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Proteomics of herpes simplex virus infected cell protein 27: association with translation initiation factors

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Abstract

The herpes simplex virus (HSV) immediate early ICP27 protein plays an essential role in stimulating viral early and late gene expression. ICP27 appears to be multifunctional in that it has been reported to stimulate viral late gene transcription, polyadenylation site usage, and RNA export. We report here on proteomic studies involving immunoprecipitation of ICP27 and mass spectrometric identification of co-precipitated proteins. These studies show an association of ICP27 with the cellular translation initiation factors poly A binding protein (PABP), eukaryotic initiation factor 3 (eIF3), and eukaryotic initiation factor 4G (eIF4G) in infected cells. Immunoprecipitation-western blot studies confirmed these associations. Finally, purified MBP-tagged ICP27 (MBP-27) can interact with eIF3 subunits p47 and p116 in vitro. These results suggest that ICP27 may also play a role in stimulating translation of certain viral and host mRNAs and/or in inhibiting host mRNA translation.

Keywords: ICP27; eIF4G; eIF3; PABP; Translation initiation

Introduction

HSV-1 gene expression is characterized by a temporal pattern of expression of three gene classes: immediate early (IE), early (E), and late (L) genes. IE genes are transcribed in the absence of de novo viral protein synthesis, E genes are activated by IE gene products, and L genes are activated by viral DNA synthesis (reviewed in Roizman and Knipe, 2001). The IE-infected cell protein 27 (ICP27) is essential for viral replication and expression of certain early and nearly all late viral genes (Rice et al., 1989; Sacks et al., 1985; Uprichard and Knipe, 1996). ICP27 is a multi-functional protein in that it increases late viral gene transcription (Jean et al., 2001), binds to RNA (Mears and Rice, 1996), associates with RNA pol II (Zhou and Knipe, 2002), and shuttles from the nucleus to the cytoplasm (Mears and Rice, 1998; Soliman et al., 1997). ICP27 has been shown to associate with cellular transcriptional proteins (Taylor and Knipe, 2004; Zhou and Knipe, 2002), as well as viral transcriptional proteins ICP4 (Panagiotidis et al., 1997) and ICP8 (Taylor and Knipe, 2004; Zhou and Knipe, 2002), and function in post-transcriptional processes, such as pre-mRNA splicing and mRNA export, through its interactions with cellular splicing and export factors involved in these pathways (Koffa et al., 2001). ICP27 directly affects the expression and stability of specific viral and cellular transcripts in both transfected (Brown et al., 1995) and infected cells (Cheung et al., 2000; Ellison et al., 2000; Pearson et al., 2004). Furthermore, ICP27 is thought to function, along with the virion host shut-off (vhs) protein, in shut-off of cellular protein synthesis (Sacks et al., 1985; Song et al., 2001), and the involvement of ICP27 in inhibition of pre-mRNA splicing provides a mechanism for shut-off of cellular protein synthesis (Sandri-Goldin, 1998). However, data presented here suggest that ICP27 may have

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a more direct role in the regulation of protein synthesis at the level of translation initiation. We show that ICP27 associates with several translation initiation factors including eIF3, eIF4G, and PABP in infected cells, and that ICP27 directly interacts with eIF3 subunits. These data suggest an additional role for ICP27 in regulation of translation.

