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Inhibition of Cellular 2′-5′ Oligoadenylate Synthetase by the Herpes Simplex Virus Type 1 Us11 Protein[▽]

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Among the many host genes induced by virus infection and interferon, the eIF2α protein kinase PKR and the 2′-5′ oligoadenylate synthetase (OAS) are both activated by double-stranded RNA (dsRNA) produced in virus-infected cells. Furthermore, each is a critical component that independently acts to inhibit virus replication and thereby contributes to the establishment of an antiviral state. As part of their tactics to foil host defense mechanisms, some viruses prevent the induction of interferon-responsive genes at the level of transcription. Other viruses, such as herpes simplex virus type 1 (HSV-1), can additionally replicate in interferon-treated cells and must also evade the actions of host defense proteins such as PKR and OAS that have been previously synthesized and merely await detection of an activating signal. Whereas HSV-1 gene products $\gamma_1$34.5 and Us11 are required for viral replication in interferon-treated cells and both act in a temporally coordinated manner during infection to counteract PKR, HSV-1 functions that target OAS have not been described. Here, we demonstrate that HSV-1 infection inhibits 2′-5′ oligoadenylate synthesis in interferon-stimulated primary human cells. The OAS-inhibiting activity is generated late in the virus' productive life cycle and requires the Us11 gene product. Moreover, we establish that the Us11 protein is sufficient to block OAS activation in extracts from uninfected, interferon-treated cells. Inhibition of OAS specifically requires the Us11 dsRNA-binding domain, suggesting a mechanism that, in part, relies on sequestering available dsRNA produced during infection. Thus, in addition to PKR and its protein activator, PACT, the HSV-1 Us11 gene product is able to counteract the activity of OAS, a third cellular protein critical for host defense.

Once exposed within the cytosol of their host, infecting viruses trigger a signal cascade that activates a set of transcription factors, including NF-κB, together with interferon (IFN) regulatory factors 3 and 7 (IRF3 and IRF7), potentially culminating in type I interferon (IFN-α/β) production and release (reviewed in reference 26). By engaging their receptors with neighboring cells, these cytokines induce expression of more than 100 distinct interferon-stimulated genes (ISGs). It is the expression of this set of genes in response to interferon that establishes an "antiviral state," where the cells are refractory to viral replication and spread. To a large extent, the failure of these interferon-treated cells to support viral replication reflects the activation of specific, latent ISG products by viral infection that directly curtails viral replication (reviewed in reference 47). While the function of this complex interferon-induced proteome is not completely understood, several key double-stranded RNA (dsRNA)-responsive effectors, notably the dsRNA-inducible protein kinase PKR and the 2′-5′ oligoadenylate synthetase (OAS), act to control protein synthesis and RNA stability in virus-infected cells.

While the eIF2α kinase PKR, which can be activated either by the PACT polypeptide or by dsRNA, contributes to host defenses by inactivating this critical translation initiation factor, 2′-5′ oligoadenylate production by a family of related synthetases can affect RNA stability globally (reviewed in references 33 and 44). Unlike PKR, the three major forms of OAS lack a canonical dsRNA-binding motif but recognize dsRNA through a positively charged groove in the molecule (17). Although the precise function of each isoform is not completely understood, each displays a characteristic set of biochemical parameters differing in the concentration of dsRNA required for maximal activation, the reaction conditions for optimal activity, the length of the 2′-5′ derivatives synthesized, and their subcellular distribution, together with their cell type specificity (29, 44). Following dsRNA binding, the enzyme undergoes a conformational change that results in the de novo synthesis of oligoadenylate (OA) derivatives with a unique 2′-5′ linkage (21). These 2′-5′ OA products subsequently associate with RNase L, a latent cellular RNase, and promote the formation of active RNase L homodimers from inactive monomer subunits (48, 49). Active RNase L cleaves at the 3′ side of UpXp sequences and enhances the decay of both mRNA and rRNA within infected cells (13, 49). Destruction of the activating signal by a phosphodiesterase terminates the response to 2′-5′ OA (18, 24, 45).

By promoting mRNA destabilization and rRNA cleavage, activated OAS poses a substantial impediment to viral replication. Indeed, single-stranded RNA (ssRNA) genomes of picornaviruses, such as mengovirus, along with encephalomyocarditis virus and a flavivirus typified by West Nile virus, are particularly susceptible to the antiviral potential of activated OAS (6, 20). In fact, picornaviral RNA has been isolated in association with OAS (14). Given the negative consequences that OAS activation would wreak on the virus' life cycle, it is not at all surprising that many viruses go to great lengths to thwart activation of this innate cellular defense molecule. Indeed, many of the same virus-encoded effectors that target PKR have also been harnessed to counteract OAS (reviewed in

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reference 25). A number of these proteins bind to dsRNA and act in part by shielding viral dsRNA from cellular defense molecules such as OAS that require this ligand for their activation. Vaccinia virus E3L (41), human cytomegalovirus TRS1/IRS1 (7), reovirus σ3 (3), and influenza virus NS1 (31) proteins all use this mechanism to prevent activation of PKR and OAS. In some cases, a specific structured element within viral RNA, like the human immunodeficiency virus (HIV) TAR sequence, can activate OAS. By binding to TAR, the HIV Tat protein prevents activation of both PKR and OAS (28). However, the means by which herpes simplex viruses (HSVs) counteract OAS have not been reported.

Early in the HSV-1 productive growth cycle, the virus is able to suppress the accumulation of ISG transcripts. This is achieved through the combined action of ICP0, an immediate-early protein that inhibits IRF3-mediated transcriptional activation of ISGs (11, 15, 27, 30), and *vhs*, an endoribonuclease delivered by incoming virions that associates with the translation initiation factor eIF4F and enhances mRNA turnover (9, 10, 12). The ability of HSV-1 to prevent ISG induction does not, however, account for its ability to replicate in cells that have been previously primed with IFN and therefore have been loaded with host defense proteins awaiting a viral trigger. Notably, why HSV-1 can replicate in IFN-treated cells without inducing a robust activation of the antiviral RNase L pathway has not been elucidated (4). Here, we demonstrate that HSV-1 infection inhibits 2′-5′ oligoadenylate synthesis in interferon-stimulated primary human cells. The OAS-inhibiting activity appears late in the virus' productive life cycle and requires the Us11 protein. Moreover, we establish that the Us11 gene product is sufficient to block OAS activation in extracts from uninfected, interferon-treated cells. Inhibition of OAS specifically requires the Us11 dsRNA-binding domain, suggesting a mechanism that, in part, could rely on sequestering available dsRNA produced during infection. Thus, in addition to PKR and PACT (35, 36), the HSV-1 Us11 gene product is able to counteract the activity of OAS, a third cellular protein critical for host defense.

MATERIALS AND METHODS

Cell culture. Normal human diploid fibroblasts (NHDF) cells (Cambrex, Walkersville, MD) were maintained by standard cell culture procedures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Vero cells were maintained in DMEM supplemented with 5% calf serum. Human IFN-α (Roche Pharmaceuticals) at a specific activity of 2.5×10^5 IU/ml was diluted in DMEM supplemented with 10% FBS.

Infections and virus. Infections of NHDF cells were carried out at a multiplicity of infection (MOI) of 5 PFU/cell. Recombinant viruses used, previously described (34.5RΔSUP virus [32] and ΔUs11 and RepUs11 [35]), were diluted in DMEM supplemented with 1% FBS. The n12 ICP4 mutant (8) was obtained from N. DeLuca (University of Pittsburgh School of Medicine, Pittsburgh, PA), and the vhsΔsma mutant (39) was provided by G. S. Read (University of Missouri, Kansas City, MO). NHDF cells were allowed to reach confluence and were then seeded at a concentration of 7.5×10^5 cells for each 100-mm-diameter plate 24 h before IFN treatment or infection. Cells treated with IFN were infected 24 h after exposure to IFN. Absorption was allowed to proceed for 1.5 h at 37°C. Unabsorbed virus was removed, and the proper medium, with or without IFN and PAA (phosphonoacetic acid), was returned to the plates for an additional 8.5 h incubation at 37°C. PAA was added directly to the medium on the monolayers at a concentration of 300 μg/ml 1 h before infection and was present at the same concentration throughout infection.

