentrations of unfolded and misfolded proteins. It has been demonstrated that the degradation of misfolded proteins prevents ER-derived oxidative stress and cell death [63]. The latter has been reported in cystic fibrosis [64], and we expect to find it in some diabetes phenotypes.

Note that a similar effect is observed if the catalytic efficiency of unfolded protein degradation is increased. The effects of increasing the fraction of unfolded protein degraded per unit time, r_u , on misfolded protein accumulation are shown in Figure 4(c). We set the parameters to $k_0 = 1\text{ s}^{-1}$, $r_f = r_m = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$. We find that a higher value in the catalytic activity (as r_u increases) diminishes the accumulation of newly synthesized protein as misfolded protein.

In summary, our model demonstrates that protein misfolding can be inhibited by increasing the catalytic efficiency of pathways operating in the unfolded state, which is the conformational ensemble preceding the misfolded state.

Fig. 5. Increasing the activities of both the ERAF and ERAD pathways minimizes the accumulation of newly synthesized protein (u_0) as misfolded protein (m) and maximizes the production of folded protein (f) in the unfolded protein response. The rapid misfolding or accumulation of misfolding protein is modeled by setting the misfolding rate to $k_0 = 10\text{ s}^{-1}$. In (a) we simulate the effects of administering pharmacological chaperones in the UPR by increasing folding efficiency (r_f). Pharmacological chaperones simultaneously decrease the accumulation of misfolding protein and increase the amount of protein folded by improving the folding efficiency. Parameter values: $r_u = r_m = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$. In (b) we simulate the effects of administering catalysts degrading misfolding proteins by increasing the misfolding degradation efficiency (r_m). This therapeutic treatment diminishes both the accumulation of misfolded protein and its timescale during the UPR. Parameter values: $r_f = r_u = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$. The equations were solved numerically with the Runge-Kutta-Rosenbrock algorithm for stiff ordinary differential equations in MAPLE 12.

Misfolded protein accumulates for longer timescales when the misfolded protein degradation efficiency diminishes

We have shown that proteins folding with a slow rate are more susceptible to the ERAD pathway during the time they are in the unfolded state. If the unfolded ensemble is misfolded, it can accumulate at higher concentrations and for a longer timescale. Our model shows that misfolding protein accumulates at higher concentration for a longer timescale when the fraction of misfolded protein degraded per unit time, r_m , diminishes. This effect is illustrated in Figure 4(d), which plots the time course of m/u_0 with progressively lower values of r_m . We set the parameters to $k_0 = 1\text{ s}^{-1}$, $r_f = r_u = 1\text{ s}^{-1}$ and $K_f = K_u = K_m = 1\text{ }\mu\text{M}$. The effect on the timescale is small when the activity for the degradation of misfolded protein is less than or equal to the activity for the degradation of unfolded proteins ($r_m \leq r_u$). However, the timescale increases significantly when the activity for the degradation of unfolded protein is much larger than the activity for the degradation of misfolded protein. This phenomenon has been reported before for enzyme catalyzed pathways competing for the same resources

[60], but it has not been reported in the ERAD pathway. This is an unanticipated and testable prediction of our model.

Predictions for therapeutic strategies

Protein misfolding diseases can generally be caused by (i) the limited production of protein to support physiological responses, (ii) the accumulation and aggregation of misfolded and unfolded protein leading to proteotoxicity, or (iii) the excessive degradation of proteins needed to support physiological functions [15]. Our mathematical formulation enables us to explore the balance between the misfolding kinetics, and the ERAF and ERAD activities to test therapeutic interventions. We consider the case for mutations leading to rapid misfolding, such as *Akita* or *Munich* mutation of the *Ins2* gene resulting in the accumulation of misfolded proinsulin. We find that the same therapeutic approaches can be applied to loss-of-function diseases resulting in disruption of the UPR, which inevitably leads to the accumulation of misfolded protein. As we have shown in the previous

section, we can model the rapid misfolding or accumulation of misfolding protein by increasing the misfolding rate to $k_0 = 10\text{s}^{-1}$. To model the protein folding, here we introduce the differential equation for the accumulation of folded protein:

$$\underbrace{\frac{df}{dt}}_{\text{Folding protein rate}} = \frac{r_f u}{\underbrace{1 + \frac{u}{K_f}}_{\text{Production due to folding of unfolded protein}}} \quad (3)$$

We calculated numerically the solution for m and f using the Runge-Kutta-Rosenbrock algorithm for stiff ordinary differential equations implemented in MAPLE 12.

The current therapeutic treatment for recessive loss-of function diseases is the administration of pharmacological chaperone [41], which improves folding efficiency by increasing the proportion of unfolded protein converted into folded protein per unit time by the ERAF. The latter efficiency is determined by r_f . In Figure 5(a) we show that when we improve the folding efficiency we can simultaneously decrease the accumulation of misfolding protein and increase the amount of protein folded. Pharmacological chaperones have this synergetic effect in the UPR because they improve folding capacity by accelerating the folding of unfolded proteins and decreasing the amount of unfolded protein available for misfolding. This mechanism explains the effectiveness of chemical chaperones in reducing ER stress and restoring glucose homeostasis in a mouse model of type 2 diabetes [65].

