REVIEW

The potential link between PML NBs and ICP0 in regulating lytic and latent infection of HSV-1

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ABSTRACT

Herpes simplex virus type 1 (HSV-1) is a common human pathogen causing cold sores and even more serious diseases. It can establish a latent stage in sensory ganglia after primary epithelial infections, and reactivate in response to stress or sunlight. Previous studies have demonstrated that viral immediate-early protein ICP0 plays a key role in regulating the balance between lytic and latent infection. Recently, It has been determined that promyelocytic leukemia (PML) nuclear bodies (NBs), small nuclear sub-structures, contribute to the repression of HSV-1 infection in the absence of functional ICP0. In this review, we discuss the fundamentals of the interaction between ICP0 and PML NBs, suggesting a potential link between PML NBs and ICP0 in regulating lytic and latent infection of HSV-1.

INTRODUCTION

Herpes simplex virus type 1 (HSV-1), the archetypal member of the alphaherpes virus subfamily, is a large, nuclear-replicating, dsDNA virus. HSV-1 is a human neurotropic virus and infects mucous membranes causing cold sores. Following primary infection in epithelial cells, HSV-1 establishes lifelong latent infection in sensory neurons of the trigeminal ganglia by getting access to nerve terminals, leading to periodic reactivation and lytic replication at the site of original infection. Latency is such a status when viral genome adopts a non-linear configuration and is transcriptionally repressed with the exception of a region encoding the latency-associated transcripts (LATs). Provoked by stress

stimuli that act on the neuron, the latent virus reactivates and switches to a productive viral replication, allowing the virus to spread. Although there are large numbers of literatures related to HSV-1 latency and reactivation, the mechanisms by which viral genomes are maintained in a repressed state during latency and the processes involved in reactivation from latency are still not fully understood (Mitchell et al., 2003; Shimomura, 2008).

Previous studies have demonstrated that viral immediate-early protein ICP0, a promiscuous activator of gene expression, is responsible for efficient entry into lytic cycle and can induce reactivation of latent or quiescent viral genomes, which have led to the suggestion that it plays a key role in regulating the balance between lytic and latent HSV-1 infection (Everett, 2000; Preston, 2000; Halford et al., 2001; Everett et al., 2004a; Hagglund and Roizman, 2004).

It is proposed that in the absence of functional ICP0, a promyelocytic leukemia (PML) nuclear bodies (NBs) related cellular repression mechanism silences viral transcription. ICP0 seems to counteract this process by stimulating the degradation of a number of cellular proteins via the ubiquitin-proteasome pathway (Table 1) (Boutell et al., 2002;

Table 1   Client proteins degraded by ICP0

Protein	Reference
PML	Chelbi-Alix and de Thé, 1999
Sp100	Chelbi-Alix and de Thé, 1999
ATRX	Everett et al., 2007
hDaxx	Everett et al., 2007
E2FBP1	Fukuyo et al., 2011
Centromeric proteins CENP-A	Lomonte et al., 2001
CENP-B	Lomonte et al., 2007
CENP-C	Everett et al., 1999
Catalytic subunit of DNA-PK	Lees-Miller et al., 1996

Everett et al., 2006; Everett et al., 2008a).

LYTIC AND LATENT INFECTION OF HSV-1

Evolutionary pressures by viral infection ensure that the host develops defense systems to eliminate virus, while viruses have adopted many strategies to outmaneuvre the host's immune and other anti-viral systems while maintaining their long term survival potential. HSV-1 has successfully developed a particularly exquisite form of accommodation within the host. Animal experiments revealed that the virus gains access to the termini of sensory neurons during primary infection in epithelial cells, and is transported by retrograde axonal transport to the sensory ganglia, where it replicates briefly and then establishes a latent infection (Mitchell et al., 2003; Shimomura, 2008) .

Lytic HSV-1 infection involves abundant transcription from the entire viral genome in a regulated cascade of immediate-early (IE), early, and late gene products (Lehman and Boehmer, 1999). The IE gene products regulate the expression of later classes of viral genes. During latent cycle, the transcription of HSV-1 genome is repressed, and only LATs, which derive from a single locus that lies countersense to the IE gene encoding ICP0, are expressed in readily detectable amounts (Wagner and Bloom, 1997; Preston, 2000; Efstathiou and Preston, 2005). HSV-1 thus establishes a life-long infection in a form that is not accessible to anti-viral defense. Periodically, episodes of lytic infection occur as a result of reactivation.

