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The role of PML nuclear bodies in the innate immune response during viral infection

Role jaderných tělísek PML ve vrozené imunitní odpovědi během virové infekce

BACHELOR'S THESIS

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Prague, 2024

Declaration / Prohlášení:

I declare that I wrote this thesis by myself and listed all used resources and bibliography. Neither this thesis nor its important part was previously used to gain any academic degree.

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V Praze, 07.08.2024

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Acknowledgement:

I would like to thank my supervisor for his patience and his help while writing this thesis.

Abstrakt:

Tématem práce je shrnout poznatky o zapojení jaderných tělísek PML do regulace vrozené imunitní odpovědi. Práce zahrnuje popis PML jaderných tělísek a immunitní odpovědi na virovou infekci. Je zde popsáno jak PML protein a další komponenty PML jaderných tělísek – SP100, DAXX/ATRX a HIRA chaperonový komplex – regulují interferonovou odpověď typu I. V práci jsou zmíněné mechanismy, jakými vybraní zástupci Herpesvirů, Adenovirů a Polyomavirů narušují tuto regulaci, a jak inhibují funkce PML jaderných tělísek, případně jak je využívají k průběhu infekce.

Klíčová slova: PML, jaderná tělíska, vrozená imunita, virus

Abstract:

The aim of this thesis is to summarize the findings on the involvement of PML nuclear bodies in the regulation of the innate immune response. The thesis includes a description of PML nuclear bodies and the immune response to viral infection. It describes how the PML protein and other components of the PML nuclear bodies - SP100, DAXX/ATRX and the HIRA chaperone complex - regulate the type I interferon response. The mechanisms by which selected representatives of Herpesviruses, Adenoviruses and Polyomaviruses interfere with this regulation and how they inhibit PML nuclear body functions or exploit them for the course of infection are discussed.

Keywords: PML, nuclear bodies, innate immunity, virus

List of Abbreviations

ASF1A	Anti-silencing function protein 1 homolog A
ATRX	Alpha-thalassemia/mental retardation syndrome X-linked
CABIN-1	Calcineurin-binding protein 1
CBP	CREB-binding protein
CDK	Cyclin-dependent kinase
cGAS	Cyclic GMP-AMP synthase
CR3	Conserved region 3
DAXX	Death domain-associated protein
E1A	Early region 1A
E1B-55K	Early region 1B-55K
E2A	Early region 2A
E4 ORF3	Early region 4 open reading frame 3
E4 ORF6	Early region 4 open reading frame 6
ERK	Extracellular signal-regulated kinase
GAF	Interferon gamma activator factor
H3.3K9me3	H3.3 histone tri-methylation on lysine 9
H3K27me3	H3 histone tri-methylation on lysine 27
H3K36me3	H3 histone tri-methylation on lysine 36
H3K9me3	H3 histone tri-methylation on lysine 9
H3.3S31ph	H3.3 histone phosphorylation at serine 31
H3K9ac	H3 histone acetylation at lysine 9
H3K14ac	H3 histone acetylation at lysine 14
H4K8ac	H4 histone acetylation at lysine 8
HAdV	Human adenoviruses
HAT	Histone acetyltransferase
HCMV	Human cytomegalovirus
HDAC	Histone deacetylase

HIPK2	Homeodomain-interacting protein kinase
HIRA	Histone cell cycle regulator A (chaperon complex)
HIRA	Histone cell cycle regulator A
HSV-1	Herpes Simplex Virus 1
ICP0	Infected cell protein 0
IE	Immediate early
IFI16	Interferon-inducible protein 16
IFN	Interferon
IFNAR	Interferon-α receptor
IKK-ε	κBkinase-related kinase
IRF3	Interferon regulation factor 3
IRF9	Interferon-regulatory factor 9
ISG	Interferon-stimulated gene
ISGF3	Interferon-stimulated gene factor 3
ISRE	Interferon-stimulated response element
JAK1	Janus kinase 1
K	lysine
Kr	Kremer bodies
LAT	Latency-associated transcript
MAVS/IPS-1	Mitochondrial antiviral signalling
MDA5	Melanoma differentiation-associated gene 5
ML	Multiple latency
MTOR	Mammalian target of rapamycin
ND10	Nuclear domains 10
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B-cells
NLR	Nucleotide oligomerisation domain-like receptor
NLS	Nuclear localisation signal
PAMP	Pathogen-associated molecular pattern

