Title: At the crossroads of survival and death: the ROS-ethylene-sugar triad and UPR

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Keywords: ER stress; ethylene; proteostasis; reactive oxygen species; sugars; unfolded protein response

Abstract

Upon stress, a trade-off between plant growth and defense responses defines the capacity for survival. Stress can result in accumulation of misfolded proteins in the endoplasmic reticulum (ER) and other organelles. To cope with these proteotoxic effects, plants rely on the unfolded protein response (UPR). The involvement of reactive oxygen species (ROS), ethylene (ETH), and sugars, as well as their crosstalk, in general stress responses is well established, yet their role in UPR deserves further scrutiny. Here, a synopsis of current evidence for ROS-ETH-sugar crosstalk in UPR is discussed. We propose that this triad acts as a major signaling hub at the crossroads of survival and death, integrating information from ER, chloroplasts and mitochondria, thereby facilitating a coordinated stress response.

Coordinated inter-organelle stress responses facilitate plant survival

The sessile nature of plants implies that they are inherently subject to changing environments. As such, they need to cope with a variety of (a)biotic stresses. These harmful conditions lead to a set of shared but also distinct responses that can include **oxidative stress (see Glossary)**, osmotic or ionic imbalances and changes in cellular components, all of which modify the physiological status. Growth and development are hindered under such conditions, either directly - for instance by oxidative damage of essential biomolecules - or indirectly, through reprogramming of energy metabolism. In particular, the functioning of chloroplasts and mitochondria, the 'powerhouses' of the cell, is disturbed upon stress. The associated changes in carbohydrate status and, ultimately, energy levels affect growth, but probably also serve as important stress signals (Figure 1, Key Figure) [1]**.** As such, mitochondria and chloroplasts act as central hubs that integrate external and internal signals to coordinate growth [2-4].

Importantly, stress perception and its downstream responses should be considered as context-dependent, and are influenced by the stress type, severity and duration. Nevertheless, an integral aspect of stress is the accumulation of unfolded or misfolded proteins (i.e. **proteotoxic stress**) [5]. The endoplasmic reticulum (ER) is essential for protein folding and secretion and has different mechanisms for protein quality control (QC). However, once the amount of un- or misfolded proteins surpasses the level that can be controlled by the ERQC, cells have to cope with the cytotoxicity of hampered **proteostasis**, called ER stress. This also occurs in chloroplasts and mitochondria [6-7]. Restoration of organellar proteostasis requires responses from both the organelle and the nucleus, and depends on intricate

crosstalk between subcellular compartments. Hence, a tight communication established via **antero-** and **retrograde** signals is necessary for coordinated gene expression to restore proteostasis (Box 1). Eukaryotes rely on the evolutionary conserved retrograde signaling pathway called the unfolded protein response (UPR) that initiates a series of transcriptional and translational changes to restore the balance between folding capacity and demand [8]. Though UPR is well described in mammals, the basic machinery present in plants has been discovered only recently. Increasing evidence underscores emerging roles for plant hormones, [e.g. salicylic acid (SA) [9], jasmonic acid (JA) [7], auxin and ethylene (ETH) [7,10]], secondary messengers (e.g. Ca^{2+}) [11], as well as other signaling molecules such as reactive oxygen species (ROS) and sugars, as important regulators of the plant UPR. The well-established intimate relation between ROS and ETH as key mediators of general stress responses, and their connection to sugar signaling prompts a reassessment of their coordinate involvement in UPR. We believe that there is significant evidence for such connections, and propose that this triad acts at the crossroads of proteotoxic stress and energy signaling. Though it is certain that other molecular players (e.g. SA, auxin, Ca^{2+}) are important drivers of UPR as well, these will not be discussed within the frame of this work.

The unfolded protein response

Upon accumulation of un- or misfolded proteins in the ER, cells trigger UPR to mitigate ER stress. This intracellular signaling mechanism aims to restore protein homeostasis by upregulating genes involved in protein folding and **ER-associated degradation (ERAD)**, or by induction of **autophagy** (Figure 1b) [8]. If ER stress persists, UPR signaling further induces the expression of autophagy-related genes,

but ultimately resorts to **programmed cell death (PCD)** (Figure 1c) [12-13]. In mammalians, UPR plays a key role in many diseases characterized by chronic ER stress [14]. In plants, UPR mitigates ER stress caused by a wide range of (a)biotic stresses overwhelming the protein folding machinery [15]. Although UPR is conserved among eukaryotes, some signaling components differ between kingdoms. In metazoans, UPR consists of three branches regulated by inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6) and protein kinase RNA-like ER kinase (PERK). In contrast, the plant UPR comprises two branches (Box 2) [12]. The first is regulated by IRE1, which induces the unconventional splicing of the BASIC LEUCINE ZIPPER 60 (bZIP60) transcription factor. The second branch relies on the transcription factors bZIP17 and bZIP28, representing ATF6 homologs. A PERK homolog has not been identified in plants [12]. Interestingly, spliced bZIP60 is able to move from cell to cell through plasmodesmata, mainly from root to shoot, supporting its involvement in non-cell autonomous, systemic UPR signaling besides its role in local, intracellular responses to ER stress [16].

The plant UPR is best characterized in response to ER stress (erUPR); however, impairment of proteostasis in other subcellular compartments (Box 1) appears to activate similar signaling mechanisms. Dogra *et al.* (2019) showed the presence of a UPR-like response in chloroplasts of the arabidopsis (*Arabidopsis thaliana*) *yellow leaf variegation 2 (var2)* mutant that accumulates damaged photosystem II proteins [6]. Defects in Clp protease activity were also shown to induce a plastidial UPR (cpUPR) [17]. Similar to erUPR, cpUPR causes the upregulation of genes encoding chaperones, proteases and proteins involved in detoxification pathways [6].

Whereas the cytoplasmic MUTANT AFFECTED IN CHLOROPLAST-TO-NUCLEUS RETROGRADE SIGNALING (MARS1) kinase was identified as a crucial player in cpUPR signal transduction in *Chlamydomonas reinhardtii*, the involved signaling molecules in higher plants remain elusive [18]. In plants, it is proposed that the mitochondrial UPR (mtUPR) activates four retrograde signaling pathways [19]. These aim to restore mitochondrial translation, protein import and folding, while maintaining sufficient growth, namely through ANAC017 [20] (Box 1), ETH (see further), auxin [21], and JA signaling [7]. Whereas erUPR is relatively well characterized in plants, less is known regarding the mechanisms underlying cpUPR and mtUPR. Nevertheless, evidence argues that the pathways originating in each subcellular compartment interact with one another, are important for survival and are governed by the well-known stress signals, ROS and ETH.

