Characterization of pUL5, an HCMV protein interacting with the cellular protein IQGAP1

Giulia Anselmi, Maria Giuliana, Giacomo Vezzana, Rossella Ferrantia, Michela Gentile, Mirko Cortese, Diego Amendolaa, Nicola Pacchiania, Romina D’Aurizio, Luca Bruno, Yasushi Uematsu, Marcello Merolaa, Marcello Merola, Domenico Maione

GSK, via Fiorentina, 1, 53100, Siena, Italy
Department of Biology, University of Naples “Federico II”, via Cintia, 80126, Naples, Italy

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ABSTRACT

Among the Herpesviridae, human cytomegalovirus (HCMV) owns the largest genome and displays a huge coding potential. Here, we characterized the UL5 gene product (pUL5) of the clinical isolate TR strain. The protein was predicted as a 166-amino-acid membrane protein with a theoretical mass of 19 kDa. Recombinant virus expressing pUL5 with a tag allowed the identification of two pUL5 non-glycosylated species of approximately 19 and 9 kDa, expressed with early and late kinetic respectively. Experiments in infection confirmed that the lower molecular weight species was translated from an internal ATG in the UL5 open reading frame. Confocal microscopy analysis showed that pUL5 localized within the assembly compartment, but is not incorporated in the virion, as shown by Western blot on purified viral particles. Finally, pull-down experiments coupled with mass spectrometry analysis identified IQGAP1 as a pUL5 interactor, giving new hints on possible roles of pUL5 during HCMV infection.

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous, highly specific herpes virus, infecting as many as 75% of adults. It establishes latent infection applying numerous immune evasion strategies. Despite being normally relatively benign, HCMV infections can induce severe disease in immunocompromised patients, and in fetuses in the case of primary maternal infection (ten Berge and van Lier, 2014). HCMV infects a wide spectrum of cell types using different sets of surface molecules. This heterogeneity suggests a very complex mechanism of recognition between the viral particle and the host cell. To obtain deeper insights of the mechanisms involved in HCMV infection, tropism, immune evasion and latency, it is yet required to study HCMV proteome.

HCMV displays a coding capacity that far exceeds that of most other Herpesviridae, having the largest genome among all known human viruses. Its 240-kb double-stranded linear DNA genome is composed of a long unique (UL) and a short unique (US) sequences, each flanked by inverted repeats, named terminal or internal based on their position (TRL/IRL and TRS/IRS), giving the overall genome configuration TRL-UL-IRL-US-TRS (Davison et al., 2003a). Within the alpha-, beta- and gamma-herpesviruses subfamilies, a set of forty-one genes are conserved as sequence and function although they differ in genomic positions and orientation. These genes are referred to as herpesvirus core genes and are involved in metabolic functions as well as structural attributes (Alba et al., 2001; Montague and Hutchison, 2000). On the other hand, each member of these subfamilies carries some unique genes in some cases conserved among them. While most of these genes are dispensable for viral replication in cell culture, they would rather have a crucial role in in vivo infection as there are often involved in viral tropism or in evasion from the host immune system, (Miller-Kittrell and Sparer, 2009). Among them, the RL11 gene family includes 11 genes present only in human and chimpanzee cytomegaloviruses (RL11, RL12, RL13, UL4, UL5, UL6, UL7, UL8, UL9, UL10, and UL11), and 3 genes only present in HCMV (UL1, RL5A, and RL6) (Chee et al., 1990).
A common characteristic of this family is the RL11 domain (RL11D), encoding a distinctive key motif consisting of a region of variable length formed around three conserved amino acid residues (a tryptophan and two cysteines) that resembles a domain shared by some members of the immunoglobulin superfamily and including potential N-linked glycosylation sites (Gabaev et al., 2011, 2014). The UL1 gene product is a hypervariable protein expressed on the surface of infected cells (Gabaev et al., 2011, 2014). UL7 encodes for a SLAM-family receptor CD229 homolog that impairs cytokine production and promote angiogenesis and reactivation of hematopoietic progenitor cells and monocytes (Crawford et al., 2018; Engel et al., 2011; MacManiman et al., 2014). In general, RL11 family members share some common features, apart from general, RL11 family members share some common features, apart from two cysteines that resembles a domain shared by some members of the immunoglobulin superfamily and including potential N-linked glycosylation sites (Chang et al., 1989a; Crawford et al., 2018; Engel et al., 2011; MacManiman et al., 2014). In the present study, we have approached the structural and functional characterization of the UL5 open reading frame (ORF). UL5 is found as a single copy in the HCMV and chimpanzee cytomegalovirus (CCMV) genomes, within the unique long region, approximately 2 kb from the junction with the terminal repeat long region (Davison et al., 2003b; Chang et al., 1989b).

