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Identification of host cell proteins which interact with herpes simplex virus type 1 tegument protein pUL37

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ABSTRACT

The herpes simplex virus type 1 (HSV-1) structural tegument protein pUL37, which is conserved across the *Herpesviridae* family, is known to be essential for secondary envelopment during the egress of viral particles. To shed light on additional roles of pUL37 during viral replication a yeast two-hybrid screen of a human brain cDNA library was undertaken. This screen identified ten host cell proteins as potential pUL37 interactors. One of the interactors, serine threonine kinase TAOK3, was subsequently confirmed to interact with pUL37 using an *in vitro* pulldown assay. Such host cell/pUL37 interactions provide further insights into the multifunctional role of this herpesviral tegument protein.

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1. Introduction

The protein–protein interactions likely to be responsible for the structural roles of herpesvirion tegument proteins during viral assembly have been primarily identified using yeast two-hybrid screens [1–9]. Tegument provides a key structural role during herpesviral assembly and egress [10,11]. In the case of the human pathogen herpes simplex virus type 1 (HSV-1), tegument proteins pUL36, pUL37 and pUL48 are essential for viral assembly [12]. In contrast, the protein–protein interactions involved in the non-structural functions of tegument proteins, which occur throughout the entire viral lifecycle, generally independently of other viral proteins, are not well characterised [13–17].

For HSV-1, a total of 23 tegument proteins have been identified [18]. The HSV-1 tegument protein pUL37 is conserved across all members of the *Herpesviridae* family [13], and is required for normal maturation of viral capsids into enveloped virions [19–24]. During viral assembly, localization and addition of pUL37 to nucleocapsids depends on binding to the major tegument protein pUL36 [12,25]. The interaction of pUL36 with pUL37 and their homologues is conserved across the *Herpesviridae* family supporting a common functional assembly role for pUL37 homologues. The interaction has been identified using both yeast two-hybrid and coimmunoprecipitation in a number of herpesviruses including HSV-1 [1,2], pseudorabies virus (PrV) [26], varicella zoster virus (VZV) [5,8], human cytomegalovirus virus (HCMV) [9] and Kaposi's sarcoma-associated herpesvirus (KSHV) [3]. Deletion of the pUL37-binding region in PrV pUL36 does not completely block viral assembly [27] although it has not been established whether the same applies to other herpesviruses. In addition to binding to pUL36, HSV-1 pUL37 has also been reported to self-associate [1,2,28]. As well, for HSV-1 and VZV, pUL37 interacts with the capsid protein pUL35 [1,8], and for HSV-1, it interacts with the tegument protein pUL46 [1,2]. In the case of HSV-1, the pUL36 binding domain [28,29], as well as a TNF receptor-associated factor 6 (TRAF6) binding motif [30], has been mapped to the C-terminus of pUL37.

In this study we sought to further define the role of HSV-1 pUL37 in herpesviral replication through identification of host cell proteins which directly interact with pUL37.

2. Materials and methods

2.1. Expression constructs of HSV-1 proteins

The HSV-1 strain 17 full-length UL37 ORF encoding amino acids 1–1123 previously cloned into displayTarget [2] was excised and

Abbreviations: BECN1, Beclin1; CCT3, chaperonin containing TCP1 subunit 3; DDX5, DEAD box family member; GARNL1, GTPase activating Rap/RanGAP domain-like 1; HSV-1, herpes simplex virus type 1; HCMV, human cytomegalovirus virus; IKAP, Iκβ kinase complex associated protein; KSHV, Kaposi's sarcoma-associated herpesvirus; NARS2, asparginyl tRNA synthetase 2; PSMB5, proteasome subunit beta type 5; TAOK3, serine/threonine kinase thousand and one kinase 3; THOC7, THOC complex 7 homolog; VZV, varicella zoster virus; ZNF350, zinc finger protein 350.

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inserted into the EcoRI site of pGBKT7 (Clontech). In addition, pUL37 was amplified from the same displayTarget pUL37 construct and inserted into the EcoRI/BamHI site of pEGFP-N1 (Clontech). A fragment of HSV-1 strain 17 pUL36 encoding amino acids 1–767 was excised from the previously described displayBait pUL36 1–767 [2] and inserted into the EcoRI site of pGADT7 AD (Clontech).

2.2. Yeast two-hybrid screening of a cDNA library

The Gal4-based Matchmaker Gold yeast-two hybrid system (Clontech) was used to identify host cell proteins which interact with HSV-1 pUL37. A Mate and Plate human adult brain normalized cDNA library cloned into the Matchmaker Gold yeast two-hybrid prey vector pGADT7-RecAB (Clontech) was used to identify pUL37 interactors. The library was supplied in the Y187 yeast strain while pGBKT7pUL37 was transformed into the Y2HGold yeast strain (Clontech) using previously described protocols [2]. Library screening was then undertaken using yeast mating according to the manufacturer's protocols (Clontech manual PT4084-1). Positive clones were initially identified on double dropout SD (synthetic dropout)/–Leu/–Trp/X-α-Gal/Aureobasidin A (DDO/X/A) plates before further selecting on higher stringency quadruple dropout SD/–Ade/–His/–Leu/–Trp/X-α-Gal/Aureobasidin A (QDO/X/A) plates. The prey plasmid was then rescued from yeast using the Easy Yeast Plasmid Isolation Kit (Clontech), transformed into Escherichia coli Novablue (Novagen) and then selected by growth on LB plus 100 μg/ml ampicillin. The cDNA clone inserts within the isolated prey plasmids were then sequenced using forward T7 (5′-TAA TAC GAC TCA CTA TAG GGC-3′) and reverse 3′AD (5′-TCT ACC ACG TGC TAC GTG TC-3′) sequencing primers. The NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was then used to identify pUL37 interactors. To confirm genuine positives and eliminate the possibility of false positives the isolated pGADT7 AD DNA constructs were retransformed into Y187. They were then mated against both pGBKT7 no insert as well as pGBKT7pUL37 and replica plated on both DDO and QDO/X/A plates.