OAS assay. The OAS assay reaction was carried out in the presence of 10 μg of total protein (soluble fraction, unless indicated otherwise), 1 μl poly(I)(C) (Amersham, GE Healthcare) diluted in water to achieve the concentration indicated in each panel, 20 mM Tris-HCl (pH 7.5), 20 mM magnesium acetate, 2.5 mM dithiothreitol (DTT), 1.5 mM ATP, 0.6 μl [α-^{32}P]ATP (800Ci/mmol; Perkin-Elmer). The final volume was adjusted with water to 10 μl. When recombinant proteins were tested (23, 36, 38), 0.3 pmol of purified protein in 100 μg/ml of nuclease-free bovine serum albumin (BSA; New England Biolabs) was mixed with 10 μg total protein extract and reaction buffer before the addition of poly(I)(C). When different extracts were mixed in the same reaction mixture, 5-μg quantities of total protein from both extracts were mixed together before the addition of poly(I)(C) and reaction buffer. To preincubate recombinant proteins with the dsRNA activator, 1 μl poly(I)(C) was incubated with 0.3 pmol purified protein (in 100 μg/ml of nuclease-free BSA) at 30°C for 10 min, followed by the addition of 10 μg of total protein extract and reaction buffer. Once all the components were present, the reaction was incubated for 2 h at 30°C. At the end of this incubation period, 2 μl of the reaction mixture was diluted in 10 μl of Gel Loading Buffer II (Ambion), boiled for 5 min, and chilled on ice for less than 5 min before running 2 μl on a 7 M urea, 20% polyacrylamide sequencing gel, in 1× TBE buffer (50 mM Tris, 20 mM boric acid, 1 mM EDTA) at 2,000 V. The gel was prerun at 2,000 V for 30 min. After it was dried, the gel was autoradiographed and quantified using a Molecular Dynamics PhosphorImager. When soluble and particulate fractions were mixed, the concentration of total protein in a 10-μl OAS reaction was maintained at 1 mg/ml.

Extract preparation. The procedure used to generate cell extracts was adapted from Schreiber et al. (46). Culture plates were washed with cold 1× phosphate-buffered saline (PBS). Cells were scraped from the plates and harvested by centrifugation at $1,000 \times g$ for 5 min at 4°C. Cells were washed in cold buffer A (10 mM HEPES [pH 7.9], 10 mM potassium chloride, 0.1 mM EDTA, 0.1 mM EGTA, 1× COMPLETE-EDTA-free protease cocktail inhibitor [Roche]) and resuspended in cold buffer A+ (10 mM HEPES [pH 7.9], 10 mM potassium chloride, 0.1 mM EDTA, 0.1 mM EGTA, 1× COMPLETE-EDTA-free protease cocktail inhibitor, 0.1 mM DTT). Cells were allowed to swell at 4°C for 15 min. After addition of Nonidet P-40 to a final concentration of 0.5% from a 10% stock, cells were mixed for 10 seconds and centrifuged for 30 seconds at 4°C in a microcentrifuge. The supernatant (soluble fraction) was aliquoted, snap-frozen in a dry-ice ethanol (EtOH) bath, and stored at −80°C. The pellet was resuspended in buffer C (20 mM HEPES [pH 7.9], 0.4 M sodium chloride, 1 mM EDTA, 1 mM EGTA, 1× COMPLETE-EDTA-free protease cocktail inhibitor, 1 mM DTT), mixed for 15 min at 4°C, and centrifuged for 5 min in a microcentrifuge at 4°C. The supernatant (particulate fraction) was aliquoted, snap-frozen in a dry-ice EtOH bath, and stored at −80°C. Protein concentration was determined by spectrophotometry using Bio-Rad protein assay.

Evaluation of OA polymer stability in HSV-1-infected cell extracts. 2′-5′ OA chains were synthesized in a standard in vitro reaction mixture containing 1 μg/ml poly(I)(C) and 10 μg of protein extract prepared from IFNα-treated NHDFs. After 2 h at 30°C, the reaction was heat inactivated at 95°C for 5 min and subsequently chilled on ice. The efficacy of the heat inactivation step was tested in a standard OAS in vitro reaction mixture using 10 μg heat-inactivated extract and 10 μg/ml poly(I)(C). Radiolabeled OA polymer contained in one-fourth of the heat-inactivated synthesis reaction mixture was subsequently incubated for an additional 2 h at 30°C in the presence of 20 mM Tris-HCl, pH 7.5, 20 mM magnesium acetate, 2.5 mM DTT, 1.5 mM ATP, and 10 ng poly(I)(C) with 5 μg of protein extract prepared from mock-infected or HSV-1-infected NHDFs in a final volume of 10 μl. At the conclusion of the incubation, reaction products were analyzed and quantified as described earlier.

Coimmunoprecipitations. Cells (293; 1.2×10^6 cells) were seeded on 60-mm dishes in DMEM plus 10% calf serum lacking penicillin and streptomycin. On the following day, cells were transfected with Lipofectamine 2000 (Invitrogen) using 8 μg of DNA from plasmid pGFP-Us11 or control vector pEGPF-C2 (Clontech) in combination with 8 μg of DNA from plasmid pFLAG-OAS1 or pFLAG-OAS2 (kindly provided by Saumedra Sarkar and Ganes Sen, Lerner Institute, Cleveland, OH) or with an equivalent volume of water. Transfections were performed according to the manufacturer's instructions and allowed to proceed at 37°C for 24 h.

Transfected cells were scraped from the plates in PBS and harvested by centrifugation ($1,000 \times g$) for 5 min at 4°C. Cells were washed in cold PBS and resuspended in 1 ml lysis buffer (50 mM HEPES-KOH [pH 7.9], 100 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, and 1× COMPLETE-EDTA-free protease cocktail inhibitor [Roche]). After rocking for 5 min at 4°C, lysates were centrifuged ($12,000 \times g$) for 10 min at 4°C, and calcium chloride was added to the cleared supernatant to a final concentration of 1 mM. Nuclease treatment was performed by adding RNase A, RNase T_1, and micrococcal nuclease to final concentrations of 50 μg/ml, 300 U/ml, and 100 U/ml, respectively, and incubating at room temperature for 20 min. Samples not treated with nuclease were incubated under the same conditions. Lysates were again centrifuged ($12,000 \times g$) for

