Review

Emerging role of the integrated stress response upon viral infection

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Abstract: The integrated stress response (ISR) is an adaptational signaling pathway that is induced in response to different stimuli, such as accumulation of unfolded and misfolded protein, hypoxia, amino acid deprivation, viral infection and ultraviolet light. It has been known that viral infection can activate ISR, but the role of ISR during viral infection is still unclear. In some cases, ISR is a protective mechanism of host cell against infection with virus whilst ISR may be hijacked by viruses for facilitating its replication. In this review, we highlighted recent advances on induction of ISR upon viral infection and the downstream responses involved such as autophagy, apoptosis, formation of stress granules and innate immunity response. We then discussed the molecular mechanism of ISR regulating viral replication and how viruses antagonize this cellular stress response resulting from ISR.

Keywords: integrated stress response; eIF2α phosphorylation; unfolded protein response; viral replication; host

1. Introduction

The integrated stress response (ISR) is an intricate signaling pathway existing in eukaryotic cells which is activated through the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) in response to different physiological changes and pathological conditions. Activation of ISR results in decrease of global protein synthesis and induction of selected genes, such as transcription factor 4 (ATF4). It is speculated that the ISR ultimate destiny is determined by intensity and duration of stress, level of eIF2α phosphorylation and ATF4 activation [1]. Initially a pro-survival effect is activated, and intracellular hemostasis is reconstructed under short-term stress. However, a cell death program is initiated when cells are exposed to a prolonged and severe stress [1-3]. It has been well known that viral infection could induce ISR, but the role of ISR is still less defined. In some cases, ISR is a protective mechanism against virus replication, while in other cases, ISR may be hijacked by the virus to facilitate its replication. In this review, we summarized current knowledge of the molecular mechanisms of ISR with an emphasis on how cells initiate ISR, how viral factors modulate the ISR and the downstream cellular responses, as well as how the ISR pathway determine cell prognosis upon viral infection.

2. Overview of the integrated stress response signaling pathway

In physiological condition, eIF2 consisting of eIF2α, eIF2β and eIF2γ, possess both phosphorylation sites and RNA binding sites. eIF2 forms a ternary complex with GTP and Met-tRNAi, and then binds 40S ribosome subunit, resulting in the formation of 43S pre-initiation complex (PIC)
with two small initiation factors (eIF1 and eIF1A) [4,5]. PIC is recruited to the 5’-methylguanine cap of mRNA through the eIF4F complex, the latter contains eIF4G and eIF4E. PIC migrates to the AUG start codon and then binds the Met-tRNAi anti-codon and the AUG start codon which facilitates protein synthesis. AUG recognition causes arrest of the scanning PIC and triggers conversion of eIF2 GDP-bound state via gated phosphate (Pi) release and GTPase-activating (GAP) factor eIF5. eIF2-GDP complex dissociates from 40S ribosomal complex and transforms to GTP with the help of eIF2B complex and enters another recycling of initiation of mRNA translation [6,7]. In stress condition, phosphorylated eIF2 is fully capable of forming an initiation-competent eIF2-TC, but following its release, phosphorylated eIF2-GDP tightly binds to and sequesters the guanine nucleotide exchange factor eIF2B, abrogating its activity. Most mRNA translation is reduced when eIF2α is phosphorylated. However, translation from certain mRNAs with at least two upstream open reading frames (uORFs) of appropriate type and position can be upregulated. The best-characterized mammalian examples are ATF4, ATF5 and CHOP. Upregulation of ATF4, ATF5 and CHOP function activates chaperon proteins to promote cellular recovery or activate cell death pathways upon sustained stress.

In mammalian cells, ISR kinases act as an early responder to restore cellular homeostasis upon different stimuli. There are four members of ISR family: general control nonrepressible 2 (GCN2), PKR-like ER kinase (PERK), the heme-regulated inhibitor (HRI) and the interferon (IFN)-induced double-stranded RNA-dependent protein kinase (PKR). Actually, each kinase can sense distinct stresses, because they share homologous catalytic domains but possess unique regulatory domains [8,9]. ISR is activated by any of the four members of eIF2α kinases family in response to various stress stimuli. GCN2 is sensitive to amino acid starvation, PERK is induced by the accumulation of unfolded or misfolded proteins in the ER, HRI is activated in response to heme deficiency, and PKR is activated by double stranded RNA (dsRNA) [10]. Schematic diagram of protein structure of eIF2α kinase family is shown in Figure 1.

![Figure 1](image)

**Figure 1.** Schematic diagram of protein structure of eIF2α kinase family. Four kinases contain different regulation structure domains and binds to various ligands to deliver signals.