HSV-1 latency is therefore a key component of its life cycle, and as such the underlying mechanisms have been a subject of considerable interest. In particular, the regulation of viral gene expression during various stages of lytic and latent infection has been a very active and productive field of research (Wagner and Bloom, 1997).

ICP0, a key activator of HSV-1 gene expression

During successional steps of HSV-1 lytic cycle gene expression, the IE genes are the first to be transcribed, and their products are required for the activation of later classes of promoters. The main viral transactivators required for the expression of the HSV-1 genes are the IE proteins ICP0, ICP4 and the late protein VP16 (Table 2).

VP16, a component of the HSV tegument which is released into the cell following fusion of viral envelope, binds to cellular factors HCF and Oct-1, initiating the whole genetic program by activating the expression of the IE genes (Mahajan et al., 2002; Wysocka and Herr, 2003).

ICP4, a factor exerting its transcriptional activity by binding specifically or nonspecifically to DNA (Smith et al., 1993), is absolutely required for the transactivation of the HSV-1 early and late genes (Watson and Clements, 1980; DeLuca et al., 1985).

ICP0, which appears to act upstream of other IE proteins, is a crucial regulator of the three classes of HSV genes and has also been shown to be able to transactivate several other heterologous promoters in transfection reporter assays. It had been established earlier that ICP0 and ICP4 produce a synergistic transactivation of those promoters, greatly exceeding that in the presence of either protein alone. ICP0 reactivates quiescent viral genomes and stimulates initiation of lytic infection by transactivating a broad range of viral and cellular promoters, suggesting crucial roles of ICP0 in different stages of HSV-1 infection (Preston and Nicholl, 1997; Samaniego et al., 1998).

Table 2 Expression time course and function of ICP0

	Expression time course	Function	Reference
ICP0	Immediate early	Activate later classes of promoters and enhance viral transcription	Cai and Schaffer, 1992
		Required for reactivation from latency	Halford and Schaffer, 2001
		E3 ubiquitin ligase activity, and degrade cellular proteins by ubiquitin-proteasome pathway	Boutell et al., 2002
		Disrupt PML NBs	Maul et al., 1993
		Relocate class II histone deacetylase	Lomonte et al., 2004
		Prevent the accumulation of histones, repress histone modifications	Ferenczy and DeLuca, 2011
		Counteract type I interferon (IFN) response	Mossman and Smiley, 2002
ICP4	Immediate early	Transactivation	Watson and Clements, 1980
		Transcription repression	Paterson and Everett, 1988
		Initiate viral DNA replication	Aslani et al., 2000
VP16	Late	Recruit general transcription factors, RNA polymerase II, histone acetyltransferases (HATs), and ATP dependent chromatin remodeling complexes to IE promoters, stimulate immediate-early gene expression	Harris and Preston, 1991; Xiao et al., 1994; Klemm et al., 1995; Memedula and Belmont, 2003; Mittler et al., 2003; Herrera and Triezenberg, 2004; von Einem et al., 2006
		Virions assembly	von Einem et al., 2006

The function of ICP0 has been extensively studied, and an increasingly detailed picture of its interactions with cellular proteins and its biochemical functions is emerging (Kawaguchi et al., 1997a, 1997b; Everett et al., 1998; Everett et al., 1997; Parkinson and Everett, 2000; Kawaguchi et al., 2001; Van Sant et al., 2001; Boutell et al., 2002; Boutell and Everett, 2003; Gu and Roizman, 2003; Jackson and DeLuca, 2003). Direct transcriptional activators either bind to specific response elements in target promoters or interact with host transcription factors that form part of the basal transcriptional machinery or associated activator complexes. Whereas ICP0 itself does not bind DNA directly, it seems that ICP0 acts as a transactivator of viral genome and a specific subset of cellular genes through interaction with some cellular proteins. Several studies reported that ICP0 interacts with various cellular factors, including cyclin D3, elongation factor EF-1σ, transcription factor BMAL1, ubiquitin-specific protease HAUSP (Everett et al., 1997; Kawaguchi et al., 1997a, 1997b) and also specific nuclear structures known as PML NBs (Gu and Roizman, 2003; Lukashchuk and Everett, 2010).

