Virology 458-459 (2014) 151–161

Contents lists available at ScienceDirect

Virology

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

Processing bodies accumulate in human cytomegalovirus-infected cells and do not affect viral replication at high multiplicity of infection

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ARTICLE INFO

Article history:
Received 16 January 2014
Returned to author for revisions
4 February 2014
Accepted 17 April 2014
Available online 13 May 2014

Keywords:
Human cytomegalovirus
P-body
Stress granule
Translation
Eukaryotic initiation factor
Translation initiation
MRNA decay

ABSTRACT

Translationally silenced mRNAs are recruited to two major classes of RNA granules in the cytoplasm, processing bodies (PBs) and stress granules (SGs). We show that PBs accumulated after human cytomegalovirus (HCMV) infection. PB assembly after HCMV infection was also detected in the presence of the protein synthesis inhibitor, cycloheximide, but required active RNA synthesis. UV-inactivated HCMV virions were sufficient to induce PB accumulation in HFF cells treated with cycloheximide. Viral IE1 RNA did not colocalize with PBs, and we could not detect an effect of PB accumulation on viral growth. These results may indicate that HCMV inhibits the colocalization of IE1 mRNA with PBs, preventing IE1 mRNA decay and translational inhibition.

Introduction

Although most individuals are infected by human cytomegalovirus (HCMV), the infection usually is asymptomatic. The virus is reactivated under immunosuppressive conditions and may cause pneumonitis, hepatitis, retinitis, and gastrointestinal diseases. HCMV replicates productively in terminally differentiated cells such as fibroblasts, epithelial and endothelial cells, and in monocyte-derived macrophages (Fish et al., 1995, 1996; Ibanez et al., 1991; Lathey and Spector, 1991; Sinzger et al., 1995, 1996; Taylor-Wiedeman et al., 1991). During productive infection, HCMV genes are expressed in a temporal cascade, designated immediate early (IE), early, and late. The major IE genes (MIE) UL123/122 (IE1/IE2) play a critical role in subsequent viral gene expression and the efficiency of viral replication (Isomura and Stinski, 2003; Isomura et al., 2004, 2005; Meier et al., 2002; Meier and Pruessner, 2000; Meier and Stinski, 1997). The early viral genes encode proteins necessary for viral DNA replication (Pari and Anders, 1993). Following viral DNA replication, delayed early and late viral genes, which encode structural proteins in the virion, are expressed.

The regulation of mRNA biogenesis and decay is important for maintaining host cell homeostasis during virus infection. mRNA decay by host factors regulates viral protein levels, eliminates aberrant viral mRNAs, and is consequently an essential host defense response to viral infection (Coller and Parker, 2004). mRNA silencing and RNA decay are closely linked via cross talk among proteins that regulate the initiation of translation. eIF4A, eIF4E, eIF4G, and certain mRNPs counteract silencing when bound to mRNPs and also regulate access to mRNA by decapping complexes and deadenylases (Parker and Sheth, 2007). mRNA decapping is a crucial step in general and specialized mRNA decay (Parker and Song, 2004). Translationally silenced mRNPs may be organized into two major classes of RNA granules in the cytoplasm, processing bodies (P-bodies, PBs) and stress granules (SGs) (Reineke and Lloyd, 2013). PBs contain translationally silenced mRNPs, which are enriched for many proteins involved in mRNA decapping and decay, such as Dcp1a, EDC4, and 4E-T (Eulalio et al., 2007). PBs have been suggested to function in many pathways of mRNA decay and repression of translation, ranging from nonsense-mediated decay and miRNA-mediated decay to mRNA storage and miRNA-mediated repression (Parker and Sheth, 2007; Seto et al., 2010). However, whether mRNP assembly into PBs is important for translational repression, decapping, or decay remains controversial. mRNA decapping and mRNA translation are thought to be competing pathways. PB-associated mRNPs are translationally repressed and can be degraded or stored for subsequent translation (Ferraiuolo et al.,

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http://dx.doi.org/10.1016/j.virol.2014.04.022
0042-6822/

2005; Franks and Lykke-Andersen, 2008). Little is known about how decapping enzymes become inactive in a PB when mRNPs are stored for subsequent translation. It has been reported that interaction of 4E-T with eIF4E is a priming event, inducing messenger ribonucleoprotein rearrangement and transition from translation to decay (Ferraiuolo et al., 2005).

Stress granules are dynamic structures that form quickly when external stresses are applied to cells and global translation rates decline, and disperse when translation conditions are restored. SGs are thought to contain stalled 43S and 48S ribosomal preinitiation complexes and have been suggested to serve as temporary repositories for these complexes (Kedersha et al., 2002). It has been suggested that there is a cytoplasmic mRNA cycle in which mRNPs move rapidly between active polysomes and the silenced compartments of PBs and SGs. This is supported by the observation that SGs and PBs are in equilibrium with actively translating mRNPs, shown by experiments involving chemical and genetic blockade of multiple steps in the process of translation initiation and elongation (Dang et al., 2006; Kedersha et al., 1999; Mokas et al., 2009). Furthermore, flux between the different RNA granules has been demonstrated by experiments showing transient docking of SGs and PBs, and many proteins have been detected in both compartments, such as Ago2, eIF4E, APOBEC3, and others (Kedersha and Anderson, 2007; Kedersha et al., 2005). Several other types of RNA granules have been described in *Caenorhabditis elegans* and *Drosophila* neurons; these contain various levels of proteins found uniquely in SGs or PBs (Buchan and Parker, 2009). Thus, a continuum of RNA granules has been suggested to exist in eukaryotic cells with degrees of similarity to SG and PBs.

In this report, we show for the first time that PBs accumulate dramatically during HCMV infection.