Normally, PERK is bound by 78-KDa glucose-regulated protein (GRP78) in the ER membrane. However, two models for PERK activation during ER stress has been put forward. In the classical model, misfolded or unfolded protein gets accumulated in the ER lemon and then GRP78 dissociates from PERK resulting in its autophosphorylation and oligomerization. Activation of PERK phosphorylates eIF2α at serine 51, which halts overall protein translation and selected gene ATF4 is upregulated to restore cellular recovery [1]. Eukaryotic cells employ three different mechanisms to deal with unfolded proteins and misfolded proteins in the ER: transcriptional induction of chaperons, protein degradation, and translational attenuation [11]. In all these processes, ATF4 is a vital point to determine cell prognosis. During amino acid starvation, ATF4 activates upregulation of several genes involved in autophagy, including ATF3, ATF5 and ATF7, which is a pro-survival
signaling [12]. Transcriptional ATF4 can induce expression of genes for pro-autophagy or anti-apoptosis to restore hemostasis upon different stress, such as regulating development and DNA damage response, anti-apoptotic myeloid cell leukemia-1 [13-15]. A cell death signaling would occur when an adaptive response failed to restore hemostasis. Transcriptional ATF4 and its downstream genes are induced, particularly ATF3 and C/EBP homologous protein (CHOP) and a pro-apoptosis signaling is generated through the formation of heterodimeric with ATF3 or CHOP. It then activate transcriptional factor expression of Bcl-2 homology3 (BH3)-only pro-apoptotic protein NOXA [16].

PKR protein which is mainly located in the cytosol and the nucleus, phosphorylate eIF2α after viral infection resulting in inhibition of viral mRNA translation and induction of apoptosis. PKR is the eIF2α kinase which is the most well studied in terms of structures and activation mechanism. PKR is comprised of kinase domain along with the other eIF2α kinases, and two dsRNA binding domains (dsRBD) modulating its activity. In non-stressed cells, PKR exists in the form of monomeric because of auto-inhibitory effect of its dsRBD. Following interaction with dsRNA molecules through the two N-terminal dsRNA-binding motifs (dsRBM) of dsRBD, PKR auto-phosphorylates on Thr446, and then phosphorylates eIF2α, which leads to a dramatic inhibition of cell protein synthesis to reduce the protein flux into the ER and viral mRNA synthesis [17-19], similarly, ATF4 also play an important role in regulating cellular and initiating downstream signaling of ISR in these processed. However, few studies have indicated that PKR is also activated by oxidation, ER stress, cytokine signaling, growth and so on [20-22].

HRI is expressed mainly in erythrocytes [23], and also present in liver and macrophages [8]. HRI ensures production of α- and β-chains of globin and balance the heme in red blood cells [8]. HRI binds to heme at its N-terminus and is activated through autophosphorylation in response to low level of heme and then phosphorylates eIF2α [24-26]. HRI can also be induced by other stresses, including heat shock, osmotic pressure, oxidative stress induced by arsenite and bacterial pathogens [27-30]. However, there is no report about induction of HRI upon viral infection in mammal animal cells.

Amino acid exhaust activates GCN2 through the increasing of deacylated tRNA molecules [31]. GCN2 is also activated upon glucose deprivation, ultraviolet irradiation, hypoxia and viral infection though the mechanism remains to be defined [32-35].

The kinases of ISR can be activated simultaneously upon stimuli, such as, viral infection can result in simultaneous activation of PERK, GCN2, as well as PKR [35-37]. In addition, four eIF2α kinases are also induced upon oxidative stress [3,38,39].

Dephosphorylation of eIF2α is a terminal signal of ISR and cell return to normal protein translation and synthesis. The process is mediated by growth arrest and DNA damage-inducible protein (GADD34) and constitutive repressor of eIF2α phosphorylation, which interact with protein phosphatase 1 (PP1) and restore protein synthesis and normal cellular function. GADD34 is a downstream production of eIF2α phosphorylation and ATF4 at late stage of the ISR, so GADD34 plays a pivotal role as a negative feedback loop in attenuating signaling of ISR [1,40].

3. Virus modulation of ISR signaling pathway

3.1. Virus modulation of ISR signaling pathway through the control of the ISR-associated kinases activity

Many viruses can activate ISR pathway in infected cells through different mechanism (Table 1). Generally, viruses hijack cellular protein synthesis mechanism for their own protein expression which may disrupt the ER homeostasis [41]. Meanwhile, viruses also develop mechanisms that manipulate host ISR signaling pathway to promote viral translation and persistence during viral infection [42,43]. In general, ISR activation is triggered by virulent or pathogenic viruses instead of inactivated viruses suggesting that the activation of ISR is associated with viral replication [44]. However, inactivated foot-and-mouth disease virus (FMDV) has been reported to induce eIF2α-ATF4 pathway [45].

