Virology 433 (2012) 157–166

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Antagonism of the protein kinase R pathway by the guinea pig cytomegalovirus US22-family gene *gp145*

Craig J. Bierle [a,b,1], Mark R. Schleiss [d,2], Adam P. Geballe [b,c,e,*]

[a] Program in Molecular and Cellular Biology, University of Washington, Seattle, WA 98115, United States
[b] Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, United States
[c] Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, United States
[d] Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455, United States
[e] Departments of Microbiology and Medicine, University of Washington, Seattle, WA 98115, United States

ARTICLE INFO

Article history:
Received 25 April 2012
Returned to author for revisions
11 May 2012
Accepted 1 August 2012
Available online 20 August 2012

Keywords:
Cytomegalovirus
Guinea pig
Protein kinase R
eIF2α
gp145
TRS1
Double-stranded RNA
US22 gene family

ABSTRACT

Viral double-stranded RNA (dsRNA) activates protein kinase R (PKR), which phosphorylates eIF2α and inhibits translation. In response, viruses have evolved various strategies to evade the antiviral impact of PKR. We investigated whether guinea pig cytomegalovirus (GPCMV), a useful model of congenital CMV infection, encodes a gene that interferes with the PKR pathway. Using a proteomic screen, we identified several GPCMV dsRNA-binding proteins, among which only gp145 rescued replication of a vaccinia virus mutant that lacks E3L. gp145 also reversed the inhibitory effects of PKR on expression of a cotransfected reporter gene. Mapping studies demonstrated that the gp145 dsRNA-binding domain has homology to the PKR antagonists of other CMVs. However, dsRNA-binding by gp145 is not sufficient for it to block PKR. gp145 differs from the PKR antagonists of murine CMV in that it functions alone and from those encoded by human CMV in functioning in cells from both primates and rodents.

Introduction

Human cytomegalovirus (HCMV) is the most common viral infection in newborns, infecting between 0.5% and 2% of infants *in utero* (Demmler, 1996). Each year in the United States there are over 5500 congenital HCMV infections that result in permanent neurologic disability or death (Bate et al., 2010). HCMV is thought to have coevolved with humans (McGeoch et al., 2006) and replicates well only in human cells. This species-specificity precludes the study of HCMV in animal models and has led to the development of several primate and rodent CMVs as models for studying pathogenesis of and immunity to HCMV (Barry et al., 2006; Powers and Fruh, 2008; Schleiss, 2006). Among these, guinea pig cytomegalovirus (GPCMV) is a particularly valuable model to study congenital infection since, like HCMV, GPCMV crosses the placenta and infects the developing fetus *in utero* (Schleiss, 2006). Application of the GPCMV model requires an understanding of the similarities and differences between GPCMV and HCMV genes and mechanisms, particularly those involved in evasion of rapidly evolving host defense systems.

Double-stranded RNA (dsRNA), which accumulates during the replication of HCMV and many other viruses (Marshall et al., 2009; Weber et al., 2006), activates several cellular antiviral pathways, including one mediated by protein kinase R (PKR). Upon binding to dsRNA, PKR dimerizes and autophosphorylates to form active PKR, which then phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF2α). Phosphorylated eIF2α inhibits the activity of the guanine nucleotide exchange factor eIF2B and thereby limits formation of the eIF2α-$tRNA_i^{Met}$-GTP ternary complex, inhibiting translation initiation and viral replication (Garcia et al., 2006).

To counteract the effects of PKR, many viruses have evolved factors that block the pathway at one or more steps (Langland et al., 2006; Mohr et al., 2007). In the case of HCMV, two genes, *IRS1* and *TRS1*, encode proteins that bind both to dsRNA and to PKR, preventing PKR activation in human cells (Child et al., 2004;

* Corresponding author at: Division of Human Biology, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., MS C2-023, Seattle, WA 98109-1024, United States. Fax: +1 206 667 6523.
E-mail addresses: cjb57@u.washington.edu (C.J. Bierle), schleiss@umn.edu (M.R. Schleiss), ageballe@fhcrc.org (A.P. Geballe).
[1] Author contact: Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., MS C2-023, Seattle, WA 98109-1024, United States.
[2] Author contact: Pediatric Infectious Diseases, Room 3-214 MTRF, 2001 6th Street SE, Minneapolis, MN 55455, United States.

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.08.005

Hakki and Geballe, 2005; Hakki et al., 2006). *TRS1* and *IRS1* are members of the β-herpesvirus US22 gene family, which also includes PKR antagonists of MCMV (*m142* and *m143*) and RhCMV (*rhTRS1*). The proteins encoded by these genes all bind to dsRNA and interact with PKR with varying species-specificities (Budt et al., 2009; Child et al., 2012,2006; Valchanova et al., 2006). Deletion of both the PKR antagonists of HCMV or either one from MCMV eliminates viral replication (Marshall et al., 2009; Valchanova et al., 2006). Productive infection can be restored to these viruses by providing an active PKR antagonist in *cis* or *trans* (Marshall et al., 2009; Valchanova et al., 2006) or, in the case of MCMV, by abolishing PKR activity in infected cells (Budt et al., 2009).

To better understand the function and evolution of PKR inhibition by CMVs, we sought to identify a PKR antagonist encoded by GPCMV. We found that gp145, a member of the US22 protein family, binds to dsRNA and inhibits the PKR pathway. gp145 shares a noncanonical dsRNA-binding domain and an ability to multimerize with TRS1 and m142/m143. Also, like TRS1, dsRNA-binding and self-association by gp145 are insufficient to antagonize PKR. Our results reveal that while dsRNA-binding is a conserved feature of the CMV PKR antagonists, mechanistic differences between GPCMV gp145 and the PKR antagonists of other rodent and primate CMVs may reflect adaptations to evolutionary changes in the PKR genes of their host species.

