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UPR signaling at the nexus of plant viral, bacterial, and fungal defenses

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In recent years there have been significant advances in our understanding of the ER stress responses in plants that are associated with virus infection, as well as bacterial and fungal diseases. In plants, ER stress induced by virus infection includes several signaling pathways that include the unfolded protein response (UPR) to promote the expression of chaperone proteins for proper protein folding. Understanding how facets of ER stress signaling broadly engage in pathogen responses, as well as those that are specific to virus infection is important to distinguishing features essential for broad cellular defenses and processes that may be specifically linked to viral infectivity and disease.

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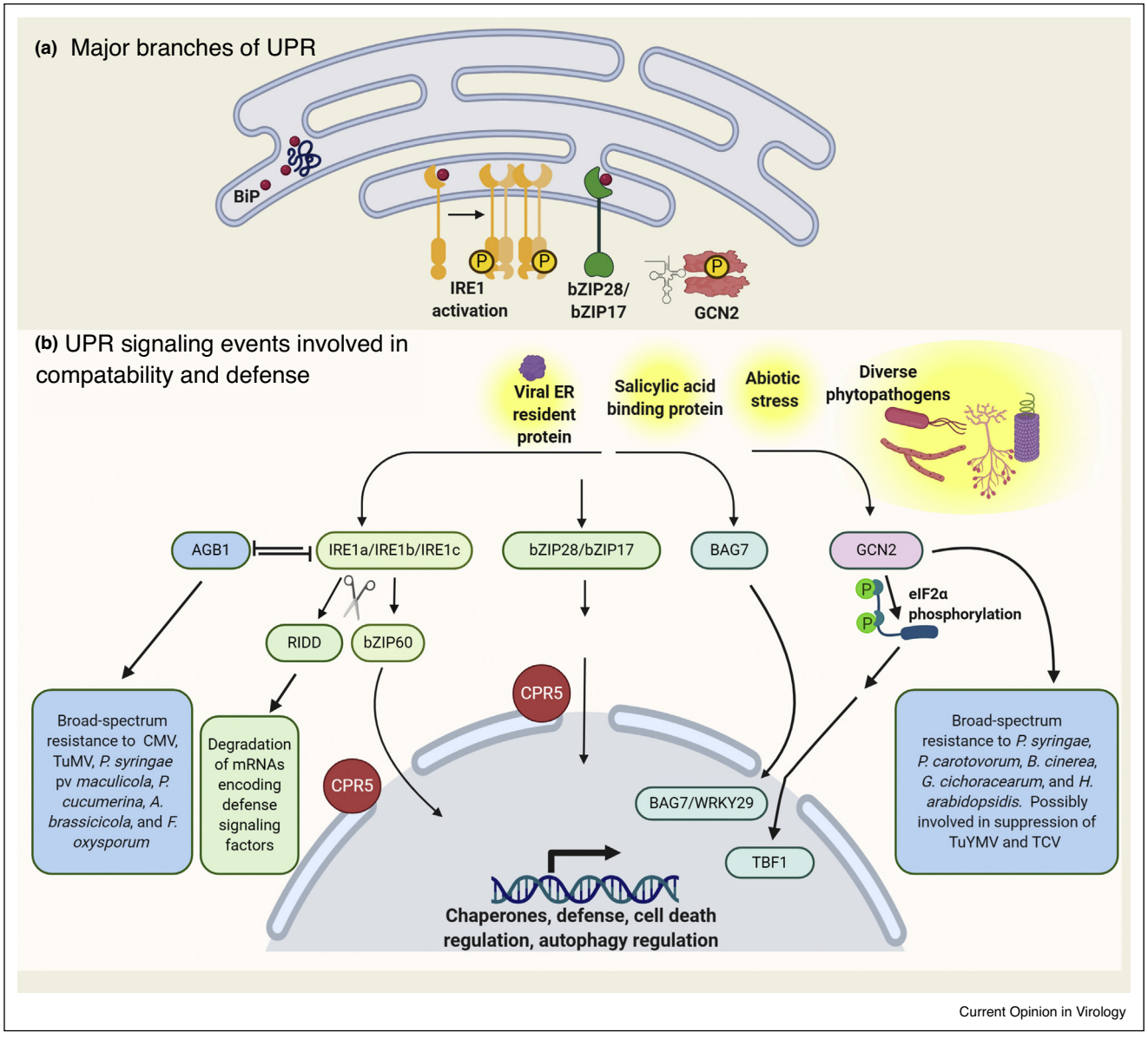
Introduction

Positive strand RNA viruses are among the largest group of viruses infecting plants and animals and contribute to some of the most critical issues in agriculture. These viruses typically create membrane-bound environments to protect replication and assembly complexes from cellular defenses. Especially for viruses that assemble complexes along the endoplasmic reticulum (ER), cellular membrane and protein synthesis become enhanced to expand the capacity of the ER to meet the needs of virus gene expression, replication, and cell-to-cell movement. Therefore, protein sensors along the ER recognize such profound changes in the functioning of the ER and activate stress responses, including the unfolded protein response (UPR) [1]. ER-resident chaperones and transmembrane transcription factors are crucial to sensing changes in the ER and contribute to adaptive changes in the cell that allows for invading pathogens [2–4].

The UPR consists of three ER stress sensors regulating separate but intertwined signaling cascades leading to the expression of ER-resident chaperones (Figure 1). In mammals, these proximal sensors include the activating transcription factor 6 (ATF6) which is a membrane-bound bZIP transcription factor; the inositol requiring enzyme 1 (IRE1, α and β isoform) which is a type 1 transmembrane protein kinase/endoribonuclease; and a group of four kinases that mediate phosphorylation of eukaryotic translation initiation factor 2α (elF2α), namely GCN2 (General Control Non-repressible 2); PERK (RNA dependent Protein Kinase like ER kinase, also known as EIF2AK4); HRI (Heme Regulated Inhibitor); and PKR (Protein Kinase R). The ER-resident sensors in plants include two transcription factors bZIP17 and bZIP28 that functionally resemble the mammalian ATF6 and three IRE1 homologs, IRE1a, IRE1b, and IRE1c [5,6,7,8••]. In plants, only one orthologue of the elF2α kinase-led branch has been identified, which is a single copy of GCN2 kinase [9]. In addition to regulating the capacity of the ER to restore misfolded proteins, the UPR associates with major cellular activities involved in innate immunity, cell death, and autophagy [5,7].