The stressed plant: a tale of many signals

Reactive oxygen species

ROS are key players in normal physiological processes and plant responses to stress. Despite their ability to damage cellular macromolecules, basal levels of ROS are indispensable for signal transduction, for instance by modifying regulatory thiols on proteins [22]. Several recent studies provide evidence for the reciprocal interaction between ROS and erUPR. The ER stress inducer tunicamycin rapidly increases hydrogen peroxide (H_2O_2) concentrations in arabidopsis (Figure 1b) [23]. This is likely related to the UPR-mediated upregulation of the ER oxidoreductase *ERO1*, which catalyzes the formation and isomerization of protein disulfide bonds in the ER, important for **oxidative protein folding**. This oxygen-consuming process

generates H_2O_2 in the ER lumen, which likely translocates to the cytosol or other subcellular compartments [24]. As such, H_2O_2 produced upon UPR activation can serve as a signal orchestrating stress responses beyond the ER. Additionally, oxidation of the ER lumen by H_2O_2 accumulation might trigger Ca^{2+} release, impacting a plethora of downstream stress-related signals, including ROS and phytohormones [24-25]. Alternatively, erroneously formed protein disulfides can be restored by electron transfer from glutathione. The resulting depletion of this crucial antioxidant can further enhance ROS generation. Moreover, ER stress induces the expression and activity of NADPH oxidases encoded by respiratory burst oxidase homologues (RBOHs) [23]. The RBOHD and RBOHF isoforms significantly contribute to superoxide and H_2O_2 production during ER stress, essential for proper activation of UPR and prevention of cell death [26]. These data imply that ROS function downstream of UPR, though they also act upstream. Low doses of up to 1 mM H_2O_2 induce the expression of UPR genes in leaves of arabidopsis, suggesting that erUPR activation depends on ROS signaling rather than damage. Interestingly, the specific transcriptional signature of ER stress-responsive genes depends on both ROS type and origin (Figure 1) [27].

The mtUPR is triggered by a transient oxidative burst that subsequently activates MITOGEN-ACTIVATED PROTEIN KINASE 6 (MPK6) and hormonal signaling [7]. Moreover, upon mitochondrial proteotoxic stress, it is suggested that release of ANAC017 from the ER (Box 1) requires mitochondrial H_2O_2 [20]. In chloroplasts, ROS accumulation under unfavorable conditions contributes to the development of proteotoxic stress [6]. Nevertheless, additional research is required to determine

their involvement in transducing the retrograde UPR signal. Lastly, ROS also play vital roles in the regulation of autophagy and PCD (Figure 1) [28].

Ethylene

A large body of work has established that the accumulation of the phytohormone ETH, as a consequence of (a)biotic stresses, leads to a series of adaptations that confer stress tolerance. Whether ETH functions in the alleviation of proteotoxic stress is, however, less well studied. The direct involvement of other stress hormones, including SA [9], JA, and auxin [7], in the regulation of proteotoxic stress prompts further detailed examination of the connection of ETH to the UPR and its interplay with other hormones. For a detailed overview on ETH biosynthesis and signaling, and its link to stress, see Box 3.

Chen *et al.* (2014) showed that ER stress does not lead to an increased expression of the ETH receptor *ETHYLENE RESPONSE 1* (*ETR1)* [10]. Nevertheless, other genes, such as the biosynthesis-related and stress-inducible *MPK3* and *MPK6* [29] could be targeted during ER stress (Figure 1b). In mitochondria, a direct MPK6-dependent link between ETH and the restoration of proteostasis was demonstrated [7]. The authors evidenced that mtUPR relies on MPK6-generated ETH, which acts as a retrograde signal together with auxin and JA, promoting the nuclear expression of MITOCHONDRIAL RIBOSOMAL PROTEINs (MRPs) and mitochondrial HEAT SHOCK PROTEINs (mtHSPs). The latter are part of the feedback anterograde signaling circuitry responsible for restoring mitochondrial protein balance. This first report on the involvement of ETH in mtUPR hints at a more general role for this major stress hormone in UPR. Moreover, ETH participates in several processes

downstream of erUPR signaling, implying broader relevance in restoring proteostasis. For instance, autophagy and PCD occurring as a consequence of mild and severe ER stress, respectively, are clearly regulated by ETH (Figure 1). In drought-stressed tomato (*Solanum lycopersicum*), ETH confers tolerance through the activation of *ERF5*, which upregulates the expression of *AUTOPHAGY*-related (*ATG*) *8* and *ATG18* [30]. Pan *et al*. (2016) found that exogenously applied 1-aminocyclopropane-1-carboxylate (ACC), the direct precursor of ETH, diminished cell death through an induction of Plant Bcl-2-associated athanogene (*BAG*) *6* and *BAG7* (Figure 1b), thereby improving salinity tolerance [31]. The latter was discovered as an important UPR transducer in the ER during heat or cold stress [32]. Altogether, it is clear that ETH is implicated in regulating various aspects of UPR, as demonstrated in mitochondria and the ER, though the connections underlying this crosstalk deserve detailed scrutiny. In addition, the role of ETH in cpUPR should not remain unexplored given that ETH also plays a role in photosynthesis, and hence sugar metabolism and signaling [33].

ROS-ETH interactions in relation to sugar and stress signaling

The concerted action of ROS and ETH

Reciprocal interactions between ROS and ETH signals have been demonstrated for different stresses and likely also function in UPR. A burst of ROS can activate downstream MAPK signaling [34], in turn upregulating ETH biosynthesis (Figure 1b) [29]. It was shown that mitochondrial ROS act as a signal upstream of ETH biosynthesis, and were required for the expression of genes that restore mitochondrial proteostasis [7]. In contrast, ETH confers salt stress tolerance in

arabidopsis by stimulating low levels of ROS production, for instance by inducing *RBOHF* expression [35]. Conversely, ETH also activates the antioxidant machinery to prevent ROS damage if their levels accumulate upon prolonged stress (Figure 1b) [36]. Hence, ROS-ETH interplay functions at the decision point for cell survival versus death, with the associated response depending on the severity and duration of the stress condition (Figure 1b-c). During drought, ETH can activate autophagy, to prevent PCD, by ERF5-mediated expression of *ATG* genes as well as via the promotion of ALTERNATIVE OXIDASE (AOX) 1a function [30]. Mitochondrial AOX1a prevents accumulation of ROS to damaging levels by restraining over-reduction of ubiquinone, maintaining low amounts of ROS to stimulate autophagy. However, upon chronic mitochondrial stress, the associated high ROS levels can ultimately lead to PCD. Noteworthy, ROS-ETH interplay can also provoke PCD in certain conditions of severe stress (Figure 1c) [37]. Thus, ROS-ETH interactions appear to play a prominent role both in the initial responses to stress, restoring proteostasis, as well as in mediating death strategies at later stages (Figure 1). This duality is likely influenced by the duration and severity of stress, tissue type, and developmental stage, and controlled by a third signaling partner, sugars.

