p32 Is a Novel Target for Viral Protein ICP34.5 of Herpes Simplex Virus Type 1 and Facilitates Viral Nuclear Egress*

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Background: HSV disrupts nuclear lamina for release from nucleus during productive infection.

Results: A cellular protein, p32, contributes to the release of HSV from nucleus.

Conclusion: p32 is hijacked by viral protein ICP34.5 to facilitate HSV nuclear egress and growth.

Significance: The discovery of a novel target for viral protein provides insight for viral propagation.

As a large double-stranded DNA virus, herpes simplex virus type 1 (HSV-1) assembles capsids in the nucleus where the viral particles exit by budding through the inner nuclear membrane. Although a number of viral and host proteins are involved, the machinery of viral egress is not well understood. In a search for host interacting proteins of ICP34.5, which is a virulence factor of HSV-1, we identified a cellular protein, p32 (gC1qR/HABP1), by mass spectrophotometer analysis. When expressed, ICP34.5 associated with p32 in mammalian cells. Upon HSV-1 infection, p32 was recruited to the inner nuclear membrane by ICP34.5, which paralleled the phosphorylation and rearrangement of nuclear lamina. Knockdown of p32 in HSV-1-infected cells significantly reduced the production of cell-free viruses, suggesting that p32 is a mediator of HSV-1 nuclear egress. These observations suggest that the interaction between HSV-1 ICP34.5 and p32 leads to the disintegration of nuclear lamina and facilitates the nuclear egress of HSV-1 particles.

Herpes simplex virus type 1 (HSV-1) is a human pathogen responsible for human diseases such as keratitis and encephalitis (1). As a member of the Alphaherpesvirinae subfamily, HSV-1 is able to establish latency or productive infection where the virus undergoes entry, viral gene expression, and DNA replication. Upon capsid assembly, viral particles exit the nucleus by budding through the inner nuclear membrane (2–4). In doing so, HSV-1 triggers conformational changes of the nuclear lamina lining the inner nuclear membrane (5), which favors nuclear egress of nucleocapsids (6).

HSV-1 egress is a complex process that requires a variety of viral proteins. Besides viral glycoproteins (such as gB, gK, gD, gE, and gM) (7–10), several tegument proteins are reported to modulate the nuclear egress of HSV-1. For example, UL31 and UL34 are required for conformational changes of nuclear lamina in HSV-1-infected cells (11–14). Of note, UL31 is thought to form a complex with UL34 and interact with lamin A/C at the nuclear rim (15, 16), which disrupts the nuclear lamina by interference with lamin-lamin interactions (5). This process also involves protein kinase C (PKC), lamin B (12), lamin B receptor (LBR), and emerin (6, 17, 18). Furthermore, Us3, UL13, and UL47 are linked to egress of the viral particles via phosphorylation of UL34 and localization of UL31–UL34 complex to the nuclear membrane (19–21). Additionally, UL12, a viral alkaline nuclease, associates with the cellular protein nucleolin, which is needed for efficient nuclear egress (22).

Previous studies suggest that nuclear egress involves HSV-1 ICP34.5 (23, 24), which is an essential protein in viral pathogenesis (25–27). HSV-1 ICP34.5 consists of 263 amino acids with an amino-terminal domain, a linker Ala-Thr-Pro repeat, and a carboxyl-terminal domain. The amino-terminal domain of ICP34.5 contains a nucleolar localization signal and a nuclear export signal, whereas the carboxyl-terminal domain contains a bipartite nuclear import signal (28). These signals direct shuttling of ICP34.5 between the nucleus and the cytoplasm, which is likely required for different functions. The multiple roles of ICP34.5 have emerged from the fact that ICP34.5 associates with various cellular proteins. For instance, ICP34.5 interacts with TBK1 and IkB kinase α/β, suppressing production of interferon (29), antigen presentation, and T cell response (30). ICP34.5 also inhibits autophagy by binding to Beclin-1 (31). In addition, ICP34.5 acts as a scaffold for protein phosphatase 1 (32) and eIF-2α to promote protein synthesis in infected cells.
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(33). Furthermore, ICP34.5 contributes to viral egress, a function independent of its anti-interferon activity (23). Nevertheless, the mechanism of ICP34.5 involved in viral egress has yet to be unveiled.