Furthermore, one of the key roles of ICP0 relates to its expression of two E3 ubiquitin ligase activities located in exons 2 and 3 (Van Sant et al., 2001; Boutell et al., 2002; Hagglund and Roizman, 2002; Hagglund et al., 2002). The ubiquitin ligase activity present in exon 2 is associated with a RING finger domain and is responsible for the proteasome-mediated degradation of cellular proteins, including major proteins associated with PML NBs such as PML and speckled protein of 100 kDa (Sp100), centromeric proteins CENP-A and CENP-C, and the catalytic subunit of DNA-dependent protein kinase (Everett et al., 1998a; Everett et al., 1999a; Lomonte et al., 2001; Gu and Roizman, 2003). The second ubiquitin ligase activity identified in exon 3 is responsible for the degradation of the E2 ubiquitin-conjugating enzyme cdc34 (Van Sant et al., 2001; Hagglund and Roizman, 2003). The present hypothesis to explain the wide transactivating or derepressing activities of ICP0, as well as its implication in the establishment of lytic replication, is the ability of ICP0 to alter the higher-order structure of chromatin by targeting a repressive factor for degradation (Everett, 2000) (Table 1).

PML NBs, small nuclear sub-structures with multifunction

PML NBs, also known as nuclear dot 10 (ND10) or PODs (PML oncogenic domains), are small nuclear sub-structures with a striking punctate appearance. The size of PML NBs is between 0.2 and 1 μm and its quantity is from 2–3 to 30 per cell, depending on the cell type and status. PML NBs are dynamic macromolecular inclusions of cellular proteins that form within the interchromosomal space in the nucleus (Sternsdorf et al., 1997a; Everett et al., 1999b; Dellaire et al., 2006), functioning in oncogenesis, the DNA damage re-

sponse (Dellaire and Bazett-Jones, 2004), the stress response, apoptosis (Bernardi and Pandolfi, 2003; Gresko et al., 2009), senescence (Bischof et al., 2002), the ubiquitin pathway (Antón et al., 1999; Lallemand-Breitenbach et al., 2001), various genetic disorders, viral infection and the interferon (IFN) response (Regad et al., 2001).

An increasing list of cellular proteins has been proven to accumulate at PML NBs; those proteins can be divided into two categories, one is permanent components, such as PML, Sp100, the death domain-associated protein (Daxx), small ubiquitin like modifier (SUMO), and the bloom syndrome helicase (BLM), and the other category is proteins that present in PML NBs only under specific circumstances (e.g. DNA repair machinery) or during overexpression (e.g. breast cancer protein BRCA1) (Negorev and Maul, 2001; Tavalai et al., 2008).

PML (also known as TRIM19), a tripartite motif family protein, is the key component of PML NBs and required for assembly of these structures (Ishov et al., 1999; Zhong et al., 2000). Posttranslational modification of PML by covalent conjugation to SUMO at K65, 160, 490, named SUMOylation, is required for proper formation of PML NBs and recruitment of PML NB-associated proteins (Sternsdorf et al., 1997b; Kamitani et al., 1998a, 1998b; Müller et al., 1998; Duprez et al., 1999; Ishov et al., 1999; Zhong et al., 2000; Seeler and Dejean, 2001; Shen et al., 2006). Sp100, another permanent component of PML NBs, is also found to be conjugated to SUMO; however, SUMOylation of Sp100 is not necessary for its localization to PML NBs (Sternsdorf et al., 1997b; Sternsdorf et al., 1999).

PML NBs confer intrinsic resistance to viral infection

PML NBs display intrinsic antiviral properties, targeting both DNA viruses and cytoplasmic replicating RNA viruses (Regad et al., 2001; McNally et al., 2008; Tavalai et al., 2008). Unlike cytokine-mediated responses, intrinsic antiviral resistance involves the actions of pre-existing cellular proteins to repress viral transcription (Saffert and Kalejta, 2008; Tavalai et al., 2008). PML NBs have been demonstrated to repress the replication of HSV-1 shortly after virus entry by mechanisms that limit early viral gene transcription, although the process is counteracted by ICP0 (Everett et al., 2006; Everett et al., 2007). Early studies also show that exogenous expression of PML isoform VI causes significant reduction of adenovirus (Doucas et al., 1996) and human cytomegalovirus (HCMV) infection (Ahn and Hayward, 2000), while depleting PML enhances varicella-zoster virus (VZV) and HCMV replication (Tavalai et al., 2006; Tavalai et al., 2008; Kyratsous and Silverstein, 2009). Daxx, another permanent component of PML NBs, was reported to be involved in transcriptional regulation. Knocking down Daxx results in increased adenoviral and HCMV replication in cells (Tavalai et al., 2008;

Schreiner et al., 2010).

