Peer-reviewed version available at *Viruses* **2019**, *12*, 17; doi:10.3390/v12010017

Review

Herpesviruses and the Unfolded Protein Response

Benjamin P. Johnston[1,2], Craig McCormick[1,2,*]

[1] Department of Microbiology & Immunology, Dalhousie University, 5850 College Street, Halifax, NS B3H 4R2, Canada
[2] Beatrice Hunter Cancer Research Institute; 5850 College Street, Halifax, NS B3H 4R2, Canada
* Correspondence: craig.mccormick@dal.ca (C.M.)

Abstract: Herpesviruses usurp cellular stress responses to avoid immune detection while simultaneously promoting viral replication and spread. The unfolded protein response (UPR) is an evolutionarily conserved stress response that is activated when the protein load in the ER saturates its chaperone folding capacity causing an accrual of misfolded proteins. Through translational and transcriptional reprogramming, the UPR aims to restore protein homeostasis; however, if this fails the cell undergoes apoptosis. It is commonly thought that many enveloped viruses, including herpesviruses, may activate the UPR due to saturation of the ER with nascent glycoproteins and thus these viruses may have evolved mechanisms to evade the potentially negative effects of UPR signaling. Over the past fifteen years there has been considerable effort to provide evidence that different viruses may reprogram the UPR to promote viral replication. Here we provide an overview of the molecular events of UPR activation, signaling and transcriptional outputs, and highlight key findings that demonstrate that the UPR is an important cellular stress response that herpesviruses have hijacked to facilitate persistent infection.

Keywords: unfolded protein response (UPR); integrated stress response (ISR); ATF6; IRE1; XBP1; PERK; ATF4; GADD34; herpesvirus; Kaposi's sarcoma-associated herpesvirus (KSHV); cytomegalovirus (CMV); herpes simplex virus (HSV).

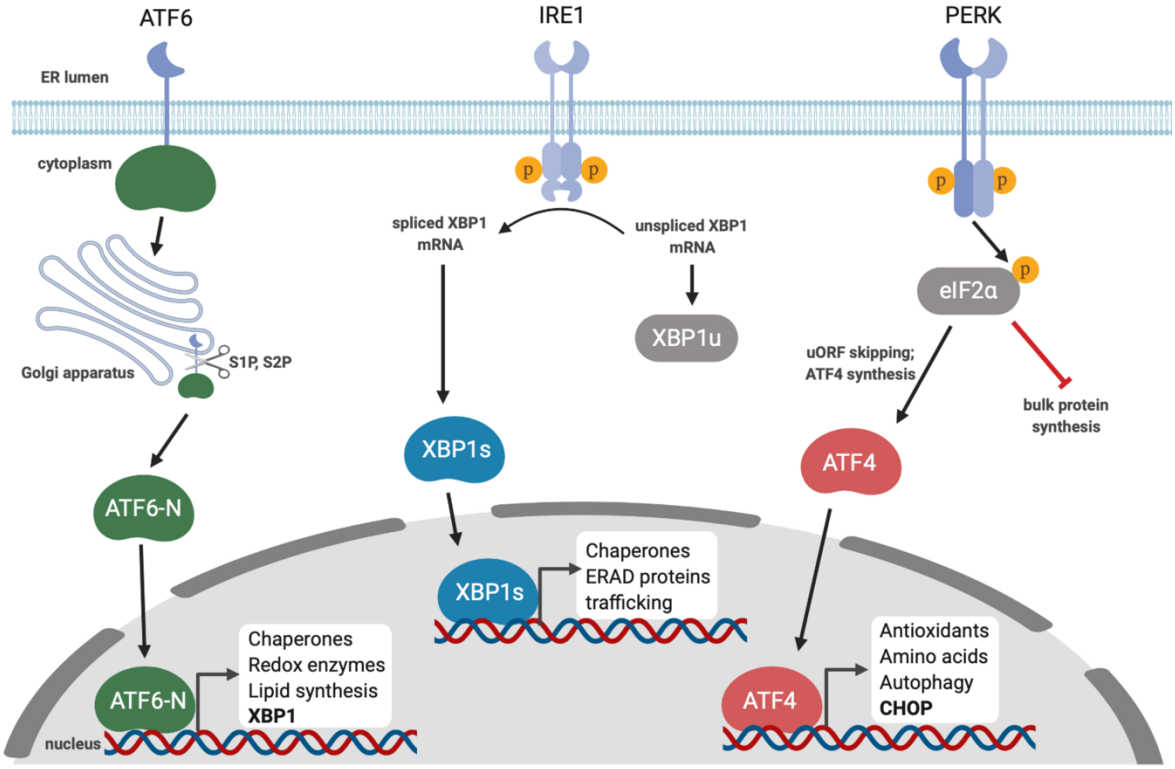
1. Overview of the unfolded protein response

The endoplasmic reticulum (ER) coordinates diverse cellular functions including lipid synthesis, calcium storage, and protein synthesis [1]. The ER is the gateway to the secretory pathway and one-third of the entire proteome is translated in the ER. These newly translated proteins need to be folded, modified, and sorted. The ER proteostasis network prevents protein misfolding by balancing the protein load with the ER folding capacity [1]. This is largely achieved through a network of enzymes that promote folding and degrade terminally misfolded or aggregated proteins through ERAD and autophagy. However, severe changes in physiology or exposure to stressful environmental factors can shift this balance, resulting in an accumulation of misfolded proteins, commonly known as ER stress. All eukaryotic cells have evolved mechanisms to sense ER stress; when stress is detected, cells attempt to restore proteostasis by increasing expression of genes that regulate ER protein folding and degradation. This evolutionarily conserved signaling response is called the unfolded protein response (UPR) [2]. In metazoans, the UPR is initiated by three ER-localized integral membrane proteins, PKR-like endoplasmic reticulum kinase (PERK), Activating Transcription Factor 6 (ATF6), and Inositol-Requiring Enzyme 1 (IRE1), that are normally maintained in an inactive state by the abundant ER chaperone Binding-Immunoglobulin Protein (BiP) (also commonly called Glucose-Regulated Protein, 78 kDa, Grp78) (Figure 1). During ER stress, BiP dissociates from the luminal domains of UPR sensors and binds hydrophobic domains of unfolded proteins to assist protein folding; this displacement of BiP triggers sensor activation [3,4]. These sensors work in concert to restore ER protein homeostasis through transcriptional and translational reprogramming. This is accomplished by transiently attenuating bulk protein translation, increasing chaperone and foldase synthesis, expanding ER surface area by increasing phospholipid synthesis, and degrading terminally misfolded proteins. Persistent or

Peer-reviewed version available at Viruses 2019, 12, 17; doi:10.3390/v12010017

irremediable ER stress causes the UPR to transition from an adaptive response to a pro-apoptotic response to prevent further damage to the host [2].

Figure 1. ER stress activates the unfolded protein response. The accumulation of misfolded proteins in the ER triggers ER stress, which activates the unfolded protein response (UPR). UPR sensor proteins ATF6, IRE1, and PERK are normally restrained by binding to the ER chaperone BiP; ER stress displaces BiP and activates the sensors, thereby promoting synthesis of UPR transcription factors that coordinate an ER stress-mitigating gene



expression program. Specifically, in response to ER stress, ATF6 translocates to the Golgi and is proteolytically cleaved by site-1 protease (S1P) and site-2 protease (S2P) releasing the N-terminal cytoplasmic transcription factor ATF6-N. IRE1 is a kinase and endoribonuclease that splices out a 26-nucleotide intron for *XBP1* mRNA, which causes a translational frameshift to generate the transcription factor XBP1s. PERK phosphorylates eIF2α, which attenuates bulk translation thereby reducing protein load in the ER. Increased eIF2α phosphorylation also causes the selective translation of the transcription factor ATF4. ATF6-N, XBP1s, and ATF4 transactivate genes involved in protein folding, degradation of misfolded proteins, lipid synthesis, and antioxidant responses.

Since the discovery of IRE1, PERK, and ATF6 as the three sensors of the UPR, there have been many studies investigating if certain viruses can trigger ER stress and/or activate the UPR. The UPR was first characterized for its ability to upregulate two highly abundant ER chaperones, BiP and Grp94, and two early studies showed that paramyxovirus Simian virus 5 or mutated hemagglutinin (HA) protein from influenza A virus could upregulate these chaperones [5,6]. Multiple studies have investigated the role of the UPR in herpesvirus replication and point towards different functions that may promote or inhibit herpesvirus replication. This review will provide an overview of the molecular signaling events of the UPR as well as some of its potential physiological roles in human health and provide examples of how different herpesviruses subvert or activate different components of the UPR to promote robust infection.

2. IRE1 is a kinase and an endoribonuclease

IRE1 is a type I ER-resident transmembrane protein that contains an ER stress-sensing N-terminal luminal domain and cytoplasmic C-terminal protein kinase and endoribonuclease domains [7]. IRE1 has two isoforms in mammals, IRE1α and IRE1β. IRE1α is expressed in all tissues, whereas IRE1β expression is primarily restricted to bronchial and intestinal epithelial cells [8]. IRE1 is maintained as an inactive monomer through the stable binding of BiP to its luminal domain. With the onset of ER stress, BiP is displaced from IRE1 due to its higher affinity for misfolded proteins, which allows dimerization of IRE1 luminal domains and kinase activation. As a result, the monomers phosphorylate one another through trans-autophosphorylation [9]. IRE1 dimers subsequently form higher-order oligomers that are essential for IRE1 activity [10]. IRE1 phosphorylation facilitates ATP binding, which induces a conformational change that activates the C-terminal ribonuclease (RNase) domain [11]. The activated RNase targets the *XBP1* mRNA for cleavage, which removes a short intron (26-nucleotides in mammalian cells) that is re-ligated by the tRNA ligase RTCB [12–16]. Splicing shifts the downstream open reading frame and generates XBP1-spliced (XBP1s), a protein with an extended C-terminus that fuses a transcription activation domain onto the basic leucine zipper (bZIP) domain, yielding a functional transcription factor. XBP1s then translocates to the nucleus and binds to consensus promoter elements that contain a core ACGT motif, found in genes that encode proteins involved in protein folding, ER-associated degradation (ERAD) and lipid biosynthesis [17,18].

The *XBP1* mRNA is the sole known substrate for IRE1-mediated splicing, with two adjacent stem-loops with IRE1 RNase cleavage sites that are positioned to enable RTCB-mediated ligation [11]. Other ER-targeted mRNAs with single XBP1-like stem-loops can be cleaved by IRE1 and subsequently degraded by the 5′-3′ exonuclease XRN1 through a process known as regulated IRE1-dependent decay (RIDD) [19–21]. The differential regulation of these two IRE1 functions remain very poorly understood, but there is evidence that they are inversely correlated to some extent. For example, IRE1 is more active in XBP1-deficient cells, which results in increased RIDD [21]. IRE1 kinase activity is required for RIDD; bypassing IRE1 kinase activity by using an IRE1 point mutant that can bind the non-hydrolysable ATP analog, 1NM-PP1, prevents RIDD despite *XBP1* splicing [19]. *In vitro* studies suggest that IRE1 levels or oligomeric state dictate function, whereby low IRE1 levels favor dimer formation and RIDD, and high IRE1 levels promote the formation of higher-order oligomers that favor *XBP1* splicing [22]. However, this observation has yet to be validated in intact living cells.

3. PERK and the integrated stress response

Eukaryotic cells attenuate global translation of non-essential genes in response to different stresses. In the absence of stress, the translation cycle of mRNAs begins with the heterotrimeric eIF2 (eukaryotic initiation factor 2) GTPase, which binds to methionine-loaded $tRNAi^{Met}$. eIF2 is a heterotrimer of eIF2α, β, and γ, where the γ subunit is responsible for direct binding of GTP and Met-$tRNAi^{Met}$ [23]. The α and β subunits stabilize the tRNA interaction, and as discussed below, the α subunit has regulatory activity [24]. When bound to GTP, eIF2 binds Met-$tRNAi^{Met}$ to form the ternary complex (TC). The TC is loaded onto the 40S small ribosomal subunit, and subsequent recruitment of eIF1, eIF1A, eIF3, and eIF5 drives formation of the 43S pre-initiation complex (PIC). The 43S PIC indirectly binds to the 5′-cap of mRNAs by binding the eIF4F heterotrimeric complex (composed of eIF4E, eIF4G, and eIF4A). eIF4F links the 5′cap to the poly(A) tail by binding the poly(A)-binding protein (PABP) to circularize the mRNA. The 43S PIC initiates scanning of the 5′UTR in a 5′-3′ direction to find the AUG start codon. Once Met-$tRNAi^{Met}$ engages with the start codon, with the help of GTPase activating protein eIF5, GTP is hydrolyzed to GDP releasing eIF2 from the ribosomal protein complex. Released eIF2 can participate in a new translation initiation cycle. However, successful recycling depends on exchange of GDP-bound eIF2 for GTP by the guanine nucleotide exchange factor (GEF) eIF2B [23].

In response to different types of stress, the α subunit of eIF2 can be phosphorylated on Serine 51 by stress-specific eIF2α kinases, which induces higher affinity binding to eIF2B, inhibiting GEF activity and

thereby reducing translation. Mammals encode four different eIF2α kinases that are activated by different types of stress: GCN2 (general control nonderepressible 2), PERK, PKR (Protein kinase R; "R" stands for RNA), and HRI (heme regulated inhibitor) [25,26]. Through these eIF2α kinases the cell can sense a wide variety of different types of stress and respond by attenuating global translation through eIF2α phosphorylation [27,28]. This initiates a cellular stress response known as the integrated stress response (ISR) [29].

Like IRE1, PERK dimerizes in response to ER stress and undergoes trans-autophosphorylation [25,26]. This induces a conformational change in PERK that allows it to bind and phosphorylate the translation initiation factor, eIF2α [30,31]. The PERK and IRE1 luminal domains are quite similar, which highlights a conserved mechanism of responding to ER stress through the disengagement of BiP with the increase in misfolded proteins [3,31,32]. A central feature of the ISR is the phosphorylation of eIF2α to attenuate bulk translation, while also specifically increasing production of stress-related proteins due to the presence of uORFs in the 5'UTR of their mRNAs [33,34]. In the absence of stress, the 43S PIC initiates protein synthesis on these uORFs, whereas eIF2α phosphorylation causes uORF bypass and downstream translation initiation on longer ORFs that encode stress-mitigating proteins like the bZIP transcription factor Activating Transcription Factor 4 (ATF4). ATF4 transactivates genes involved in amino acid biosynthesis, the anti-oxidant response and autophagy [29]. It is a member of the ATF/CREB family of transcription factors and can bind to ATF/CRE-like sequences with a consensus sequence of 5'-TGACGTCA-3', and amino acid response elements (AAREs) that have a conserved core sequence 5'-ATTGCATCA-3' [35]. ATF4 amplifies ISR transcriptional responses by transactivating genes encoding the bZIP transcription factors Activating Transcription Factor 3 (ATF3) and C/EBP-Homologous Protein (CHOP) [33,36]. ATF4 can also heterodimerize with CHOP and change the repertoire of transactivated genes [25,37,38]. Recently, a small molecule called ISRIB (ISR inhibitor) was shown to bind eIF2B and promote GEF activity, which attenuated translation inhibition following activation of an eIF2α kinase, thereby suppressing ATF4 and CHOP synthesis [39–42]. CHOP transactivates *Growth Arrest and DNA-Damage-Inducible 34 (GADD34)* [43]; GADD34 recruits protein phosphatase 1α (PP1α) to phospho-eIF2α to enforce eIF2α dephosphorylation and restore bulk translation [44]. However, these GADD34-PP1α phosphatase complexes operate irrespective of stress resolution, which can have an important impact on cell fate (discussed below). Like ATF4, both CHOP and GADD34 contain uORFs, which are required for maximal translation following engagement of the ISR, which includes UPR activation from ER stress [45–47].