Biotrophic bacterial and fungal pathogens also induce changes in the plant ER and Golgi networks resulting in increased synthesis of host proteins and lipids acting at the plant-biotroph interface to accommodate as well as restrict microbial proliferation [10,11]. There are additional ER-resident factors that are known to be involved in cell death regulation, autophagy, and calcium signals, such as Bax inhibitor-1 (BI-1), B-cell lymphoma-2 (Bcl-2) associated athanogene 7 (BAG7), NAC089, NAC103, GAAP1, and GAAP3. Their activities appear to coordinate with UPR responses creating a complex network of molecular interactions that can either benefit viruses or certain bacterial and fungal infections to achieve compatibility, or support plant defense responses and innate immunity [5,12,13••,14,15]. Studies involving potato virus X (PVX), potato virus Y (PVY), plantago asiatica mosaic virus (PlAMV), and turnip mosaic virus (TuMV) have uncovered how the ER and UPR machinery creates an environment that is restrictive to infection but also suppresses oxidative stress and cell death [14,16]. Similarly, studies in plants involving *Pseudomonas syringae*, *Piriformospora indica*, *Alternaria alternata*, and *Phytophthora sojae* demonstrate the UPR machinery

Figure 1



(a) Model depiction of the IRE1, bZIP28/bZIP17, and GCN2 led branches of the UPR. BiP is a molecular chaperone required for folding proteins and also binds IRE1 or bZIP28 monomers in the ER lumen. Phosphorylation controls the activation of IRE1 and GCN2.
(b) Model demonstrates the UPR signaling pathways are involved that respond to viral proteins, abiotic stress, SA binding proteins, and several other bacterial and fungal pathogens. The major branches are regulated by AGB1 and BAG7 that are known to activate cellular defenses involved in broad-spectrum resistance to pathogen infection. GCN2 regulates eIF2α phosphorylation associated with broad-spectrum resistance to bacterial and fungal pathogens. Its role in viral pathogenesis is not known. The IRE1 leads two divergent pathways that control the bulk degradation of cellular mRNAs (RIDD) or activate the bZIP60 transcription factor. The bZIP60, bZIP28, and bZIP17 separately respond to ER stress but coordinate in the nucleus to activate the expression of cellular chaperones and cell fate-determining genes. BAG7 transfers into the nucleus to function as a cofactor of the WRKY29 transcription factor. The GCN2 pathway activates TBF1. Both WRKY29 and TBF1 regulate the expression of defense genes.

is vital for host defenses and the establishment of mutualistic interactions [11,17,18,19••,21]. Here, we will discuss the current understanding of the mechanisms underlying molecular plant-pathogen interactions involving ER stress and UPR. We will explore how plant viruses engage with the UPR machinery in ways that are similar or different from bacterial and fungal pathogens.

IRE1-bZIP60 pathway modulates plant virus infection

Mammals and plants have two or three IRE1 isoforms, respectively, whereas yeast has a single gene. In mammals and yeast, the ER-resident chaperone immunoglobulin binding protein BiP (also known as GRP78) binds to the N-terminal ER lumen domain (NLD) and interferes with their dimerization. During ER stress, BiP releases and IRE1s dimerize, resulting in the activation of the cytosolic IRE1 kinase domain and endoribonuclease activity for UPR (Figure 1a, see below for details). Regarding the Arabidopsis isoforms IRE1a and IRE1b, no binding partner has been identified experimentally for the NLD region. The IRE1c isoform lacks the ER lumen domain but cooperates with IRE1b in ER stress sensing for growth and development [8••]. Its role in pathogen responses is unknown at this time. In Arabidopsis, the endoribonuclease activity catalyzes the splicing of a 23-nt segment of the *bZIP60* mRNA to produce a functional transcription factor. The IRE1-bZIP60 pathway activates the expression of genes involved in managing ER stress, UPR essential chaperones for protein folding, cell fate determination, and innate immunity (Figure 1).

The IRE1-bZIP60 pathway is known to be supportive and restrictive of virus infection [14]. For example, the TRV vector was used to deliver *bZIP60* transcript fragments for *bZIP60*-silencing in *N. benthamiana* plants, and these silenced plants were then inoculated with an infectious clone of PVX containing the green fluorescent protein (GFP). There were fewer PVX-GFP infection foci on the inoculated leaves and the virus spread more slowly to the upper leaves compared to wild-type plants. It was not clear from these studies if reduced *bZIP60* expression specifically compromised virus replication, genome expression, or cell-to-cell movement. We were also concerned that possible interactions between PVX and TRV, or TRV and host cellular interactions could have obscured the results that would be achieved by PVX alone in a *bZIP60*-knockout background. Therefore, to better understand the role of the IRE1-bZIP60 pathway we used Arabidopsis knockout mutations disrupting *IRE1* and *bZIP60* genes [14,16,22] to inoculate with a related potexvirus, PlAMV, and the potyvirus, TuMV, for which Arabidopsis serves as a host [14,16,22]. Experiments showed that *bZIP60* mRNA splicing occurs within three days following inoculation with PlAMV or TuMV, or following expression of the potexvirus TGB3 and potyvirus 6K2 proteins in wild-type Arabidopsis. In the study by Zhang *et al.*, T-DNA mutant lines of Arabidopsis known as *bzip60-1* and *bzip60-2* which produce 5' or 3' terminal truncated *bZIP60* transcripts had different effects on TuMV infection [22]. At 18 days post-inoculation (dpi), TuMV RNA levels were lower in systemically infected tissues of *bzip60-2* plants than *bzip60-1* plants. TuMV infection also resulted in fewer and shorter stems above the rosette of basal leaves in the *bzip60-1* plants than in *bzip60-2* inoculated with TuMV and in buffer treated plants, suggesting that *bzip60-2* had less severe disease [22]. While these studies linked *AtbZIP60* to virus pathogenesis, they did not provide an in-depth analysis of the effects of the IRE1-bZIP60 pathway on virus accumulation in the inoculated and systemic leaves. Also, the Arabidopsis plants were flowering at 18 dpi that is often associated with a decline virus titer. To follow up on these investigations, Gaguancela *et al.* used GFP-tagged TuMV and PlAMV to track the pattern of virus movement in *ire1a-2*, *ire1b-4*, *ire1a-2/ire1b-4*, and *bzip60-2* mutant plants using a time-course study [16]. In this study TuMV-GFP accumulation was higher in the inoculated leaves of *ire1a-2*, *ire1b-4*, *ire1a-2/ire1b-4*, and *bzip60-2* mutant plants than in wild-type inoculated leaves. We used GFP to track the systemic spread, and TuMV-GFP reached higher levels in the upper leaves of *ire1a-2/ire1b-4* mutant plants than in *ire1a-2* or *ire1b-4* suggesting there is some functional redundancy in how they regulate phloem transport of TuMV-GFP. In parallel experiments, PlAMV-GFP reached higher levels in the *ire1a-2* or *ire1a-2/ire1b-4* knockout lines than in *ire1b-4* and wild-type Arabidopsis plants. Immunoblot analysis also reported higher levels of PlAMV coat protein in *ire1a-2* and *ire1a-2/ire1b-4* knockout lines than in *ire1b-4* and wild-type Arabidopsis. In this case, IRE1a and IRE1b were not functionally overlapping in how they regulated the PlAMV-GFP movement through the phloem. These separate observations suggest that the IRE1-bZIP60 signaling network includes activities that promote or suppress infection [16,22]. The IRE1a-led and IRE1b-led pathways seem to differently recognize these unrelated viral proteins, which may have different outcomes affecting virus replication, cell-to-cell movement, or systemic movement through the phloem.