Sugar signaling translates cellular energy status

Disturbed energy metabolism is a direct consequence of many stress conditions, leading to either starvation or “sweetening” [1]. A reduction in sugars as well as cytosolic ATP levels likely results from malfunctioning chloroplasts and mitochondria, for instance caused by ROS accumulation (Figure 1b) [38]. Sugars and ATP are essential for basic metabolism, but also facilitate protein folding and post-translational modifications [5]. Hence, the level of soluble sugars confers

information about the plant's physiological status, and should be tightly monitored. In plants, two main energy sensors exist, Target of rapamycin (TOR) and sucrose-non-fermenting-related protein kinase 1 (SnRK1), regulating cellular homeostasis [39]. For instance, upon energy abundance (sugar availability), TOR is activated, stimulating growth in **sink** organs (i.e. young growing leaves). It is important to note that TOR is not exclusively activated by sugars. Readers are referred to Ingargiola et al. (2020) for a detailed overview on the regulation of TOR [40]. In contrast, stresses like nutrient starvation, pathogen attack and oxidative stress often lead to sugar starvation in sink tissues. Upon energy deficiency, SnRK1 is activated, stimulating catabolism and repressing biosynthetic pathways [41]. Conversely, SnRK1 is inhibited by sugar phosphates including trehalose-6-phosphate, glucose-1-phosphate and glucose-6-phosphate [41].

In animals, energy status and metabolism are intricately linked with UPR [42]. Direct evidence in plants is scarce, though, given the prime role of sugars, crosstalk with UPR signaling is plausible. UDP-Glucose (UDP-Glc) serves as a precursor for glycosylation as well as sucrose synthesis. Expression of a UDP-Glc transporter (AtUTr1) in the ER was upregulated by UPR [43] and disturbances in UDP-Glc levels induced PCD [44]. Protein folding requires ATP, and low levels of ATP are correlated with UPR induction [45]. In ER-LOCALIZED ADENINE NUCLEOTIDE TRANSPORTER 1 (ER-ANT1) rice (*Oryza sativa*) mutants unable to transport ATP into the ER lumen, UPR is triggered [46]. Deprivation of Glc in *er-ant1* loss-of-function mutants also activated IRE1, further supporting a link with UPR. Additionally, *er-ant1* mutants exhibited induced expression of SnRK1. Induction of UPR responses by lowered ATP levels could play a broader role in the response to

stress (Figure 1). During mild stress, normal functioning of chloroplasts and mitochondria, major sites for sugar synthesis and ATP production, is generally impeded. Disturbed proteostasis caused by ROS accumulation within these organelles and a concomitant decrease in cytosolic ATP levels likely trigger a retrograde signaling network to restore protein folding in all subcellular compartments (Figure 1b). Communication between organelles (Box 1), either directly via membrane contact sites (MCS) or through the expression of nuclear genes, is assumed to orchestrate a coordinated stress response. The sugar sensor SnRK1 could play a vital role in this retrograde signaling network, as suggested by other reports [47]. Lastly, it was demonstrated that ER stress-induced autophagy requires SnRK1 as well [48].

Further research on SnRK1 during sugar excess in mature leaves (**source** tissues) is warranted, since many abiotic stresses (drought, cold, salt) lead to leaf sweetening and trehalose-6-phosphate has no inhibitory action on SnRK1 activity, *in vitro,* derived from mature leaves [49-50]. It is possible that SnRK1 is also activated by stresses causing sugar excess, likely mediated by abscisic acid (ABA), since it was recently shown that ABA leads to the dissociation of the SnRK1-SnRK2 complex in seedlings [51]. Disassembly of the complexes releases SnRK1 and SnRK2 to trigger stress responses and inhibit growth. This is partly accomplished through direct TOR repression by SnRK1. In absence of stress, SnRK2 promotes growth by inhibiting SnRK1. However, it is not clear whether ABA is able to overrule the inhibition of SnRK1 by sugars. Moreover, it remains to be demonstrated whether these interactions also exist in mature tissues. Furthermore, it needs to be proven

whether the SnRK1-TOR interactions are truly sugar-specific, not representing osmotic effects that can also be accomplished by other molecules.

Overall, we are just on the verge of understanding the regulation of SnRK1 and its interaction with TOR. The latter was found to be significantly more active in mature leaves photosynthesizing a surplus of sugars as compared to young, growing leaves [52]. The concomitant increase in TOR activity correlates with decreased rates of plasmodesmatal (PD) sugar transport. Thus, leaf cells appear to regulate PD trafficking in response to altered carbohydrate availability in a TOR-dependent pathway. Nevertheless, since TOR is classically known as a growth-promoting factor, it remains to be seen whether plants contain an alternative TOR complex, as demonstrated in mammalian cells [53].

The role of respiratory pathways in UPR responses should be evaluated as well. Both photorespiration, connecting plastids, mitochondria and cytosol, as well as alternative respiration through AOX in mitochondria serve as important - likely intertwined - mechanisms for stress adaptation [54], by limiting the amount of reducing equivalents and consequently preventing ROS accumulation. Since these pathways consume, respectively limit ATP production, activation of photorespiration and AOX probably induces UPR pathways (Figure 1b). Moreover, crosstalk with H_2O_2 [54] and ETH signaling [30,55] is likely to mediate or fine-tune this response.

It is clear that SnRK1 functions as a sensory hub coordinating stress and energy signaling (Figure 1) [56]. Multiple connections with both ROS and ETH signaling have been demonstrated. Sugar signaling, through SnRK1, and ROS/ETH converge to stimulate stress responses at the expense of growth (Figure 1). For

instance, SnRK1 expression is induced in ETH-insensitive mutants [57], and SnRK1 positively regulates ETH synthesis during catabolism-driven senescence [58], suggesting feedforward loops. Excess intracellular Glc enhances EIN3 degradation [59], ultimately leading to lowered ETH signaling together with activation of TOR. In contrast, SnRK1 inhibits EIN3 to limit ETH-induced senescence [58], suggesting a context-dependent ETH-sugar interaction. Furthermore, high extracellular Glc levels were shown to activate ROS-generating NADPH oxidases [60]. In addition, it has been shown *in vivo* that low ROS levels might activate SnRK1 under starvation stress in sinks [61], whereas *in vitro* experiments suggest that excessive ROS can inactivate it by oxidation (Figure 1) [62], urging the need for further research. As SnRK1 is a central metabolic hub, these interactions allow for fine-tuned stress responses, balancing with the TOR kinase signaling complex.

Lastly, it is important to mention the emerging evidence for the involvement of TOR in abiotic stress responses [63]. Specifically, the reciprocal interaction with ABA signaling is important in the adaptation to unfavorable conditions and the retuning of growth. As such, a direct link between TOR signaling and UPR might exist and should be evaluated. In yeast (*Saccharomyces cerevisiae*), for instance, a hyperactive TORC1 led to an enhanced sensitivity to ER stress [64]. It is conceivable that both SnRK1 and TOR have specific roles in the regulation of UPR signaling, which likely depend on intricate crosstalk with internal and external signals, and on the severity and type of stress.

