Supporting Information

The rate of the unfolded proteins is:

$$\frac{dU}{dt} = \alpha e - \delta \cdot C \frac{U}{U + K_U} - \frac{U}{\tau}$$

where αe is the translation rate of newly synthesized polypeptides into the ER, which it is proportional to the amount of unphosphorylated eIF2α, e.

If the translation is not regulated in response to the increase in unfolded proteins, then the amount of unphosphorylated eIF2α is a constant,E_t, and the rate of the unfolded proteins is

$$\frac{dU}{dt} = E_t - \delta C \frac{U}{U + K_U} - \frac{U}{\tau}$$

If PERK is present, then the translation rate — proportional to the amount of unphosphorylated eIF2α — depends on the amount of unfolded proteins, $e(U)$.

$$\frac{dU}{dt} = \alpha e(U) - \delta C \frac{U}{U + K_U} - \frac{U}{\tau}$$

Total eIF2α,

$$e_t = e + e_p$$

where e_p is the amount of phosphorylated eIF2α, which if we assume Michaelis-Menten kinetics is:$e_p = \zeta[U{:}P]\frac{e}{e+K_e}$or in the linear regime

$$e_p = \zeta[U{:}P]\frac{e}{K_e} = e_t - e \rightarrow e = \frac{e_t}{1 + \frac{\zeta[U{:}P]}{K_e}}, \qquad (S1)$$

where $[U{:}P]$ is the concentration of the unfolded proteins bound to PERK , which determines the amount of activated PERK. ζ is the coefficient of proportionality which takes into account the rate of dephosphorylation of eIF2α through phosphatases, (e.g. PP1C phosphatase[1]). The activation mechanisms of both PERK and Ire1 are thought to be similar, thus to derive the expression for $[U{:}P]$ and $[U{:}I]$ let's consider the activation of the Ire1. The law of mass conservation for our main components is stated below.

$$U_t = U_f + [C{:}U] + [I{:}U] \qquad (S2)$$

$$I_t = I_f + [I{:}C] + [I{:}U] \qquad (S3)$$

$$C_t = C_f + [C{:}U] + [I{:}C], \qquad (S4)$$

where the notation $[A{:}B]$ stand for the concentration of the AB complex, A_t is the total amount of species A and A_f is the free amount of A. In the main text we denote the **free amount of unfolded proteins** by U, $U \equiv U_f$; and the **total amount of chaperones** by C, $C \equiv C_t$. The copy number of yeast Ire1 is low, about 200 copies per cell [2], whereas the chaperones (KAR2) are the most abundant proteins in the ER, they amount to about 300000 copies per cell [2]. This allows us to ignore the $[I{:}C]$ term in Eq. (4), $[I{:}C] \ll [C{:}U] and [I{:}C] \ll C_f$ and Eq. (4) becomes

$$C_t = C_f + \frac{C_f U_f}{K_{CU}} \rightarrow C_f = \frac{C_t}{1 + U_f/K_{CU}} , \qquad (S5)$$

where K_{CU} is the dissociation constant of $[C{:}U]$ complex.

Equation (3) can be rewritten as

$$I_t = I_f + \frac{C_f I_f}{K_{CI}} + \frac{U_f I_f}{K_{IU}} \rightarrow I_f = \frac{I_t}{1 + C_f/K_{CI} + U_f/K_{IU}} .$$

And thus from Eq. (3) the amount of the activated Ire1, I^{act}, which we assume to be proportional to the $[I{:}U]$ is

$$I^{act} \propto [I{:}U] = \frac{U_f I_f}{K_{IU}} \rightarrow$$

$$I^{act} \propto [I{:}U] = I_t \frac{U_f/K_{IU}}{1 + C_f/K_{CI} + U_f/K_{IU}} \qquad (S6)$$

When there is no ER stress, U_f is at its minimal level ($U_f/K_{IU} \ll 1$) and thus the sequestering of chaperones by unfolded proteins is minimal resulting in maximal levels of C_f which bind and inhibit Ire1, $I^{act} \propto I_t \frac{U_f/K_{IU}}{1+ C_f/K_{CI}}$.

Under ER stress, large amounts of U_f ($U_f/K_{IU} \gg 1$ and $U_f/K_{CU} \gg 1$) sequester chaperones, so that $C_f \rightarrow 0$, see Eq. (5). Thus under ER stress Ire1 is activated to its maximal level $I^a \propto I_t$.