PI3K	Phosphatidylinositol-3 kinase
PKC-δ	Protein kinase C-delta
PKM2	Pyruvate kinase 2
PML	Promyelocytic leukaemia protein
PML NBs	Promyelocytic leukaemia nuclear bodies
PRMT1	Arginine methyltransferase 1
PRR	Pattern recognition receptor
PTM	Post-translational modification
PTM	Post-translational modification
PTP	Protein tyrosine phosphatases
RBBC	RING finger domain–B-box domain–Coiled-coil region
RIG-I	Retinoic acid-inducible gene-I
RLR	Retinoic acid-inducible gene-like receptor
S	single
shRNA	Short hairpin RNA
SIM	SUMO-interacting motif
SLIM	STAT-interacting LIM
SOCS	Suppressor of cytokine signalling
SP100	Speckled protein 100 nuclear antigen
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
SUMO	Small ubiquitin-like modifier
TBK1	TANK-binding kinase 1
TF	Transcription factor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TRIM	Tripartite motif
TYK2	Tyrosine kinase 2

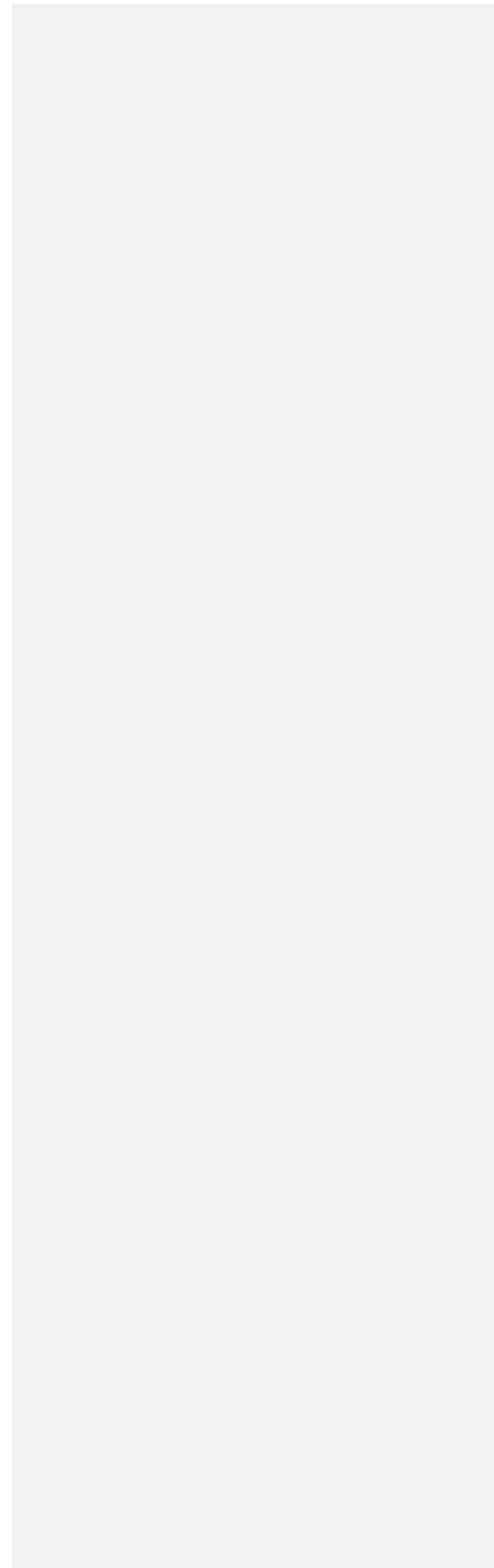|	
UBN1	Ubinuclein 1
USP18	Ubiquitin-specific peptidase 18
USP18	Ubiquitin-specific peptidase 18
vDCP NBs	Viral DNA-containing PML NBs
VSV	Vesicular stomatitis Indiana virus

Table of Contents

1 Introduction

The interplay between viral infections and the host's immune system is a complex and dynamic battle. The innate immune system serves as a first line of defence against pathogens, including viruses. It aims to prevent the virus from spreading through the organism. During cell infection, the innate immune system detects the presence of a virus through various pattern recognition receptors. These receptors then activate signalling cascades, sending the information about the virus in the cell to the nucleus to start the production of type I interferon. Interferon molecules inform about the infection autocrine and paracrine via specific receptors. Activated interferon receptors initiate both the canonical JAK-STAT signalling and noncanonical pathways to promote the expression of interferon-stimulated genes that promote an antiviral state (reviewed in (*1*)).