In this study, we report that ICP34.5 targets a cellular protein, p32, also known as component 1q receptor (C1qR) or hauronic acid-binding protein (HABP1). p32 is an abundant protein primarily located in mitochondrial matrix and associates with a number of cellular proteins including LBR (34). We show that ICP34.5 binds to and redirects p32 to the LBR in HSV-1(F)-infected cells. The interaction between ICP34.5 and p32 leads to the redistribution of nuclear lamina and facilitates the nuclear egress of HSV-1. These results suggest that p32 is a novel target for ICP34.5 and plays a significant role in the productive infection of HSV-1.

EXPERIMENTAL PROCEDURES

Cells and Viruses—HeLa, HEK293T, and Vero cells were originally obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin. U251 cells were kindly provided by Prof. S. Yu (Tianjin Medical University Cancer Institute and Hospital, China) and cultured in DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin. HSV-1(F) and its derived γ,34.5-null virus, R3616, were described previously (25).

Plasmids—To construct FLAG-ICP34.5, a DNA fragment encoding ICP34.5 was obtained by PCR and cloned into the BamHI/Xhol site of pCMV-Tag2B vector. To construct GFP-p32, p32 coding region was obtained by PCR and cloned into the Xhol/BamHI site of pEGFP-C1 vector. The short hairpin RNA (shRNA) targeting p32 and a random sequence RNA were obtained from Xu et al. (35). Oligonucleotides were synthesized and cloned into the AgeI/EcoRI site of pLKO.1 vector. Control plasmid pLKO.1-shVav2 was from our laboratory.

Reagents—Anti-mouse p32 antibody (sc-271200), anti-rabbit p32 antibody (sc-48795), anti-lamin A/C antibody (sc-7292), agarase conjugated with protein G (sc-271200), goat anti-mouse IgG-FITC (sc-2012), goat anti-mouse IgG-TRITC (sc-2092), anti-PKCε antibody (sc-937), the PKC inhibitors RO-31-7549 and RO-31-7549, goat anti-rabbit IgG-FITC (sc-2012), goat anti-rabbit IgG-TRITC (sc-2092), anti-lamin A/C antibody (sc-7292), and anti-GFP antibody (sc-2092) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-lamin A/C (Ser(P)-22) (2026S) antibody was provided by Cell Signaling Technology (Danvers, MA). Anti-HSV-1 antibody (B0114) was purchased from Dako, Inc. (Carpinteria, CA). Anti-FLAG antibody (F1804), anti-α-tubulin antibody (T6074), and anti-GFP antibody (G6539) were purchased from Sigma. Anti-LBR antibody (398L) (ab32535) and anti-collin antibody (ab11822) were purchased from Abcam (Cambridgeshire, UK). MitoTracker Red CMXRos was purchased from Invitrogen.

Immunoblotting—Cells were harvested, washed with phosphate-buffered saline (PBS), and lysed with ice-cold radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1.0% Triton X-100, and protease inhibitor mixture) for 30 min on ice. After centrifugation, supernatants were boiled in 1× loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS (w/v), 0.1% bromphenol blue, 10% glycerol, and 100 mM β-mercaptoethanol). The protein contents were separated by 12% SDS-PAGE, transferred to PVDF membranes, and identified with the indicated antibodies.

Immunoprecipitation Analysis—To examine protein interactions, transfected or infected HeLa cells were harvested and lysed with ice-cold radioimmune precipitation assay buffer for 30 min on ice. After centrifugation, cell extracts were obtained and incubated with antibody and agarose conjugated with protein G for 2 h at 4 °C. The beads were washed four times with wash buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% Triton X-100, and protease inhibitor mixture) and boiled in 1× loading buffer. The immobilized proteins were then subjected to immunoblotting analysis.

Virus Growth Assay—HeLa cell monolayers were infected with HSV-1(F) or R3616 at the indicated multiplicity of infection (m.o.i.). The free viral particles in the culture medium and cell-associated viruses were collected at the indicated hours postinfection (hpi), frozen, and thawed three times followed by infection of Vero cells with serial dilution. Virus titers were determined by oncolytic plaques on a monolayer of Vero cells.

