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Role of Promyelocytic Leukemia Protein in Host Antiviral Defense

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Several pathways have been implicated in the establishment of antiviral state in response to interferon (IFN), one of which implicates the promyelocytic leukemia (PML) protein. The *PML* gene has been discovered 20 years ago and has led to new insights into oncogenesis, apoptosis, cell senescence, and antiviral defense. *PML* is induced by IFN, leading to a marked increase of expression of PML isoforms and the number of PML nuclear bodies (NBs). PML is the organizer of the NBs that contains at least 2 permanent NB-associated proteins, the IFN-stimulated gene product Speckled protein of 100 kDa (Sp100) and death-associated dead protein (Daxx), as well as numerous other transient proteins recruited in these structures in response to different stimuli. Accumulating reports have implicated PML in host antiviral defense and revealed various strategies developed by viruses to disrupt PML NBs. This review will focus on the regulation of PML and the implication of PML NBs in conferring resistance to DNA and RNA viruses. The role of PML in mediating an IFN-induced antiviral state will also be discussed.

Introduction

The establishment of an antiviral state in cells is the defining property of interferons (IFNs) as well as the property that permitted their discovery. IFNs act on target cells to confer resistance to viral infection at many stages of viral replication, including entry, transcription, RNA stability, initiation of translation, maturation, assembly, and release. IFNs are also recognized as key regulators of cell growth, apoptosis, and immune response. All activities of IFNs are believed to be mediated by IFN-upregulated cellular proteins. Promyelocytic leukemia (PML) protein and several other proteins such as dsRNA-dependent protein kinase, 2′5′ oligoadenylate synthetase, Mx proteins, and Viperin (see the reviews in this issue) are effectors of IFN action and display intrinsic antiviral activities. PML has been discovered 20 years ago and has been shown later to be induced by IFNs. Accumulating reports implicate PML in host antiviral defense and reveal various strategies developed by viruses to alter PML expression and/or localization within the nuclear bodies (NBs). In this review, we will focus on the regulation of PML at transcriptional and post-translational levels and the capacity of PML and permanent NB-associated proteins Speckled protein of 100 kDa (Sp100) and death-associated dead protein (Daxx) to confer resistance to different DNA and RNA viruses. Their roles in mediating an IFN-induced antiviral state against these viruses will also be discussed.

Discovery of PML

The *PML* gene was originally identified in acute promyelocytic leukemia (APL), where it is fused to the RARα gene as a result of the t(15;17) chromosomal translocation (de The and others 1990; Kakizuka and others 1991). In over 98% cases of APL, this leads to the formation of a fusion protein, PML-RARα, which blocks differentiation of hematopoietic progenitor cells. In normal cells, PML forms discrete nuclear dots named PML NBs, which are dispersed in a large number of microspeckles in APL cells due to expression of PML-RARα (Dyck and others 1994; Weis and others 1994). Treatment of APL patients with *all-trans* retinoic acid (ATRA) or arsenic trioxide (As_2O_3), 2 agents that reverse the disease phenotype, promotes PML-RARα degradation, leading to PML NB reformation (Zhu and others 1997, 1999). Disruption of PML NBs is believed to play a key role in the development of APL, which is consistent with the fact that the PML protein has growth- and transformation-suppressing properties (Mu and others 1994; Bernardi and others 2008). Since the discovery of PML in APL, numerous studies have been conducted to link PML and PML NBs with various cellular functions, including DNA damage, senescence, apoptosis, protein degradation, or antiviral defense.

PML is the organizer of NBs, which are small nuclear substructures that exist in almost all mammalian cells (Ishov and others 1999). They have a striking punctuate appearance when examined by immunofluorescence microscopy and appear as empty spheres with an electron-light core by electron microscopy (Puvion-Dutilleul and others 1995). These structures are also known as nuclear domain 10, PML oncogenic domains or Kr (for Krüppel) bodies. PML oncogenic domains, Kr bodies, and nuclear domain 10 are inappropriate names since they do not take into consideration that PML is required for the formation of these structures and is not

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associated with oncogenic functions. The PML NBs range in size between 0.2 and 1 μm and in number between 1 and 30 bodies per cell. The composition of PML NBs can change during the cell cycle, and indeed PML NBs undergo dramatic rearrangement during mitosis (Everett and others 1999).

Structure and Isoforms of PML

PML, also known as TRIM19, is a member of the tripartite motif (TRIM) family. PML contains a really interesting new gene (RING) domain followed by two B-boxes and a α-helical coiled-coil domain that defines the characteristic RING-B-boxes-coiled-coil (RBCC)/TRIM motif (Jensen and others 2001) (Fig. 1). The RING domain (C4HC3) contains a regular arrangement of cysteine and histidine residues that coordinates 2 zinc atoms in a cross brace structure. The RING is found to associate with UBC9, the small ubiquitin modifier (SUMO)-E2-conjugating enzyme, suggesting that PML could be involved in SUMO modification (Duprez and others 1999). The 2 B-boxes are also zinc-binding motifs involved in protein–protein interactions and the coiled-coil region is required for PML multimerization and heterodimerization with PML-RARα. The integrity of the RBCC motif is required for the localization of PML within the NBs. Further, the RBCC motif contains important residues for PML regulation, including 2 lysine residues (K65 and K160) located in the RING and the B-box1, which are critical for PML SUMOylation (Ishov and others 1999) and PML NB formation.

The single *PML* gene consists of 9 major exons and several alternatively spliced PML transcripts lead to expression of a multitude of different PML isoforms (Fig. 1). These are classified into 7 groups, designated PMLI-VII, which share a common N-terminal region that includes the RBCC motif but differ in their C termini due to alternative splicing of exons 7 to 9 (Jensen and others 2001). Several motifs have been identified in the C-terminus of PML: a nuclear localization signal (NLS) (found in PMLI-VI at position 476–490), a nuclear exclusion signal (found only in PMLI at position 704–713) (Henderson and Eleftheriou 2000) and a phospho-regulated SUMO-interacting motif (SIM) (only present in PMLI-V at position 508–511) (Shen and others 2006; Stehmeier and Muller 2009). PMLI has both a nuclear and a cytoplasmic distribution, which is consistent with the presence of a nuclear exclusion signal (Condemine and others 2006). The NLS contains a SUMOylation site at position 490, which is essential for the nuclear localization of PML (Kamitani and others 1998). The SIM has been proposed to mediate non-covalent interactions with other SUMOylated proteins and to promote their recruitment in PML NBs (Shen and others 2006; Stehmeier and Muller 2009). However, the recent finding that PMLVI isoform, which does not contain a SIM, is still able to form NBs in PML$^{-/-}$ cells (Brand and others 2010) suggests that this domain is not essential for NB formation. In addition, the variability of the C-terminal part of PML isoforms is important for the recruitment of interacting partners of PML and therefore their functions. As an example, PMLIV harbors binding site for p53 (Fogal and others 2000) that is required for PMLIV-induced apoptosis. A further classification into 3 subgroups, named a, b, and c, represents PML isoforms without exon 5, exons 5 and 6, or exons 4, 5, and 6, respectively. The b and c variants are likely to be cytoplasmic as they lack the NLS as observed for PMLVII.