Subnuclear structures, known as PML nuclear bodies, have been shown to participate in numerous cellular processes, including the antiviral response. They modulate the signalling molecules and transcription factors, enhancing the expression of interferon-stimulated genes that encode proteins with direct antiviral effects. PML nuclear bodies help limit viral replication and spread through these mechanisms, highlighting their importance in the cellular antiviral defence machinery (reviewed in (*2*)).

Viruses have developed various methods to evade the immune response to infection by disrupting interferon signalling. PML nuclear bodies and numerous other cellular proteins are targeted and modified by viral proteins to enable the virus to produce progeny successfully. Interestingly, evidence of a positive role played by PML nuclear bodies on the viral infection has also surfaced (reviewed in (*3*)).

This thesis aims to summarize the known information about the role of PML nuclear bodies in regulating innate antiviral immunity. It will provide a detailed description of PML NBs: their structure and function. Furthermore, key components of innate immunity recognizing viral infection will be introduced, emphasising the type I interferon response. The mechanism of regulation of the type I interferon response by PML NBs and how certain DNA viruses interfere with this mechanism will be described.

2 PML Nuclear Bodies

Promyelocytic leukaemia nuclear bodies (PML NBs), also known as nuclear domains 10 (ND10) or Kremer (Kr) bodies, are nuclear, membrane-less, multiprotein structures. Their existence was first documented in the year 1960 by de Thé (*4*). In 1991, they were re-discovered and characterised in more detail when they were found to be involved in the development of acute promyelocytic leukaemia (*5*, *6*). Since then, PML NBs have been intensively studied and found to participate in various cellular processes, including apoptosis, antiviral defence and regulation of gene expression (*7*).

2.1 Structure and Composition of PML Nuclear Bodies

PML NBs are structures localised in the nucleus of most mammalian cells (*5*). Under the electron microscope, they appear as dense spherical structures that can be either empty or granular inside (Figure 1), with a diameter of 0.3 μm (*4*, *5*).

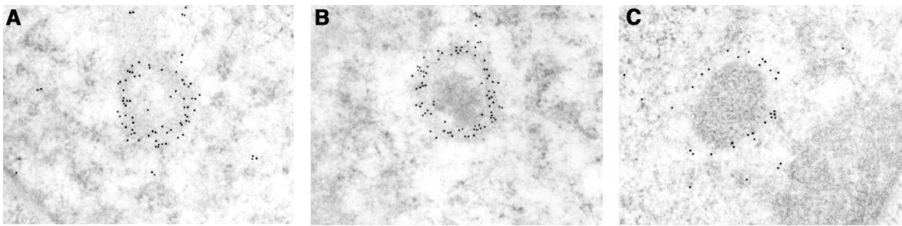*Figure 1. Different types of PML bodies. Immuno-gold electron microscopy of PML NBs in CHO cell line stably overexpressing PML. (A, B, C) "Classical" PML NBs. PML is distributed on a dense electron shell (A, B) or on light halo (C), that can contain a microgranular inner core (B, C) or not (A). Images courtesy of Edmond and Francine Puvion (CNRS, Villejuif, France) Taken from: (8); edited.*

In the normal state, 5-30 PML NBs can be found in the nucleus (*5*). PML NBs are commonly associated with transcriptionally active genomic regions and are bound to the chromatin (*9*, *10*). During the S-phase, chromatin rearrangements lead to the fission of PML NBs and, thus, to an approximately 2-fold increase in their number (*11*). Completion of cell division results in an equal distribution of PML NBs among the daughter cells and their increase to their original size (*12*). PML NBs disperse into microbodies in cells exposed to heavy metals or heat stress (*13*).