PML deficiency renders mice more susceptible to some viral infection, including lymphocytic choriomeningitis virus, vesicular stomatitis virus (VSV) (Bonilla et al., 2002), encephalomyocarditis virus (El McHichi et al., 2010), resulting in an increased viral replication. Mouse embryonic fibroblasts (MEFs) derived from these mice are also more sensitive than wild type MEFs to rabies virus infection, while a high level of PML isoform IV leads to a reduction in rabies virus replication (Blondel et al., 2002). SUMOylation of PML IV is required for the antiviral effect (Blondel et al., 2010). Knocking down all PML isoforms by siRNA also significantly enhanced the propagation of influenza A virus strains PR8(H1N1), ST364(H3N2) (Li et al., 2009) and influenza virus in cells (Iki et al., 2005). Exogenous expression of PML III can also confer resistance to human foamy virus (HFV) (Rolley et al., 1995), VSV, influenza virus (Chelbi-Alix et al., 1998; Regad et al., 2001) and poliovirus infection (Pampin et al., 2006).

Although multiple PML isoforms are reported due to alternative splice of transcript, and most of isoforms localize in PML NBs, a subset of PML isoforms, lacking exons 5 & 6, named PML Ib, exist in cytoplasm and is enriched during HSV-1 infection. PML Ib is demonstrated to sequestrate ICP0 and mediate the intrinsic cellular defense against HSV-1 (McNally et al., 2008). Three of Sp100 isoforms could prevent the transcription of HSV-1 proteins ICP0 and ICP4 at the promoter level, and IFN could change the splice of the Sp100 mRNA in favor of the inhibitor Sp100C (Negorev et al., 2009).

Association of PML NBs with the viral genomes contributes to intrinsic resistance

PML NBs have been implicated to inhibit the replication of adenovirus (Doucas et al., 1996) and limit early viral gene transcription of HSV-1 and HCMV shortly after virus entry (Everett et al., 2006; Tavalai et al., 2006; Everett et al., 2007). A number of studies show that PML NBs associate with the genome of several DNA viruses. Maul et al., for the first time, observed that the parental genomes of HSV-1, HCMV and adenovirus are associated with PML NBs (Ishov and Maul, 1996; Maul et al., 1996). Via examining newly-infected cells at the edges of developing virus plaques, PML NB component proteins were found to accumulate in PML NB like structures that are closely associated with the viral genomes, which only just enter the nucleus. PML and other PML NBs components were recruited to PML NB like structures during initial stages of infection, rather than the migration of pre-existing PML NBs (Wiesmeijer et al., 2002; Everett et al., 2004a; Everett and Murray, 2005). The process occurs extremely rapidly and does not depend on expression of viral protein, implying an intrinsic antiviral response to viral genome entry (Everett and Murray, 2005). The SUMO interaction motifs of PML, Sp100 and hDaxx are necessary for recruitment of these repressive proteins to HSV-1 genomes

(Cuchet-Lourenço et al., 2011).

The infection of mutant HSV-1 ICP0, ICP4 and/or VP16 causes quiescent infection which is stable and similar to latent infection. However, quiescent infection could be reversed only by provision of herpes viral proteins such as ICP0 and not by alteration of cell physiological state. By establishing quiescent infection of mutant HSV-1 which inactivates ICP0, ICP4 and VP16, Everett et al. found that quiescent HSV-1 genomes in human fibroblast nucleus are associated with enlarged PML NB like structures, and the foci viral genomes were apparently enveloped within a sphere of PML and other ND10 proteins (Everett et al., 2007). During the initial stages of establishment of a quiescent infection in such cells, other ND10 proteins such as Sp100, hDaxx, and alpha thalassemia/mental retardation syndrome X-linked (ATRX) were recruited into PML NB like structures which associated with HSV-1 genomes. Accumulations of conjugated ubiquitin were also observed in PML NB like structures. Viral gene expression can be reactivated by superinfection with a virus that expresses ICP0. PML deficiency decreases the repression of ICP0-null mutant HSV-1(Everett and Chelbi-Alix, 2007) (Fig. 1).