Results

Co-immunoprecipitation of translation initiation factors with ICP27

We used a proteomics approach (Taylor and Knipe, 2004) to define cellular proteins that interact with ICP27. Lysates from mock- or wt virus-infected HEp-2 cells were incubated with the ICP27-specific H1119 monoclonal mouse antibody (Ackermann et al., 1984). Proteins in the immunoprecipitates were resolved by SDS-PAGE, and the gels were stained with Coomassie blue stain or silver stain to visualize protein bands (Fig. 1). Bands that were present in the wt-infection lanes but not in the mock-infection lanes were excised for analysis by tandem mass spectrometry (MS). Using this procedure, from one band of a doublet that migrated slightly higher than 220 kDa (Fig. 1A), the translation initiation factor eIF4G was identified with 20 peptide matches. Additional proteins identified in this band were alpha-filamin, beta-filamin, nucleoporin Nup214, myosin, splicing factor PRP8, and polyubiquitin. From a band migrating slightly higher than 70 kDa (Fig. 1B), eIF3 subunits 3b (eIF3-9) and 3c (eIF3-8) were identified by tandem MS with three and seven peptide matches, respectively. Because eIF3b and eIF3c were identified from a band that was resolved at approximately 70 kDa, which is less than the molecular weights of eIF3b and eIF3c, 116 and 110 kDa, respectively, these bands may represent degradation products. From a separate immunoprecipitate and gel, we excised two bands migrating at and above an apparent MW of 70 k (Fig. 1C). In these we identified PABP1 by tandem MS with 11 peptide matches. Additional proteins identified in these bands were hnRNP K, HSP70 protein 1, PABP4, alpha-actin, beta-actin, DEAD-box protein p72, TKT, HSC71, FUB1, and hnRNP R.

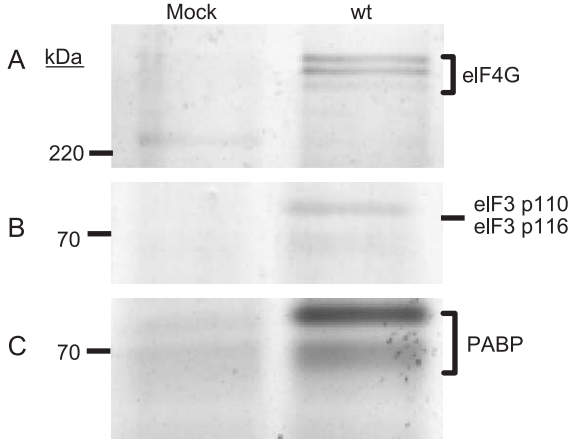

Fig. 1. Co-precipitation of cellular proteins with ICP27. Immunoprecipitates of ICP27 and associated proteins from mock-infected and wt HSV-1-infected HEp-2 cells were resolved by SDS-PAGE, and the gels were then stained with Coomassie blue or Silver stain. Shown are portions of three separate gels containing proteins larger than 220 kDa (A) and approximately 70 kDa (B and C). The bands labeled as eIF4G were excised from the wt lane and identified by mass spectrometry (A). The bands labeled as eIF3 p110 and eIF3 p116 (B), and PABP (C) were excised from the wt lane and identified by mass spectrometry.

To confirm the association of the translation factors with ICP27, we performed immunoprecipitations with H1119 ICP27-specific monoclonal antibody, or polyclonal antibodies specific for the translation initiation factors, followed by Western blotting to identify the co-precipitated proteins. Immunoprecipitation of ICP27 from wt virus-infected cells led to the co-precipitation of eIF4G, eIF3 p116 subunit, and PABP (Fig. 2A, lane 6), as shown by Western blotting. The translation factors did not immunoprecipitate with H1119 Mab using lysates from cells infected with the *d*27-1 null mutant virus (Fig. 2A, lane 5). By the reverse approach, immunoprecipitation of eIF4G, PABP, or eIF3 p116 subunit led to co-precipitation of ICP27 (Fig. 2C, lanes 3, 6, and 9, respectively). ICP27 did not precipitate when wt virus lysates were incubated with beads alone (data not shown). Thus, the immunoprecipitation-Western blotting studies confirmed the IP-MS results showing interaction of ICP27 with translation initiation factors.

To determine if the associations of ICP27 with initiation factors were mediated via RNA, we incubated the cell lysates with a mixture of RNases prior to conducting the immunoprecipitation. RNase treatment eliminated the co-precipitation of eIF4G with ICP27 (Fig. 2B, lane 6), indicating that the association likely involved RNA. RNase treatment reduced the co-precipitation of PABP with ICP27 by approximately 6-fold (lane 6), indicating that RNA was involved for part of the association but that part of the association may involve protein–protein interactions. Similarly, RNase treatment reduced the co-precipitation of eIF3 p116 subunit with ICP27 by 2.5-fold, (Fig. 2B, lane 6) but a significant portion of the signal remained, indicating that p116 may associate in part with ICP27 by protein–protein interactions.

