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REVIEW ARTICLE

The unfolded protein response and translation attenuation: a modelling approach

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Unfolded protein response (UPR) is a stress response to increased levels of unfolded proteins in the endoplasmic reticulum (ER). To deal with this stress, all eukaryotic cells share a well-conserved strategy – the upregulation of chaperons and proteases to facilitate protein folding and to degrade the misfolded proteins. For metazoans, however, an additional and seemingly redundant strategy has been evolved – translation attenuation (TA) of proteins targeted to the ER via the protein kinase PERK pathway. PERK is essential in secretory cells, such as the pancreatic β-cells, but not in non-secretory cell types. We have recently developed a mathematical model of UPR, focusing on the interplay and synergy between the TA arm and the conserved Ire1 arm of the UPR. The model showed that the TA mechanism is beneficial in highly fluctuating environment, for example, in the case where the ER stress changes frequently. Under highly variable levels of ER stress, tight regulation of the ER load by TA avoids excess amount of chaperons and proteases being produced. The model also showed that TA is of greater importance when there is a large flux of proteins through the ER. In this study, we further expand our model to investigate different types of ER stress and different temporal profiles of the stress. We found that TA is more desirable in dealing with the translation stress, for example, prolonged stimulation of proinsulin biosynthesis, than the chemical stress.

Keywords: diabetes, feedback loops, modelling, translation attenuation, unfolded protein response

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Introduction

The endoplasmic reticulum (ER) is an organelle where the secretory and transmembrane proteins are folded. The ER is of crucial importance to the functioning of the 'professional' secretory cells such as the insulin-producing pancreatic β-cells. Proteins in the ER are folded with the help of chaperones. A sudden increase in the unfolded protein levels causes ER stress and activates the unfolded protein response (UPR) – a molecular network that evolved to keep the concentrations of unfolded proteins low. A number of diseases are associated with the malfunctioning of the UPR system, such as diabetes mellitus, atherosclerosis, and neo-plasia [1]. All eukaryotes share a similar mechanism to deal with increased levels of unfolded proteins: they upregulate chaperones to augment folding and proteases to degrade the irreversibly misfolded ER proteins [2] (figure 1A). ER chaperones are upregulated by a conserved Ire1 pathway in both yeasts and mammals. In yeast, an increase in unfolded proteins (U) activates Ire1 [2], which in turn splices Hac1 mRNA. Spliced Hac1 mRNA, Hac1s, is translated to produce the transcription factor Hac1, which activates the transcription of chaperones and ER degradation components – for brevity, these activities in their aggregate will henceforth be referred to simply as chaperones (C). Chaperones (C) reduce the levels of unfolded proteins (U) and inhibit the activity of Ire1, thereby restoring homeostasis (arrows for U and Ire1 in figure 1A). In mammalian cells, upregulation of chaperones is mainly controlled by two pathways: Ire1α, homologous to the yeast Ire1, fulfils this function by splicing the Xbp1 mRNA, whereas another ER stress transducer, ATF6, is activated by ER unfolded proteins as well, producing downstream targets whose functions overlap extensively with those of Ire1α (figure 1B).

Metazoans have an additional mechanism to deal with ER stress: they attenuate translation and thus reduce the flux of newly translated proteins into the ER [2] (figure 1B). This translation attenuation (TA) pathway is mediated by the ER kinase PERK: increased level of unfolded proteins activates PERK, which in turn phosphorylates the translation initiation factor eIF2α. The increase in the levels of phosphorylated eIF2α, eIF2α-P, attenuates translation, as eIF2α-P cannot form the ribosomal preinitiation complex that is necessary for translation initiation. PERK plays an important role in secretory cells: in mice lacking PERK, the major secretory cells of the pancreas and the skeletal system display a rough ER distended with protein aggregates and accelerated cell death, which result in skeletal defects at birth, and progressive diabetes mellitus [3]. A growing body of evidence indicates that optimal function and survival of diverse secretory cells depends on translational regulation via this PERK/eIF2α branch of the UPR [4]. By mathematical modelling we have previously shown that differences in the cell functions and in their environments

