Biochimica et Biophysica Acta 1833 (2013) 2458–2463

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Evolution of the unfolded protein response ☆

Julie Hollien *

Department of Biology and the Center for Cell and Genome Science, University of Utah, Salt Lake City, UT 84112, USA

ARTICLE INFO

Article history:
Received 13 November 2012
Received in revised form 7 January 2013
Accepted 13 January 2013
Available online 28 January 2013

Keywords:
ER stress
Protein secretion
Ire1
Perk
Atf6

ABSTRACT

The unfolded protein response (UPR) is a network of signaling pathways that responds to stress in the endoplasmic reticulum (ER). The general output of the UPR is to upregulate genes involved in ER function, thus restoring and/or increasing the capacity of the ER to fold and process proteins. In parallel, many organisms have mechanisms for limiting the load on the ER by attenuating translation or degrading ER-targeted mRNAs. Despite broad conservation of these signaling pathways across eukaryotes, interesting variations demonstrate a variety of mechanisms for managing ER stress. How do early-diverging protozoa respond to stress when they lack traditional transcriptional regulation? What is the role of the ER stress sensor Ire1 in fungal species that are missing its main target? Here I describe how diverse species have optimized the UPR to fit their needs. This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

1. Introduction

Maintenance of homeostasis in the endoplasmic reticulum relies on a collection of signaling pathways known as the unfolded protein response (UPR). These pathways sense disturbances in protein folding in the ER through transmembrane proteins that then initiate signaling pathways affecting many aspects of gene expression. Together they decrease the load of proteins entering the ER and increase the capacity of the ER to fold and process these proteins. In mammals, these pathways are essential for survival not only during infection and disease, but also during normal development, especially during the differentiation of professional secretory cells.

Reviews over the last two years have offered perspectives on many interesting and fundamental facets of the UPR, including its role in ER homeostasis [1,2], apoptosis [3], disease [4,5], inflammation [6], secretory cell function [7], and aging [8]. This abundance of reviews does not seem completely unwarranted; a recent Pubmed search yielded well over 500 research articles pertaining to the UPR in 2012 alone. A special issue focused on the ER would therefore not be complete without some mention of the UPR. Inspired by recent discoveries in plants and fission yeast, and in an effort to offer a non-redundant review of the UPR, this article will highlight similarities across eukaryotes and variations on these pathways that have evolved in organisms other than the well-studied mammalian and budding yeast model systems.

2. An overview of UPR signaling pathways in budding yeast and metazoa

Stress in the ER, generally considered to be the result of an imbalance between the protein folding load and the capacity of the ER, occurs in a variety of circumstances. Pathological ER stress can result from infection or diseases linked to the ER, whereas physiological stress is thought to activate the UPR during differentiation and maintenance of secretory cells in metazoans. A classic example of the latter is the activation of certain aspects of the UPR during the differentiation of antibody-secreting plasma cells [9,10]. Because of the central role of the UPR in regulating the capacity of the ER and managing stress, several of the signal transducers are required for normal development and survival in mammals.

One of the main mechanisms governing UPR signaling was originally worked out in *Saccharomyces cerevisiae*, where a single transmembrane protein, Ire1, is responsible for sensing ER stress [11,12] (Fig. 1A). Oligomerization of Ire1 leads to autophosphorylation and activation of an endoribonuclease domain on the cytosolic side of the membrane. This nuclease cleaves at two specific sites in the mRNA encoding Hac1 [13,14], removing a regulatory intron from the message, which is then spliced back together to form the template for the active Hac1 protein. Hac1p, a bZip transcription factor, upregulates many genes associated with the secretory pathway, including the major ER chaperone BiP [15]. This pathway thereby enhances ER function and is conserved in most eukaryotes, with the known exceptions discussed below.

The metazoan UPR is decidedly more complex that that seen in budding yeast. Mammals possess two copies of Ire1; Ire1α is expressed ubiquitously [16] and is essential for embryonic development [17,18], whereas Ire1β is expressed specifically in intestinal epithelial cells and its deletion sensitizes mice to colitis [19]. Both isoforms of Ire1 can

☆ This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

* Tel.: +1 801 587 7783; fax: +1 801 581 2174.
E-mail address: hollien@biology.utah.edu.