The results above demonstrate that ICP27 interactions with eIF3 and PABP protein were only partially mediated by RNA, and suggest that ICP27 interacts directly, and/or through additional protein–protein interactions, with these translation factors. This conclusion was supported by results showing that the PABP precipitated equally with wt ICP27 and *d*4-5, an ICP27 RGG box mutant (data not shown). Finally, it is known that during normal translation eIF4G acts as a scaffolding protein for the cap-binding complex, and interacts directly with both PABP and eIF3 (reviewed in Kawaguchi and Bailey-Serres, 2002). Thus, our data suggested that ICP27 associates with eIF4G through interactions with PABP and eIF3 and/or additional protein–protein interactions, and through interactions with RNA.

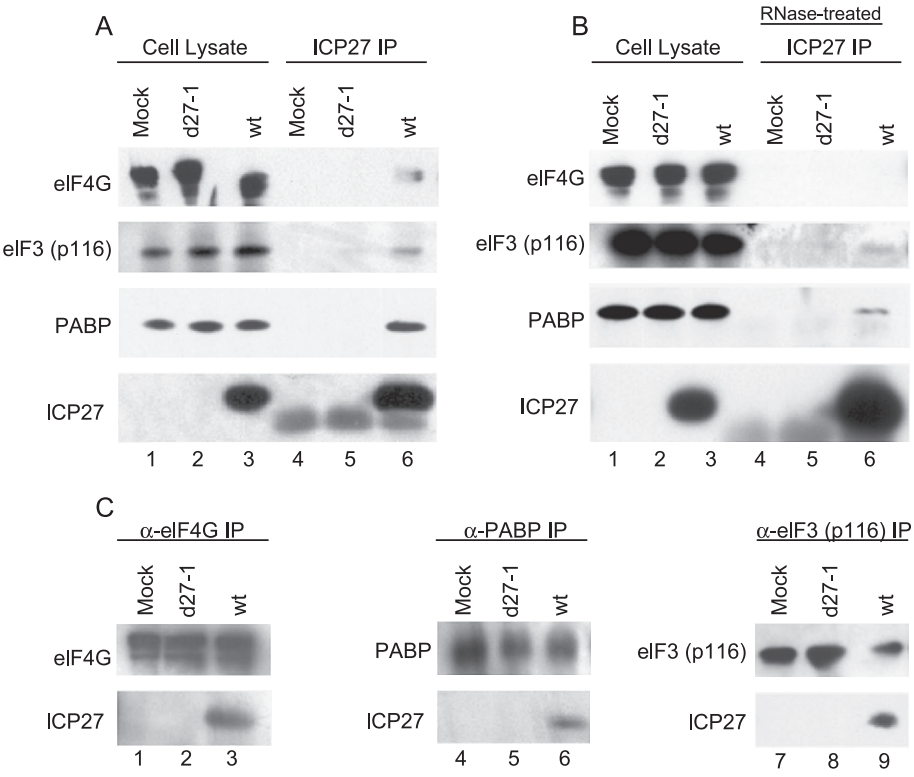

Fig. 2. Coprecipitation of cellular translation initiation factors with ICP27 from HSV-infected cells. Immunoprecipitations of ICP27 or translation factors were performed with lysates from mock-infected cells or cells infected with wt HSV or *d*27-1 virus. Shown are Western blots detecting specific proteins in the immunoprecipitates. (A) Immunoprecipitations of ICP27 with anti-ICP27 monoclonal antibody H1119. (B) Immunoprecipitations of ICP27 with anti-ICP27 monoclonal antibody H1119 from lysates not treated or treated with RNase. (C) Immunoprecipitations of eIF4G, eIF3, and PABP with specific antibodies.