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detection were performed by using the BrightStar BioDetect kit (Life Technologies) according to the manufacturer’s instructions. Briefly, UL5-specific DNA hybridization probes were labeled with biotin-16-dUTP (Roche) by PCR using UL5-specific primers UL5-NB-F and UL5-NB-R (Table 1) as well as TR BAC as a template. The PCR mix was prepared with 40% of biontinated dUTP and 60% of dUTP.

2.5. Preparation of eukaryotic expression constructs and DNA transfection

A human codon-optimized UL5 sequence, from HCMV TR strain, was synthesized by Geneart (Life Technologies) and cloned in plasmid pcDNA3.1(-)/c-myc/6HisA (Life Technologies) in frame with C-term c-myc and six histidine tag sequences. Mutation of the second ATG in UL5 open-reading frame (Met87) on the TR-UL5-2StrepII-2FLAG was obtained using primers UL5-M87A-F and UL5-M87A-R. The complete list of the primers used in this study is provided in Table 1. Lipofectamine 2000 (Life Technologies) was used to transfect HEK-293T cells according to manufacturer’s instructions.

2.6. Confocal microscopy analysis

HFF cells grown on glass in 8-well chamberslide were infected at MOI 1. The cover slips were fixed 6 days post infection after infection, the cover slips were washed and fixed in Cytofix/Cytoperm buffer (BD Biosciences). The fixed cells were incubated in blocking buffer (phosphate-buffered saline - PBS + 5% bovine serum albumin - BSA) for 60 min before antibody staining. Primary and secondary antibodies were always diluted in blocking buffer and incubated for 60 and 30 min, respectively at room temperature. The cover slides were mounted using ProLong Gold Antifade Mountant with DAPI (Life Technologies). The intracellular staining was examined under laser illumination in a Zeiss LSM 710 confocal microscope and images were captured using ZEN software (Carl Zeiss). Images were processed using ImageJ software.

2.7. SDS-PAGE and immunoblotting

Proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 4–12% polyacrylamide pre-cast gels (Life Technologies) under standard conditions. Proteins were transferred to nitrocellulose membranes (iBlot system – Life Technologies), and the membranes were blocked with PBS containing 0.05% Tween 20 and 10% powdered milk. Antibodies and sera were diluted in PBS containing 0.05% Tween 20 and 1% powdered milk. For detection of primary antibody binding, horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody and the West Pico Chemoluminescent Substrate (Thermo Scientific) were used according to the manufacturer’s instructions. Removal of N-linked oligosaccharides was carried out using recombinant peptide:N-glycosidase F (PNGase F) and endoglycosidase H (Endo H) (NEB) according to the manufacturer’s specification. Removal of O-linked oligosaccharides was performed by Endo-α-N-Acetylgalactosaminidase (O-glycosidase) (NEB).

2.8. HCMV virions purification and virion proteins fractionation

Mature HCMV virions were purified from T175 cm² flasks of infected HFF culture supernatant collected at 6 dpi and subjected to 4000 rpm centrifugation for 20 min at 4 °C. Clear supernatant was transferred to polycarbonate ultracentrifuge tubes under lied with 20% sucrose cushion and centrifuged at 230000 rpm in a Beckman SW32Ti rotor for 60 min. The virus-containing pellet was solubilized in 1% Triton X-110 in PBS and cOmplete protease inhibitor cocktail (merck) and finally equilibrated in SDS-PAGE loading buffer for Western blot analysis.