Due to the homology in their sensing domains, and the fact that chaperones bind both Ire1 and PERK in mammalian UPR, it is believed that PERK and Ire1 sense ER stress in the same way. We thus use exactly the same expression for PERK activation, keeping all dissociation constant equal to those in Ire1 pathway

$$P^{act} \propto [P{:}U] = P_t \frac{U_f/K_{IU}}{1 + C_f/K_{CP} + U_f/K_{IU}} \qquad (S7)$$

Substituting Eq. (S7) back into Eq. (S1) and redefining $P_t \equiv \frac{\zeta}{K_e} P_t$, we obtain final value for the translation rate,

$$e = \frac{e_t}{1 + P_t \frac{U_f/K_{IU}}{1 + C_f/K_{CP} + U_f/K_{IU}}}.$$

By substituting C_f with equation (5) and denoting $U_f \equiv U$; and $C_t \equiv C$ we obtain the final equation for the change in unfolded proteins, presented in Figure 1D

$$\frac{dU}{dt} = \frac{E_t}{1 + P_t \frac{U_f/K_{IU}}{1 + \frac{C_t}{K_{CP}} \frac{1}{1 + U_f/K_{CU}} + U_f/K_{IU}}} - \delta \cdot C \frac{U}{U + K_U} - \frac{U}{\tau}.$$

The amount of Hac1/Xbp1 transcription factor at any time point isproportional to the amount of its Hac1$_s$ mRNA. This is possible due to very short half –life of Hac1 protein, with is estimated to be 1.5[3] minutes and is much shorter than the half-life of the mRNA, $\tau_H \approx 30$min [4].

The increase in Hac1$_s$ mRNA is proportional to the amount of active Ire1, and using Eq. (S6)

$$\frac{dH}{dt} = \beta \frac{U_f/K_{IU}}{1 + C_f/K_{CI} + U_f/K_{IU}} - \frac{H}{\tau_H},$$

Where β is a rescaled total Ire1, $\beta \equiv aI_t$, here a reflects the production rate of Hac1, and depends on the splicing and translation rates.

The increase in chaperones is proportional to the amount if Hac1 transcription factor, with coefficient of proportionality α; for simplicity we assume linear dependence, and no cooperativity.

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

Here γ is the basal level of C production and $\tau = 120$ min.The parameteres were chosen to produce the response to the ER traffic in the range of $10^4 - 10^6$ proteins per minute (10^6 is an estimated scale of translation rate into the ER, see main text) as well as $10^5 - 10^6$chaperones (the number of ER chaperones is

estimated to be 3×10^5 in yeast cells [2]). The basal level of C production, γ, as well as α, the rate of upregulation of chaperones mediated by H, was chosen such as to provide the estimated amount of chaperones.Another restriction to the choice of parameteres is the steady state level of free unfolded proteins, U, which we chose to keep below 20 000 at steady states.

As seen in Figure 3C, yellow region, the –TA UPR is indeed slightly less efficient under the conditions of high traffic in our model. The parameter controlling this is the Michaelis-Menten constant K_{CU} in the $-\delta \cdot C\frac{U}{U+K_{CU}}$ term in Eq. 1.The rationale for such a behavior is following: To accommodate higher traffic ($E_{t2} > E_{t1}$) the steady state level of chaperones has to be higher ($C_2^{ss} > C_1^{ss}$). This results in a higher amount of unfolded proteins ($U_2^{ss} > U_1^{ss}$) at steady state since the only way to match the amount of chaperones, C, to the traffic, E_t is through unfolded proteins, see Equations 2 and 3. Generally the term -$\delta \cdot C\frac{U}{U+K_{CU}}$ will depend linearly on U when it is small ($U_1^{ss} \ll K_{CU}$) and will saturate as U increases ($U_2^{ss} \gg K_{CU}$). Thus at very low traffic $E_{t1}, C_1^{ss}, U_1^{ss}$, the chaperones are far from saturation and can accommodate initial sudden increase in unfolded proteins immediately without increase in C , whereas at high traffic, $E_{t2}, C_2^{ss}, U_2^{ss}$, the chaperones are closer to saturation, and the only way to provide adequate folding capacity is through a slower process of chaperone upregulation.
We would like to stress that this effect has a minor contribution to our results (see Figure 3C yellow region) since the main contribution to the beneficial effect of TA under the conditions of high traffic comes from the fact that +TA UPR is less effective under the condition of low traffic (Et = $100x10^3$) and is more effective when the traffic is high (Et=$1000x10^3$), see Figure 3, blue region.