Direct interaction of ICP27 with eIF3 subunits

To determine if ICP27 was interacting directly with eIF3, we performed an in vitro pull-down assay using tagged ICP27 and eIF3 subunit proteins. Maltose binding protein (MBP)-tagged ICP27 and MBP were immobilized on amylose resin, and His-tagged eIF3 subunits p47 and p116 were incubated with the resin. Bound proteins were eluted by boiling in gel loading buffer. Both eIF3 subunits p47 and p116 bound to MBP-tagged ICP27 (Fig. 3A, lanes 5 and 6) but not to MBP (Fig. 3A, lanes 3 and 4). As a control, GST-tagged RNAPII C-terminal domain (GSTPOL) was immobilized on a glutathione Sepharose resin, and eIF3 subunits were incubated with this resin. The eIF3 subunits p47 and p116 did not bind to GSTPOL (Fig. 3A, lanes 7 and 8), arguing for specificity of the interactions with ICP27. The 70-kDa band observed in the GSTPOL pull-downs was probably a bacterial protein that was recognized nonspecifically by the anti-His antibody used to probe the Western blot. As an additional control, partially purified reovirus μ2 protein was incubated with eIF3 subunits p47 or p116 immobilized on a nickel affinity resin, and MBP or MBP27 immobilized on an amylose resin (Fig. 3B). Reovirus μ2 did not bind to p116-His, p47-His, MBP27, or MBP (Fig. 3B, lanes 2–5), providing further evidence of the specificity. Two nonspecific bands, migrating just above or below μ2 were observed for each pull-down reaction (Fig. 3B, lanes 2, 3, 4, and 5). Again, these bands were probably bacterial proteins, which are recognized nonspecifically by the anti-μ2 antibody, because they were apparent in Western blots of bacterial extracts (results not shown). In total, these in vitro results argued that ICP27 binds specifically to the eIF3 p47 and p116 subunits.

Discussion

ICP27 has been shown to interact with pol II holoenzyme (Zhou and Knipe, 2002), RNA transport proteins (Chen et al., 2002; Koffa et al., 2001) and in this study, with translation factors. Thus, ICP27 has been shown to stimulate transcription (Jean et al., 2001), mRNA export from the nucleus (Koffa et al., 2001), and gene expression at several posttranscriptional levels (Hardwicke and Sandri-Goldin, 1994; McLauchlan et al., 1992; Perkins et al., 2003). The interactions of ICP27 with translation factors demonstrated here raise the idea of a novel function for ICP27 in promoting the translation of specific viral and cellular mRNAs and/or inhibiting the translation of host mRNAs by direct interactions with the cellular translation machinery.

We hypothesize that, as a result of its localization to the RNA pol II holoenzyme complex (Zhou and Knipe, 2002), ICP27 can stimulate transcription and then load onto nascent transcripts. Interactions of ICP27 with host

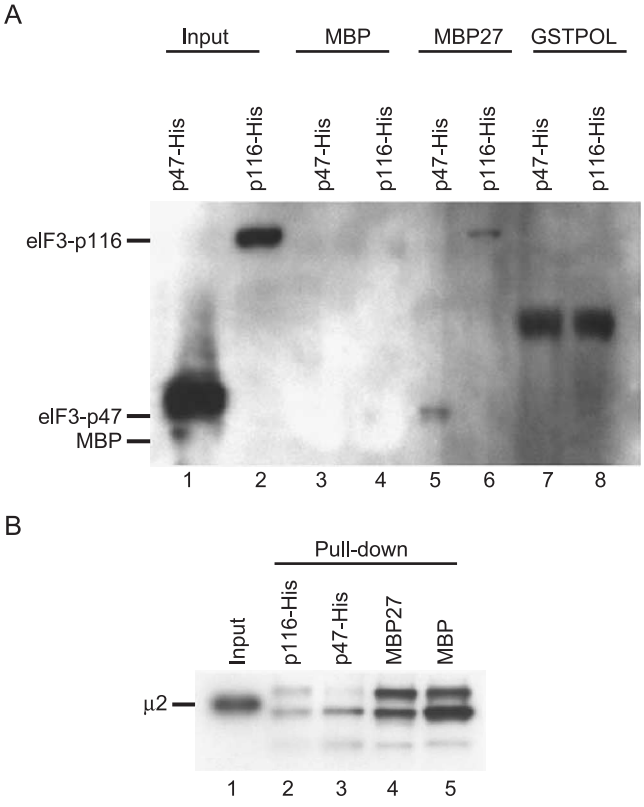

Fig. 3. In vitro binding studies of ICP27 and eIF3 p47 and p116 subunits. (A) His-tagged eIF3 subunits were expressed and purified in *E. coli* cells and run on an amylose column loaded with MBP or MBP-ICP27 fusion protein. The bound proteins were resolved by SDS-PAGE. Shown is an Anti-His Western blot detecting His-tagged eIF3 subunits p47 and p116 proteins in the bound fractions. (B) Lack of binding of ICP27 or translation factors to reovirus μ2 protein. HIS-p116 or HIS-p47 bound to a nickel affinity column, and MBP27 or MBP bound to an amylose column, were incubated with μ2 protein. Shown is a Western blot to detect μ2 protein in the input sample or in the pull-downs.