Through its dynamic localization (cytosol, nucleus and ER) [65], it can be hypothesized that ER-localized SnRK1 integrates ROS, ETH and sugars as a

central triad of signals mediating UPR responses emerging in all subcellular compartments, essential for plants at the crossroads of survival and death. Nevertheless, it is probable that other molecular players, such as the aforementioned signals SA, auxin, and Ca^{2+}, among others, interact with this triad, adding additional layers of complexity.

Concluding remarks

Significant progress has been made in elucidating the molecular basis for erUPR in plants. However, research efforts to unravel mtUPR and cpUPR are still in their infancy. Furthermore, the signals operating upstream and downstream of these UPR pathways remain elusive. Current evidence shows important roles for ROS and ETH – closely intertwined regulators of stress responses – in activating and modulating UPR, but their connection to key UPR players remains unclear. Studying responses of UPR mutants in relation to altered ROS and ETH accumulation or signaling would shed light on this issue. Furthermore, recently developed fluorescence-based approaches to identify heterologously expressed proteins involved in UPR regulation provide powerful tools to untangle the involvement of ROS and ETH therein [66]. As important determinants of the energy status and stress signaling, sugars and ATP levels are likely also involved in defining UPR, with SnRK1 playing a key role. Multiple connections between sugar signaling, ROS and ETH exist. Therefore, we propose that these act in concert during UPR pathways, triggered upon proteotoxic stress, perceived in different subcellular compartments and essentially orchestrating the decision between cell survival or death (Figure 1). Furthermore, the unexplored role of photorespiratory and alternative respiration pathways as additional inducers of UPR responses

represents an interesting avenue for future research. The challenge to unravel the complexity and significance of the ROS-ETH-sugar triad in plant UPR pathways lies ahead (Outstanding Questions Box). In this context, it is crucial to focus research efforts on responses in individual organelles, through site-specific pharmacological interference of redox state or by genetic disruption of protein quality control or known UPR components. Indeed, the communication between subcellular compartments is pivotal for a harmonious response across the entire cell, tissue or plant.

BOX 1: Organellar stress responses require antero- and retrograde signaling cascades

Stress sensing and response can occur at the plasma membrane and in different organelles, including the ER, mitochondria and chloroplasts [67]. For instance, stress signals can disrupt electron transport chains, causing ROS accumulation, severe metabolic imbalances and disturbed proteostasis [38]. Integration of signals emerging from subcellular compartments is especially relevant for mitochondria and chloroplasts, given their endosymbiont origin. Over the course of evolution, these organelles have become semi-autonomous due to the large number of “organellar” functions now encoded on the nuclear genome. Consequently, their development and performance depend on intricate communication with the nucleus. Anterograde (nucleus-to-organelle) and retrograde (organelle-to-nucleus) signaling routes are indispensable to steer nuclear expression of organelle-localized proteins in adaptation to stress (Figure I). In chloroplasts, stress-induced ROS production causes the accumulation of several retrograde signals, including carotenoid derivatives, the isoprenoid precursor methylerythritol cyclodiphosphate (MEcPP)

and 3′-phosphoadenosine-5′-phosphate (PAP) leading to the induction of “stress genes” in the nucleus (Figure I) [2]. The pentatricopeptide repeat (PPR) protein GENOMES UNCOUPLED 1 (GUN1), another well-known retrograde signaling component, was recently shown to be involved in plastidial proteostasis [4]. Upon environmental stress, GUN1 functioning is associated with improved protein import and reduced accumulation of unfolded plastid proteins in the cytosol. In mitochondria, ROS, PAP and other unknown signals act as retrograde signals (Figure I), though a well-defined mechanistic understanding of these pathways is lacking. Ng *et al.* (2013) demonstrated that mitochondrial stress activates the proteolytic cleavage of the ER-bound ANAC017 transcription factor. ANAC017 is essential for the nuclear induction of *ALTERNATIVE OXIDASE 1a (AOX1a)* [68], an important marker for mitochondrial retrograde regulation, supporting metabolic homeostasis by avoiding over-reduction of ubiquinone (Figure I). This mechanism illustrates the importance of inter-organelle communication under stress, in addition to canonical retrograde signaling. Other examples include the role of MEcPP in ER stress [69], or the presence of PAP in different subcellular compartments [70]. The exchange of these signaling molecules can even be further facilitated by the presence of membrane contact sites (MCS) between the ER and other organelles (Figure I) [71]. Altogether, it is clear that plants have evolved an intricate inter-organelle signaling network to respond to stress.

BOX 2: Basic UPR machinery in plants

The core UPR machinery has been mainly characterized in arabidopsis. It relies on three transcription factors belonging to the bZIP family and consists of two main branches (Figure I). The first is the most conserved in eukaryotes and is regulated

by IRE1. This transmembrane protein contains an ER-luminal protein-protein interaction domain and a cytosolic tail with kinase and RNase domains. In response to ER stress, IRE1 homodimerizes and trans-autophosphorylates its kinase domain [72]. The resulting conformational change activates the RNase domain that subsequently catalyzes unconventional splicing of bZIP60 in a process termed regulated IRE-dependent splicing (RIDS). This causes a frameshift removing the ER anchor, which allows translocation of the activated bZIP60 to the nucleus, inducing the expression of ER stress-responsive genes [8,73]. IRE1 also engages in cleavage and bulk degradation of specific mRNAs during regulated IRE-dependent decay (RIDD). This process might relieve ER stress by degrading mRNAs encoding ER-resident proteins, thereby decreasing the protein folding load [74]. Alternatively, RIDD can guide cells toward autophagy by eliminating mRNAs encoding negative regulators of this process [75].

The main players of the second UPR branch are the bZIP17 and bZIP28 transcription factors (Figure I). These transmembrane proteins contain a cytosolic N-terminal part harboring a transcription factor domain and a C-terminal part residing in the ER lumen. Under unstressed conditions, bZIP28 is retained in the ER due to binding of its C-terminal domain to the ER chaperone binding protein (BiP). Upon perceiving ER stress, BiP binds to unfolded proteins to prevent their aggregation, causing bZIP28 dissociation and translocation to the Golgi [76,77]. Here, regulated intermembrane proteolysis by proteases releases the active bZIP28 transcription factor domain into the cytosol, enabling its nuclear translocation [78]. Although the activation mechanism of bZIP17 might be similar,

404 the interacting protein responsible for its retention in the ER under non-stressed
405 conditions is currently unknown [12].

406 In the nucleus, bZIP28 and bZIP60 bind to conserved ER stress response element
407 (ERSE) and unfolded protein response element (UPRE) *cis*-regulatory motifs in the
408 promoter region of ER stress-responsive genes to regulate their expression [8]. For
409 a comprehensive overview of the UPR machinery in plants and its comparison to
410 that in other eukaryotes, readers are referred to Pastor-Cantizano *et al.* (2020) [12].