The 6 human nuclear PML isoforms are all able to form NBs in PML-negative cells (Brand and others 2010), but it is becoming increasingly clear that each isoform may have a specific function. Most of the studies implicating PML in apoptosis and senescence were performed with PMLIV, whereas those implicating PML in antiviral defense have been done with PMLIII, IV, or VI. Further, endogenous ex-

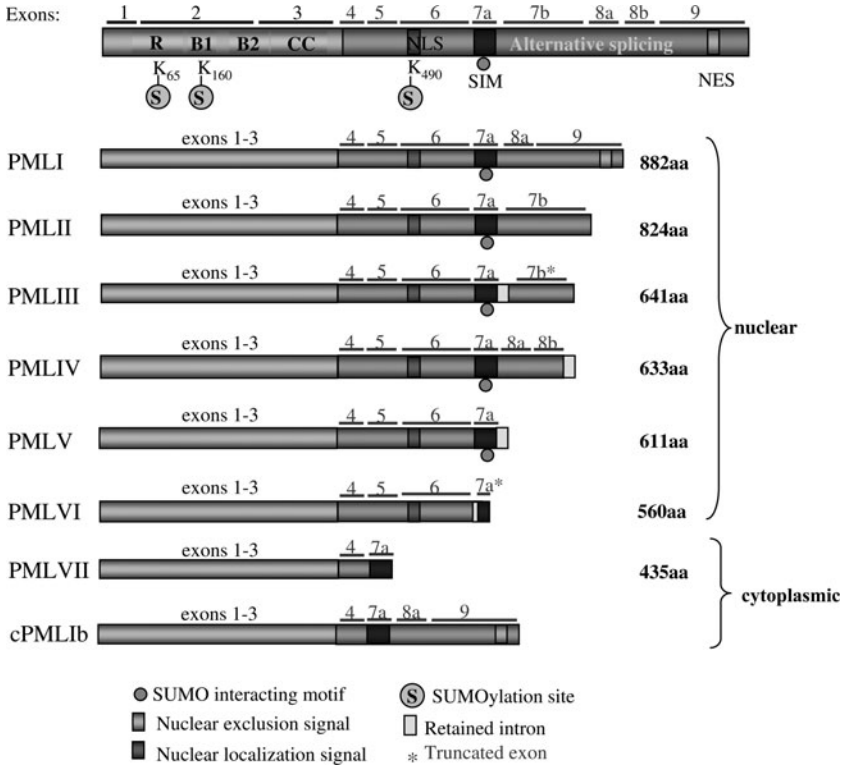

FIG. 1. Structure of PML isoforms. All PML isoforms share the first 3 exons, including the RBCC motif (R), 2 B-box (B1 and B2), and the coil-coil region (CC). PMLI to PMLVII differ in their C-termini due to an alternative splicing of exons 7 to 9, whereas cPMLIb results from an alternative splicing of exon 4–6. PML, promyelocytic leukemia; RBCC, RING-B-boxes-coiled-coil.

pression of PML isoforms differs in various cell lines as it has been reported that PMLIII, PMLIV, and PMLV are quantitatively minor isoforms compared to PMLI and PMLII (Condemine and others 2006). Paradoxically, very few studies have been performed with PMLI and PMLII. Therefore, it remains to determine whether functions attributed to one PML isoform are also shared by other isoforms.

Transcriptional Regulation of PML

Both IFN-dependent and IFN-independent pathways enhance *PML* gene expression. However, IFNs are the best characterized inducers of *PML*. All IFNs (α, β, and γ) sharply enhance mRNA and protein levels of PML, leading to a marked increase in the number and size of PML NBs (Chelbi-Alix and others 1995). In APL, in which the *PML* gene is disrupted in one allele, IFNs induce both PML and PML/RARα expression, resulting in an increased sequestration of PML in the microspeckles, out of the NBs, induced by the fusion protein (Chelbi-Alix and others 1995).

In various cell lines, PML isoforms arising from alternative splicing of a single gene are increased in response to IFN. *PML* gene expression is directly induced by IFNs through identified IFN-stimulated response elements in its promoter (ISRE; -GAGAATCGAAACT-) and gamma-activated site (GAS; -TTTACCGTAAG-) (Stadler and others 1995). Deletion of the GAS element only modestly alters the response to type II IFN, whereas ISRE motif is required for both type I and type II IFN responses (Stadler and others 1995). Indeed, deletion of ISRE in *PML* promoter abolishes the response to type I IFN and considerably decreases induction by type II IFN. The binding of the IFN regulatory factor 8 (IRF-8) to *PML* ISRE is also involved in upregulation of PML in response to type II IFN (Dror and others 2007).

Additional mechanisms independent of IFN regulate also PML expression (Kim and others 2007). The IRF-3, a key inducer of type I IFN, increases PML expression by transcriptional activation that requires both ISRE and GAS elements in *PML* promoter. The *PML* induction by IRF-3 is direct and does not implicate IFN synthesis. The tumor suppressor p53 regulates also PML expression as the first intron of *PML* contains a p53-binding site. In p53-deficient cells, overexpression of p53 upregulates *PML* expression, leading to an increase in PML NB number and size. Further, PML levels increase during oncogenic stimuli like Ras overexpression (Ferbeyre and others 2000). However, this increase is observed at a period coincident with senescence, suggesting that Ras signaling to PML is indirect. Altogether these data show that IFN, IRF-3, and p53 regulate PML through distinct mechanisms.

Post-Translational Modifications of PML

PML is subjected to multiple post-translational modifications, which include SUMOylation, phosphorylation, ubiquitinylation, and acetylation. More recently, it has been reported that PML could be also the target of IFN-stimulated gene of 15 kDa (ISG15), another ubiquitin-like modifier (Shah and others 2008).

SUMOylation, one of the most-studied post-translational modifications of PML, has important consequences on PML functions as it can affect its localization, its stability or its ability to interact with other partners. PML is modified with SUMO-1 and SUMO-2/-3 on 3 lysines residues (K65, K160, and K490) (Kamitani and others 1998) (Fig. 1). SUMOylation of PML is critical for the formation of NBs as PML mutants that cannot be SUMOylated are unable to form these structures (Ishov and others 1999). The involvement of SUMO in NB formation is further demonstrated in cells that do not express SUMO-1 or the SUMO conjugating enzyme, UBC9. Indeed, cells from *SUMO-1* or *UBC9* knockout mice have dramatic defects in PML NB formation, demonstrating that SUMOylation is essential for the maintenance of PML NB integrity (Nacerddine and others 2005; Evdokimov and others 2008).