0167-4889/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2013.01.016

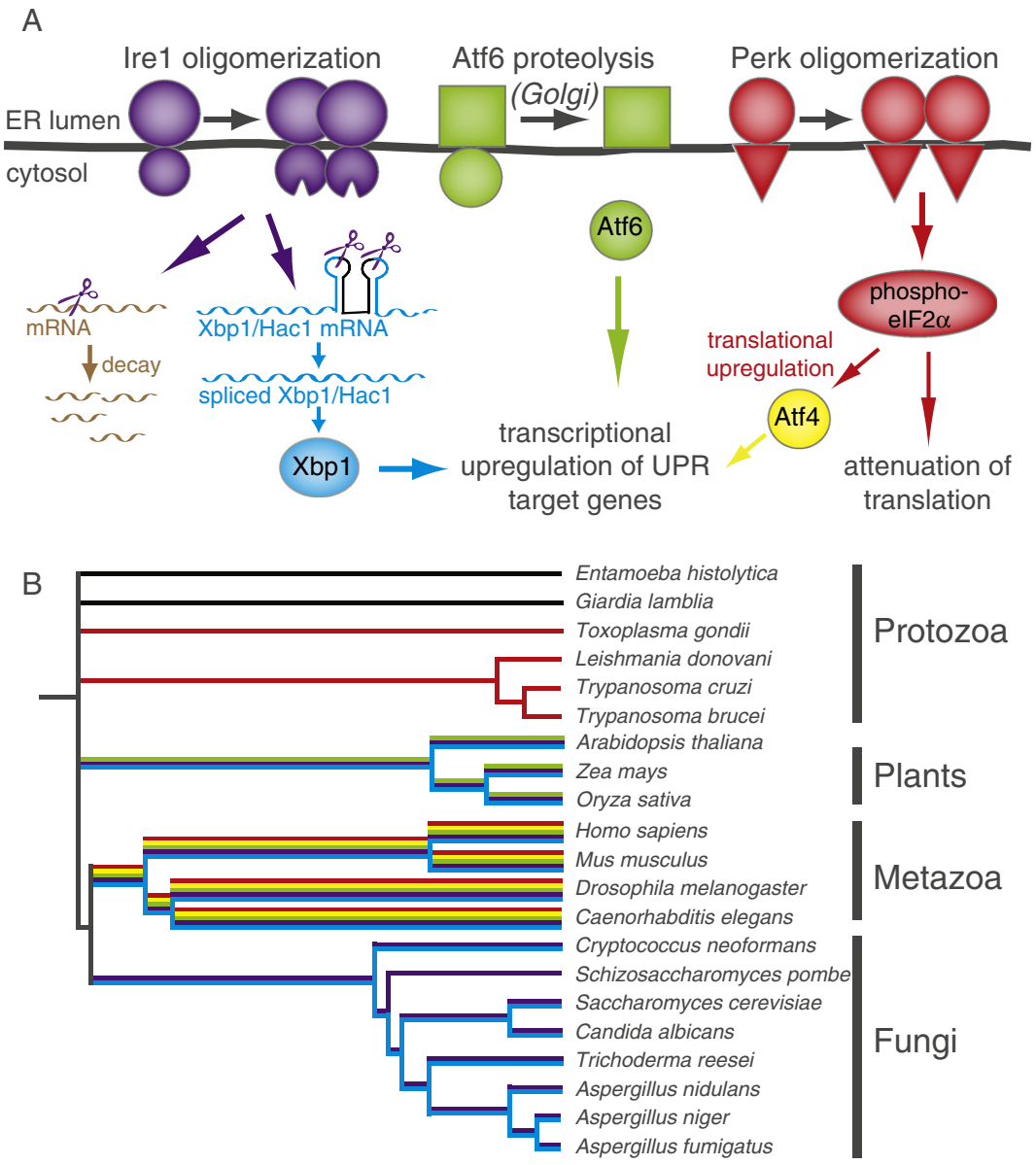

Fig. 1. A. An overview of the signaling mechanisms of the unfolded protein response. During ER stress, Ire1 oligomerizes, which activates its cytosolic kinase and nuclease. The outputs of Ire1 include cleavage of the mRNA encoding Xbp1 (Hac1 in yeast) and degradation of mRNAs. Atf6 travels to the Golgi apparatus during ER stress, where its cytosolic domain is released by proteolysis. Perk oligomerizes and phosphorylates eIF2alpha, leading to translational attenuation in general, and translational activation of certain proteins, including Atf4. Xbp1, Atf6, and Atf4 then coordinate a broad transcriptional response to ER stress. B. A tree diagram of the species mentioned in this review. Branches are color coded (same colors as in A) according to whether these UPR pathways exist in these organisms. Note that for some protozoa the evidence for Perk is not clear (see text). Black indicates that there is evidence against a functional UPR in these organisms. The tree was constructed using the online tool iTOL [99].

initiate the splicing of a Hac1-like bZip transcription factor, X-box binding protein (Xbp1) [20–22], but there is some degree of specialization, as the metazoan Ire1 has been shown to regulate other functions in addition to splicing. For example, in mammals and flies, Ire1 mediates the degradation of many other mRNAs through a pathway referred to as regulated Ire1-dependent decay, or RIDD [23,24]. Comparisons of Ire1α and β in mammals suggest that Ire1α is more effective in Xbp1 splicing whereas Ire1β is more promiscuous [25].

In addition to Ire1, metazoans express two other main sensors of ER stress, Perk and Atf6 (Fig. 1A). Atf6 is a transcription factor that travels from the ER to the Golgi during stress, where it is activated by intramembrane proteolysis, mediated by Site-1 and Site-2 proteases (S1P and S2P) [26,27]. The cytosolic domain released by this cleavage is an active bZip transcription factor, capable of inducing UPR target gene expression on its own and of heterodimerizing with Xbp1 [28]. As with Ire1, there are two isoforms of Atf6 in mammals, and deletion of both results in embryonic lethality in mice [28]. Perk, the third main branch of the UPR in metazoans, is an ER transmembrane kinase that phosphorylates eukaryotic initiation factor 2alpha (eIF2α) in response to ER stress [29,30]. This leads to a general translational attenuation, thought to limit the protein folding load on the ER and conserve resources. Conversely, specific mRNAs that harbor short open reading frames in their 5′ UTRs can be upregulated translationally by eIF2α phosphorylation. Examples of such mRNAs include those encoding Atf4 [31], a third bZip transcription factor that regulates UPR target genes important for stress recovery, and Gadd34 [32], which serves as a negative feedback regulator by dephosphorylating eIF2α [33,34].

The basic features of the UPR appear to be well conserved throughout metazoa. Most species have homologs of the three main signaling

pathways, Ire1, Atf6 and Perk. The UPR has been examined in several vertebrates, including mammals, fish and frogs, which appear to have conserved the dual copies of both Ire1 and Atf6 [35–37], whereas flies and worms have one copy of each. The importance of these pathways for normal development and function also appears to be a general theme for metazoa; Xbp1 in flies is an essential gene [38,39], as it is in mammals [40], and worms lacking Ire1 and either of the other two main UPR branches arrest during larval development [21,41].

Overall these signaling pathways cooperate to restore and/or expand ER function, largely through upregulation of many components of the protein folding and quality control machinery within the ER, and to limit the burden on the ER, through attenuation of translation and possibly through RIDD. When ER stress is not alleviated, further signaling through these pathways also induces apoptosis [3]. The exact outcome of UPR activation varies for different cells and in different situations. While chemical induction of stress usually activates all branches of the UPR, specific cell types express different levels of the UPR sensors, which also display different intrinsic sensitivities to different types of ER perturbation [7]. Thus the UPR transducers impact tissues differentially and tissue-specific knockouts display a variety of phenotypes. For example, Ire1 and Xbp1 in mammals are critical for plasma cell function, whereas Perk is more important for function in the endocrine and exocrine pancreas [42,43]. The customization of the UPR to suit the needs of a particular cell can also be seen, not surprisingly, in comparisons across different species (Fig. 1B).