2.9. Pull-down coupled with mass spectrometry and immunoprecipitations

For pull-down experiments HFF cells were infected with the recombinant or wild-type viruses at MOI 3. Cell lysates were prepared with Celllytic M (Sigma-Aldrich) according to manufacturer’s specifications. Pull-down was carried out using anti-FLAG M2 magnetic beads (Sigma-Aldrich) according to manufacturer’s instructions; the binding step was prolonged overnight and protein complexes were eluted by competition with the FLAG peptide. Eluted fractions were subjected to SDS-PAGE and stained with colloidal Coomassie G-250. Bands of interest were excised, dehydrated with acetonitrile for 15 min and digested with trypsin overnight at 37 °C, then spotted on the target 1:1 with recrystallization buffer (70% ethanol, 1% trifluoroacetic acid) and analyzed by MALDI-TOF. The trypsin spectrum was subtracted from the resulting spectra and the final peaks were searched on Mascot database (MatrixScience). For immunoprecipitations (IP), HEK-293T cells were transfected with Lipofectamine 2000 (Life Technologies) and lysates were prepared using Celllytic M (Sigma-Aldrich). IPs were carried out using Dynabeads Protein G (Life Technologies) according to manufacturer’s specifications, using 5 μg of antibody for IP reaction.

2.10. Sequence analysis

The amino-acid sequences for the UL5 coding sequences from laboratory adapted HCMV strains (AD169 and Towne) and clinical isolates have been assigned the following accession numbers from GenBank: AGL96607.1 (TR), AAC49222.1 (Toledo), AFR56339.1 (HAN31), AFR56172.1 (HAN28), AFR56005.1 (HAN22), AFR4510.1 (6397), AGT36439.1 (Towne-BAC-der), ACZ80259.1 (AF1), ACZ80094.1 (U1), ACZ79929.1 (VR1814), ACZ79764.1 (U8), ACS92108.1 (JP), ACM47996.1 (Towne), ACL51086.1 (AD169), AAR31292.1 (3157), AAR31275.1 (3301). Multiple sequence alignments were performed using CLUSTALW 2.1 (Larkin et al., 2007); N-, O- and glycosylation sites, signal peptides and transmembrane regions were predicted using NetNGlyc 1.0 (Gupta et al., 2004), NetOglyc 4.0 (Steentoft et al., 2013), SignalP 4.1 (Petersen et al., 2011) and PSORT 2.1 (Nakai and Horton, 1999) servers, respectively.

3. Results

3.1. UL5 open reading frame and its predicted protein product

In order to investigate UL5 ORF and the similarity of amino-acid sequence among different HCMV strains, a multiple alignment was performed using CLUSTALW server (Larkin et al., 2007). UL5 sequences derived from both laboratory-adapted strains and clinical isolates were compared, showing that the overall sequence is highly conserved and major differences belong to the N-terminal part of the predicted protein (Fig. 1).

The UL5 ORF is predicted to encode a 166 amino-acid protein. Analysis with PSORT server (Nakai and Horton, 1999) identified a transmembrane region between amino-acids 118 and 134, suggesting that pUL5 is a type Ib membrane protein with the C-terminus exposed in the cytoplasmic region and the N-terminus exposed towards the lumen or extracellular space. PSORT analysis also excluded the presence of a signal peptide, result in agreement with SIGNALP server prediction (Petersen et al., 2011). Since many RL11 family members are postulated or have been identified as membrane glycoproteins (Cortese et al., 2012; Shikhagaie et al., 2012; Gabaev et al., 2011), glycosylation predictions were also performed using NetNGlyc (Gupta et al., 2004) and NetOglyc (Steentoft et al., 2013) servers. The results show that pUL5 is not predicted to have N-glycosylation sites, while 7 potential sites of O-glycosylation are present (Fig. 1).
3.2. UL5 transcription

