MINIREVIEW

The Unfolded Protein Response and Autophagy: Herpesviruses Rule!†

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The unfolded protein response (UPR) and autophagy are two cellular environmental responses that affect a cell's life or death. The UPR begins on the sensing of an excess of unfolded proteins in the endoplasmic reticulum (ER). Autophagy, originally discovered as a response to nutrient depletion, is involved in development, in the degradation of cellular components, and in the reaction to intracellular bacteria and viruses. Some herpesviruses now appear to modify one or both of these responses to their own advantages during productive and latent infections. We know that the UPR can be mechanistically linked to autophagy, prompting the notion that herpesviruses may modify both responses by regulating one or that they may even uncouple them.

The UPR is induced when the ER responds to an overload of unfolded proteins. For example, malfolded forms of immunoglobulin heavy chain-binding protein, a chaperone termed BiP, which is diagnostic of the UPR (17). When the capacities of BiP and other ER-resident chaperones are surpassed, the UPR is induced, leading eventually to increases in the levels of these chaperones. Three regulatory paths central to the UPR are activated in the ER upon its induction. One involves activating transcription factor 6 (ATF6), which is transported to the Golgi compartment, where it is cleaved and released to translocate to the nucleus and there induce transcription of genes such as the BiP gene (5). The second path uses PKR-like ER kinase (PERK), which, when induced, phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), which inhibits general protein synthesis and, along with ATF4, induces expression of the CCAAT/enhancer-binding protein-homologous protein (CHOP) (31). Under some conditions, CHOP can lead to apoptosis in cells undergoing the UPR (39). A third path is mediated by inositol-requiring kinase 1 (IRE-1), which is induced to splice the RNA that encodes X-box-binding protein 1 (XBP-1) in an enzymatically unconventional process. The spliced XBP-1 message is translated into a transcription factor, which moves to the nucleus to transcribe multiple genes whose products ultimately home to the ER, including p58′IPK, an inhibitor of PERK (19, 36). Termination of PERK signaling and dephosphorylation of eIF2α in the later stages of the UPR permit the synthetic phase of the UPR, which requires new protein synthesis.

Autophagy is a response dissected genetically in yeast that leads to the envelopment of cytoplasmic organelles and potentially to their degradation. It is characterized by the formation of double-membrane-bound vesicles whose formation is dependent on multiple genes conserved from Saccharomyces cerevisiae to mammals (24). These double-membrane-bound vesicles, termed autophagosomes, can fuse with lysosomes to allow their contents, including whole organelles, to be degraded. The products of this degradation include amino acids that can both be used in protein synthesis and contribute to energy metabolism (22). Thus, when induced by nutrient deprivation, autophagy can lead to the redistribution of synthetic components and the energy needed for the cell to survive. Autophagy is regulated by multiple signals. It is inhibited, for example, by TOR kinase, so growth factors that affect TOR kinase also affect autophagy (22).

It is clear that the UPR can induce autophagy in S. cerevisiae such that portions of the ER are contained within and help form its double-membrane vesicles (2, 38). Other evidence supports this mechanistic linkage of the UPR to autophagy in mammalian cells. For example, the activation of PERK, a kinase central to the UPR, is required for a malfolded protein modeled on those of polyglutamine diseases to induce autophagy (16). In addition, treatment of cells to block posttranslational modifications of proteins can induce both the UPR and autophagy. The formation of autophagosomes under these conditions is inhibited in mouse embryo fibroblasts (MEFs) with deletions of IRE-1, a mediator of one arm of the UPR, indicating that this facet of the UPR is required for autophagy (27, 37). How do herpesviruses cope with these cellular responses and their mechanistic linkage?