PML localization is intimately linked to its SUMOylation and its phosphorylation. Within the nucleus, most of PML is expressed in the diffuse nuclear fraction of the nucleoplasm and only a small fraction is found in the matrix-associated NBs. The transfer of PML from the nucleoplasm to NBs depends on the phosphorylation and the SUMOylation of PML. Indeed, in response to As_2O_3 or poliovirus infection, PML is phosphorylated through the mitogen-activated protein kinase pathway, leading to the transfer of PML from the nucleoplasm to the nuclear matrix and to the increase of PML SUMOylation and NB size (Lallemand-Breitenbach and others 2001; Hayakawa and Privalsky 2004; Pampin and others 2006). In addition, a PML mutant deficient for SUMOylation is still transferred to the nuclear matrix in response to As_2O_3 (Lallemand-Breitenbach and others 2001), suggesting that PML phosphorylation may regulate the shift of PML to the nuclear matrix where PML SUMOylation is believed to occur.

Phosphorylation of PML controls also other aspects of PML functions. Recently, the serine/threonine kinase homeodomain-interacting protein kinase (HIPK2) has been shown to phosphorylate PML at serines 8 and 38 in response to DNA damage, leading to SUMOylation of PML and to the induction of apoptosis (Gresko and others 2009). Other kinases lead also to the phosphorylation of PML on several serine or tyrosine residues. PML is phosphorylated by the ataxia telangiectasia-mutated and Rad3-related kinase (ATR), a Chk2 activating kinase in cells exposed to DNA damaging agent such as doxorubicin (Bernardi and others 2004). Further, in response to γ-irradiation, PML is phosphorylated by the proapoptotic checkpoint kinase Chk2, leading to γ-irradiation-induced apoptosis (Yang and others 2002). However, it is not known whether these modifications are required to promote PML SUMOylation. Further studies remain to be done to better understand the importance of the cross-talk between phosphorylation and SUMOylation on PML functions.

A new link between SUMOylation and ubiquitinylation has emerged with the identification of the SUMO-dependent ubiquitin E3 ligase, RNF4, that targets PML for a proteasome-mediated degradation (Lallemand-Breitenbach and others 2008; Tatham and others 2008; Geoffroy and Hay 2009). RNF4 harbors multiple SIM in its N-terminus region that allow a strong interaction with SUMO. This leads to the ubiquitinylation of poly-SUMO chains conjugated to PML and also of several lysine residues in PML. In the absence of RNF4, SUMO-modified PML accumulates resulting in the increase of the number and the size of PML NBs (Fig. 2). In APL cells, RNF4 targets the PML moiety of PML-RARα, leading to its degradation in response to As_2O_3. These studies shed a new light on the mechanism of arsenic-induced remission for APL patients. They reveal PML as the first example of a protein degraded by the ubiquitin-proteasome pathway in a SUMO-dependent manner and demonstrate that RNF4 is an important regulator of PML stability.

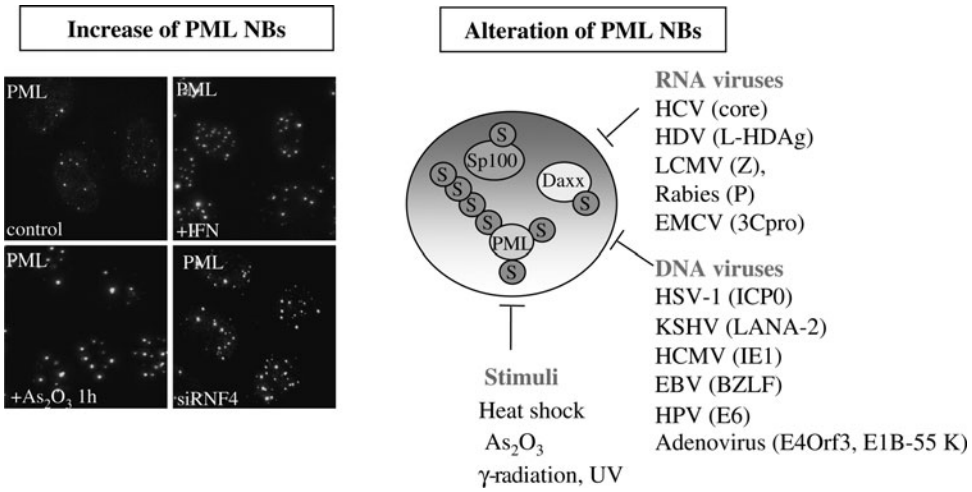

FIG. 2. Homeostasis of PML NBs. The size and the number of PML NBs increase upon interferon treatment or down-regulation of RNF4 by small interfering RNA (siRNA). Short exposure (<1 h) with As_2O_3 leads to the accumulation of SUMO modified PML within NBs, which is followed later by PML degradation. At the opposite, cellular stress such as heat shock, long exposure to As_2O_3, gamma-irradiation, UV, or viral infection alter the structures of PML NBs. As_2O_3, arsenic trioxide; NBs, nuclear bodies.

The oncogenic casein kinase 2 (CK2) has also been shown to regulate PML protein level by promoting its ubiquitin-mediated degradation dependent on phosphorylation at Ser517 (Scaglioni and others 2006). PML mutants that are resistant to CK2 phosphorylation display increased tumor suppressive functions (Scaglioni and others 2006). However, how CK2 mediates PML degradation and whether RNF4 is involved in this process are still unknown.

Additional post-translational modifications of PML have also been reported. PML is acetylated by trichostatin A, a histone deacetylase inhibitor, leading to increased PML SUMOylation and trichostatin A-induced apoptosis (Hayakawa and others 2008). Further investigations are needed to determine whether acetylation is involved in PML NB formation and/or other functions of PML. Moreover, PML has been reported to be a target of another ubiquitin-like modifier named ISG15 (Shah and others 2008). Expression of ISG15 and its ubiquitin-activating enzyme-E1 is enhanced upon ATRA treatment, leading to the modification of PML by ISG15. However, the mechanism leading to PML ISGylation is still unknown. As ATRA upregulates type I IFN and many ISG products (Pelicano and others 1999), the increase of ISG15 expression could be a consequence of ATRA-induced IFN. Therefore, it remains to be determined whether PML ISGylation is observed in IFN-treated cells.

Involvement of TRIM Proteins in Antiviral Defense

The TRIM protein family, which includes PML/TRIM19, comprises >70 members that have emerging role in IFN responses (Ozato and others 2008). TRIM protein family harbors a highly conserved RBCC motif, with a RING domain, 1 or 2 B-boxes and a coiled-coil region, found in its N-terminal part. The RING domain contributes to the biological diversity of TRIM proteins as it can mediate the conjugation of ubiquitin or ubiquitin-like modifiers such as SUMO. As an example, TRIM5α, TRIM11, and TRIM22 harbor a RING-dependent E3 ubiquitin ligase activity on different substrates restricting lentivirus infection (Ozato and others 2008), whereas other proteins such as TRIM19/PML interact with UBC9 in a RING-dependent manner, suggesting a possible role of PML in promoting SUMO conjugation (Duprez and others 1999). The associated B-box and coiled-coil domains are involved in protein–protein interactions and in the formation of large protein complexes that define specific cytoplasmic clusters for TRIM5α or NBs for TRIM19/PML. The C-terminal region of TRIM proteins is highly variable and contains specific regions involved in protein-protein interactions such as the B30.2 or SPRY domains found in almost 60% of TRIM proteins (Ozato and others 2008).