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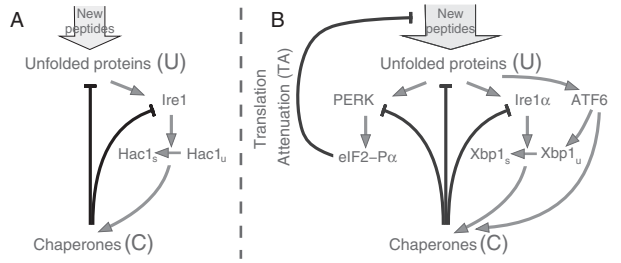

Figure 1. The unfolded protein response networks in (A) yeast (or non-secretory) and (B) secretory (B) cells. Arrows indicate positive regulation and short bars negative regulation.

can explain why the TA mechanism is important for one cell type and not the other [5]. In this paper, we further investigate the role of the TA branch of UPR in a more detailed manner. In particular, we investigate how the duration between bursts of increased flux affects our previous conclusions. We also show that TA is even more beneficial when the stress is modelled as a multiplicative stress, which better reflects the nature of the ER stress in secretory cells (translational stress), than the additive stress (chemical stress) that we used in our previous model.

Reduced Model of the UPR

The UPR system involves multiple pathways, interactions of many proteins and genes, splicing of mRNAs, and biophysical processes such as ER volume expansion. Rather than building a model with all the detailed interactions and processes included (with some unknown regulations and many unknown kinetic parameters), we take an opposite approach to construct a simple mathematical model that captures certain key features of the overall dynamic behaviour of the UPR [5]. The overall philosophy in our model construction is to model two distinct time scales of UPR: the slow homeostatic response of Ire1α/ATF6 pathways and the fast transient response of translational attenuation.

Upregulation of chaperones happens on a slower time scale compared to the time scale of the TA mechanism. Increase in chaperone requires several transcriptional and translational steps, which take hours in mammalian cells, whereas it takes two phosphorylation steps to reduce the translation of polypeptides into the ER, which is a much faster process ($\sim$10 min). It was shown experimentally that there is a time separation in activities of each of the three pathways in mammalian cells: TA is activated first within the first 30 min after stress induction, next ATF6 is turned on in about 2 h, followed by Ire1α in 6–8 h after the stress induction [6].

We model the unfolded protein level, the Ire1/Ire1α/ATF6 activation, the consequent production of the active downstream transcription factors, and the target (chaperone) gene upregulation in response to the ER stress with ordinary differential equations as shown below [5]:

$$\frac{dU}{dt} = E + S_c - \delta \cdot C \cdot \frac{U}{U + K_U} - \frac{U}{\tau} \tag{1a}$$

$$I^{act} \propto [I : U] = I_t \frac{U_f/K_{IU}}{1 + C_f/K_{CI} + U_f/K_{IU}} \tag{2}$$

$$\frac{dH}{dt} = \beta I^{act} - \frac{H}{\tau_H} \tag{3}$$

$$\frac{dC}{dt} = \gamma + \alpha H - \frac{C}{\tau} \tag{4}$$

The above equations model the Ire1/Ire1α/ATF6 arm of UPR, where S_C models the amount of chemical stress. With the TA arm present, Eq. (1a) becomes

$$\frac{dU}{dt} = \frac{E}{1 + P^{act}} + S_c - \delta \cdot C \cdot \frac{U}{U + K_U} - \frac{U}{\tau} \tag{1b}$$

where

$$P^{act} \propto [P : U] = P_t \frac{U_f/K_{IU}}{1 + C_f/K_{CP} + U_f/K_{IU}} \tag{5}$$

is the activated PERK. For further details on the derivation of the equations and the choice of model parameters, please see Ref. [5].