According to literature, the UL5 coding sequence is included in five transcripts: three containing also the UL4 ORF and transcribed from three promoters with different kinetics (Chang et al., 1989a, 1989b; Gao et al., 2015), and two low-abundance UL5 transcripts identified in late class RNAs (Gao et al., 2015). HCMV lytic gene expression is conventionally divided into three major kinetic classes of the viral genes, immediate-early, early, and late. The UL4 protein product, also called gp48, is a glycoprotein expressed in the early phase of infection that seems to have a very complex transcriptional and translational regulations, due to an upstream ORF present in the bicistronic transcripts (Alderete et al., 1999, 2001). To date, no information is available on the protein product of the UL5 ORF.

To define the transcription profile of the UL5 ORF, total RNA was isolated from HFF cells at different time points after infection with HCMV TR and subjected to Northern blot analysis using a UL5 mRNA-specific biotinylated DNA probe. The probe detected a single species of about 1.6 kb already visible at 6 h post-infection (Fig. 2A). This finding is in line with a previous report from Gao et al. in which a transcript of 1600–1800 nt, corresponding to a transcription unit containing both UL4 and UL5, was found in the initial IE phase (Gao et al., 2015). Conversely, we were not able to detect the shorter late transcript of ~650 nt identified by Gao with UL5 specific probes. This difference could be ascribed to sensitivity of the probe used or to the virus tested, in our case the TR strain while Gao et al. analyzed the low passage strain H. Interestingly, Gao et al. (2015) reported the presence of non-canonical TATA elements upstream of the UL5 initiation site, supporting the hypothesis that the short transcript is a conventional mRNA with the potential to encode for 9 kDa protein.

3.3. UL5 protein expression

In order to investigate the UL5 protein product in the context of infection, we applied a two-step Red-Gam mediated mutagenesis on a bacterial artificial chromosome (BAC) containing the entire genome of HCMV TR strain (Tischer et al., 2006) to obtain a recombinant virus carrying two StrepII and two FLAG tags at the C-terminus of UL5 protein (TR-UL5-2StrepII-2FLAG). The recombinant TR-UL5-2StrepII-2FLAG infectious virus was used to infect HFF cells at MOI 5, lysates were prepared at different times post-infection (p.i.) were prepared and subjected to immunoblot using the anti-FLAG monoclonal antibody to assess the presence of UL5 (Fig. 2B). Monoclonal antibodies against HCMV immediate-early antigen 1 and glycoprotein B (late gene) were used as references for the expression kinetics while GAPDH taken as loading control. Two species, approximately 24 and 15 kDa (~19 kDa and 9 kDa considering the 2StrepII-2FLAG tags, respectively), were detected as early as 24 h p.i (UL5-H and UL5-L, respectively) and steadily increased during the course of the infection. Both species were absent at 12 h p.i. while the immediate-early antigen 1 was already detected. To determine the expression kinetics of the two UL5 forms, infected cells were treated with either cycloheximide (CHX), or with phosphonoacetic acid (PAA), and cell lysates were prepared at different time points. Treatment with CHX, an inhibitor of protein translation,
restricts the expression of IE (immediate early) genes. On the contrary, treatment with PAA, a viral DNA synthesis inhibitor, affects the expression of the late proteins without influencing IE genes expression. The expression profiles of the viral proteins IE1 and gB, an immediate early and a late phase protein respectively, confirmed the effectiveness of the treatments (Fig. 2B). While both forms of UL5 were sensitive to CHX treatment, only UL5-H was clearly detectable upon PAA treatment. Thus, UL5-H and UL5-L proteins exhibit differential expression profiles: the former showed a profile compatible with an early/delayed early (E/DE) kinetic while the latter was clearly detected only in the absence of any inhibitor, a profile comparable to a late gene expression kinetic such as gB.