The expression of another ancient and evolutionarily conserved UPR player, *AtBI-1* (Arabidopsis BCL2-Associated X (BAX) Inhibitor-1; see details below), was elevated and dependent upon IRE1a/IRE1b in leaves expressing the TuMV 6K2 or PVY 6K2 proteins; however, *AtBI-1* expression was independent of IRE1 in leaves expressing PlAMV TGB3 or PVX TGB3 [11]. These viral TGB3 and 6K2 proteins induced *bZIP60* mRNA splicing suggesting that regulation of *AtBI-1* expression may depend upon additional factors that combine with *bZIP60*. In *atbi-1* Arabidopsis plants, or *bZIP60*-silenced and *BI-1*-silenced *N. benthamiana*, potyvirus and potexvirus accumulation were higher in locally inoculated and systemic leaves. The effects of AtBI-1 were far greater than bZIP60 suggesting that bZIP60 may be acting in concert with other transcription factors to regulate infection [16]. This explanation could also account for the seemingly contrasting effects of bZIP60 silencing and genetic mutations on various virus infections [16].

Regulated IRE1-dependent decay of mRNA (RIDD) in plant immunity

IRE1 catalyzes the endonucleolytic cleavage and bulk degradation of specific mRNAs in a negative-regulation

process called Regulated IRE1-Dependent Decay (RIDD; Figure 1b) [23]. The mammalian XBP1 is the orthologue of plant bZIP60, and RIDD cleavage occurs at an XBP1-like consensus site but with an activity divergent from RIDD-based XBP1 mRNA splicing [24]. While the exact nature and roles of the RIDD-cleaved mRNAs are not completely understood, research shows that RIDD is integral to the molecular signaling during IRE1-mediated cellular transitions between pro-survival and pro-death programs [25].

While the cell is in the pro-survival state, IRE1 degrades mRNAs encoding ER-resident proteins leading to a decrease in the protein folding load in the ER. The RIDD targets predominantly exhibit ER-membrane associated localization and transitory functions. Researchers have speculated that in mammals, viral RNAs may be RIDD targets as a host defense strategy. As a counter-defense, the viruses may evolve cleavage resistance [26,27]. In addition, Japanese encephalitis virus (JEV) infection hijacks and triggers the RIDD pathway which exerts a pro-viral outcome by enabling higher viral protein production and higher virus titers [28••]. Regardless of the final infection outcomes, all of these scenarios require further investigation. XBP1 is a strong inhibitor of the RIDD mechanism and consequently, some immune cells, such as cross-presenting lung DCs and NK cells, spontaneously turn on IRE1 to inhibit viral-induced RIDD [29]. Whether an analogous mechanism involving bZIP60 exists in plants remains to be explored. In sum, modulating the host ER stress response to decrease viral replication could constitute a promising strategy to combat infection.

The RIDD pathway is a profoundly understudied area in plant UPR with only two reports documented to date. A recent study in Arabidopsis shed light on plant RIDD mechanisms in response to two ER stress-inducing agents, tunicamycin (Tm) and dithiothreitol (DTT), as well as an abiotic stress factor heat [30•]. Interestingly, 49 immune-associated transcripts were identified as potential direct RIDD targets. Such targets include well-characterized immune-responsive genes against bacterial and fungal infections, such as peroxidase PRX34, EDS1, WRKY33, WRKY53, WRKY70, MLO4, several heat shock proteins, and heat shock factors, chitinases (including pathogenesis-related protein PR-4), and other PR proteins from the β-glucosidase and defensin families [30•]. In the second study, IRE1b RNase activity was required for tunicamycin-induced autophagy in Arabidopsis through RIDD-mediated degradation of mRNAs [31••,32]. This is particularly interesting considering a recent study showing that TuMV activates autophagy in a manner that depends upon the IRE1/bZIP60 pathway and promotes virus infection. While this study reports that bZIP60 is responsible for the upregulation of NBR1, which an autophagy cargo adaptor protein, TuMV co-opts the NBR1 and ATG8f to help direct the viral replication complex to the tonoplast membrane to create a protective environment for the viral replication machinery [33]. Given the evidence that IRE1b can also induce autophagy independent of bZIP60 through RIDD mediated degradation, TuMV may rely on both IRE1-mediated pathways to stimulate autophagy in a manner that benefits virus infection and reduces cell death.

Intriguingly, several RIDD targets are genes contribute to antiviral defenses such as Hsp70A, NDR1/HIN1-like protein 3 (NHL3), Argonaute 2 (AGO2), plasmodesmata-located protein 1 (PDLP1), and PR-4 [5,34]. The presence of immune-related mRNAs among the RIDD targets implies that plant IRE1 plays a role in altering infection outcomes by rewiring the immune signaling cascades and modifying the cellular abundance of PR proteins, an area of future investigation. Moreover, it would be useful to understand the crosstalk between IRE1-dependent splicing (RIDS) and RIDD during the pro-survival to pro-death transition and elucidate how diverse viruses and other pathogens interfere with this nexus for their benefit. Conversely, additional viral-host interaction RIDD studies will allow us to understand whether the plant hosts have evolved to degrade viral RNAs through RIDD.

bZIP60 along with bZIP28 and bZIP17 respond differently to potexvirus and potyvirus infection

Two ER transmembrane transcription factors, bZIP28 and bZIP17, represent an alternative branch of the UPR (Figure 1). The bZIP28 occurs as a complex in the ER with the Bcl-2 associated athanogene 7 (BAG7) and BiP. During heat, drought, or salt stress, these bZIP factors shuttle from the ER to the Golgi where the SITE-1 protease (S1P) and S2P remove their transmembrane domains to enable nuclear migration. Studies revealed that bZIP60, bZIP28, and bZIP17 form homodimers and heterodimers in the nucleus. BAG7 has a transmembrane domain that is also proteolytically removed before its transfer into the nucleus where it interacts with WRKY29 to induce transcription of cytoprotective stress-responsive genes. Gayral *et al.* [14] showed that the potexvirus TGB3 and potyvirus 6K2 induce expression of bZIP60, bZIP28, and bZIP17. The Arabidopsis *bzip17*, *bzip28*, *bzip60*, *bzip17bzip60*, *and bzip28bzip60* knockout lines were inoculated with PlAMV-GFP or TuMV-GFP. PlAMV-GFP infection was elevated in the inoculated and systemic leaves of *bzip17*, *bzip60*, and *bzip17bzip60* plants compared to *bzip28*, *bzip28bzip60*, or wild-type plants. These data suggest that virus infection was downregulated by bZIP17 and bZIP60. On the other hand, TuMV-GFP accumulated to higher levels in the inoculated and systemic leaves of *bzip28*, *bzip60*, *bzip28/bzip60*, and *bzip17/bzip60* knockout plants compared to *bzip17* and wild-type Arabidopsis plants [14]. Gayral *et al.* [14] proposed a

