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Guinea pig cytomegalovirus GP84 is a functional homolog of the human cytomegalovirus (HCMV) UL84 gene that can complement for the loss of UL84 in a chimeric HCMV

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ABSTRACT

The guinea pig cytomegalovirus (GPCMV) co-linear gene and potential functional homolog of HCMV UL84 (GP84) was investigated. The GP84 gene had delayed early transcription kinetics and transient expression studies of GP84 protein (pGP84) demonstrated that it targeted the nucleus and co-localized with the viral DNA polymerase accessory protein as described for HCMV pUL84. Additionally, pGP84 exhibited a transdominant inhibitory effect on viral growth as described for HCMV. The inhibitory domain could be localized to a minimal peptide sequence of 99 aa. Knockout of GP84 generated virus with greatly impaired growth kinetics. Lastly, the GP84 ORF was capable of complementing for the loss of the UL84 coding sequence in a chimeric HCMV. Based on this research and previous studies we conclude that GPCMV is similar to HCMV by encoding single copy co-linear functional homologs of HCMV UL82 (pp71), UL83 (pp65) and UL84 genes.

Introduction

Human cytomegalovirus (HCMV) is a ubiquitous pathogen that causes asymptomatic infection in normal healthy individuals. However, it has emerged as a serious infection in immune suppressed individuals, including organ transplant and AIDS patients as well as the fetus during pregnancy (Pass, 2001). Indeed, congenital infection of the fetus by HCMV (1–2% of live births in the US) is a major cause of mental retardation and hearing loss in surviving newborns in the developed world (Ross and Boppana, 2005; Griffiths and Walter, 2005). Although there are candidate vaccines in clinical trials there is currently no effective vaccine to HCMV (Schleiss and Heineman, 2006). Antivirals are available but these act at late stages of the virus life cycle and result in the development of resistant strains emerging during prolonged antiviral therapy (Biron, 2006; Chou, 2008; McGregor, 2010). Consequently, there is a need for the development of new vaccine strategies as well as the development of novel antiviral strategies that act at an earlier stage of the virus life cycle and less likely to lead to the development of resistant strains of virus.

In human cytomegalovirus (HCMV) the UL82, UL83 and UL84 genes are thought to have evolved from a common ancestor by gene duplication with subsequent differentiation to fulfill unique functions associated with the virus life cycle (Davison and Stow, 2005). Both UL82 and UL83 encode tegument proteins whereas UL84 encodes a non-structural protein (He et al., 1992; Hensel et al., 1992; Schmolke et al., 1995). Both tegument proteins, pp71 (UL82) and pp65 (UL83), have roles in initiating the viral life cycle in the infected cell as well as avoiding the cellular innate immune response (Abate et al., 2004; Baldick et al., 1997; Cantrell and Bresnahan, 2006). The function of UL84 protein in the HCMV viral life cycle is not entirely understood but it has an essential role insofar as knockout HCMV UL84 mutants are nonviable (Dunn et al., 2003; Xu et al., 2004; Yu et al., 2003). The UL84 proteins are unique to betaherpesviruses and in HCMV it is required in an auxiliary role for HCMV DNA replication (Sarisky and Hayward, 1996; Xu et al., 2004). Specific interaction of pUL84 with the DNA polymerase accessory protein (pUL44) has been demonstrated (Strang et al., 2009). However, Reid et al. (2003) using HSV-1 DNA replication fork proteins showed that replication from the HCMV origin site is independent of UL84 protein. Initially identified as a

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nuclear protein, pUL84 can shuttle between the nucleus and the cytoplasm and multimerize with itself (Colletti et al., 2004; He et al., 1992; Lischka et al., 2003; Lischka et al., 2006; Xu et al., 2002). The purpose of pUL84 shuttling has yet to be defined but involves the alpha beta importin pathway (Lischka et al., 2003). Potentially, shuttling may be linked to pUL84 homology with DExD/H box family of helicases, which shuttle between the nucleus and cytoplasm (Colletti et al., 2005; Lischka et al., 2006). The protein is also suggested to have UTPase activity (Colletti et al., 2005) and other pUL84 homologs, including GPCMV, carry UTPase motifs (Davison and Stow, 2005). Additionally, pUL84 exhibits a transdominant inhibitory growth effect on HCMV and interaction studies determined that the inhibitory effect is linked to specific interaction with IE2-p86 that impairs transactivation (Gebert et al., 1997; Colletti et al., 2004). Since IE2-p86 is important for the initiation of HCMV viral replication, this transdominant inhibitory effect could potentially be developed as a novel antiviral strategy, which would impact on virus replication at an earlier stage than current antivirals. Therefore in addition to identifying GPCMV GP84 as a functional homolog we sought to identify a minimal peptide domain with a transdominant inhibitory effect on GPCMV growth for potential development as an antiviral in the guinea pig model.