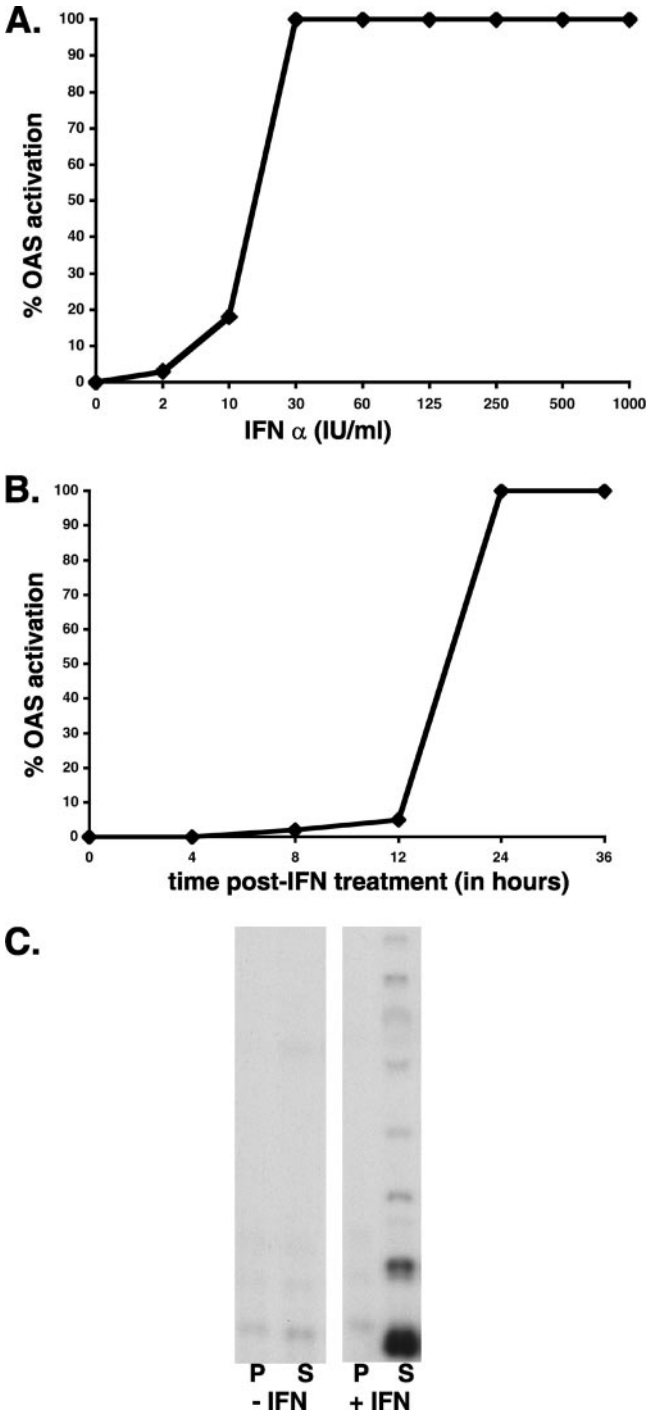

FIG. 1. Characterization of OAS activity in NHDF cell extracts. (A) Following a 24-h exposure to the indicated concentration of IFN-α, cell extracts were prepared from NHDF cells, and the soluble fraction was tested for 2′-5′ OA synthesis as a measure of OAS activation in the presence of 10 μg/μl of poly(I)(C). Maximum OAS activity (100%) was achieved by treating cells with 30 IU/ml IFN-α, as greater concentrations of IFN-α did not result in a corresponding rise in OAS activity. (B) NHDF cells were treated with one single dose of IFN-α (1,000 IU/ml), and cell extracts were prepared after harvesting the cells at the indicated time points. OAS activity from the soluble fraction was tested in the presence of 10 μg/μl of poly(I)(C), and maximum activity (100%) was set when 2′-5′ OA products stopped accumulating as a function of time. (C) Comparison of OAS activity present in soluble (S) and particulate (P) fractions from NHDF cells either treated with IFN-α (1,000 IU/ml for 24 h) or untreated. The OAS assay was performed in the presence of 10 μg/μl of poly(I)(C). Reaction products were fractionated by electrophoresis in a denaturing polyacrylamide gel and visualized by autoradiography.

10 min at 4°C. Protein G Sepharose (Amersham, GE Healthcare) was washed and resuspended in 10 volumes of buffer A (20 mM HEPES [pH 7.9], 100 mM potassium chloride, 5 mM magnesium acetate) and bound to 4.6 μg of anti-FLAG M2 monoclonal antibody (Sigma) for 1 h at room temperature. The protein G Sepharose-anti-FLAG slurry was washed once in 10 volumes of buffer A, resuspended in modified lysis buffer (50 mM HEPES-KOH [pH 7.9], 100 mM NaCl, 1 mM EDTA, 3% fraction V BSA [Sigma], and 1× COMPLETE-EDTA-free protease cocktail inhibitor), and incubated for 1 h at room temperature to block nonspecific binding sites on the matrix. Protein G Sepharose (10 μl) bound to anti-FLAG antibody was resuspended in the transfected cell lysate and incubated at room temperature for 1 h. Immune complexes were collected by centrifugation, washed three times in 100 volumes of 1× PBS, and resuspended in 20 μl of 2× sample buffer (125 mM Tris-HCl [pH 6.8], 4% sodium dodecyl sulfate [SDS], 20% glycerol, 1.4 M β-mercaptoethanol, 0.02% bromophenol blue). The samples were boiled for 5 min, resolved by SDS-polyacrylamide gel electrophoresis (PAGE), and immunoblotted following standard procedures.

RESULTS

Inhibition of 2′-5′ oligoadenylate accumulation in HSV-1-infected cells. To define the impact of HSV-1 infection on OAS, we first needed to establish the parameters for detecting enzyme activity in the strain of primary NHDFs utilized in this investigation. Cell extracts were prepared from untreated and IFN-treated NHDFs, and the dsRNA-dependent synthesis of oligoadenylate chains, reflected by the incorporation of [α-^{32}P]ATP, was monitored via electrophoresis in denaturing polyacrylamide gels. This allowed us to evaluate both the extent of OAS activity and the size distribution of the oligoadenylate products. Whereas OAS activity was reproducibly detected above background levels in cells treated with as little as 2 U/ml of IFN for 24 h, concentrations of 30 U/ml and greater were all capable of maximally activating the enzyme in a manner dependent upon dsRNA (Fig. 1A and data not shown). In addition, OAS activity was detected 8 h following IFN reatment and reached maximal levels 24 h posttreatment (Fig. 1B). Providing fresh medium along with fresh IFN after 24 h did not increase OAS activity or the length of the oligoadenylate products over the next 24 h (data not shown). Finally, to evaluate the efficiency of our extraction protocol and to verify that we were not assaying a small fraction of soluble OAS, a 0.4 M salt wash was prepared from the particulate fraction, and the OAS activity was compared to that of the initial soluble fraction. Figure 1C clearly shows that all of the detectable OAS activity was effectively confined to the soluble fraction prepared from IFN-treated cells. Moreover, reaction mixtures in which proportionately equal fractions of soluble and particulate extracts were mixed showed the same activity as that of soluble extracts (data not shown), suggesting that fractionation did not segregate functions that may regulate 2′-5′ OA synthesis.

To determine how OAS activity is affected by HSV-1 infection, cell extracts were prepared from untreated and IFN-treated NHDFs which were either mock infected or infected with wild-type virus. Whereas OAS activity was detectable in mock-infected IFN-treated cells supplemented with as little as

0.2 μg/ml dsRNA or a maximum of 0.5 μg/ml, OAS activity above background levels was not detected in HSV-1-infected, IFN-treated cells until the reactions were supplemented with 1 μg/ml dsRNA (Fig. 2A). Thus, stimulation of the RNase L antiviral pathway, as measured by the accumulation of its specific 2′-5′ oligoadenylate activator, was dramatically suppressed in extracts prepared from IFN-treated, HSV-1-infected cells. The inability to detect any OAS activity in virus-infected cells not exposed to IFN is consistent with both the absence of OAS in untreated cells and the documented ability of HSV-1 to prevent the induction of ISGs including OAS (11, 15, 27, 30). However, extracts prepared from HSV-1-infected cells not subjected to prior interferon treatment contained an activity that can completely inhibit detection of OAS activity in cell extracts from IFN-treated uninfected NHDFs (Fig. 5A).

The absence of detectable 2′-5′ OA products in HSV-1-infected cell extracts could be attributed to their rapid degradation. To test whether infected cell extracts contain an activity capable of catalyzing 2′-5′ OA degradation, radiolabeled 2′-5′ OA products were first synthesized in standard OAS assays containing extract from IFN-treated cells supplemented with 1 μg/μl poly(I)(C), and then the reactions were subsequently incubated for 5 min at 95°C. These conditions for heat inactivation were sufficient to eliminate detectable OAS activity (Fig. 2B, lane 1). Incubation of presynthesized, radiolabeled OA polymers for 2 h at 30°C with cell extracts prepared from mock-infected or HSV-1-infected cells resulted in a decrease in their overall abundance (Fig. 2B, compare lane 2 with lanes 3 and 4). However, the presynthesized OA polymers incubated with extracts prepared from HSV-1-infected cells (lane 4, Fig. 2B) were at least as stable as the OA chains incubated with extracts prepared from mock-infected cells (Fig. 2B, lane 3). Thus, we were unable to detect any activity in HSV-1-infected cell extracts that destabilized presynthesized OA chains below those levels observed in mock-infected cell extracts.

Taken together, these data suggest that the absence of detectable OAS activity in extracts prepared from HSV-1-infected cells or supplemented with viral gene products is not due to the rapid degradation of the 2′-5′ OA products; instead, data reflect the inhibition of oligoadenylate synthesis. That the observed suppression of 2′-5′ OA accumulation is in part overcome by the addition of excess dsRNA to the in vitro reactions supports this hypothesis (Fig. 2A); moreover, it is consistent with the possibility that HSV-1-infected cells might contain an OAS inhibitor whose activity can be effectively titrated by increasing concentrations of the dsRNA activator.