Table 1. Molecular Mechanism of ISR upon Viral Infection.
| Virus | Type of virus | Activation of UPR branch | Activation of ISR kinases | Activation mechanism | References |
|-------|--------------|--------------------------|--------------------------|---------------------|------------|
| TGEV  | A single-stranded positive-sense RNA | PERK, IRE1a, ATF6 | PERK, PKR | TGEV replication is inhibited through activation of NF-κB, which facilitate type I IFN production, and PERK-eIF2α-P induce overall attenuation of protein translation. | Mei Xue, et al. 2018; Jazmina L. G. Cruz, et al. 2010. |
| SARS-CoV | A single-stranded positive-sense RNA | PERK, PKR | Viral replication is do not influenced by both kinases, apoptosis is induced by PKR kinase. | Verena Kra‘hling, et al. 2009. |
| IBV   | A single-stranded positive-sense RNA | PERK, PKR | IBV infection causes ER stress and induces PERK phosphorylation. Phosphorylation of eIF2α by PERK and PKR induces the expression of ATF4, ATF3, and GADD153. GADD153 exerts its proapoptotic activities via suppressing Bcl2 and antagonizing the survival kinases (ERKs) by inducing TRIB3. | Ying Liao, et al. 2013 |
| PEDV  | A single-stranded positive-sense RNA | PERK, IRE1a, ATF6 | PERK | Inhibit viral replication through PERK-eIF2α-P induced overall attenuation of protein translation. | Yue Wang, et al. 2014. |
| BTV   | Double stranded RNA | PERK, PERK | BTV induce ER stress mediates autophagy via the PERK-eIF2α pathway and contributes to BTV1 replication. | Shuang Lv, et al. 2015 |
| HCV   | A single-stranded positive-sense RNA | PERK, ATF6 | PERK, | HCV core protein induce autophagy and UPR induced autophagy promote viral replication through PERK and ATF6 pathways. | J. Wang, et al. 2015. |
| EMCV  | A single-stranded positive-sense RNA | PERK, ATF6 | PERK | 2C and 3D protein of EMCV induce autophagy and UPR induced autophagy promote viral replication through PERK and ATF6 pathways. | Lei Hou, et al. 2014. |
| BVDV  | A single-stranded positive-sense RNA | PERK | BVDV infection induce pro-apoptosis process through PERK-eIF2α pathway, which leads to expression of CHOP, caspase12 and PARP. | Jordan, R. et al. 2002. |
| Virus | Stranded RNA | PERK, IRE1α, ATF6 | PERK | Description |
|---|---|---|---|---|
| **DENV** | Single-stranded positive-sense RNA | PERK | PERK | PERK signaling is induced at the early stage of DENV2 infection, IRE1α and ATF6 pathways are activated at late stage, which leads to the expression of GADD34 and CHOP that resulting in apoptosis. DENV-induced autophagy promotes viral replication through forming the autophagosome, which provide a dock and energy for viral replication. |
| **WNV** | Single-stranded positive-sense RNA | PERK, IRE1α, ATF6 | PERK | Limit viral replication. |
| **HSV** | Double-stranded DNA | PERK, PKR | PERK, PKR | PKR is induced firstly, and PERK is activated when viral protein accumulates in ER. Activation of PERK and PKR phosphorylate eIF2α to block translation of viral protein. However, γ34.5 protein of HSV promote viral replication through recruiting PP1 to dephosphorylate eIF2α. Us11 and ICP34.5 protein of HSV-1 can block activation of PKR-eIF2α signaling pathway and regulate autophagy by binding directly PKR-binding domain and binding to Beclin1 respectively to promote viral replication. |
| **PCV2** | Single-stranded circular DNA | PERK | PERK | Cap protein of PCV2 activate UPR-induced apoptosis via PERK-eIF2α-ATF4-CHOP-Bcl-2 axis. Meanwhile, PCV2 can utilize UPR to promote viral replication and expression of Cap protein. |
| **JEV** | Single-stranded positive-sense RNA | | PKR | JEV infection induce PKR at the late stage. |
| **WNV** | Single-stranded positive-sense RNA | | PKR | WNV infection induce PKR at the late stage. |
| Virus | Type | Kinase | Description | Reference |
|-------|------|--------|-------------|-----------|
| SINV | A single-stranded positive-sense RNA | PKR GCN2 | SINV infection induce PKR kinase. SINV infection induce GCN2. Meanwhile, GCN2 inhibit early viral translation and prevent viral replication through activation of eIF2α phosphorylation. | Domingo-Gil E, et al. 2011; Gorchakov R, et al. 2004; Juan J Berlanga, et al. 2006. |
| SFV | A single-stranded positive-sense RNA | PKR | SFV infection induce PKR kinase. | Ventoso I, et al. 2006. |
| EV71 | A single-stranded positive-sense RNA | PKR | EV71 infection induce typical SGs through PKR pathway. However, EV71 induced SG-like structures are antiviral RNA granules to suppress viral propagation. | Yuanmei Zhu, et al. 2016; Hua Zhang, et al. 2016. |
| PFV | A positive sense single-stranded RNA virus | PERK, IRE1α, ATF6 | PFV induce a complete autophagic process through UPRs, increasing activation of autophagy inhibit viral replication. | Peipei Yuan, et al. 2017. |
| MNV | A positive sense single-stranded RNA virus | PKR | MNV infection induce PKR pathway and eIF2α phosphorylation. NS3 protein of MNV control host protein translation. Meanwhile, MNV recruit G3BP1 to promote viral replication and prevent SGs formation. | Svenja Fritzlar, et al. 2019. |