Recently, PML NBs are reported to sequester newly assembled VZV nucleocapsids in neurons and satellite cells of human dorsal root ganglia and skin cells infected with VZV *in vivo*. PML fibers of those PML NBs form spherical cages that enclose mature and immature VZV nucleocapsids. Only PML IV could enhance the sequestration of nucleocapsids by interacting with VZV capsid surface protein, and significantly inhibit the viral infection by inhibiting nuclear egress and formation of infectious virus particles (Reichelt et al., 2011). Therefore PML cages were suggested to play a crucial role in the intrinsic antiviral defense, and the efficient sequestration of virion capsids in PML cages appears to be a basic cytoprotective function of PML NBs (Reichelt et al., 2011).

These observations illustrate a crucial role of PML NBs in the intrinsic antiviral defense and the potential link between PML NBs and quiescent infections. Recruitment of PML NBs components to sites associated with HSV-1 genomes and VZV nucleocapsids contributes to the intrinsic defense against invading viral genome (Everett et al., 2007; Cuchet-Lourenço et al., 2011; Reichelt et al., 2011), which suggests a crucial role of PML NBs in resistance to viral infection. However, the association of PML NBs with viral genome is complex in nature and requires further study. For example, Epstein-Barr virus genomes in latently infected cells do not appear to be associated with PML NBs (Bell et al., 2000).

Genomes of adenoviruses, simian virus 40, papillomaviruses and polyomavirus were also shown to be associated with PML NBs. After entrance into the nucleus, parental genomes of those viruses move to the sites of pre-existing PML NBs, and the replication compartments of all these viruses become associated with PML NB like structures when viral
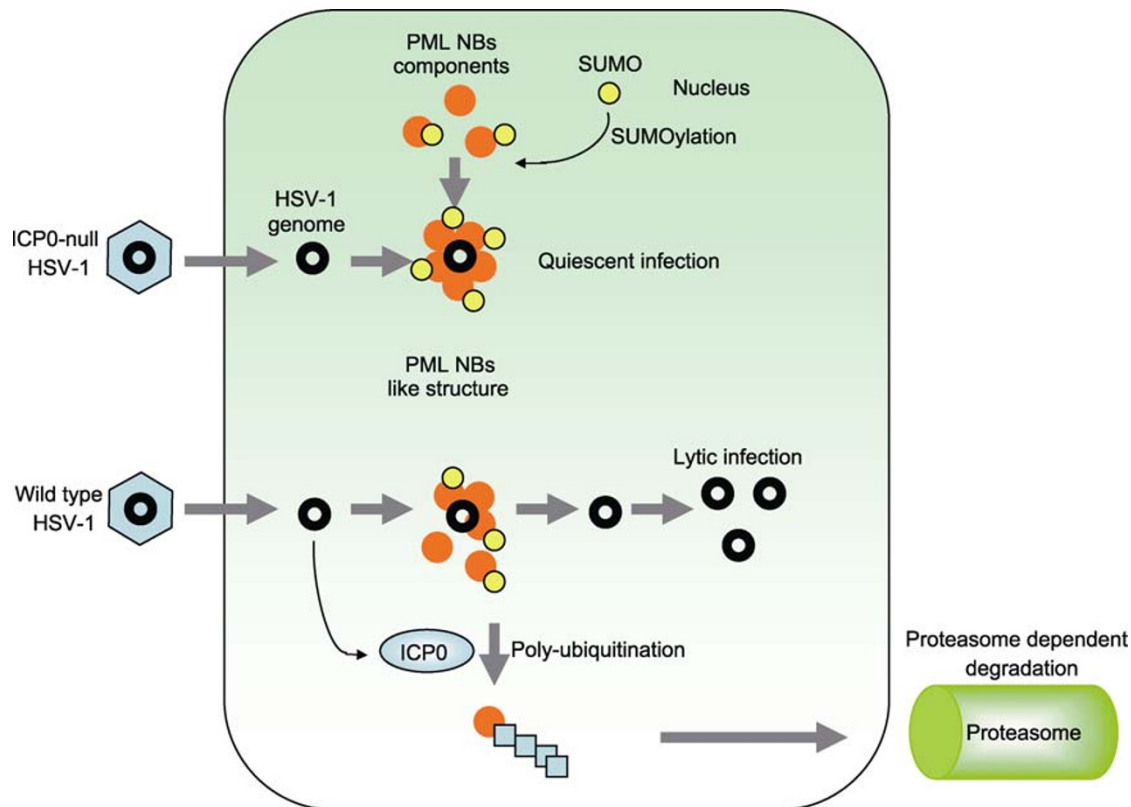
Figure 1.   Association of PML NBs components with HSV-1 genomes contributes to intrinsic resistance. A sphere of PML NBs components in human fibroblast nucleus forms enlarged PML NBs like structures enveloping the HSV-1 genomes, and leading to the repression of HSV-1 genomes. The inhibition is counteracted by proteasome dependent degradation of PML NBs mediated by ICP0.