How is chaperone translation affected by TA mechanism?

As was mentioned in the introduction, phosphorylation of translation initiation factor eIF2α leads to general translation inhibition with only few proteins, such as for example, ATF4, escaping the translational block. The model presented in the main part of the paper is based on the assumption that the translation of chaperones is constant and is not affected by the TA mechanism. The main objective was to reproduce the known experimental data where chaperone concentration increases in response to ER stress. The transient partial inhibition of chaperone translation would transiently change the rate at which this concentration of chaperones is increased due to increased transcription of chaperone mRNA through Hac1/Xbp1 transcription factor. This should not change the qualitative outcome of the model as long as translation inhibition is moderate and does not prevent the overall increase in C in response to ER stress.

To validate our results for the case when chaperone translation is downregulated by TA,

we have included the TA regulation of chaperone translation by making the rate of chaperone production proportional to the amount of non-phosphorylated eIF2α:

$$\frac{dC}{dt} = \gamma + \alpha He - \frac{C}{\tau}$$

where e is given by equation *S1*.

In Figure 1S we compare the benefits of TA mechanism when TA has a very weak effect (the effect on C is about 100 times less as that on U) on chaperone translation, see Figure 1S A and B, with the case when TA has the same effect on C as on U, see Figure 1S C and D. As one can see there is a slight decrease in TA benefits , B_C

and B_U when C is the subject to TA regulation, but general trend did not change much.

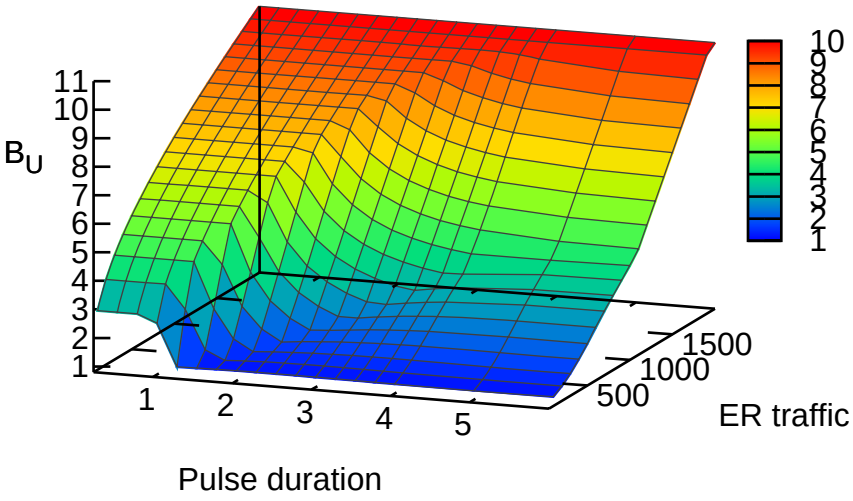
Table S1*: Table of parameters used in the model*

Figure S1: The qualitative outcome of the model does not change when the effect of TA mechanism on chaperone translation is added. *The benefit of TA in reducing unfolded proteins, B_U, (A and C) and excess chaperones, B_C, (B and D) is larger when translation of chaperones is close constant, i.e. nearly not affected by TA.*

1. Novoa, I., et al., Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. J Cell Biol, 2001. **153**(5): p. 1011-22.
2. Ghaemmaghami, S., et al., Global analysis of protein expression in yeast. Nature, 2003. **425**(6959): p. 737-741.
3. Pal, B., et al., SCFCdc4-mediated degradation of the Hac1p transcription factor regulates the unfolded protein response in Saccharomyces cerevisiae. Molecular Biology of the Cell, 2007. **18**(2): p. 426-440.
4. Ruegsegger, U., J.H. Leber, and P. Walter, Block of HAC1 mRNA translation by long-range base pairing is released by cytoplasmic splicing upon induction of the unfolded protein response. Cell, 2001. **107**(1): p. 103-14.
5. Axelsen, J.B. and K. Sneppen, Quantifying the benefits of translation regulation in the unfolded protein response. Physical Biology, 2004. **1**(3): p. 159-165.
6. Ruegsegger, U., J.H. Leber, and P. Walter, Block of HAC1 mRNA translation by long-range base pairing is released by cytoplasmic splicing upon induction of the unfolded protein response. Cell, 2001. **107**(1): p. 103-114.