For translational stress, that is, stress induced by increased flux E of the incoming peptides, the TA acts directly on the stress itself. In this case, Eqs (1a) and (1b) are replaced by

$$\frac{dU}{dt} = E \cdot S_t - \delta \cdot C \cdot \frac{U}{U + K_U} - \frac{U}{\tau} \tag{1c}$$

$$\frac{dU}{dt} = \frac{E \cdot S_t}{1 + P^{act}} - \delta \cdot C \cdot \frac{U}{U + K_U} - \frac{U}{\tau} \tag{1d}$$

where S_t is the fold increase of the ER incoming flux.

Results

TA Buffers Against Both Chemical and Translational Stress, But Is More Beneficial for Translational Stress

Experimental studies show that the accumulation of misfolded proteins results in protein aggregates that cause cell toxicity and eventual cell death [7]. Chaperones and proteases, unregulated by the UPR, maintain the concentration of unfolded proteins at low levels. However, excessive accumulation of chaperones can be toxic when out of proportion to the amount of unfolded proteins [8,9]. To capture these two physiological aspects in our model, we compare the quality of the response in the presence and absence of the TA mechanism by monitoring (i) how well it can minimize the levels of the unfolded protein (U) and (ii) how effective the response is in preventing excessive accumulation of chaperones (C).

In figure 2, we show the outcome of the model simulation with the stress induced at time $t = 0$. With and without the TA mechanism, an acute stress resulting in a million-fold increase in the production rate of unfolded proteins (measured in molecules per minute) (figure 2A, F) leads to a sudden increase in the number of molecules of unfolded proteins (U) (figure 2B, G). We consider two types of stresses, a chemical stress typically used in laboratory conditions, for example, DTT, and translational stress, which would correspond to a sudden increase in the flux of newly translated polypeptides into the ER. In our model, these two types of stresses differ by how they appear in the rate equation of the unfolded protein production (Eqs (1a)–(1d)).

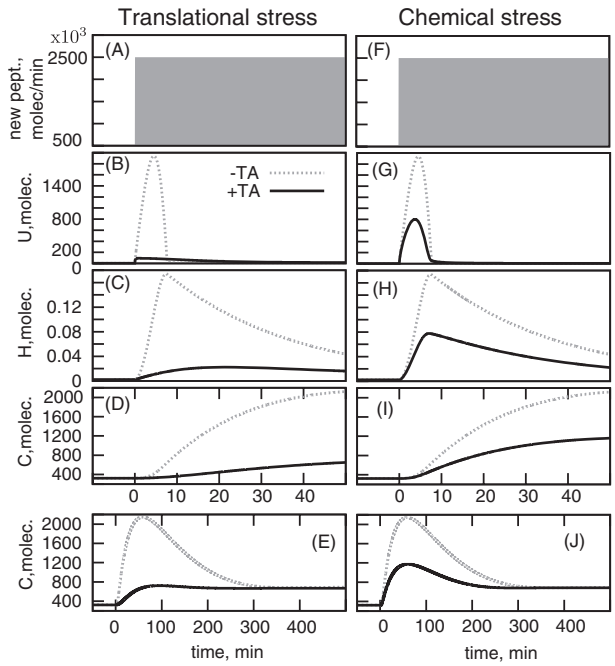

Figure 2. Translation attenuation buffers against both chemical (additive) and translational (multiplicative) stresses. (A) and (F) show the level of stress. Here, translation stress was normalized such that it corresponds to the same increase in the rate of unfolded proteins as in the case of chemical stress. (B) and (G) show how the concentration of unfolded proteins changes with time after stress induction. (C) and (H) show the corresponding changes in Hac1 protein amounts, and (D) and (I) are the chaperone concentrations. Shown in different time scale in (E) and (J) are chaperone concentrations approaching their new steady states corresponding to the stress. The simulation was performed for $E = 500 \times 10^3$ molec/min.

We only consider remediable stresses to which cells could adapt through the UPR. After stress initiation (figure 2A, F), U in figure 2B, G reaches its maximal level, then decreases to a low, steady-state level. Translation stress was normalized such that it corresponds to the same sudden increase in the rate of unfolded proteins as in the case of chemical stress. As a result of such normalization, as expected, there is no difference between chemical and translation stresses in the temporal profile of the unfolded proteins (U), when there is no TA (compare the dashed curves in figure 2B, G). However, when TA is at work, the difference is remarkable (compare the solid black curves in figure 2B, G).

