View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Carolina Digital Repository

Human cytomegalovirus regulation of eIF2α kinases

Heather A Vincent[1] & Nathaniel J Moorman[*,1]

[1]Department of Microbiology & Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

* Author for correspondence: nmoorman@med.unc.edu

First draft submitted: 30 May 2017; Accepted for publication: 14 June 2017; Published online: 12 October 2017

Keywords • eIF2α • GCN2 • gene expression • HCMV • herpesvirus • HRI • human cytomegalovirus • mRNA translation • PERK • PKR • protein kinase R • protein synthesis

Viral infection is an inherently stressful event. Virus replication taxes cellular biosynthetic machinery and produces ligands that activate antiviral responses which impair normal cell function. These virus-induced stressors alter cell function, in part by activating a family of four kinases that phosphorylate the translation initiation factor eIF2α, leading to a dramatic reduction in protein synthesis. While eIF2α kinase activation typically inhibits virus replication, human cytomegalovirus (HCMV) efficiently replicates in the presence of persistent cell stress. Here we review the ways in which HCMV infection induces cellular stress responses and the mechanisms HCMV uses to evade and manipulate these responses to its own ends.

Human cytomegalovirus

Human cytomegalovirus (HCMV) is a large (>230 kB) dsDNA virus of the betaherpesvirus family, encoding over 200 open reading frames [1]. HCMV has a long replication cycle that is characterized by extensive manipulation of host signaling processes. Infection activates multiple cell stress response pathways, including the antiviral interferon pathway and the unfolded protein response [2], which if left unchecked, would inhibit HCMV replication. However, HCMV heavily regulates each of these stress response pathways, constraining aspects that limit virus replication while activating others that benefit the virus [3]. Induction of stress response pathways often activates at least one of four kinases that phosphorylate and inactivate a critical translation factor, the eIF2 complex. Here, we review how HCMV regulates the activity of eIF2α kinases to enhance virus replication and discuss remaining questions regarding the control of cellular stress responses by HCMV.

eIF2α kinases & mRNA translation

eIF2α kinases play a critical role in matching the cell's protein synthesis capacity to the cellular environment. A variety of stressors activate eIF2α kinases (described below), resulting in phosphorylation of their substrate, the eIF2α subunit of the eIF2 translation initiation factor complex. The eIF2 complex plays a critical role in translation initiation as part of the ternary complex, which consists of eIF2, GTP and a charged methionyl tRNA. The ternary complex binds the 40S ribosomal subunit to form the 43S preinitiation complex, which is then recruited to the 5′ end of an mRNA by the eIF4F cap-binding complex. Together these factors constitute the 48S complex, which scans the 5′ untranslated region (5′UTR) of the mRNA until recognizing a translation start site. Upon start site recognition, the GTP in the ternary complex is hydrolyzed and eIF2-GDP is released into the cytosol. The eIF2B subunit of eIF2 then exchanges GDP for GTP, allowing recharged eIF2 to participate in a subsequent round of initiation. Phosphorylation of eIF2α by an eIF2α kinase greatly increases its affinity for eIF2B, preventing GTP exchange. As eIF2α is present in significant excess to eIF2B, even small increases in eIF2α phosphorylation rapidly deplete the pool of active eIF2 available for ternary complex formation. Thus, eIF2α kinase activation leads to a significant inhibition of translation initiation and overall levels of protein synthesis. The mechanisms used by HCMV to prevent eIF2α phosphorylation during infection, despite significant cellular stress, are discussed below.

Human cytomegalovirus & eIF2α kinases

Throughout infection, HCMV generates cellular stresses that are potent activators of eIF2α kinases. However, very little eIF2α phosphorylation is observed in infected cells and only during the late stage of the virus lytic cycle [4], suggesting that HCMV actively prevents eIF2α kinase activation. The mechanism(s) used by HCMV to inhibit or counteract activation of two eIF2α kinases, protein kinase R (PKR) and PKR-like endoplasmic reticulum kinase (PERK), is well described [4–9]. The potential role of the other eIF2α kinases, HRI and GCN2, during HCMV infection is less clear, however they could play beneficial or inhibitory roles in virus replication.

Protein kinase R

Perhaps the best characterized eIF2α kinase during HCMV infection is PKR, a critical component of the cell intrinsic antiviral response [10]. PKR is activated upon binding to dsRNAs produced during viral infection. Binding to dsRNA induces PKR homodimerization and subsequent activating autophosphorylation. Activated PKR then binds and phosphorylates eIF2α, leading to a significant decrease in the translation of both cellular and viral mRNAs and thus, decreased virus replication.