model suggesting that bZIP60 and bZIP28 overlap in their ability to activate genes that support PlAMV while bZIP17 and bZIP60 work in concert to suppress infection. Conversely, the bZIP28 and bZIP60 appear to restrict TuMV while bZIP17 does not seem to impact TuMV infection. Surprisingly, PlAMV-GFP, but not TuMV-GFP, is restricted in the inoculated leaves in a manner that requires BAG7. These data are interesting since BAG7 is a co-factor with WRKY29 engaged in pattern-triggered immunity, suggesting that TGB3-activation of UPR may contribute to the maintenance of antiviral immunity [13••,14].

The *bZIP17*, *bZIP28*, and *bZIP60* genes are attributed to activating expression of ER-resident protein chaperones and foldases that are central for UPR activity. But it is also reasonable to consider that there may be other genes that contribute to cell defenses whose expression is also impacted by these transcription factors and important for regulating virus infection. To understand if ER-resident protein chaperones and foldases are implicated in virus infection, leaves were treated with chemical UPR modulators DTT or tauroursodeoxycholic acid (TUDCA). DTT causes ER stress by reducing protein disulfide bonds and decreases the protein folding capacity of the cell whereas TUDCA alleviates ER stress by mitigating protein aggregation and stabilizes protein conformation. When plants were inoculated with PlAMV-GFP, we noted higher viral loads in the DTT-treated leaves but lower levels in TUDCA-treated leaves compared to untreated controls [14]. Although the ways that bZIP17, bZIP28, and bZIP60 cooperate to regulate potyvirus and potexvirus infection are not yet understood, they do serve to reinforce the levels of ER-resident chaperones contributing to cellular defenses that limit virus accumulation [14].

Diverse arms of UPR converge with innate immunity networks

Salicylic acid (SA) mediates antiviral defenses including systemic acquired resistance (SAR) [35–37]. Treating leaves with synthetic SA restricts systemic virus movement through the phloem [38]. There are approximately 30 SA-binding proteins in Arabidopsis, including the non-expresser of pathogenesis-related protein 1 (NPR1), which is a transcriptional co-factor activating the expression of *PR* genes essential for SAR [35]. SA signaling overlaps with several antiviral mechanisms including, RNA silencing, and influences the activation of RNA-dependent RNA polymerase 1 (RDR1) and AGO [38–40]. Moreover, while RDR1-mediated and AGO-mediated defenses can reduce infection by PVX, PVY, turnip crinkle virus (TCV), and tobacco mosaic virus (TMV), SA induces viral resistance mechanisms that are independent of the antiviral silencing pathways [38]. For example, alternative oxidase (AOX) is a mitochondrial enzyme that regulates SA-induced resistance to plant viruses such as cucumber mosaic virus (CMV), PVX, TCV, TMV, and tomato spotted wilt virus (TSWV), in an NPR1-independent manner [38].

SA treatment activates the IRE1/bZIP60 and bZIP28 arms of the UPR (Figure 1) in an NPR1-independent manner [41]. SA treatment leads to transcriptional activation of bZIP60 [41]. It is not known if any SA-binding proteins mediate the activation of the IRE1-bZIP60 and bZIP28 pathways or if IRE1 and bZIP28 directly bind SA. Some researchers speculated that SA elicits changes in the phospholipid composition of the ER membrane and that activates both pathways [42,43].

CPR5 is a crucial inhibitor of effector-triggered immunity that anchors to the nuclear pore complex (Figure 1) and modulates SA-dependent UPR signaling [44,45••,46]. *CPR5* associates with recessive *rym1* resistance to rice yellow mottle virus (RYMV) and *Arabidopsis cpr5* mutants show enhanced resistance to cauliflower mosaic virus, pepper mild mottle virus, and tobacco mild green mosaic virus [47,48]. Overexpression of CPR5 limits the nuclear entry of stress and defense-related proteins such as NPR1, JAZ1, ABI5 and, compromises effector-triggered resistance to bacterial pathogens. Double mutants *cpr5 bzip28* and *cpr5 bzip60*, as well as triple mutants *cpr5 bzip28 bzip60*, showed reduced transcript levels for BiP3 and the spliced *bZIP60* products indicating CPR5, is linked to UPR regulation through the IRE1-bZIP60 and bZIP28 arms [45••,49]. Experiments also showed that CPR5 interacts with bZIP60 and bZIP28, although it is not evident that CPR5 restricts nuclear entry of these bZIP factors [44]. Further research is needed to understand if CPR5 alters host susceptibility to infection through modulating effector-triggered immunity, SA-mediated defense, UPR signaling, or combinations of these pathways.

The Arabidopsis heterotrimeric G protein β subunit, AGB1, was also shown to trigger UPR-related cell death [50]. G proteins are eukaryotic GTP hydrolases that transduce signaling in response to biotic and abiotic stresses as well as developmental cues. Arabidopsis plants lacking functional AGB1 support higher loads of CMV and TuMV, which was further corroborated by the diminished spread of necrosis and reduced ion leakage [50]. In a study by Lee *et al.*, Arabidopsis *agb1-2* plants displayed enhanced disease susceptibility towards *Pseudomonas syringae* pv. *maculicola*, as well as defects in stomatal immunity in response to a non-host bacterial pathogen [51]. Although the underlying molecular mechanisms of AGB1's involvement in disease resistance are not yet fully understood, it was proposed that AGB1 operates in concert with other subunits in a heterotrimeric complex, where individual subunits might have more specialized roles in plant immunity [51]. Consistent with AGB1's broader role in immunity to diverse pathogens, two independent studies confirmed its positive contribution

towards defense against necrotrophic fungi including *Plectosphaerella cucumerina*, *Alternaria brassicicola*, and *Fusarium oxysporum* [11,20]. Intriguingly, genetic interaction studies determined an antagonistic relationship between IRE1 and AGB1 [52]. Future mechanistic studies will shed more light on the importance of AGB1 and its connection with UPR and plant immunity.