translation factors may recruit these factors to viral mRNA and facilitate their translation in the cytoplasm. One situation where this may have already been demonstrated is in the case of the U_L24 gene short transcript, which is expressed in the absence of ICP27 but not translated efficiently into U_L24 protein (Pearson et al., 2004). Alternatively, interactions of ICP27 with translation initiation factors may inhibit their ability to promote host mRNA translation. Obviously, these two effects are not mutually exclusive.

In these studies, a small fraction, 0.3–1%, of the translation factors co-precipitated with ICP27. Although these levels are low, similar levels of co-precipitation of the various translation factors with each other, 0.1–1%, were observed. These results show that under our conditions the extent of co-precipitation of ICP27 with the translation factors approximated the levels of co-precipitation of translation factors known to interact physically and functionally, for example, PABP and eIF4G. Therefore, there was sufficient co-precipitation of ICP27 and the translation factors for the interactions to be biologically relevant. Genetic studies will be required to demonstrate the essentiality of these interactions.

The interaction of eIF4G and PABP is thought to facilitate the interaction between the 5′cap and 3′polyadenylated end of the mRNA, which enhances translation both in vitro and in vivo, and facilitates recruitment of the 40S ribosomal subunit to the 5′end of the mRNA molecule [(reviewed in Prevot et al., 2003) and (Sonenberg and Dever, 2003)]. eIF3 is a multi-subunit component of the 40S ribosome, and interaction of eIF4G with eIF3 leads to recruitment of mRNA to the 43S complex (reviewed (Gallie, 2002). Thus, the interaction of ICP27 with both eIF3 and PABP could lead to the recruitment of these translation initiation factors to viral mRNA and stimulation of translation of these mRNAs. Moreover, both PABP and eIF3 p47 subunit have been shown to localize to both the cytoplasm and the nucleus (Afonina et al., 1998; Shi et al., 2003). Therefore, ICP27 could recruit these proteins to nascent viral transcripts, which may facilitate viral mRNA export out of the nucleus, and increase the efficiency of translational initiation on these mRNAs.

PABP, eIF3, and eIF4G are known targets for modification by viruses. These cellular translation factors are altered by specific viral proteins, and as a result, host cell protein synthesis is shut down (reviewed in Bushell and Sarnow, 2002; Daughenbaugh et al., 2003). Translation initiation factor eIF4G acts as a scaffolding protein for the cap-binding complex (eIF4F), and interacts with multiple translation initiation proteins including PABP and eIF3 (reviewed in Kawaguchi and Bailey-Serres, 2002). Furthermore, each of these translation initiation factors have been shown to function in viral translation regulatory mechanisms, which require specific binding to viral proteins (reviewed in Gallie, 2002).

Our findings in this study raise a potential new role for ICP27 in directly regulating translation of viral and host mRNAs. To clarify this function of ICP27, future studies could determine if specific mutations in the ICP27 gene can lead to defects in translation of viral mRNAs, or in shutoff of host protein synthesis without affecting levels of cytoplasmic viral mRNAs. In addition, studies of the effect of ICP27 on the size of polysomes assembled on specific viral mRNAs in infected cells, or on the effect of translation of mRNAs in vitro should be informative about this potential role for ICP27. Thus, future studies of ICP27 and its role in gene expression will need to distinguish potential effects on transcription, posttranscriptional processes, and translation.