4. ATF6 is activated by regulated intramembrane proteolysis

Another sensor of ER stress is ATF6, a 90 kDa ER-localized type II transmembrane glycoprotein with two isoforms known as ATF6α and ATF6β [48–50]. Both isoforms are expressed in all tissue types, and may have some redundant roles, but ATF6α predominates in UPR signaling in response to ER stress [51]. Similar to IRE1 and PERK, in the absence of stress BiP binds ATF6 to inhibit signaling [52]. There are likely other modes of maintaining ATF6 in an inactive state, including the formation of intra- and inter-molecular disulfide bonds with other ATF6 proteins, creating ATF6 dimers and oligomers [53]. In response to ER stress, the disulfide bonds are reduced and BiP is released, which allows ATF6 to translocate to the Golgi in a CopII-dependent manner where it is cleaved at the luminal side of the ER by Site-1 protease (S1P) and the cytoplasmic side of the ER by S2P in a process referred to as regulated intramembrane proteolysis (RIP) [54,55]. These cleavage events release the N-terminal cytosolic fragment, ATF6-N, which is an active bZIP transcription factor that traffics to the nucleus to transactivate genes to mitigate the ER stress.

ATF6-N homodimers bind to conserved consensus motif called ERSE (for ER stress response element; consensus sequence CCAAT-N9-CCACG) and ERSE-II (consensus sequence ATTGG-N-CCACG),

Peer-reviewed version available at Viruses 2019, 12, 17; doi:10.3390/v12010017

which are found in a variety of UPR dependent genes that encode chaperones, quality control proteins and ERAD proteins, as well as components of the redox pathway [56–58]. Two of the best-characterized genes that ATF6 upregulates are BiP and Grp94 [57,59,60], two of the most abundant ER chaperones [61]. ATF6 also interacts with the heterotrimeric transcription factor NF-Y (composed of NF-YA, NF-YB, and NF-YC) and this interaction is required for ATF6 binding to the promoter [58]. With regard to the ERSE consensus motif, the ATF6 dimer binds to CCACG while NF-Y occupies CCAAT [62]. ATF6 can upregulate the ISR gene CHOP due to the presence of an ERSE in the CHOP promoter [62,63]. ATF6 has also been shown to upregulate *XBP1* mRNA expression [13]. ATF6 can also heterodimerize with XBP1s to upregulate a different set of UPR genes, as well as likely synergistically activating ATF6- or XBP1s-dependent genes [51,57].

The mechanism of how ATF6 trafficking and cleavage is regulated following ER stress is not well understood. It was found that only the luminal domain of ATF6 is involved in ER stress sensing and trafficking to the Golgi; swapping the C-terminal luminal domain of ATF6 onto the constitutively transported protein Sec22 caused the hybrid protein to be retained in the ER, followed by release to the Golgi upon ER stress [64]. COPII is located on the cytoplasmic side of the ER and therefore it is not known how the luminal domain of ATF6 signals to COPII for trafficking [54,65]. This method of activating a transcription factor through ER-to-Golgi trafficking to undergo proteolytic cleavage is not unique to ATF6. Sterol Regulatory Element Binding Transcription Factor 1 (SREBP1) and SREBP2 are ER membrane bound transcription factors that undergo RIP by S1P and S2P in response to low cholesterol and the released cytosolic transcription factors upregulate genes involved in cholesterol biosynthesis. The SREBPs interact with SCAP (SREBP cleavage activating protein), which is the protein that actually senses sterol levels. SREBP and SCAP are retained in the ER by INSIG (insulin-induced gene) binding of SCAP. Low levels of sterols cause release of INSIG from SCAP, allowing SCAP and SREBP to translocate to the Golgi in COPII vesicles [66]. ATF6 processing does not require SCAP [55], and as of yet, the molecular events that control ATF6 release to the Golgi continue to remain elusive.

There are also other ER transmembrane proteins that share similar domain structure to ATF6 and belong to the OASIS subfamily of bZIP transcription factors including Luman/LZIP/CREB3, OASIS/CREB3L1, BBF2H7/CREB3L2, CREBH/CREB3L3, and CREB4/AIbZIP/Tisp40/CREB3L4 [67]. Like ATF6 and SREBP1/2, these proteins are also processed by RIP to release their N-terminal bZIP transcription factor. Other than Luman, these proteins have cell or tissue-specific expression indicating that they have different physiological roles. Interestingly, some of these ATF6-like proteins can also be activated by ER stress, indicating that the UPR in different tissues may have a different gene expression profile depending on the presence of other ATF6-like transcription factors [67].

5. Crosstalk among the branches of the UPR

Although activation of the three UPR sensors during ER stress elicits distinct transcriptional outputs, there is accumulating evidence of coordination and co-regulation between the different branches. Furthermore, the type and duration of stress seems to impact the output of each branch of the UPR. For example, Peter Walter's group showed that lethal doses of ER stress-inducing molecules thapsigargin or tunicamycin elicit sustained, long-term PERK activity, but only transient IRE1 and ATF6 activity [68]. Thus, it appears that UPR resolution mechanisms are quite asynchronous between the different branches of the pathway.

The attenuation of prolonged IRE1 signaling is not fully understood but a recent study showed that the phosphatase RNA Polymerase II Associated Protein 2 (RPAP2) is activated in a PERK-dependent manner and dephosphorylates IRE1 [69]. PERK has also been shown to silence XBP1 expression through the upregulation of microRNA 30c-2-3p, which binds and diminishes translation of the *XBP1* mRNA; this

may serve as another mechanism of attenuating IRE1 signaling after prolonged ER stress [70]. Alternatively, IRE1 has also been shown to bind the XBP1 target gene hsp40 co-chaperone ERdj4 to facilitate BiP recruitment and stabilize IRE1 monomers [71]. This may indicate that the IRE1-XBP1 pathway may institute a negative feedback loop through XBP1 upregulation of ERdj4 to control IRE1 signaling.

Another potential mechanism of crosstalk between the PERK and IRE1 branch is that XBP1s can transcriptionally upregulate another hsp40 family member p58^{IPK}, which was reported to repress PERK activity [72]. p58^{IPK} (also known as DNAJC3) was originally identified as an inhibitor of PKR [73], but since these two eIF2α kinases share sequence similarity in the cytoplasmic domain, it is not surprising that p58^{IPK} could also repress PERK. p58^{IPK} has also been shown to act as a co-chaperone for BiP and thus p58^{IPK} may indirectly control PERK activity through BiP binding to PERK [74]. Thus, prolonged XBP1s expression may inactivate PERK through p58^{IPK} upregulation, although this has not been demonstrated experimentally.

There is evidence that PERK activation can suppress IRE1 signaling, but there has also been a report that demonstrated that the ISR can promote IRE1-XBP1 axis through either ATF4 transactivation of *IRE1* [75] or by increasing mRNA stability of spliced *XBP1* [76]. These conflicting observations may be due to genetic variations in cell models or from differences in the conditions used to study the regulation of the UPR signaling in these studies. Therefore, further analysis is required to clarify how PERK coordinates IRE1 activity.

As previously mentioned, ATF6 transactivates *XBP1* and *CHOP*, and therefore impacts both IRE1 and PERK pathways, respectively [13,62]. ATF6 and XBP1s can form heterodimeric complexes that can either enhance gene expression or broaden the transcriptional landscape compared to XBP1 and ATF6 homodimers [57]. ATF6 primarily upregulates BiP, but ATF4, with the help of ATF1 and CREB1 has also been reported to promote BiP transcription [77]. The ISR has also been shown to be important for regulating BiP through the presence of uORFs in the 5'UTR of BiP mRNA [78]. However, this uORF-dependent translation of BiP is dependent on eIF2A rather than eIF2α; eIF2A is a functional homolog of eIF2 that does not rely on eIF2B for GTP cycling, and promotes translation initiation on non-canonical CUG start codons [79]. These examples clearly indicate that crosstalk among the branches of the UPR is important for coordinating a robust and tightly regulated response to ER stress and influence cell fate decisions. Disruption of coordinated UPR signaling among the three branches may have a deleterious impact on mitigating ER stress, resulting in disease onset.

6. The UPR and cell fate

Sustained, unresolved ER stress causes the UPR to switch from an adaptive response to a pro-apoptotic response [2]. This switch from adaptation to apoptosis depends on the strength, type, and duration of the stimuli. The precise mechanisms that govern this switch are not well understood. Furthermore, some of the pro-apoptotic factors induced by the UPR, such as CHOP, are induced during the adaptive phase and it is not clear how these pro-apoptotic factors are regulated to ensure that their apoptotic functions are implemented only after the cell has reached a "tipping point" after which the stress cannot be resolved [80]. Nonetheless, there has been some considerable effort to elucidate mechanisms of UPR-mediated apoptosis and identify factors that govern the switch from adaptive responses to apoptosis. Generally, it is thought that ATF6 plays a lead role in proteostasis, whereas IRE1 and PERK heavily influence cell fate [81]. However, confidence in these assignments is undermined by the intertwined nature of UPR regulation.

Sustained IRE1 signaling elicits apoptosis through multiple mechanisms. IRE1 can bind TRAF2 and recruit and activate the MAP3K apoptosis signal-regulating kinase 1 (ASK1) [82,83]. Activated ASK1 phosphorylates the MAPK JNK, which then phosphorylates and activates the transcription factor c-Jun.

IRE1-dependent activation of JNK promotes apoptosis via inhibitory phosphorylation of the anti-apoptotic Bcl-2 protein, and activating phosphorylation of the pro-apoptotic Bim protein [84,85]. IRE1 can also promote apoptosis via RIDD. RIDD cleaves a variety of microRNAs, including miR-17, -34a, -96, and -125, all of which target pro-caspase-2; thus, RIDD-mediated destruction of key miRNAs causes accumulation of pro-caspase-2 [86]. This larger available pool of caspase-2 increases the capacity for cleavage of BID into active tBID, which activates the pro-apoptotic Bax protein [87]. However, RIDD does not immediately induce apoptosis in response to ER stress; in the early stages of the UPR, RIDD circumvents the extrinsic apoptosis pathway by cleaving the mRNA encoding Death Receptor 5 (DR5) and preventing caspase-8 activation [88]. However, recently the role of DR5 and caspase-8 activation in ER stress-induced apoptosis has been disputed [89]. Therefore, further work is needed to elucidate precise mechanisms that regulate IRE1-dependent cell death mechanisms in response to chronic ER stress.

PERK-mediated eIF2α phosphorylation arrests global protein synthesis while enabling selective translation of uORF-containing mRNAs encoding ATF4 and CHOP. CHOP is generally considered a pro-apoptotic transcription factor and transactivates *DR5* to promote apoptosis by activating caspase-8 [90]. CHOP has also been shown to upregulate Bim transcription through heterodimerization with C/EBPα [85]. Increased Bim promotes activation of Bax to trigger mitochondrial dysfunction. Normally the anti-apoptotic protein Bcl-2 can antagonize Bim activity, and CHOP has also been reported to repress Bcl-2 expression [84]. CHOP has also been shown to be important for inducing apoptosis following prolonged exposure to ER stress through the upregulation of Endoplasmic Reticulum Oxidoreductase 1 alpha (Ero1α), which leads to increased ROS and hyperoxidation of ER resident proteins that causes additional protein misfolding [43]. Increased Ero1α also activates the inositol-1,4,5-triphosphate (IP3) receptor (IP3R) leading to calcium release from the ER [91]. Large increases in cytoplasmic calcium can be taken up by the mitochondria, which has been shown to induce mitochondrial permeabilization and cytochrome c release [92]. ER calcium is required for protein folding and therefore secretion of calcium through the IP3R may also potentiate protein misfolding, leading to further toxicity [93].

As previously mentioned, CHOP transactivates *GADD34*, which directs dephosphorylation of eIF2α by PP1a, thereby restoring translation [43,44]. This is an important step during the adaptive phase of the UPR that accompanies stress resolution. However, apoptosis can be promoted by inappropriate resumption of global protein synthesis and translocation of nascent proteins into an ER that is already burdened by misfolded proteins [43]. GADD34 expression likely also facilitates translation of pro-apoptotic proteins [94]. It has also been shown that the combined action of ATF4 and CHOP, through both homo- and hetero-dimerization, transcriptionally upregulates genes that promote protein synthesis, including GADD34, and it is a combination of this increase in protein synthesis and oxidative stress that drives apoptosis [38]. Blocking the restoration of translation by inhibiting GADD34 during chronic ER stress can protect cells from apoptosis [95]. ATF4 and CHOP are upregulated during the adaptive phase of the UPR and therefore it is not entirely clear why early induction of these transcription factors does not pre-destine the cell to apoptosis. Both the mRNAs and proteins of ATF4 and CHOP are quite labile. Therefore, one theory is that if the stress was resolved then ATF4 and CHOP signaling would be rapidly quenched; however chronic activation of PERK allows these proteins to accumulate to promote apoptosis [80].

Much of what we know regarding the UPR signaling that controls cell fate is from the use of ER stress-inducing drugs that have gross deleterious effects on the ER and the cell. These include tunicamycin, which inhibits N-linked glycosylation; thapsigargin, which depletes ER Ca^{2+} levels by inhibiting the SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) pump; and reducing agents dithiothreitol (DTT) and β-mercaptoethanol, which disrupt disulfide bonds. Therefore, many of these studies describing how the UPR controls the switch from restoring protein homeostasis may not be fully reflective of what is happening *in vivo* following a disruption in ER protein homeostasis. Developing better models of ER stress, both *in vitro*

and *in vivo,* that are more reflective of physiological forms of ER stress will help us better understand the precise contributions of the different UPR sensors in regulating apoptosis.

7. The UPR in health and disease

The UPR governs a variety of physiological processes and associated disease states [96]. Certain cells require the UPR and an augmented ER to meet increased demands on the secretory system during their differentiation; these include plasma cells [97], pancreatic β cells [98], and granulocytic eosinophils [99]. In the intestinal epithelium, UPR signaling is required for differentiation and maintenance of cells with strong secretory phenotypes that guard against infection, including Paneth cells that secrete antimicrobial peptides and goblet cells that secrete large amounts of mucins to prevent pathogen infiltration past the intestinal barrier. Accordingly, knocking out *XBP1* in mouse intestines eliminated Paneth cells and decreased levels of goblet cells, leaving them more susceptible to spontaneous enteritis and *Listeria monocytogenes* infection [100]. Paneth cell dysfunction also contributes to inflammatory bowel disease (IBD), including Crohn's disease or ulcerative colitis [101]. *XBP1* deletion in Paneth cells leads to increased ER stress, which can be mitigated by increased protein catabolism through autophagy [100].