3.1.1. PERK kinase

Many viral proteins are synthesized in the ER, consequently, the induction of ER stress is a common outcome. It was found that PERK axis of unfolded protein response (UPR) plays an important role in regulation of viral protein synthesis under ER stress. Bluettongue virus (BTV), Hepatitis C virus (HCV), encephalomyocarditis virus (EMCV), dengue virus (DENV) infection were able to induce UPR-induced autophagy to promote viral replication [46-50]. Porcine circovirus type 2 (PCV2) cap protein was involved in activating UPR-induced apoptosis and deploys UPR to enhance viral replication through PERK-eIF2α-ATF4-CHOP-Bcl2 signaling pathway [51,52]. Likewise, Bovine viral diarrhea virus (BVDV) and WNV also activate UPR-induced apoptosis through PERK pathway, however its effect on the replication of WNV and BVDV is yet unclear [53,54]. It is suggested that the synthesis of these viral proteins is associated with ER and ISR initiates downstream signaling pathways to restore cellular hemostasis. Meanwhile, viruses utilize the activation of ISR to promote viral replication. Coronavirus replication is also closely associated with ER stress. Gastroenteritis virus (TGEV) infection was found to activate all three UPR pathways through upregulation of GRP78, but PERK-eIF2α branch mainly suppress viral replication through inducing IFN-I production and
eIF2α phosphorylation-mediated global attenuation of protein translation occurred during TGEV infection in vitro and in vivo [55].

In contrast, another study showed that TGEV infection was only able to induce the PKR-eIF2α signaling pathway [56]. The reason for this discrepancy was unclear but it might be due to the different TGEV strains used in those two studies. In addition, both PERK and PKR pathway axis are induced by other coronaviruses such as severe acute respiratory syndrome coronavirus (SARS-CoV), but the viral replication is not influenced by these kinases [57]. Infectious bronchitis virus (IBV) infection activated PERK/PKR kinases and viral replication may be promoted through GADD153-dependent apoptosis [55,58,59]. HSV-1 also regulates autophagy to promote its replication through PKR pathway [37].

3.1.2. PKR kinase

Among ISR-associated four kinases, PKR has been found to play an important role in antiviral host defense due to its induction by IFN [60]. PKR is also activated by dsRNA but mostly from by-products of viral replication or transcription. In RNA virus, dsRNA replicative formations are obligatory intermediates for synthesis of new genomic RNA copies. For DNA viruses, vaccinia virus (VV), adenovirus and herpes simplex virus (HSV) possess ORFs in opposite orientation and produce overlapping mRNA transcripts and then fold to form dsRNA stretches that activate PKR [36,60]. Japanese encephalitis virus (JEV) and West Nile virus (WNV), belonging to positive-sense RNA, induce PKR at the late stage of viral infection [61,62], this could be due to the time required for the conversion of ssRNA to dsRNA and the activity of PKR is blocked upon viral infection. PKR is also induced by Sindbis virus (SINV) and Semliki Forest virus (SFV) [63-65]. In general, global protein translation is blocked upon viral infection, including viral protein replication. However, Murine norovirus (MNV) infection induces PKR signaling pathway and leads to eIF2α phosphorylation and global protein translation shutoff which does not impact viral replication because NS3 protein of MNV disturb in host protein translational efficiency. It is suggested that viruses control host translational mechanism for itself. Meanwhile, MNV promote viral replication and prevent SGs formation through recruiting G3BP1 protein to the site of viral replication complex [66].

3.1.3. HRI kinase

To date, there is no evidence that HRI can be activated upon virus infection in mammal cells. HRI of Epinephelus coioides (EcHRI), a homologue gene in fish, is changed at the transcription level upon red-spotted grouper nervous necrosis virus (RGNNV) infection and inhibit viral replication through upregulating the expression of IFN-related cytokines which indicates the potential role of HRI in antiviral response [67].

3.1.4. GCN2 kinase

The role of GCN2 against RNA viruses is yet to be known. GCN2 is specifically induced through phosphorylation of eIF2α at early stage of SINV infection. During that mechanism, two nonadjacent regions of SINV genomic bind to histidyl-tRNA synthetase-related domain of GCN2 and meanwhile, GCN2 block early viral translation of genomic SINV by eIF2α phosphorylation induced by the activation of GCN2 [35,68].

3.2. Viral proteins which directly regulate integrated stress response signaling axis

Viruses develop different mechanisms to manipulate ISR signaling pathway to promote viral translation and persistence during viral infection [42,43]. In order to facilitate replication, both DNA and RNA viruses encode proteins to selectively regulate ISR pathway, enhance ER protein folding capacity and metabolic regulation of cells. M protein of vesicular stomatitis virus (VSV) is responsible for counteracting antiviral response of eIF2α phosphorylation [69]. NS5A protein and E2 protein of HCV were found to interfere PKR and PERK kinase respectively which leads to inhibition of downstream eIF2α phosphorylation and helps in viral replication [70,71], whereas in JEV, NS2A

