Disruption of PML-associated nuclear bodies mediated by the human cytomegalovirus major immediate early gene product

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The PML gene product is associated with a defined nuclear structure (10–20 per cell) known variously as PML-bodies, ND10, PODs or Kr bodies. Certain conditions are known to compromise the integrity of PML-bodies; these include environmental stress (e.g. heat shock), a chromosomal translocation-associated acute promyelocytic leukaemia, and infection with certain viruses [including human cytomegalovirus (HCMV), herpes simplex virus type 1 and adenovirus]. Expression of the HCMV major immediate early (IE) protein (IE1491aa) is by itself sufficient to cause disruption of PML-bodies, resulting in the dispersal of the PML antigen uniformly throughout the nucleus. In uninfected cells undergoing mitosis PML is excluded from chromatin. However, both IE1491aa and PML were observed to associate with mitotic chromosomes in cells infected with HCMV or transfected with the IE1 gene. A series of in-frame IE1 deletion mutants was used in DNA transfection experiments to identify two large sequence elements (aa 132–274 and the C-terminal aa 347–491) not required for dispersal of the PML antigen. However, a putative leucine-zipper domain (aa 105–139), a putative zinc-finger domain (aa 267–286) and exon 2 and 3 coding sequences (aa 6–85) were required. The association of the IE1 gene product with chromatin required an acidic domain near the C terminus (aa 421–486). The interaction of IE1491aa with chromatin was therefore not required for the disruption of PML-bodies. Exon 2 (aa 1–24) was shown to encode a nuclear localization signal.

Introduction

Human cytomegalovirus (HCMV) replicates efficiently in human fibroblasts in vitro, where gene expression is conventionally divided into three phases: immediate early (IE), early and late. The IE gene products activate early and late phases of gene expression and thus regulate the transition to the lytic phase of the replication cycle. The major IE promoter drives expression of both the IE1 and IE2 genes. IE1 encodes a 1.95 kb mRNA which is transcribed from exons 1, 2, 3 and 4, whereas multiple IE2 gene products are encoded by sequences within exons 1, 2, 3, 5, 6 and 7 by differential splicing (Akrigg et al., 1985; Stenberg et al., 1985; Kerry et al., 1995). The IE1 gene product (IE1491aa) is a ~72 kDa phosphoprotein which is a strong activator of its own promoter and a weak transactivator of heterologous promoters. However, IE1 acts synergistically with a strong promiscuous transactivator encoded by IE2 (IE2791aa) to stimulate expression from both viral and cellular promoters (Pizzorno et al., 1988; Malone et al., 1990). An indirect interaction between the IE1 gene product (IE1491aa) and the transcription factor E2F is associated with transcriptional activation via an E2F-binding site (Hayhurst et al., 1995; Margolis et al., 1995). The Rb-related cellular protein p107 is both a suppresser of cell proliferation and a repressor of E2F-responsive promoters. Recently, IE1491aa has been shown to interact directly with p107 to relieve repression of an E2F-responsive promoter (Poma et al., 1996). IE1 expression is associated with induced cell cycle progression in quiescent cells (Poma et al., 1996).