411 **BOX 3: Ethylene biosynthesis and signaling in *Arabidopsis***

412 Ethylene biosynthesis is characterized by a two-step reaction situated in the cytosol
413 (Figure I) [79,80]. First, S-adenosyl-methionine (SAM) is converted to ACC by ACC
414 synthases (ACS). Being a soluble precursor, ACC is often applied to probe ethylene
415 responses in *in vitro* studies. Subsequently ACC is converted to ETH in an oxygen-
416 dependent reaction catalyzed by ACC oxidases (ACO). Both intracellular levels of
417 ACC and ETH are tightly controlled via a plethora of transcriptional and post-
418 translational mechanisms[79]. Expression of *ACS* can be promoted by a broad
419 range of stress stimuli [81]. Furthermore, various post-translational control
420 mechanisms modulate ETH biosynthesis by altering ACS enzyme stability and/or
421 activity [79]. For instance, phosphorylation of type I ACS isozymes (e.g. ACS2/6)
422 by MAPKs is responsible for a rapid burst of ETH synthesis by stabilization of ACSs
423 in response to (a)biotic stress, bypassing the need for transcriptional changes
424 (Figure I) [29]. It should be mentioned that ACC homeostasis is also guided by its
425 conjugation to malonyl-, γ-glutamyl- and jasmonyl-derivatives, degradation through
426 deamination, and by local and systemic ACC transport through specific carriers, all

of which add further layers of complexity, fine-tuning ETH metabolism [82]. Ethylene is perceived at the ER membrane by a family of five receptors, including ETR1 (Figure I), ETR2, ETHYLENE RESPONSE SENSOR 1 (ERS1), ERS2 and ETHYLENE INSENSITIVE 4 (EIN4) [83]. The Raf-like kinase, CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) forms a complex with these receptors, which inactivates downstream signaling in the absence of the hormone [84]. Upon ETH binding, a conformational change in the receptors inactivates CTR1, promoting the proteolytic cleavage of the C-terminal end of the central signal transducer EIN2 [85], which subsequently migrates to the nucleus where it stimulates the accumulation of the major transcription factors EIN3 and EIN3-LIKE 1 (EIL1; Figure I) [86]. Both EIN2 and EIN3/EIL1 levels are targeted by the F-box proteins EIN2-TARGETING PROTEIN 1 (ETP1) and ETP2 [87] and EIN3 BINDING F-BOX 1 (EBF1) and EBF2 [86], respectively, for degradation by the 26S proteasome, adding another layer of control to the signaling pathway. One class of primary ETH response genes that contain EIN3 binding sites (EBS) in their promoters, are the APETALA2/ETHYLENE RESPONSIVE FACTORs (AP2/ERFs), a large family of transcription factors that mediate a plethora of defense responses (Figure I) [88]. Several studies report on additional signaling routes, such as the controversial, CTR1-dependent, MKK9-MPK3/MPK6 pathway [89] that need further scrutiny.

Glossary

Anterograde signaling: signaling route in eukaryotes that mediates nucleus-to-organelle communication. Nuclear-encoded proteins that function in organelles and affect the expression of organellar genes are called anterograde signals. These include, but are not limited to, signals involved in the regulation of plastid

transcription, such as sigma factors (SIGs) and pentatricopeptide repeat (PPR) proteins, and regulators of protein-protein interactions, such as tetratricopeptide repeat (TPR) proteins.

Autophagy: recycling mechanism in eukaryotes in which cellular components are transported to vacuoles and lysosomes for subsequent degradation. It is an essential part of cellular metabolism, providing energy and recycling cellular components for cell renewal. In non-stressed conditions, autophagy is essential for cellular homeostasis. In addition, it is often stimulated by stress, e.g. upon nutrient starvation.

ERAD: endoplasmic reticulum-associated degradation is a process integral to ER quality control assisting in the maintenance of proteostasis. ERAD comprises multiple steps that translocate misfolded proteins from the ER to the cytosol and target them for proteasome-assisted degradation.

Ethylene: volatile 2-carbon atom molecule classified as one of the traditional plant hormones. Ethylene regulates a plethora of developmental and physiological processes, including vegetative growth, fruit ripening, leaf and flower senescence and abscission, and is important in response to certain biotic and most abiotic stresses.

Oxidative protein folding: ER-localized process of disulfide bond formation, essential for optimal protein folding and stability, which depends on electron transfer by the ER oxidoreductase - protein disulfide isomerase (ERO-PDI) system, generating hydrogen peroxide.

Oxidative stress: imbalance between reactive oxygen species (ROS) and antioxidants in favor of the former, which imposes cellular stress by damaging organelles and important biomolecules such as proteins, lipids, DNA and carbohydrates.

Programmed cell death: process that is an integral part of cell physiology. It consists of an active mechanism initiating cellular death, as part of the developmental program under physiological conditions, or in response to stress, to avoid broad tissue or organ damage.

Proteostasis: cellular protein homeostasis associated with healthy steady-state levels of functional proteins. Proteostasis is the result of protein biogenesis, folding and degradation, and is essential to sustain cellular processes.

Proteotoxic stress: type of cellular stress consequent to an accumulation of un- or misfolded proteins, ultimately leading to protein dysfunction and disruption of metabolic processes.

Reactive oxygen species: reactive chemical species produced upon electron transfer to oxygen (superoxide, hydrogen peroxide and hydroxyl radicals) or upon excitation energy transfer to oxygen (singlet oxygen). They are able to damage cellular macromolecules, but also serve as important signals during stress adaptation.

Retrograde signaling: signaling route in eukaryotes that mediates organelle-to-nucleus communication. Retrograde signals are produced in the organelle and relay information to the nucleus via various pathways, ultimately affecting nuclear gene expression.

Sinks: tissues or organs including growing vegetative (e.g. young leaves) and reproductive tissues that utilize carbohydrates supplied from source tissues; thus at least in part fueled by sugars exported from sources.

Sources: tissues or organs including mature photosynthetic leaves and storage organs, from which carbohydrates are mobilized to sink tissues.

Sugars: generic term for any disaccharide or monosaccharide used by organisms to store energy. In addition to their key role in metabolism, soluble sugars regulate a plethora of physiological and developmental processes.

Acknowledgements

DVDS and AC gratefully acknowledge the Research Foundation Flanders (FWO project G032717N and G0C7518N respectively) and the Special Research Fund (BOF) of Ghent and Hasselt University for financial support. All images were created with BioRender.com

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Figure legends

Figure I (Box 1): Simplified overview of the inter-organelle stress response. Various external stimuli can induce a stress response which is both sensed and transduced in different organelles, including the ER, mitochondria and chloroplasts. A network of retrograde signaling pathways (blue arrows), transduced by signaling molecules including ROS, MEcPP, PAP, GUN1 and yet undiscovered players, is responsible for the appropriate nuclear expression of stress response genes. Subsequent anterograde signals (red arrows) function in the restoration of organellar and cellular homeostasis. In the ER, stress leads to a distinct signaling pathway called UPR, which is required for the expression of genes that restore ER proteostasis. Apart from communication with the nucleus, inter-organelle communication also occurs, mediated for instance by MEcPP or by the mitochondrial stress-induced cleavage of the ER-localized ANAC017 transcription factor. Black stars represent the presence of membrane contact sites that facilitate inter-organelle exchange of compounds between ER and mitochondria, chloroplasts, or the cell membrane. Putative signaling routes (Box 1) are depicted with dashed arrows. Abbreviations: *AOX1a*, *ALTERNATIVE OXIDASE 1a*; GUN1, GENOMES UNCOUPLED 1; MEcPP, methylerythritol cyclodiphosphate; PAP, 3′-phosphoadenosine 5′-phosphate; ROS, reactive oxygen species; UPR, unfolded protein response.