While the UPR and specifically the transcription factor XBP1s is essential for plasma cell differentiation [97,102,103], dysregulated XBP1s signaling can also promote disease progression as overexpression of XBP1s can promote the plasma cell malignancy multiple myeloma [104]. XBP1 and the UPR are implicated in other cancers, such as breast cancer [105] and ovarian cancer [106], as well as protein misfolding neurodegenerative disorders like Huntington's disease [107]. Due to the importance of the UPR in insulin secretion by pancreatic β cells, dysregulated UPR signaling is also linked to type II diabetes [108].

The IRE1-XBP1 pathway is also important for the development and survival of many different immune cells, including dendritic cells [109]. Depending on the location or type of dendritic cell, XBP1 can be essential for survival or impact DC function, such as MHC-I surface expression. Constitutive expression of XBP1s can promote lipid accumulation in DCs, which can inhibit antigen presentation [110,111]. IRE1-XBP1 signaling was also reported to be activated in CD8+ T cells in response to infection and loss of XBP1 abrogated effector T cell differentiation [112].

The IRE1-XBP1 pathway also likely plays an important role in regulating inflammation in response to infection. Activation of TLR2 or TLR4 in macrophages specifically engaged the IRE1-XBP1 pathway and XBP1 was required to augment expression of pro-inflammatory cytokines IL-6, TNFα, and IFN-β [113]. Engagement of TLR2 and TLR4 was reported to promote IRE1 polyubiquitination by TRAF6, which blocked IRE1 dephosphorylation by PP2A to prolong IRE1 signaling [114]. TLR signaling was specific to IRE1 activation while ATF6 and PERK were inhibited [113]. PERK and the ISR was also inhibited by TLR signaling following treatment with tunicamycin [115]. TLR signaling was shown to inhibit the ISR by PP2A-mediated dephosphorylation of the epsilon subunit of eIF2B [116]. Our understanding of the role for the UPR in immunity and inflammation is only in its infancy and employing different mouse models for studying inflammation or using new technologies like single cell sequencing will surely help advance this field.

There is also accumulating evidence for UPR subversion during virus infection. Naturally, most studies to date have focused on enveloped viruses, since the synthesis of envelope glycoproteins in the ER could burden ER folding machinery to the point where it activates the UPR. Certain viruses have been shown to activate all three UPR sensors, whereas other viruses appear to selectively activate or inhibit one of the sensors. It is plausible that certain features of the UPR could aid viral replication. For example, UPR activation in response to infection stress could promote efficient viral protein synthesis and cell survival through increased synthesis of chaperone proteins and ER expansion. However, other aspects of the UPR

could hinder viral replication by attenuating bulk translation and increasing degradation of viral proteins via ERAD. UPR-mediated apoptosis could limit replication of certain viruses, but others may benefit from apoptosis induction to aid dissemination of progeny [117]. The UPR can also impact broader responses to viral infection like autophagy and inflammation [118]. Because the UPR can exert complex virus-specific and cell type-specific effects on viral replication, it makes sense that certain viruses appear to "fine-tune" the UPR by selectively blocking one or more branches to aid viral replication. For the most part, we lack a mechanistic understanding of how the UPR impacts viral replication, or how viruses usurp the UPR to create an optimal environment for productive viral replication. However, important advances have been made through the study of herpesviruses over the past decade. Here, we will review the current understanding of herpesvirus interactions with the UPR, and viral mechanisms for UPR subversion.

8. Gammaherpesviruses and the UPR

Herpesviruses establish life-long persistent infection through latency, a quiescent state wherein viral gene expression is restricted to a handful of genes and the viral genome is usually maintained as a circular episome associated with host chromatin [119]. During mitosis, the viral episome is replicated by the cellular DNA polymerase and segregated to daughter cells. An essential feature of latency is reversibility; host signal transduction causes episome decondensation and initiates the full lytic viral gene expression program, which proceeds in an ordered, temporal cascade. The lytic cycle also features replication of the viral genome by a viral DNA polymerase, yielding a linear DNA product that is packaged into capsids to generate infectious virions. These newly replicated virions can spread to infect other cells.

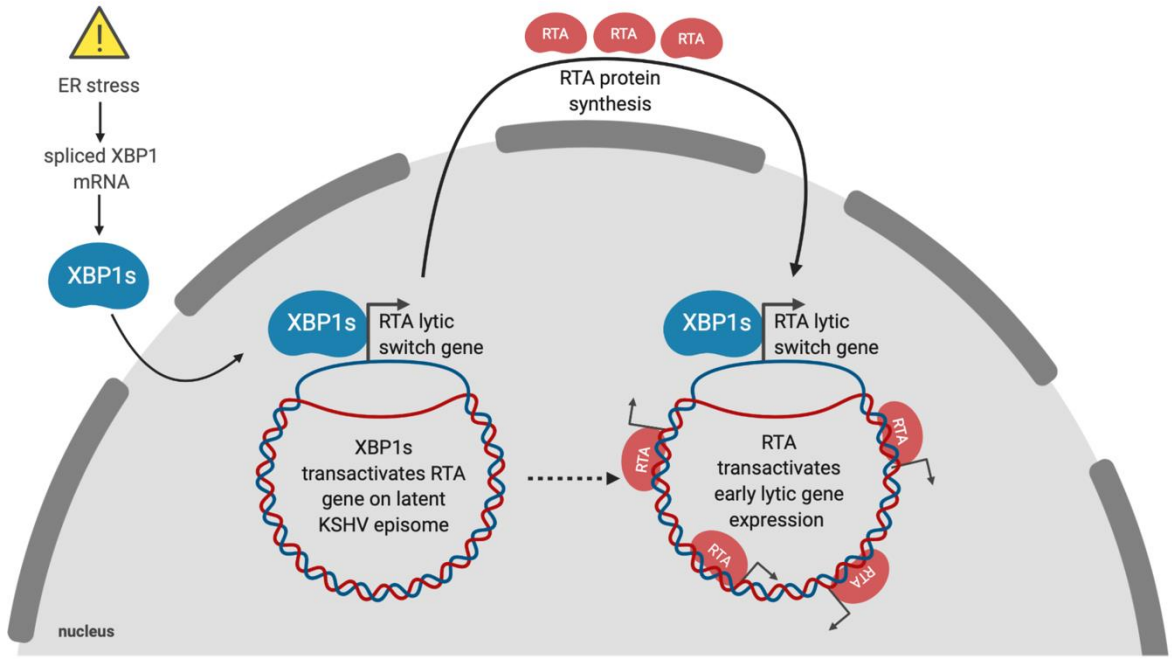


Figure 2. KSHV reactivates from latency in response to ER stress. In response to ER stress, *XBP1* mRNA is spliced and the XBP1s protein accumulates and translocates to the nucleus, where it transactivates the *RTA* gene encoding the lytic switch protein. RTA binds and transactivates viral early gene promoters throughout the genome and commits the virus to the lytic gene expression program and virion production.

Latency is the default replication program for gammaherpesviruses, which include the human herpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) [119]. The

Peer-reviewed version available at Viruses 2019, 12, 17; doi:10.3390/v12010017

physiologic signals that control the switch from latency to lytic replication are unclear, but in cell culture viral reactivation has often been linked to exposure to stress-inducing stimuli. ER stress can trigger reactivation of EBV, KSHV, and the close relative of KSHV, murine herpesvirus 68 (MHV68) [120–123]. Lytic reactivation in response to ER stress is primarily due to XBP1s (Figure 2). The ER stress-sensing mechanism involves the presence of XBP1s target sequences in the promoters of immediate early viral genes. KSHV and MHV68 express the immediate early protein RTA, which is essential and sufficient to induce lytic replication [124,125]. The RTA promoter in KSHV contains at least one XBP1s response element with an ACGT core motif [126]. The RTA promoter also contains hypoxia-inducible factor 1 (HIF-1) response elements, which also contain ACGT core sequences and thus KSHV can reactivate in response to hypoxia [127]. Hypoxia was also shown to induce *XBP1* splicing, and robust RTA expression was dependent on both HIF-1 and XBP1s [126].

EBV lytic replication requires the immediate early proteins BRLF1 and BZLF1. While ER stress has been shown to trigger EBV reactivation from latency, XBP1s only minimally induces BRLF1 and BZLF1 expression, and robust expression requires coincident protein kinase D activation [120]. ER stress also induces the EBV oncoprotein Latent Membrane Protein 1 (LMP1) through direct transactivation by XBP1s and possibly via ATF4 as well [128]. The UPR can drive LMP1 expression, and newly synthesized LMP1 can sustain UPR activity, which may provide a feed-forward mechanism to further increase LMP1 production during EBV latency [129]. Thus, the UPR is tightly linked to EBV oncogenesis by inducing the expression of oncoproteins and controlling the reactivation and spread of the virus.

KSHV and MHV68 are lymphotropic viruses that infect primary B cells that differentiate into B cell tumors called primary effusion lymphoma (PEL) and present with a pre-plasma cell (or plasmablast) phenotype [130–132]. As previously mentioned, XBP1s is essential for differentiation of B cells into non-dividing plasma cells (PCs) [97]. Normally, plasma cells develop in response to B cell receptor (BCR) activation following antigen recognition. Lytic reactivation was induced following BCR activation from IgM crosslinking in a KSHV-infected, EBV-negative Burkitt's lymphoma cell line in part through XBP1s [133]. Since PEL cells have a plasmablast-like phenotype [134], further differentiation into PCs may allow KSHV to escape from cell cycle arrest through XBP1s transactivation of *RTA*. Conversely, KSHV may actively repress XBP1 activity to maintain tight control of latency, which may halt the differentiation process at a pre-plasma cell differentiation stage. PEL cells have also been shown to reactivate following prolonged ER stress through caspase-dependent cleavage of RAD21 [135]. RAD21 is a component of the cohesin complex that partners with CTCF to silence viral gene expression and maintain latency [136]. MHV68 RTA expression also responds to XBP1s expression *in vitro*. However, XBP1s is dispensable for lytic reactivation *in vivo*, and is instead governed by another plasma cell differentiation factor known as interferon regulatory factor 4 (IRF4) [121]. MHV68 encodes the ER-localized M1 protein that is induced by RTA and IRF4 and may play a role in reactivation from latency [137,138]. M1 was also shown to induce low levels of *XBP1* splicing [139], and therefore may promote a feedforward response to reactivate MHV68 by increasing levels of XBP1. M1 also upregulates ATF6-dependent chaperones BiP and Grp94, while preventing PERK phosphorylation of eIF2α, potentially as a mechanism to facilitate an increase in viral glycoprotein synthesis and folding.

Collectively, these studies demonstrate a link between the UPR and gammaherpesvirus reactivation from latency and suggest that activation of XBP1s may be an evolutionary conserved mechanism for reactivation. However, the subsequent interplay between these viruses and the UPR during lytic replication is less well understood.

Nonetheless, details are slowly emerging about the effects of KSHV lytic replication on the UPR. We recently demonstrated that lytic replication activates all three UPR sensors, which supports productive KSHV replication; inhibiting each UPR sensor via chemical inhibitors or RNA silencing diminished yield

of infectious virions [140] (Figure 3). Despite clear UPR sensor activation, downstream UPR transcription was blunted in all three branches of the pathway. Specifically, we observed that PERK was activated during the lytic cycle and eIF2α was phosphorylated, but ATF4 did not accumulate, and accordingly, ATF4 target genes were not transcribed. Furthermore, ATF6 was proteolytically cleaved to release the ATF6-N bZIP transcription factor, but ATF6-N target genes were not transcribed. Finally, we observed that IRE1 was activated and *XBP1* mRNA was efficiently spliced during KSHV lytic replication, but XBP1s protein did not accumulate, and neither did products of XBP1s target genes. To determine whether UPR transcription might impact KSHV lytic replication, we ectopically expressed the spliced isoform of *XBP1*, which potently inhibited virion production in epithelial cells in a dose-dependent manner. This suggests that even though XBP1s plays an important role in reactivation from latency, the virus silences it's expression during the lytic cycle to circumvent deleterious effects on viral replication. Currently we are unsure of how precisely XBP1s is blocking virus production, but it appears to be at a late step in the replication cycle.

There is supporting evidence that excessive UPR signaling inhibits KSHV lytic replication; strong pharmacologic induction of the UPR with various drugs including 2-deoxyglucose (inhibits glycosylation), brefeldin A (inhibits vesicular transport), tunicamycin, or treatment with ER stress-inducing proteasome inhibitors (bortezomib, MG132, lactacystin, proteasome inhibitor I) can induce lytic reactivation but inhibit virion production and trigger apoptosis [141–144]. We speculate that the virus may induce low levels of UPR activation to remodel the host cell and promote efficient lytic replication, but acute ER stress may still be detrimental to the virus due to the ensuing terminal pro-apoptotic UPR. This idea merits further investigation, but we should be cautious in interpreting studies that employ molecules that induce ER stress as a by-product of their primary mode of action.

It remains largely unclear how KSHV lytic replication activates UPR sensors while simultaneously inhibiting downstream UPR transcription. It is possible that coordinated action of multiple viral gene products is required to arrive at this outcome, as is the case for the alpha- and beta-herpesviruses. KSHV encodes the viral host shutoff endonuclease SOX that targets the majority of host mRNAs for degradation [145,146]. As a result, this causes an indirect widespread transcriptional attenuation by repressing RNA polymerase II recruitment to host promoters [147]. We originally hypothesized that SOX-mediated repression of transcription during lytic replication was responsible for inhibiting the downstream transcriptional responses of the UPR; however, we showed that ectopic expression of SOX had no effect on UPR-responsive genes [140].