3.4. UL5 protein analysis

The predicted molecular weight for the tagged protein is 24.5 kDa, consistent with the higher band present in the Western blot. This suggests that neither form undergoes glycosylation. To confirm this finding, HEK-293T cells were transfected with pcDNA-UL5-c-myc-6his (calculated molecular weight 22 kDa). Lysates were treated with EndoH, PNGaseF and O-glycosidase, subjected to SDS-PAGE followed by immunoblot using an anti-his tag monoclonal antibody. As expected, no band shift was observed after the treatments, demonstrating that neither form of pUL5 is glycosylated (Fig. 3A).

We speculated that there are two possible explanations justifying the production of two different UL5 isoforms maintaining the same ORF: a protease cleavage or the presence of a second ATG within the UL5 coding sequence. Nucleotide sequence of the TR UL5 gene showed a unique internal methionine at position 87 in frame with Met1. To verify if methionine 87 represents the second initiation codon for the shorter UL5 product we introduce a Met to Ala substitution in the TR-UL5-2StrepII-2FLAG and analyze the lysate from infected HFF cells of this mutant in Western blot. As shown in Fig. 3B, removal of the internal methionine abrogates the expression of the lower molecular weight UL5. This data strongly suggests that the UL5 Met87 is an active translation initiation codon. Interestingly, the expression profile of UL5-L is consistent with the appearance of the short transcript described by Gao and co-workers at late infection phase (Gao et al., 2015). Moreover, the starting codon of the putative ORF identified by Gao et al. corresponds to UL5 Met87. Indeed, the MW observed for the UL5-L form, which is 15 kDa including the ~6 kDa of the 2StrepII-2FLAG tags, exactly coincides with the 9 kDa MW calculated for the protein encoded by the short UL5 mRNA. Thus, the UL5-H and UL5-L would share the same C-terminus (as shown by the reactivity against the C-terminal flag tag), but different N-termini, with the latter starting from an internal ATG corresponding to the Met87 of the UL5-H. Although these data do not rule out completely the possibility of a protease cleavage, they strongly suggest that an internal transcription/translation site is active.

3.5. Localization of UL5

To analyze the subcellular localization of pUL5 during infection we performed a confocal analysis on HFF infected with either wt TR virus or TR-UL5-2StrepII-2FLAG. Six day post infection, cells were fixed and stained with anti-FLAG monoclonal antibody, to detect UL5, and a marker of the trans-Golgi network (TGN46). The HCMV glycoprotein B (gB) was also used to visualize formation and localization of the assembly complex (AC). As displayed in Fig. 4, the UL5 protein was rather diffused into the cells but accumulated with TGN46 and gB suggesting its massive presence into the viral AC, where the virus particles are...
generated. HCMV infection changes the morphology of the nucleus which becomes larger and wraps around this juxtanuclear compartment through reorganization of cellular organelles including the endoplasmic reticulum, the Golgi apparatus, secretory vesicles and the early endosomes, as well as the lysosomes (Alwine, 2012). In this compartment, the nucleocapsids transported from the nucleus associate with tegument and envelope proteins with a mechanism that is not yet well understood (Britt et al., 2007).

Since pUL5 localized in the AC during infection, we wondered whether it is also incorporated into the viral particles. To address this question, supernatants from TR-UL5-2StrepII-2FLAG HCMV infected fibroblasts were subjected to ultracentrifugation on a 20% sucrose cushion and virions were isolated and subjected to Western blot analysis. Both lysates from infected cells (WCL) and purified virions were positive for the structural proteins gB and pp65. However, upon probing with anti-FLAG antibody, we were able to detect the UL5 protein only in the total lysate from infected cells but not in the lysate from purified virions (Fig. 5) suggesting that UL5 is not a HCMV structural protein but it may have cytoplasmic roles.