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**protein was found to inhibit PKR-induced eIF2α phosphorylation [61,71]. Influenza A virus non-structural protein 1 (IAV-NS1) limit eIF2α phosphorylation through hampering PKR dimerization and autophosphorylation [72]. Upon DENV infection, PERK-induced eIF2α phosphorylation is suppressed through upregulating expression of GADD34, which interacts with PP1 to dephosphorylate eIF2α [73]. Protein 7 of TGEV and M protein of VSV antagonize eIF2α phosphorylation during infection [56,69]. PKR is induced at early stage of HSV infection, activation of PKR phosphorylates eIF2α and block protein translation, including viral protein, PERK is activated during viral protein accumulation, however, γ34.5 protein of HSV inhibit PERK phosphorylation to promote viral replication. Meanwhile, the expression of GADD34 binds in an eIF2α-independent mechanism to PP1 and mediate in dephosphorylation of eIF2α. It is speculated that γ34.5 protein may recruit PP1 to dephosphorylate eIF-2 and antagonizes the activities of both PKR and PERK [37].**

### 3.3. Modulation of down-streaming signaling of ISR upon viral infection

ISR is activated through eIF2α phosphorylation during viral infection, which leads to translation arrest of both cellular protein and viral protein, meanwhile, phosphorylated eIF2α initiate downstream signaling of ISR to regulate viral replication and restore cellular homeostasis, such as autophagy, formation of SGs, apoptosis and innate immunity response.

#### 3.3.1. Induction of autophagy through ISR

Autophagy exerts a pro-survival signaling to facilitate cell metabolic homeostasis. Although the functions between UPR and autophagy are independent, many studies showed that the activation of UPR regulates autophagy and further control viral replication and pathogenic mechanism during viral infection.

Autophagy could be activated through UPR signaling pathway during viral infection. It was found that autophagy response could benefit viral replication during DENV2 infection. Further analysis showed that PERK-eIF2α-ATF4-ATG12 and IRE1α-JNK-BECLIN1 signaling pathway was mainly involved in this process. IRE1α-JNK induced Bcl-2 phosphorylation to release Beclin1 which triggered autophagic activity. PERK-eIF2α-ATF4-ATG12 signaling pathway partly effect on autophagy at early stage of DENV infection [49]. Another report showed that PERK signaling pathway participates in DENV-induced autophagy and enhance viral replication in dog MDCK and mouse MEFs [50], mounting results also demonstrated that DENV-induced autophagy promotes viral replication through forming the autophagosome, which provide a dock and energy for viral replication. Meanwhile, DENV capsid protein promote viral replication by altering lipid biosynthesis derived from ER membrane which alleviates ER stress and promote cell survival [50,74,75]. This phenomenon is also common in other members of flaviviruses family, such as HCV, JEV and WNV [76-78] indicating that DEVN and other members of flaviviruses family are ER-tropic viruses that accomplish translation, replication and package in the ER.

Other viruses also induce autophagy through UPRs to enhance viral replication. BTV infection induce autophagy through PERK-eIF2α pathway and in turn autophagy promotes viral replication [46,79]. Similarly duck enteritis virus (DEV) activates autophagy to benefit its replication through PERK-eIF2α-ATF4 and IRE1-XBP1 signaling pathways [80]. Autophagy is induced during Newcastle disease virus (NDV) infection and promotes its replication. Studies have further shown that P and NP proteins of NDV induce autophagy via PERK and ATF6 pathways [81]. A complete autophagy is induced by HCV core protein and CHOP plays a vital role in UPR autophagy signaling. [82]In addition, UPR associated autophagy has been found to promote viral replication through PERK-eIF2α-ATF4 and ATF6 signaling pathways activating ATF4 and CHOP enhance the expression of ATG12 and LC3B, which benefits autophagic process [11,70,83]. EMCV infection induces autophagy through PERK and ATF6 pathways via viral 2C and 3C protein, which promote viral replication [47,84]. Autophagy is induced via ER stress during coxsackievirus (CV) B3 infection, and three branches of UPR participate in regulation of autophagy [85]. These results suggest that viruses can induce autophagy through UPR to promote mainly viral replication. It is speculated that UPR-induced autophagy may promote cellular survival and restore homeostasis.
However, prototype foamy virus (PFV) infection can induce a complete autophagic process through ER stress containing PERK, IRE1 and ATF6 branches, and increasing activation of autophagy inhibit PFV replication which implies that PFV-induced autophagy has a novel mechanism and plays an antiviral role in viral replication [86]. elf2α-ATF4 pathway is induced through inhibition of AKT-MTOR signaling pathway during FMDV infection by VP2 protein interacting with heat shock protein family B small member1 (HSPB1) in mammal cells [45].

PKR is induced by dsRNA and reduces translation of viral mRNAs to protect cells [36]. PKR is also required for nutrient deprivation and viral-induced autophagy [10]. PKR-elf2α signaling is activated during HSV-1 infection but the role of autophagy in HSV-1 replication is unclear. However, Us11 protein of HSV-1 can block activation of PKR-elf2α signaling by binding directly to PKR-binding domain [10,87], and ICP34.5 of HSV-1 also regulate autophagy through dephosphorylation of elf2α and binding to Beclin1 to promote viral replication [88,89]. These findings indicate that autophagy has an antiviral effect against HSV-1.