Despite the unmistakable parallels between UPR signaling in *S. cerevisiae* and mammals, there are interesting variations on these pathways that are now being uncovered in other organisms, including surprising outcomes of Ire1 activation in fission yeast and UPR-like gene expression changes in organisms that rarely regulate transcription. Because Ire1 is the only major branch of the UPR that is present in budding yeast, plants, and metazoans, it is sometimes referred to as the most ancient, or most conserved, UPR signaling pathway. However, protozoans generally do not have recognizable orthologs of Ire1 and Xbp1 [44], whereas there is evidence for Perk-like control of translation in at least some protozoans [44,45], as described below. Thus while both Ire1 and Perk may be ancient ER signaling proteins, they are each lost in certain lineages.

3. Protozoa

Protozoans represent some of the earliest-diverging eukaryotes, and encompass organisms with diverse lifecycles and niches. They include many parasites that infect humans and other animals, and their ability to respond to various forms of stress is thought to be important for the developmental transitions that allow them to switch hosts, move between intracellular and extracellular lifestyles, and enter dormancy [46]. In addition, some of these organisms maintain high levels of protein secretion, also thought to be critical for survival in their hosts. The general organization and molecular mechanisms of protein secretion are conserved between these early eukaryotes and the more familiar metazoa [47]. Consistently, the ER appears to be the main sensor of secretory protein folding burden; stress signals emanating from the ER membrane can lead to both upregulation of ER chaperones and reduction in ER protein load even in these early eukaryotes.

Toxoplasma gondii is a parasite of the apicomplexa phylum; its primary host is feline but it infects humans as well, leading to birth defects if infection takes place during pregnancy and causing serious complications for immunocompromised individuals. *T. gondii* phosphorylates eIF2α in response to several types of stress, including ER stress induced by tunicamycin or the calcium ionophore A23187 [45]. This leads to a reduction in protein synthesis and also induces expression of bradyzoite-specific genes leading to development of these quiescent and immune-evasive cysts [45]. These parasites express an ER-membrane-associated eIF2α kinase that associates with BiP and releases upon ER stress [45], suggesting that this mechanism of regulating translation during ER stress was adopted very early in eukaryotic evolution, then lost in certain lineages such as plants and yeast.

ER stress responses have also been observed in trypanosomatids, parasites that can cycle between insect and mammalian hosts and are the causative agents of sleeping sickness (*Trypanosoma brucei*), Chagas disease (*Trypanosoma cruzi*), and leishmaniasis (several species of *Leishmania*). Like *T. gondii*, trypanosomatids have a transmembrane kinase that phosphorylates eIF2α. At least in *Leishmania donovani*, this phosphorylation occurs in response to dithiothreitol (DTT) [44], a reducing agent and potent inducer of ER stress, and results in a decrease in protein synthesis [48]. In *T. brucei* the only identified membrane-bound eIF2α kinase is localized to the flagellar pocket rather than the ER [49]. As the flagellar pocket is a critical gate for protein secretion in these organisms, it is possible that this kinase is involved in sensing changes in the load on the secretory pathway, but this has yet to be examined.

Transcriptional regulation, a key aspect of the UPR in higher eukaryotes, is not widely employed by protozoans, which appear to lack Ire1 and Xbp1 [44]. For trypanosomatids the lack of transcriptional regulation is reflected in the fact that mRNAs are synthesized as long polycistronic RNAs that are then trans-spliced onto a common 5′ leader sequence. Nonetheless, ER stress in *T. brucei* does result in gene expression changes similar to those seen during the UPR in other eukaryotes, including upregulation of many genes involved in protein secretion [50]. Although the proteins that mediate this response are not known, upregulation of several targets is accomplished through mRNA stabilization [50]. Interestingly, a large fraction of RNAs down-regulated by stress in *T. brucei* encode proteins destined for the ER, reminiscent of the RIDD pathway in higher eukaryotes [50]. In a further parallel with the mammalian UPR, these organisms respond to prolonged ER stress with programmed cell death. In *T. brucei* this is induced by the spliced leader RNA silencing (SLS) pathway [50], which reduces production of the spliced leader sequence necessary for maintaining normal levels and functions of most mRNAs.

Although there may be some organisms among these deeply rooted eukaryotes that do not have a recognizable UPR (including the pathologically significant *Entamoeba histolytica* [51] and *Giardia lamblia* [52]), overall the ability to phosphorylate eIF2α and attenuate protein synthesis in response to stress is a very ancient feature of eukaryotes. Whether this phosphorylation event leads to translational upregulation of specific proteins during ER stress, as it does in mammals, is currently not known. However, some protozoa have been shown to use upstream open reading frames to regulate translation [53,54], and BiP is upregulated (for example, in *T. brucei* [50]), suggesting that this mechanism may be employed during ER stress.

4. Plants

The original description of a functional UPR in plants occurred not long after the discovery of the initial clues indicating the existence of a UPR in mammalian cells [55]. Seed storage proteins are produced during maturation of the endosperm, the tissue surrounding the developing embryo that not only provides nutrition for the embryo but also is an important food source for humans. A large fraction of protein in corn kernels consists of storage proteins known as zeins. These are deficient in the essential amino acid lysine, prompting efforts to find mutants with increased lysine content. Mutants such as *opaque-2* and *floury-2* had decreased zein content but also starchy endosperm. Interestingly, several of these mutants also dramatically induced expression of BiP and other ER chaperones during seed maturation [56–59]. The *floury-2* mutant was subsequently shown to produce a storage protein with a defect in the processing of its ER-targeting signal sequence, causing it to be abnormally retained in the ER [60,61].