Results

Identification of GPCMV dsRNA-binding proteins

To test whether GPCMV encodes a PKR antagonist, we initially investigated whether GPCMV infection could rescue a vaccinia virus mutant (VVΔE3L) that lacks its PKR antagonist E3L and thus replicates poorly in many cell types (Beattie et al., 1996). VVΔE3L replication can be rescued by coinfection with a second virus that blocks PKR (Jacobs et al., 1998), as was observed previously by coinfection with HCMV and VVΔE3L (Child et al., 2002). However, VVΔE3L replication was much less restricted in guinea pig lung fibroblasts (GPL) and other GPCMV-permissive cell lines than it is in other cell types such as human fibroblasts or HeLa cells. Prior infection with GPCMV did not further increase VVΔE3L replication in GPL cells (data not shown). We also tested whether a second vaccinia protein, K3L (Beattie et al., 1991), might be acting as the primary PKR antagonist in GPL cells but found that VVΔK3L also displayed only a modest growth defect in GPL cells and prior infection with GPCMV resulted in no substantial rescue of VVΔK3L (data not shown). Thus we were unable to utilize rescue of VVΔE3L in GPCMV-permissive cell lines to determine whether GPCMV encodes a PKR antagonist.

The unusually weak phenotypes of VVΔE3L and VVΔK3L viruses in GPCMV-permissive cell lines led us to explore alternative means to screen for a GPCMV PKR antagonist. Because the PKR antagonists of many viruses are dsRNA-binding proteins (Child et al., 2012,2006; Hakki and Geballe, 2005; Langland et al., 2006; Mohr et al., 2007), we hypothesized that a GPCMV PKR antagonist might also bind dsRNA. To identify GPCMV dsRNA-binding proteins, we incubated lysates from mock or GPCMV-infected GPL cells with poly I:C agarose beads (Fig. 1) as described in Materials and Methods. Several bands unique to GPCMV infection were detected, and these likely represented either viral or virally-induced host dsRNA-binding proteins. To identify these proteins, the prominent bands were excised, digested with trypsin, and subjected to liquid chromatography–tandem mass spectrometry (Table 1). Several cellular dsRNA-binding proteins were detected in this experiment, including

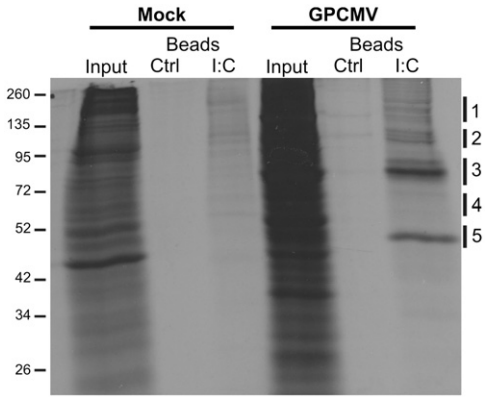

Fig. 1. dsRNA-binding proteins produced during GPCMV infection. GPL cells infected with GPCMV were radiolabeled with ^{35}S at 4 days post infection, lysed, and the proteins bound to control or poly I:C conjugated agarose beads were visualized by autoradiography following SDS-PAGE. Regions indicated on the right (1–5) correspond to samples isolated from a second experiment that were analyzed by mass spectrometry (see Table 1).

mammalian Staufen and ILF3 (Buaas et al., 1999; Ramos et al., 2000), supporting the specificity of the poly I:C pull-down assay. The most intense bands (~72 and ~47 kDa) corresponded to the GPCMV proteins gp145 and GP44. gp145 is a member of the US22 gene family and has been annotated as a possible homologue of HCMV TRS1, while GP44 is homologous to UL44, a HCMV DNA polymerase accessory protein that has been previously shown to interact with TRS1 (Schleiss et al., 2008; Strang et al., 2010). gp145 was detected in multiple samples that migrated above and below the predicted molecular weight of the protein, suggesting that multiple or modified forms of gp145 might be produced during infection. Alternatively, the smaller fragments could be degradation products generated during sample preparation. Other abundant GPCMV proteins identified in this screen included gp3, a US22 family protein with no obvious homologue in HCMV, and GP122, the GPCMV homologue of the HCMV IE2 transcriptional transactivator. A third US22 family protein identified in this experiment, gp139 (Schleiss et al., 2008), was not detected when this experiment was repeated.

We next sought to differentiate direct dsRNA-binding by GPCMV proteins from indirect binding as part of a multi-protein complex. In addition to testing gp3, gp44, and gp145, we also tested the dsRNA-binding of three additional US22 family proteins that have some sequence similarity to TRS1 (gp141, gp144, and gp146) but that were not detected in the proteomic analysis. The GPCMV proteins were expressed by *in vitro* translation and analyzed by the dsRNA-binding assay. With the exception of gp141, which failed to express either by *in vitro* translation or transient transfection, all of the GPCMV proteins tested bound to the poly I:C beads (Fig. 2 and data not shown). To test the specificity of the observed dsRNA-binding, the *in vitro* translation products were preincubated with either DNA or poly I:C prior to incubation with poly I:C beads. As expected, E3L binding to the poly I:C beads was competed by dsRNA but not by DNA, and gp145 binding was also more strongly competed by preincubation with dsRNA. The opposite pattern was observed from gp3 and GP44, suggesting that these proteins preferentially bind to DNA. These experiments demonstrated GPCMV encodes several dsRNA-binding proteins, although only gp145 appears to preferentially bind dsRNA over DNA.

gp145 rescues VVΔE3L

Having identified GPCMV proteins capable of dsRNA-binding, we next tested their ability to inhibit the PKR pathway by