Peer-reviewed version available at *Viruses* **2019**, *12*, 17; doi:10.3390/v12010017

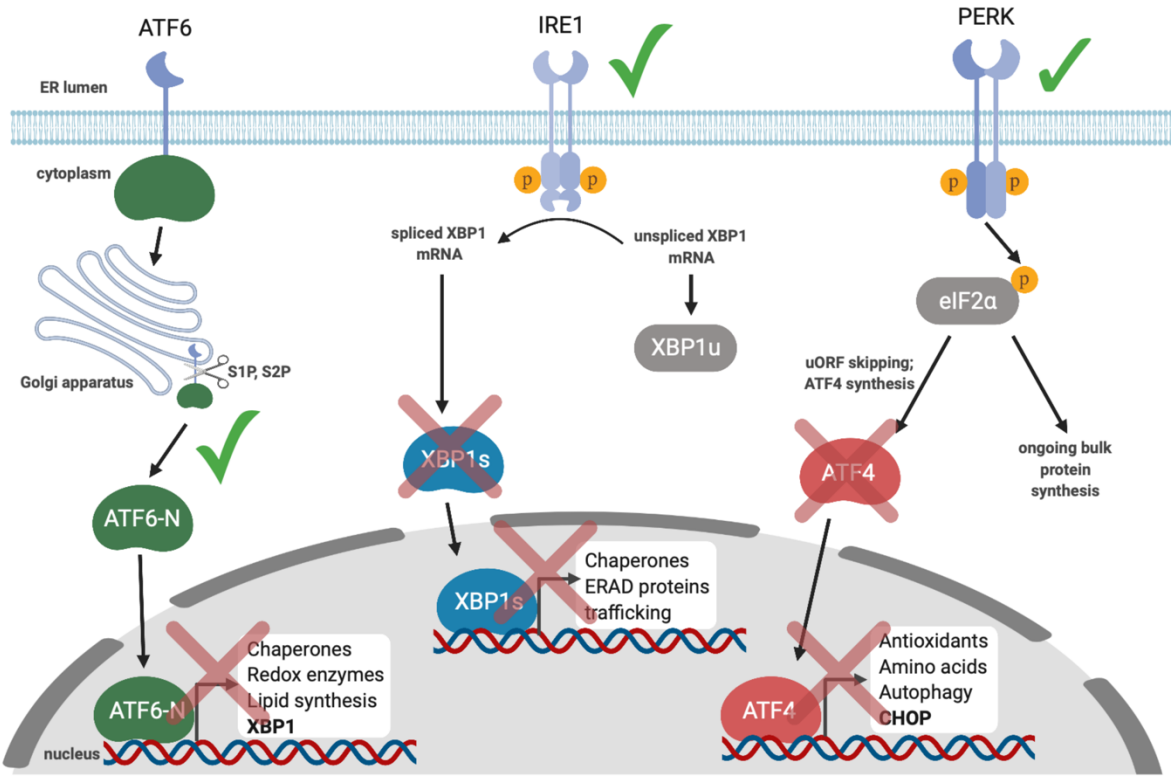


Figure 3. KSHV activates UPR sensors but limits UPR gene expression during the lytic cycle. XBP1s is required for reactivation from latency in response to ER stress. All three UPR sensor proteins are activated in the early stages of lytic replication, but downstream UPR transcription is inhibited. ATF6-N is produced via proteolytic cleavage in the Golgi, but ATF6-N-responsive genes are not transcribed. IRE1 is activated and *XBP1* is spliced, but XBP1s protein does not accumulate and XBP1s-responsive genes are not transcribed. eIF2α is phosphorylated in a PERK-dependent manner, but ATF4 protein does not accumulate and ATF4 responsive genes are not transcribed.

The uORFs in *ATF4* mRNA are essential for ATF4 translation following eIF2α phosphorylation [34]. There also uORFs in the KSHV genome, notably ones that regulate the expression of the ORF35-ORF36-ORF37 locus [148,149]. Currently it is not known how ISR activation impacts the translation of these uORF-containing viral mRNAs. Potentially KSHV disrupts translation of host mRNAs that contains uORFs (like ATF4) as a mechanism to control expression of viral uORF-containing mRNAs. XBP1s, ATF6-N, and ATF4 are bZIP transcription factors and heterodimerization is a notable characteristic of this class of transcription factors, which can change the repertoire of genes that they regulate [150]. KSHV expresses the lytic ORF K8, which encodes K-bZIP, which is named accordingly due to a basic-leucine zipper domain in its C-terminus [151]. It would be worth investigating if any of the UPR transcription factors can be inhibited or their target genes altered from dimerizing with K-bZIP. K-bZIP also contains SUMO ligase activity [152,153], while RTA has been shown to promote ubiquitination of SUMOylated proteins [154]. Therefore, the lack of XBP1s or ATF4 during lytic replication may be due to a concerted effort by K-bZIP and RTA to facilitate their proteasomal degradation.

Another potential mechanism of why the UPR transcriptional responses are stymied is that the transcription factors may be sequestered into viral replication compartments due to the presence of targeting sequences within the KSHV genome, similar to XBP1s binding to the immediate early promoter of RTA to promote reactivation, as discussed previously. This would not be a unique feature for herpesviruses as even the RNA virus porcine reproductive and respiratory syndrome virus was recently shown to recruit ATF4 to replication compartments in the cytoplasm [155]. These examples are mere speculations of how KSHV proteins might suppress the downstream transcriptional responses and should be tested experimentally.

One KSHV protein that may modulate UPR signalling during the lytic cycle is viral interleukin-6 (vIL-6; also called K2). vIL-6 has an amino-terminal ER signal sequence that directs its translocation into the ER lumen and subsequent secretion. However, a significant fraction of v-IL6 is retained in the ER where it interacts with hypoxia upregulated 1 (HYOU1, also called Grp170) [156]. HYOU1 is a nucleotide exchange factor that promotes ADP release from BiP, which allows for sustained BiP association with unfolded or misfolded proteins [157]. vIL-6 may interfere with HYOU1-BiP interactions, resulting in increased protein misfolding and UPR activation. However, there is also evidence that vIL-6 may promote protein folding by binding components of the calnexin cycle, including UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1) and glucosidase II (GlucII) [158]. Therefore, vIL-6 may play a role in fine-tuning UPR signaling by directly controlling proteostasis. Another study showed that there are XBP1 targeting sequences in the promoter of vIL-6, suggesting that vIL-6 can be upregulated in response to ER stress in a manner similar to RTA [159]. This arrangement could allow vIL-6 to quickly respond to changes in ER proteostasis.

In addition to vIL-6, products of the ORF47-ORF46-ORF45 locus may stimulate the UPR during the KSHV lytic cycle. P.-J. Chang *et al.* discovered new products of the tri-cistronic mRNA that encodes ORF47 (glycoprotein L), ORF46 (uracil DNA glycosylase), and ORF45 (multifunctional tegument protein), that activate the UPR [160]. *In silico* analysis of these alternatively spliced mRNAs suggested that they had the potential to encode two new variants of ORF45 dubbed ORF47/ORF45A and ORF47/ORF45B, which contain 114 and 56 amino acids from the N-terminus of gL, respectively. Effectively, ORF47/ORF45A and ORF47/ORF45B are fusions of the amino-terminal ER signal sequence of gL with the soluble tegument protein ORF45. Accordingly, ectopic expression of these two new gL/ORF45 fusion proteins revealed ER localization, whereas ORF45 remained in the nucleus and cytoplasm. Ectopic expression of ORF47/ORF45A and ORF47/ORF45B, but not ORF45, triggered *XBP1* splicing and BiP accumulation in HEK293T cells, whereas PERK and ATF6 were largely unaffected. It was proposed that the upregulation of these UPR markers was due to ER localization of these new ORF45 isoforms. Although the role that these alternative ORF45 isoforms play during lytic replication remains unclear, silencing BiP expression significantly reduced production of progeny virions, despite having little impact on viral gene expression [160]. This suggests that BiP may be important for viral glycoprotein folding in the ER. However, BiP silencing also triggers ER stress and activates the UPR, so the diminished virion production may be due to increased UPR signaling rather than viral protein folding activity of BiP. Therefore, further investigations into the functions of the products of this viral gene locus are warranted.

9. Cytomegalovirus (CMV) and the UPR

Human CMV (HCMV) and murine CMV (MCMV) are betaherpesviruses that cause dramatic rearrangement of the cellular secretory pathway into a large perinuclear replication compartment that is visible by light microscopy[161–164]. Such changes would be expected to impact the ER proteostasis network and ER stress response. Multiple studies have shown that HCMV and MCMV subvert the UPR during infection, likely as a means to support robust lytic replication. The first comprehensive study of

Peer-reviewed version available at Viruses 2019, 12, 17; doi:10.3390/v12010017

UPR signaling in HMCV infection showed that the virus triggered IRE1-dependent splicing of *XBP1*, but the XBP1s gene target *EDEM* did not accumulate, suggesting a defect in downstream UPR transcription [165] (Figure 4). Likewise, HCMV infection activated PERK but elicited minimal eIF2α phosphorylation and bulk translation was largely unaffected; however, the ISR transcription factor ATF4 did accumulate, suggesting that HCMV exerts additional control over this arm of UPR. HCMV activation of PERK was also reported to promote virus replication through activation of SREBP1 by regulated intramembrane proteolysis, which then transactivates genes involved in lipid biosynthesis [166]. The mechanism of how HCMV infection triggers PERK-dependent SREBP1 cleavage is not known.

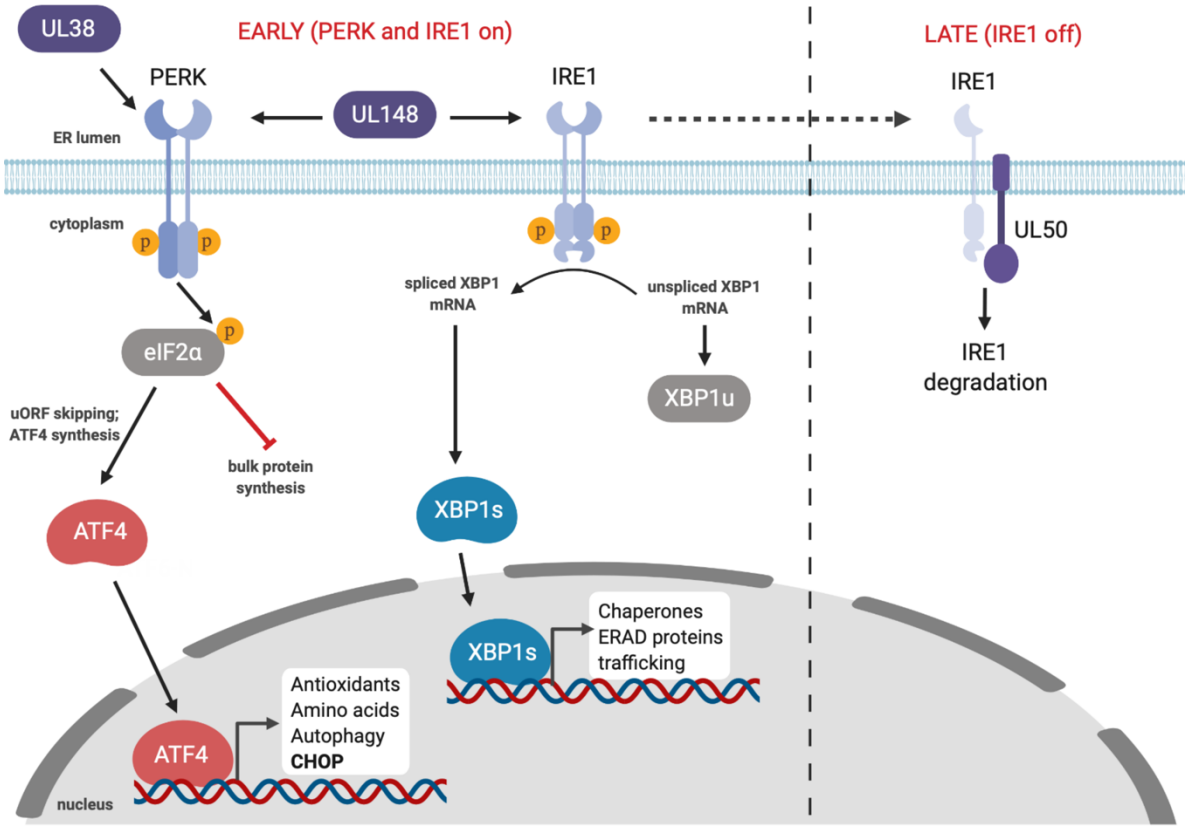


Figure 4. Differential control of the unfolded protein response by HCMV. In the early stages of HCMV lytic replication, PERK is activated by UL148 and UL38, which causes eIF2α phosphorylation and diminished global protein synthesis. At the same time, stress-dependent uORF skipping enables translation of the ATF4 bZIP transcription factor, which translocates to the nucleus and transactivates a variety of UPR genes. UL148 activates PERK as well as IRE1, which enables *XBP1* splicing and synthesis of the XBP1s bZIP transcription factor that transactivates a distinct set of UPR genes. Later in the lytic cycle, the UL50 protein binds and downregulates IRE1 through an unknown mechanism, effectively shutting down IRE1-dependent UPR gene expression.

Multiple HCMV proteins have been shown to influence UPR responses; UL38 triggers PERK phosphorylation, eIF2α phosphorylation and ATF4 synthesis, which promotes survival of infected cells [167] and the ER-resident UL148 protein also activates PERK and promotes ATF4 accumulation [168] (Figure 4). Beyond PERK activation, UL148 also triggers IRE1 activation and *XBP1* splicing. UL148 remodels the ER and recruits components of the ERAD machinery to discrete compartments [169], most likely as a mechanism to increase the stability of its glycoproteins, like gO [170]. This remodeling of the ER by UL148 may be responsible for activating the UPR via inhibiting proteasomal degradation of misfolded proteins. Interestingly, this ER remodeling is enhanced by PERK activation as treatment with ISRIB or a PERK inhibitor delayed the formation of UL148 foci [169]. This remodeling of the ER also appears to be an evolutionary distinct mechanism of human UL140 to control ER reorganization and UPR activation, as the chimpanzee of macaque UL148 do not perform this function [171].

MCMV control of PERK is less nuanced, with PERK activation causing robust eIF2α phosphorylation, attenuation of bulk translation, and ATF4 accumulation, which aids virus production [172]. However, MCMV infection does not induce the accumulation of the ATF4 target protein CHOP, suggesting that the virus may intervene at this downstream step in the pathway.

Interestingly, MCMV and HCMV induce IRE1 activation and *XBP1* splicing early in infection, but at later stages of lytic replication IRE1 is downregulated, which impedes *XBP1* splicing (Figure 3). This block in *XBP1* splicing was also observed following pharmacologic induction of ER stress. IRE1 downregulation is mediated by the orthologous type II transmembrane MCMV M50 and HCMV UL50 proteins [173]. These proteins are already known to assemble with M53 and UL53, respectively, and accumulate in the inner nuclear membrane, where they act as a nuclear egress complex that helps newly packaged capsids breach the nuclear envelope and access the cytoplasm [174]. M50 and UL50 are sufficient for IRE1 downregulation without their NEC partner proteins, but the precise mechanisms of IRE1 suppression remain unknown. It is also not known how IRE1 downregulation might provide an advantage for HCMV or MCMV at late stages of infection. Interestingly, release of MCMV progeny virions is diminished in XBP1-deficient cells [175] indicating that IRE1 activation early in the lytic cycle is likely required for efficient viral replication. Recently, Hinte *et al.* showed that the early IRE1 activation following MCMV infection previously observed is important for viral replication by depleting the cells of XBP1u [176]. They showed that *IRE1* knockout cells displayed reduced viral replication, which was restored in XBP1/IRE1 double knockout cells. XBP1u has a short half-life due to the presence of protein destabilizing domains in the C-terminus and can bind to XBP1s and ATF6 to promote proteasomal degradation [177,178]. Hinte *et al.* showed that XBP1s and ATF6 can bind to the promoter of the MCMV major immediate early protein (MIEP) and induce expression to promote early replication [176]. Therefore, activating IRE1 early in infection depletes the cell of XBP1u allowing XBP1s and ATF6 to transactivate the *MIEP* ORF.