Molecular characterization of the UPR in plants began in 2001, when it was found that *Arabidopsis thaliana* encodes two Ire1 proteins with different expression patterns [62], similar to the situation in vertebrates. As in mammals, there may be some degree of functional diversification between the two plant Ire1 proteins [63,64], although there are some conflicting data regarding the extent of functional overlap [63,65,66]. Ire1 proteins from *A. thaliana* and *Oryza sativa* (rice) are localized to the ER, functional as kinases, and capable of sensing disturbances in ER homeostasis through their luminal domains [62,67,68]. However, an Xbp1-like target in plants was not identified until 2011–2012, when it was shown that AtbZIP60 in *A. thaliana* and OsBZIP50 in *O. sativa* are spliced in response to ER stress [63,65,69,70].

BZIP60 was already recognized as a key transcription factor in the plant UPR, as disruption of this gene by a transposable-DNA insertion led to impaired upregulation of several UPR target genes [71,72]. However, it was originally thought to be activated through proteolysis in a manner similar to Atf6. ER stress resulted in a shift of the bZIP60 protein to a smaller size, with a concomitant shift in localization from the ER to the nucleus [71,73]. The protein also has a transmembrane domain, removal of which was sufficient to cause upregulation of UPR target genes [73]. Perplexingly, however, bZIP60 does not contain the RxxL or RxL motifs normally necessary for intramembrane S1P proteolysis and neither S1P nor S2P were required for activation [71]. The discovery that bZIP60 is actually activated by Ire1-mediated splicing explained these observations. The mRNA for bZIP60 has two stem-loops that are quite similar to those found in mammalian Xbp1 [63,65]. These sites are located just upstream of the region coding for the transmembrane domain, so that the frame shift induced by splicing abolishes the transmembrane domain, resulting in a nuclear-localized protein. Unlike Xbp1, both the bZip domain and the transactivation domain of bZIP60 are located upstream of the splice sites [74], suggesting that this mechanism for sequestering bZIP60 at the ER membrane is important for preventing sporadic activation in the absence of ER stress.

In addition to the Ire1-bZIP60 pathway, plants also express homologs of the Atf6 branch of the UPR described in mammals. In *Arabidopsis*, both bZIP28 and bZIP17 are cleaved by S1P and S2P in response to ER stress [75–77], and mutants of bZip28 fail to fully induce BiP, especially at early times following tunicamycin treatment [75]. A Perk homolog has yet to be identified in plants, and neither translational attenuation nor upregulation of eIF2α phosphorylation is seen in response to tunicamycin treatment in *Arabidopsis* [78]. ER stress does induce a homolog of $P58^{IPK}$, a negative regulator of Perk in mammalian cells [79,80], but this appears to result in an overall ER stress-dependent decrease in eIF2α phosphorylation compared to untreated plants [78]. It may be that the RIDD pathway compensates for the lack of translational regulation in plants. Downregulation of mRNAs encoding secreted proteins has been observed in response to ER stress in *Arabidopsis* [81], suggesting that this aspect of Ire1 function is conserved across kingdoms.

Both abiotic and biotic stresses appear to trigger the UPR in plants. In *Arabidopsis*, Ire1-mediated splicing of bZIP60 and activation of bZIP28 and bZIP17 occur in response to heat [63,76,82], and bZIP17 (but not bZIP28) responds to salt stress [75,83]. The Ire1/bZIP60 pathway also is important in mounting an effective response to bacterial pathogens in *Arabidopsis* [64] and *Nicotiana benthamiana* plants [84].

The UPR in mammals responds to endogenous forms of stress resulting from high levels of secretion in certain cell types, and there is evidence that the UPR plays a similar role in plant secretory cells. For example, the processed form of bZIP60 is observed in anthers [71], which contain the highly secretory cells producing pollen surface proteins. Furthermore, double mutants of Ire1 in *Arabidopsis* have defects in elongation of root cells, a process characterized by rapid secretory pathway-dependent synthesis of cell wall material [66]. Mutants of S2P also display impaired root growth, which can be restored by expression of the active nuclear form of bZIP17 or 28 [76]. Lastly, as discussed earlier, endosperm production of seed storage proteins also places a large burden on the ER, and endosperm mutants apparently induce the UPR. Surprisingly, BiP induction is not seen during seed maturation in wild-type maize [56], but the fact that seed development in several other types of plants is accompanied by upregulation of BiP [85–88] suggests that the UPR may indeed be important in regulating the ER capacity during production of seed storage proteins.

5. Fungi

Some of the earliest descriptions of the UPR, including the discovery of Ire1, were carried out in the budding yeast *S. cerevisiae*. In general, fungi use similar mechanisms to manage stress in the ER: activation of Ire1, removal of a regulatory intron in the mRNA encoding Hac1/Xbp1, and transcriptional upregulation of target genes. However, recent studies of the UPR in filamentous fungi and the fission yeast *Schizosaccharomyces pombe* have uncovered interesting variations on this theme. For example, the long Hac1 intron in *S. cerevisiae*, important for preventing premature translation of the unspliced mRNA [89], is conserved only in *Saccharomyces* and a few related yeasts [90]. Many other fungi, including several species of filamentous fungi, possess a Hac1 homolog with a short regulatory intron more like the Xbp1 intron in most animals and plants, suggesting that the ancestral intron was short [90–93]. Surprisingly, although *Candida albicans* splice Hac1 in response to stress [93], a small group of *Candida*-related species has Hac1 homologs that are completely missing the regulatory intron [90]. This suggests that there are other mechanisms for inducing Hac1 in response to stress that have yet to be discovered. One possibility could be regulation of the 5′UTR of Hac1: *C. albicans*, *Trichoderma reesei*, *Aspergillus nidulans*, and *Aspergillus niger* all express Hac1 mRNAs with truncated 5′UTRs in response to stress [91,93,94], suggesting that translational upregulation may be important for Hac1 activation.

Interestingly, *Schizosaccharomyces* and several other fungal phyla appear to lack Hac1 homologs altogether [90]. *Schizosaccharomyces pombe* does possess an Ire1 gene, deletion of which leads to sensitivity to ER stress [95,96] as it does in *S. cerevisiae* and other fungi. However, whereas Ire1 and Hac1 in budding yeast have highly correlated patterns of genetic interactions, the genes most similar to Ire1 in *S. pombe* are not transcription factors but other sensors of protein misfolding, namely UDP-glucose-glycoprotein glucosyltransferase and calnexin [95].