We demonstrated that an HCMV gene expressed with IE kinetics affects the integrity of a nuclear body associated with the PML antigen (Kelly et al., 1995). More recently, it has been demonstrated that IE1 alone can cause disruption of PML-associated nuclear bodies and that IE2 associates with PML-bodies without causing disruption (Kelly, 1996; Korioth et al., 1996; Ahn et al., 1997; Ishov et al., 1997). A number of cellular proteins are now known to be associated...
with PML-bodies, including: Sp100, NDP-55 (Ascoli & Maul, 1991), PML (Stuurman et al., 1992), NCP-52 (Koriol et al., 1995), PIC-1 (Body et al., 1996), Int-ß (Desbois et al., 1996) and the herpesvirus-associated ubiquitin-specific protease HAUSP (Everett et al., 1997). PML-bodies are spherical structures (0.3–1.0 µm in diameter) which are present in virtually all cell types (10–20 per cell) and consist of a central core surrounded by an electron-dense capsule. Characteristically, PML-bodies are located within interchromatin regions and are associated with the nuclear matrix. The function of PML is of particular interest because a t(15:17) chromosomal translocation between the genes encoding PML and RARα is associated with acute promyelocytic leukaemia. The PML–RARα fusion protein dominantly delocalizes the PML-body-associated antigens PML and Sp100 (Daniel et al., 1993; Dyck et al., 1994; Weis et al., 1994). Although the normal function of PML is unknown, recent studies have provided strong evidence that the PML protein functions as a growth and a tumour suppressor (Mu et al., 1994; Koken et al., 1995; Liu et al., 1995).

A number of viral proteins have now been shown to interact with PML-bodies. During herpes simplex virus type 1 (HSV-1) infection, the IE transcriptional transactivator protein Vmw110 is responsible for dispersal of PML-body-associated antigens PML and Sp100 (Daniel et al., 1993; Dyck et al., 1994; Maul & Everett, 1994). Expression of adenovirus (Ad) type 5 E4-ORF3 results in reorganization of PML-bodies such that PML is associated with filamentous nuclear structures or ‘tracks’ (Puvion-Dutilleul et al., 1995; Carvalho et al., 1995; Doucas et al., 1996). Furthermore, human T-lymphotropic virus type I Tax binds to Int-ß and causes it to be redistributed from PML-bodies into the cytosol (Desbois et al., 1996). Additional virus-encoded proteins have been found associated with PML-bodies without causing disruption (Carvalho et al., 1995; Doucas et al., 1996; Szekely et al., 1996). Recent studies suggest that PML-bodies are associated with the site of early HCMV, HSV-1 and Ad5 transcription (Doucas et al., 1996; Ishov & Maul, 1996; Ishov et al., 1997).

The functional significance of the virus interactions with PML-bodies is as yet poorly understood. This study therefore analyses the interaction between HCMV IE1 and PML-bodies in more detail and by exploiting IE1 deletion mutants in transient DNA transfection experiments to map functional elements within the major IE protein.

Methods

Cells and viruses. Human foreskin fibroblasts (HFF cells) and CV1 cells, kindly provided by Graham Farrar and ECACC (CAMR, Porton Down, UK) respectively, were cultured on modified Eagle’s medium containing 10% foetal calf sera. HCMV strain AD169 was obtained from J. Booth (St George’s Hospital, London, UK). DNA transfection was performed either using LipofectAMINE (GibcoBRL) or SuperFect transfection reagent (Qiagen).

Immunohistochemistry. Indirect immunofluorescent staining for the PML and IE1 antigens was performed as described previously (Kelly et al., 1995). The monoclonal antibodies specific for the PML antigen (5E10; Stuurman et al., 1992) and IE1 exon12 (1D6-ß) were kindly provided by R. Van Driel (University of Amsterdam, The Netherlands) and B. Rogers (Murex, UK), respectively. A polyclonal antibody specific for IE1 was generated by a single intraperitoneal immunization of a guinea pig with 10³ p.f.u. of a replication-deficient adenovirus recombinant encoding IE1 (RAD31; Wilkinson & Akigg, 1992) with sera being collected 12 days post-immunization. An FITC-conjugated rat anti-mouse IgG monoclonal antibody (Zymed) and a Texas red-conjugated goat anti-guinea pig polyclonal antibody (EY Laboratories) were used as second antibodies. DNA was stained with 1 µg/ml DAPI (4',6-diamidino-2-phenylindole; Sigma) with the second antibody. Immunofluorescence analysis was done with a Leica DM LB microscope with filter cubes A (DAPI, BP 340–380), L4 (FITC, BP 450–490) and TX (Texas red, BP 530–595). Images were captured either using a 35 mm camera or a Hamamatsu CCD camera combined with a Leica Q500-A image analysis system. PAG and Western transfer were performed as described previously (Foaks et al., 1993) using the ECL detection system according to the manufacturer’s instructions (Amersham). Electronic images were compiled using Adobe Photoshop 3.0.