Several members of the TRIM superfamily are involved in various cellular processes associated with inhibition of cell growth, apoptosis, and innate immunity (Ozato and others 2008; Carthagena and others 2009). Among the 72 known human TRIM genes, 16 are upregulated at the mRNA level by type I IFN and 8 by type II IFN (Carthagena and others 2009). So far, only TRIM5α, TRIM19/PML, and TRIM25 have been shown to be directly induced by IFN as they have the ISREs in their promoters (Carthagena and others 2009). Further, TRIM members are implicated in antiviral defense as observed for TRIM19/PML. For example, TRIM22 confers resistance to hepatitis B virus by acting as a transcriptional suppressor (Gao and others 2009) and to encephalomyocarditis virus by targeting the viral 3C protease for ubiquitin-mediated degradation (Eldin and others 2009). In the case of infection with the human immunodeficiency virus (HIV), TRIM5α interferes with uncoating of the viral preintegration complex, TRIM11 and TRIM32 repress viral gene expression, and TRIM15 and TRIM22 inhibit virus assembly (Stremlau and others 2004; Uchil and others 2008). It has been suggested that TRIM5α and TRIM22 mediate their antiviral effects via their B30.2 and SPRY domains, respectively (Ozato and others 2008). However, TRIM proteins without these domains, like PML/TRIM19, can be also involved in antiviral defense, suggesting that these domains are not critical for this activity.

Further, some TRIM proteins are important regulators of IFN pathway as they alter IFN production or IFN signaling.

For example, the E3 ubiquitin ligase TRIM21 is induced upon RNA viral infection and interacts with IRF-3, leading to the alteration of IRF-3 stability in a RING-independent and SPRY-dependent manner (Yang and others 2009). Knockdown of TRIM21 by small interfering RNA (siRNA) impairs IRF-3-mediated gene expression, thus blocking virus-induced IFN production. TRIM25 has been shown to be essential for retinoic acid-inducible gene-1 ubiquitinylation and for retinoic acid-inducible gene-mediated IFN synthesis in response to RNA viral infection (Gack and others 2007). TRIM8 binds to suppressor of cytokine signaling-1 and alters IFN-γ signaling mediated by suppressor of cytokine signaling-1 (Toniato and others 2002).

Altogether, the involvement of IFN-induced TRIM proteins in antiviral defense and signaling pathways underlines the importance of the TRIM protein family in IFN responses and has opened a new field of research.

Lessons from Knockout PML Mice and Their Derived Cells

The multiple functions of PML in various cellular processes have been revealed from genetic studies. Ablation of the *PML* gene by homologous recombination has shown that mice are viable and develop normally. However, loss of PML alters hemopoietic differentiation, cell growth, and tumorigenesis (Wang and others 1998a, 1998b). In addition, these mice present abnormal mammary gland development (Li and others 2009a) and have defects in the ratio of progenitor cells that affects adult brain development (Regad and others 2009).

Cells derived from these mice are defective in the induction of apoptosis induced by type I and type II IFNs (Wang and others 1998b). Similar results were observed in human cells as PML downregulation by siRNA blocks IFN-α-induced apoptosis (Crowder and others 2005). In addition, $PML^{-/-}$ mouse embryonic fibroblasts (MEFs) are also resistant to the induction of apoptosis by other stimuli such as Fas, tumor necrosis factor, ceramides (Wang and others 1998b), and transforming growth factor (TGF)-β (Lin and others 2004). PML has been shown to be an essential modulator of TGF-β signaling by controlling the phosphorylation and nuclear translocation of the TGF-β signaling proteins, Smad2 and Smad3 (Lin and others 2004). However, the involvement of PML in other signaling pathways remains to be determined.

Analysis of $PML^{-/-}$ mice also shows that they are more susceptible to lymphocytic choriomeningitis virus (LCMV) and vesicular stomatis virus (VSV) infections (Bonilla and others 2002). These observations corroborate with findings demonstrating that fibroblasts derived from PML knockout mice, $PML^{-/-}$ MEFs, exhibit enhanced LCMV multiplication (Djavani and others 2001). They are more sensitive to infection with EMCV (El Mchichi and others 2010) and rabies virus (Blondel and others 2002). However, replication of other virus, ie, herpes simplex type 1 (HSV-1) (Chee and others 2003) or human foamy virus (HFV) (Regad and others 2001), is not affected by the loss of endogenous PML. The capacity of IFN to protect cells against these viruses was more drastic in wild-type MEFs than in $PML^{-/-}$ MEFs, demonstrating the importance of PML to establish an IFN-induced antiviral state against HSV-1 and HFV (Table 1).

Altogether, the involvement of PML in cell development, apoptosis, cell signaling, and antiviral defense underline the multiple functions of PML due to its ability to interact with various partners either in the cytoplasm or in the nucleus.

Functions of PML NBs

PML NBs are involved in DNA damage, stress response, senescence, apoptosis, protein degradation, viral infection, and IFN response (Regad and Chelbi-Alix 2001; Everett and Chelbi-Alix 2007; Bernardi and others 2008; Tavalai and Stamminger 2008). These dynamic structures move within the nucleus in intimate contact with the surrounding chromatin and harbor permanent (Sp100, Daxx) and numerous transient proteins (such as p53, CREB binding protein [CBP] HIPK2, and ataxia telangiectasia mutated [ATM]) depending on different conditions, ie, transformation, stress, IFN treatment, and viral infections (Negorev and Maul 2001). PML is the organizer of PML NBs as the permanent NB-associated proteins are unable to form PML-like structures in PML-negative cells (Ishov and others 1999).

On the basis of databases analysis, PML partners were recently brought together in a comprehensive network containing 166 proteins (www.ua.ac.be/ppse) (Van Damme and others 2010). In the literature, almost 40% of PML partners have been confirmed to be SUMOylated, suggesting that PML NBs are enriched sites for SUMOylated proteins (Van Damme and others 2010). Further evidence of links between PML NBs and SUMO pathways comes from the detection of SUMO-specific proteases and SUMO E3 ligases of the protein inhibitor of activated STAT family within PML NBs, suggesting that PML NBs may function as a nuclear SUMOylation hotspot (Sachdev and others 2001; Best and others 2002). PML NBs could also act as a nuclear platform for other post-translational modifications. Phosphorylation and/or

Table 1. Lessons from Knockout Promyelocytic Leukemia Mice and Their Derived Cell Lines

	Virus	*Viral replication*	*References*
Mice $PML^{-/-}$	VSV	Increase	Bonilla and others (2002)
	LCMV	Increase	Bonilla and others (2002)
MEFs $PML^{-/-}$	Rabies	Increase	Blondel and others (2002)
	EMCV	Increase	El Mchichi and others (2010)
	HSV-1	No effect	Chee and others (2003)
	HSV-1+IFN	Drastic reduction of antiviral effect of IFN	Chee and others (2003)
	HFV	No effect	Regad and others (2001)
	HFV+IFN	Drastic reduction of antiviral effect of IFN	Regad and others (2001)

IFN, interferon; HFV, human foamy virus; HSV-1, herpes simplex type 1; LCMV, lymphocytic choriomeningitis virus; MEFs, mouse embryonic fibroblasts; PML, promyelocytic leukemia; VSV, vesicular stomatis virus.