Figure S1

A)


B_U
11
10
9
8
7
6
5
4
3
2
1
1
2
3
4
5
Pulse duration
500
1000
1500
ER traffic
10
9
8
7
6
5
4
3
2
1

B)

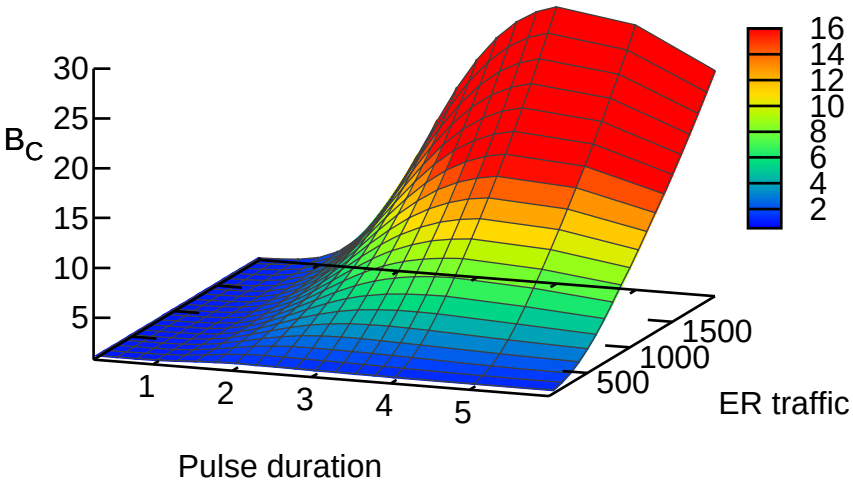
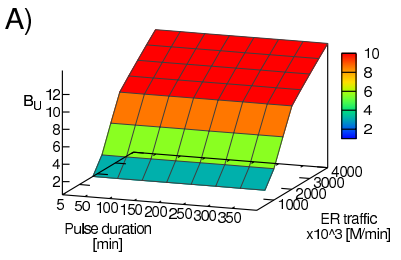
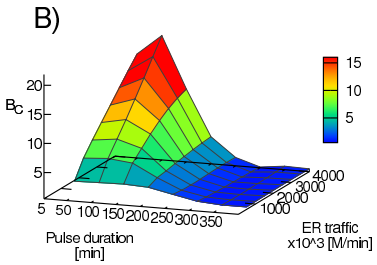

B_C
30
25
20
15
10
5
1
2
3
4
5
Pulse duration
500
1000
1500
ER traffic
16
14
12
10
8
6
4
2

Figure S2

Chaperones escape TA regulation

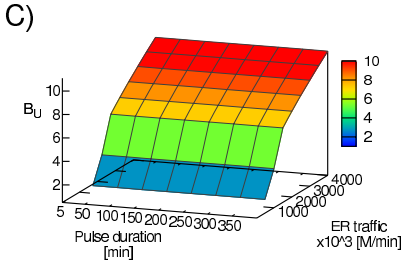
(TA decreases translation of C 100 times less than that of U)





Chaperones under TA regulation

(TA decreases translation of C and U equally)



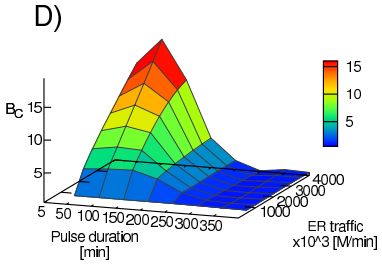


Table S1: Table of parameters used in the model

P_t mol	$K_{IU} = K_{PU}$ mol	$K_{CP} = K_{CI}$ mol	K_U mol	K_{CU} mol	τ min	τ_H min	γ mol/min	α 1/min	δ 1/min
100	10^6	$6x10^3$	$10x10^3$	$200x10^3$	120	30	1.5	500	6