Overall, elf2α phosphorylation serves as the central link between viral infection and induction of autophagy. Interestingly, the other branches of UPR also involve in activation of autophagy during viral infection thereby indicating that viral-induced autophagy mediated by ER stress is a pro-survival cellular process at the early stage of viral infection and a cell death signaling may be initiated in case of severe viral infection. However, the underlying mechanism of how UPR stress regulates viral induced autophagy remains unclear. The above findings are summarized (Figure 2).

**Figure 2.** Diagram of the ISR signaling pathway, autophagy and apoptosis during viral infection. Autophagy: autophagy is activated through PERK/PKR-elf2α pathway upon TGEV, HSV-1, ARV, CV, HCV, PFV, BTV, CVB3, EMCV and DEV infection, FMDV-VP2 induces autophagy through interaction with HSPB1 and activation of the elf2α-ATF4 pathway. In turn, HSV-Us11, HCV-NS5A and HCV-E2 protein block autophagy (red). Apoptosis: apoptosis is induced via PERK/PKR-elf2α pathway under IBV, PCV2, BVDV, JEV, WNV infection. Oppositely, JEV-NS2A inhibit apoptosis (yellow). In addition, autophagy and apoptosis simultaneously are induced through PERK -elf2α pathway during the same virus infection, such as DENV and NDV (pink).

### 3.3.2. Formation of SGs through ISR

SGs are the accumulation of cytoplasmic non-membrane structures of mRNA-binding proteins (mRNPs) and related proteins in response to stress stimuli. The mechanism for protein translation inhibition mainly by elf2α phosphorylation at Ser-51 leads to an increase of mRNA released from polysomes [90]. It has been proposed that four kinases phosphorylate elf2α and lead to the formation of SGs. On the other hand, SGs formation is independent of elf2α phosphorylation, such as disruption of elf4A helicase by patamaine A and the elf4F complex by H2O2 which implies that SGs composition and assembly differ in a stress-dependent manner [91-93]. SGs are formed in response...
to various stresses in mammalian cells, including oxidative stress, energy depletion, UV irradiation, hypoxia, ER stress and viral infection [92-94].

The virus requires cellular translation machinery to synthesize its proteins in host cells. However, SGs formation results from global translation repression of mRNAs and blocks viral gene expression during viral infection. Thus, SGs formation may be the result of an innate immune response [95]. Moreover, virus also take measures to confront these adverse conditions and maximizes replication efficiency through inhibition of SGs formation and disruption of PBs assembly [96]. Therefore, the illumination of the relationship between SGs and virus is very important to understand the interaction of host and viruses [96].

\[\text{eIF2}a\] is phosphorylated through PKR branch during MNV infection causing stoppage of protein translation but without inhibiting viral protein replication because MNV suppress cytokine translation and the formation of SGs to promote viral replication. MNV infection does not induce SGs formation. However, MNV recruits SGs nucleating protein G3BP1 to enhance viral replication and prevent SGs formation. This suggests that MNV promote viral replication through PKR-P-eIF2a-SGs axis and evade innate immune response [66]. The similar phenomenon was observed in FMDV where FMDV infection does not induce SGs formation. Instead L\(^{po}\) of FMDV actively antagonize SGs formation and cleave SGs scaffold proteins Ras-GAP SH3 domain binding protein1 (G3BP1) and G3BP2 but not T-cell-restricted intracellular antigen1 (TIA-1). Additionally, L\(^{po}\) had no effect on PKR activation and eIF2a phosphorylation. Enteroviruses71 (EV71) infection was able to induce formation of typical SGs (tSGs) via the PKR-eIF2a pathway. Further, SG-like structures also are induced which is a different canonical SGs and is an antiviral structures to suppress EV71 propagation [97]. However, 2A\(^{po}\) of EV71 block tSGs formation and transforms from tSGs to atypical SGs (aSGs) through cleaving eIF4GI. 2Apro regulates SGs formation which is also common in picornaviruses [55,98]. Several studies demonstrated that the composition of SGs is different from FMDV L\(^{po}\) and EV 2A\(^{po}\) infection, but the exact composition of aSGs remains unclear [97,99,100].

IAV deficient in NS1, induce cytoplasmic granules termed as antiviral stress granules (avSGs) which is different from the canonical SGs. IAVA-NS1 infection inhibit formation of avSGs and production of IFN through PKR-eIF2a signaling pathway [101]. SINV, EMCV, Adenovirus, HCV and NDV also trigger aSGs implying that aSGs may play an important role in detection of viruses to initiate antiviral signaling. NS1 protein of IAV block the formation of SGs and the activation of IFN genes [94]. Interestingly, SGs might provide a platform for interaction between virus and host antiviral molecules and help in the removal of viruses.

EMCV was able to transiently induce SGs formation through PKR signaling at early infection, however, EMCV 3C protein was found to inhibit SGs formation via cleaving G3BP1 at late stage of infection. Similarly, 3C protein of poliovirus (PV) and L protein of Theiler’s murine encephalomyelitis virus (TMEV) were able to inhibit SGs formation [100,102,103]. These findings indicate that picornaviruses also use the same strategy to evade immune response by targeting G3BP1 which is essential for efficient induction of IFN-\(\beta\).