Figure I (Box 2): Simplified overview of the basic UPR machinery in plants. The plant UPR machinery consists of two main branches. The first depends on IRE1, which homodimerizes and autophosphorylates in response to ER stress. Subsequently, IRE1 mediates the cytosolic splicing of the transcription factor

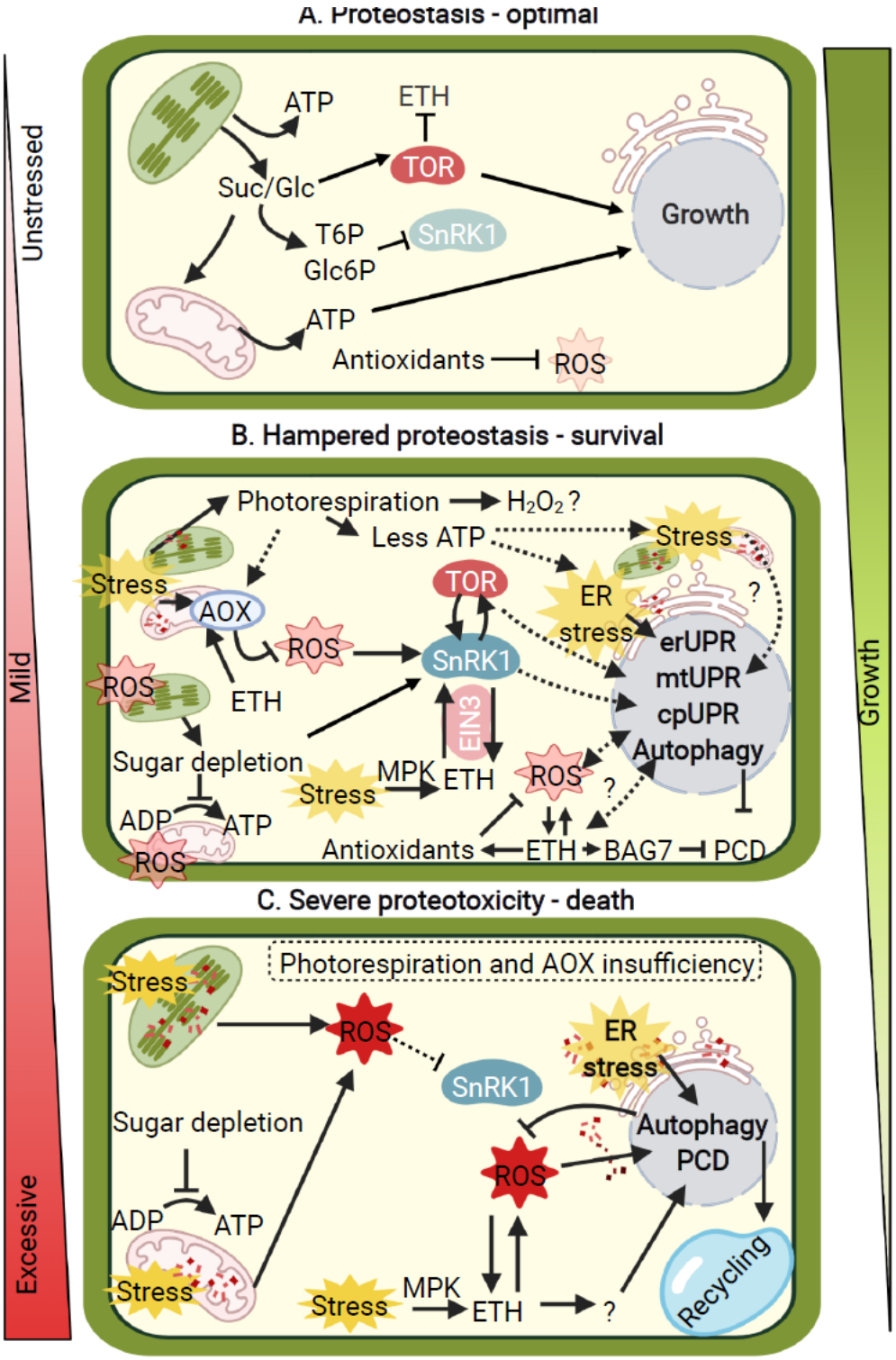
bZIP60, causing the removal of its ER anchor, enabling its translocation to the nucleus. The central players of the second plant UPR branch are the bZIP17 and bZIP28 transcription factors. Under non-stressed conditions, bZIP28 is retained in the ER through binding to the ER chaperone BiP. In case of ER stress, BiP binds to unfolded or misfolded proteins to prevent their aggregation, thereby releasing bZIP28. The latter moves to the Golgi, where it is cleaved by a set of proteases. Its transcription factor domain subsequently translocates to the nucleus. Although a similar mechanism is likely responsible for bZIP17 activation, the interacting protein governing its retention in the ER is still unknown. Inside the nucleus, bZIP17, bZIP28 and bZIP60 regulate the expression of their target genes through binding to conserved *cis*-regulatory motifs in their promoter region. Abbreviations: BiP, BINDING PROTEIN; bZIP, BASIC ZIPPER LEUCINE; bZIP60u, unspliced *bZIP60* mRNA; bZIP60s, unconventionally spliced *bZIP60* mRNA; ER, endoplasmic reticulum; IRE1, INOSITOL-REQUIRING ENZYME 1; UPR, unfolded protein response.