HCMV lytic replication selectively activates PERK and IRE1, but not ATF6 [165]. Nevertheless, the ATF6 target gene BiP is upregulated at the protein level in infected cells, likely through IRES-dependent translation [179]. BiP was also shown to colocalize with HCMV replication compartments, and decreasing BiP levels by RNA silencing or treatment with SubAB Shiga toxin disrupts formation of the replication complex leading to a block in trafficking of viral nucleocapsids [180]. Therefore, it appears that HCMV may bypass ATF6 activation to upregulate BiP; potentially this is because there are other ATF6 target genes that are deleterious to HCMV.

Collectively, these studies indicate that PERK activation is pro-viral for both HCMV and MCMV via multiple mechanisms, but roles for IRE1 and ATF6 are less well understood and may differ significantly between these viruses.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 December 2019 doi:10.20944/preprints201912.0040.v1

Peer-reviewed version available at Viruses 2019, 12, 17; doi:10.3390/v12010017

10. HSV-1 and the UPR

HSV-1 is an alphaherpesvirus that also controls the UPR [181,182]. The best-known example of control over the UPR is that HSV-1 expresses an ortholog of GADD34 called γ34.5. Like GADD34, γ 34.5 binds and recruits protein phosphatase 1 (PP1) to eIF2α, causing eIF2α dephosphorylation to ensure ongoing bulk protein synthesis as a precaution that eIF2α kinases like PKR and PERK become activated during viral infection [182,183] (Figure 5). HSV-1 Us11 protein can bind dsRNA and block PKR activation [184]. Ian Mohr's group observed that a Us11 and γ34.5 double-knockout virus was still resistant to PERK activation in response to thapsigargin treatment, indicating that there may be other viral inhibitors of the UPR and/or the ISR that act directly on PERK [185]. They went on to show that the HSV-1 glycoprotein gB bound to PERK and this interaction was important for blocking eIF2α phosphorylation in response to stress, likely to enable viral protein translation to occur even in the presence of ER stress [186]. Thus, in contrast to CMV, PERK and activation of the ISR is likely inhibitory to HSV-1 replication.

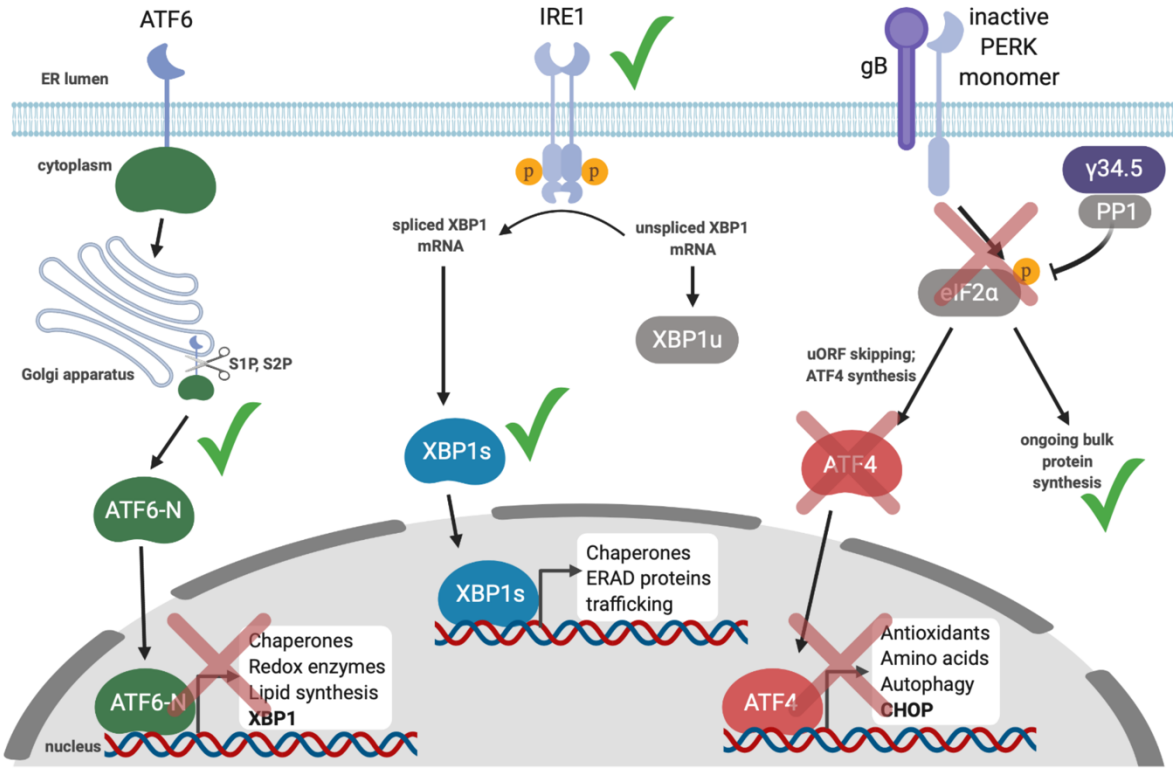


Figure 5. HSV-1 inhibits the UPR and the ISR. HSV-1 infection causes translocation of ATF6 to the Golgi apparatus, where is it cleaved by S1P and S2P to yield the ATF6-N bZIP transcription factor. Despite this, ATF6-N responsive genes are not transcribed in HSV-1 infected cells. HSV-1 activates IRE1, triggers *XBP1* splicing and XBP1s synthesis, and subsequent XBP1s-dependent transcription. By contrast, HSV-1 glycoprotein B (gB) binds to PERK and prevents its activation, thereby limiting eIF2α phosphorylation and maintaining bulk protein synthesis. HSV-1 γ34.5 protein acts similarly to GADD34, recruiting the cellular PP1 phosphatase to eIF2a and reinforcing the blockade on ISR activation. As a result, ATF4 is not synthesized and ATF4-responsive genes remain dormant.

HSV-1 replication has been shown to induce ATF6 cleavage but there is no impact on the ATF6 target gene BiP suggesting that HSV-1 blocks signaling of the ATF6 branch of the UPR [181]. IRE1 has also been reported to be activated during HSV-1 infection but its RNase activity is suppressed through an unknown mechanism [187]. Alternatively, IRE1 kinase activity and the IRE1-dependent phosphorylation

of JNK was reported to promote virus replication, whereas XBP1s overexpression inhibits virus production. This suggests that HSV-1 directly fine-tunes IRE1 signaling to suppress XBP1s activation while promoting activation of the JNK pathway in order to promote HSV-1 replication [187]. Since XBP1s inhibits HSV-1, restoring IRE1 RNase activity may be one potential avenue of investigation for inhibiting HSV-1.

12. Conclusions and outstanding questions

The UPR is an evolutionarily conserved cellular stress response that senses and responds to perturbations in ER protein homeostasis. Acute or chronic disruptions in ER protein folding can have drastic effects on the host if gone unchecked. Therefore, in response to increases in ER protein misfolding the UPR transiently attenuates protein translation and selectively upregulates stress-responsive genes through the combined action of the transcription factors ATF4, XBP1 and ATF6. The end goal is to try to increase protein folding capabilities and degrade terminally misfolded proteins. Enveloped viruses may strain the protein folding machinery in the ER due to requirements for viral glycoprotein synthesis. As a result, these viruses likely have evolved mechanisms to ensure that different signaling responses of the UPR do not disrupt viral replication. Each of the branches of the UPR has a different transcriptional response while also coordinating expression of different genes. Depending on the virus in question, the induced UPR genes could either be proviral or antiviral. Herpesviruses are large enveloped viruses and are one family of viruses that appear to have evolved to directly usurp UPR signaling pathways likely to dampen the antiviral transcriptional responses while simultaneously promoting transcription of genes that promote virus replication. The pathways that are activated or inhibited greatly depend on the specific herpesvirus as well as the cell model being used.

UPR activation is the most reliable way to determine if a cell is experiencing ER stress. Concurrent activation of all three UPR sensors during infection suggests that the virus is inducing *bona fide* ER stress that causes BiP displacement from UPR sensor proteins. Alternatively, selective activation of UPR sensors could result from engagement with viral gene products in an ER stress-independent fashion. The development of new biochemical assays and reporter assays to detect ER protein misfolding will be helpful in differentiating between UPR-stimulating and UPR-inhibiting viral gene products. These studies will also be aided by the recent revolution in single-cell analysis of gene expression, which will provide richer information about the UPR transcriptional output during infection.

Potent ER stress-inducing drugs thapsigargin and tunicamycin have played a large role to date in UPR studies, but these drugs are often used at doses that do not recapitulate physiologic ER stress, and they also have known off-target effects. Thus, for studies that show modest activation of UPR sensors during virus infection and little UPR transcriptional output, it is unclear if UPR activation is too modest to elicit the anticipated transcriptional responses, or if the widespread use of thapsigargin and tunicamycin has left us with unreasonable expectations of transcriptional output. These studies will benefit from the development of more sensitive assays of UPR output and employing control molecules that more faithfully recapitulate the impact of infection stress on the ER.

As described above, there are links between UPR and innate and adaptive arms of the immune response. In this light, it is likely that herpesviral UPR modulation not only aids lytic viral replication but also aids viral evasion of immune surveillance. Improved understanding of the fundamental mechanistic links between the UPR and immunity should inform future studies of viral UPR subversion. Conversely, the study of UPR-modulating viral proteins could provide new tools to investigate functional interplay between the UPR and immunity.

Author Contributions: writing—original draft preparation, B.P.J; writing—review and editing, B.P.J., C.M.

Funding: This work was supported by Canadian Institutes for Health Research Operating Grant MOP-84554.

Acknowledgments: We thank members of the McCormick laboratory for helpful discussions.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the writing of the manuscript.

References

1. Klaips, C.L.; Jayaraj, G.G.; Hartl, F.U. Pathways of cellular proteostasis in aging and disease. *J. Cell Biol.* **2018**, *217*, 51–63.
2. Walter, P.; Ron, D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **2011**, *334*, 1081–1086.
3. Bertolotti, A.; Zhang, Y.; Hendershot, L.M.; Harding, H.P.; Ron, D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* **2000**, *2*, 326–332.
4. Ma, K.; Vattem, K.M.; Wek, R.C. Dimerization and Release of Molecular Chaperone Inhibition Facilitate Activation of Eukaryotic Initiation Factor-2 Kinase in Response to Endoplasmic Reticulum Stress. *J. Biol. Chem.* **2002**, *277*, 18728–18735.
5. Gething, M.J.; McCammon, K.; Sambrook, J. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell* **1986**, *46*, 939–50.
6. Peluso, R.W.; Lamb, R.A.; Choppin, P.W. Infection with paramyxoviruses stimulates synthesis of cellular polypeptides that are also stimulated in cells transformed by Rous sarcoma virus or deprived of glucose. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75*, 6120–4.
7. Credle, J.J.; Finer-Moore, J.S.; Papa, F.R.; Stroud, R.M.; Walter, P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc. Natl. Acad. Sci.* **2005**, *102*, 18773–18784.
8. Bertolotti, A.; Wang, X.; Novoa, I.; Jungreis, R.; Schlessinger, K.; Cho, J.H.; West, A.B.; Ron, D. Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. *J. Clin. Invest.* **2001**, *107*, 585–593.
9. Lee, K.P.K.; Dey, M.; Neculai, D.; Cao, C.; Dever, T.E.; Sicheri, F. Structure of the Dual Enzyme Ire1 Reveals the Basis for Catalysis and Regulation in Nonconventional RNA Splicing. *Cell* **2008**, *132*, 89–100.
10. Welihinda, A.A.; Kaufman, R.J. The unfolded protein response pathway in Saccharomyces cerevisiae. Oligomerization and trans-phosphorylation of Ire1p (Ern1p) are required for kinase activation. *J. Biol. Chem.* **1996**, *271*, 18181–18187.
11. Sidrauski, C.; Walter, P. The Transmembrane Kinase Ire1p Is a Site-Specific Endonuclease That Initiates mRNA Splicing in the Unfolded Protein Response. *Cell* **1997**, *90*, 1031–1039.
12. Calfon, M.; Zeng, H.; Urano, F.; Till, J.H.; Hubbard, S.R.; Harding, H.P.; Clark, S.G.; Ron, D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **2002**, *415*, 92–96.
13. Yoshida, H.; Matsui, T.; Yamamoto, A.; Okada, T.; Mori, K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **2001**, *107*, 881-891.
14. Jurkin, J.; Henkel, T.; Nielsen, A.F.; Minnich, M.; Popow, J.; Kaufmann, T.; Heindl, K.; Hoffmann, T.; Busslinger, M.; Martinez, J. The mammalian tRNA ligase complex mediates splicing of *XBP1* mRNA and controls antibody secretion in plasma cells. *EMBO J.* **2014**, *33*, 2922–2936.
15. Lu, Y.; Liang, F.-X.; Wang, X. A Synthetic Biology Approach Identifies the Mammalian UPR RNA Ligase RtcB. *Mol. Cell* **2014**, *55*, 758–770.
16. Kosmaczewski, S.G.; Edwards, T.J.; Han, S.M.; Eckwahl, M.J.; Meyer, B.I.; Peach, S.; Hesselberth, J.R.; Wolin, S.L.; Hammarlund, M. The RtcB RNA ligase is an essential component of the metazoan unfolded protein response. *EMBO Rep.* **2014**, *15*, 1278–85.
17. Ron, D.; Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 519–529.
18. Yamamoto, K.; Yoshida, H.; Kokame, K.; Kaufman, R.J.; Mori, K. Differential Contributions of ATF6 and XBP1 to the Activation of Endoplasmic Reticulum Stress-Responsive cis-Acting Elements ERSE, UPRE and ERSE-II. *J. Biochem.* **2004**, *136*, 343–350.
19. Hollien, J.; Lin, J.H.; Li, H.; Stevens, N.; Walter, P.; Weissman, J.S. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biol.* **2009**.
20. Hollien, J.; Weissman, J.S. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **2006**, *313*, 104–7.