HSV-1

During the productive stage of an infection, viral protein synthesis may push the ER's folding capacity to its upper limit. It would not be surprising to observe the activation of UPR under these conditions. However, both PERK and IRE-1 remain inactive in herpes simplex virus type 1 (HSV-1)-infected cells (25). Glycoprotein B (gB) of HSV-1 appears to manipulate PERK by binding to it and leading neither to the phosphorylation of eIF2α nor to the activation of PERK itself but conferring control on the levels of accumulation of multiple viral proteins in the infected cell (25) (Fig. 1). This blocking of the activation of PERK by gB extends to infected cells in which the UPR is experimentally induced. However, IRE-1 is still...
activated by this experimental induction of the UPR, indicating that HSV-1 selectively modifies the UPR to its own ends (25). HSV-1 devotes at least two additional genes to regulating the phosphorylation of eIF2. As a response to viral infection, the host cell can induce the phosphorylation of eIF2 by activating PKR. The ICP34.5 gene of HSV-1 encodes a protein that homes the cellular phosphatase protein phosphatase 1 alpha (PP1) to eIF2 to mediate its dephosphorylation (9). The US11 protein of HSV-1 binds PKR to block its phosphorylation of eIF2 as well (6, 26). HSV-1 blocks phosphorylation of eIF2, ensuring support for the efficient synthesis of its own proteins, and subverts PERK to control the accumulation of those proteins. By not activating PERK, HSV-1 gains the additional advantage of not inducing the synthesis of CHOP and its subsequent induction of apoptosis (23, 39).

HSV-1 uses the same ICP34.5 gene to block autophagy in infected cells (Fig. 1). Infection of MEFs with ICP34.5-null HSV-1 induces autophagy, while infection of PKR-null MEFs with the same virus does not (34). However, ICP34.5 apparently acts not only through eIF2 to block autophagy but also does so by binding and inhibiting Beclin-1, known too as Atg-6, the first regulator of autophagy identified in mammalian cells (28). Infection with wild-type HSV-1 yields 1,000-fold-more progeny than an ICP34.5-null strain within 3 days of infection of MEFs (35); this advantage appears to be mediated by ICP34.5’s blocking the UPR independently of its role in blocking autophagy (1). HSV-1 thus encodes a multifunctional protein to block both the UPR and autophagy to create an environment favorable for its own replication.

HCMV

HCMV also induces the UPR during its productive infection, as evidenced by the accumulation of ATF4, a transcription factor translated when eIF2 is phosphorylated, and by the accumulation of the spliced form of the XBP-1 message (11). During this infection, ATF6 is not found to be cleaved, indicating that this herpesvirus, too, controls the UPR (11). HCMV infection stimulates the accumulation of BiP; treating cells shortly after infection with a toxin that cleaves BiP blocks the assembly of HCMV particles (5). Stimulation of BiP synthesis is therefore needed to support virus assembly. HCMV uses BiP to its own advantage in a second way. Two viral gene products, US2 and US11, bind BiP to mediate the degradation of major histocompatibility complex class I proteins, thus protecting HCMV’s infected host cell from being recognized by a cytotoxic immune response (10). It is not now known if infection with HCMV induces any facets of auto-
It remains possible that HCMV’s fine control of the UPR unlinks these two cellular responses.