Staining for β-galactosidase activity. Transfected cells on 35 mm diameter coverslips were fixed with 0.5% glutaraldehyde in PBS for 15 min and stained using X-Gal (Melford Laboratories), the chromogenic substrate for β-galactosidase, at 200 µg/ml in 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, 1:3 mM MgCl₂ at 37 °C for 3 h.

Recombinant plasmids. The plasmid pSG5IE1 contains a cDNA copy of IE1 under the control of the SV40 promoter (Hagemeier et al., 1992) while a series of in-frame deletion mutants of IE1 constructed from pSG5IE1 by means of in vitro mutagenesis have been described previously (Hayhurst et al., 1995). For consistency we have now re-named them as follows: pAL132 has a deletion of exon 3 (AAa 25–85); pAL138 a deletion involving exons 2 and 3 (Δ6–85); pAL134 a deletion in a putative leucine-zipper motif (Δ105–139); pAL136 a deletion of a putative zinc-finger motif (Δ267–286); pAL137 a deletion adjacent to the C terminus (Δ351–420) and pAL135 a deletion which comprises the C-terminal acidic domain (Δ421–486). Additional IE deletion mutants were produced specifically for this study, pAL150 (Δ132–274); pAL151 (Δ118–180) and pAL152 (Δ162–332) have in-frame deletions of fragments within the IE1 gene generated by excising EcoRV, NarI and BstEII fragments respectively from the plasmid pSG5IE1. pAL139, which has a C-terminal truncation of IE1 (Δ347–491), was constructed from pMV31 (Wilkinson & Akigg, 1992) by subcloning a BamHII–BglII fragment under the control of the HCMV major IE promoter. Numbering of sequences is according to Akigg et al. (1985).

pAL184 (described in Results) was constructed by fusing the N terminus of IE1 (from pAL182) with lacZ (from pMV10) using this Sphi–KpnI adaptor to repair IE1 aa 18–24 and maintain the open reading frame into lacZ:

\[ 5'\ CTTCCCTCCAGGGTCTCCAGGGTAC 3' \]
\[ 3'\ CCGGAGAGGTTGTCACCGGTG 5' \]

Results

Association of PML with chromatin

In HCMV-infected cells, both IE1Δ118a and PML are typically evenly dispersed throughout the nucleus, exhibiting a similar granular immunofluorescence staining pattern (Kelly et al., 1995). An unusual feature of IE1Δ118a is its association...
with mitotic chromosomes. This event is only occasionally observed during a productive HCMV infection as the virus arrests the majority of cells in a premitotic phase of the cell cycle (Bresnahan et al., 1996; Jault et al., 1995), but can be readily observed in actively replicating, transfected cells expressing IE1 (Lafemina et al., 1989). In contrast, PML is excluded from chromatin in mitotic cells (Stuurman et al., 1992) not infected with HCMV. The fate of PML in mitotic cells expressing IE1Δ91aa was therefore investigated in an immunofluorescence assay. In the same cells, IE1Δ91aa expression was visualized with a IE1-specific guinea pig polyclonal antibody combined with a Texas red-conjugated second antibody, while PML distribution was visualized using the monoclonal anti-PML antibody, while DNA stained directly with DAPI. In HCMV-infected human fibroblasts it was clear that both IE1Δ91aa and PML were associated with chromatin in cells undergoing mitosis (Fig. 1a–c). The experiment was repeated in CV-1 cells transfected with the major IE gene. The association of both IE1Δ91aa and PML with chromatin was again clear (Fig. 1d–f); thus expression of IE1 alone is sufficient to cause PML to associate with mitotic chromatin.