21. Maurel, M.; Chevet, E.; Tavernier, J.; Gerlo, S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* **2014**, *39*, 245–254.
22. Tam, A.B.; Koong, A.C.; Niwa, M. Ire1 has distinct catalytic mechanisms for XBP1/HAC1 splicing and RIDD. *Cell Rep.* **2014**, *9*, 850–8.
23. Sonenberg, N.; Hinnebusch, A.G. Regulation of Translation Initiation in Eukaryotes: Mechanisms and Biological Targets. *Cell* **2009**, *136*, 731–745.
24. Kashiwagi, K.; Takahashi, M.; Nishimoto, M.; Hiyama, T.B.; Higo, T.; Umehara, T.; Sakamoto, K.; Ito, T.; Yokoyama, S. Crystal structure of eukaryotic translation initiation factor 2B. *Nature* **2016**, *531*, 122–125.
25. Pakos-Zebrucka, K.; Koryga, I.; Mnich, K.; Ljujic, M.; Samali, A.; Gorman, A.M. The integrated stress response. *EMBO Rep.* **2016**, *17*, 1374–1395.
26. Taniuchi, S.; Miyake, M.; Tsugawa, K.; Oyadomari, M.; Oyadomari, S. Integrated stress response of vertebrates is regulated by four eIF2α kinases. *Sci. Rep.* **2016**, *6*, 32886.
27. Krishnamoorthy, T.; Pavitt, G.D.; Zhang, F.; Dever, T.E.; Hinnebusch, A.G. Tight Binding of the Phosphorylated Subunit of Initiation Factor 2 (eIF2) to the Regulatory Subunits of Guanine Nucleotide Exchange Factor eIF2B Is Required for Inhibition of Translation Initiation. *Mol. Cell. Biol.* **2001**, *21*, 5018–5030.
28. Pavitt, G.D.; Ramaiah, K. V; Kimball, S.R.; Hinnebusch, A.G. eIF2 independently binds two distinct eIF2B subcomplexes that catalyze and regulate guanine-nucleotide exchange. *Genes Dev.* **1998**, *12*, 514–26.
29. Harding, H.P.; Zhang, Y.; Zeng, H.; Novoa, I.; Lu, P.D.; Calfon, M.; Sadri, N.; Yun, C.; Popko, B.; Paules, R.; et al. An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress. *Mol. Cell* **2003**, *11*, 619–633.
30. Cui, W.; Li, J.; Ron, D.; Sha, B. The structure of the PERK kinase domain suggests the mechanism for its activation. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2011**, *67*, 423–428.
31. Harding, H.P.; Zhang, Y.; Ron, D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **1999**, *397*, 271–274.
32. Ma, Y.; Brewer, J.W.; Alan Diehl, J.; Hendershot, L.M. Two Distinct Stress Signaling Pathways Converge Upon the CHOP Promoter During the Mammalian Unfolded Protein Response. *J. Mol. Biol.* **2002**, *318*, 1351–1365.
33. Harding, H.P.; Novoa, I.; Zhang, Y.; Zeng, H.; Wek, R.; Schapira, M.; Ron, D. Regulated Translation Initiation Controls Stress-Induced Gene Expression in Mammalian Cells. *Mol. Cell* **2000**, *6*, 1099–1108.
34. Vattem, K.M.; Wek, R.C. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 11269–74.
35. Hai, T.; Hartman, M.G. The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene* **2001**, *273*, 1–11.
36. Jiang, H.-Y.; Wek, S.A.; McGrath, B.C.; Lu, D.; Hai, T.; Harding, H.P.; Wang, X.; Ron, D.; Cavener, D.R.; Wek, R.C. Activating transcription factor 3 is integral to the eukaryotic initiation factor 2 kinase stress response. *Mol. Cell. Biol.* **2004**, *24*, 1365–77.
37. Gachon, F.; Gaudray, G.; Thébault, S.; Basbous, J.; Koffi, J.A.; Devaux, C.; Mesnard, J. The cAMP response element binding protein-2 (CREB-2) can interact with the C/EBP-homologous protein (CHOP). *FEBS Lett.* **2001**, *502*, 57–62.
38. Han, J.; Back, S.H.; Hur, J.; Lin, Y.-H.; Gildersleeve, R.; Shan, J.; Yuan, C.L.; Krokowski, D.; Wang, S.; Hatzoglou, M.; et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat. Cell Biol.* **2013**, *15*, 481–490.
39. Sekine, Y.; Zyryanova, A.; Crespillo-Casado, A.; Fischer, P.M.; Harding, H.P.; Ron, D. Stress responses. Mutations in a translation initiation factor identify the target of a memory-enhancing compound. *Science* **2015**, *348*, 1027–1030.
40. Sidrauski, C.; McGeachy, A.M.; Ingolia, N.T.; Walter, P. The small molecule ISRIB reverses the effects of eIF2α phosphorylation on translation and stress granule assembly. *Elife* **2015**, *4*.
41. Sidrauski, C.; Acosta-Alvear, D.; Khoutorsky, A.; Vedantham, P.; Hearn, B.R.; Li, H.; Gamache, K.; Gallagher, C.M.; Ang, K.K.-H.; Wilson, C.; et al. Pharmacological brake-release of mRNA translation enhances cognitive memory. *Elife* **2013**, *2*, e00498.
42. Zyryanova, A.F.; Weis, F.; Faille, A.; Akeel, †; Alard, A.; Crespillo-Casado, A.; Sekine, Y.; Harding, H.P.; Allen, F.; Parts, L.; et al. Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. *Science* **2018**, *359*, 1533-1536.
43. Marciniak, S.J.; Yun, C.Y.; Oyadomari, S.; Novoa, I.; Zhang, Y.; Jungreis, R.; Nagata, K.; Harding, H.P.; Ron, D.

CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* **2004**, *18*, 3066–3077.

44. Novoa, I.; Zeng, H.; Harding, H.P.; Ron, D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J. Cell Biol.* **2001**, *153*, 1011–22.
45. Jousse, C.; Bruhat, A.; Carraro, V.; Urano, F.; Ferrara, M.; Ron, D.; Fafournoux, P. Inhibition of CHOP translation by a peptide encoded by an open reading frame localized in the chop 5'UTR. *Nucleic Acids Res.* **2001**, *29*, 4341–51.
46. Palam, L.R.; Baird, T.D.; Wek, R.C. Phosphorylation of eIF2 Facilitates Ribosomal Bypass of an Inhibitory Upstream ORF to Enhance *CHOP* Translation. *J. Biol. Chem.* **2011**, *286*, 10939–10949.
47. Lee, Y.-Y.; Cevallos, R.C.; Jan, E. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. *J. Biol. Chem.* **2009**, *284*, 6661–73.
48. Haze, K.; Okada, T.; Yoshida, H.; Yanagi, H.; Yura, T.; Negishi, M.; Mori, K. Identification of the G13 (cAMP-response-element-binding protein-related protein) gene product related to activating transcription factor 6 as a transcriptional activator of the mammalian unfolded protein response. *Biochem. J.* **2001**, *355*, 19–28.
49. Haze, K.; Yoshida, H.; Yanagi, H.; Yura, T.; Mori, K. Mammalian Transcription Factor ATF6 Is Synthesized as a Transmembrane Protein and Activated by Proteolysis in Response to Endoplasmic Reticulum Stress. *Mol. Biol. Cell* **1999**, *10*, 3787–3799.
50. Zhu, C.; Johansen, F.E.; Prywes, R. Interaction of ATF6 and serum response factor. *Mol. Cell. Biol.* **1997**, *17*, 4957–66.
51. Yamamoto, K.; Sato, T.; Matsui, T.; Sato, M.; Okada, T.; Yoshida, H.; Harada, A.; Mori, K. Transcriptional Induction of Mammalian ER Quality Control Proteins Is Mediated by Single or Combined Action of ATF6α and XBP1. *Dev. Cell* **2007**, *13*, 365–376.
52. Shen, J.; Snapp, E.L.; Lippincott-Schwartz, J.; Prywes, R. Stable Binding of ATF6 to BiP in the Endoplasmic Reticulum Stress Response. *Mol. Cell. Biol.* **2005**, *25*, 921–932.
53. Nadanaka, S.; Okada, T.; Yoshida, H.; Mori, K. Role of Disulfide Bridges Formed in the Luminal Domain of ATF6 in Sensing Endoplasmic Reticulum Stress. *Mol. Cell. Biol.* **2007**, *27*, 1027–1043.
54. Schindler, A.J.; Schekman, R. In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles. *Proc. Natl. Acad. Sci.* **2009**, *106*, 17775–17780.
55. Ye, J.; Rawson, R.B.; Komuro, R.; Chen, X.; Davé, U.P.; Prywes, R.; Brown, M.S.; Goldstein, J.L. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* **2000**, *6*, 1355–64.
56. Kokame, K.; Kato, H.; Miyata, T. Identification of ERSE-II, a New *cis* -Acting Element Responsible for the ATF6-dependent Mammalian Unfolded Protein Response. *J. Biol. Chem.* **2001**, *276*, 9199–9205.
57. Shoulders, M.D.; Ryno, L.M.; Genereux, J.C.; Moresco, J.J.; Tu, P.G.; Wu, C.; Yates, J.R.; Su, A.I.; Kelly, J.W.; Wiseman, R.L. Stress-Independent Activation of XBP1s and/or ATF6 Reveals Three Functionally Diverse ER Proteostasis Environments. *Cell Rep.* **2013**, *3*, 1279–1292.
58. Yoshida, H.; Okada, T.; Haze, K.; Yanagi, H.; Yura, T.; Negishi, M.; Mori, K. Endoplasmic Reticulum Stress-Induced Formation of Transcription Factor Complex ERSF Including NF-Y (CBF) and Activating Transcription Factors 6 and 6 That Activates the Mammalian Unfolded Protein Response. *Mol. Cell. Biol.* **2001**, *21*, 1239–1248.
59. Li, M.; Baumeister, P.; Roy, B.; Phan, T.; Foti, D.; Luo, S.; Lee, A.S. ATF6 as a transcription activator of the endoplasmic reticulum stress element: thapsigargin stress-induced changes and synergistic interactions with NF-Y and YY1. *Mol. Cell. Biol.* **2000**, *20*, 5096–5106.
60. Wu, J.; Rutkowski, D.T.; Dubois, M.; Swathirajan, J.; Saunders, T.; Wang, J.; Song, B.; Yau, G.D.-Y.; Kaufman, R.J. ATF6α Optimizes Long-Term Endoplasmic Reticulum Function to Protect Cells from Chronic Stress. *Dev. Cell* **2007**, *13*, 351–364.
61. Shiu, R.P.C.; Pouyssegur, J.; Pastan, I. Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts. *Proc. Natl. Acad. Sci.* **1977**, *74*, 3840–3844.
62. Yoshida, H.; Okada, T.; Haze, K.; Yanagi, H.; Yura, T.; Negishi, M.; Mori, K. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol. Cell. Biol.* **2000**, *20*, 6755–67.
63. Ma, Y.; Brewer, J.W.; Diehl, J.A.; Hendershot, L.M. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J. Mol. Biol.* **2002**, *318*, 1351–65.
64. Sato, Y.; Nadanaka, S.; Okada, T.; Okawa, K.; Mori, K. Luminal domain of ATF6 alone is sufficient for sensing endoplasmic reticulum stress and subsequent transport to the Golgi apparatus. *Cell Struct. Funct.* **2011**, *36*, 35–47.

65. Gallagher, C.M.; Walter, P. Ceapins inhibit ATF6α signaling by selectively preventing transport of ATF6α to the Golgi apparatus during ER stress. *Elife* **2016**, *5*, e11880.
66. Yang, T.; Espenshade, P.J.; Wright, M.E.; Yabe, D.; Gong, Y.; Aebersold, R.; Goldstein, J.L.; Brown, M.S. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* **2002**, *110*, 489–500.
67. Asada, R.; Kanemoto, S.; Kondo, S.; Saito, A.; Imaizumi, K. The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology. *J. Biochem.* **2011**, *149*, 507–518.
68. Lin, J.H.; Li, H.; Yasumura, D.; Cohen, H.R.; Zhang, C.; Panning, B.; Shokat, K.M.; Lavail, M.M.; Walter, P. IRE1 signaling affects cell fate during the unfolded protein response. *Science* **2007**, *318*, 944–949.
69. Chang, T.-K.; Lawrence, D.A.; Lu, M.; Tan, J.; Harnoss, J.M.; Marsters, S.A.; Liu, P.; Sandoval, W.; Martin, S.E.; Ashkenazi, A. Coordination between two branches of the unfolded protein response determines apoptotic cell fate. *Mol. Cell* **2018**, *71*, 629-636.
70. Byrd, A.E.; Aragon, I. V.; Brewer, J.W. MicroRNA-30c-2* limits expression of proadaptive factor XBP1 in the unfolded protein response. *J. Cell Biol.* **2012**, *196*, 689–698.
71. Amin-Wetzel, N.; Saunders, R.A.; Kamphuis, M.J.; Rato, C.; Preissler, S.; Harding, H.P.; Ron, D. A J-Protein Co-chaperone Recruits BiP to Monomerize IRE1 and Repress the Unfolded Protein Response. *Cell* **2017**, *171*, 1625-1637.e13.
72. Yan, W.; Frank, C.L.; Korth, M.J.; Sopher, B.L.; Novoa, I.; Ron, D.; Katze, M.G. Control of PERK eIF2 kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. *Proc. Natl. Acad. Sci.* **2002**, *99*, 15920–15925.
73. Polyak, S.J.; Tang, N.; Wambach, M.; Barber, G.N.; Katze, M.G. The P58 cellular inhibitor complexes with the interferon-induced, double-stranded RNA-dependent protein kinase, PKR, to regulate its autophosphorylation and activity. *J. Biol. Chem.* **1996**, *271*, 1702–7.
74. Rutkowski, D.T.; Kang, S.-W.; Goodman, A.G.; Garrison, J.L.; Taunton, J.; Katze, M.G.; Kaufman, R.J.; Hegde, R.S. The role of p58IPK in protecting the stressed endoplasmic reticulum. *Mol. Biol. Cell* **2007**, *18*, 3681–91.
75. Tsuru, A.; Imai, Y.; Saito, M.; Kohno, K. Novel mechanism of enhancing IRE1alpha-XBP1 signalling via the PERK-ATF4 pathway. *Sci. Rep.* **2016**, *6*, 24217.
76. Majumder, M.; Huang, C.; Snider, M.D.; Komar, A.A.; Tanaka, J.; Kaufman, R.J.; Krokowski, D.; Hatzoglou, M. A novel feedback loop regulates the response to endoplasmic reticulum stress via the cooperation of cytoplasmic splicing and mRNA translation. *Mol. Cell. Biol.* **2012**, *32*, 992–1003.
77. Luo, S.; Baumeister, P.; Yang, S.; Abcouwer, S.F.; Lee, A.S. Induction of Grp78/BiP by Translational Block. *J. Biol. Chem.* **2003**, *278*, 37375–37385.
78. Starck, S.R.; Tsai, J.C.; Chen, K.; Shodiya, M.; Wang, L.; Yahiro, K.; Martins-Green, M.; Shastri, N.; Walter, P. Translation from the 5′ untranslated region shapes the integrated stress response. *Science* **2016**, *351*, aad3867–aad3867.
79. Komar, A.A.; Gross, S.R.; Barth-Baus, D.; Strachan, R.; Hensold, J.O.; Goss Kinzy, T.; Merrick, W.C. Novel Characteristics of the Biological Properties of the Yeast *Saccharomyces cerevisiae* Eukaryotic Initiation Factor 2A. *J. Biol. Chem.* **2005**, *280*, 15601–15611.
80. Rutkowski, D.T.; Arnold, S.M.; Miller, C.N.; Wu, J.; Li, J.; Gunnison, K.M.; Mori, K.; Sadighi Akha, A.A.; Raden, D.; Kaufman, R.J. Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. *PLoS Biol.* **2006**, *4*, e374.
81. Hetz, C.; Papa, F.R. The Unfolded Protein Response and Cell Fate Control. *Mol. Cell* **2018**, *69*, 169–181.
82. Nishitoh, H.; Matsuzawa, A.; Tobiume, K.; Saegusa, K.; Takeda, K.; Inoue, K.; Hori, S.; Kakizuka, A.; Ichijo, H. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev.* **2002**, *16*, 1345–55.
83. Urano, F.; Wang, X.; Bertolotti, A.; Zhang, Y.; Chung, P.; Harding, H.P.; Ron, D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **2000**, *287*, 664–6.
84. McCullough, K.D.; Martindale, J.L.; Klotz, L.O.; Aw, T.Y.; Holbrook, N.J. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol. Cell. Biol.* **2001**, *21*, 1249–59.
85. Puthalakath, H.; O'Reilly, L.A.; Gunn, P.; Lee, L.; Kelly, P.N.; Huntington, N.D.; Hughes, P.D.; Michalak, E.M.; McKimm-Breschkin, J.; Motoyama, N.; et al. ER Stress Triggers Apoptosis by Activating BH3-Only Protein Bim. *Cell* **2007**, *129*, 1337–1349.
86. Upton, J.-P.; Wang, L.; Han, D.; Wang, E.S.; Huskey, N.E.; Lim, L.; Truitt, M.; McManus, M.T.; Ruggero, D.; Goga, A.; et al. IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic

Caspase-2. *Science* **2012**, *338*, 818–22.
87. Upton, J.-P.; Austgen, K.; Nishino, M.; Coakley, K.M.; Hagen, A.; Han, D.; Papa, F.R.; Oakes, S.A. Caspase-2 Cleavage of BID Is a Critical Apoptotic Signal Downstream of Endoplasmic Reticulum Stress. *Mol. Cell. Biol.* **2008**, *28*, 3943–3951.
88. Lu, M.; Lawrence, D.A.; Marsters, S.; Acosta-Alvear, D.; Kimmig, P.; Mendez, A.S.; Paton, A.W.; Paton, J.C.; Walter, P.; Ashkenazi, A. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. *Science* **2014**, *345*, 98–101.
89. Glab, J.A.; Doerflinger, M.; Nedeva, C.; Jose, I.; Mbogo, G.W.; Paton, J.C.; Paton, A.W.; Kueh, A.J.; Herold, M.J.; Huang, D.C.; et al. DR5 and caspase-8 are dispensable in ER stress-induced apoptosis. *Cell Death Differ.* **2017**, *24*, 944–950.
90. Yamaguchi, H.; Wang, H.-G. CHOP Is Involved in Endoplasmic Reticulum Stress-induced Apoptosis by Enhancing DR5 Expression in Human Carcinoma Cells. *J. Biol. Chem.* **2004**, *279*, 45495–45502.
91. Li, G.; Mongillo, M.; Chin, K.-T.; Harding, H.; Ron, D.; Marks, A.R.; Tabas, I. Role of ERO1-α–mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress–induced apoptosis. *J. Cell Biol.* **2009**, *186*, 783–792.
92. Hajnóczky, G.; Davies, E.; Madesh, M. Calcium signaling and apoptosis. *Biochem. Biophys. Res. Commun.* **2003**, *304*, 445–54.
93. Michalak, M.; Robert Parker, J.M.; Opas, M. Ca2+ signaling and calcium binding chaperones of the endoplasmic reticulum. *Cell Calcium* **2002**, *32*, 269–78.
94. Szegezdi, E.; Logue, S.E.; Gorman, A.M.; Samali, A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep.* **2006**, *7*, 880–885.
95. Tsaytler, P.; Harding, H.P.; Ron, D.; Bertolotti, A. Selective Inhibition of a Regulatory Subunit of Protein Phosphatase 1 Restores Proteostasis. *Science* **2011**, *332*, 91–94.
96. Wang, S.; Kaufman, R.J. The impact of the unfolded protein response on human disease. *J. Cell Biol.* **2012**, *197*, 857–867.
97. Reimold, A.M.; Iwakoshi, N.N.; Manis, J.; Vallabhajosyula, P.; Szomolanyi-Tsuda, E.; Gravallese, E.M.; Friend, D.; Grusby, M.J.; Alt, F.; Glimcher, L.H. Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **2001**, *412*, 300–307.
98. Akiyama, M.; Liew, C.W.; Lu, S.; Hu, J.; Martinez, R.; Hambro, B.; Kennedy, R.T.; Kulkarni, R.N. X-Box Binding Protein 1 Is Essential for Insulin Regulation of Pancreatic -Cell Function. *Diabetes* **2013**, *62*, 2439–2449.
99. Bettigole, S.E.; Lis, R.; Adoro, S.; Lee, A.-H.; Spencer, L.A.; Weller, P.F.; Glimcher, L.H. The transcription factor XBP1 is selectively required for eosinophil differentiation. *Nat. Immunol.* **2015**, *16*, 829–837.
100. Adolph, T.E.; Tomczak, M.F.; Niederreiter, L.; Ko, H.-J.; Böck, J.; Martinez-Naves, E.; Glickman, J.N.; Tschurtschenthaler, M.; Hartwig, J.; Hosomi, S.; et al. Paneth cells as a site of origin for intestinal inflammation. *Nature* **2013**, *503*, 272–276.
101. Elphick, D.A.; Mahida, Y.R. Paneth cells: their role in innate immunity and inflammatory disease. *Gut* **2005**, *54*, 1802–1809.
102. Iwakoshi, N.N.; Lee, A.-H.; Vallabhajosyula, P.; Otipoby, K.L.; Rajewsky, K.; Glimcher, L.H. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nat. Immunol.* **2003**, *4*, 321–329.
103. Shaffer, A.L.; Shapiro-Shelef, M.; Iwakoshi, N.N.; Lee, A.-H.; Qian, S.-B.; Zhao, H.; Yu, X.; Yang, L.; Tan, B.K.; Rosenwald, A.; et al. XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* **2004**, *21*, 81–93.
104. Carrasco, D.R.; Sukhdeo, K.; Protopopova, M.; Sinha, R.; Enos, M.; Carrasco, D.E.; Zheng, M.; Mani, M.; Henderson, J.; Pinkus, G.S.; et al. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. *Cancer Cell* **2007**, *11*, 349–60.
105. Chen, X.; Iliopoulos, D.; Zhang, Q.; Tang, Q.; Greenblatt, M.B.; Hatziapostolou, M.; Lim, E.; Tam, W.L.; Ni, M.; Chen, Y.; et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway. *Nature* **2014**, *508*, 103–107.
106. Song, M.; Sandoval, T.A.; Chae, C.-S.; Chopra, S.; Tan, C.; Rutkowski, M.R.; Raundhal, M.; Chaurio, R.A.; Payne, K.K.; Konrad, C.; et al. IRE1α–XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. *Nature* **2018**, *562*, 423–428.
107. Roussel, B.D.; Kruppa, A.J.; Miranda, E.; Crowther, D.C.; Lomas, D.A.; Marciniak, S.J. Endoplasmic reticulum dysfunction in neurological disease. *Lancet Neurol.* **2013**, *12*, 105–118.
108. Back, S.H.; Kaufman, R.J. Endoplasmic reticulum stress and type 2 diabetes. *Annu. Rev. Biochem.* **2012**, *81*, 767–

93.

109. Tavernier, S.J.; Osorio, F.; Vandersarren, L.; Vetters, J.; Vanlangenakker, N.; Van Isterdael, G.; Vergote, K.; De Rycke, R.; Parthoens, E.; van de Laar, L.; et al. Regulated IRE1-dependent mRNA decay sets the threshold for dendritic cell survival. *Nat. Cell Biol.* **2017**, *19*, 698–710.
110. Cubillos-Ruiz, J.R.; Silberman, P.C.; Rutkowski, M.R.; Chopra, S.; Perales-Puchalt, A.; Song, M.; Zhang, S.; Bettigole, S.E.; Gupta, D.; Holcomb, K.; et al. ER Stress Sensor XBP1 Controls Anti-tumor Immunity by Disrupting Dendritic Cell Homeostasis. *Cell* **2015**, *161*, 1527–1538.
111. Herber, D.L.; Cao, W.; Nefedova, Y.; Novitskiy, S. V; Nagaraj, S.; Tyurin, V.A.; Corzo, A.; Cho, H.-I.; Celis, E.; Lennox, B.; et al. Lipid accumulation and dendritic cell dysfunction in cancer. *Nat. Med.* **2010**, *16*, 880–886.
112. Kamimura, D.; Bevan, M.J. Endoplasmic reticulum stress regulator XBP-1 contributes to effector CD8+ T cell differentiation during acute infection. *J. Immunol.* **2008**, *181*, 5433–41.
113. Martinon, F.; Chen, X.; Lee, A.-H.; Glimcher, L.H. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* **2010**, *11*, 411–8.
114. Qiu, Q.; Zheng, Z.; Chang, L.; Zhao, Y.-S.; Tan, C.; Dandekar, A.; Zhang, Z.; Lin, Z.; Gui, M.; Li, X.; et al. Toll-like receptor-mediated IRE1α activation as a therapeutic target for inflammatory arthritis. *EMBO J.* **2013**, *32*, 2477–90.
115. Woo, C.W.; Cui, D.; Arellano, J.; Dorweiler, B.; Harding, H.; Fitzgerald, K.A.; Ron, D.; Tabas, I. Adaptive suppression of the ATF4–CHOP branch of the unfolded protein response by toll-like receptor signalling. *Nat. Cell Biol.* **2009**, *11*, 1473–1480.
116. Woo, C.W.; Kutzler, L.; Kimball, S.R.; Tabas, I. Toll-like receptor activation suppresses ER stress factor CHOP and translation inhibition through activation of eIF2B. *Nat. Cell Biol.* **2012**, *14*, 192–200.
117. Roulston, A.; Marcellus, R.C.; Branton, P.E. Viruses and apoptosis. *Annu. Rev. Microbiol.* **1999**, *53*, 577–628.
118. Senft, D.; Ronai, Z.A. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem. Sci.* **2015**, *40*, 141–8.
119. Speck, S.H.; Ganem, D. Viral Latency and Its Regulation: Lessons from the γ-Herpesviruses. *Cell Host Microbe* **2010**, *8*, 100–115.
120. Bhende, P.M.; Dickerson, S.J.; Sun, X.; Feng, W.-H.; Kenney, S.C. X-Box-Binding Protein 1 Activates Lytic Epstein-Barr Virus Gene Expression in Combination with Protein Kinase D. *J. Virol.* **2007**, *81*, 7363–7370.
121. Matar, C.G.; Rangaswamy, U.S.; Wakeman, B.S.; Iwakoshi, N.; Speck, S.H. Murine gammaherpesvirus 68 reactivation from B cells requires IRF4 but not XBP-1. *J. Virol.* **2014**, *88*, 11600–10.
122. Wilson, S.J.; Tsao, E.H.; Webb, B.L.J.; Ye, H.; Dalton-Griffin, L.; Tsantoulas, C.; Gale, C. V; Du, M.-Q.; Whitehouse, A.; Kellam, P. X Box Binding Protein XBP-1s Transactivates the Kaposi's Sarcoma-Associated Herpesvirus (KSHV) ORF50 Promoter, Linking Plasma Cell Differentiation to KSHV Reactivation from Latency. *J. Virol.* **2007**, *81*, 13578–13586.
123. Yu, F.; Feng, J.; Harada, J.N.; Chanda, S.K.; Kenney, S.C.; Sun, R. B cell terminal differentiation factor XBP-1 induces reactivation of Kaposi's sarcoma-associated herpesvirus. *FEBS Lett.* **2007**, *581*, 3485–3488.
124. Lukac, D.M.; Renne, R.; Kirshner, J.R.; Ganem, D. Reactivation of Kaposi's sarcoma-associated herpesvirus infection from latency by expression of the ORF 50 transactivator, a homolog of the EBV R protein. *Virology* **1998**, *252*, 304–312.
125. Sun, R.; Lin, S.F.; Gradoville, L.; Yuan, Y.; Zhu, F.; Miller, G. A viral gene that activates lytic cycle expression of Kaposi's sarcoma-associated herpesvirus. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 10866–10871.
126. Dalton-Griffin, L.; Wilson, S.J.; Kellam, P. X-box binding protein 1 contributes to induction of the Kaposi's sarcoma-associated herpesvirus lytic cycle under hypoxic conditions. *J. Virol.* **2009**, *83*, 7202–7209.
127. Cai, Q.; Lan, K.; Verma, S.C.; Si, H.; Lin, D.; Robertson, E.S. Kaposi's Sarcoma-Associated Herpesvirus Latent Protein LANA Interacts with HIF-1 To Upregulate RTA Expression during Hypoxia: Latency Control under Low Oxygen Conditions. *J. Virol.* **2006**, *80*, 7965–7975.
128. Hsiao, J.-R.; Chang, K.-C.; Chen, C.-W.; Wu, S.-Y.; Su, I.-J.; Hsu, M.-C.; Jin, Y.-T.; Tsai, S.-T.; Takada, K.; Chang, Y. Endoplasmic Reticulum Stress Triggers XBP-1-Mediated Up-regulation of an EBV Oncoprotein in Nasopharyngeal Carcinoma. *Cancer Res.* **2009**, *69*, 4461–4467.
129. Lee, D.Y.; Sugden, B. The LMP1 oncogene of EBV activates PERK and the unfolded protein response to drive its own synthesis. *Blood* **2008**, *111*, 2280–9.
130. Chadburn, A.; Hyjek, E.M.; Tam, W.; Liu, Y.; Rengifo, T.; Cesarman, E.; Knowles, D.M. Immunophenotypic analysis of the Kaposi sarcoma herpesvirus (KSHV; HHV-8)-infected B cells in HIV+ multicentric Castleman disease (MCD). *Histopathology* **2008**, *53*, 513–524.
131. Hassman, L.M.; Ellison, T.J.; Kedes, D.H. KSHV infects a subset of human tonsillar B cells, driving proliferation

and plasmablast differentiation. *J. Clin. Invest.* **2011**, *121*, 752–768.