A recent paper from Peter Walter's lab revealed a surprising role for Ire1 in regulating the response to ER stress in *S. pombe* [96]. In contrast to *S. cerevisiae*, ER stress in *S. pombe* results in minimal upregulation of mRNAs encoding residents of the secretory pathway. However, ER stress does induce widespread Ire1-dependent degradation of mRNAs encoding ER-targeted proteins, in essence the RIDD pathway that is observed in fly and mammalian cells but not in budding yeast. The targets of this degradation are selective and highly enriched for mRNAs encoding proteins involved in lipid metabolism, especially sterol metabolism. Kimmig et al. [96] also identified a new twist to this pathway, in which Ire1 specifically cleaves BiP mRNA in its 3′UTR. This truncated BiP mRNA, despite lacking a polyA tail, is highly stable and more translationally active than its full-length precursor, leading to upregulation of this important ER chaperone. How BiP mRNA escapes degradation has yet to be discovered, but it will be fascinating to see whether similar mechanisms exist in other species.

Finally, although Ire1 and Hac1 in *S. cerevisiae* appear to be required for growth only during ER stress, the UPR is important for various aspects of daily life for other fungi. For example, pathogenic fungi such as *Aspergillus fumigatus* [97] and *Cryptococcus neoformans* [98] require a functional UPR for virulence. The UPR in *A. fumigatus* and *C. albicans* also appears to be important for cell wall maintenance and hyphal formation [93,97].

6. Concluding remarks

Here I have attempted to highlight the mechanisms and effects of the UPR in a variety of organisms across eukarya. Overall, it appears that the ability to respond to ER stress evolved along with the ER itself. Even the simplest eukaryotes, which lack extensive transcriptional regulation, respond with changes in gene expression mediated by translation and/or mRNA stability. Examination of the widely conserved Ire1 arm of the UPR suggests that Ire1's originally identified function in mediating the splicing of Hac1 may not be its ancestral function. The nuclease activity of Ire1 is important even in organisms without Hac1/Xbp1, suggesting that degradation of ER-localized mRNAs is an effective mechanism for limiting the folding burden on the ER. As some species of *Candida* and two *Ciona* (sea squirt) genomes contain clear Hac1/Xbp1 homologs lacking the regulatory intron [90], it may be that this transcription factor can be activated by other mechanisms, and at some point took advantage of Ire1's underlying sequence preferences to optimize its own cleavage and splicing in response to stress [96]. As more species are examined, we will undoubtedly learn of other interesting variations on this ancient collection of pathways.