HCV infection triggers eIF2a phosphorylation and SGs formation depending on PKR branch [104]. In addition, SGs formation is induced through PKR-P-eIF2a-SGs pathway by respiratory syncytial virus (RSV), VV, measles virus (MeV) and human immunodeficiency virus (HIV), C protein-deficient Sendai virus (SeV), tick-borne encephalitis virus (TBEV), SINV, EV71 and PV infection [105-111]. It is suggested that PKR-P-eIF2a-SGs pathway is an important signaling for SGs formation. SGs formation is activated through eIF2a phosphorylation upon reovirus infection, however, which kinase is induced in this process remain unknown [112]. PERK-P-eIF2a-SGs signaling pathway is induced during human cytomegalovirus (HCMV) infection and GCN2-P-eIF2a-SGs branch is activated during SINV infection [35,113]. Hence, ISR kinases are involved in SGs formation during viral infection and play an important role in antiviral defense and restoring cell homeostasis.

Recent studies have demonstrated that different viral infection can induce or inhibit the formation of SGs such as Flaviviridae virus, rotavirus, Junin virus, mouse hepatitis virus (MHV), HIV-1, nervous necrosis virus (NNV), VSV, MRV and HSV [105,114-120]. Furthermore, SGs
formation is induced or inhibited at different stage of a viral replication cycle or via different signaling pathway such as SFV, HCV and RSV [121]. Emerging evidence suggests that eIF2α-SGs signaling pathway plays a pivotal role in innate immunity maintaining cell hemostasis. The summary of SGs formation during viral infection is shown (Figure3).

![Diagram](image)

**Figure 3.** Diagram of the ISR signaling pathway and SGs formation under viral infection. EV71, VV, HIV, HCV, SeV, EMCV, RSV, TBEV, MeV, TMEM, Adenovirus, NDV and SINV infection promote SGs formation through PKR-eIF2α-p signaling pathway. MeV, Reovirus, SFV, RV, HSV, VSV, MHV and MRV trigger eIF2α phosphorylation and benefit for SGs formation (blue). However, IAV-NS1, MNV, NNV, FMDV-Lpro, PV-3Cpro, TMEM-Lpro and EMCV-3C infection inhibit SGs formation (red). SGs formation is increased via PERK-eIF2α-P branch during HCMV infection. SINV infection enhance SGs formation through GCN2-eIF2α-P branch.

3.3.3. Activation of Apoptosis through ISR

Apoptosis, a programing cell death, is supposed to be a host strategy to combat viral infection. PERK/PKR-eIF2α-ATF4 pathway is activated during the early stage of IBV infection in Vero cells and H1299 cells which results in expression of ATF4, ATF3 and GADD153. Activated GADD153 induce a ER stress-mediated pro-apoptotic pathway through suppressing Bcl2 and antagonizing the survival kinases (ERKs) by inducing tribbles homolog3 (TRIB3) [59]. Similarly, studies have shown that ER stressor IRE1α is also activated in IBV-infected cells and serves as a survival factor during coronavirus infection [59,122,123]. HCV triggers apoptosis through induction of GADD153 and ER calcium depletion [113,124-126]. JEV infection triggers UPR and apoptosis through expression of GADD153 and p38 kinase. However, which branch is induced remains unknown [127]. Cap protein of PCV2 induces UPR and then results in apoptosis through PERK-eIF2α-ATF4-CHOP-Bcl-2 signaling pathway which reduces Bcl2 expression and increases caspase3 enhancing the viral replication [51,52]. Some viruses of Flaviviridae family also induce apoptosis. A pro-apoptosis response is induced through PERK-eIF2α pathway which leads to expression of CHOP, caspase12 and poly ADP ribose polymerase (PARP) and downregulation of Bcl2 such as NS protein of WNV, BVDV and DENV [53] [54,73,128]. Three branches of UPR involve in NDV-induced apoptosis and CHOP is initiated by PERK/PKR-eIF2α signaling to promote viral proliferation [129]. Overall, UPR-induced apoptosis is activated through PERK-eIF2α-ATF4-CHOP signaling upon viral infection. Activation of CHOP leads to suppression of Bcl2 and induction of GADD34 and plays a pro-survival function. However, eIF2α-ATF4-GADD153 pathway may inhibit viral replication during a prolong stress condition. Hence, CHOP may serve as a pro-apoptosis or pro-survival function depending on the condition of stress. In addition, IRE1α modulates Akt and JNK kinases to promote cell survival. UPR-induced apoptosis is summarized (Figure2).
3.4. Role of ISR in antiviral response

Apart from the importance of ISR on restoring cell homeostasis, ISR kinases also play a vital role in innate immunity during viral infection which is thought to function as an antiviral pathway [95,130]. Kinases of ISR phosphorylate eIF2α and block overall protein translation including viral protein. Hence, this process is an antiviral response. SGs formation results in global translation repression of mRNAs including viral protein. In this process, PKR plays important role because it can directly recruit the formation of SGs. Thus, SGs formation is also an innate immune response to viral infection [95]. However, some viruses were able to avoid antiviral response of ISR to promote its replication. MNV infection leads to eIF2α phosphorylation through PKR pathway and block translational shutoff of host cell protein. At the same time, the translation of IFN-α, IFN-β and IL-6 are suppressed to promote MNV replication which is also an immune evasion strategy [66]. Furthermore, other members of ISR kinases are also involved in antiviral immune response. NF-κB is activated and IFN-1 are produced through PERK-eIF2α axis during TGEV infection which represents a characterizing role of virus-induced UPR in NF-κB activation during other viral infection [55]. On the other hand, GCN2 plays a novel role in the antiviral response to certain RNA viruses. As mentioned above, GCN2 block the translation of viral protein and further prevent replication of SINV through eIF2α phosphorylation [35].