Figure I (Box 3): Simplified overview of ethylene biosynthesis and signaling. ETH is synthesized in the cytosol in a two-step reaction. SAM is converted to ACC by ACS. The second enzyme, ACO, converts ACC to ETH. Various stressors are known to stimulate *ACS* and *ACO* expression and stability. MPK3 and MPK6 enhance ACS stability upon stress. Additionally, ACC can be conjugated to MACC, GACC, or JA-ACC. In the absence of ETH, the ER-localized ethylene receptors (only ETR1 shown here) block signaling through their interaction with CTR1 (see inset), which inactivates the positive regulator EIN2 via phosphorylation of its C-END, blocking signal transduction. Upon ETH binding, the receptors and CTR1 are

inactivated. The dephosphorylated cytosolic EIN2 C-END is cleaved off and translocated to the nucleus, promoting the accumulation of transcription factors EIN3 and EIL1. The latter bind to EIN3 binding sites (EBS) of ETH response genes, including the AP2/ERF transcription factor family, triggering multiple responses downstream. Negative feedback occurs at the level of EIN2, via ETP1 and ETP2, and EIN3/EIL1, via EBF1 and EBF2. CTR1 inactivation is also proposed to stimulate the MKK9-MPK3/MPK6 signaling cascade (dashed arrows) in parallel to EIN2, activating EIN3/EIL1 as well. Arrow-headed lines represent stimulatory interactions; bar-headed lines indicate inhibitory interactions. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; AP2/ERF, APETALA2/ETHYLENE RESPONSE FACTOR; C-END, C-terminal end; CTR, CONSTITUTIVE TRIPLE RESPONSE; EBF, EIN3-BINDING F-BOX; EIL, EIN3-LIKE; EIN, ETHYLENE INSENSITIVE; ETH, ethylene; ETP, EIN2-TARGETING PROTEIN; ETR, ETHYLENE RESPONSE; GACC, γ-glutamyl-ACC; JA-ACC, jasmonyl-ACC; MACC, malonyl-ACC; MKK, MITOGEN-ACTIVATED PROTEIN KINASE KINASE; MPK, MITOGEN-ACTIVATED PROTEIN KINASE; SAM, S-adenosylmethionine.

Key Figure/Figure 1. ROS-ETH-sugar interplay at different levels of stress. In unstressed conditions (A), plastidial and mitochondrial metabolism provide sugars and ATP, inhibiting SnRK1 and stimulating growth. At high intracellular glucose levels, TOR is activated and ETH signaling inhibited. These conditions sustain proteostasis, concomitant with limited ER stress. Upon mild stress (B), the balance between protein folding capacity and demand is disturbed. Accumulation of ROS in organelles is stimulated, affecting their functioning, and causing sugar and ATP

deprivation. This results in damage to proteins and other cellular components and induces the UPR gene expression. Elevated ROS and low sugar levels activate SnRK1, promoting catabolism. Stress-generated ETH, mediated by MPK3/6, regulates ROS levels, retaining them at signaling doses, and interacts with SnRK1 through EIN3. The triad of interactions, likely converging through SnRK1, supports restoration of proteostasis in all subcellular compartments, by promoting UPR. This includes autophagy to recycle cellular components, provide energy and remove excess ROS, preventing PCD. Abiotic stress responses also rely on balanced SnRK/TOR signaling. Hence, a putative role for TOR in the regulation of UPR is conceivable. An indirect role of photorespiration and AOX in UPR is proposed, through limitation of ROS accumulation and depletion of ATP. Hydrogen peroxide production during photorespiration could act as a signal in UPR. Under excessive stress (C), cells are unable to regulate ROS levels, causing damage to organelles. Eventually UPR is unable to cope with excessive misfolded proteins. Autophagy is further stimulated. As a last resort, the cell enters PCD, mediated by ROS-ETH crosstalk. The scheme focuses on stresses that induce sugar starvation and sugar signalling in sink tissues. Full lines: established interactions. Dashed lines: hypothetical interactions. Arrows: stimulatory interactions. Bar-headed lines: inhibitory interactions. Abbreviations: AOX, ALTERNATIVE OXIDASE; EIN, ETHYLENE INSENSITIVE; Glc, glucose; Glc6P, glucose-6-phosphate; MPK, MITOGEN-ACTIVATED PROTEIN KINASE; SnRK1, sucrose-non-fermenting-related protein kinase 1; Suc, sucrose; TOR, Target of rapamycin; T6P, trehalose-6-phosphate; UPR, unfolded protein response.



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831 Figure 1

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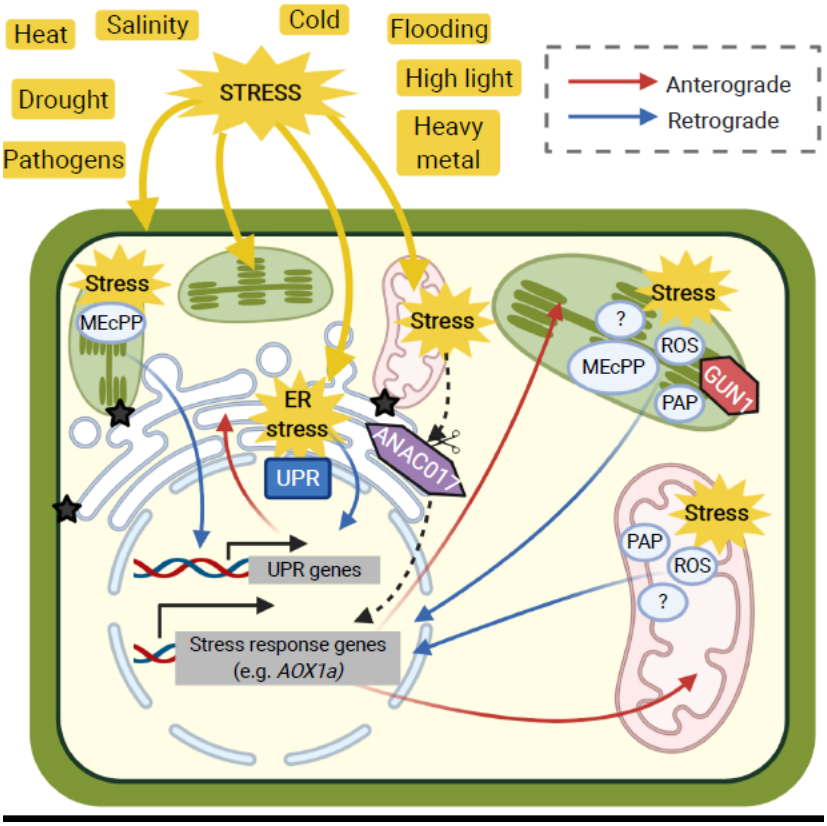