The presence of TA in our model decreases the maximal level of unfolded proteins, U_{max}, by about threefold in the case of chemical stress and for $E = 500 \times 10^3$ molec/min. We see that TA is even more efficient in the case of translation stress, decreasing U_{max} by about 10-fold. In our previous work [5], we have shown that the maximal amount of unfolded proteins (U_{max}) determines the 'chaperone overshoot' (excess production), that is, the higher the U_{max}, the faster the increase in the unfolded proteins, the larger the Hac1/Xbp1 and chaperone overshoot. We also argued that such chaperone overshoot can potentially be toxic to cells because of the long chaperone half-life. The large difference in maximal levels of unfolded proteins in the cases of translation and chemical stresses (solid black curves in figure 2B, G) results in very different Hac1/Xbp1 (figure 2C, H) and chaperone (figure 2D, E, I, K; note the different time scales in these plots) profiles – the chaperone overshoot is completely eliminated in the case of translational stress.

Thus, the TA mechanism provides an even tighter response to translational stress, that is, it is more potent at keeping the amount of unfolded proteins low and minimizing the amount of chaperones during the response to the translational stress compared to chemical stress. Note that in the case of chemical stress, the TA branch of the UPR acts like a fast source of new chaperones: it attenuates the influx of peptides into the ER, thus freeing up chaperones that would be otherwise required to help with the folding of the new peptides. This effect is especially pronounced with a high ER traffic, as shown in our previous work [5] and in figure 2. Therefore, without quantitative modelling, it is not completely obvious why TA should be much more potent in dealing with the translational stress.

TA Is Dispensable Under Pulsatile Stress of High Frequency

Insulin-secreting pancreatic β-cells respond to highly variable patterns of the blood glucose level. Rapidly changing glucose levels lead to correspondingly rapid changes in insulin production and secretion – glucose directly upregulates both transcription and translation of preproinsulin, which has to be processed and folded in the ER [10]. This creates a highly fluctuating ER load over time. Therefore, it is important to study the role of the TA mechanism under conditions of transient, recurrent stress. We model the recurrent stress conditions as periodic pulses of stress.

In our previous work [5] we showed that TA is beneficial in dealing with pulsatile stresses. We studied how the benefit of TA increases with decreasing pulse duration. Here, we study the case in which the duration of the pulse is fixed but the interval between pulses changes. In figure 3, we show simulations where the transient recurrent stresses (pulse) arrive with different frequencies. The simulations are carried out for the case of chemical stress, but the main conclusion also holds for the translational stress. In figure 3A, where the stresses come with 400-min interval, each pulse of stress generates a transient increase in unfolded proteins. Interestingly, the pattern in unfolded proteins (U) changes with an increasing pulse frequency (figures 3B–D). In figure 3B, D, the significantly large transient increase in unfolded proteins is occurring only every other pulse (e.g. there is no transient increase in unfolded proteins in response to the stress pulse at $t = 250$ min in figure 3C). This behaviour is even more prominent if the frequency of stress pulses is increased further. A significant amount of unfolded proteins is generated only in response to the first pulse, but not to the consecutive ones (figure 3D). This is because of the high level of the residual chaperons in response to the first pulse. In the case of very high frequency pulses, the system reaches the equilibrium of chaperones with the stress level, so that it does not respond anymore. Thus, regular