Although, as described above, the *bona fide* PERK ortholog is absent in plants and regulatory mechanisms of GCN2 activation are largely unknown, plant GCN2 can phosphorylate eIF2α under a wide range of abiotic, chemical, and pathogen treatments. This includes amino acid starvation, herbicide glyphosate [53], UV and cold stress, wounding, and salicylic acid (SA), as well as bacterial infection [54,55] indicative of the functional conservation of GCN2's role in UPR (Figure 1). AtGCN2-mediated phosphorylation of eIF2α leads to the translational derepression of TBF1 [56••,57], a transcription factor activating the expression of *TL1 cis*-regulatory motif-containing secretory genes including *BiP2*, *CNX1*, *CNX2*, *PDI*, *DAD1*, *CRT1*, *CRT3*, etc. [57], similar to translational regulatory mechanisms of mammalian ATF4 and yeast GCN4.

Akin to AGB1, AtGCN2 has also been implicated in broad-spectrum disease resistance against bacteria *P. syringae* and *Pectobacterium carotovorum*, fungi *Golovinomyces cichoracearum* and *Botrytis cinerea*, and oomycete *Hyaloperonospora arabidopsidis* [58]. Recently, opposing roles of AtGCN2 in pre-and post-invasive immunity against *P. syringae* were uncovered that involve abscisic acid homeostasis and stomatal immunity [56••]. Although preliminary work indicated that *atgcn2* did not exhibit any phenotypic difference to turnip yellow mosaic virus and TCV [9], the plant virus-GCN2 nexus remains wide open. Given the importance of host translational machinery in viral replication [59], and the involvement of mammalian GCN2 in host responses to a variety of viruses, it is plausible that plant GCN2 homologs play crucial roles in viral immunity, an area of future study.

Viral-triggered or bacterial-triggered UPR modulates host cell death responses through BiP and AtBI-1

Few studies point to a dual role for BiP and AtBI-1 in modulating cell death responses across a variety of pathological conditions. Besides interacting with IRE1, BiP exhibits molecular chaperone activities, is involved in protein folding and maturation in the ER lumen, and is recognized as a cytoprotective factor required for normal cell physiology and support of host immunity [5,14,60,61••]. The Arabidopsis genome encodes three members of the BiP family, of which BiP1/2 are ubiquitously expressed proteins sharing 99% identity, whereas BiP3 exclusively expresses during ER stress conditions. The loss of function of *BiP2* combined with another secretory pathway mutation, such as *sec61α* or *dad1*, in Arabidopsis diminishes the secretion of pathogenesis-related-1 (PR-1) protein and establishment of SAR. Initial studies in *N. benthamiana* plants showed that the PVX TGB3, the garlic virus X (GarVX) P11, the P34 of lettuce infectious yellows virus (LIYV), and the P6 of citrus tristeza virus (CTV) are ER-resident proteins that cause visible necrosis on *N. benthamiana* leaves when they are overexpressed and activate NbbZIP60 leading to the upregulation of ER-resident foldases including BiP [60,62]. Aguilar *et al.* reported that PCD seen as the result of PVX and PVY synergism or in tissues co-expressing the PVX TGB1 and PVY HC-Pro proteins is likely due to collapse of the ER [63]. For each of these examples where viral protein interactions with the ER caused cell death, transient overexpression of BiP in the same tissues protects against PCD [64]. We hypothesize that overexpression of BiP attenuates the viral-induced UPR, and in turn, abrogates the induction of cell death-like symptoms indirectly. Indeed, soybean and tobacco transgenics plants overexpressing *BiP* genes exhibited downregulation of PCD-related transcriptome as well as marked delay in the onset of leaf senescence under normal physiological conditions. This negative regulation of PCD was attributed, at least in part, to the inhibition of UPR and cell death signaling pathways [65]. Consistent with its protein folding and negative cell death functions, overexpression of rice BiP3 significantly decreased the accumulation of a rice receptor-like kinase, XA21, and consequently compromised plant immunity triggered against a bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*. In contrast, overexpression of BiP exhibited accelerated hypersensitive response (HR; a hallmark of PCD) triggered by *P. syringae* pv. *tomato* in soybean and tobacco suggesting the positive contribution of BiP in promoting cell death [65,66]. Similarly, BiP expression is necessary for HRT-mediated hypersensitive response to Turnip crinkle virus infection [67].

The eukaryotic BI-1 also exhibits both pro-and anti-apoptotic properties under diverse ER-stress inducing conditions [66,68]. In animals, the dual roles of BI-1 were attributed to intensity and duration of UPR signaling, that is, under adaptive and prolonged/severe ER stress conditions, respectively (Figure 1). Plant BI-1 shares structural and functional similarity to the mammalian BI-1, although plant genomes lack the counterparts of BI-1 interacting proteins such as the BAX and Bcl2-related proteins [69], AtBI-1 was shown to be implicated in PCD in response to viral, bacterial, and fungal pathogens [16,66]. Consistently, *atbi-1* mutant and *N. benthamiana* plants silenced for *BI-1* differentially contributed to potyvirus-induced and potexvirus-induced necrosis, that was possibly linked to their modes for local and systematic spread [16]. In another instance, BI-1 was dual-function in TMV-*N. benthamiana* interactions [68]. While the underlying molecular mechanisms are unknown, both the BI-1-silenced and overexpressing *N. benthamiana* plants exhibited enhanced HR-like cell death

phenotypes in response to TMV infection. Similarly, ectopic overexpression of barley BI-1 resulted in both disease susceptibility and resistance to diverse fungal pathogens. While the frequency of HR-like cell death not reduced upon infection with *Blumeria graminis*, a biotrophic fungal pathogen, young seedlings overexpressing BI-1 were significantly more resistant to *Fusarium graminearum* [66,69,70]. These findings point to the fact that BI-1 operates as both a negative and positive regulator of cell death in response to pathogens with diverse lifestyles and at different plant developmental stages. Likewise, bacteria-induced cell death via *P. syringae* pv. *tomato* DC3000-AvrRpt2 strain was enhanced in *atbi-1* knockdown plants [71]. It is important to note that AtB-1 overexpression did not result in any differential cell death phenotype to this bacterial strain presumably due to AtBI-1-dependent activation of different arms as well as intensity or duration of UPR under viral or bacterial infection.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Tam AB, Roberts LS, Chandra V, Rivera IG, Nomura DK, Forbes DJ, Niwa M: **The UPR activator ATF6 responds to proteotoxic and lipotoxic stress by distinct mechanisms**. *Dev Cell* 2018, **46**:327-343.e7.

2. Liebrand TWH, Smit P, Abd-El-Haliem A, de Jonge R, Cordewener JHG, America AHP, Sklenar J, Jones AME, Robatzek S, Thomma BPHJ *et al.*: **Endoplasmic reticulum-quality control chaperones facilitate the biogenesis of Cf receptor-like proteins involved in pathogen resistance of tomato**. *Plant Physiol* 2012, **159**:1819-1833.

3. Sasvari Z, Alatriste Gonzalez P, Nagy PD: **Tombusvirus-yeast interactions identify conserved cell-intrinsic viral restriction factors**. *Front Plant Sci* 2014, **5**:1-14.