Table 2. Promyelocytic Leukemia Isoforms Confer Resistance to DNA and RNA Viruses from Different Families

Viruses	*Protective effect of PML isoforms*		*Mechanisms*	*References*
DNA viruses				
HSV-1	cPMLIb	Yes	Sequestration of ICP0 in the cytoplasm	McNally and others (2008)
HCMV	PMLVI	Yes	Inhibition of viral mRNA and protein	Tavalai and others (2008)
Adenovirus	PMLVI	Yes	ND	Doucas and others (1996)
RNA viruses				
HFV	PMLIII	Yes	Inhibition of transcriptional activity of Tas	Regad and others (2001)
HIV-1	ND	ND	ND	
VSV	PMLIII	Yes	Inhibition of transcription	Chelbi-Alix and others (1998)
Rabies	PMLIV	Yes	Inhibition of transcription	Blondell and others (2010)
LCMV	ND	Yes	ND	
Influenza A	PMLIII	Yes	ND	Chelbi-Alix and others (1998)
	PMLIV	Yes	ND	Iki and others (2005)
	PMLVI	Yes	ND	Iki and others (2005) Li and others (2009b)
Poliovirus	PMLIII	Yes	Activation of p53 and induction of p53-target genes	Pampin and others (2006)

HCMV, human cytomegalovirus; HIV-1, human immunodeficiency virus type 1; ND, not determined.

acetylation of p53 upon DNA damage, cellular stress, and viral infection require PML NBs (Pearson and others 2000; Moller and others 2003; Pampin and others 2006). Finally, PML NBs could be a site of protein degradation for PML itself or the PML NB-associated protein p53. Indeed, components of the proteasome are present in the NBs (Lallemand-Breitenbach and others 2001) and PML protein accumulates in the NBs in the presence of proteasome inhibitors (Bailey and O'Hare 2005). Further, the recent findings showing that RNF4 targets PML for proteasome-mediated degradation by adding polyubiquitin chains to the SUMO moieties conjugating to PML, strongly suggest that the degradation of PML occurs in the NBs (Lallemand-Breitenbach and others 2008; Tatham and others 2008). The recruitment of p53 and murine double minute 2 (MDM2) within PML NBs is observed in poliovirus infected cells, resulting in the degradation of p53 in a proteasome- and MDM2-dependent manner. This process, which requires PML suggests that PML NBs could be a site for protein degradation during viral infection (Pampin and others 2006).

Altogether, these studies demonstrate that PML NBs have multiple roles depending on the proteins recruited within these structures upon different stimuli.

PML NB-Associated Proteins and IFN response

In response to IFN and others stimuli, many cellular proteins colocalize with PML within the NBs. Among these proteins, some are known to be directly induced by IFN, such as Sp100 and p53 and their recruitment appears to depend on PML SUMOylation. The participation of the NB-associated proteins Sp100, p53, and Daxx in the antiviral defense is discussed in virus section.

Sp100

The Speckled protein of 100 kDa (Sp100) is a permanent PML NB-associated protein that was the first NB protein identified by using the sera from patients suffering from primary biliary cirrhosis (Szostecki and others 1990). Like PML, Sp100 is SUMOylated, but SUMO modification of Sp100 is not required for its PML NB localization (Sternsdorf and others 1997).

Sp100 comprises a family of 4 proteins, namely, Sp100A, Sp100B, Sp100C, and Sp100HMG, which are produced by alternative splicing of a single primary transcript. These isoforms share the same N-terminal region, but contain different motifs in their C-terminal part. Except for Sp100A, all Sp100 isoforms harbor a SAND domain that is required for direct binding to DNA. In addition to SAND, Sp100C harbors PHD and Bromo motifs, whereas Sp100HMG contains only HMG boxes (Negorev and others 2009). All these Sp100 isoforms are increased in cells treated with IFN in addition to other proteins of the Sp family, Sp140 and Sp110 (Bloch and others 1999, 2000).

Sp100 is a primary target gene of both type I and type II IFNs as functional ISRE (-ACTTTCACTTCTCT-) and GAS (-TTCCAGGAA-) domains are present in its promoter (Guldner and others 1992; Grotzinger and others 1996). In addition, Sp100 and PML seem to be coregulated as the downregulation of Sp100 by siRNA reduces also PML expression (Negorev and others 2009). This suggests that Sp100, in addition to RNF4 and SUMO, is important for the integrity of PML NBs.

p53

The tumor suppressor p53 is not a permanent PML NB-associated protein, but is recruited in these structures only under certain conditions, including Ras activation, exposure to UV light, ionizing radiation, poliovirus infection, or exogenous expression of PMLIV. Other proteins involved in the post-translational modification of p53, such as the acetylase CBP, the protein kinase HIPK2, the E3 ubiquitin ligase MDM2, and the herpesvirus-associated ubiquitin-specific protease (HAUSP) ubiquitin protease, are also recruited to

PML NBs, indicating that these structures are an important regulatory site for p53 function (Bernardi and others 2008). In contrast to PML, SUMO modification of p53 on lysine residue 386 is not required for its targeting to PML NBs (Kwek and others 2001).

P53 was discovered in 1980, but its connection to the IFN pathway was only revealed >20 years later by Taniguchi and colleagues (Takaoka and others 2003). Type I IFN directly induces p53 expression due to the presence of an active ISRE motif in its promoter, but do not lead to its activation (Takaoka and others 2003). Also, p53 is not associated with PML NBs in MCF7 or U2OS cells treated with IFN (Porta and others 2005), indicating that IFN is not sufficient to promote the recruitment of p53 within PML NBs. However, poliovirus infection, which induces PML SUMOylation, leads to the recruitment and activation of p53 within PML NBs, resulting in apoptosis of infected cells (see in virus section) (Pampin and others 2006). Further, PML is required for p53 acetylation and/or p53 phosphorylation upon DNA damage and cellular stress (Bernardi and others 2008). Therefore, the cross-talk between PML and p53 is important for the induction of apoptosis and antiviral defense.

Daxx

The death-associated dead protein (Daxx), a transcriptional corepressor, is permanently associated with PML NBs and is also found diffusely throughout in the nucleoplasm. It has been suggested that type I IFN increases xpression of Daxx in murine B cell progenitors (Gongora and others 2001). However, in our hands, in different human cells, type I IFN increases expression of PML without affecting the protein level of Daxx as tested by Western blot (data not shown). The sequestration of Daxx within PML NBs depends on its ability to bind to SUMOylated PML via a SIM domain (Lin and others 2006). IFN and short exposure to As_2O_3 increase PML expression and PML SUMOylation, respectively, leading to the recruitment of Daxx to PML NBs (Kawai and others 2003). In contrast, cellular stress that induces PML deSUMOylation leads to the release of Daxx from PML NBs (Nefkens and others 2003). Taken together, these results suggest that Daxx is not directly induced by IFN but is recruited through its SIM domain within PML NBs due to the increase of PML in response to IFN.