Materials and methods

Cells and viruses

Human epidermoid (HEp-2) cells (American type Culture Collection; Manassas, VA) were grown and

maintained in Dulbecco's modified Eagle's medium (DMEM; Media Tech Inc., Herndon, Va.) supplemented with 5% fetal bovine serum (Gibco, Carlsbad, CA), 5% bovine calf serum (Hyclone, Logan, UT), streptomycin (100 μg/ml), and penicillin (100 U/ml). The HSV-1 wild-type (wt) KOS1.1 strain was propagated and assayed on Vero cells. The KOS1.1 *d*27-1 virus is an *ICP27* gene deletion virus and was characterized previously (Rice and Knipe, 1990). The KOS1.1 d4-5 virus is an ICP27 deletion virus and was characterized previously (Mears et al., 1995).

Antibodies

The H1119 anti-ICP27 mouse monoclonal antibody was purchased from the Goodwin Institute for Cancer Research Inc. (Plantation, FL). The following antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): rabbit polyclonal anti-eIF4G (H-300), goat polyclonal eIF3η (A-20), goat polyclonal PABP (F-20), His-probe (H-15). Mouse monoclonal anti-PABP was purchased from ImmunoQuest (United Kingdom). The μ2-specific rabbit polyclonal antibody was a gift from Max Nibert (Harvard Medical School, Boston, MA).

Viral infections and immunoprecipitations

HEp-2 cells were grown in 100-mm tissue culture dishes to 90% confluence at time of infection and infected at a multiplicity of infection of 20 PFU per cell with either wt or *d*27-1 virus diluted in cold phosphate-buffered saline (PBS) containing 0.1% glucose. After 1 h of adsorption at 37 °C, cells were switched into Dulbecco's modified Eagle's medium containing 1% heat-inactivated bovine calf serum. Cells were harvested at 6 h. p.i. by scraping them into the media. After two washes in cold PBS, cells from each dish were incubated on ice for 30 min in 1 ml of immunoprecipitation (IP) buffer (20 mM Tris–HCl [pH 7.5], 50 mM NaCl, 0.1% NP-40, 10 mM β-glycerophosphate, 5 mM NaF, 1 mM PMSF, 2.5% glycerol, and 1 Complete mini protease inhibitor cocktail tablet [Roche Applied Science, Indianapolis, Ind.] per 10 ml). Cell lysates were clarified by centrifugation at 10K X g at 4 °C for 5 min, and precleared overnight by incubating with a mixture of protein A- and protein G-agarose beads at 4 °C. Ten microliters of precleared cell lysate was set aside, and immunoprecipitation was carried out with appropriate antibodies and a mixture of protein A- and protein G-agarose beads at 4 °C for 2–4 h. After four washes in washing buffer (20 mM Tris–HCl [pH 7.5], 50 mM NaCl, 0.1% NP-40, and 1 mM PMSF), the immunoprecipitates were dissolved in gel sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). All RNase incubations were performed with cell lysates at 30 °C for 30 min prior to immunoprecipitation. The RNase cocktail (Ambion, Austin, TX) used included 25 U of RNase A/ml and 1000 U of RNase T_1/ml as recommended by the manufacturer. Quantifications of the translation factors were performed by calculating the density of the bands representing the cell lysate and the co-immunoprecipitated translation factors. All quantifications were calculated using NIH Image 1.62 software.

SDS-PAGE and Western blotting

Proteins in the immunoprecipitates were resolved by SDS-PAGE in diallyltartardiamide cross-linked 9.25% (unless stated otherwise) polyacrylamide gels at 14 mA overnight and transferred onto a nitrocellulose membrane by electroblotting at 40 V for 2 days. The membranes were blocked in 2% milk in Tris-buffered saline with 0.1% Tween (TBST), probed with appropriate antibodies in TBST, and stained with ECL Western blotting detection reagents (Amersham Pharmacia Biotech Inc., Piscataway, NJ) in accordance with the manufacturer's procedure.

Mass spectrometry

Bands excised from Coomassie stained gels were analyzed at the Taplin Biological Mass Spectrometry (MS) Facility, Harvard Medical School, by microcapillary liquid chromatography-tandem MS with a LCQ DECA ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA).