4. Kaido M, Abe K, Mine A, Hyodo K, Taniguchi T, Taniguchi H, Mise K, Okuno T: **GAPDH—a recruits a plant virus movement protein to cortical virus replication complexes to facilitate viral cell-to-cell movement**. *PLoS Pathog* 2014, **10**:e1004505.

5. Afrin T, Diwan D, Sahawneh K, Pajerowska-Mukhtar K: **Multilevel regulation of endoplasmic reticulum stress responses in plants: where old roads and new paths meet**. *J Exp Bot* 2020, **71**:1659-1667.

6. Deng Y, Srivastava R, Quilichini TD, Dong H, Bao Y, Horner HT, Howell SH: **IRE1, a component of the unfolded protein response signaling pathway, protects pollen development in Arabidopsis from heat stress**. *Plant J* 2016, **88**:193-204.

7. Bao Y, Howell SH: **The unfolded protein response supports plant development and defense as well as responses to abiotic stress**. *Front Plant Sci* 2017, **8**:1-6.

8. •• Pu Y, Ruberti C, Angelos ER, Brandizzi F: **AtIRE1C, an unconventional isoform of the UPR master regulator AtIRE1, is functionally associated with AtIRE1B in Arabidopsis gametogenesis**. *Plant Direct* 2019 http://dx.doi.org/10.1002/pld3.187.

In addition to the two *bona fide* IRE1 homologs, IRE1A and IRE1B, another IRE1 isoform designated as IRE1C was discovered. This new member possesses an intact and functional C-terminal domain but lacks the ER sensing luminal domain, indicating that IRE1C might operate as an ER stress coreceptor with roles in plant development and reproduction.

9. Zhang Y, Wang Y, Kanyuka K, Parry MAJ, Powers SJ, Halford NG: **GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2α in Arabidopsis**. *J Exp Bot* 2008 http://dx.doi.org/10.1093/jxb/ern169.

10. Breeze E, Vale V, McLellan H, Godiard L, Grant M, Frigerio L: **The plant endoplasmic reticulum is both receptive and responsive to pathogen effectors**. *bioRxiv* 2020.

11. Xu Z, Song N, Ma L, Wu J: **IRE1-bZIP60 pathway is required for *Nicotiana attenuata* resistance to fungal pathogen *Alternaria alternata***. *Front Plant Sci* 2019, **10**:1-10.

12. Guo K, Wang W, Fan W, Wang Z, Zhu M, Tang X, Wu W, Yang X, Shao X, Sun Y *et al.*: **Arabidopsis GAAP1 and GAAP3 modulate the unfolded protein response and the onset of cell death in response to ER stress**. *Front Plant Sci* 2018, **9**:1-16.

13. •• Li Y, Williams B, Dickman M: **(BAG7)-mediated heat tolerance requires translocation, sumoylation, and binding to WRKY29**. *New Phytol* 2017, **2**:695-705.

This paper links the ER-resident BAG7 protein to ER quality control pathways and to defense regulation through the WRKY29 transcription factor. This work links UPR regulation and transcriptional control of defense-related gene expression.

14. Gayral M, Arias Gaguancela O, Vasquez E, Herath V, Flores FJ, Dickman MB, Verchot J: **Multiple E.R.-to-nucleus stress signaling pathways are activated during *Plantago asiatica* mosaic virus and Turnip mosaic virus infection in *Arabidopsis thaliana***. *Plant J* 2020, **103**:1233-1245.

15. Krajewska M, Xu L, Xu W, Krajewski S, Kress CL, Cui J, Yang L, Irie F, Yamaguchi Y, Lipton SA *et al.*: **Endoplasmic reticulum protein BI-1 modulates unfolded protein response signaling and protects against stroke and traumatic brain injury**. *Brain Res* 2011, **1370**:227-237.

16. Gaguancela OA, Źuñiga LP, Arias AV, Halterman D, Flores FJ, Johansen IE, Wang A, Yamaji Y, Verchot J: **The IRE1/bZIP60 pathway and Bax inhibitor 1 suppress systemic accumulation of potyviruses and potexviruses in arabidopsis and *Nicotiana benthamiana* plants**. *Mol Plant-Microbe Interact* 2016, **29**.

17. Chakraborty R, Uddin S, Macoy DM, Park SO, Van Anh DT, Ryu GR, Kim YH, Lee JY, Cha JY, Kim WY *et al.*: **Inositol-requiring enzyme 1 (IRE1) plays for AvrRpt2-triggered immunity and RIN4 cleavage in Arabidopsis under endoplasmic reticulum (ER) stress**. *Plant Physiol Biochem* 2020, **156**:105-114.

18. Jing M, Wang Y: **Plant pathogens utilize effectors to hijack the host endoplasmic reticulum as part of their infection strategy**. *Engineering* 2020, **6**:500-504.

19. •• Jing M, Guo B, Li H, Yang B, Wang H, Kong G, Zhao Y, Xu H, Wang Y, Ye W *et al.*: **A *Phytophthora sojae* effector suppresses endoplasmic reticulum stress-mediated immunity by stabilizing plant binding immunoglobulin proteins**. *Nat Commun* 2016, **7**.

This study highlights the discovery of an important pathogen virulence factor PsAvh262 from *Phytophthora sojae* that directly interacts and manipulates the activities of BiP proteins, leading to the suppression of ER stress-triggered cell death and promoting pathogen infection.

20. Moreno AA, Mukhtar MS, Blanco F, Boatwright JL, Moreno I, Jordan MR, Chen Y, Brandizzi F, Dong X, Orellana A *et al.*: **IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses**. *PLoS One* 2012, **7**:e31944.

21. Tateda C, Ozaki R, Onodera Y, Takahashi Y, Yamaguchi K, Berberich T, Koizumi N, Kusano T: **NtbZIP60, an endoplasmic reticulum-localized transcription factor, plays a role in the defense response against bacterial pathogens in Nicotiana tabacum**. *J Plant Res* 2008, **121**:603-611.

22. Zhang L, Chen H, Brandizzi F, Verchot J, Wang A: **The UPR branch IRE1-bZIP60 in plants plays an essential role in viral infection and is complementary to the only UPR pathway in yeast**. *PLoS Genet* 2015, **11**:e1005164.

23. Coelho DS, Domingos PM: **Physiological roles of regulated Ire1 dependent decay**. *Front Genet* 2014 http://dx.doi.org/10.3389/fgene.2014.00076.

24. Maurel M, Chevet E, Tavernier J, Gerlo S: **Getting RIDD of RNA: IRE1 in cell fate regulation**. *Trends Biochem Sci* 2014 http://dx.doi.org/10.1016/j.tibs.2014.02.008.