2.3. Expression constructs of pUL37 interactors

Identified pUL37 host cell interactors (generally corresponding to partial cDNA clones) were amplified from the isolated pGADT7 cDNA constructs. These were inserted into either EcoRI, BamHI/XhoI or EcoRI/XhoI sites of the glutathione-S-transferase (GST) expression vector pGEX5X-1 (GE Health Sciences).

2.4. In vitro pulldown assay

GST-tagged proteins were expressed and harvested as previously described [2] with the exception that induction was at 30 °C for 3 h. In addition, Bugbuster protein extraction reagent (Novagen) was used according to the manufacturer's instructions (instead of sonication) to generate E. coli lysates. Conditions for binding and washing were as previously described [2]. Immobilized GST fusion proteins were then incubated with rocking at 4 °C for 3 h with transfected HeLa cell lysates. Beads were pelleted by spinning at 1000g for 5 min then washed, as previously described [2], to remove unbound proteins. Bound protein complexes were then eluted by heating in 50 μl of 2× reducing Laemmli buffer for 4 min at 95 °C.

2.5. Transfections

HeLa cells were maintained in T-75 Falcon flasks at 37 °C (5% CO_2) in Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 9% (v/v) fetal calf serum (JRH Bioscience). HeLa cells in 6 well plates were transiently transfected using Lipoafectamine 2000 transfection reagent (Invitrogen). Cells were processed for the in vitro pulldown assay at 24 h post transfection. At time of harvest, cells were washed twice with PBS before the addition of 250 μl per well of lysis buffer (PBS, 0.1% (v/v) Triton X-100, 1× mammalian protease inhibitors (Sigma)) and incubation at 4 °C for 10 min with gentle rocking. After incubation, cells were scraped into lysis buffer and transferred to prechilled eppendorf tubes. Cells were lysed by incubation on ice for 30 min before pelleting of cell debris for 20 min at 10000g at 4 °C.

2.6. Analysis of protein complexes

Proteins were separated by SDS–PAGE and the gels were further processed for either total protein staining or immunoblotting as previously described [2]. Primary antibody used was mouse monoclonal against GFP (1:800 dilution; Santa Cruz). Secondary antibody for the Odyssey system was goat anti-mouse IRDye 680-conjugated IgG (1:5000 dilution; Licor).

3. Results

3.1. Yeast two-hybrid screen

A Gal4-based Matchmaker Gold yeast two-hybrid screen was employed to identify host cell interactors of HSV-1 pUL37. We initially screened, using HSV-1 pUL37 cloned into bait vector pGBKT7, a Mate and Plate human adult brain cDNA library cloned into the Matchmaker Gold yeast two-hybrid prey vector pGADT7. Full-length HSV-1 pUL37 (1123 amino acids) was chosen as bait since attempts in our laboratory to map functional domains have been unsuccessful [29]. Furthermore, only one report to date has documented limited mapping of functional domains in HSV-1 pUL37 [28]. Positive pUL37 interactors from the yeast two-hybrid screen were identified after rescue of prey library plasmids in bacteria. The rescued isolated prey library plasmids were then reintroduced into the Matchmaker Gold yeast two-hybrid assay to confirm a genuine positive interaction with pGBKT7 HSV-1 pUL37 (Fig. 1A). All of the pUL37 cellular interactors were confirmed as positives when rescreened against pUL37 (Fig. 1A). To validate our yeast two-hybrid assay, we confirmed the known interaction of HSV-1 pUL36 with pUL37 [1,2,29] (Fig. 1B). The possibility of false positives due to autoactivation of reporter genes was eliminated by testing pUL37 interactors in target (pGADT7) against bait (pGBKT7) no insert (Fig. 1A) or bait (pGBKT7) pUL37 against target (pGADT7) no insert (Fig. 1C). A summary of the yeast two-hybrid screen indicating the domain structure of identified pUL37 interactors illustrates the potential diverse host cell binding partners of HSV-1 pUL37 (Fig. 2). Only in the case of BECN1 was an internal in-frame cDNA fragment identified as a pUL37 interactor (Fig. 2). For both DDX5 and GARNL1 two overlapping cDNA fragments were identified as pUL37 interactors (Fig. 2). In the case of THOC7 a full-length cDNA clone was identified as a pUL37 interactor (Fig. 2).

3.2. In vitro pulldown assay

To confirm the binary interactions identified by the yeast two-hybrid screen we used an *in vitro* pulldown assay. We generated GST-tagged expression constructs of all the HSV-1 pUL37 interactors identified by the yeast two-hybrid screen (Figs. 1A and 2). These expression constructs corresponded to the shortest cDNA fragments encoding each pUL37 interactor identified in the screen (Fig. 2). Each of the GST-tagged constructs were expressed in total bacterial lysates but only GST-TAOK3 and GST-THOC7 were found to be present in the soluble fraction (result not shown). We subsequently tested these soluble constructs in the in vitro pulldown

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