### Analysis of IE1 deletion mutants

To identify sequences within the IE1 gene involved in the disruption of PML-bodies, a series of IE1 deletion mutants was used in transient DNA transfection experiments. All of the plasmids used are described in Methods and the location of deletions is illustrated in Fig. 2. Plasmids identified here as pAL132, pAL134, pAL135, pAL136, pAL137 and pAL138 have been previously demonstrated to express a truncated IE1 gene product (Hayhurst et al., 1995). An additional series of novel constructs, pAL139(Δ347–491), pAL150(Δ132–274), pAL151(Δ118–180) and pAL152(Δ162–332), was generated for this study. The ability of these novel constructs to promote expression of a truncated IE1 gene product of the appropriate size was assessed. Since human fibroblasts are not transfected efficiently using standard techniques, CV1 cells were used. CV1 cells were transfected with each new construct and total cell extracts prepared for analysis in a Western transfer experiment. The relative size of the major protein species recognized by a monoclonal antibody to IE1 in pAL150-, pAL151- and pAL152-transfected cells was in agreement with the predicted size of the deletion in IE1. However, the size of the IE1 deletion in pAL139 (145 aa) was similar to that in pAL150 (143 aa) and much smaller than that in pAL152 (170 aa), yet it migrates much faster (Fig. 3). Molecular mass estimations of the HCMV major IE protein by SDS–PAGE (~ 72 kDa) are significantly greater than that predicted from the sequence (55,634 Da). The result suggests that the acidic region at the C terminus of the protein may be making a significant contribution to the anomalous migration of IE1Δ91aa on SDS–PAGE.

Each of the IE1 deletion mutants was transfected into cells and assayed for expression of IE1 and PML by immunofluorescence. Representative results for each transfected plasmid are illustrated in Fig. 4, in which the same field of cells is viewed through an appropriate filter to reveal IE1 expression (Texas red-conjugated second antibody) or the distribution of PML (FITC-conjugated second antibody). There was no detectable cross-reactivity between the two antibody detection systems although some background cytoplasmic staining is observed with PML-detection due to a weak reaction of the FITC-conjugated rat anti-mouse monoclonal antibody with a cellular protein.

Transfection of CV-1 with the complete IE gene (pAL133) resulted in expression of IE1 which was invariably associated with the efficient disruption of PML-bodies. Two transfected cells exhibit the characteristic nuclear IE1 expression in Fig. 4(a), while the same field in Fig. 4(b) shows normal distribution of PML in non-transfected cells but a diffuse nuclear PML distribution in the IE1-expressing cells. The results of immunofluorescence experiments indicated that all but one of the truncated IE1 gene products were expressed as a diffuse nuclear protein (Fig. 4e, i, k, m, o, q, s, u, w). The IE1 gene product expressed following pAL138 (Δ6–85) transfection routinely exhibited a cytoplasmic distribution (Fig. 4c). In the vast majority of cells transfected with the plasmids pAL138 (Fig. 4c, d), pAL132 (e, f), pAL134 (g, h), pAL151 (i, j), pAL152 (o, p) and pAL136 (q, r) PML-bodies remained intact, thus indicating that the deletion contained within these constructs effectively disabled the ability of IE to disrupt PML-bodies. The inability of the pAL138 (Δ exon 2/3) gene product to cause disruption of PML-bodies could be due to the protein not being transported to the nucleus. However, the product of pAL132 (Δ exon 3) was transported efficiently to the nucleus and it also was not associated with PML-body disruption (Fig. 4c, f). The plasmid construct pAL150 was interesting in that it resulted in an intermediate phenotype in which an equal proportion of cells expressing the truncated IE gene exhibited intact and disrupted PML-bodies. Fig. 4(k, l) illustrates a pAL150-transfected cell in which PML-bodies remained discernible while Fig. 4(m, n) illustrates a pAL150-transfected cell where the domains were disrupted completely. Even when not disrupted, the pAL150-encoded IE1 protein was preferentially associated with PML-bodies (Fig. 4k, l). The deletion in pAL150 therefore impairs but does not inactivate the capacity of the encoded protein to disrupt PML-bodies.