Lentivirus-based Transduction—To generate HeLa cells with knockdown of the p32 protein or an unrelated Vav2 protein, HEK293T cells were transfected with plasmids including pLKO.1-shRNA, pCMV-VSV-G, pMDLg/pRRE, and pRSV-REV. At 48 h post-transfection, the viral particles in the supernatants were pelleted by centrifugation at 20,000 × g for 1.5 h, resuspended in DMEM supplemented with 10% (v/v) FBS, and incubated with HeLa cells at 37 °C for 24 h. After selection with 1.0 μg/ml puromycin for 7 days, HeLa cells with knockdown of p32 or Vav2 were obtained.

Confluent Microscopy—Infected or transfected HeLa cells were incubated with 100 mM MitoTracker Red CMXRos in DMEM for 30 min at 37 °C. Cells were then washed with PBS, fixed with ice-cold 4% paraformaldehyde for 30 min, permeabilized with 0.5% Triton X-100 in PBS, and incubated with primary antibodies at 4 °C overnight. Cells were then washed three times and incubated with fluorescence (FITC or TRITC)-conjugated secondary antibodies for 2 h at room temperature. After washing five times with PBS, cells were mounted and visualized with a TCS SP5 confocal microscope.

Cell Fractionation—Infected cells were harvested, washed with PBS, and lysed with 0.2% Nonidet P-40 in PBS and protease inhibitor mixture for 15 min on ice. After centrifugation for 5 min, supernatants were transferred to a new tube. The nuclei were pelleted and resuspended with 0.2% Nonidet P-40 in PBS. The viral particles in cytoplasmic and nuclear fractions were collected, and the yield was determined on Vero cells as described above.

Electron Microscopy Analysis—HeLa cells were infected with HSV-1(F) or R3616 at an m.o.i. of 5. At 16 hpi, cells were harvested and then washed twice with PBS and one time with FBS. Then the samples were fixed in 6% glutaraldehyde for 2 h followed by 1% osmium tetroxide for 1 h. After washing two times with PBS, the samples were dehydrated in a series of 30, 70, 90, and 100% ethanol and embedded in Epon 812. The ultrathin sections were prepared with Leica UC7 and stained with a mixture of uranyl acetate and lead citrate. Thin sections were
viewed with an H600 transmission electron microscope. The numbers of viral particles in the nucleus and extranucleus were counted in electron micrographs of 10 randomly sampled HeLa cells. The numbers shown in the tables represent the average numbers of viral particles in different cellular compartments and the total viral particles in 10 randomly sampled HeLa cells. The numbers in parentheses represent the percentage of viral particles per cellular compartment. The quantitative data are presented as mean value ± S.D. of three independent experiments.

Statistical Analysis—The quantitative data are presented as mean value ± S.D. of three independent experiments. Data were analyzed using Student’s t test. A p value <0.05 (*) was considered to be statistically significant.

RESULTS

Identification of Cellular p32 as an HSV-1 ICP34.5-interacting Protein—HSV-1 ICP34.5 is a virulence factor that has multiple roles in virus-infected cells, such as reducing interferon β production (29), promoting protein synthesis (36), blocking autophagy (31), and facilitating viral egress (23). To study ICP34.5, we set out to identify cellular proteins that may interact with ICP34.5. In brief, the plasmid encoding FLAG-tagged ICP34.5 was transfected into HEK293T cells, and a pulldown was performed with anti-FLAG antibody. The immunoprecipitates were fractionated by SDS-PAGE (Fig. 1A), and a distinguished band was isolated and identified to be p32 protein. B, HeLa cells transfected with plasmid encoding FLAG-ICP34.5 or FLAG-Ctrl were processed for immunoprecipitation (IP) with anti-FLAG antibody. Precipitated proteins (top two panels) and cell lysates (bottom three panels) were analyzed by immunoblotting with antibodies against p32, ICP34.5, and tubulin as a loading control, respectively.