**EBV**

It is clear, however, that Epstein-Barr virus (EBV) regulates both the UPR and autophagy to foster the latent phase of its life cycle. The latent membrane protein 1 (LMP-1) oncogene of EBV induces the UPR dose dependently (21) (Fig. 2). LMP-1 mimics the cellular CD40 receptor and activates NF-κB, AP1, and the Jak/Stat pathways through its carboxy-terminal signaling domain to promote cell proliferation (4, 14). It uses its amino-terminal, six-membrane-spanning domain to regulate its signaling in lieu of a ligand, and it is this latter domain that induces the UPR, activating PERK, ATF6, and IRE-1 (21). Different cells within clonal populations of EBV-transformed B cells express levels of LMP-1 that range over 100-fold, and those with the highest levels have phosphorylated eIF2α and decreased levels of protein synthesis and are inhibited in their proliferation (13, 18, 32). LMP-1’s dose-dependent activation of PERK stimulates the translation of ATF4 dose-dependently, which drives transcription from LMP-1’s promoter and contributes to the accumulation of the LMP-1 protein (21). Given that the prolonged activation of PERK in cells can lead to apoptosis (23, 39), how do EBV-infected cells tolerate its increasing activation? LMP-1 also activates autophagy dose dependently through its last four membrane-spanning domains (20) (Fig. 2). Neither naïve B cells nor their protein A-stimulated blasts show signs of autophagy, which indicates that LMP-1’s induction of cell proliferation alone does not induce autophagy (20). EBV-infected B cells that support the most-advanced stages of autophagy also have the highest levels of activated PERK, and in these cells, LMP-1 is degraded rapidly (20). The rapid degradation observed in these cells is inhibited when the cells are treated with lysosome inhibitors (J. Lee and B. Sugden, unpublished data). This finding is also supported by studies in which autophagy is inhibited in EBV-transformed B cells. Inhibiting the expression of Beclin-1 with short hairpin RNA, for example, leads to supraphysiological levels of LMP-1 and inhibits the proliferation and/or survival of these cells (20). EBV thus controls both the UPR and autophagy to regulate the levels of its LMP-1 oncogene so that this ligand-independent mimic of a cellular growth receptor signals in a cell autonomous manner.

EBV’s control of the UPR does lead to the induction of XBP-1’s spliced form of RNA, a molecule required for the differentiation of plasma cells and the secretion of immuno-

**FIG. 2.** EBV infects B lymphocytes and induces and maintains their proliferation while expressing only a small subset of viral genes. One of these, LMP-1, is essential for driving their proliferation. It is expressed at widely different levels in individual cells within clonal populations of infected cells. These levels of expression are controlled by LMP-1’s regulation of the UPR and autophagy. LMP-1 activates PERK, which phosphorylates eIF2α. This phosphorylation promotes expression of ATF4, which increases transcription of LMP-1’s promoter. LMP-1’s induction of the UPR leads to IRE-1’s splicing of XBP-1, which is required for the secretion of the immunoglobulins observed in EBV-infected B cells. High levels of LMP-1 lead to the inhibition of general protein synthesis associated with the UPR and to the final stages of autophagy. This latter response fosters degradation of the high levels of LMP-1, lowering these levels to reset the cell’s physiology such that LMP-1 can again drive proliferation and increase its own expression.
globulins (12, 21, 30). These EBV-infected cells do accordingly secrete immunoglobulins at levels of $1 \times 10^{-4}$ to $3 \times 10^{-7}$ molecules per cell per hour (21). The virus likely benefits from this induction in an unexpected way. In different studies, XBP-1 has been found to stimulate the transcription of EBV’s BZLF1 and BRLF1 genes, activators of its productive cycle (3, 33). By controlling the UPR dose dependently with its range of expression of LMP-1, EBV thus positions a subset of infected cells to support its productive cycle when given further appropriate stimulation.

**CONCLUDING REMARKS**

HSV-1, HCMV, and EBV regulate the UPR and/or autophagy. We now know that HSV-1 and HCMV benefit from these cellular responses during productive infections, while EBV makes use of them during latent infections and likely during its escape from latency. By having evolved in the midst of these two linked responses, these and other herpesviruses have probably adapted to them to benefit themselves. The UPR and autophagy were studied initially as responses to adverse environmental conditions, but now there is growing appreciation that these pathways also affect normal mammalian development and disease. Constituents of the UPR contribute to plasma cell development (30), while their activation is linked to type 2 diabetes (29). Autophagy is required to limit neurodegeneration (7, 15). Herpesviruses infect the differentiated cell types in which the UPR and autophagy contribute to normal function: EBV infects B cells, and HSV-1 infects neurons. Additional studies with herpesviruses should help reveal how the UPR and autophagy contribute to their host cell’s life or death in both normal and diseased states.

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