25. Abdullah A, Ravanan P: **The unknown face of IRE1α – Beyond ER stress**. *Eur J Cell Biol* 2018, **97**:359-368.

26. Hollien J: **Evolution of the unfolded protein response**. *Biochim Biophys Acta - Mol Cell Res* 2013 http://dx.doi.org/10.1016/j.bbamcr.2013.01.016.

27. Bhattacharyya S: **Can't RIDD off viruses**. *Front Microbiol* 2014 http://dx.doi.org/10.3389/fmicb.2014.00292.

28. •• Bhattacharyya S, Sen U, Vrati S: **Regulated IRE1-dependent decay pathway is activated during Japanese encephalitis virus-induced unfolded protein response and benefits viral replication**. *J Gen Virol* 2014, **95**:71-79.
Many features of potexvirus interactions with the UPR in plants resemble flavivirus interactions with the UPR in mammalian cells. The authors demonstrate activation of the IRE1 dependent decay (RIDD) pathway is beneficial for the virus life cycle but does not target viral genomes. This pathway remains poorly understood in regard to plant-virus interactions.

29. Mehrbod P, Ande SR, Alizadeh J, Rahimizadeh S, Shariati A, Malek H, Hashemi M, Glover KKM, Sher AA, Coombs KM *et al.*: **The roles of apoptosis, autophagy and unfolded protein response in arbovirus, influenza virus, and HIV infections**. *Virulence* 2019, **10**:376-413.

30. • Mishiba KI, Nagashima Y, Suzukia E, Hayashi N, Ogata Y, Shimada Y, Koizumi N: **Defects in IRE1 enhance cell death and fail to degrade mRNAs encoding secretory pathway proteins in the Arabidopsis unfolded protein response**. *Proc Natl Acad Sci U S A* 2013, **110**:5713-5718.
Plant viruses depend on the secretory pathway for a number of functions and so this study demostrating that the mRNAs associated with secretory proteins are degraded during ER stress is particularly noteworthy and opens the possibilty that viruses may use the UPR in plants to modify the secretory pathway.

31. •• Bao Y, Pu Y, Yu X, Gregory BD, Srivastava R, Howell SH, Bassham DC: **IRE1B degrades RNAs encoding proteins that interfere with the induction of autophagy by ER stress in *Arabidopsis thaliana***. *Autophagy* 2018, **14**:1562-1573.
This is one of the pioneer studies to identify RIDD targets in plants. IRE1B specifically degrades mRNAs that interfere with autophagy, thereby linking ER stress to the onset of autophagy.

32. Yang X, Srivastava R, Howell SH, Bassham DC: **Activation of autophagy by unfolded proteins during endoplasmic reticulum stress**. *Plant J* 2016, **85**:83-95.

33. Li F, Zhang C, Tang Z, Zhang L, Dai Z, Lyu S, Li Y, Hou X, Bernards M, Wang A: **A plant RNA virus activates selective autophagy in a UPR-dependent manner to promote virus infection**. *New Phytol* 2020, **228**:622-639.

34. Zeng Y, Li B, Zhang W, Jiang L: **ER-phagy, and ER stress response (ERSR) in plants**. *Front Plant Sci* 2019, **10**:1-8.

35. Klessig DF, Tian M, Choi HW: **Multiple targets of salicylic acid and its derivatives in plants and animals**. *Front Immunol* 2016, **7**:1-10.

36. Boatwright JL, Pajerowska-Mukhtar K: **Salicylic acid: an old hormone up to new tricks**. *Mol Plant Pathol* 2013, **14**:623-634.

37. Liu X, Rockett KS, Kørner CJ, Pajerowska-Mukhtar KM: **Salicylic acid signaling: new insights and prospects at a quarter-century milestone**. *Essays Biochem* 2015, **58**:101-113.

38. Carr JP, Murphy AM, Tungadi T, Yoon JY: **Plant defense signals: players and pawns in plant-virus-vector interactions**. *Plant Sci* 2019, **279**:87-95.

39. Kørner CJ, Pitzalis N, Peña EJ, Erhardt M, Vazquez F, Heinlein M: **Crosstalk between PTGS and TGS pathways in natural antiviral immunity and disease recovery**. *Nat Plants* 2018, **4**:157-164.

40. Donaire L, Burgyán J, García-Arenal F: **RNA silencing may play a role in but is not the only determinant**. *J Virol* 2016, **90**:553-561.

41. Nagashima Y, Iwata Y, Ashida M, Mishiba KI, Koizumi N: **Exogenous salicylic acid activates two signaling arms of the unfolded protein response in arabidopsis**. *Plant Cell Physiol* 2014, **55**:1772-1778.

42. Piña F, Yagisawa F, Obara K, Gregerson JD, Kihara A, Niwa M: **Sphingolipids activate the endoplasmic reticulum stress surveillance pathway**. *J Cell Biol* 2018, **217**:495-505.

43. Park WJ, Park JW: **The role of sphingolipids in endoplasmic reticulum stress**. *FEBS Lett* 2020 http://dx.doi.org/10.1002/1873-3468.13863.

44. Gu Y, Zebell SG, Liang Z, Wang S, Kang B, Dong X: **Nuclear pore permeabilization is a convergent signaling event in effector-triggered immunity**. *Cell* 2017, **166**:1526-1538.

45. •• Meng Z, Ruberti C, Gong Z, Brandizzi F: **CPR5 modulates salicylic acid and the unfolded protein response to manage tradeoffs between plant growth and stress responses**. *Plant J* 2017, **89**:486-501.
CPR5 is one of the well-known repressors of salicylic acid signaling that modulates plant growth. This study sheds light on CPR5's involvement through two different arms of UPR.

46. Orjuela J, Thiémélé Deless EF, Kolade O, Chéron S, Ghesquiére A, Albar L: **A recessive resistance to rice yellow mottle virus is associated with a rice homolog of the CPR5 Gene, a regulator of active defense mechanisms**. *Mol Plant-Microbe Interact* 2013, **26**:1455-1463.

47. Love AJ, Laval V, Geri C, Laird J, Tomos AD, Hooks MA, Milner JJ: **Components of Arabidopsis defense- and ethylene-signaling pathways regulate susceptibility to cauliflower mosaic virus by restricting long-distance movement**. *Mol Plant-Microbe Interact* 2007 http://dx.doi.org/10.1094/MPMI-20-6-0659.

48. Fujisaki K, Iwahashi F, Kaido M, Okuno T, Mise K: **Genetic analysis of a host determination mechanism of bromoviruses in *Arabidopsis thaliana***. *Virus Res* 2009, **140**:103-111.

49. Angelos E, Ruberti C, Kim S-J, Brandizzi F: **Maintaining the factory: the roles of the unfolded protein response in cellular homeostasis in plants**. *Plant J* 2017, **90**:671-682.