Implication of PML and PML NBs in Antiviral Defense

It is well established that several DNA and RNA viruses encode proteins that colocalize with PML and disorganize the NBs, suggesting that PML NB alteration could be a viral strategy to evade a cellular resistance mechanism. This field has been covered in numerous reviews (Everett 2001; Everett and Chelbi-Alix 2007; Tavalai and Stamminger 2008) and is summarized in Fig. 2, so this topic will be described only briefly.

The ICP0 protein of HSV-1 (Everett and Maul 1994), the IE1 protein of human cytomegalovirus (HCMV) (Ahn and Hayward 1997), the LANA2 of Kaposi's sarcoma-associated herpesvirus (Marcos-Villar and others 2009), the E4orf3 protein of adenovirus 5 (Doucas and others 1996), and the BLZF protein of Epstein Barr virus (Adamson and Kenney 2001) all disorganize PML NBs. In addition, at least 5 early proteins (E1, E2, E5, E6, and E7) and 3 late proteins (E1–E4, L1, and L2) of human papillomavirus are all associated with PML NBs, suggesting that PML NBs could be the site for the initiation of viral infection (Tavalai and Stamminger 2008). However, only E6 has been reported to promote proteasome-dependent PML degradation (Louria-Hayon and others 2009).

Some RNA viruses whose replication take place in the cytoplasm and is inhibited by PML have developed different strategies to counteract PML NBs (Chelbi-Alix and others 2006). LCMV and rabies viral infections result in alteration of PML NBs mediated by a small nonstructural protein named Z and the phosphoprotein P, respectively (Blondel and others 2002; Kentsis and others 2001) (see virus section). Recently, it has been shown that EMCV induces PML degradation in a proteasome- and SUMO-dependent pathways. Reduction of PML is mediated by the viral 3C protease which colocalizes with PML within the NBs early post infection (El Mchichi and others 2010). Like other RNA viruses, the L-HDAg protein of hepatitis delta virus alters also PML NB structures and the distribution of NB-associated proteins (Bell and others 2000). In the case of Hepatitis C virus, the protein core interacts with PMLIV (Herzer and others 2005) in transfected cells and abrogates both p53 acetylation and phosphorylation, leading to inhibition of PMLIV-induced apoptosis. However, it remains to be determined whether this effect is also observed in Hepatitis C virus-infected cells.

Various reports revealed that PML has an inhibitory effect against DNA and RNA viruses and mediates an IFN-induced antiviral response (Table 2) (Regad and Chelbi-Alix 2001; Everett and Chelbi-Alix 2007). We will focus on the capacity of PML and NB-associated Sp100 and Daxx to confer resistance to different DNA and RNA viruses listed below.

PML and DNA Viruses

HSV-1 and HSV-2

HSV-1, a member of the neurotropic α-subfamily of herpesviruses, was the first virus reported to disrupt PML NBs during infection (Maul and others 1993). This effect is mediated by the ubiquitin E3 ligase ICP0, an immediate early viral protein that induces the proetasome-mediated degradation of PML in a RING-dependent manner (Everett and others 1998; Everett and Chelbi-Alix 2007; Tavalai and Stamminger 2008). Sp100, another NB-associated protein, is also degraded in HSV-1-infected cells (Chelbi-Alix and de The 1999). However, the apparent loss of various isoforms of Sp100 during HSV-1 infection seems to be a consequence of ICP0-induced PML degradation rather than a direct effect of ICP0 on Sp100 itself (Negorev and others 2009). The replication of ICP0-null mutant HSV-1 is accelerated when either PML or Sp100 expression is downregulated by siRNA (Everett and others 2008) and this effect is enhanced with simultaneous depletion of both proteins. However, it has been recently suggested that Sp100 acts through PML as all Sp100 isoforms protect PML from ICPO-induced degradation, keeping PML available for anti-HSV-1 inhibition (Negorev and others 2009).

PML is an important mediator of IFN to protect cells from HSV-1 infection as revealed by analysis of $PML^{-/-}$ MEFs derived from knockout mice. Replication of HSV-1 is similar

in wild-type PML MEFs and PML$^{-/-}$ MEFs, indicating that endogenous PML expression is not sufficient to inhibit this virus. However, the capacity of IFN to inhibit HSV-1 (Chee and others 2003) is greatly reduced in PML$^{-/-}$ MEFs, underlining the role of PML in IFN-mediated antiviral response. Recently, the antiviral activity of PML against HSV-1 has been found to be mediated by a cytoplasmic variant of PMLIb, providing the first example for DNA viruses of a protective effect of PML out of the nucleus. Indeed, HSV-1 infection leads to a change in splicing of PML pre-mRNA resulting in the selective enrichment of the cytoplasmic isoform cPMLIb variant lacking exons 5 and 6 (McNally and others 2008). Expression of this cytoplasmic isoform mediates resistance against HSV-1 via an ICP0-dependent mechanism, whereas expression of the nuclear PML isoforms (III, IV, or VI) fails to protect cells from HSV-1 infection (McNally and others 2008). The capacity of PML to inhibit HSV-1 replication seems to be specific to this cytoplasmic PML isoform. However, the antiviral effect of cPMLIb need expression of other isoforms as this effect is stronger in MEFs PML$^{+/+}$ compared to MEFs PML$^{-/-}$ (McNally and others 2008). It remains to determine whether other cytoplasmic isoforms of PML such as PMLVII could also have a protective effect against HSV-1. Splicing of PML pre-mRNA occurs also during HSV-2 infection (Nojima and others 2009). Expression of PMLII is switched to PMLV in the early stage of infection, leading to a slight reduction of viral replication. This effect is mediated by ICP27 that act as a splicing silencer and could contribute to the establishment of HSV-2 latency.

Human cytomegalovirus

HCMV is the prototype of the β-subgroup of herpesviruses. Early after HCMV infection, the major immediate-early protein IE1 colocalizes with PML causing NB reorganization by preventing or removing SUMO adducts on PML (Lee and others 2004). Knockdown of PML in human fibroblasts results in enhanced HCMV replication with a considerable increase in the number of immediate-early (IE) protein-positive cells (Tavalai and others 2006). At the opposite, expression of exogenous PMLVI in U373MG cells inhibits viral protein accumulation and DNA replication (Ahn and Hayward 2000). Another viral protein localizes within PML NBs before the production of IE proteins. Indeed, the transactivator tegument pp71 accumulates within PML NBs by inducing the SUMOylation and proteasome-independent ubiquitin degradation of Daxx (Hwang and Kalejta 2009). Daxx knockdown by siRNA leads to increased gene expression and virus replication (Preston and Nicholl 2006). Moreover, downregulation of both PML and Daxx results in an additional increase in HCMV replication (Tavalai and others 2008). These results strongly suggest that PML and the PML NB-associated protein Daxx function as part of the antiviral defense mechanism against HCMV infections.