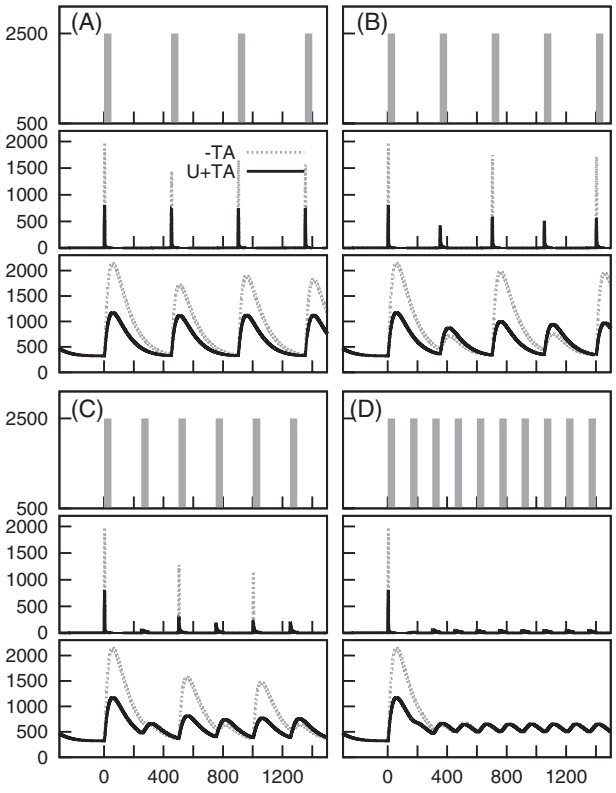

Figure 3. The role of TA in reducing the amount of chaperones diminishes as the time between pulses decreases. Shown are the response profiles to a pulsatile stress where the duration of the pulse is 50 min and the time between pulses are: (A) 400 min, (B) 300 min, (C) 200 min, and (D) 100 min.

frequent pulses are less toxic for cells because they both reduce the amount of unfolded proteins as well as the excess chaperons.

This result is particularly interesting in light of the recent findings showing that there is a correlation between the irregularity of insulin ultradian pulsatility and the onset of type 2 diabetes. In healthy people, about 75% of insulin is released in a very regular pulsatile manner with a period of around 6–10 min. In contrast, type 2 diabetic patients exhibit irregular pulsatile patterns of insulin secretion. Furthermore, the irregular pulsatility seems to be a common feature in people prone to developing diabetes [11,12].

Summary

Failure of the ER-folding machinery results in protein aggregation and eventual cell death. The TA branch of the UPR is particularly important for insulin-producing β-cells, as evidenced by transgenic mice models lacking the UPR components PERK/eIF2α [3], and the PERK repressor, P58IPK [13]. With the help of mathematical modelling, we investigated the role of the TA component of the UPR. In particular, we monitored and compared the quality of the UPR with and without the TA mechanism. Earlier [5], we had studied and compared the stress conditions physiologically encountered by β-cells – high rates of basal ER polypeptide production (ER traffic) punctuated further by transient and recurrent ER stress – to those more typical for non-secretory cells and yeast where ER traffic is low. In this study, we expanded the model to assess the effects of chemical vs. translational stress. We found that the role of TA is even greater under the conditions of translational stress – both the amount of unfolded proteins and the amount of chaperones are minimized to much greater extents, when compared to chemical stresses.

The characteristic of stress is, however, not limited to only its magnitude or how it affects the rate of increase in unfolded proteins. Another important property is the temporal profile of the stress. We had found earlier that professional secretory cells may benefit from the presence of a TA mechanism because of its ability to minimize the amount of chaperones needed to deal with transient stresses. Chaperones are generally long-lived proteins, so once they become upregulated in response to transient stress they will be present in the ER long after the stress is gone. There are numerous reports suggesting that an excess of chaperones in the absence of folding stress imposes a burden that may even cause cell toxicity [8]. Our model suggested that the presence of a TA mechanism is especially important for minimizing the excess chaperones when the duration of the transient stress is approximately the same or smaller than the time it takes for chaperones to be induced [5]. In this study, we found that it is not only the duration of the stress pulse but also the time between consecutive pulses that determines the necessity of TA mechanism. If the frequency of stress pulses is high enough, such that the time between the consecutive pulses is shorter than the chaperone half-life, then a significant amount of unfolded proteins is generated only in response to the first pulse, but not to the consecutive ones. In this case, TA only provides benefits for the first pulse, but not for all the consecutive ones.

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Conflict of Interests

The authors do not declare any conflict of interest relevant to this manuscript.

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