PML NBs consist of an outer shell, composed predominantly of polymerised PML protein and an inner core, where either permanently or transiently PML NB-associated proteins are located (*14*). To date, 271 proteins have been identified to interact with PML NBs (*15*). Permanently associated proteins include speckled protein 100 (SP100), small ubiquitin-like modifier (SUMO) proteins, death-associated protein 6 (DAXX), and alpha-thalassemia/mental retardation syndrome X-linked (ATRX), which will be described later in work (*14*, *16–18*). Protein molecules rapidly exchange between PML NBs and the nucleoplasm (*19*). The immunofluorescent image of PML NBs in the nucleus of 3T6 cell presented in

Figure 2 shows the PML protein assembled at the surface of the spherical structure and the SP100 protein colocalising with it. Interestingly, the PML arrangement captured in the image suggests that PML protein forms an icosahedral structure. This arrangement could participate in the trafficking of partner proteins between the PML NBs and the nucleoplasm, as was reviewed in (*20*). PML deficiency tested in mice results in aberrant diffusion of PML NBs-associated protein in the nucleus (*21*).
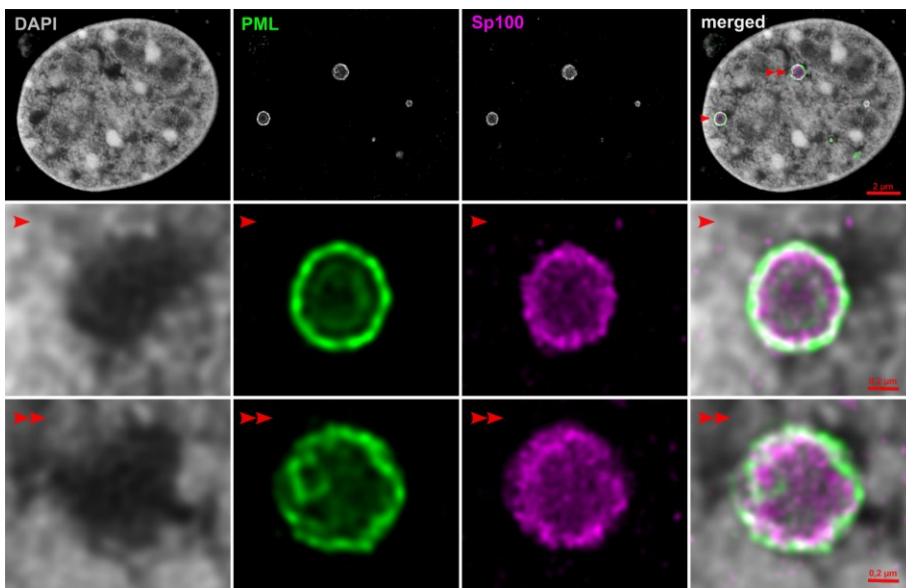

Figure 2. Section through the centre of the nucleus of a mouse fibroblast (3T6) cell line showing localisation of nuclear chromatin and two constitutive members of PML NB, PML and SP100 proteins (upper panel). Nuclear chromatin is visualised by DAPI staining (confocal microscopy), while PML protein and SP100 protein (which was transiently expressed as SP100-EGFP fusion protein) are visualised by indirect immunofluorescence (STED nanoscopy). Areas indicated by red arrows are enlarged and presented in the middle and bottom panels. Taken from: (3).

2.1.1 PML protein

PML protein, or TRIM19, is the main organiser of PML NBs (*14*, *21*). PML can be present in the cytosol and the nucleus (reviewed in *21*) In the nucleus, PML protein accumulates in PML NBs (*5*). The synthesis of the PML protein depends on the tissue and cell type and the cell cycle phase (*5*, *23*). This leads to high inter- and intra-organ variability.