132. Jenner, R.G.; Maillard, K.; Cattini, N.; Weiss, R.A.; Boshoff, C.; Wooster, R.; Kellam, P. Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphoma has a plasma cell gene expression profile. *Proc. Natl. Acad. Sci.* **2003**, *100*, 10399–10404.
133. Kati, S.; Tsao, E.H.; Günther, T.; Weidner-Glunde, M.; Rothämel, T.; Grundhoff, A.; Kellam, P.; Schulz, T.F. Activation of the B-cell antigen receptor triggers reactivation of latent Kaposi's Sarcoma-associated Herpesvirus. *J. Virol* **2013**, *87*, 8004-8016.
134. Chadburn, A.; Said, J.; Gratzinger, D.; Chan, J.K.C.; de Jong, D.; Jaffe, E.S.; Natkunam, Y.; Goodlad, J.R. HHV8/KSHV-Positive Lymphoproliferative Disorders and the Spectrum of Plasmablastic and Plasma Cell Neoplasms: 2015 SH/EAHP Workshop Report-Part 3. *Am. J. Clin. Pathol.* **2017**, *147*, 171–187.
135. De Leo, A.; Chen, H.-S.; Hu, C.-C.A.; Lieberman, P.M. Deregulation of KSHV latency conformation by ER-stress and caspase-dependent RAD21-cleavage. *PLoS Pathog.* **2017**, *13*, e1006596.
136. Chen, H.-S.; De Leo, A.; Wang, Z.; Kerekovic, A.; Hills, R.; Lieberman, P.M. BET-Inhibitors Disrupt Rad21-Dependent Conformational Control of KSHV Latency. *PLoS Pathog.* **2017**, *13*, e1006100.
137. O'Flaherty, B.M.; Soni, T.; Wakeman, B.S.; Speck, S.H. The murine gammaherpesvirus immediate-early Rta synergizes with IRF4, targeting expression of the viral M1 superantigen to plasma cells. *PLoS Pathog.* **2014**, *10*, e1004302.
138. Clambey, E.T.; Virgin, H.W. 4th; Speck, S.H. Disruption of the murine gammaherpesvirus 68 M1 open reading frame leads to enhanced reactivation from latency. *J. Virol.* **2000**, *74*, 1973–1984.
139. Feng, J.; Gong, D.; Fu, X.; Wu, T.; Wang, J.; Chang, J.; Zhou, J.; Lu, G.; Wang, Y.; Sun, R. M1 of Murine Gamma-Herpesvirus 68 Induces Endoplasmic Reticulum Chaperone Production. *Sci. Rep.* **2015**, *5*, 17228.
140. Johnston, B.P.; Pringle, E.S.; McCormick, C. KSHV activates unfolded protein response sensors but suppresses downstream transcriptional responses to support lytic replication. *PLoS Pathog.* **2019**, *15*, e1008185.
141. Granato, M.; Romeo, M.A.; Tiano, M.S.; Santarelli, R.; Gonnella, R.; Gilardini Montani, M.S.; Faggioni, A.; Cirone, M. Bortezomib promotes KHSV and EBV lytic cycle by activating JNK and autophagy. *Sci. Rep.* **2017**, *7*, 13052.
142. Saji, C.; Higashi, C.; Niinaka, Y.; Yamada, K.; Noguchi, K.; Fujimuro, M. Proteasome inhibitors induce apoptosis and reduce viral replication in primary effusion lymphoma cells. *Biochem. Biophys. Res. Commun.* **2011**, *415*, 573–578.
143. Leung, H.J.; Duran, E.M.; Kurtoglu, M.; Andreansky, S.; Lampidis, T.J.; Mesri, E.A. Activation of the unfolded protein response by 2-deoxy-D-glucose inhibits Kaposi's sarcoma-associated herpesvirus replication and gene expression. *Antimicrob. Agents Chemother.* **2012**, *56*, 5794–803.
144. Shigemi, Z.; Baba, Y.; Hara, N.; Matsuhiro, J.; Kagawa, H.; Watanabe, T.; Fujimuro, M. Effects of ER stress on unfolded protein responses, cell survival, and viral replication in primary effusion lymphoma. *Biochem. Biophys. Res. Commun.* **2016**, *469*, 565–572.
145. Glaunsinger, B.; Ganem, D. Lytic KSHV infection inhibits host gene expression by accelerating global mRNA turnover. *Mol. Cell* **2004**, *13*, 713–723.
146. Chandriani, S.; Ganem, D. Host Transcript Accumulation during Lytic KSHV Infection Reveals Several Classes of Host Responses. *PLoS One* **2007**, *2*, e811.
147. Abernathy, E.; Gilbertson, S.; Alla, R.; Glaunsinger, B. Viral Nucleases Induce an mRNA Degradation-Transcription Feedback Loop in Mammalian Cells. *Cell Host Microbe* **2015**, *18*, 243–253.
148. Arias, C.; Weisburd, B.; Stern-Ginossar, N.; Mercier, A.; Madrid, A.S.; Bellare, P.; Holdorf, M.; Weissman, J.S.; Ganem, D. KSHV 2.0: A Comprehensive Annotation of the Kaposi's Sarcoma-Associated Herpesvirus Genome Using Next-Generation Sequencing Reveals Novel Genomic and Functional Features. *PLoS Pathog.* **2014**, *10*, e1003847.
149. Kronstad, L.M.; Brulois, K.F.; Jung, J.U.; Glaunsinger, B.A. Dual Short Upstream Open Reading Frames Control Translation of a Herpesviral Polycistronic mRNA. *PLoS Pathog.* **2013**, *9*, e1003156.
150. Reinke, A.W.; Baek, J.; Ashenberg, O.; Keating, A.E. Networks of bZIP protein-protein interactions diversified over a billion years of evolution. *Science* **2013**, *340*, 730–4.
151. Lin, S.F.; Robinson, D.R.; Kung, H.J.; Miller, G. Kaposi's sarcoma-associated herpesvirus encodes a bZIP protein with homology to BZLF1 of Epstein-Barr virus. *J. Virol.* **1999**, *73*, 1909–1917.
152. Yang, W.-S.; Hsu, H.-W.; Campbell, M.; Cheng, C.-Y.; Chang, P.-C. K-bZIP Mediated SUMO-2/3 Specific Modification on the KSHV Genome Negatively Regulates Lytic Gene Expression and Viral Reactivation. *PLoS Pathog.* **2015**, *11*, e1005051.
153. Chang, P.C.; Izumiya, Y.; Wu, C.Y.; Fitzgerald, L.D.; Campbell, M.; Ellison, T.J.; Lam, K.S.; Luciw, P.A.; Kung,

H.J. Kaposi's Sarcoma-associated Herpesvirus (KSHV) encodes a SUMO E3 ligase that is SIM-dependent and SUMO-2/3-specific. *J. Biol. Chem.* **2010**, *285*, 5266–5273.

154. Izumiya, Y.; Kobayashi, K.; Kim, K.Y.; Pochampalli, M.; Izumiya, C.; Shevchenko, B.; Wang, D.-H.; Huerta, S.B.; Martinez, A.; Campbell, M.; et al. Kaposi's Sarcoma-Associated Herpesvirus K-Rta Exhibits SUMO-Targeting Ubiquitin Ligase (STUbL) Like Activity and Is Essential for Viral Reactivation. *PLoS Pathog.* **2013**, *9*, e1003506.
155. Gao, P.; Chai, Y.; Song, J.; Liu, T.; Chen, P.; Zhou, L.; Ge, X.; Guo, X.; Han, J.; Yang, H. Reprogramming the unfolded protein response for replication by porcine reproductive and respiratory syndrome virus. *PLoS Pathog.* **2019**, *15*, e1008169.
156. Giffin, L.; Yan, F.; Ben Major, M.; Damania, B. Modulation of Kaposi's sarcoma-associated herpesvirus interleukin-6 function by hypoxia-upregulated protein 1. *J. Virol.* **2014**, *88*, 9429–41.
157. Behnke, J.; Feige, M.J.; Hendershot, L.M. BiP and its nucleotide exchange factors Grp170 and Sil1: mechanisms of action and biological functions. *J. Mol. Biol.* **2015**, *427*, 1589–608.
158. Chen, D.; Xiang, Q.; Nicholas, J. Human herpesvirus 8 interleukin-6 interacts with calnexin cycle components and promotes protein folding. *J. Virol.* **2017**, *91*, e00965-17.
159. Hu, D.; Wang, V.; Yang, M.; Abdullah, S.; Davis, D.A.; Uldrick, T.S.; Polizzotto, M.N.; Veeranna, R.P.; Pittaluga, S.; Tosato, G.; et al. Induction of Kaposi's Sarcoma-Associated Herpesvirus-Encoded Viral Interleukin-6 by X-Box Binding Protein 1. *J. Virol.* **2016**, *90*, 368–378.
160. Chang, P.-J.; Hung, C.-H.; Wang, S.-S.; Tsai, P.-H.; Shih, Y.-J.; Chen, L.-Y.; Huang, H.-Y.; Wei, L.-H.; Yen, J.-B.; Lin, C.-L.; et al. Identification and characterization of two novel spliced genes located in the orf47-orf46-orf45 gene locus of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **2014**, *88*, 10092–10109
161. Sanchez, V.; Sztul, E.; Britt, W.J. Human cytomegalovirus pp28 (UL99) localizes to a cytoplasmic compartment which overlaps the endoplasmic reticulum-golgi-intermediate compartment. *J. Virol.* **2000**, *74*, 3842–3851.
162. Das, S.; Vasanji, A.; Pellett, P.E. Three-dimensional structure of the human cytomegalovirus cytoplasmic virion assembly complex includes a reoriented secretory apparatus. *J. Virol.* **2007**, *81*, 11861–11869.
163. Das, S.; Pellett, P.E. Spatial relationships between markers for secretory and endosomal machinery in human cytomegalovirus-infected cells versus those in uninfected cells. *J. Virol.* **2011**, *85*, 5864–5879.
164. Karleusa, L.; Mahmutefendic, H.; Tomas, M.I.; Zagorac, G.B.; Lucin, P. Landmarks of endosomal remodeling in the early phase of cytomegalovirus infection. *Virology* **2018**, *515*, 108–122.
165. Isler, J.A.; Skalet, A.H.; Alwine, J.C. Human cytomegalovirus infection activates and regulates the unfolded protein response. *J. Virol.* **2005**, *79*, 6890–6899.
166. Yu, Y.; Pierciey, F.J.; Maguire, T.G.; Alwine, J.C. PKR-Like Endoplasmic Reticulum Kinase Is Necessary for Lipogenic Activation during HCMV Infection. *PLoS Pathog.* **2013**, *9*, e1003266.
167. Xuan, B.; Qian, Z.; Torigoi, E.; Yu, D. Human cytomegalovirus protein pUL38 induces ATF4 expression, inhibits persistent JNK phosphorylation, and suppresses endoplasmic reticulum stress-induced cell death. *J. Virol.* **2009**, *83*, 3463–74.
168. Siddiquey, M.N.A.; Zhang, H.; Nguyen, C.C.; Domma, A.J.; Kamil, J.P. The Human Cytomegalovirus Endoplasmic Reticulum-Resident Glycoprotein UL148 Activates the Unfolded Protein Response. *J. Virol.* **2018**, *92*.
169. Zhang, H.; Read, C.; Nguyen, C.C.; Siddiquey, M.N.A.; Shang, C.; Hall, C.M.; von Einem, J.; Kamil, J.P. A cytomegalovirus immunevasin triggers integrated stress response-dependent reorganization of the endoplasmic reticulum. *bioRxiv* **2019**.
170. Nguyen, C.C.; Siddiquey, M.N.A.; Zhang, H.; Li, G.; Kamil, J.P. Human Cytomegalovirus Tropism Modulator UL148 Interacts with SEL1L, a Cellular Factor That Governs Endoplasmic Reticulum-Associated Degradation of the Viral Envelope Glycoprotein gO. *J. Virol.* **2018**, *92*.
171. Nguyen, C.C.; Domma, A.J.; Zhang, H.; Kamil, J.P. ER reorganization and intracellular retention of CD58 are functionally independent properties of the human cytomegalovirus ER resident glycoprotein UL148. *bioRxiv* **2019**.
172. Qian, Z.; Xuan, B.; Chapa, T.J.; Gualberto, N.; Yu, D. Murine Cytomegalovirus Targets Transcription Factor ATF4 To Exploit the Unfolded-Protein Response. *J. Virol.* **2012**, *86*, 6712–6723.
173. Stahl, S.; Burkhart, J.M.; Hinte, F.; Tirosh, B.; Mohr, H.; Zahedi, R.P.; Sickmann, A.; Ruzsics, Z.; Budt, M.; Brune, W. Cytomegalovirus Downregulates IRE1 to Repress the Unfolded Protein Response. *PLoS Pathog.* **2013**, *9*, e1003544.
174. Sam, M.D.; Evans, B.T.; Coen, D.M.; Hogle, J.M. Biochemical, biophysical, and mutational analyses of subunit interactions of the human cytomegalovirus nuclear egress complex. *J. Virol.* **2009**, *83*, 2996–3006.

175. Drori, A.; Messerle, M.; Brune, W.; Tirosh, B. Lack of XBP-1 impedes murine cytomegalovirus gene expression. *PLoS One* **2014**, *9*, e110942.
176. Hinte, F.; van Anken, E.; Tirosh, B.; Brune, W. Repression of Viral Gene Expression and Replication by the Unfolded Protein Response Effector XBP1u. *bioRxiv* **2019**, 763227.
177. Yoshida, H.; Oku, M.; Suzuki, M.; Mori, K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. *J. Cell Biol.* **2006**, *172*, 565–575.
178. Yoshida, H.; Uemura, A.; Mori, K. pXBP1(U), a negative regulator of the unfolded protein response activator pXBP1(S), targets ATF6 but not ATF4 in proteasome-mediated degradation. *Cell Struct. Funct.* **2009**, *34*, 1–10.
179. Buchkovich, N.J.; Yu, Y.; Pierciey, F.J.; Alwine, J.C. Human Cytomegalovirus Induces the Endoplasmic Reticulum Chaperone BiP through Increased Transcription and Activation of Translation by Using the BiP Internal Ribosome Entry Site. *J. Virol.* **2010**, *84*, 11479–11486.
180. Buchkovich, N.J.; Maguire, T.G.; Paton, A.W.; Paton, J.C.; Alwine, J.C. The Endoplasmic Reticulum Chaperone BiP/GRP78 Is Important in the Structure and Function of the Human Cytomegalovirus Assembly Compartment. *J. Virol.* **2009**, *83*, 11421–11428.
181. Burnett, H.F.; Audas, T.E.; Liang, G.; Lu, R.R. Herpes simplex virus-1 disarms the unfolded protein response in the early stages of infection. *Cell Stress Chaperones* **2012**, *17*, 473–83.
182. Cheng, G.; Feng, Z.; He, B. Herpes Simplex Virus 1 Infection Activates the Endoplasmic Reticulum Resident Kinase PERK and Mediates eIF-2 Dephosphorylation by the 134.5 Protein. *J. Virol.* **2005**, *79*, 1379–1388.
183. He, B.; Gross, M.; Roizman, B. The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 843–8.
184. Poppers, J.; Mulvey, M.; Khoo, D.; Mohr, I. Inhibition of PKR activation by the proline-rich RNA binding domain of the herpes simplex virus type 1 Us11 protein. *J. Virol.* **2000**, *74*, 11215–21.
185. Mulvey, M.; Arias, C.; Mohr, I. Resistance of mRNA Translation to Acute Endoplasmic Reticulum Stress-Inducing Agents in Herpes Simplex Virus Type 1-Infected Cells Requires Multiple Virus-Encoded Functions. *J. Virol.* **2006**, *80*, 7354–7363.
186. Mulvey, M.; Arias, C.; Mohr, I. Maintenance of endoplasmic reticulum (ER) homeostasis in herpes simplex virus type 1-infected cells through the association of a viral glycoprotein with PERK, a cellular ER stress sensor. *J. Virol.* **2007**, *81*, 3377–3390.
187. Su, A.; Wang, H.; Li, Y.; Wang, X.; Chen, D.; Wu, Z. Opposite Roles of RNase and Kinase Activities of Inositol-Requiring Enzyme 1 (IRE1) on HSV-1 Replication. *Viruses* **2017**, *9*.