FIGURE 1. Interaction between ICP34.5 and p32. A, HEK293T cells were transfected with a plasmid encoding FLAG-ICP34.5 or a control plasmid, and cells were processed for immunoprecipitation with antibody against FLAG. Proteins in precipitations (lanes 1 and 2), supernatants (lanes 3 and 4), and the whole cell lysates (WCL; lanes 5 and 6) were resolved by SDS-PAGE and stained with Coomassie Blue. The specific band co-precipitated with ICP34.5 was subjected to mass spectrophotometer analysis and identified to be p32 protein. B, HeLa cells transfected with plasmid encoding FLAG-ICP34.5 were processed for immunoprecipitation with anti-ICP34.5 antibody (C) or anti-p32 antibody (D). Precipitated proteins (top two panels) and cell lysates (bottom three panels) were analyzed by immunoblotting with antibodies against p32, ICP34.5, and tubulin as a loading control, respectively.
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A mutant lacking the γ34.5 gene exhibits a growth defect in cells and experimental animals (25–27). Therefore, to determine whether the host protein p32, as an interactor of ICP34.5, plays a role in HSV-1 replication, we carried out shRNA knockdown analysis. For this purpose, we established cells stably expressing an shRNA to suppress the endogenous expression of p32 or with a random sequence RNA (shCtrl) as controls, respectively. The expression of p32 and Vav2 were significantly reduced as monitored by immunoblotting analysis (Fig. 2A). We next determined viral growth in a time course. As shown in Fig. 2B, p32 knockdown reduced virus yield at 48 hpi. A similar pattern was seen with the yield of cell-associated virus (Fig. 2C). As infection progressed, the yield of cell-free virus was reduced significantly in p32 knockdown cells (Fig. 2D). These results suggest that viral release or egress is impaired in the absence of cellular p32.

ICP34.5 and p32 Are Necessary for Viral Nuclear Egress—HSV egress from the infected cells includes two steps: 1) budding from the nucleus where the viral capsids are enveloped and 2) release from the host cell (39). Previous studies suggest that ICP34.5 is required for viral nuclear egress in MEF 3T6 cells (23, 24). To further study this phenotype, we examined the nuclear egress of HSV-1 in HeLa cells by electron microscopy (EM) (Fig. 3). Accordingly, HeLa cells were infected with HSV-1(F) (Fig. 3A) or R3616 (Fig. 3B), and the viruses located inside (inset a) or outside (inset b) the nucleus were visualized by EM, respectively. The distribution of viral particles was also quantitated in 10 randomly selected HeLa cells for each infection and is summarized in Table 1. In cells infected with HSV-1(F), approximately equal distribution of viral particles was seen in the nucleus and the extranucleus. In comparison, in cells infected with R3616, about 79% of viral particles were restrained in the nucleus, and only 21% were seen outside the nucleus. Consistent with the previous observations in MEF 3T6 cells, ICP34.5 is indeed involved in nuclear egress of viral particles in HeLa cells infected with HSV-1(F).

Because the p32 protein is a host target of ICP34.5, we examined whether p32 was also involved in nuclear egress of viral particles in HSV-1(F)-infected cells. As seen in Fig. 2, knockdown of p32 impeded viral release, so we speculated that one of the steps for viral export was affected. As such, we assessed nuclear egress of HSV-1(F) by EM in shCtrl and shP32 HeLa cells. The representative EM images of the two cells are shown in Fig. 3, C and D, with the zoomed in images for inside (inset a) and outside (inset b) the nucleus, respectively. The distribution of viral particles was counted in 10 randomly selected cells for each infection and is summarized in Table 2. In HeLa-shCtrl cells (Fig. 3C), 54% of HSV-1 particles were in the nucleus, and
46% were in the extranucleus. By contrast, in HeLa-shP32 cells (Fig. 3D), 75% of viral particles were present in the nucleus, whereas only 25% egressed to the extranucleus. Furthermore, we examined the nuclear egress of HSV-1 in different cell fractions of shCtrl or shP32 cells (Fig. 3E). Control proteins coilin and tubulin were used as indicators for the nuclear and cytoplasmic fractions, respectively. Quantitation of viral particles in nuclear and cytoplasmic fractions was analyzed by titration and is presented in the bar graph (Fig. 3F). In HeLa-shP32 cells (open bars), nearly 90% of viral particles were restrained in the nucleus. In comparison, less than 70% of viral particles were in the nuclear fraction of HeLa-shCtrl cells (solid bars). Thus, the host cellular protein p32 is favorable for efficient nuclear egress of viral particles in HSV-1(F)-infected cells.

ICP34.5 recruits p32 to LBR—Cellular p32 is a multifunctional protein that disperses in the cytoplasm, mitochondria, and nucleus (40). To assess whether p32 localization is affected by HSV-1 infection, we first performed confocal microscopy analysis (Fig. 4A). In mock-infected cells (left column) and cells infected with R3616 (right column), p32 stain overlapped with the mitochondrial marker, indicating the colocalization of p32 with mitochondria. In contrast, in cells infected with HSV-1(F) (center column), p32 converged profoundly to the nuclear rim as shown in the merge row. Moreover, p32 colocalized to the nuclear rim with ICP34.5 in HSV-1(F)-infected cells (Fig. 4B). The above results implicate that HSV-1 infection results in the relocalization of p32.