Adenovirus

Adenoviruses are a family of nonenveloped nuclear replicating viruses. Infection with adenovirus type 5, the most studied subtype, results early during infection in the reorganization of PML NBs from punctuate structures into elongated nuclear tracks (Puvion-Dutilleul and others 1995). This effect is mediated by E4orf3, which specifically targets the PMLII (Hoppe and others 2006) (Fig. 2). A region of 40 amino acids in the C-terminal part of PMLII (645–684 aa) is essential for E4orf3 binding. Among nuclear PML forms, this sequence encoded by exon 7b of the *PML* gene is unique to PMLII (Leppard and others 2009) thus reinforcing the specific role of PMLII in the reorganization of PML NBs.

E4orf3 is required for efficient adenovirus growth as the replication of a mutant lacking this orf, unable to alter PML NBs, is severely compromised (Ullman and others 2007). In addition, in cells treated with IFN, the replication of this mutant is more inhibited in comparison with the wild-type adenovirus (Ullman and others 2007). This indicates that the integrity of PML NBs may be required for the antiviral state induced by IFN. The replication of the E4orf3 mutant is restored when PML is downregulated by siRNA in IFN-treated cells. On the other hand, expression of the PMLVI isoform blocks or severely delays adenovirus replication (Doucas and others 1996). In addition, the role of Sp100 and Daxx has been determined during adenovirus infection (Ullman and Hearing 2008). Knockdown of Daxx by siRNA in IFN-treated cells restores the replicative capacity of the E4orf3 mutant adenovirus, whereas that of Sp100 does not. A recent study has also shown that depletion of Daxx in human hepatocyte cells by siRNA results in a significantly increased of adenovirus replication, demonstrating the importance of Daxx in adenovirus growth restriction (Schreiner and others 2010). The antiviral effect of Daxx is counteracted by the viral protein E1B-55K, which targets Daxx for proteasome-dependent degradation (Schreiner and others 2010). Altogether, these studies show that both PML and Daxx expression are critical for the inhibition of adenovirus replication.

PML and RNA Viruses

PML confers resistance to RNA viruses in a p53-independent way by interacting with viral proteins to inhibit their functions or in a p53-dependent way by inducing apoptosis in infected cells.

HIV type 1

HIV type 1 (HIV-1) is a retrovirus, member of the lentivirus genus. Upon infection, the RNA genome of HIV-1 is reverse transcribed into a linear double-stranded DNA molecule, which integrates into the cellular genome to produce new viral RNA. It has been suggested that the HIV-1 preintegration complex triggers the delocalization of PML into the cytoplasm early during the infection. However, other groups have found that HIV-1 does not modify PML NBs at early or late times of infection but resides in close association with SC35 domains in the case of unintegrated HIV-1 DNA (see review Tavalai and Stamminger 2008). A recent report reveals that HIV-1 leads to PML aggregation in syncytia present in the brain or lymph nodes of infected patients or in syncytia elicited by the envelope glycoprotein complex of HIV-1 *in vitro* (Perfettini and others 2009). The formation of syncytium induced by HIV-1 triggers an apoptotic pathway that requires PML for the phosphorylation of ATM which colocalizes with PML in NBs. PML knockdown by siRNA inhibits the ATM-dependent DNA damage response and p53-dependent cell death, leading to the suppression of syncytial apoptosis in HIV-infected CD4 cells (Perfettini and others 2009). Further studies need to be car-

ried out to confirm the involvement of PML in the antiviral response against HIV.

Human foamy virus

Foamy viruses are complex retroviruses isolated from different animal species, mainly nonhuman primates. HFV encodes 2 auxiliary proteins, Tas and Bet, in addition to the structural and enzymatic Gag, Pol, and Env proteins. Tas, a nuclear phosphoprotein, transactivates viral gene expression by binding directly to the long-terminal repeat and an internal promoter. HFV infection does not alter expression or localization of PML (unpublished data) and replication of HFV is similar in wild-type PML MEFs and $PML^{-/-}$ MEFs, indicating that endogenous PML expression is not sufficient to inhibit HFV (Regad and others 2001). Further, PML does not play an important role in mediating HFV latency (Meiering and Linial 2003). However, overexpression of PMLIII represses HFV transcription by complexing the HFV transactivator, Tas, preventing its binding to DNA (long-terminal repeat and internal promoter) (Regad and others 2001). This interaction requires the N-terminal region of Tas and the RING finger of PML, but does not necessitate PML modification with SUMO. The antiviral effect of PML against HFV is further demonstrated in IFN-treated cells as IFN inhibits viral protein expression and HFV replication in wild-type MEFs but not in $PML^{-/-}$ MEFs. Other IFN mediators such as MxA, Mx1, and PKR do not alter HFV multiplication (Regad and others 2001), demonstrating the specific role of PML in mediating an IFN-induced antiviral state against HFV.

Vesicular stomatitis virus

VSV, a member of the rhabdoviridae viral family, possesses a relatively small, negative-sense RNA genome encoding the 5 viral proteins necessary for replication, assembly, and budding of its enveloped virions. VSV, whose replication takes place entirely in the cytoplasm, does not disrupt PML NBs (data not shown). The antiviral effect of PML against this virus has been demonstrated *in vivo* as PML knockout mice are more susceptible to VSV infection (Bonilla and others 2002). These data are in agreement with previous findings showing that expression of exogenous PMLIII, but not of Sp100, confers resistance to VSV by inhibiting both VSV mRNA and protein synthesis (Chelbi-Alix and others 1998). This inhibition is observed by both nuclear PMLIII or a cytoplasmic PMLIII mutant. However, how PML inhibits VSV replication in the relevant compartment is still unknown.

Rabies virus

Rabies virus, another of rhabdoviridae family, has a linear, single-strand RNA genome of negative polarity. This virus whose replication takes place entirely in the cytoplasm reorganizes the PML NBs, which become larger and appear as dense aggregates. Among the rabies viral proteins (P, N, M, G, and L), only expression of the phosphoprotein P delocalizes PMLIII from the NBs into cytoplasmic dots where both proteins colocalize. P binds to PMLIII in transfected or in infected cells through their C-terminal domain and the PML RING finger. However, despite the fact that rabies virus alters PMLIII localization, the replication of the virus is not affected by the overexpression of this isoform (Blondel and others 2002). $PML^{-/-}$ MEFs are more susceptible to rabies infection than wild-type MEFs (Blondel and others 2002), suggesting that other PML isoforms are involved in this antiviral defense. Recently, it has been shown that only PMLIV confers the resistance to rabies virus (Blondel and others 2010). This antiviral effect requires PMLIV SUMOylation. Further investigations are needed to determine how PML confers resistance to rabies virus in IFN-treated cells.