DNA replication begins (Doucas et al., 1996; Ishov et al., 1997; Maul, 1998; Everett, 2001; Fraefel et al., 2004; Jul-Larsen et al., 2004; Smith and Helenius, 2004).

ICP0 targets PML NBs

The herpes viruses encode regulatory proteins that localize to and in many cases disrupt PML NB structure; the disruption of PML NBs by herpes virus regulatory proteins correlates with their functions in augmenting viral gene expression. Early studies have shown that ICP0 localized in the nucleus at the onset of infection, with a punctate staining pattern in a diffuse background. It is also suggested that these ICP0 foci corresponded to pre-existing cellular nuclear sub-structures PML NBs and that as infection progressed, ICP0 had the amazing effect of completely disrupting them (Maul et al., 1993; Everett and Maul, 1994; Maul and Everett, 1994). Since PML staining disappears after HSV infection (Maul et al., 1993), it demonstrates that virus replication affects PML NBs.

The localization of ICP0 to PML NBs is observed within a few hours post infection, followed by complete disruption of these structures, a process that requires both its RING finger domains conferring ICP0 the ubiquitin E3 ligase activity. The

C-terminal region of ICP0 is required for efficient localization of ICP0 to PML NBs (Everett and Maul, 1994; Maul and Everett, 1994). The degradation of PML and the SUMO-modified isoforms of Sp100 is mediated by ICP0 in a proteasome-dependent manner (Boutell et al., 2002). Therefore, it is partly explained why exogenous expression of PML III or PML VI could not inhibit HSV-1 infection due to the proteasome dependent degradation of PML mediated by ICP0 (Chelbi-Alix and de Thé, 1999; Lopez et al., 2002). This process closely correlates with the role of ICP0 in stimulating HSV-1 gene expression and lytic HSV-1 infection (Everett, 1988, 1989, 2000; Everett and Maul, 1994; Meredith et al., 1995; Hagglund and Roizman, 2004). It is reasonable to conclude that there is an intimate link between the ability of ICP0 to interact with and disrupt PML NBs, and the regulation of latent and lytic infection by ICP0 was in some way connected.

PML/Sp100-related repression mechanism in the absence of ICP0

To further investigate the relationship between ICP0 and PML NBs, ICP0-null mutant viruses were constructed (Stow and Stow, 1986; Sacks and Schaffer, 1987). It was found then

that in the absence of ICP0, the virus was still replication competitive but grew poorly and also reactivated from latency at much lower levels than the wild type virus when the multiplicity of infection (MOI) was low (Stow and Stow, 1986; Sacks and Schaffer 1987; Leib et al., 1989; Cai et al., 1993). Virus mutants lacking the ICP0 gene have increased particle-to-PFU ratios, substantially lower yields, and decreased levels of gene expression and thus decreased probability of initiating lytic infection. ICP0-null mutant HSV-1 exhibits a greatly reduced plaque forming efficiency, but this defect is partially reversed in cells depleted of PML, Sp100, hDaxx or ATRX (Everett et al., 2006; Everett et al., 2008a).