Human p32 interacts with various cellular and pathogen proteins such as pre-mRNA splicing factor in the nucleus (41), BH3-only protein harakiri in the mitochondria (42), HIV Tat and Rev (43, 44), human cytomegalovirus (CMV) pUL97 at the nuclear membrane (45), and hepatitis C virus core protein on
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TABLE 2
Subcellular localization of viral particles in infected cells

| Type of infected cells | Average no. of viral particles* (percentage of total particles counted) | Total no. of particles/total no. of cells |
|------------------------|-----------------------------------------------------------------------|-----------------------------------------|
| shCtrl                 | 48.4 ± 7.8 (54%)                                                      | 904/10                                  |
| shP32                  | 57.6 ± 9.1 (75%)                                                      | 765/10                                  |

*The numbers of viral particles in the nucleus and extranucleus were counted in electron micrographs of 10 randomly sampled HeLa-shCtrl or HeLa-shP32 cells infected with HSV-1(F).

FIGURE 4. Colocalization of ICP34.5 and p32 with LBR. A, HeLa cells were infected with HSV-1(F) (m.o.i. = 5). At 16 hpi, cells were collected, stained with MitoTracker Red CMXRos (red), p32 antibody (green), and DAPI (blue) prior to confocal microscopy. The merged images are shown in the bottom row. B, mock-(upper row) or HSV-1 (lower row)-infected cells (m.o.i. = 5) were processed for confocal microscopy analysis with the indicated dyes for the viral ICP34.5, endogenous p32, and nuclei, respectively. The merged images are shown in the right column. C, overexpression of GFP-p32 fusion protein alone (upper row) or together with FLAG-ICP34.5 (lower row) in HeLa cells was achieved by transient transfection and analyzed by confocal microscopy. D, HeLa cells were infected with HSV-1(F) or R3616 (m.o.i. = 5). At 16 hpi, cells lysates were prepared for immunoprecipitation (IP) with anti-p32 antibody. Co-precipitated proteins (top three panels) and cell lysates (bottom four panels) were analyzed by immunoblotting with the indicated antibodies, respectively. E, as indicated at the top, HeLa cells transiently transfected with vector alone as a control (left lane), GFP-p32 alone (center lane), or together with FLAG-ICP34.5 (right lane) were processed for immunoprecipitation with anti-GFP antibody. Immobilized proteins (top three panels) and cell lysates (bottom four panels) were analyzed by immunoblotting with antibodies as indicated.

the cell surface (46). Besides, by yeast two-hybrid assay, p32 was identified to interact with ectopically expressed LBR in vitro (47, 48). Because wild type virus, but not the ICP34.5 null mutant, redirected p32 to the nuclear rim, we wondered whether ICP34.5-p32 associated with the nuclear lamina proteins. By co-immunoprecipitation assay, LBR was co-precipitated with p32 in HSV-1(F)-infected cells (Fig. 4D), whereas LBR co-precipitation was not detectable in R3616-infected cells. Moreover, when ectopically expressed, ICP34.5 stimulated the association of p32 and LBR, but such binding was barely detectable in the absence of ICP34.5 (Fig. 4E). We conclude from these experiments that the translocation and association of p32 with LBR require ICP34.5 of HSV-1(F).

p32 Is Indispensable for ICP34.5-induced Phosphorylation and Redistribution of the Nuclear Lamina—An important step in HSV-1 nuclear egress is the disruption of nuclear lamina. To examine the impact of p32 and ICP34.5, we carried out infection analysis with wild type virus and the ICP34.5 null mutant, respectively. The organization of nuclear lamina, represented by lamin A/C, was visualized by confocal microscopy (Fig. 5). In mock-infected cells (top row) and cells infected with R3616 (bottom row), lamin A/C lined the nuclear rim, whereas in cells infected with HSV-1(F), it appeared diffuse at the nuclear rim and punctate in the nucleus (middle row). These results indicate that ICP34.5 is required to perturb the integrity of nuclear lamina.