Almost all known viruses express factors that inhibit PKR activation or limit eIF2α phosphorylation in infected cells. Some viruses produce RNAs that bind PKR and prevent its activation, such as the adenovirus VAI RNA [11] and Epstein-Barr virus (EBV) EBER RNAs [12]. Other viruses encode proteins that inhibit PKR activation and eIF2α phosphorylation. The herpes simplex virus 1 (HSV-1) US11 protein prevents PKR activation [13,14], while the HSV-1 ICP 34.5 protein recruits the host protein phosphatase 1 (PP1) to de-phosphorylate eIF2α in order to maintain sufficient levels of active ternary complex [15]. Thus, inhibition of PKR activation is a conserved strategy used by many viruses to ensure efficient replication.

dsRNA ligands for PKR accumulate during HCMV replication [14], likely due to transcription from overlapping regions of both strands of the viral genome. HCMV encodes two PKR antagonists, pTRS1 and pIRS1, that limit PKR activation through two distinct mechanisms. Both pTRS1 and pIRS1 contain a noncanonical RNA binding domain that binds dsRNA [5], although with relatively low affinity compared with PKR. However, the combined levels of pTRS1 and pIRS1 exceed that of PKR in HCMV infected cells, possibly allowing the two proteins to compete with PKR for dsRNA ligand binding [5]. In addition, both proteins directly bind PKR, independent of an RNA intermediate [7–8,16]. The ability of pTRS1 to bind PKR is necessary for inhibition of PKR activation during HCMV infection, in the absence of pIRS1. In transfected cells, pTRS1 binds the PKR eIF2α contact site and inhibits PKR kinase activity [7]. Thus, HCMV encodes two viral proteins, pTRS1 and pIRS1, that together prevent PKR activation to ensure the continued synthesis of viral proteins.

Protein kinase R-like endoplasmic reticulum kinase

High levels of protein synthesis, as occurs during the late phase of HCMV infection, can overwhelm the folding capacity of the endoplasmic reticulum (ER). The presence of unfolded proteins in the ER initiates a coordinated cellular response, called the unfolded protein response (UPR). The UPR is activated when unfolded proteins accumulate in the ER, competing the ER chaperone BiP away from its normal binding partners, the ER sensors ATF6, IRE-1 and PERK [17]. Loss of BiP binding, activates each sensor and initiates a series of events designed to re-establish cellular homeostasis. UPR activation triggers cleavage of the membrane bound ATF6 precursor protein releasing the mature ATF6 transcription factor to induce the expression of ER chaperones, including BiP itself [18]. The UPR also stimulates the IRE-1 protein, leading to activation of the Xbp-1 transcription factor, which drives expression of protein degradation factors such as EDEM. HCMV manipulates these arms of the UPR to its advantage [2,3]. For example, HCMV induces the expression of ATF6 dependent genes, yet ATF6 is not processed in infected cells. Similarly, while Xbp-1 is activated after infection, Xbp-1 dependent transcription of EDEM does not occur [4]. Thus, while infection activates the UPR, HCMV specifically manipulates the UPR to best benefit virus replication.

The third arm of the UPR involves activation of the PERK. Dissociation from BiP activates PERK, which then phosphorylates eIF2α. This results in a global reduction in translation, preventing the synthesis of new proteins into the already overtaxed ER. In addition, eIF2α phosphorylation enhances the translation of a specific subset of mRNAs that encode proteins involved in resolving ER stress [19]. Thus, eIF2α phosphorylation by PERK allows the cell to both prevent further accumulation of unfolded proteins and reduce their levels within the ER. PERK levels increase during infection and PERK expression is necessary for efficient HCMV replication. Depletion of PERK prevents the increase in lipogenesis observed during infection and inhibits viral growth [9]. Despite increased PERK

abundance, it remains inactive until late in infection. These data again demonstrate how HCMV manipulates complex signaling pathways to ensure successful virus replication.

Heme-regulated inhibitor kinase

In addition to activating the UPR, ER stress increases the levels of reactive oxygen species and overall levels of oxidative stress, which activate the eIF2α kinase, heme-regulated inhibitor kinase (HRI) [20]. Oxidative stress is observed within 30 min of HCMV infection and is critical for *HCMV* gene expression and virus replication [21]. Infection also stimulates NADPH oxidase, the enzyme responsible for production of superoxide [22]. However, the effect of HCMV-induced oxidative stress on HRI expression or activity has not been examined. As HCMV induces oxidative stress and limits eIF2α phosphorylation, it seems likely that the virus regulates HRI activation or its ability to phosphorylate eIF2α. Perhaps, the elevated expression of antioxidants such as glutathione during infection is sufficient to limit HRI activation. Alternatively, HCMV may express viral factors that limit HRI activity. In transfected cells, HCMV pTRS1 limits eIF2α phosphorylation in response to arsenite [7], an HRI agonist, suggesting that pTRS1 and pIRS1 may inhibit the activation of multiple eIF2α kinases. Further studies are needed to understand if and how HCMV-induced stress impacts HRI signaling.