In vitro pull-down assays

All bacterial constructs were expressed in and purified from BL21 Star Chemically Competent *Escherichia coli* cells (Invitrogen, Carlsbad, CA). The eIF3-subunit His fusion constructs (pET28c-p47 and p116) were provided by J. W. Hershey (Asano et al., 1997; Methot et al., 1997) and purified using EZview Red His-Select HC Nickel Affinity Gel according to the manufacturer's procedure (Sigma, Saint Louis, MO). MBP-27 construction, expression, and purification will be described (Olesky, McNamee Zhou, Taylor, and Knipe, in press). MBP pull-down assays were conducted as described (Olesky, McNamee, Zhou, Taylor, and Knipe, in press). The GSTPOL construct (GST-CTD) was a gift from Jeff Parvin (Brigham and Women's Hospital, Boston, MA) and GST-CTD was purified using Glutathione Sepharose 4 Fast Flow resin using the same procedure as described for His purifications above (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The reovirus μ2 protein was a gift from Max Nibert (Harvard Medical School, Boston, MA).

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References

Ackermann, M., Braun, D.K., Pereira, L., Roizman, B., 1984. Characterization of herpes simplex virus 1 alpha proteins 0, 4, and 27 with monoclonal antibodies. J. Virol. 52, 108–118.

Afonina, E., Stauber, R., Pavlakis, G.N., 1998. The human poly(A)-binding protein 1 shuttles between the nucleus and the cytoplasm. J. Biol. Chem. 273, 13015–13021.

Asano, K., Vornlocher, H.P., Richter-Cook, N.J., Merrick, W.C., Hinnebusch, A.G., Hershey, J.W., 1997. Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits. Possible roles in RNA binding and macromolecular assembly. J. Biol. Chem. 272, 27042–27052.

Brown, C.R., Nakamura, M.S., Mosca, J.D., Hayward, G.S., Straus, S.E., Perera, L.P., 1995. Herpes simplex virus trans-regulatory protein ICP27 stabilizes and binds to 3′ ends of labile mRNA. J. Virol. 69, 7187–7195.

Bushell, M., Sarnow, P., 2002. Hijacking the translation apparatus by RNA viruses. J. Cell Biol. 158, 395–399.

Chen, I.H., Sciabica, K.S., Sandri-Goldin, R.M., 2002. ICP27 interacts with the RNA export factor Aly/REF to direct herpes simplex virus type 1 intronless mRNAs to the TAP export pathway. J. Virol. 76, 12877–12889.

Cheung, P., Ellison, K.S., Verity, R., Smiley, J.R., 2000. Herpes simplex virus ICP27 induces cytoplasmic accumulation of unspliced polyadenylated alpha-globin pre-mRNA in infected HeLa cells. J. Virol. 74, 2913–2919.

Daughenbaugh, K.F., Fraser, C.S., Hershey, J.W., Hardy, M.E., 2003. The genome-linked protein VPg of the Norwalk virus binds eIF3, suggesting its role in translation initiation complex recruitment. EMBO J. 22, 2852–2859.

Ellison, K.S., Rice, S.A., Verity, R., Smiley, J.R., 2000. Processing of alpha-globin and ICP0 mRNA in cells infected with herpes simplex virus type 1 ICP27 mutants. J. Virol. 74, 7307–7319.

Gallie, D.R., 2002. Protein–protein interactions required during translation. Plant Mol. Biol. 50, 949–970.

Hardwicke, M.A., Sandri-Goldin, R.M., 1994. The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. J. Virol. 68, 4797–4810.

Jean, S., LeVan, K.M., Song, B., Levine, M., Knipe, D.M., 2001. Herpes simplex virus 1 ICP27 is required for transcription of two viral late (gamma2) genes in infected cells. Virology 283, 273–284.

Kawaguchi, R., Bailey-Serres, J., 2002. Regulation of translational initiation in plants. Curr. Opin. Plant Biol. 5, 460–465.

Koffa, M.D., Clements, J.B., Izaurralde, E., Wadd, S., Wilson, S.A., Mattaj, I.W., Kuersten, S., 2001. Herpes simplex virus ICP27 protein provides viral mRNAs with access to the cellular mRNA export pathway. EMBO J. 20, 5769–5778.

McLauchlan, J., Phelan, A., Loney, C., Sandri-Goldin, R.M., Clements, J.B., 1992. Herpes simplex virus IE63 acts at the posttranscriptional level to stimulate viral mRNA 3′ processing. J. Virol. 66, 6939–6945.