Suppression of OAS activity requires HSV-1 late-gene expression. Having demonstrated that OAS is repressed in cells productively infected with HSV-1, we sought to delineate which kinetic class of viral genes mediated this effect. To discern when during the HSV-1 replicative cycle the virus-specified OAS repressor is expressed, OAS activity was first measured in extracts prepared from IFN-treated cells infected with an ICP4-deficient virus. Critical viral transcriptional control proteins required for progression beyond the immediate-early (IE) stage of the viral life cycle, ICP4 mutants produce a limited subset of viral polypeptides before arresting (8). One of the proteins produced in cells infected with an *ICP4* mutant virus is ICP0, an inhibitor of ISG induction (27). Figure 3A demonstrates that an ICP4-deficient virus fails to suppress

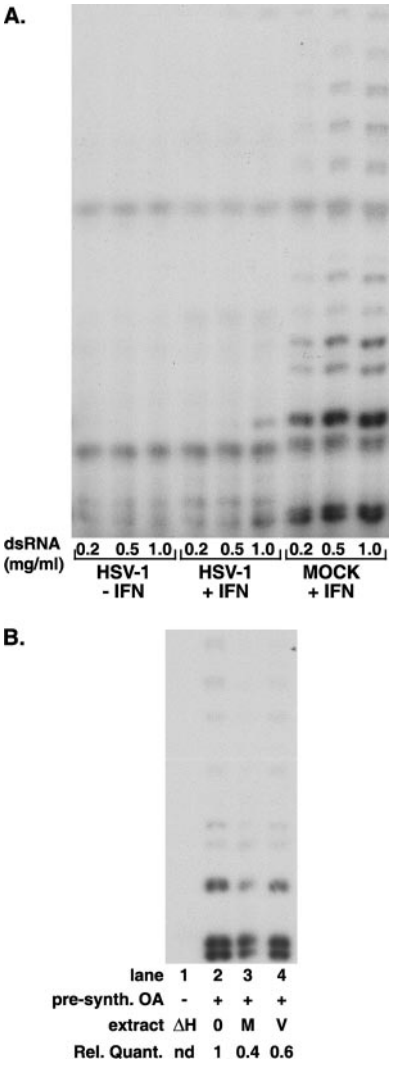

FIG. 2. Inhibition of OAS activity in extracts prepared from HSV-1-infected cells. (A) NHDF cells treated with IFN-α (1,000 IU/ml for 24 h) or untreated were infected (MOI of 5) with wild-type HSV-1 (Patton strain). An IFNα-treated culture was also mock infected. At 10 h postinfection, soluble extracts were prepared and their OAS activity was evaluated in the presence of increasing concentrations of dsRNA activator. Reaction products were fractionated by electrophoresis in a denaturing polyacrylamide gel and visualized by autoradiography. (B) Radiolabeled 2′-5′ OA products were synthesized in a standard in vitro OAS reaction mixture. After heat inactivation, a fraction of this reaction mixture was incubated for 2 h at 30°C with soluble cell extracts prepared from mock-infected (M) or HSV-1-infected (V) NHDFs (MOI of 5; harvested at 10 h postinfection). An equivalent fraction of the initial heat-inactivated OAS reaction mixture was diluted without extract (lane 2). OAS activity was not detected in heat-inactivated (ΔH) extracts (lane 1). The abundance of OA products (Rel. Quant.) relative to that in the initial starting material (lane 2, normalized to 1) was quantified and is indicated. nd, not detected.

OAS activity and suggests that the expression of viral IE genes other than ICP4 is not sufficient to inhibit OAS. Whereas extracts prepared from cells infected with an ICP4 mutant are unable to suppress OAS activity, they exhibit a reduced sensitivity to the dsRNA activator (Fig. 3A). This could potentially reflect the introduction of small quantities of the virus-en-

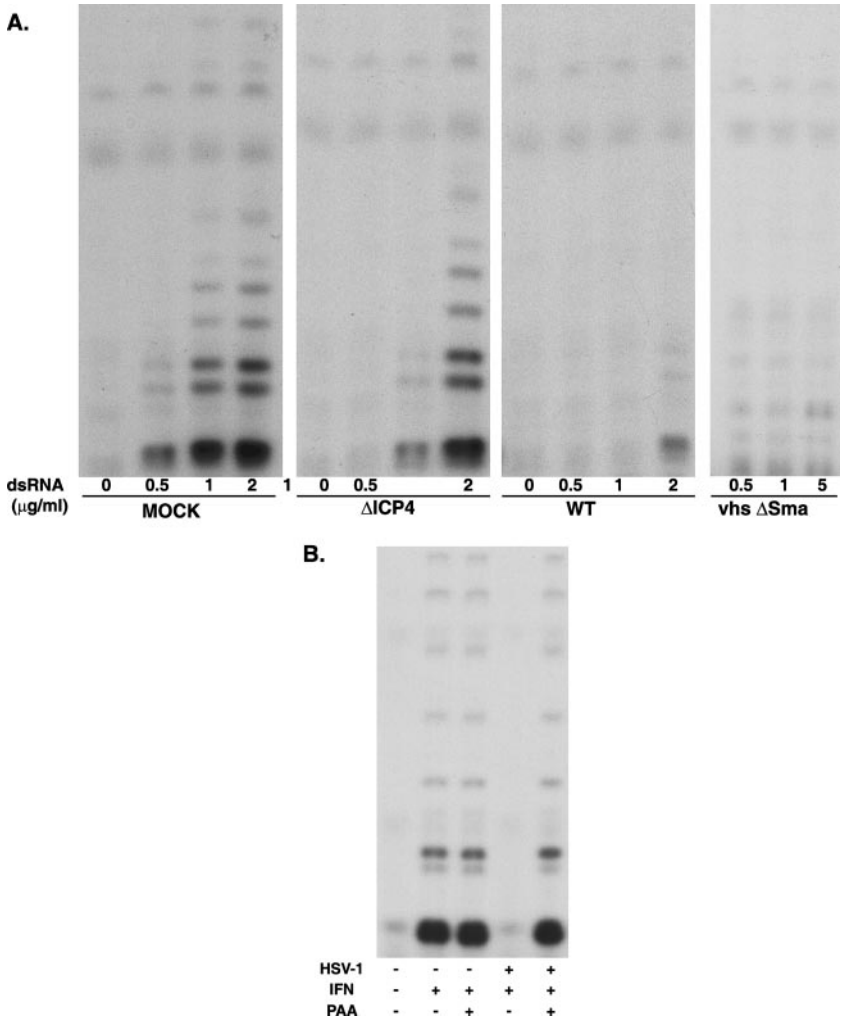

FIG. 3. Inhibition of OAS activity in HSV-1-infected cells requires late gene expression. (A) IE gene expression is not sufficient to completely inhibit OAS activity. NHDF cells treated with IFN-α (1,000 IU/ml for 24 h) or untreated were either mock infected or infected (MOI of 5) with the indicated HSV-1 mutant strains. At 10 h postinfection, soluble extracts were prepared and their OAS activity was evaluated in the presence of increasing concentrations of dsRNA activator. Reaction products were processed as described in the legend to Fig. 2. (B) NHDF cells (untreated or treated with 1,000 IU/ml IFN-α for 24 h) were either mock infected or infected with wild-type HSV-1 (Patton strain). Cultures were treated with PAA (300 μg/ml) as indicated, and OAS activity was evaluated in the presence of 0.1 μg/μl of poly(I)(C).

coded inhibitor within the tegument layer of infecting virions. Finally, OAS activity was effectively inhibited in extracts prepared from cells infected with a *vhs*-defective mutant (Fig. 3A). While the *vhs* and *ICP4* mutant viruses both produce ICP0 and inhibit ISG induction (27), only the *ICP4* mutant virus is unable to suppress OAS (Fig. 3A). Thus, the HSV-1-encoded OAS antagonist and its ability to suppress the induction of ISGs are likely to be separable, independent functions.

To differentiate whether the OAS repressor was likely to be an early or a late viral gene product, OAS activity was next assessed in extracts prepared from IFN-treated cells that were infected and maintained in the presence or absence of PAA, an inhibitor of HSV-1 DNA replication. Blocking viral DNA synthesis prevents entry into the late phase of the productive growth cycle, reducing transcription of early-late (γ_1) genes and inhibiting transcription of true-late (γ_2) genes. Importantly, PAA treatment had no impact on the abundance or length of OA products synthesized in mock-infected, IFN-treated cells (Fig. 3B). However, while extracts from HSV-1-infected, IFN-treated cells displayed a marked reduction in OAS activity, extracts from IFN-treated cells that were infected and maintained in the presence of PAA exhibited OAS activity similar to that of extracts isolated from mock-infected cells (Fig. 3B). Thus, PAA treatment prevents the virus-induced suppression of OAS activity observed in extracts prepared from IFN-treated, HSV-1-infected NHDFs. This strongly suggests that viral DNA replication or an event closely linked to viral DNA replication, such as true-late (γ_2) gene transcription or polypeptide production, is required to suppress OAS activity.