References

[1] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science 334 (2011) 1081–1086.
[2] C. Hetz, The unfolded protein response: controlling cell fate decisions under ER stress and beyond, Nat. Rev. Mol. Cell Biol. 13 (2012) 89–102.
[3] I. Tabas, D. Ron, Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress, Nat. Cell Biol. 13 (2011) 184–190.
[4] S. Wang, R.J. Kaufman, The impact of the unfolded protein response on human disease, J. Cell Biol. 197 (2012) 857–867.
[5] S.H. Back, R.J. Kaufman, Endoplasmic reticulum stress and type 2 diabetes, Annu. Rev. Biochem. 81 (2012) 767–793.
[6] S.Z. Hasnain, R. Lourie, I. Das, A.C. Chen, M.A. McGuckin, The interplay between endoplasmic reticulum stress and inflammation, Immunol. Cell Biol. 90 (2012) 260–270.
[7] K.A. Moore, J. Hollien, The unfolded protein response in secretory cell function, Annu. Rev. Genet. 46 (2012) 165–183.
[8] M.K. Brown, N. Naidoo, The endoplasmic reticulum stress response in aging and age-related diseases, Front. Physiol. 3 (2012) 263.
[9] N.N. Iwakoshi, A.H. Lee, P. Vallabhajosyula, K.L. Otipoby, K. Rajewsky, L.H. Glimcher, Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1, Nat. Immunol. 4 (2003) 321–329.
[10] A.M. Reimold, N.N. Iwakoshi, J. Manis, P. Vallabhajosyula, E. Szomolanyi-Tsuda, E.M. Gravallese, D. Friend, M.J. Grusby, F. Alt, L.H. Glimcher, Plasma cell differentiation requires the transcription factor XBP-1, Nature 412 (2001) 300–307.
[11] J.S. Cox, C.E. Shamu, P. Walter, Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase, Cell 73 (1993) 1197–1206.
[12] K. Mori, W. Ma, M.J. Gething, J. Sambrook, A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus, Cell 74 (1993) 743–756.
[13] K. Mori, T. Kawahara, H. Yoshida, H. Yanagi, T. Yura, Signalling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the unfolded protein-response pathway, Genes Cells 1 (1996) 803–817.
[14] J.S. Cox, P. Walter, A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response, Cell 87 (1996) 391–404.
[15] K.J. Travers, C.K. Patil, L. Wodicka, D.J. Lockhart, J.S. Weissman, P. Walter, Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation, Cell 101 (2000) 249–258.
[16] W. Tirasophon, A.A. Welihinda, R.J. Kaufman, A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells, Genes Dev. 12 (1998) 1812–1824.
[17] T. Iwawaki, R. Akai, S. Yamanaka, K. Kohno, Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 16657–16662.
[18] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science 287 (2000) 664–666.
[19] A. Bertolotti, X. Wang, I. Novoa, R. Jungreis, K. Schlessinger, J.H. Cho, A.B. West, D. Ron, Increased sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient mice, J. Clin. Invest. 107 (2001) 585–593.
[20] M. Calfon, H. Zeng, F. Urano, J.H. Till, S.R. Hubbard, H.P. Harding, S.G. Clark, D. Ron, IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature 415 (2002) 92–96.
[21] X. Shen, R.E. Ellis, K. Lee, C.Y. Liu, K. Yang, A. Solomon, H. Yoshida, R. Morimoto, D.M. Kurnit, K. Mori, R.J. Kaufman, Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development, Cell 107 (2001) 893–903.
[22] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 107 (2001) 881–891.
[23] J. Hollien, J.H. Lin, H. Li, N. Stevens, P. Walter, J.S. Weissman, Regulated Ire1-dependent decay of messenger RNAs in mammalian cells, J. Cell Biol. 186 (2009) 323–331.
[24] J. Hollien, J.S. Weissman, Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response, Science 313 (2006) 104–107.
[25] Y. Imagawa, A. Hosoda, S. Sasaka, A. Tsuru, K. Kohno, RNase domains determine the functional difference between IRE1alpha and IRE1beta, FEBS Lett. 582 (2008) 656–660.
[26] Y. Wang, J. Shen, N. Arenzana, W. Tirasophon, R.J. Kaufman, R. Prywes, Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response, J. Biol. Chem. 275 (2000) 27013–27020.
[27] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol. Biol. Cell 10 (1999) 3787–3799.
[28] K. Yamamoto, T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada, K. Mori, Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1, Dev. Cell 13 (2007) 365–376.
[29] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature 397 (1999) 271–274.
[30] Y. Shi, K.M. Vattem, R. Sood, J. An, J. Liang, L. Stramm, R.C. Wek, Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control, Mol. Cell. Biol. 18 (1998) 7499–7509.
[31] H.P. Harding, I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira, D. Ron, Regulated translation initiation controls stress-induced gene expression in mammalian cells, Mol. Cell 6 (2000) 1099–1108.
[32] Y.Y. Lee, R.C. Cevallos, E. Jan, An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation, J. Biol. Chem. 284 (2009) 6661–6673.
[33] Y. Ma, L.M. Hendershot, Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress, J. Biol. Chem. 278 (2003) 34864–34873.
[34] I. Novoa, H. Zeng, H.P. Harding, D. Ron, Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha, J. Cell Biol. 153 (2001) 1011–1022.
[35] T. Ishikawa, Y. Taniguchi, T. Okada, S. Takeda, K. Mori, Vertebrate unfolded protein response: mammalian signaling pathways are conserved in Medaka fish, Cell Struct Funct 36 (2011) 247–259.
[36] L. Yuan, Y. Cao, F. Oswald, W. Knochel, IRE1beta is required for mesoderm formation in Xenopus embryos, Mech. Dev. 125 (2008) 207–222.
[37] J.Y. Zhang, K.S. Lee, J.S. Kim, B.S. Song, D.I. Jin, D.B. Koo, K. Yu, Functional characterization of the ER stress induced X-box-binding protein-1 (Xbp-1) in the porcine system, BMC Mol Biol 12 (2011) 25.
[38] H.D. Ryoo, P.M. Domingos, M.J. Kang, H. Steller, Unfolded protein response in a Drosophila model for retinal degeneration, EMBO J. 26 (2007) 242–252.
[39] S. Souid, J.A. Lepesant, C. Yanicostas, The xbp-1 gene is essential for development in Drosophila, Dev. Genes Evol. 217 (2007) 159–167.
[40] A.M. Reimold, A. Etkin, I. Clauss, A. Perkins, D.S. Friend, J. Zhang, H.F. Horton, A. Scott, S.H. Orkin, M.C. Byrne, M.J. Grusby, L.H. Glimcher, An essential role in liver development for transcription factor XBP-1, Genes Dev. 14 (2000) 152–157.
[41] X. Shen, R.E. Ellis, K. Sakaki, R.J. Kaufman, Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in *C. elegans*, PLoS Genet. 1 (2005) e37.
[42] H.P. Harding, H. Zeng, Y. Zhang, R. Jungries, P. Chung, H. Plesken, D.D. Sabatini, D. Ron, Diabetes mellitus and exocrine pancreatic dysfunction in perk−/− mice reveals a role for translational control in secretory cell survival, Mol. Cell 7 (2001) 1153–1163.
[43] P. Zhang, B. McGrath, S. Li, A. Frank, F. Zambito, J. Reinert, M. Gannon, K. Ma, K. McNaughton, D.R. Cavener, The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas, Mol. Cell. Biol. 22 (2002) 3864–3874.
[44] S.J. Gosline, M. Nascimento, L.I. McCall, D. Zilberstein, D.Y. Thomas, G. Matlashewski, M. Hallett, Intracellular eukaryotic parasites have a distinct unfolded protein response, PLoS One 6 (2011) e19118.
[45] J. Narasimhan, B.R. Joyce, A. Naguleswaran, A.T. Smith, M.R. Livingston, S.E. Dixon, I. Coppens, R.C. Wek, W.J. Sullivan Jr., Translation regulation by eukaryotic initiation factor-2 kinases in the development of latent cysts in *Toxoplasma gondii*, J. Biol. Chem. 283 (2008) 16591–16601.
[46] N. Vonlaufen, S.M. Kanzok, R.C. Wek, W.J. Sullivan Jr., Stress response pathways in protozoan parasites, Cell. Microbiol. 10 (2008) 2387–2399.
[47] B. Becker, M. Melkonian, The secretory pathway of protists: spatial and functional organization and evolution, Microbiol. Rev. 60 (1996) 697–721.
[48] T. Lahav, D. Sivam, H. Volpin, M. Ronen, P. Tsigankov, A. Green, N. Holland, M. Kuzyk, C. Borchers, D. Zilberstein, P.J. Myler, Multiple levels of gene regulation mediate differentiation of the intracellular pathogen Leishmania, FASEB J. 25 (2011) 515–525.
[49] M.C. Moraes, T.C. Jesus, N.N. Hashimoto, M. Dey, K.J. Schwartz, V.S. Alves, C.C. Avila, J.D. Bangs, T.E. Dever, S. Schenkman, B.A. Castilho, Novel membrane-bound eIF2alpha kinase in the flagellar pocket of Trypanosoma brucei, Eukaryot. Cell 6 (2007) 1979–1991.
[50] H. Goldshmidt, D. Matas, A. Kabi, S. Carmi, R. Hope, S. Michaeli, Persistent ER stress induces the spliced leader RNA silencing pathway (SLS), leading to programmed cell death in *Trypanosoma brucei*, PloS Pathog. 6 (2010) e1000731.