PML protein is encoded by a single *Pml* gene (*6*, *24*). PML belongs to the tripartite motif (TRIM) protein family (reviewed in *19*). In humans, there are seven known isoforms of the PML protein, designated PML-I to PML-VII. The isoforms share the same N-terminal region with the TRIM motif, also known as RBBC, but vary in their C-terminal region. The TRIM consists of three zinc-binding domains: a RING (R), a B-box type 1 (B1), and a B-box type 2 (B2), followed by a coiled-coil (CC) region (reviewed in *19*). Most PML isoforms are localised in the nucleus, as their C-terminus contains

a nuclear localisation signal (NLS). However, due to alternative splicing, pre-mRNA may have their NLS spliced, resulting in the production of an isoform localised in the cytoplasm (*6, 24, 26, 27*). The C-terminal region also contains a SUMO-interacting motif (SIM) (*28*). In the PML protein, SUMO proteins are bound covalently to three lysine (K) residues with the assistance of the UBC9 enzyme (*29, 30*).

2.1.2 SUMO-SIM interactions

The SIM domain enables PML proteins to interact non-covalently with each other and with partner proteins (*31*). The domain with SUMOylated proteins (*31*). Partner proteins like SP100 and DAXX can be SUMOylated or contain the SIM domain, or both, and thus can interact with PML proteins (*32*). Taken together, SUMO-SIM interactions mediate the formation of the PML outer shell and the recruitment of partner proteins to the nuclear bodies (*31*). The process of SUMOylation will be discussed in more detail later in the chapter. Apart from the cooperating enzymes that mediate SUMOylation, PML protein also has the SUMO ligase activity, thus promoting SUMOylation of itself and other proteins (*33, 34*).

2.2 Functions of PML NBs

PML NBs are implicated in multiple cellular pathways such as DNA damage sensing and repair, apoptosis, regulation of gene expression, and tumour suppression (reviewed in *31*). The involvement of PML NBs is mediated via a variety of mechanisms in which PML NBs can be considered as depots for proteins, transcription factors and co-factors, as sites of post-translational modification of various proteins, modulators of histone chaperone function, and as protein compartmentalisation centres (reviewed in *32*).

PML NBs also regulate the innate immune responses to viral infection (*7, 37*). The genes encoding the PML and some of the PML NB-associated proteins belong to the group of interferon-stimulated genes (ISGs). The cell's PML and SP100 protein levels increase with the interferon stimuli (*38, 39*). Chapter 4 of the work will discuss the role of PML nuclear bodies in regulating the interferon response.

The PML-/- mice are viable but more susceptible to infections and tumorigenesis (*40*). Moreover, PML deficiency in mice protects the cells from apoptotic stimuli (*37*).

2.3 PML NB-associated proteins

This work exclusively discusses PML NB-associated proteins that are involved in regulation of type I interferon response and engage in viral infection. This chapter describes the selection of proteins.

SUMO

Small ubiquitin-like modifier (SUMO) proteins are constitutive partner proteins of PML NBs (*16*). They are covalently bound to lysine (K) residues as a post-translational modification, SUMOylation (*41*, *42*). Like ubiquitination, the binding is done through the action of three cooperating enzymes: an E1 activating enzyme (*43*, *44*), an E2 conjugating enzyme (*45*), and an E3 ligase (*46*, *47*). SUMO proteins participate in forming PML NBs; they mediate interactions of PML and PML NB-associated proteins by interacting with proteins' SIM domains (*31*).

SP100

Speckled protein 100 nuclear antigen (SP100) is a constitutive partner protein of PML NBs, the first discovered partner protein of the PML protein (*18*). The *Sp100* gene encodes eleven isoforms, but only four of them, SP100A, SP100B, SP100HBM and SP100C, have been investigated (*48*). The protein serves both as an activator and repressor of gene expression of cellular and viral genes (*49*, *50*).

DAXX/ATRX

The DAXX/ATRX complex is an H3-H4 chaperon, H3.3 specific (*51*). It is made up of two constitutive PML NBs-associated proteins: death-associated protein 6 (DAXX) and an ATP-dependent chromatin remodelling factor, alpha-thalassemia/mental retardation syndrome X-linked (ATRX). The ATRX localisation in PML NBs is DAXX-dependent (*17*). The histone chaperon mediates H3.3 histone deposition on heterochromatic regions of chromosomes, maintaining its silence (*52*).