Suppression of OAS activity requires the Us11 gene product. Among the HSV-1 genes whose expression is PAA sensitive, reflecting their dependence upon virus DNA synthesis, only the product of the Us11 gene binds dsRNA and contributes to viral growth in IFN-treated cells (23, 34). It is also a tegument protein delivered into the host cell cytosol in limited

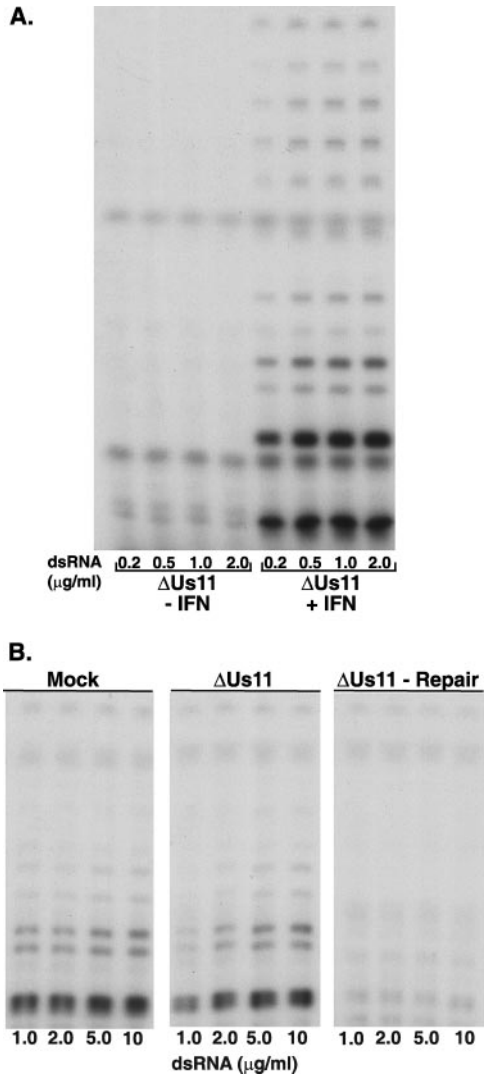

FIG. 4. The Us11 gene product is required to inhibit OAS. (A) NHDF cells (untreated or treated with 1,000 IU/ml IFNα for 24 h) were infected with a Us11-deficient virus (ΔUs11). At 10 h postinfection, soluble extracts were prepared and their OAS activity was evaluated in the presence of increasing concentrations of dsRNA activator. Reaction products were processed as described in the legend to Fig. 2. (B) Cell extracts from IFN-α-treated, uninfected NHDF cells were supplemented with NHDF extracts prepared at 10 h postinfection from cultures infected (MOI of 5) with a Us11-deficient virus (ΔUs11) or a virus in which the Us11 mutation had been repaired to a wild-type state (ΔUs11-Repair). OAS activity was evaluated in the presence of increasing concentrations of dsRNA activator, and the reaction products were processed as described in the legend to Fig. 2.

quantities by infecting virus particles (42). Since OAS is a dsRNA-activated enzyme and its expression is induced by IFN, it was conceivable that Us11 might be required to suppress OAS activity in virus-infected cells. To determine if Us11 was necessary to suppress OAS activity in virus-infected cells, cell extracts were prepared from untreated and IFN-treated NHDFs infected with a Us11-deficient virus, and OAS activity was evaluated. Whereas OAS activity above background levels was not observed in extracts prepared from untreated cells infected with a Us11 deficient virus, extracts from IFN-treated cells exhibited OAS activity over a broad range of dsRNA concentrations (Fig. 4A). That Us11 is only required to suppress OAS activity in IFN-treated cells, compared to untreated cells (Fig. 4A), suggests that Us11 does not participate in repressing the induction of OAS in virus-infected cells, which requires ICP0 and *vhs* (10, 11, 15, 27, 30). Instead, the Us11 gene product is required to inhibit OAS activity in cells previously exposed to IFN.

To rule out any potential effects of IFN on the expression of viral genes, extracts from HSV-1 infected cells, which were never exposed to IFN, were mixed with uninfected extracts isolated from IFN-treated, mock-infected NHDFs prior to measuring OAS activity. This scheme allowed us to evaluate the effect of adding infected cell components to an uninfected cell extract rich in OAS. In addition to the Us11-deficient virus, a viral recombinant in which the Us11 mutant allele was repaired to its wild-type state was included in this experiment. The latter control is critical, ensuring that any phenotypes observed using the Us11-deficient virus are in fact corrected upon restoring the Us11 gene. Significantly, whereas extracts prepared from untreated cells infected with a virus harboring a wild-type Us11 gene effectively suppressed OAS over a broad range of dsRNA concentrations, extracts identically prepared from cells infected with a Us11-deficient virus cannot (Fig. 4B). In fact, the extent of OAS activation seen in mock-infected extracts is similar to that observed in extracts prepared from NHDFs infected with a Us11-deficient virus (Fig. 4B). Thus, the HSV-1 Us11 gene is required to suppress OAS activity in extracts from IFN-treated, normal primary human cells.

Us11 is sufficient to suppress OAS activity in HSV-1-infected cells. Experiments in the preceding section establish that Us11 loss-of-function mutants are unable to suppress OAS activity in vitro. While this suggests that Us11 is necessary to inhibit OAS, Us11 is one of many late proteins whose synthesis is blocked by PAA treatment and it may be a key contributor to a complex process that involves multiple PAA-sensitive genes. To discern if expression of PAA-sensitive true-late genes other than Us11 are required to suppress OAS, a gain-of-function mutant that expresses Us11 from an IE promoter was enlisted (32). IFN-treated NHDFs were infected with either a Us11-deficient virus or the IE-Us11 recombinant in the presence and absence of PAA. As expected, the Us11-deficient virus (ΔUs11) was unable to suppress OAS activity in extracts prepared from IFN-treated NHDFs in the presence or absence of PAA (Fig. 5). In contrast, IFN-treated cells infected with the IE-Us11 recombinant exhibited background levels of OAS activity similar to those seen in non-IFN-treated, mock-infected cells irrespective of PAA treatment (Fig. 5). Remarkably, the IE Us11 recombinant expressed an activity that completely suppressed OAS function even in the presence of PAA (Fig. 5), whereas extracts from wild-type HSV-1-infected cells treated with PAA failed to inhibit OAS activity (Fig. 3B). Since PAA prevents HSV-1-mediated suppression of OAS (Fig. 3B), this strongly implies that Us11 is the only PAA-sensitive component required for this effect. Furthermore, it demonstrates that IE expression of Us11 is sufficient to repress OAS in extracts prepared from IFN-treated, HSV-1 infected cells maintained in PAA. The Us11 gene product therefore appears to be necessary and sufficient to repress OAS activity in HSV-1-infected cells.

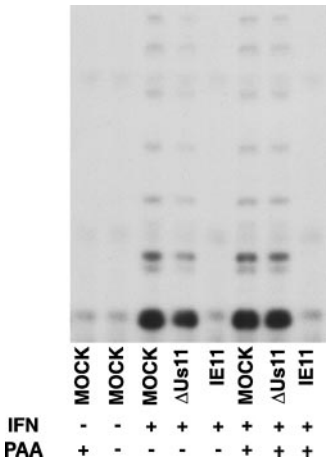

FIG. 5. Us11 expression is sufficient to inhibit OAS activity in HSV-1-infected cells. NHDF cells (untreated or treated with 1,000 IU/ml IFN-α for 24 h) were either mock infected, infected (MOI of 5) with a Us11 deficient virus (ΔUs11), or infected (MOI of 5) with a virus in which the true-late, γ_2 Us11 gene was expressed as an IE gene (IE11). Cultures were treated with PAA (300 μg/ml) as indicated. Extracts were prepared 10 h postinfection and OAS activity was evaluated in the presence of 0.1 μg/μl of poly(I)(C). Reaction products were processed as described in the legend to Fig. 2.

The Us11 protein and its dsRNA binding domain are sufficient to inhibit OAS activity in vitro. As Us11 is necessary to suppress OAS and its IE expression is sufficient to inhibit OAS activity in extracts prepared from PAA-treated cells, this raised the possibility that the Us11 polypeptide was in fact the only virus-specified component both necessary and sufficient to directly inhibit the cellular OAS protein. To test whether the Us11 gene product alone was sufficient to repress OAS or if other viral factors were required for Us11 function, extracts from IFN-treated cells were supplemented with increasing concentrations of bacterially expressed, affinity-purified, recombinant Us11 protein (Fig. 6A). The addition of increasing quantities of Us11 efficiently repressed OAS activity, reaching approximately 90% inhibition at 5 μg/ml (Fig. 6A). Moreover, while 1 μg/ml of Us11 was insufficient to detectably reduce OAS activity in extracts previously supplemented with dsRNA, preincubation of Us11 with dsRNA dramatically enhanced its ability to suppress OAS activity and resulted in near total inhibition of OAS function (Fig. 6A). Taken together, these data establish that Us11 is the only viral component required to inhibit OAS activity in extracts from IFN-treated primary human cells and suggest that the mechanism of this inhibition could involve, in part, an interaction with the dsRNA activator.