50. Brenya E, Trusov Y, Dietzgen RG, Botella JR: **Heterotrimeric G-proteins facilitate resistance to plant pathogenic viruses in *Arabidopsis thaliana* (L.) Heynh**. *Plant Signal Behav* 2016 http://dx.doi.org/10.1080/15592324.2016.1212798.

51. Lee S, Rojas CM, Ishiga Y, Pandey S, Mysore KS: **Arabidopsis heterotrimeric G-proteins play a critical role in host and nonhost resistance against *Pseudomonas syringae* pathogens**. *PLoS One* 2013 http://dx.doi.org/10.1371/journal.pone.0082445.

52. Chen Y, Brandizzi F: **AtIRE1A/AtIRE1B and AGB1 independently control two essential unfolded protein response pathways in Arabidopsis**. *Plant J* 2012 http://dx.doi.org/10.1111/j.1365-313X.2011.04788.x.

53. Faus I, Zabalza A, Santiago J, Nebauer SG, Royuela M, Serrano R, Gadea J: **Protein kinase GCN2 mediates responses to glyphosate in Arabidopsis**. *BMC Plant Biol* 2015 http://dx.doi.org/10.1186/s12870-014-0378-0.

54. Li N, Zhang SJ, Zhao Q, Long Y, Guo H, Jia HF, Yang YX, Zhang HY, Ye XF, Zhang ST: **Overexpression of tobacco GCN2 stimulates multiple physiological changes associated with stress tolerance**. *Front Plant Sci* 2018 http://dx.doi.org/10.3389/fpls.2018.00725.

55. Liu X, Kørner CJ, Hajdu D, Guo T, Ramonell KM, Argueso CT, Pajerowska-Mukhtar KM: **Arabidopsis thaliana AtGCN2 Kinase is involved in disease resistance against pathogens with diverse lifestyles**. *Int J Phytopathol* 2015 http://dx.doi.org/10.33687/phytopath.004.02.1342.

56. •• Liu X, Afrin T, Pajerowska-Mukhtar KM: **Arabidopsis GCN2 kinase contributes to ABA homeostasis and stomatal immunity**. *Commun Biol* 2019, **2**.
The diverse roles of abscisic acid (ABA) in plant immunity are known for decades. This article provided a mechanistic understanding of how GCN2 fine-tunes ABA synthesis and signaling and participates in pre-invasive and post-invasive stages of infection with *Pseudomonas syringae*.

57. Pajerowska-Mukhtar KM, Wang W, Tada Y, Oka N, Tucker CL, Fonseca JP, Dong X: **The HSF-like transcription factor TBF1 is a major molecular switch for plant growth-to-defense transition**. *Curr Biol* 2012 http://dx.doi.org/10.1016/j.cub.2011.12.015.

58. Berrocal-Lobo M, Toribio R, Castellano MM: **Eif2α phosphorylation by gcn2 is induced in the presence of chitin and plays an important role in plant defense against b. Cinerea infection**. *Int J Mol Sci* 2020, **21**:1-11.

59. Merchante C, Stepanova AN, Alonso JM: **Translation regulation in plants: an interesting past, an exciting present, and a promising future**. *Plant J* 2017 http://dx.doi.org/10.1111/tpj.13520.

60. Lu Y, Yin M, Wang X, Chen B, Yang X, Peng J, Zheng H, Zhao J, Lin L, Yu C *et al.*: **The unfolded protein response and programmed cell death are induced by expression of Garlic virus X p11 in *Nicotiana benthamiana***. *J Gen Virol* 2016, **97**:1462-1468.

61. •• Ruberti C, Lai YS, Brandizzi F: **Recovery from temporary endoplasmic reticulum stress in plants relies on the tissue-specific and largely independent roles of bZIP28 and bZIP60, as well as an antagonizing function of BAX-Inhibitor 1 upon the pro-adaptive signaling mediated by bZIP28**. *Plant J* 2018, **93**:155-165.
BAX-Inhibitor 1 (BI-1) plays dual roles as a cell death modulator in both plants and animals. This study demonstrated the negative roles of BI-1 in bZIP28-mediated UPR signaling; an evolutionarily diverged function compared to its animal counterparts.

62. Qiao W, Helpio EL, Falk BW: **Two crinivirus-conserved small proteins, P5 and P9, are indispensable for efficient Lettuce infectious yellows virus infectivity in plants**. *Viruses* 2018, **10**:1-16.

63. Aguilar E, del Toro FJ, Brosseau C, Moffett P, Canto T, Tenllado F: **Cell death triggered by the P25 protein in Potato virus X -associated synergisms results from endoplasmic reticulum stress in *Nicotiana benthamiana***. *Mol Plant Pathol* 2019, **20**:194-210.

64. Herath V, Gayral M, Miller RK, Verchot J: **BIP, and the unfolded protein response are important for potyvirus and potexvirus infection**. *Plant Signal Behav* 2020, **15**.

65. Carvalho HH, Silva Pa, Mendes GC, Brustolini OJB, Pimenta MR, Gouveia BC, Valente MAS, Ramos HJO, Soares-Ramos JRL, Fontes EPB: **The endoplasmic reticulum binding protein BiP displays dual function in modulating cell death events**. *Plant Physiol* 2014, **164**:654-670.

66. Williams B, Verchot J, Dickman MB: **When supply does not meet demand-ER stress and plant programmed cell death**. *Front Plant Sci* 2014, **5**:1-9.

67. Moon JY, Lee JH, Oh CS, Kang HG, Park JM: **Endoplasmic reticulum stress responses function in the HRT-mediated hypersensitive response in *Nicotiana benthamiana***. *Mol Plant Pathol* 2016, **17**:1382-1397.

68. Xu G, Wang S, Han S, Xie K, Wang Y, Li J, Liu Y: **Plant Bax Inhibitor-1 interacts with ATG6 to regulate autophagy and programmed cell death**. *Autophagy* 2017, **13**:1161-1175.

69. Mukhtar MS, McCormack ME, Argueso CT, Pajerowska-Mukhtar KM: **Pathogen tactics to manipulate plant cell death**. *Curr Biol* 2016 http://dx.doi.org/10.1016/j.cub.2016.02.051.

70. Lu P-P, Yu T-F, Zheng W-J, Chen M, Zhou Y-B, Chen J, Ma Y-Z, Xi Y-J, Xu Z-S: **The Wheat Bax Inhibitor-1 protein interacts with an aquaporin TaPIP1 and enhances disease resistance in Arabidopsis**. *Front Plant Sci* 2018, **9**.

71. Kawai-Yamada M, Hori Z, Ogawa T, Ihara-Ohori Y, Tamura K, Nagano M, Ishikawa T, Uchimiya H: **Loss of calmodulin binding to Bax inhibitor-1 affects pseudomonas-mediated hypersensitive response-associated cell death in *Arabidopsis thaliana***. *J Biol Chem* 2009 http://dx.doi.org/10.1074/jbc.M109.037234.