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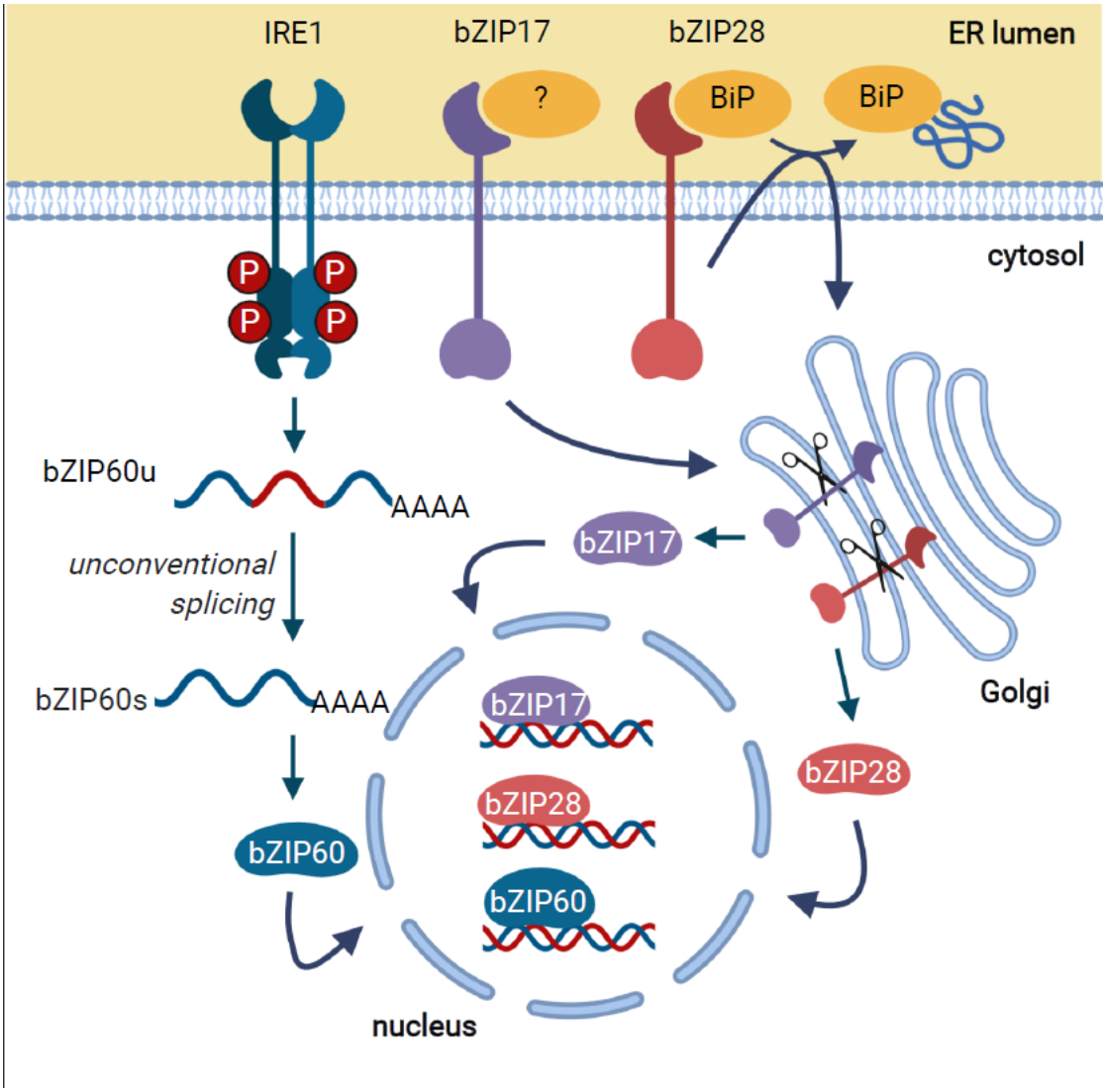

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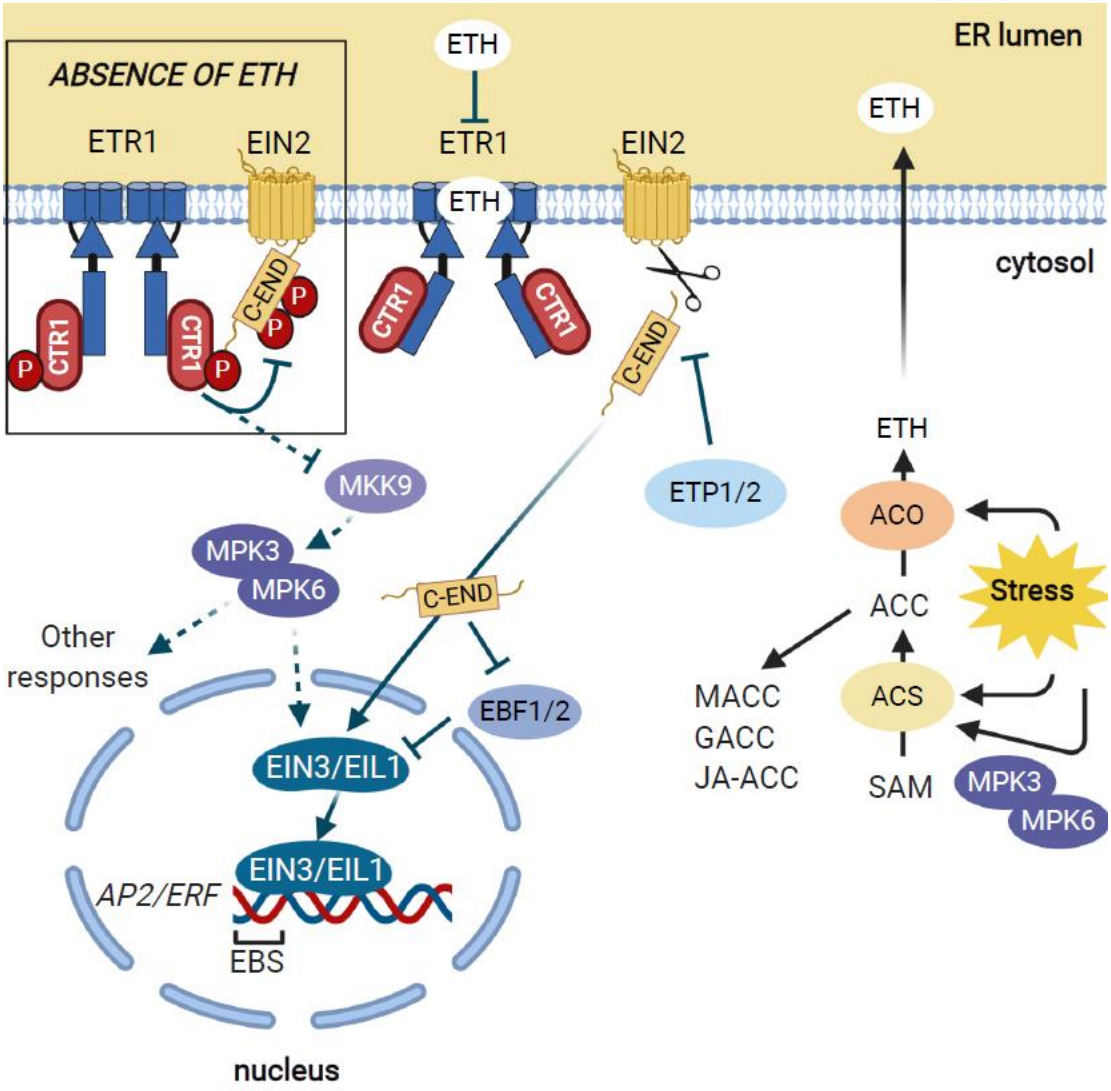
Box 3


ABSENCE OF ETH
ETR1
EIN2
CTR1
C-END
P
ETH
ER lumen
cytosol
MKK9
MPK3
MPK6
Other
responses
C-END
ETP1/2
EBF1/2
EIN3/EIL1
AP2/ERF
EBS
nucleus
ACO
ACC
ACS
SAM
MACC
GACC
JA-ACC
Stress