Since the 68 C-terminal residues of Us11 contain a canonical, proline-rich dsRNA-binding motif (32, 38), the potential of this isolated protein domain to inhibit OAS was evaluated. Whereas purified glutathione *S*-transferase (GST) fused to the C-terminal 68 residues of Us11 was as effective on a molar basis as full-length Us11 in inhibiting OAS, a GST fusion protein fused to the N-terminal 87 residues of Us11 was as ineffective as BSA, buffer alone, or GST (Fig. 6B and data not shown). Thus, the 68 residues from the Us11 C terminus that contain the proline-rich, dsRNA-binding domain are sufficient to inhibit OAS activity in extracts from IFN-treated, primary

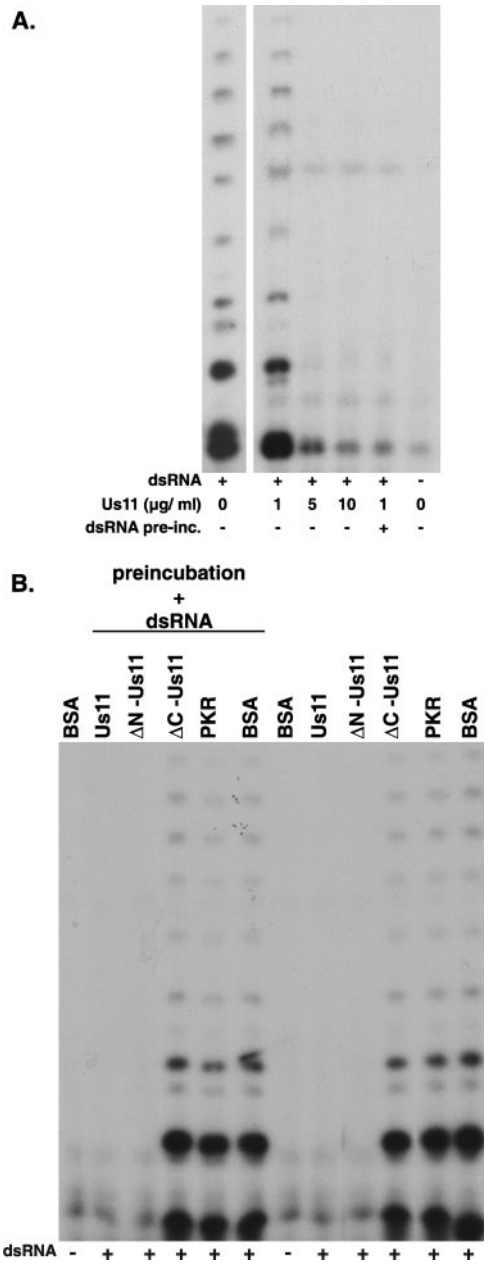

FIG. 6. Purified Us11 protein is necessary and sufficient to inhibit OAS activity in uninfected cell extracts. (A) Cell extracts from IFN-α-treated (1,000 IU/ml; 24 h) NHDFs were supplemented with increasing quantities of purified, recombinant His-tagged Us11 and their OAS activity was evaluated in the presence of 2 μg/μl poly(I)(C). One sample was preincubated with dsRNA (dsRNA pre-inc., lane +) for 10 min prior to being added to the cell extract. The only exogenously added dsRNA in this reaction (dsRNA pre-inc., lane +) was that previously incubated with the Us11 protein. The dsRNA − lane did not contain any exogenous dsRNA. Reaction products were processed as described in the legend to Fig. 2. (B) Cell extracts (as in panel A) were supplemented with the indicated purified proteins and OAS activity was evaluated as described above. At the left side of the panel, proteins were first preincubated with the dsRNA ligand prior to their addition to the extract. All components were added directly to the extract in the experiment whose results are shown in the right panel. ΔN-Us11 (0.3 pmol), C-terminal 68 residues of Us11 fused to GST; ΔC-Us11 (0.3 pmol), N-terminal 87 residues of Us11 fused to GST; Us11 (0.3 pmol), His-tagged, wild-type, full-length Us11; PKR (0.3 pmol), full-length, His-tagged wild-type PKR.

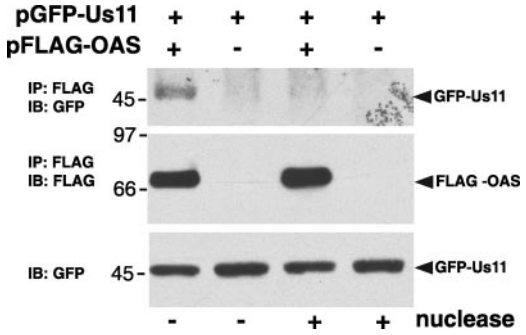

FIG. 7. Association of Us11 with OAS in a nuclease-sensitive complex. Cells (293) were transfected with a Us11 expression plasmid (pGFP-Us11) in the presence and absence of an OAS expression plasmid (pFLAG-OAS). Twenty-four hours posttransfection, soluble cell-free lysates were prepared and immunoprecipitated with anti-FLAG monoclonal antibody (IP: FLAG). Immune complexes were fractionated by SDS-PAGE and analyzed by immunoblotting with anti-GFP (IB: GFP) or anti-FLAG (IB: FLAG). The bottom panel displays the overall abundance of the GFP-Us11 antigen in the starting lysates. Lysates treated with a nuclease cocktail (RNase A, T_1, micrococcal nuclease) that degrades both single- and double-stranded RNA are designated by a + beneath the bottom panel.

human cells. While this suggests a mechanism for preventing OAS activation that in part relies on interaction with and possibly sequestration of the dsRNA activator, it is noteworthy that the potent dsRNA-binding domain in PKR was not observed to inhibit OAS activity (Fig. 6B). Identical results were obtained when the purified proteins were first preincubated with the dsRNA activator before their addition to the extract (Fig. 6B). This raises the possibility that other mechanisms, in addition to dsRNA binding, might contribute to the Us11-mediated inhibition of OAS in HSV-1 infected cells.

OAS and Us11 associate in an RNA-containing complex. To evaluate the possibility that Us11 and OAS might physically associate, 293 cells were transfected with plasmids expressing FLAG-OAS and/or green fluorescent protein (GFP)-Us11. Soluble cell extracts were prepared and immunoprecipitated with an anti-FLAG monoclonal antibody. Immune complexes were subsequently fractionated by SDS-PAGE and analyzed by immunoblotting using anti-GFP or anti-FLAG antibodies. Whereas equal quantities of GFP-Us11 are present in the different lysates, GFP-Us11 is present in anti-FLAG immune complexes only when the OAS-encoding plasmid is transfected and the OAS polypeptide is expressed (Fig. 7). The association of GFP-Us11 with FLAG-OAS was disrupted by nuclease treatment (Fig. 7), suggesting that both proteins are part of a higher order complex that contains RNA. While both GFP-Us11 and FLAG-OAS may physically interact in an RNA-dependent manner, these experiments do not rule out the possibility that both proteins may simply contact the same RNA molecule independently.

DISCUSSION

Innate host responses comprise the frontlines of defense against viral invaders by imposing powerful barriers that restrict viral replication. The execution of this process requires the induction of interferon-stimulated genes, the products of which act in diverse ways to independently hamper virus replication (reviewed in reference 43). That many viruses effectively block ISG induction is a testament to their importance. Irrespective of their capabilities to block ISG activation, some viruses, such as HSV-1, can replicate in IFN-treated cells and must therefore effectively neutralize the action of already existing ISG products. Within this constellation of cellular defense functions are two key components activated by dsRNA produced in virus-infected cells: the eIF2α kinase PKR and the 2′-5′ OAS. Whereas PKR globally prevents translation initiation by phosphorylating and consequently inactivating eIF2 on its α subunit, the 2′-5′ oligoadenylate products produced by OAS activate RNase L (reviewed in reference 33). Once activated, RNase L inhibits translation, cleaving both rRNA and mRNA. Prior studies demonstrated that HSV-1 gene products $\gamma_1$34.5 and Us11 are both required for replication in IFN-treated cells; moreover, each acts at discrete times in the virus developmental program to prevent the accumulation of phosphorylated eIF2α (5, 34, 35). However, the means by which the virus evades OAS remained obscure. Here, we establish that the dsRNA-binding protein product of the HSV-1 Us11 gene is both necessary and sufficient to inhibit the activity of OAS.