General control nonderepressible 2

High levels of translation increase the levels of deacetylated or uncharged, tRNAs in the cell. The eIF2α kinase general control nonderepressible 2 (GCN2) is activated by binding to uncharged tRNAs [20]. Thus, as levels of translation and uncharged tRNAs increase, so do the levels of eIF2α phosphorylation. The resulting inhibition of translation allows the cell to restore a sufficient pool of charged tRNAs to support normal levels of protein synthesis. GCN2 is also activated by glucose deprivation, matching the translation capacity of the cell to nutrient availability. To date, the role of GCN2 during HCMV infection has not been examined, however it seems likely that HCMV regulates GCN2 signaling to allow for continued viral protein expression. The high levels of viral protein synthesis together with the ongoing synthesis of host proteins would be expected to decrease the available pool of charged tRNAs, resulting in GCN2 activation. The metabolic demands of HCMV replication results in at least mild nutrient deprivation, as evidenced by activation of the nutrient-sensitive kinase AMPK [23]. In addition, the related murine cytomegalovirus caused more severe disease in mice lacking GCN2 compared with wild-type mice [24], demonstrating an antiviral role for GCN2 *in vivo*. Because HCMV induces multiple stresses that could activate GCN2 and GCN2 plays an antiviral role in murine cytomegalovirus infection, it is likely that HCMV has mechanisms in place to limit GCN2 activation.

Future perspective

As noted above, HCMV replication induces multiple cellular stress pathways that should lead to eIF2α phosphorylation. Yet during early infection, no eIF2α phosphorylation occurs, due in part to HCMV proteins like pTRS1 and pIRS1 that inhibit eIF2α kinases [6,8]. It seems plausible, if not likely, that HCMV encodes additional proteins that regulate eIF2α kinases. Future studies to identify if and how additional eIF2α kinases such as GCN2 and HRI are regulated during infection will likely help in the identification of other viral eIF2α kinase antagonists.

HCMV also regulates eIF2α phosphorylation independent of its effect on eIF2α kinases by an unknown mechanism. eIF2α must be dephosphorylated by protein phosphatases when cell stress is resolved in order to resume translation and re-establish homeostasis. Previous studies show that phosphatase activity is required to limit eIF2α phosphorylation during HCMV infection [25], suggesting that HCMV employs additional strategies to maintain sufficient supplies of ternary complex in addition to expression of viral eIF2α kinase antagonists. HCMV induces protein phosphatase 1 (PP1) expression [25], which could help limit eIF2α phosphorylation. Other herpesviruses encode viral proteins that bind and recruit PP1 to phosphorylated eIF2α, such as the HSV-1 protein, ICP 34.5 [15]. Perhaps, HCMV proteins also recruit PP1 to reverse eIF2α phosphorylation and facilitate viral protein synthesis. In addition to regulating eIF2α phosphorylation, such viral proteins could impact other cell signaling events as PP1 regulates multiple signaling pathways. Clearly, more work is needed to understand how HCMV manipulates PP1 activity and target specificity and how this affects protein synthesis and cell signaling during infection.

Interestingly, eIF2α is phosphorylated during the late stage of HCMV infection, however overall levels of protein synthesis are not affected [4]. Further, protein synthesis becomes refractile to chemicals such as thapsigargin that potently induce eIF2α phosphorylation late in infection [26]. The mechanism(s) by which cellular and viral

mRNAs continue to translate despite eIF2α phosphorylation is currently unknown. Perhaps, infection increases the expression of translation factors needed for ternary complex formation. For example, infection could increase eIF2B levels, such that low levels of eIF2α phosphorylation would not completely deplete the pool of active eIF2 complex. Alternatively, HCMV could redirect mRNAs to locations where active ternary complex accumulates or sequestrates eIF2α kinases away from translating mRNAs, thus physically separating mRNA translation from sites where eIF2α phosphorylation occurs. In any case, more work is needed to uncover the mechanisms that allow HCMV mRNAs to efficiently translate in the face of eIF2α stress.

Like all viruses, HCMV infection induces a series of cell stresses that if left unchecked would inhibit viral protein synthesis. HCMV both manipulates and mitigates cell stress responses that activate eIF2α kinases to fine-tune the intracellular environment to support efficient virus replication. Unraveling the mechanisms HCMV uses to cope with stressors that induce eIF2α phosphorylation that will likely provide valuable insights into both viral regulation of cell stress and the cellular mechanisms that regulate mRNA translation.

Acknowledgements

We apologize to the many authors whose important work was not referenced herein due to space limitations. We wish to acknowledge members of the Moorman Lab for helpful discussions and comments.