Provision of exogenous ICP0 allows reactivation of the quiescent genomes and entry into a normal productive cycle (Preston, 2000), strengthening the hypothesis that ICP0 might be involved in the control of the balance between lytic and latent infection, such that in its absence the latent state is favored (Samaniego et al., 1998). The extent of this multiplicity dependent defect was cell-type dependent and can be overcome by cell cycle status (Cai and Schaffer, 1991; Yao and Schaffer, 1995), being particularly marked in limited-passage human fibroblasts, while less severe in BHK and Vero cells (Everett et al., 2004a) and negligible in osteosarcoma cell lines such as U2OS (Yao and Schaffer, 1995). Thus the defect could not be simply explained by damaged or faulty virus particles since apparently defective virus in one cell type was infectious in another (Yao and Schaffer, 1995). Furthermore, once lytic infection had been initiated at high MOI, the mutant viruses produced normal levels of viral transcripts, proteins and progeny particles (Everett, 1989). However, using an equivalent number of viral particles could reduce the proportion of successfully infected cells by as much as 10,000 fold. It seemed that the ICP0 mutant results in low probability of initiating lytic infection, and once the lytic infection had progressed beyond the early stages, it proceeded normally (Everett, 1989).

Cells have intrinsic defenses against virus infection, acting before the innate or the adaptive immune response. Pre-existing antiviral proteins such as PML, Sp100, and Daxx are stored in specific PML NBs. The antagonistic relationship between ICP0 and components of PML NBs has implied the existence of certain link. During HSV-1 lytic infection, the viral regulatory protein ICP0 localizes to PML NBs and induces the degradation of PML, thereby disrupting PML NBS and dispersing their constituent proteins whereas HSV mutants that fail to express ICP0 are defective in their abilities to modify and degrade PML NBs components (Maul et al., 1993), leading to the consequence of latent infection.

The strong correlation between the effects of ICP0 on PML NBs and its requirement for lytic virus infection prompted the hypothesis that PML NBs might have a repressive effect on HSV-1 gene expression and thereby constitute an intrinsic antiviral defense. ICP0-null mutant viruses are defective in PML degradation and PML NBs disruption, and concomi-

tantly they initiate productive infection very inefficiently. The evidence that depletion of PML from human fibroblasts increases the probability of plaque formation and enhances gene expression of ICP0-null mutant HSV-1 (Everett et al., 2006) further supported the possibility that PML NBs structures have a repressive effect on viral infection, and viral regulatory protein ICP0 that disrupts these structures does so to relieve this repression. Parallel studies using HCMV add weight to the hypothesis that PML and PML NBs contribute to an intrinsic cellular defense that represses herpes virus gene expression, which is countered by the activity of ICP0 during lytic infection (Everett, 2006; Everett and Chelbi-Alix, 2007). However, it is clear that PML cannot be the sole cellular factor involved in HSV-1 genome repression because the enhancement of ICP0-null mutant replication in PML-depleted cells is modest compared to that expected if repression were completely lifted (Everett et al., 2006).

Sp100, yet another major PML NBs component, has been implicated in repression of HSV-1 gene expression (Negorev et al., 2006) and in the regulation of Epstein-Barr virus transcription (Ling et al., 2005). It is confirmed that Sp100 is also involved in HSV-1 genome repression in the absence of ICP0 in human fibroblasts by the observation that depletion of Sp100 results in a similar increase in ICP0-null mutant gene expression (Everett et al., 2008a).

Furthermore, depletion of PML or Sp100 from human fibroblasts modestly enhances ICP0-null mutant HSV-1 infection and gene expression. Deficiency of both proteins complements the mutant virus to a greater degree, but does not restore the plaque formation to wild type HSV-1 levels (Everett et al., 2006, 2008a). Those observations can be explained as both PML and Sp100 contribute to a cellular mechanism of HSV-1 genome repression; meanwhile, additional factors must be required for the remaining repression of ICP0-null mutant HSV-1 genomes that occurs in cells depleted of both PML and Sp100. Recently, Lukashchuk et al. revealed that knock down ATRX or Daxx by RNAi increases both gene expression and ICP0-null mutant HSV-1 and plaque formation. ATRX and Daxx act as a complex functioning in intrinsic antiviral resistance to HSV-1 infection. This process is also counteracted by ICP0 (Lukashchuk and Everett, 2010).