Lymphocytic choriomeningitis virus

LCMV is a negative-stranded virus in the arenavirus family, which encodes a nucleocapsid, an envelope glycoprotein, an RNA polymerase, and a RING finger protein Z. LCMV infection results in redistribution of PML from NBs to the cytoplasm (Borden and others 1998). This effect is mediated by the protein Z, which interacts with endogenous PML and the elongation factor eIF4E, reducing its cap-binding activity and leading to the inhibition of translation (Kentsis and others 2001). The most relevant results of the antiviral effect of PML have also been observed *in vivo*, since PML knockout mice are more susceptible to LCMV infection (Bonilla and others 2002). In addition, the capacity of IFN to protect against LCMV is higher in wild-type MEFs than in $PML^{-/-}$ MEFs (Djavani and others 2001). However, IFN still inhibits LCMV replication in $PML^{-/-}$ MEFs, indicating the involvement of other IFN-induced mediators (Djavani and others 2001). Exogenous expression of PMLIII does not counteract LCMV replication (Asper and others 2004), suggesting the implication of other PML isoforms. Also, overexpression of Sp100 does not alter the growth of this virus (Asper and others 2004), underlining the importance of PML in the antiviral state against LCMV.

Influenza A virus

Influenza A is an orthomyxovirus that is highly contagious and causes seasonal epidemic or pandemic human influenza. Expression of different nuclear PML isoforms has been shown to confer resistance to this virus known to replicate in the cell nucleus. Cells expressing PMLIII (Chelbi-Alix and others 1998), PMLIV, or PMLVI (Iki and others 2005; Li and others 2009b) showed a significant reduction in the rate of influenza A viral propagation compared with control cells transfected with empty vector. In contrast, downregulation of PML expression by siRNA in human cells enhanced influenza A viral replication (Iki and others 2005; Li and others 2009b). However, the antiviral effect of PML on influenza A viruses seems to be viral subtype/strain-specific as the protective effect of PML is not observed in all influenza strain (Li and others 2009b). In influenza A virus-infected cells, the matrix protein M1 and the nonstructural polypeptides NS1 and NS2 have been shown to associate with PML NBs (Iki and others 2005). The functional significance of these findings is still unclear and it remains to be determined how PML inhibits influenza A viral propagation.

Poliovirus

Poliovirus, the etiological agent of paralytic poliomyelitis, belongs to the Picornaviridae family. This virion is composed of a single-stranded RNA molecule of positive polarity.

PMLIII expression confers resistance to poliovirus in p53 wild-type cells and not in p53-inactive cells (Pampin and others 2006). Infection with poliovirus induces ERK activation, which triggers PML phosphorylation. This modification is required for poliovirus-induced PML SUMOylation resulting in the transfer of PML from the nucleoplasm to the nuclear matrix and in an increase in PML NB size. These events lead to the recruitment of p53 to PML NBs and its phosphorylation on Ser15 in a PML-dependent manner (Pampin and others 2006). Infection by poliovirus of p53 wild-type cells induces the activation of p53 target genes, *Mdm2* and *Noxa*, leading to the induction of apoptosis and the inhibition of viral replication. All these effects are increased by exogenous expression of PMLIII and abolished when endogenous PML expression is downregulated by siRNA. Moreover, the knockdown of p53 by siRNA results in higher poliovirus replication, indicating that both PML and p53 participate in antiviral defense. The protective effect mediated by PML and p53 is transient since poliovirus targets p53 by inducing its degradation in a proteasome- and an MDM2-dependent manner (Pampin and others 2006). These data demonstrate that both PML and p53 cooperate to inhibit poliovirus replication and that PML NBs could be a site for p53 activation to trigger apoptosis in infected cells. It would be interesting to determine whether other virus could be inhibited in a similar way.

Concluding Remarks

In this review we have focused on the regulation of PML and its role in antiviral defense. The regulation of PML at the post-translational level has been extensively studied this last decade. Indeed, phosphorylation, SUMOylation, or ubiquitinylation of PML play an important role for PML functions. Links between PML phosphorylation and SUMOylation have been demonstrated in the case of arsenic and poliovirus. Poliovirus infection induces sequentially phosphorylation and SUMOylation of PML, leading to the inhibition of viral replication in a p53-dependant way. It remains to be determined whether post-translational modifications of PML are observed during other viral infections and are important for its antiviral effect.

PML, the organizer of the NBs, is involved in various cellular processes, including antiviral defense. The recruitment of a growing number of partners within the NBs contributes probably to the pleiotropic functions of PML. PML and Sp100 are both induced by IFN but do not equally contribute to antiviral defense as downregulation of Sp100 by siRNA or its overexpression does not impair the replication of many viruses. However, Sp100 could contribute indirectly to antiviral defense against HSV-1 as its expression is important for the stability of PML. In addition, p53, known to be also induced by IFN, is recruited within NBs upon poliovirus infection and contributes with PML to inhibit viral replication. Finally, the recent finding showing that the NB-associated Daxx restricts adenovirus and HCMV growth reinforces the protective role of PML NBs during viral infection. Much has been done in 20 years of research but there are still many unresolved questions. As an example, IFN treatment or exogenous expression of PML are known to play a role in apoptosis, inhibition of cell growth, and antiviral defense. However, further investigations are needed to determine the contribution of PML in these IFN-induced biological effects.

The role of PML in antiviral defense has been elucidated only for few viruses. PML impairs viral replication by sequestering viral protein either in the cytoplasm (ICP0 of HSV-1) or in the nucleus (Tas and HFV), by inhibiting the synthesis of viral mRNA (HCMV, rabies virus and VSV) or by inducing apoptosis in infected cells through the recruitment and the activation of p53 in the NBs (poliovirus). A central question is whether PML NBs are sites for a specific activity of PML that does not occur out of the NBs. Among all the viruses presented in this review, some virus replicate entirely in the cytoplasm (VSV, rabies virus and poliovirus) and are inhibited by nuclear PML, whereas another virus replicates in the nucleus (HSV-1) and is inhibited by a cytoplasmic isoform of PML. This indicates that PML can exert its antiviral effect either in the nucleus or in the cytoplasm. In addition, the antiviral effect of PML against poliovirus requires PML localization within the NBs, whereas the inhibitory effect of PML against HSV-1, VSV, or HFV can occur out of the NBs.

The antiviral activity of PML against a specific virus can involve one or several isoform(s) and can be enhanced by the permanent NB-associated protein Daxx or by the recruitment of p53 within these structures. Recent reports have revealed that infection with herpes viruses lead to change in splicing of PML pre-mRNA splicing. HSV-1 infection results in a switch of PMLI to cPMLIb and HSV-2 infection in a switch of PMLII to PMLV, leading to inhibition of viral replication. It remains to be determined whether other viruses regulate also PML splicing.

Finally, PML and NB-associated proteins represent one of the IFN protective pathways during viral infection. PML uses different mechanisms to inhibit the replication of DNA and RNA viruses. Elucidating the role of PML for each virus will probably lead to the discovery of new functions of PML and PML NB-associated proteins.

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