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Mini Review

Unfolded Protein Independent UPR Activation: Challenges and Perspectives

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The Unfolded Protein Response is the first described retrograde signalling pathway from the endoplasmic reticulum to the nucleus in eukaryotes. It was initially described as a specific stress response to proteostasis defects within the ER lumen, but emerging data from the last two decades has challenged this description, since it is now clear that the response can also be triggered by membrane lipid composition and/or lipid metabolism alterations. In the present work I aimed to gather the most recent descriptions of the unfolded protein independent Ire1p activation and discuss possible limitations that may arise when working with this stress response. The unfolded protein response (UPR) is a retrograde signaling pathway that promotes cellular transcriptome alterations in response to endoplasmic reticulum (ER) stress, increasing the protein folding and lipid metabolism capacity of the organelle [1,2]. In mammals, the UPR starts with ER membrane sensor proteins (Ire1a, PERK and ATF6 in its full-length state), that are able to recognize alterations in the lumen of the ER and activate independently the pathway effectors (XBP1s, ATF4 and ATF6 Golgi-cleaved, respectively) via different mechanisms in the cytosol. These effectors are then imported into the nucleus, where they act as transcription factors, increasing the expression of genes related to the organelle functions (protein and lipid synthesis, protein folding and degradation etc.) [3]. In this work, I'll focus on the Ire1a-XBP1 branch since it is the most studied in regard to unfolded protein independent activation.

From the late 80's until the beginning of the 00's this stress response pathway was associated almost exclusively with the accumulation of misfolded proteins within the ER lumen – hence its "unfolded protein response" name – and a large bulk of research rapidly stablished the mechanisms driving Ire1a activation and further processing of XBP1 mRNA. It was stablished, mostly in the yeast Saccharomyces cerevisiae (which possesses only the Ire1p-Hac1p axis of this response, homologous to the mammalian Ire1a-XBP1), that:

a) The ER lumen chaperone BiP dissociates from Ire1a in the presence of accumulated unfolded proteins [4-6].

b) Ire1a is able to directly bind unfolded regions of proteins in the ER lumen [7].

c) The binding of Ire1a with unfolded proteins stabilizes the dimerization/oligomerization of Ire1a molecules [8].

d) When Ire1a is in its dimer/oligomer state, one copy can phosphorylate the other, promoting structural alterations in this protein [9].

e) These structural changes activate a RNAse site in the cytosolic portion of the protein that is able to recognize an immature mRNA of XBP1 and to catalyze an alternative cytosolic splicing reaction, producing a mature mRNA that can be translated into a transcription factor (XBP1s) and finally modulate the cell transcriptome in the nucleus [10,11]. Experimentally, one can easily probe Ire1a dimerization/oligomerization or its phosphorylation by western blot, and/or monitor the cleavage of XBP1 mRNA by southern blot or RT-PCR, and/or quantify the transcription induction of XBP1s target genes by qRT-PCR [12].

More recently, though, it has been observed that Ire1a (both yeast and mammalian proteins) is responsive to lipid metabolism alterations, especially modifications in the ER membrane composition. Promlek et al. showed that the activation of Ire1p in yeast is dependent on the presence of its luminal domain III, thought to generate a binding site for unfolded proteins in the dimer/oligomer state of Ire1p, when cells are challenged with the protein-unfolding agent DTT, but it is independent of this luminal domain III when activated by inositol depletion – a previously known "metabolic" way of activating the UPR. Interestingly, in this paper the authors showed that inhibition of the sphingolipid de novo biosynthesis with myriocin (an inhibitor of the serine palmitoyltransferase, SPT, complex) hampers Ire1p activation under inositol starvation, but not when cells were challenged with DTT, suggesting that this lipid-induced mode of action of Ire1p is somewhat different from its canonical mechanism, observed in the presence of accumulated misfolded proteins within the ER [13]. These results were conveniently in accordance with the observations that yeast mutants of ORM1/2 genes show persistent UPR activation [14,15]. Orm1/2p are master regulators of the same SPT complex in eukaryotes, therefore there must exist a still elusive role for sphingolipid metabolism on the ER physiology and UPR activation. Accordingly, inositol is a precursor of phosphatidylinositol, a lipid building block for sphingolipids and other lipids, further connecting lipid metabolism and Ire1p activation. Finally, it was recently shown that myriocin can also inhibit the unfolded protein-independent activation of Ire1p, in yeast models of the human genetic disease classic galactosemia [16], bringing the sphingolipid de novo synthesis pathway and the UPR even closer together in a human disease context. Romain Volmer and David Ron detailed thoroughly the possible mechanisms that could underline this lipid dependent Ire1p activation [17].