Mears, W.E., Rice, S.A., 1996. The RGG box motif of the herpes simplex virus ICP27 protein mediates an RNA-binding activity and determines in vivo methylation. J. Virol. 70, 7445–7453.

Mears, W.E., Rice, S.A., 1998. The herpes simplex virus immediate-early protein ICP27 shuttles between nucleus and cytoplasm. Virology 242, 128–137.

Methot, N., Rom, E., Olsen, H., Sonenberg, N., 1997. The human homologue of the yeast Prt1 protein is an integral part of the eukaryotic initiation factor 3 complex and interacts with p170. J. Biol. Chem. 272, 1110–1116.

Panagiotidis, C.A., Lium, E.K., Silverstein, S.J., 1997. Physical and functional interactions between herpes simplex virus immediate-early proteins ICP4 and ICP27. J. Virol. 71, 1547–1557.

Pearson, A., Knipe, D.M., Coen, D.M., 2004. ICP27 selectively regulates the cytoplasmic localization of a subset of viral transcripts in herpes simplex virus type 1-infected cells. J. Virol. 78, 23–32.

Perkins, K.D., Gregonis, J., Borge, S., Rice, S.A., 2003. Transactivation of a viral target gene by herpes simplex virus ICP27 is posttranscriptional and does not require the endogenous promoter or polyadenylation site. J. Virol. 77, 9872–9884.

Prevot, D., Darlix, J.L., Ohlmann, T., 2003. Conducting the initiation of protein synthesis: the role of eIF4G. Biol. Cell 95, 141–156.

Rice, S.A., Knipe, D.M., 1990. Genetic evidence for two distinct transactivation functions of the herpes simplex virus alpha protein ICP27. J. Virol. 64, 1704–1715.

Rice, S.A., Su, L.S., Knipe, D.M., 1989. Herpes simplex virus alpha protein ICP27 possesses separable positive and negative regulatory activities. J. Virol. 63, 3399–3407.

Roizman, B., Knipe, D.M., 2001. Herpes simplex viruses and their replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, 4th ed. Lippincott, Williams and Wilkins, Philadelphia, PA, pp. 2399–2460.

Sacks, W.R., Greene, C.C., Aschman, D.P., Schaffer, P.A., 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. 55, 796–805.

Sandri-Goldin, R.M., 1998. ICP27 mediates HSV RNA export by shuttling through a leucine-rich nuclear export signal and binding viral intronless RNAs through an RGG motif. Genes Dev. 12, 868–879.

Shi, J., Feng, Y., Goulet, A.C., Vaillancourt, R.R., Sachs, N.A., Hershey, J.W., Nelson, M.A., 2003. The p34cdc2-related cyclin-dependent kinase 11 interacts with the p47 subunit of eukaryotic initiation factor 3 during apoptosis. J. Biol. Chem. 278, 5062–5071.

Soliman, T.M., Sandri-Goldin, R.M., Silverstein, S.J., 1997. Shuttling of the herpes simplex virus type 1 regulatory protein ICP27 between the nucleus and cytoplasm mediates the expression of late proteins. J. Virol. 71, 9188–9197.

Sonenberg, N., Dever, T.E., 2003. Eukaryotic translation initiation factors and regulators. Curr. Opin. Struct. Biol. 13, 56–63.

Song, B., Yeh, K.C., Liu, J.J., Knipe, D.M., 2001. Herpes simplex virus gene products required for viral infection of expression of G1-phase functions. Virology 290, 320–328.

Taylor, T.J., Knipe, D.M., 2004. Proteomics of herpes simplex virus replication compartments: association of cellular DNA replication, repair, recombination, and chromatin remodeling proteins with ICP8. J. Virol. 78, 5856–5866.

Uprichard, S.L., Knipe, D.M., 1996. Herpes simplex virus ICP27 mutant viruses exhibit reduced expression of specific DNA replication genes. J. Virol. 70, 1969–1980.

Zhou, C., Knipe, D.M., 2002. Association of herpes simplex virus type 1 ICP8 and ICP27 proteins with cellular RNA polymerase II holoenzyme. J. Virol. 76, 5893–5904.