4. Discussion

ISR is a protective response and is induced by different kinds of stresses which leads to the inhibition of overall protein translation and the preferential transcription of targeting genes through eIF2α phosphorylation to restore cellular hemostasis. ISR is firstly observed in 2002 because ISR is a hub for many signaling pathways that converge on eIF2α phosphorylation such as autophagy, apoptosis, SGs formation, cell hemostasis and innate immunity response. However, there is a great gap about ISR and how eIF2α kinases are activated and the genes of downstream of eIF2α determine cell prognosis during viral infection. Generally, a pro-survival response is induced in a short and mild stress to restore cell homeostasis but a cell death signaling is activated during long and severe stress. However, the mechanism of the switch between pro-survival and cell death signaling needs to be further illuminated.

eIF2α phosphorylation is the core of ISR and inhibit viral protein synthesis to restore cellular homeostasis during viral infection. Activation of eIF2α phosphorylation initiate downstream mechanism through UPR, autophagy, apoptosis and formation of SGs. UPR-induced these responses regulate viral replication to alleviate stress. PERK-eIF2α pathway plays an important role in benefiting viral replication and other branches of UPR are also involved. It is speculated that viruses may take some strategies to promote viral protein synthesis and UPR-related autophagy is a novel mechanism for regulating viral replication. These phenomena infer that autophagy has the dual role in regulating viral replication. PKR plays a vital role in virus-induced SGs formation. Not only in eIF2α phosphorylation and inhibition of viral replication, but it also provides a platform to promote IFN gene production [114,131]. The leader protein of EMCV can inhibit IFN gene activation [103] and some viruses can disturb in the formation of SGs [102]. PERK-eIF2α signaling pathway and CHOP-mediated apoptosis are very vital during viral infection. The levels of viral protein expression and virus titer are increased in CHOP-deficient cells thereby indicating that both ISR pathway and CHOP expression enhance antiviral response [54].

On contrary, viruses use different strategies to promote viral protein synthesis. For example, M protein of VSV can counteract antiviral response. Chikungunya virus (CHIKV) and VSV antagonize phosphorylation of eIF2α [69,132]. In addition, some viruses switch translation mode from an eIF2-dependent to an eIF2-independent process to ensure efficient replication such as PV and EV [133,134]. It was reported that DENV infection inhibit PERK-mediated eIF2α phosphorylation by elevating the expression of GADD34 which interacts with PPI to dephosphorylate eIF2α [73]. However, the conversion mechanism of viral replication through ISR remains unclear.

Recently, emerging evidence showed that ISR, autophagy and apoptosis are induced simultaneously during viral infection [71,135]. It was reported that a complete autophagy could be
induced during HCV infection and CHOP played a pivotal role in the ISR autophagy pathway [82]. Further study showed that HCV core protein activated autophagy through PERK-eIF2α-ATF4 and ATF6 pathways and facilitated ATG12 and LC3 protein expression via ATF4 and CHOP [48]. Hence, ISR is a complicated and integrated signaling response.

Activation of PERK phosphorylates eIF2α to induce ISR upon ER stress and ATF4 is a vital point to determine cell prognosis in this process [1]. We speculate that ISR is a part of UPRER, and both are induced upon stresses including viral infection but the mechanism between UPRER and ISR remains unknown. Numerous studies demonstrated that upregulation of eIF2α phosphorylation and induction of ISR during UPRMT which inhibits protein synthesis and is required for preferential synthesis of ATF4, ATF5 and CHOP to recover mitochondria function [136-138]. Hence, the role of eIF2α in UPRMT and the mechanism of UPRMT under viral infection also need to be illuminated [139]. We speculate that ISR, synergizing with UPRER and UPRMT plays an important and integrated role in maintaining cellular homeostasis and promoting cell survival against viral infection.

In conclusion, the role of ISR is becoming more and more important during viral infection. ISR is a complicated, integrated and pro-survival cellular response that converge on eIF2α phosphorylation. PERK-eIF2α signaling pathway plays vital role in regulation of viral replication and UPR-induced autophagy. PKR-eIF2α signaling pathway mediates mainly in the formation of SGs and CHOP controls UPR-induced apoptosis. Meanwhile, other branches of UPR is also involved in this process. However, many mechanisms of ISR remain unclear. Hence, further investigations are highly essential to understand the mechanism of ISR upon viral infection. In addition, it is also necessary to understand that how viruses take measures to modulate ISR to promote own replication and virulence which is vital to illuminate the interaction between host and viruses as a therapeutic target to enhance host defense against viruses.

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