With the exception of pAL139, which uses the HCMV major IE promoter, expression from all of the plasmid constructs used in Fig. 4 is driven by the SV40 promoter. However, the stability of the truncated IE1 gene products expressed in transfected cells may vary and thus levels of expression achieved may not be consistent. It is possible that an IE deletion mutant assessed here as being unable to disrupt PML-bodies may yet be able to do so if expressed at a high enough level.
An outstanding feature of the transfection experiment was that constructs with deletions restricted to the C terminus of IE1 (pAL135; pAL137; pAL139) were able to disrupt PML-bodies with an efficiency comparable with that of wild-type IE1 (Fig. 4s–x). The association of IE1 with chromatin is dependent on sequences within the acidic C terminus (aa 421–486). The C terminus of IE1 is therefore required for the association with chromatin but is not required for disruption of PML-bodies. The majority of plasmids used in this study (pSG5IE1, pAL132, pAL134, pAL136, pAL137, pAL150, pAL151 and pAL152) expressed a form of IE1 which could be found associated with mitotic chromosomes (not shown).

A nuclear localization signal associated with exon 2

The gene product expressed from pAL138 (Δ6–85) was unexpectedly found to be localized entirely to the cytoplasm (Fig. 4c). Translation of IE1 is initiated within exon 2 which encodes the N-terminal 24 aa of the protein. Interestingly, the IE1 gene product expressed by pAL132, in which only exon 3

Fig. 1 (a, b, d, e). For legend see facing page.
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Fig. 1. IE1 causes PML to associate with chromatin. HFFF were infected with HCMV (m.o.i. 5) for 24 h or CV-1 cells were transfected with plasmid pAL133 encoding IE1. Cells were simultaneously assayed for the expression of IE1 using guinea pig polyclonal sera and for PML using the monoclonal antibody SE10. IE1 expression was detected using a Texas red-conjugated goat anti-guinea pig antibody and PML with an FITC-conjugated rat anti-mouse monoclonal antibody. DNA was stained with DAPI. The same field was photographed through appropriate filters to reveal (a) IE1 expression in HCMV-infected cells, (b) PML distribution in HCMV-infected cells and (c) DNA staining in HCMV-infected cells (× 40 objective). Likewise, the same field of CV-1 cells transfected with pAL133 was photographed through appropriate filters to reveal (d) IE1 expression, (e) PML distribution and (f) DNA staining (× 100 objective).

Fig. 2. Schematic representation of deletions made in the IE1-coding region. The IE1 gene was derived from a cDNA clone (Akrigg et al., 1985) thus eliminating any requirement for splicing; numbering at the top is for the cDNA clone. The SV40 promoter was used for all constructs except pAL139, which uses the HCMV major IE promoter (−299 to +69). The bars indicate the regions which have been deleted from each expression construct and the inset numbers refer to deleted amino acid sequences. The construction of pAL139(Δ347–491), pAL150(Δ132–274), pAL151(Δ118–180), and pAL152(Δ162–332) is described in Methods. The construction of the exon 2/3 [pAL138(Δ6–85)], exon3 [pAL132(Δ25–85)], leucine-zipper motif [LZ; pAL134(Δ105–139)], zinc-finger motif [pAL136(Δ267–286)], aa 350–420 (pAL137) and acidic domain [pAL135(Δ25–85351–420)] deletions in IE1 have been described previously (Hayhurst et al., 1995).
has been deleted (aa 25–85), is efficiently transported to the nucleus (Fig. 4e). Thus, the short sequence (aa 6–24) encoded by exon 2, which is present in pAL132 but absent in pAL138, was predicted to have a role in transport of IE1<sub>1491aa</sub> to the nucleus. To test this prediction, exon 2 coding sequences were fused to the N terminus of β-galactosidase (Fig. 5). Briefly, pAL139 contains a cDNA copy of IE1 (aa 1–347) under the control of the major IE promoter. IE1 coding sequences from aa 18–347 were excised from pAL139 by digestion with Apal/KpnI, with the deletion of aa 18–24 repaired and the KpnI site regenerated by insertion of an adapter (see Methods).