The instability of nuclear lamina is characterized by the phosphorylation of nuclear lamina, and Ser-22 is one of the phosphorylated residues of lamin A/C (49–52). By immunoblotting analysis (Fig. 5B), we noted elevated phosphorylation of lamin A/C (Ser(P)-22) in HSV-1(F)-infected HeLa cells (left panels, lane 2). In contrast, in R3616-infected cells, the phosphorylation of lamin A/C (Ser(P)-22) remained the same as that in mock-infected cells (left panels, lanes 1 and 3). As HSV-1 is a
neurotropic virus, we also tested the phosphorylation of lamin A/C (Ser(P)-22) in a human neuroglioblastoma cell line U251 (right panels), and the data showed that HSV-1 ICP34.5 induced the phosphorylation of lamin A/C (Ser(P)-22) as well.

The above results led us to hypothesize that p32 triggers the integration of nuclear lamina. To address this issue, we visualized the organization of lamin A/C by confocal microscopy. As shown in Fig. 5C, lamin A/C lined the nuclear rim in mock-infected cells regardless of the presence of p32 (top two rows). However, when infected by HSV-1 (bottom two rows), lamin A/C appeared diffuse around the nuclear rim and punctate in the nucleus in shCtrl cells, whereas it remained intact in cells lacking p32 (shP32). The data here strongly suggested that p32 influenced the redistribution of nuclear lamina during HSV-1 infection. Furthermore, we tested the phosphorylation of lamin A/C (Ser(P)-22) in shCtrl and shP32 cells. As shown in Fig. 5D, the basal phosphorylation of lamin A/C (Ser(P)-22) was at comparable levels in mock-infected shCtrl and shP32 cells (left two lanes), whereas upon HSV-1(F) infection, the phosphorylated level of lamin A/C (Ser(P)-22) was more elevated in shCtrl cells (lane 3) than that in shP32 cells (lane 4), implying that p32 was involved in the phosphorylation of nuclear lamina induced by HSV-1 infection. Therefore, the disruption of nuclear lamina during HSV-1 infection coincides with the localization of ICP34.5 and p32 at the nuclear rim, which induces the phosphorylation of lamin A/C (Ser(P)-22).

PKCδ Is Responsible for the Phosphorylation of Lamin A/C (Ser(P)-22) Induced by HSV-1—Previous studies reveal that p32 binds to several PKC isoforms in vitro (53). Because neither p32 nor ICP34.5 is a kinase, we hypothesized that p32 may chaperone one PKC to the nuclear rim along with ICP34.5. We first attempted to identify the isoform(s) of PKC involved in HSV-1-induced phosphorylation of lamin A/C (Ser(P)-22) by a panel of inhibitors for conventional PKCs, novel PKCs, and atypical PKCs, respectively. As shown in Fig. 6A, compared with HSV-1(F)-infected sample (lane 2 from left), RO-31-7549, which preferentially inhibits PKCa isoform, and PKCζ pseudosubstrate displayed no apparent inhibition toward the phosphorylation of lamin A/C (Ser(P)-22), whereas rottlerin, which is a widely used selective inhibitor for PKCδ, exhibited efficient inhibition toward the phosphorylation of lamin A/C (Ser(P)-22) (lane 4 from left). Furthermore, the distribution of lamin A/C was analyzed by confocal microscopy. As shown in Fig. 6B, in contrast with HSV-1(F)-infected cells in which lamin A/C was in a diffuse pattern in the nucleus (middle row), rottlerin exhibited efficient inhibition toward the redistribution of lamin A/C (bottom row). In addition to this observation, a similar distribution pattern of PKCδ with p32 was observed by confocal microscopy analysis (not shown), and the complex of p32 with PKCδ by co-immunoprecipitation was detected in cells infected with HSV-1(F) but not R3616 (Fig. 6C). These results suggest that ICP34.5 triggers the redistribution of p32 and PKCδ to the nuclear rim where the phosphorylation of lamin A/C (Ser(P)-22) occurs.