3.6. UL5 interacts with IQGAP1

To obtain hints on the potential intracellular role of the pUL5, we performed pull-down experiments coupled with mass spectrometry analysis to identify protein interactors. To this end, fibroblasts were

![Figure 3](image1.png)

**Fig. 3.** (A) Glycosylation analysis of pUL5. HEK-293T cells were transfected with plasmid pcDNA3.1(−)/UL5-c-myc/6HisA expressing pUL5 with myc and two 6xHis C-terminal tail. Cell-lytic extracts were prepared from the transfectants after 48 h incubation at 37 °C. Ten μg of proteins from the cellular extracts were treated with Endoglycosidase H (EndoH), peptide:N-glycosidase F (PNGase F), Endo-α-N-Acetylgalactosaminidase (O-glycosidase) or in absence of enzymes. Western blot analysis was performed using anti-His as probe. Purified gH/UL116 (described in (Calo et al., 2016)) was used as control of glycosidases enzymatic activity. (B) Expression profile of the pUL5-M87A mutant. Western blot on lysates of HFF cells infected with TR UL5-2StrepII-2FLAG or TR UL5-2StrepII-2FLAG M87A revealed with an anti-FLAG mouse monoclonal antibody.

![Figure 4](image2.png)

**Fig. 4.** Subcellular localization of pUL5 in infected cells. Confocal imaging of HFF cells infected with the TR wild-type virus (TR) or with the recombinant UL5-2StrepII-2FLAG virus (TR UL5-FLAG) at MOI of 1 and fixed at 96 h p.i. The intracellular localization was determined by comparing the signal from anti-FLAG-specific antibody to detect UL5 with those of antibodies specific for HCMV envelope glycoprotein B and TGN46, a marker of the trans-Golgi network. The merge panel shows the colocalization of the signals, cell nuclei stained with DAPI are shown in blue. Scale bar: 5 μm.

![Figure 5](image3.png)

**Fig. 5.** pUL5 cellular and viral localization. Two 175 cm² plates of sub-confluent HFF cells were infected with UL5-2StrepII-2FLAG virus at MOI of 1. After 6 days at 37 °C, culture media were collected and treated for sucrose cushion viral particles purification as detailed in M&M. Pelleted viruses (virions) and cells (WCL) were lysed in PBS containing 1% Triton X-110 and protease inhibitor cocktail, equilibrated in SDS-PAGE loading buffer and submitted to Western blot analysis using the indicated antibodies as probe.
infected with TR-UL5-2strepII-2FLAG and with TR wild-type (negative control) HCMV at MOI 3. Lysates were prepared at four days p.i. and subjected to immuno-precipitation (IP) using anti-FLAG monoclonal antibody-coupled magnetic beads as described in the materials and methods section. The eluted fractions were then separated on SDS-PAGE and stained with colloidal Coomassie. A fraction of the samples was also analyzed by Western blot to verify the presence of the immunoprecipitated pUL5 only in the TR-UL5-2strepII-2FLAG sample and its absence in the control using an anti-FLAG monoclonal antibody (data not shown).

Differentially detected bands, present in the TR-UL5-2strepII-2FLAG but not in the TR control sample, were analyzed by mass spectrometry. The corresponding regions in the control sample were used as control and processed in parallel. The resulting spectra were consulted on Mascot database (MatrixxScience v. 2.5.1) and a positive score was obtained for the cellular protein IQGAP1, identified by 18 peptides in the sample and none in the control.

In order to confirm this interaction HEK-293T cells were transfected with pcDNA-UL5-c-myc-6his and reciprocal immuno-precipitations (IPs) were carried out using Dynabeads protein G with anti-his or anti-IQGAP1 antibodies as described in the materials and methods. Samples were separated by SDS-PAGE and revealed by immunoblotting using the same antibodies for IQGAP1 and the anti-myc for UL5-c-myc-6his. As control, cells transfected with the empty vector were submitted to identical procedures (Fig. 6A).

Targeting pUL5 in IP co-precipitated IQGAP1, confirming the pull-down. The IP with the anti-IQGAP1 antibody co-immunoprecipitated mainly the high MW form of pUL5 (Fig. 6A). To further confirm the interaction between pUL5 and IQGAP1, we infected HFF cells with TR-UL5-2strepII-2FLAG at moi 3, using TR-UL119-FLAG infected HFF as control. At 4 days post infection, anti-FLAG immunoprecipitation performed using the cell lysates of the infected cells also revealed by Western blot that pUL5 interacts with IQGAP1 and not the anti-myc for UL5-c-myc-6his. As control, control protein UL119-FLAG (Fig. 6B).