The CMV IE promoter/exon 2 sequences were excised from the resultant plasmid (pAL182) on a SpH1–KpnI fragment and inserted into pMV10, reconstituting the CMV promoter, to generate pAL184. Thus, pMV10 contains the IE promoter driving expression of LacZ, while in pAL184 the N terminus of LacZ is fused in-frame with aa 1–24 of IE1 (Fig. 5).

HFFF cells were transfected with pMV10 and pAL184 and stained for β-galactosidase expression with the chromogenic substrate X-Gal. While the β-galactosidase protein expressed from pMV10 exhibited predominantly cytoplasmic staining (Fig. 6a), the pAL184-encoded protein was preferentially associated with the nucleus (Fig. 6b). The monoclonal antibody for IE1 (1D6-6) specifically recognizes an epitope in exon 2. An immunofluorescence assay using this monoclonal antibody demonstrated directly that the gene product of pAL184 exhibits a predominant nuclear localization (Fig. 6c). It is therefore proposed that the sequence aa 1–24 of IE1 contains a nuclear localization signal.

**Discussion**

HCMV IE1<sub>1491aa</sub>, HSV-1 Vmw110 and Ad E4 ORF3 all share a capacity to affect the nuclear distribution of the PML antigen. Following transient association with PML-bodies, Vmw110 causes PML and Sp100 to be dispersed with a proportion of PML being relocated to the nuclear membrane (Everett & Maul, 1994; Maul & Everett, 1994), and adenovirus infection redistributes PML-body associated proteins into nuclear tracks (Doucas et al., 1996). The effect of HCMV IE1<sub>1491aa</sub> on PML-bodies is distinct resulting in the dispersal of the PML antigen throughout the nucleus and, more remarkably, the association of the PML antigen with mitotic chromosomes. HSV-1 Vmw110 and Ad E4 ORF3 deletion mutants have a similar phenotype in that they replicate efficiently following infection at high m.o.i. but are severely disabled at low m.o.i. A similar phenotype has recently been assigned to an HCMV IE1 exon 4 deletion mutant (Mocarski et al., 1996).

While IE phase gene expression is most commonly associated with regulating transcription from the virus genome during the latent and productive phases of virus infection, recent studies have highlighted their importance in establishing a cellular environment conducive for efficient virus replication. The US3 gene product is believed to enable immune evasion of the infected cell by downregulating surface expression of MHC class I molecules (Ahn et al., 1996), the IE1 and IE2 gene products have been reported to inhibit TNF-mediated apoptosis in HeLa cells (Zhu et al., 1995) and the interaction of IE2<sub>578aa</sub> with Rb (Hagemeier et al., 1994; Jault et al., 1995) together with the interaction of IE1<sub>1491aa</sub> with p107 (Poma et al., 1996) may promote progression of the infected cell towards the G1/S phase of the cell cycle. The functional significance of the interaction of IE1 with PML domains is not yet clear. PML in association with its nuclear domain may constitute an intranuclear barrier to virus replication, a hypothesis supported by the observation that interferon upregulates expression of PML (Chelbi-Alix et al., 1995; Studler et al., 1995). Furthermore, both interferon treatment and overexpression of PML have been shown to inhibit Ad early phase gene expression (Doucas et al., 1996). Insight into the potential nature of this barrier is provided by the observation that the initial sites of SV40, Ad and HSV-1 DNA replication and transcription (Maul et al., 1996; Ishov & Maul, 1996) and HCMV transcription are associated with PML-bodies (Ishov et al., 1997). A function of PML may be to suppress access to cellular factors required by virus to initiate transcription. However, additional cellular proteins are known to be associated with PML-bodies (e.g. Sp100, NDP-55, NDP-52, PIC-1, Int-6 and HAUSP) and it will also be important to investigate further the effect IE1<sub>1491aa</sub> has on other components of this nuclear domain.