Results

HCMV infection caused accumulation of PBs

Viral and non-viral single-stranded RNAs can induce innate immune responses (Brencicova and Diebold, 2013; Diebold et al., 2004, 2006). It has been reported that SGs contain viral RNA and the cytoplasmic RNA sensor RIG-I, which initiates antiviral interferon responses in influenza A-infected cells (Onomoto et al., 2012; Yoo et al., 2014). It is possible that PBs and/or SGs sense robust expression of viral IE and cellular mRNAs to induce antiviral host defenses in HCMV-infected cells. Because SGs are not formed during HCMV infection (Isler et al., 2005), we sought to investigate whether PBs accumulate during HCMV infection. Dcp1a and EDC4 have been well characterized as markers for visualizing PBs and play a role in decapping and enhancing decapping, respectively (Franks and Lykke-Andersen, 2008). HFF cells were infected with HCMV at an moi of 3 and then stained with antibodies specific for Dcp1a and EDC4 at 6, 24, and 48 hpi. To detect PB formation, confocal images consisting of eight 1 mm slices along the *z*-axis were digitally merged. As shown in Fig. 1A, PBs were detected by relocalization of Dcp1a and EDC4 to discrete cytoplasmic puncta in the mock-infected cells, as described previously (Parker and Sheth, 2007). The proportion of cells containing more than three PB foci increased after HCMV infection (Fig. 1A–E). The number of PB foci per cell also increased at 6, 24, and 48 hpi, and larger PB foci accumulated to cover large areas of the entire cytoplasm by 48 hpi (Fig. 1A–D and F). These results suggested that HCMV infection induced the accumulation of PBs.

De novo protein synthesis was not required for PB accumulation during HCMV infection.

Because Fig. 1 showed increased accumulation of PBs at 24 and 48 hpi, we sought to determine whether this accumulation was associated with an increase in the abundance of PB proteins or simply redistribution of existing PB proteins. HFF cells were infected with HCMV at an moi of 3 and harvested before infection and at 24 hpi in the presence or absence of the protein synthesis inhibitor, cycloheximide, and at 6 and 48 hpi in the absence of cycloheximide for Western blot analysis. As shown in Fig. 2A, IE1 and IE2 proteins were detected at 6 hpi and increased at 24 and 48 hpi. In addition to the PB markers, Dcp1a and EDC4, DDX6 and Lsm14A (also known as Rck/p54 and RAP55, respectively), which play a role in suppression of translation, 4E-T, which has been suggested to contribute to inhibition of cap-dependent translation, and Lsm1, an mRNA decapping enzyme, were tested (Ferraiuolo et al., 2005; Kufel et al., 2003; Yang et al., 2006). HCMV infection induced a small increase in several of the factors in the absence of cycloheximide (Fig. 2A).

Subsequently, to further determine whether protein synthesis is required for the accumulation of PBs, HFF cells were infected with HCMV at an moi of 3 or treated with 0.5 mM Arsenite for 30 min in the presence or absence of cycloheximide, and stained with antibodies specific for Dcp1a or Lsm14A, and EDC4, before infection and at 24 hpi. Accumulation of PBs was induced as demonstrated by the relocalization of Dcp1a or Lsm14A with EDC4 to discrete cytoplasmic puncta in the cells treated with Arsenite (Fig. 2B). At 24 hpi, HCMV induced the relocalization of Dcp1 or Lsm14 with EDC4 proteins in the absence or presence of cycloheximide (Fig. 2B). Despite cycloheximide blocking *de novo* protein synthesis, HCMV infection still induced accumulation of PBs. PB formation was completely inhibited before infection in the presence of cycloheximide (Fig. 2B) as described previously (Andrei et al., 2005). Cycloheximide presumably trapped mRNAs in polysomes, which depleted the cellular pool of ribosome-free mRNPs in the uninfected cells (Franks and Lykke-Andersen, 2008). HFF cells infected with HCMV in the presence of cycloheximide did not result in an increase in Dcp1 and EDC4 protein (Fig. 2A); thus, these PB markers assembled independent of *de novo* protein synthesis.

PBs have been implicated in miRNA-mediated post-transcriptional gene silencing, and Ago2 is known to be a key mediator that selectively recruits miRNAs and their target mRNAs to PBs (Holley-Guthrie et al., 2005). To determine whether Ago2 is recruited to PBs formed by HCMV infection, HFF cells were infected with HCMV in the presence or absence of cycloheximide, and stained with antibodies to EDC4 and Ago2 before infection and at 24 hpi. In HCMV-infected cells, foci formation by Ago2 was not clearly detected and Ago2 did not accumulate in PBs, as demonstrated by colocalization with EDC4 foci, during HCMV infection in the presence or absence of cycloheximide (Fig. 2C).

Inhibition of RNA transcription abrogated PB accumulation after HCMV infection

To determine whether active transcription is required for the accumulation of PBs induced by HCMV infection, HFF cells were infected in the absence or presence of Actinomycin D (ActD), which inhibits RNA synthesis, then immunostained with Lsm14A and EDC4. As shown in Fig. 3A, inhibition of transcription for 24 h after HCMV infection resulted in little to no PBs. ActD may have inhibited PB accumulation altogether or induced PB dispersion after HCMV infection. The histogram in Fig. 3B shows the average number of PBs/cell (*Y* axis). After treatment with ActD for 24 h, PBs had fallen to the level before HCMV infection. These results clearly show that HCMV-induced accumulation of PBs was dependent on active RNA synthesis.

UV-inactivated HCMV virions induced PB accumulation in HFF cells

To determine whether HCMV gene expression is essential for PB accumulation, HFF cells were infected with HCMV, UV-inactivated virions, or the culture supernatant after removal of virions at an moi of 3. HFF cells were maintained in the absence or presence of