[51] J. Santi-Rocca, S. Smith, C. Weber, E. Pineda, C.C. Hon, E. Saavedra, A. Olivos-Garcia, S. Rousseau, M.A. Dillies, J.Y. Coppee, N. Guillen, Endoplasmic reticulum stress-sensing mechanism is activated in *Entamoeba histolytica* upon treatment with nitric oxide, PLoS One 7 (2012) e31777.
[52] D.S. Reiner, J.M. McCaffery, F.D. Gillin, Reversible interruption of Giardia lamblia cyst wall protein transport in a novel regulated secretory pathway, Cell. Microbiol. 3 (2001) 459–472.
[53] B. Amulic, A. Salanti, T. Lavstsen, M.A. Nielsen, K.W. Deitsch, An upstream open reading frame controls translation of var2csa, a gene implicated in placental malaria, PloS Pathog. 5 (2009) e1000256.
[54] S.M. Teixeira, L.V. Kirchhoff, J.E. Donelson, *Trypanosoma cruzi*: suppression of tuzin gene expression by its 5′-UTR and spliced leader addition site, Exp. Parasitol. 93 (1999) 143–151.
[55] Y. Kozutsumi, M. Segal, K. Normington, M.J. Gething, J. Sambrook, The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins, Nature 332 (1988) 462–464.
[56] R.S. Boston, E.B. Fontes, B.B. Shank, R.L. Wrobel, Increased expression of the maize immunoglobulin binding protein homolog b-70 in three zein regulatory mutants, Plant Cell 3 (1991) 497–505.
[57] E.B. Fontes, B.B. Shank, R.L. Wrobel, S.P. Moose, O.B. GR, E.T. Wurtzel, R.S. Boston, Characterization of an immunoglobulin binding protein homolog in the maize floury-2 endosperm mutant, Plant Cell 3 (1991) 483–496.
[58] A. Marocco, A. Santucci, S. Cerioli, M. Motto, N. Di Fonzo, R. Thompson, F. Salamini, Three high-lysine mutations control the level of ATP-binding HSP70-like proteins in the maize endosperm, Plant Cell 3 (1991) 507–515.
[59] C.P. Li, B.A. Larkins, Expression of protein disulfide isomerase is elevated in the endosperm of the maize floury-2 mutant, Plant Mol. Biol. 30 (1996) 873–882.
[60] C.E. Coleman, M.A. Lopes, J.W. Gillikin, R.S. Boston, B.A. Larkins, A defective signal peptide in the maize high-lysine mutant floury 2, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 6828–6831.
[61] J.W. Gillikin, F. Zhang, C.E. Coleman, H.W. Bass, B.A. Larkins, R.S. Boston, A defective signal peptide tethers the floury-2 zein to the endoplasmic reticulum membrane, Plant Physiol. 114 (1997) 345–352.
[62] N. Koizumi, I.M. Martinez, Y. Kimata, K. Kohno, H. Sano, M.J. Chrispeels, Molecular characterization of two Arabidopsis Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases, Plant Physiol. 127 (2001) 949–962.
[63] Y. Deng, S. Humbert, J.X. Liu, R. Srivastava, S.J. Rothstein, S.H. Howell, Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in Arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7247–7252.
[64] A.A. Moreno, M.S. Mukhtar, F. Blanco, J.L. Boatwright, I. Moreno, M.R. Jordan, Y. Chen, F. Brandizzi, X. Dong, A. Orellana, K.M. Pajerowska-Mukhtar, IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses, PLoS One 7 (2012) e31944.
[65] Y. Nagashima, K. Mishiba, E. Suzuki, Y. Shimada, Y. Iwata, N. Koizumi, Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor, Sci. Rep. 1 (2012) 29.
[66] Y. Chen, F. Brandizzi, AtIRE1A/AtIRE1B and AGB1 independently control two essential unfolded protein response pathways in Arabidopsis, Plant J. 69 (2011) 266–277.
[67] Y. Okushima, N. Koizumi, Y. Yamaguchi, Y. Kimata, K. Kohno, H. Sano, Isolation and characterization of a putative transducer of endoplasmic reticulum stress in *Oryza sativa*, Plant Cell Physiol. 43 (2002) 532–539.
[68] S.J. Noh, C.S. Kwon, W.I. Chung, Characterization of two homologs of Ire1p, a kinase/endoribonuclease in yeast, in *Arabidopsis thaliana*, Biochim. Biophys. Acta 1575 (2002) 130–134.
[69] S. Hayashi, Y. Wakasa, H. Takahashi, T. Kawakatsu, F. Takaiwa, Signal transduction by IRE1-mediated splicing of bZIP50 and other stress sensors in the endoplasmic reticulum stress response of rice, Plant J. 69 (2012) 946–956.
[70] S.J. Lu, Z.T. Yang, L. Sun, Z.T. Song, J.X. Liu, Conservation of IRE1-regulated bZIP74 mRNA unconventional splicing in rice (*Oryza sativa* L.) involved in ER stress responses, Mol. Plant 5 (2012) 504–514.
[71] Y. Iwata, N.V. Fedoroff, N. Koizumi, Arabidopsis bZIP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response, Plant Cell 20 (2008) 3107–3121.
[72] D.P. Lu, D.A. Christopher, Endoplasmic reticulum stress activates the expression of a sub-group of protein disulfide isomerase genes and AtbZIP60 modulates the response in *Arabidopsis thaliana*, Mol. Genet. Genomics 280 (2008) 199–210.
[73] Y. Iwata, N. Koizumi, An Arabidopsis transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 5280–5285.
[74] Y. Iwata, M. Yoneda, Y. Yanagawa, N. Koizumi, Characteristics of the nuclear form of the Arabidopsis transcription factor AtbZIP60 during the endoplasmic reticulum stress response, Biosci. Biotechnol. Biochem. 73 (2009) 865–869.
[75] J.X. Liu, R. Srivastava, P. Che, S.H. Howell, An endoplasmic reticulum stress response in Arabidopsis is mediated by proteolytic processing and nuclear relocation of a membrane-associated transcription factor, bZIP28, Plant Cell 19 (2007) 4111–4119.