Recently, E2FBP1/hDril1, an AT-rich interaction domain family protein, is reported to regulate the activation of PML NBs. E2FBP1 is capable of disintegrating PML NBs by specific deSumoylation of PML (Fukuyo et al., 2004). E2FBP1 could repress ICP0 expression at the level of transcription, inhibiting accumulation of ICP0 RNA; however, this process is counteracted by ICP0 via polyubiquitylation of E2FBP1 mediated by RING/HUL-2 (herpes ubiquitin ligase 2) domain of ICP0. E2FBP1 interacts with ICP0 *in vivo*, and affects ICP0's nuclear distribution (Fukuyo et al., 2011). Those observations suggest that E2FBP1 functions as an intrinsic cellular defense factor in spite of its PML NB dissociation

function, also outmaneuvered by ICP0.

A logical explanation for this phenomenon that HSV-1 originates the lytic infection from latent state in virtue of ICP0 may lie in resisting the host suicide or self-protection system, because cell death is detrimental for viral proliferation. On the one hand, ICP0 inhibits the breakage of PML NBs structure by E2FBP1 to repress the premature senescence; on the other hand, ICP0 could mediate the dissociation of PML NBs itself in HSV-1 infected cells. The direct or indirect relationship between ICP0 and PML NBs may be out of HSV-1 life-cycle demand. The key point for ICP0 to regulate the PML NBs function is exactly mediated during various HSV-1 life periods.

The orthologues of ICP0 expressed by other alphaherpes viruses have similar biological functions and can disrupt PML NBs, including BICP0 of bovine herpes virus 1, Eg63 of equine herpes virus 1, ORF61 protein in VZV and EP0 in pseudorabies virus (PRV) (Parkinson and Everett, 2000).

In a word, all the previous studies and observations establish the following inferences: (1) Components of PML NBs contribute to intrinsic anti-HSV defense mechanism; (2) E3 ubiquitin ligase activity of ICP0 counterbalances the cellular repression by proteasomal degradation of PML NBs, promoting viral gene expression and switching into lytic cycle; (3) Cells infected with ICP0-null mutant viruses fail to degrade repressive factors, resulting in quiescent status of viral genomes, which is the characteristic of latency; (4) The evidence that depletion of PML enhances gene expression of ICP0-null mutant HSV-1 demonstrates that the link between PML NBs and ICP0 in regulating lytic and latent infection of HSV-1 does exactly exist.

CONCLUSIONS

The observations that HSV-1 mutants, failing to express the viral immediate-early protein ICP0, have a pronounced defect in viral gene expression and efficient progression of infected cells into lytic infection, especially at low MOI, have led to the inference that ICP0 produces a marked effect on the regulation of the balance between lytic and latent HSV-1 infection.

ICP0, acting as a wide-spectrum transactivator of gene expression and the ubiquitin E3 ligase activity conferred by the RING finger domain, is one of the most prominent activities, with the ability to localize to and disrupt discrete nuclear structures known as PML NBs or ND10. This disruption occurs through ICP0-induced degradation of PML. HSV-1 mutants that fail to express ICP0 or that express mutant ICP0 proteins that lack RING finger activity are unable to disrupt PML NBs or to degrade PML. Such mutants have a profound defect in HSV-1 gene expression after infection of limited-passage human fibroblasts. With the data above, a hypothesis that degradation of PML NBs plays a key role in progressing infected cells into productive infection arose, to which the accumulating evidence that several PML NBs pro-

teins, including PML and Sp100, are involved in the repression or regulation of viral gene expression lends support. All together, we conclude that there is a potential link between PML and Sp100—contributing to repression of HSV-1 gene expression and the functions of ICP0 in regulating lytic and latent infection. Understanding the mechanisms by which ICP0 represses intrinsic resistance will provide new insight into the pivotal role of ICP0 during HSV-1 infection, making ICP0 an attractive target for designing antiviral drug to prevent HSV-1 diseases.

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ABBREVIATIONS

BLM, the Bloom syndrome helicase; Daxx, the death domain-associated protein; HCMV, human cytomegalovirus; HFV, human foamy virus; HSV-1, herpes simplex virus type 1; IE, immediate-early; LATs, latency-associated transcripts; MEFs, mouse embryonic fibroblasts; MOI, multiplicity of infection; PML NBs, promyelocytic leukemia (PML) nuclear bodies (NBs); PRV, pseudorabies virus; SUMO, small ubiquitin-like modifier; VSV, vesicular stomatitis virus; VZV, varicella-zoster virus

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