A series of deletion mutants in the IE1 gene were analysed in transient DNA transfection experiments. An ‘acidic region’ near the C terminus of IE1 (aa 424–486) was required for the
Fig. 4 (a–h). For legend see page 1242.
Fig. 4 (i–p). For legend see page 1242.
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Fig. 4 (q–x). For legend see next page.
association of IE1491aa with chromatin. A construct with C-terminal truncation extending from aa 347–491 was capable of efficiently disrupting PML-bodies. Consequently, the association of IE1491aa with mitotic chromatin and nuclear dispersal of the PML antigen appear to be encoded by functionally distinct domains of the protein.

All the in-frame deletions of IE1-coding sequences between aa 6–332 that were tested were impaired in their ability to disrupt PML-bodies (Fig. 2). In particular, specific deletions which affected three distinct non-overlapping elements (exon 3, the putative leucine-zipper and the putative zinc-finger domains) disabled IE1 function. It is thus conceivable that more than one element may contribute to the interaction between PML-bodies and IE1491aa. Indeed this appears to be the case with HSV-1 Vmw110 where both a RING finger domain and a C-terminal region of the protein contribute to the interaction with PML-bodies (Maul & Everett, 1994; Merideth et al., 1995). Attempts to identify sequences in IE1 required for its transactivation function have been frustrated and led to the suggestion that the gene was ‘hypersensitive’ to mutation (Stenberg, 1993). Deletions affecting C-terminal proximal amino acids have a relatively small effect on the ability of IE1 to function as a transactivator, whereas the in-frame deletion of exons 2 and 3, the leucine-zipper and the zinc-finger domains were severely impaired (Hayhurst et al., 1995). That a series of deletions spanning aa 6–286 impairs the capacity of IE1 to function both as a transactivator and to disrupt PML-bodies supports the contention that this region of protein may be very sensitive to modifications. However, these two functions of IE1 may yet overlap: one mechanism by which IE1 may conceivably function as a transactivator is by sequestering PML; PML has been reported to be a repressor of both growth and transcription (Liu et al., 1995; Mu et al., 1994).

Sequences coded by exons 2 and 3 are critical for IE1-mediated disruption of PML-bodies but are unable to induce disruption when expressed in the context of IE2 (Maul & Everett, 1994; Merideth et al., 1995; Ishov et al., 1997). In DNA transfection studies, the deletion of exon 2 sequences (aa 6–24) was associated with the expression of a cytosolic form of the IE1 gene product. The short proline-rich sequence within exon 2 has a high surface probability and contains clusters of basic amino acids, features associated with NLS function (Dingwall et al., 1987; Silver, 1991). Two sequences within exon 5 of IE2579aa have been demonstrated to function as NLS sequences and can convert a cytosolic form of the HSV-1 IE175 protein into a nuclear form (Pizzorno et al., 1991). Our result is therefore consistent with the presence of an additional NLS sequence in IE2. Exon 2 may have a role in the transport of both IE1 and IE2 gene products to the nucleus.

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Fig. 6. Identification of a nuclear localization signal. (a) HFFF cells transfected with pMV10 and stained with X-Gal to detect β-galactosidase expression; (b) HFFF cells transfected with pAL184 and stained with X-Gal to detect β-galactosidase expression; (c) HFFF cells transfected with pAL184 with the expression of the N-terminal sequences in the β-galactosidase fusion protein detected in an immunofluorescence assay with a IE1-specific monoclonal antibody (1D6-6).
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