[76] P. Che, J.D. Bussell, W. Zhou, G.M. Estavillo, B.J. Pogson, S.M. Smith, Signaling from the endoplasmic reticulum activates brassinosteroid signaling and promotes acclimation to stress in Arabidopsis, Sci. Signal. 3 (2010) ra69.
[77] H. Tajima, Y. Iwata, M. Iwano, S. Takayama, N. Koizumi, Identification of an Arabidopsis transmembrane bZIP transcription factor involved in the endoplasmic reticulum stress response, Biochem. Biophys. Res. Commun. 374 (2008) 242–247.
[78] S. Kamauchi, H. Nakatani, C. Nakano, R. Urade, Gene expression in response to endoplasmic reticulum stress in *Arabidopsis thaliana*, FEBS J. 272 (2005) 3461–3476.
[79] R. van Huizen, J.L. Martindale, M. Gorospe, N.J. Holbrook, P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling, J. Biol. Chem. 278 (2003) 15558–15564.
[80] W. Yan, C.L. Frank, M.J. Korth, B.L. Sopher, I. Novoa, D. Ron, M.G. Katze, Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15920–15925.
[81] I.M. Martinez, M.J. Chrispeels, Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes, Plant Cell 15 (2003) 561–576.
[82] H. Gao, F. Brandizzi, C. Benning, R.M. Larkin, A membrane-tethered transcription factor defines a branch of the heat stress response in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 16398–16403.
[83] J.X. Liu, R. Srivastava, P. Che, S.H. Howell, Salt stress responses in Arabidopsis utilize a signal transduction pathway related to endoplasmic reticulum stress signaling, Plant J. 51 (2007) 897–909.
[84] C. Tateda, R. Ozaki, Y. Onodera, Y. Takahashi, K. Yamaguchi, T. Berberich, N. Koizumi, T. Kusano, NtbZIP60, an endoplasmic reticulum-localized transcription factor, plays a role in the defense response against bacterial pathogens in *Nicotiana tabacum*, J. Plant Res. 121 (2008) 603–611.
[85] B.S. Forward, S. Misra, Characterization and expression of the Douglas-fir luminal binding protein (PmBiP), Planta 212 (2000) 41–51.
[86] A. Kalinski, D.L. Rowley, D.S. Loer, C. Foley, G. Buta, E.M. Herman, Binding-protein expression is subject to temporal, developmental and stress-induced regulation in terminally differentiated soybean organs, Planta 195 (1995) 611–621.
[87] D.G. Muench, Y. Wu, Y. Zhang, X. Li, R.S. Boston, T.W. Okita, Molecular cloning, expression and subcellular localization of a BiP homolog from rice endosperm tissue, Plant Cell Physiol. 38 (1997) 404–412.
[88] K. Hatano, T. Shimada, N. Hiraiwa, M. Nishimura, I. Hara-Nishimura, A rapid increase in the level of binding protein (BiP) is accompanied by synthesis and degradation of storage proteins in pumpkin cotyledons, Plant Cell Physiol. 38 (1997) 344–351.
[89] U. Ruegsegger, J.H. Leber, 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 107 (2001) 103–114.
[90] K.B. Hooks, S. Griffiths-Jones, Conserved RNA structures in the non-canonical Hac1/Xbp1 intron, RNA Biol. 8 (2011) 552–556.
[91] M. Saloheimo, M. Valkonen, M. Penttila, Activation mechanisms of the HAC1-mediated unfolded protein response in filamentous fungi, Mol. Microbiol. 47 (2003) 1149–1161.
[92] M.H. Oh, S.A. Cheon, H.A. Kang, J.Y. Kim, Functional characterization of the unconventional splicing of *Yarrowia lipolytica* HAC1 mRNA induced by unfolded protein response, Yeast 27 (2010) 443–452.
[93] T.T. Wimalasena, B. Enjalbert, T. Guillemette, A. Plumridge, S. Budge, Z. Yin, A.J. Brown, D.B. Archer, Impact of the unfolded protein response upon genome-wide expression patterns, and the role of Hac1 in the polarized growth, of *Candida albicans*, Fungal Genet. Biol. 45 (2008) 1235–1247.
[94] H.J. Mulder, M. Saloheimo, M. Penttila, S.M. Madrid, The transcription factor HACA mediates the unfolded protein response in *Aspergillus niger*, and up-regulates its own transcription, Mol. Genet. Genomics 271 (2004) 130–140.
[95] A. Frost, M.G. Elgort, O. Brandman, C. Ives, S.R. Collins, L. Miller-Vedam, J. Weibezahn, M.Y. Hein, I. Poser, M. Mann, A.A. Hyman, J.S. Weissman, Functional repurposing revealed by comparing *S. pombe* and *S. cerevisiae* genetic interactions, Cell 149 (2012) 1339–1352.
[96] P. Kimmig, M. Diaz, J. Zheng, C.C. Williams, A. Lang, T. Aragon, H. Li, P. Walter, The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis, elife 1 (2012) e00048.
[97] D.L. Richie, L. Hartl, V. Aimanianda, M.S. Winters, K.K. Fuller, M.D. Miley, S. White, J.W. McCarthy, J.P. Latge, M. Feldmesser, J.C. Rhodes, D.S. Askew, A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in *Aspergillus fumigatus*, PloS Pathog. 5 (2009) e1000258.
[98] S.A. Cheon, K.W. Jung, Y.L. Chen, J. Heitman, Y.S. Bahn, H.A. Kang, Unique evolution of the UPR pathway with a novel bZIP transcription factor, Hxl1, for controlling pathogenicity of Cryptococcus neoformans, PLoS Pathog 7 (2011) e1002177.
[99] I. Letunic, P. Bork, Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy, Nucleic Acids Res. 39 (2011) W475–W478.