While Us11 is not required for HSV-1 replication in Vero cells, an established, immortalized line of African green monkey kidney cells that is unable to produce endogenous interferon, it significantly contributes to replication in normal human diploid fibroblasts exposed to type I interferon (34). An HSV-1-specified true-late polypeptide whose expression is strictly dependent upon viral DNA synthesis, Us11 is also a virion component delivered into the host cytosol, where it can associate with 60S ribosome subunits prior to the onset of immediate-early gene expression (42). Although the precise function of tegument-derived Us11 is not clear, it is insufficient to prevent PKR activation even when the input dose of infecting virions is exceedingly high. Here, we demonstrate that Us11 is the only true-late gene required to inhibit OAS-activity in virus-infected cell extracts. Thus, it appears that this newest function of Us11 also operates late in the virus developmental program. This likely reflects the fact that late genes, whose open reading frames are located on opposing DNA strands, produce mRNAs with the potential to form dsRNA (19), as the ability of Us11 to inhibit OAS together with PKR activation requires the unusual Us11 dsRNA-binding segment. However, this does not exclude the possibility that tegument-derived Us11 brought into cells by incoming virions also contributes to the observed inhibition of OAS, as extracts prepared from cells infected with ICP4-deficient viruses exhibit reduced sensitivity to dsRNA activators (Fig. 3A). Construction of a viral recombinant containing a Us11 null allele introduced into an ICP4-deficient genetic background would be required to definitively test this hypothesis.

Composed of a repetitive Arg- and Pro-rich sequence that occupies many of the 68 C-terminal residues, the Us11 dsRNA-binding domain inhibits OAS and prevents PKR activation in response to both PACT (36) and dsRNA (38). As both OAS and PKR can be activated by dsRNA, a single simple mechanism that involves sequestering the dsRNA ligand by Us11 might be operative in its activity against both of these enzymes. Indeed, preincubation of Us11 with the RNA ligand enhances its effectiveness in inhibiting OAS. However,

Us11 also prevents PKR activation by PACT, a protein ligand that does not require dsRNA (36). Moreover, other effective dsRNA-binding domains are not effective in inhibiting OAS, even following preincubation with the RNA ligand, suggesting that the Us11 RNA-binding domain is particularly adept at preventing OAS activation. Certainly, interaction between Us11 and OAS could contribute to this effect as both proteins can be isolated in an RNA-dependent complex. The interaction between Us11 and OAS is therefore different from the nuclease-resistant complexes formed between Us11 and either PKR or PACT (37). Further work is required to determine if the RNA-dependent association of OAS with Us11 merely reflects the interaction of both proteins with the same piece of RNA or if actual protein-protein contacts are formed during RNA assembly. Neither of these mechanisms, however, is mutually exclusive, and both may in fact synergize to achieve optimal inhibition of the Us11 target enzymes.

Although PKR and OAS are both major host defense components activated by dsRNA in HSV-1-infected cells, they do not necessarily always make equal contributions. Whereas the inhibition of HSV-1 replication in trigeminal ganglia cultures by an IFN-β transgene requires both PKR and OAS (1), the predominant antiviral pathway at work in L929 cells involves PKR only (16). Similarly, HSV-1 reportedly replicates 100-fold better in $PKR^{-/-}$ mouse embryo fibroblasts (MEFs) and only 3.6-fold better in $PKR^{-/-}$ RNase $L^{-/-}$ MEFs (22). However, in a mouse model of HSV-1 ocular infection, an IFN-β transgene selectively upregulates OAS mRNA and confers protection against HSV-1. RNase L is required for this protection, as extensive virus-induced damage is observed in RNase L-deficient animals (2). Thus, the contribution of the RNase L pathway to the antiviral state appears to vary in different tissue types following infection with HSV-1. Exactly why different host innate defense pathways predominate in distinct tissue types, however, remains a mystery. Conceivably, this might reflect the varied expression levels and subcellular localization of the three different size classes of OAS, all of which are induced by IFN and whose precise role is also incompletely understood. With the exception of the largest OAS, the small and intermediate-sized classes of OAS are composed of multiple isoforms generated by differential splicing and are catalytically active as oligomers, while only a single isoform of the large OAS, which is active as a monomer, has been identified (reviewed in reference 43). Furthermore, whereas the small and intermediate classes synthesize 2′-5′ oligoadenylates of various lengths, the product of the large class is predominately pppA2′-pA5′ molecules, which are inefficient RNase L activators (40). Nevertheless, by utilizing the same dsRNA-binding effector molecule to counteract both OAS and PKR, HSV-1, like other viruses with dsRNA-binding proteins, seems to have maximally utilized its finite genomic coding capacity to permit replication in a broad variety of tissues, irrespective of dsRNA-responsive host defenses.

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REFERENCES

1. **Al-Khatib, K., B. R. Williams, R. H. Silverman, W. Halford, and D. J. Carr.** 2003. The murine double-stranded RNA-dependent protein kinase PKR and the murine 2′,5′-oligoadenylate synthetase-dependent RNase L are required for IFN-beta-mediated resistance against herpes simplex virus type 1 in primary trigeminal ganglion culture. Virology **313:**126–135.
2. **Austin, B. A., C. James, R. H. Silverman, and D. J. Carr.** 2005. Critical role for the oligoadenylate synthetase/RNase L pathway in response to IFN-B during acute ocular herpes simplex virus type 1 infection. J. Immunol. **175:**1100–1106.
3. **Beattie, E., K. L. Denzler, J. Tartaglia, M. E. Perkus, E. Paoletti, and B. L. Jacobs.** 1995. Reversal of the interferon-sensitive phenotype of a vaccinia virus lacking E3L by expression of the reovirus S4 gene. J. Virol. **69:**499–505.
4. **Cayley, P. J., J. A. Davies, K. G. McCullagh, and I. M. Kerr.** 1984. Activation of the ppp(A2′p)nA system in interferon-treated, herpes simplex virus-infected cells and evidence for novel inhibitors of the ppp(A2′p)nA-dependent RNase. Eur. J. Biochem. **143:**165–174.
5. **Cerveny, M., S. Hessefort, K. Yang, G. Cheng, M. Gross, and B. He.** 2003. Amino acid substitutions in the effector domain of the $\gamma_1$34.5 protein of herpes simplex virus 1 have differential effects on viral response to interferon-α. Virology **307:**290–300.
6. **Chebath, J., P. Benech, M. Revel, and M. Vigneron.** 1987. Constitutive expression of 2′-5′ oligo A synthetase confers resistance to picornavirus infection. Nature **330:**587–588.
7. **Child, S. J., M. Hakki, K. L. De Niro, and A. P. Geballe.** 2004. Evasion of cellular antiviral responses by human cytomegalovirus *TRS1* and *IRS1*. J. Virol. **78:**197–205.
8. **DeLuca, N., and Schaffer, P.** 1988. Physical and functional domains of the herpes simplex virus. J. Virol. **62:**732–743.
9. **Doepker, R. C., W.-L. Hsu, H. A. Saffran, and J. R. Smiley.** 2004. Herpes simplex virus virion host shutoff protein is stimulated by translation initiation factors eIF4B and eIF4H. J. Virol. **78:**4684–4699.
10. **Duerst, R. J., and L. A. Morrison.** 2004. Herpes simplex virus 2 virion host shutoff protein interferes with type I interferon production and responsiveness. Virology **322:**158–167.
11. **Eidson, K. M., W. E. Hobbs, B. J. Manning, P. Carlson, and N. A. DeLuca.** 2002. Expression of herpes simplex virus ICP0 inhibits the induction of interferon-stimulated genes by viral infection. J. Virol. **76:**2180–2191.
12. **Feng, P., D. N. Everly, Jr., and G. S. Read.** 2005. mRNA decay during herpes simplex virus (HSV) infections: protein-protein interactions involving the HSV virion host shutoff protein and translation factors eIF4H and eIF4A. J. Virol. **79:**9651–9664.
13. **Floyd-Smith, G., E. Slattery, and P. Lengyel.** 1981. Interferon action: RNA cleavage pattern of a 2′-5′ oligoadenylate-dependent endonuclease. Science **212:**1030–1032.
14. **Gribaudo, G., D. Lembo, G. Cavallo, S. Landolfo, and P. Lengyel.** 1991. Interferon action: binding of viral RNA to the 40-kilodalton 2′-5′-oligoadenylate synthetase in interferon-treated HeLa cells infected with encephalomyocarditis virus. J. Virol. **65:**1748–1757.
15. **Härle, P., B. Sainz, Jr., D. J. Carr, and W. P. Halford.** 2002. The immediate-early protein, ICP0, is essential for the resistance of herpes simplex virus to interferon-alpha/beta. Virology **293:**295–304.
16. **Härle. P., V. Cull, M.-P. Agbaga, R. Silverman, B. R. G. Williams, C. James, and D. J. J. Carr.** 2002. Differential effect of murine alpha/beta interferon transgenes on antagonization of herpes simplex virus type 1 replication. J. Virol. **76:**6558–6567.
17. **Hartmann, R., J. Justesen, S. N. Sarkar, G. C. Sen, and V. C. Yee.** 2003. Crystal structure of the 2′-specific and double-stranded RNA-activated interferon-induced antiviral protein 2′-5′-oligoadenylate synthetase. Mol. Cell **12:**1173–1185.
18. **Hovanessian, A. G., J. Wood, E. Meurs, and L. Montagnier.** 1979. Increased nuclease activity in cells treated with pppA2′p5′A2′p5′ A. Proc. Natl. Acad. Sci. USA **76:**3261–3265.
19. **Jacquemont, B., and B. Roizman.** 1975. RNA synthesis in cells infected with herpes simplex virus. X. Properties of viral symmetric transcripts and of double-stranded RNA prepared from them. J. Virol. **15:**707–713.
20. **Kajaste-Rudnitski, A., T. Mashimo, M. P. Frenkiel, J. L. Guenet, M. Lucas, and P. Despres.** 2006. The 2′,5′ oligoadenylate synthetase 1b is a potent inhibitor of West Nile virus replication inside infected cells. J. Biol. Chem. **281:**4624–4637.
21. **Kerr, I. M., and R. E. Brown.** 1978. pppA2′p5′A2′p5′A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc. Natl. Acad. Sci. USA **75:**256–260.
22. **Khabar, K. S., M. Dhalla, Y. Siddiqui, A. Zhou, M. N. Al-Ahdal, S. D. Der, R. H. Silverman, and B. R. Williams.** 2000. Effect of deficiency of the double-stranded RNA-dependent protein kinase, PKR, on antiviral resistance in the presence or absence of ribonuclease L: HSV-1 replication is particularly sensitive to deficiency of the major IFN-mediated enzymes. J. Interferon Cytokine Res. **20:**653–659.