We therefore concluded that pUL5 is a newly identified IQGAP1 interactor.

4. Discussion

In this study, we have investigated the protein product of UL5, a previously uncharacterized ORF of HCMV genome. UL5 is classified as an RL11 family gene and is predicted to encode for a type Ib membrane protein with no signal peptide and 7 potential O-glycosylation sites.
result of a fortuitous incorporation of pUL5 into the mature virion. In fact, it is very common that several cellular and viral elements, including proteins, small molecules and RNAs, are randomly included in the viral particles (Mocarski et al., 2013). In 1991, Ripalti et al. published that all products of HCMV genes UL1-UL7 are dispensable for viral growth in cell culture (Ripalti and Mocarski, 1991) and later on this observation was confirmed by other authors (Dunn et al., 2003; Yu et al., 2003). Thus, UL5 could be one of the “accessory” genes required in vivo for productive infection.

Pull-down experiments coupled with mass spectrometry analysis allowed us to identify the cellular scaffold protein IQGAP1 as a UL5 protein interactor. Reciprocal IPs in transfection and UL5 pull down of IQGAP1 in infection further confirmed this further interaction.

IQGAP1 (IQ-motif-containing GTPase Activating protein 1) is a ubiquitously expressed scaffold protein which participates in multiple cellular processes (White et al., 2012; Jacquemet and Humphries, 2013), including Ca2+/Calmodulin signaling, cytoskeletal organization, CDC42 and Rac signaling, E-cadherin-mediated cell–cell adhesion and β-catenin-mediated transcription. All members of the family, that includes IQGAP2 and IQGAP3 with tissue specific expression in human, are evolutionary conserved from yeast to mammals (Abel et al., 2015). IQGAP1 is one of the largest scaffold protein that has been found to interact with a very broad and growing list of factors. This huge variety of interactors is due to the six domains organization of the protein, each domain presenting identified recognition motif. From the N-terminus, IQGAP1 presents: 1) a calponin homology domain (CHD) that binds F-actin and N-WASP, 2) a coiled-coiled domain that binds ezrin, 3) the WW domain recognizing pro-rich motives among which Erk1/2, 4) four small GTPases activating site (GAP) activity, in fact it inhibits CDC42 GTPase activity stabilizing the protein in its active state (Zhang et al., 1997). Overall, IQGAP1 has a key role in the modulation of actin cytoskeleton through its interaction with small GTPases controlling the reshuffling of the actin filaments and microtubules. Most recently IQGAP1 has been found to be deeply involved in tight junction control and exocytosis (Noordstra and Akhmanova, 2017; Tanos et al., 2018). It is, therefore, of no surprise that IQGAP1 is a common target for intracellular pathogens. For example, Salmonella typhimurium modulates IQGAP1 and its binding to both Rac1/Cdc42 and actin to gain entry into host cells. Furthermore, Ca2+/calmodulin signaling by enteropathogenic E. coli (EPEC) to induce actin pedestal formation is mediated entirely through IQGAP1 (Kim et al., 2011). IQGAP1 has also been identified as a viral target during Ebola egress from the host cell (Lu et al., 2013). Since this protein is involved in so many cellular mechanisms, pUL5 interaction with IQGAP1 could have several purposes. We observed that pUL5 is located in the AC during infection, this could suggest a role of pUL5 in the formation of this compartment by interacting with a cytoskeleton regulator or in to the virus egress. Although we did not explore the exact role played in infection and further experiments are needed in order to understand the biological and pathological meaning of this interaction, it is reasonable to speculate an active role of UL5 in the rearrangement of cellular cytoskeleton towards an efficient viral assembly and spreading.

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Appendix A. Supplementary data

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