The strict species specificity of HCMV precludes studying this virus in a non-human animal model. Consequently, animal CMVs are used in their respective hosts for viral pathogenicity or candidate vaccine studies. Various animal models have been explored in the study of CMV (Schleiss, 2006; Barry et al., 2006). The guinea pig model of CMV is uniquely useful for the study of congenital infection and the development of intervention strategies that prevent infection of the fetus (Kumar and Nankervis, 1978; Kern, 2006; Schleiss, 2002). One focus of vaccines against HCMV has been directed towards the pp65 viral tegument protein (UL83), the major CTL target (Gonczol and Plotkin, 2001). The homolog protein, GP83, in GPCMV has been successfully used as protective vaccine in the guinea pig model (McGregor et al., 2004; Schleiss et al., 2007). In contrast, the M84 gene in murine cytomegalovirus (MCMV), while co-linear to UL84, encodes a functional homolog to pp65 (UL83) and is a major CTL target (Ye et al., 2004). Accordingly, M84 vaccines provide protection against MCMV challenge in mice (Holtappels et al., 2001; Morello et al., 2000). Hence, from the standpoint of vaccine development studies in the guinea pig model it was important to more properly define the function of the co-linear homolog to UL84, GP84, in the GPCMV life cycle. We previously identified proteins encoded by the GPCMV genes GP82 and GP83 as tegument proteins and direct functional homologs of HCMV encoded proteins pp71 (UL82) and pp65 (UL83) (McGregor et al., 2004; Schleiss et al., 1999). Preliminary studies suggest that the predicted pGP84 is potentially a functional homolog of pUL84 based on the presence of conserved UTPase motifs not found in the MCMV homolog (Davison and Stow, 2005). Therefore in this paper we attempted to demonstrate that pGP84 is a functional homolog of pUL84.

Results

GP84 expression and cellular location of pGP84 protein

The GP84 ORF encodes a protein of 483 aa in length that exhibits the highest homology to HCMV UL84 protein (22% identity by BLAST analysis). Analysis of the kinetics of gene expression in wild type virus infected cells reveals the GP84 gene expression to be delayed early kinetics (Fig. 1), which is similar to HCMV UL84 (He et al., 1992). This conclusion is based on comparative RT-PCR assays also performed for different classes of GPCMV genes previously characterized (Fig. 1): immediate early, IE2, unique exon GP122 (Yamada et al., 2009); early, GP54, the viral polymerase (Schleiss, 1995); late, GP83, encoding the homolog to pp65 (Schleiss et al., 1999). Additionally, the class of kinetics was also confirmed by the inclusion of protein inhibitor cycloheximide (for IE transcripts) or DNA replication inhibitor phosphonoacetic acid, PAA (for detection of IE and E transcripts) in virus infected cultures. GP84 transcripts were detected in the presence of PAA but not in the presence of cycloheximide which confirmed GP84 as an E transcript (see supplementary Figure S1).

The full length GP84 ORF was cloned in frame into the expression vector pCMV2A (Promega), which tagged the N-terminal domain of the ORF with an epitope FLAG tag. Transient expression studies of pGP84 were carried out on GPL cells. The cellular distribution of pGP84 was determined at various times between 3 and 48 h post transfection via immunofluorescence assay. Fig. 1(ii), image A, indicates that pGP84 is a nuclear protein and this result is consistent with the cellular location reported for HCMV pUL84 (Xu et al., 2002). Using convenient restriction enzyme sites a series of collapses were made to the full length GP84 ORF to generate N- and C-terminal deletions as described in Materials and methods (and shown in Supplementary Figure S2). In transient cellular expression assays, the N-terminal collapsed pGP84 (lacking the first 211 aa of full length pGP84) encoded on plasmid pFLAGGP84(B) appeared only in the cytoplasm and not in the nucleus (Fig. 1(ii), image C). In contrast, the C-terminal and combined N- and C-terminal truncated versions of pGP84 (encoded on plasmids pFLAGGP84(S) and pFLAGGP84(B) respectively) were found in both nuclear and cytoplasmic compartments (Supplementary Figure S3). This indicated that pGP84 carries a N-terminal NLS as described for HCMV pUL84 (Xu et al., 2002). However, further study would be required to determine if pGP84 is dependent upon the alpha and beta importin pathway for nuclear targeting as described for HCMV (Lischka et al., 2006).

pGP84 co-localizes with the viral DNA polymerase accessory protein (pGP44)

HCMV pUL84 plays an accessory role in viral DNA replication and has recently been shown to interact with the viral DNA polymerase accessory protein, pUL44, (Strang et al., 2009). The UL44 homolog gene (GP44) in GPCMV (Schleiss et al., 2008) encodes a protein of 407 amino acids that exhibits 57% identity with HCMV pUL44 by BLAST analysis (McGregor, unpublished data). In an effort to demonstrate similar interactions between GPCMV homolog proteins (pGP84 and pGP44) the GP44 coding sequence was cloned in frame into a FLAG tag expression plasmid to epitope tag the GP44 ORF. Transient expression studies were carried out in the presence of GFP tagged pGP84 expression plasmid or control GFP plasmids (expressing GFP only or a HCMV pp65 GFP fusion). Interactions were followed by cellular co-localization studies via immunofluorescence assay. Fig. 2, panels A–F, show the co-localization of pGP84 and pGP44 in the nucleus of cells expressing both proteins. Identical patterns of nuclear aggregates were observed under GFP filter for pGP84 (panels A and D) or Texas red filter for pGP44 (panels B and E). Co-expression of pGP44 and GFP protein did not indicate any specific co-localization (Fig. 2, panels J and K). The GFP protein was seen evenly distributed in both nuclear and cytoplasmic compartments, whereas pGP44 was only located in the nucleus and was uniform in the nucleus without aggregates seen in the presence of pGP84. In another control experiment, co-expression of pGP44 with HCMV pp65-GFP fusion protein did not give rise to pGP44 nuclear aggregates (Fig. 2, panels G and H). The pp65 protein produced characteristic punctate nuclear fluorescence but the pGP44 protein was evenly distributed in the nucleus. The expression pattern of pGP44 only (Fig. 2, panel M) is the same pattern seen in GFP and GFP-pp65 co-transfection cells (K and H respectively). Overall the transient GP84 expression studies suggest that the pGP84 has similar cell localization properties to pUL84 and potentially interacts with the viral DNA replication machinery as demonstrated for HCMV pUL84 (Strang et al., 2009; Sarisky and Hayward, 1996).