23. **Khoo, D., C. Perez, and I. Mohr.** 2002. Characterization of RNA determinants recognized by the arginine- and proline-rich region of Us11, a herpes simplex virus type 1-encoded double-stranded RNA binding protein that prevents PKR activation. J. Virol. **76:**11971–11981.
24. **Kubota, K., K. Nakahara, T. Ohtsuka, S. Yoshida, J. Kawaguchi, Y. Fujita, Y. Ozeki, A. Hara, C. Yoshimura, H. Furukawa, H. Haruyama, K. Ichikawa, M. Yamashita, T. Matsuoka, and Y. Iijima.** 2004. Identification of 2′-phosphodiesterase, which plays a role in the 2-5A system regulated by interferon. J. Biol. Chem. **279:**37832–37841.
25. **Langland, J. O., J. M. Cameron, M. C. Heck, J. K. Jancovich, and B. L. Jacobs.** 2006. Inhibition of PKR by RNA and DNA viruses. Virus Res. **119:**100–110.
26. **Levy, D. E., I. Marie, and A. Prakash.** 2003. Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity. Curr. Opin. Immunol. **15:**52–58.
27. **Lin, R., R. S. Noyce, S. E. Collins, R. D. Everett, and K. L. Mossman.** 2004. The herpes simplex virus ICP0 RING finger domain inhibits IRF3- and IRF7-mediated activation of interferon-stimulated genes. J. Virol. **78:**1675–1684.
28. **Maitra, R. K., N. A. McMillan, S. Desai, J. McSwiggen, A. Hovanessian, G. Sen, B. R. Williams, and R. H. Silverman.** 1994. HIV-1 TAR RNA has an intrinsic ability to activate interferon-inducible enzymes. Virology **204:**823–827.
29. **Marie, I., J. Svab, N. Robert, J. Galabrn, and A. G. Hovanessian.** 1990. Differential expression and distinct structure of 69- and 100-kDa forms of 2-5A synthetase in human cells treated with interferon. J. Biol. Chem. **265:**18601–18607.
30. **Melroe, G. T., N. A. DeLuca, and D. M. Knipe.** 2004. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. J. Virol. **78:**8411–8420.
31. **Min, J. Y., and R. Krug.** 2006. The primary function of RNA binding by the influenza A virus NS1 protein in infected cells: inhibiting the 2′-5′ oligo (A) synthetase/RNase L pathway. Proc. Natl. Acad. Sci. USA **103:**7100–7105.
32. **Mohr, I., S. Sternberg, D. Ward, M. Leib, M. Mulvey, and Y. Gluzman.** 2001. A herpes simplex virus type 1 γ34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. J. Virol. **75:**5189–5196.
33. **Mohr, I. J., T. Pe'ery, and M. B. Mathews.** 2006. Protein synthesis and translational control during viral infection. *In* N. Sonenberg, J. W. B. Hershey, and M. B. Mathews (ed.), Translational control. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
34. **Mulvey, M., V. Camarena, and I. Mohr.** 2004. Full resistance of HSV-1 infected primary human cells to interferon α require both the Us11 and $\gamma_1$34.5 gene products. J. Virol. **78:**10193–10196.
35. **Mulvey, M., J. Poppers, D. Sternberg, and I. Mohr.** 2003. Regulation of eIF2α phosphorylation by different functions that act during discrete phases in the herpes simplex virus type 1 life cycle. J. Virol. **77:**10917–10928.
36. **Peters, G., D. Khoo, I. Mohr, and G. C. Sen.** 2002. Inhibition of PACT-mediated activation of PKR by the herpes simplex virus type 1 Us11 protein. J. Virol. **76:**11054–11064.
37. **Poppers, J., M. Mulvey, C. Perez, D. Khoo, and I. Mohr.** 2003. Identification of a lytic Epstein-Barr virus gene product that can regulate PKR activation. J. Virol. **77:**228–236.
38. **Poppers, J., M. Mulvey, D. Khoo, and I. Mohr.** 2000. Inhibition of PKR activation by the proline-rich, RNA binding domain of the HSV-1 Us11 protein. J. Virol. **74:**11215–11221.
39. **Read, G. S., B. M. Karr, and K. Knight.** 1993. Isolation of a herpes simplex virus type 1 mutant with a deletion in the virion host shutoff gene and identification of multiple forms of the *vhs* (UL41) polypeptide. J. Virol. **67:**7149–7160.
40. **Rebouillat, D., A. Hovnanian, I. Marie, and A. G. Hovanessian.** 1999. The 100-kDa 2′,5′-oligoadenylate synthetase catalyzing preferentially the synthesis of dimeric pppA2′p5′A molecules is composed of three homologous domains. J. Biol. Chem. **274:**1557–1565.
41. **Rivas, C., J. Gil, Z. Melkova, M. Esteban, and M. Diaz-Guerra.** 1998. Vaccinia virus E3L protein is an inhibitor of the interferon (i.f.n.)-induced 2-5A synthetase enzyme. Virology **243:**406–414.
42. **Roller, R. J., and B. Roizman.** 1992. The herpes simplex virus 1 RNA binding protein Us11 is a virion component and associates with 60S ribosomal subunits. J. Virol. **66:**3624–3632.
43. **Sarkar, S. N., and G. C. Sen.** 2004. Novel functions of proteins encoded by viral stress-inducible genes. Pharmacol. Ther. **103:**245–259.
44. **Sarkar, S. N., and G. C. Sen.** 1998. Production, purification and characterization of recombinant 2′5′-oligoadenylate synthetases. Methods **15:**233–242.
45. **Schmidt, A., Y. Chernajovsky, L. Shulman, P. Federman, H. Berissi, and M. Revel.** 1979. An interferon-induced phosphodiesterase degrading (2′-5′) oligoisoadenylate and the C-C-A terminus of tRNA. Proc. Natl. Acad. Sci. USA **76:**4788–4792.
46. **Schreiber, E., P. Matthias, M. M. Müller, and W. Schaffner.** 1989. Rapid detection of octamer binding proteins with "mini extracts," prepared from a small number of cells. Nucleic Acids Res. **17:**6419.
47. **Sen, G. C.** 2000. Novel functions of interferon-induced proteins. Semin. Cancer Biol. **10:**93–101.
48. **Silverman, R. H., D. D. Jung, N. L. Nolan-Sorden, C. W. Dieffenbach, V. P. Kedar, and D. N. SenGupta.** 1988. Purification and analysis of murine 2-5A-dependent RNase. J. Biol. Chem. **263:**7336–7341.
49. **Wreschner, D. H., J. W. McCaule, J. J. Skehel, and I. M. Kerr.** 1981. Interferon action sequence specificity of the ppp(A2′p)nA-dependent ribonuclease. Nature **289:**414–417.