DISCUSSION

Upon productive infection, HSV-1 undergoes viral gene expression, DNA replication, capsid assembly, and egress. In the present study, we found that the viral protein ICP34.5 interacted with cellular p32 to form a complex, and the redistribu-
tion of this complex was linked to the viral budding from nuclei. Notably, knockdown of p32 by shRNA caused a restriction of HSV-1 nuclear egress by 75% (Table 2), whereas 79% of R3616 was trapped in the nucleus in normal HeLa cells (Table 1), similar to that in MEF 3T6 cells in which the majority of viruses are retained in the nucleus of cells infected with ICP34.5 deletion mutants (23). These results suggest that p32 is a mediator of HSV-1 nuclear egress by interaction with ICP34.5.

p32 (HABP1) is a ubiquitous protein in most cell types and is reported to interact with pathogen proteins as a surface receptor of immune cells, 

\[ \text{human immunodeficiency virus type 1 (Rev) and Tat (43), hepatitis C virus core protein (46), EBNA-1 of Epstein-Barr virus (54), and open reading frame P of HSV (55).} \]

The intracellular localization and function of p32 have been demonstrated primarily as a mitochondrial membrane protein in harakiri-mediated apoptosis (42), a mitochondrial antiviral signaling protein-interacting protein in RNA-induced retinoic acid-inducible gene I signaling (35), an interactor of splicing factor SF2/alternative splicing factor for RNA splicing (41), and an RNA-interacting protein for the translation of mitochondrial RNA (56). To date, the only viral kinase reported to associate with intracellular p32 is human CMV pUL97, a kinase that phosphorylates nuclear lamina at the inner nuclear membrane (45). Kinase pUL97 is recruited to the nuclear rim by p32, directly phosphorylates lamin A/C, and induces lamin A/C disassembly to promote viral nuclear egress (57). In this context, in both HeLa and human neuroglioblastoma cells infected with HSV-1(F), the similar phenotypes including p32 relocalization to the nuclear rim (Fig. 4) and phosphorylation of nuclear lamina occurred only in the presence of ICP34.5 (Fig. 5). Although viral kinase Us3 has been suggested to phosphorylate lamin A by an \textit{in vitro} assay (58), the phosphorylation at Ser-22 in our study was well correlated with the presence of ICP34.5 (Fig. 5B). However, ICP34.5 is by no means a kinase. These results suggest that a host kinase must be recruited to the complex at the nuclear rim. Furthermore, the kinase responsible for ICP34.5-mediated phosphorylation of lamin A/C at Ser-22 was identified to be PKCδ.

Our work suggests that p32 forms a complex through inter- actions with ICP34.5, LBR, and PKCδ at the inner nuclear membrane. In this respect, p32 probably serves as an adaptor to bridge viral and host proteins. This notion is consistent with the observation noted in cytomegalovirus infection (48, 53). Although mostly present in the cytoplasm, p32 was recruited to the nuclear rim by ICP34.5 in HSV-1-infected cells. The precise nature of the p32 complex is unknown. We speculate that the interaction between p32 and ICP34.5 is required for the phosphorylation and reorganization of nuclear lamina.

HSV-1 infection modifies the architecture of nuclear lamina to allow nuclear egress and envelopment of viral particles (6, 11–14). Prior studies demonstrate that UL31 and UL34 localize to the nuclear rim and induce conformational changes of the nuclear lamina via association with lamin A/C or indirect recruitment of PKCα and PKCδ to the nuclear membrane (12). However, it is unclear whether this process involves other viral proteins. In this study, a cellular protein, p32, was identified to cooperatively recruit PKCδ to disrupt nuclear lamina together with ICP34.5. In this context, ICP34.5 appeared to play a critical
role. This was supported by the following evidence. 1) Without ICP34.5, the mutant virus was mostly restrained inside the nucleus, although all other viral proteins including UL31, UL34, Us3, and others were present. 2) The phosphorylation of nuclear lamina and the recruitment of PKCδ relied on the efficient expression of p32 in association with ICP34.5. Based on these data, we speculate that ICP34.5, UL31/UL34, p32, and PKCδ may coordinately work to promote viral egress. Further investigation is needed to address the formation of such complex.

In summary, we identified cellular p32 as a novel HSV-1 virulence factor ICP34.5-interacting protein and revealed that p32 was required for the nuclear egress of HSV-1 capsids. Based on these results, we postulate a model whereby ICP34.5 facilitates viral nuclear egress (Fig. 7). In HSV-1-infected cells, ICP34.5 binds to and induces the redistribution of p32 along with PKCδ to the nuclear rim, forming a complex that phosphorylates the nuclear lamina components. As a result, this dismantles the nuclear lamina, promoting HSV nucleocapsid egress from the nucleus (right).

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