Financial & competing interests disclosure

This work was supported by NIH grants AI03311 and AI123811 to NJ Moorman, and the North Carolina University Cancer Research Fund. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1. Mocarski E Jr. Betaherpes viral genes and their functions. In: *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G, Mocarski E *et al.* (Eds). Cambridge University Press, Cambridge, UK (2007).
2. Shenk T, Alwine JC. Human cytomegalovirus: coordinating cellular stress, signaling, and metabolic pathways. *Ann. Rev. Virol.* 1(1), 355–374 (2014).
3. Alwine JC. Modulation of host cell stress responses by human cytomegalovirus. *Curr. Top. Microbiol. Immunol.* 325, 263–279 (2008).
4. Isler JA, Skalet AH, Alwine JC. Human cytomegalovirus infection activates and regulates the unfolded protein response. *J. Virol.* 79(11), 6890–6899 (2005).
5. Bierle CJ, Semmens KM, Geballe AP. Double-stranded RNA binding by the human cytomegalovirus PKR antagonist TRS1. *Virology* 442(1), 28–37 (2013).
6. Braggin JE, Child SJ, Geballe AP. Essential role of protein kinase R antagonism by TRS1 in human cytomegalovirus replication. *Virology* 489, 75–85 (2016).
7. Vincent HA, Ziehr B, Moorman NJ. Mechanism of protein kinase R inhibition by human cytomegalovirus pTRS1. *J. Virol.* 91(5), (2017).
8. Ziehr B, Vincent HA, Moorman NJ. Human cytomegalovirus pTRS1 and pIRS1 antagonize protein kinase R to facilitate virus replication. *J. Virol.* 90(8), 3839–3848 (2016).
9. Yu Y, Pierciey FJ Jr, Maguire TG, Alwine JC. PKR-like endoplasmic reticulum kinase is necessary for lipogenic activation during HCMV infection. *PLoS Pathog.* 9(4), e1003266 (2013).
10. Sadler AJ, Williams BR. Structure and function of the protein kinase R. *Curr. Top. Microbiol. Immunol.* 316, 253–292 (2007).
11. Kitajewski J, Schneider RJ, Safer B *et al.* Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2 alpha kinase. *Cell* 45(2), 195–200 (1986).
12. Sharp TV, Schwemmle M, Jeffrey I *et al.* Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein-Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. *Nucleic Acids Res.* 21(19), 4483–4490 (1993).
13. Cassady KA, Gross M, Roizman B. The herpes simplex virus US11 protein effectively compensates for the gamma1(34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. *J. Virol.* 72(11), 8620–8626 (1998).
14. Marshall EE, Bierle CJ, Brune W, Geballe AP. Essential role for either TRS1 or IRS1 in human cytomegalovirus replication. *J. Virol.* 83(9), 4112–4120 (2009).
15. He B, Gross M, Roizman B. The gamma (1) 34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1 alpha 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. USA* 94(3), 843–848 (1997).

16. Hakki M, Marshall EE, De Niro KL, Geballe AP. Binding and nuclear relocalization of protein kinase R by human cytomegalovirus TRS1. *J. Virol.* 80(23), 11817–11826 (2006).

17. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2(6), 326–332 (2000).

18. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334(6059), 1081–1086 (2011).

19. Harding HP, Novoa I, Bertolotti A *et al.* Translational regulation in the cellular response to biosynthetic load on the endoplasmic reticulum. *Cold Spring Harb. Symp. Quant. Biol.* 66, 499–508 (2001).

20. Donnelly N, Gorman AM, Gupta S, Samali A. The eIF2alpha kinases: their structures and functions. *Cell. Mol. Life Sci.* 70(19), 3493–3511 (2013).

21. Speir E, Shibutani T, Yu ZX, Ferrans V, Epstein SE. Role of reactive oxygen intermediates in cytomegalovirus gene expression and in the response of human smooth muscle cells to viral infection. *Circ. Res.* 79(6), 1143–1152 (1996).

22. Tilton C, Clippinger AJ, Maguire T, Alwine JC. Human cytomegalovirus induces multiple means to combat reactive oxygen species. *J. Virol.* 85(23), 12585–12593 (2011).

23. Mcardle J, Moorman NJ, Munger J. HCMV targets the metabolic stress response through activation of AMPK whose activity is important for viral replication. *PLoS Pathog.* 8(1), e1002502 (2012).

24. Won S, Eidenschenk C, Arnold CN *et al.* Increased susceptibility to DNA virus infection in mice with a GCN2 mutation. *J. Virol.* 86(3), 1802–1808 (2012).

25. Hakki M, Geballe AP. Cellular serine/threonine phosphatase activity during human cytomegalovirus infection. *Virology* 380(2), 255–263 (2008).

26. Isler JA, Maguire TG, Alwine JC. Production of infectious human cytomegalovirus virions is inhibited by drugs that disrupt calcium homeostasis in the endoplasmic reticulum. *J. Virol.* 79(24), 15388–15397 (2005).