The analysis carried out in the previous section demonstrates that incrementing the fraction of misfolded protein that is degraded per unit time by the ERAD pathway (r_m) causes a sharp reduction in the accumulation and persistence of misfolded protein during the UPR. In Figure 5(b) we show the effect of administering a catalyst to increment the degradation of misfolded protein. These catalysts decrease the accumulation of misfolded protein and, more importantly, reduce significantly the timescale of misfolded protein accumulation in the UPR. Unfortunately, these catalysts do not improve protein folding, and therefore are ineffective for treating loss-of-function diseases. However, these catalysts may be very effective in the treatment for gain-of-toxic-function diseases, such as disruption of disulfide bond pairing in proinsulin leading to young adult onset [24, 25] and neonatal [26] diabetes.

We can make an additional prediction of a therapeutic strategy for treating misfolding diseases. Our model suggests that simultaneously administering degradation catalysts of misfolding proteins and pharmacological chaperones is the most effective therapy

to reduce ER stress and increase production of the folded protein [Figures 5(a) and 5(b)]. It has been shown that ERAD pathway has the capacity of reducing ER stress directly [66]. It is, therefore, important to investigate the ERAD for potential targets to treat misfolding diseases.

Discussion

The UPR is a complex system of pathways which adapts to fluctuations in the demand for protein synthesis. An understanding of the principles regulating the UPR is important for the development of treatments for protein misfolding diseases [15], among which diabetes mellitus can be considered a case study [20]. The UPR is influenced by the interaction of the ERAF and ERAD pathways. These pathways consist of many components. For example, there is a large body of evidence demonstrating that the ERAD machinery consists of numerous enzymes and proteins which recognize unfolded and misfolded proteins. These components of the ERAD pathway deliver both the unfolded and misfolded protein to the ER retrotranslocon for retrotranslocation and degradation [13]. Understanding complex systems such as the UPR can be enhanced through combined experimental and mathematical analysis.

In this paper we have developed a mathematical formulation of the UPR, where ERAF and ERAD pathways are each modeled as a single enzyme with the unfolding or misfolded proteins as their substrates. We have modeled the rates of both pathways with a Michaelis-Menten equation. Each pathway has a maximum velocity and a Michaelis-Menten constant measuring the affinity of the pathways for its substrate. Simplified Michaelis-Menten kinetics of both ERAF and ERAD pathways has previously been successfully employed to investigate the exocytic pathways for protein homeostasis control in the ER [39]. Here we develop a new model with the objective of investigating the factors affecting the accumulation of newly synthesized protein as misfolded protein, and suggest new therapeutic strategies for misfolding diseases. The analysis of our model shows that the accumulation of misfolded protein can change sharply with small variations in the misfolding rate [see, Figure 4 (a)] and with the fraction of misfolded protein that is degraded per unit time by the ERAD pathway [see, Figure 4 (d)]. In agreement with experimental data [41], we show that increasing the catalytic activity of the ERAF improves the folding efficiency. We also find that increasing the fraction of

unfolded protein converted to folding protein per unit time simultaneously enhances folding and partially inhibits ERAD [see, Figure 5 (a)]; this result was recently reported [39] in the literature. Our model provides a mechanistic explanation for the effectiveness of chemical chaperones in reducing ER stress and restoring glucose homeostasis in a mouse model of type 2 diabetes [65]. More importantly, we can make a prediction for therapeutic strategies. Our model suggests that increasing simultaneously the ERAD capacity for degrading misfolded protein and the ERAF of folding protein is the most effective therapy to reduce ER stress and increase folded protein production [see, Figures 5 (a) and (b)].

We recognize that our model is a simplification; it could be further elaborated in many ways. As mentioned previously, the UPR transducers signal the ERAF to increase its folding capacity, signal the ERAD to increase its degradation capacity, and prevent mRNA translation of new proteins. Extending our model to incorporate the UPR transducers will permit us to explore the role of specific genes in the etiology of some diabetes phenotypes [20], such as type-2 diabetes in Dutch [22] and Pima Indian cohorts [23] and type 1 diabetes in the NOD mouse model [67]. Proteotoxicity in the Akita mouse is linked to the aggregation of misfolded proinsulin with folded insulin [16]. Protein aggregation impairs trafficking and the availability of folded protein for physiological processes. Models of protein misfolding and aggregation [62, 68] could be used to gain further insights in therapeutic approaches. The UPR regulates the expression of a large set of chaperones and foldases [43, 50]. These proteins are engaged in elaborate pathways, which have been modeled to understand up-regulation and the rate limiting steps of the folding process [69, 70]. We plan to use these models to optimize insulin folding and secretion. These

additional features may make the model more realistic, but it will certainly also make it more complex to analyze.

Since the 1960, numerous mathematical models have been developed to investigate the role of β -cell in diabetes mellitus [71]. These models have focused on diverse mechanisms of β -cell failure. However no attention has been paid to the problem of quality control of insulin production in β -cells. We have developed a simple mathematical model to investigate the role of the protein quality control pathways in the restoration of insulin production. The current advantage of our model and its simplicity is that it can explain experimental data and make predictions where data are still limited. We are making the unanticipated and testable prediction that diminishing the fraction of unfolded protein that is degraded per unit time by the ERAD pathway leads to both an accumulation and an increase in the persistence of misfolded proteins during the UPR. Our model also predicts that the ERAD pathway is a potential target for the treatment of misfolded protein diseases, such as diabetes mellitus.

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