In 2017, Halbleib and colleagues described an important role for an amphipatic helix proximal to the ER membrane that could act as the Ire1p sensor for lipid bilayer stress in the ER. In this work, the authors were able to generate a minimal construct in vitro containing the transmembrane and the amphipathic helixes from Ire1p and observed a correlation between membrane bilayer composition and oligomerization of this minimal sensor, allowing the conclusion that these Ire1p structural features are sufficient for membrane composition sensing, in a mechanism that resembles the known mode of action of Ire1p in living cells i.e., the dimerization/oligomerization step [18]. Accordingly, it was shown that mammalian Ire1a can also be activated in vivo following palmitic acid exposure and, at least partially, this activation was not dependent on the Ire1a luminal domain [19], highlighting a role for the transmembrane portion of the protein as a lipid sensor in the ER. Finally, it has been shown that the transcriptome alterations mediated by Hac1p in the nucleus can differ depending on the nature of ER stress [20], suggesting a still far from understood mechanism that ensures that the correct information is transmitted from the ER to the nucleus. Taken together these observations emphasize a new complexity layer of regulation of the physiology of the endoplasmic reticulum, especially in regard to ER stress responses. It has always been challenging to directly measure unfolded proteins in the ER lumen, therefore several results acquired during the 90's and early 00's that showed Ire1a/Ire1p activation and/or XBP1/HAC1 splicing were interpreted as evidence of proteostasis failure within the organelle. Thus, the present work should also serve as a caution note for researches evaluating the UPR across the world, since it is now clear that this response can also be activated in the absence of direct binding of Ire1p to unfolded proteins.

References

1. Shamu CE, Cox JS, Walter P (1994) The unfolded-protein-response pathway in yeast. Trends in Cell Biology 4(2): 56-60.
2. Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J (1988) The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 332(6163): 462-464.
3. Walter P, Ron D (2011) The unfolded protein response: From stress pathway to homeostatic regulation. Science 334(6059): 1081-1086.
4. Kimata Y, Kimata YI, Shimizu Y, Abe H, Farcasanu IC, et al. (2003) Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins. Molecular Biology of the Cell 14(6): 2559-2569.
5. Kimata Y, Oikawa D, Shimizu Y, Ishiwata KY, Kohno K (2004) A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. The Journal of Cell Biology 167(3): 445-456.
6. Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K (2000) Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. Biochemical and Biophysical Research Communications 279(2): 445-450.
7. Kimata Y, Ishiwata KY, Ito T, Hirata A, Suzuki T (2007) Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. The Journal of Cell Biology 179(1): 75-86.
8. Oikawa D, Kimata Y, Takeuchi M, Kohno K (2005) An essential dimer-forming subregion of the endoplasmic reticulum stress sensor Ire1. The Biochemical Journal 391(Pt 1): 135-142.

9. Bernales S, Papa FR, Walter P (2006) Intracellular signaling by the unfolded protein response. Annual Review of Cell and Developmental Biology 22: 487-508.

10. Welihinda AA, Tirasophon W, Kaufman RJ (1999) The cellular response to protein misfolding in the endoplasmic reticulum. Gene Expression 7(4-6): 293-300.

11. Kohno K, Normington K, Sambrook J, Gething MJ, Mori K (1993) The promoter region of the yeast KAR2 (BiP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum. Molecular and Cellular Biology 13(2): 877-890.

12. Souza DEA, Pimentel FS, Machado CM, Martins LS, Silva DWS, et al. (2014) The unfolded protein response has a protective role in yeast models of classic galactosemia. Disease Models & Mechanisms 7(1): 55-61.

13. Promlek T, Ishiwata KY, Shido M, Sakuramoto M, Kohno K, et al. (2011) Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Molecular Biology of the Cell 22(18): 3520-3532.

14. Liu M, Huang C, Polu SR, Schneiter R, Chang A (2012) Regulation of sphingolipid synthesis through Orm1 and Orm2 in yeast. Journal of Cell Science 125(Pt 10): 2428-2435.

15. Han S, Lone MA, Schneiter R, Chang A (2010) Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. Proceedings of the National Academy of Sciences of the United States of America 107(13): 5851-5856.

16. Pimentel F, Machado CM, De-Souza EA, Fernandes CM, De-Queiroz ALFV, et al. (2022) Sphingolipid depletion suppresses UPR activation and promotes galactose hypersensitivity in yeast models of classic galactosemia. Biochimica Et Biophysica Acta Molecular Basis of Disease 1868(6): 166389.

17. Volmer R, Ron D (2015) Lipid-dependent regulation of the unfolded protein response. Current Opinion in Cell Biology 33: 67-73.

18. Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, et al. (2017) Activation of the unfolded protein response by lipid bilayer stress. Molecular Cell 67(4): 673-684.e8.

19. Volmer R, Ploeg KVD, Ron D (2013) Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proceedings of the National Academy of Sciences of the United States of America 110(12): 4628-4633.

20. Nurulain H, Wei SY, Jiaming X, Haoxi W, Jhee HK, et al. (2020) Stress sensor Ire1 deploys a divergent transcriptional program in response to lipid bilayer stress. The Journal of Cell Biology 219(7): e201909165.