HIRA

The Histone cell cycle regulator A (HIRA) complex is another H3-H4 histone chaperon which consists of three proteins: the histone cell cycle regulator A (HIRA), ubinuclein 1 (UBN1), and calcineurin-binding protein 1 (CABIN-1) (*53*, *54*). It also cooperates with a histone-binding protein, the anti-silencing function protein 1 homolog A (ASF1A) (*55*). Unlike DAXX/ATRX, the HIRA complex is a transient partner protein of PML NBs and associates with nuclear bodies in response to the type I IFN treatment and to the entry of a naked histone-free DNA to the nucleus (*56*).

H3.3

H3.3 histone is a non-canonical variant of histone H3, that differs in only four amino acid sites (*57*). Unlike the canonical forms, H3.1 and H3.2, which are expressed during the S-phase, H3.3 is expressed throughout the cell cycle (*58*). DAXX/ATRX or HIRA chaperon complexes deposit the H3.3 histone onto DNA in a DNA-synthesis-independent manner (*59*). The replacement of canonical H3 by H3.3 histone can be followed by tri-methylation of H3.3 at lysine 9 (H3.3K9me3) or phosphorylation at serine 31 (H3.3S31ph). The presence of H3.3K9me3 in chromatin is associated with inhibition of transcription (*60*). H3.3S31ph acts as a cofactor of acetyltransferase p300 and is associated with transcriptional activation (*61*).

3 Antiviral innate immunity

3.1 Pattern Recognition Receptors

Viruses encounter various barriers during the infection, such as epithelial surfaces and protective mucus layers. These barriers prevent the virus from adhering to cell surfaces and entering them. Once the virus breaches these barriers, the innate immune system detects the virus through pattern recognition receptors (PRRs) (Figure 3). Pattern recognition receptors are protein molecules that can bind specific "pattern" molecules characteristic of pathogens. The anti-viral PRRs identify viruses mainly through their nucleic acid. These PRRs are represented by Toll-like receptors (TLRs), retinoic acid-inducible gene-like receptors (RLRs), nucleotide oligomerisation domain-like receptors (NLRs), and a group of DNA receptors (reviewed in(*62*)).

TLRs recognise viral DNA and viral RNA in endosomes. Signal transduction leads to the activation of transcription factors: nuclear factor kappa-light-chain enhancer of activated B-cells (NF-κB), interferon regulation factor 3 (IRF3) and/or interferon regulation factor 7 (IRF7) to stimulate the expression of type I interferon and other cytokines (reviewed in(*63*)).

The RLR family of receptors, retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), recognise viral RNA. After sensing, they interact with an adaptor protein mitochondrial antiviral signalling (MAVS, also known as IPS-1), followed by activation of the IRF3 and NF-κB (*64*).

Viral DNA sensors, such as IFN-inducible protein 16 (IFI16) and cyclic GMP-AMP synthase (cGAS), relay signals through an adaptor protein known as a stimulator of interferon genes (STING) to activate the IRF3 (*65*).

Activated transcription factors are translocated to the nucleus and stimulate the expression of type I interferon and other cytokines.
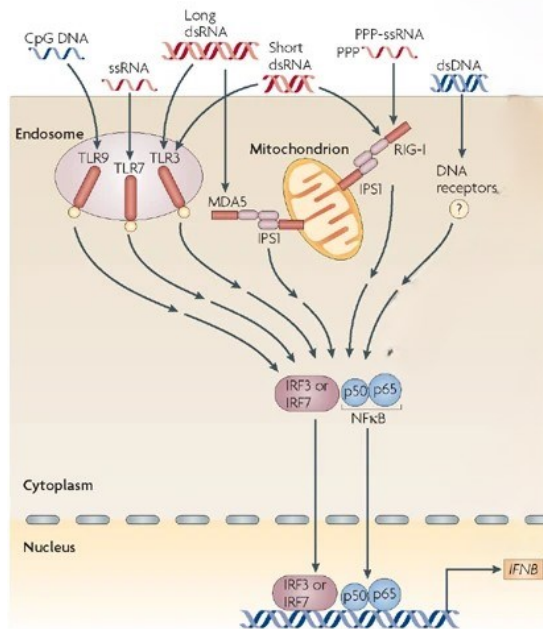
Figure 3. Anti-viral pattern recognition receptors. The figure shows the various pattern recognition receptors that respond to viral infection from DNA and RNA. The major forms of nucleic acids that act as PAMPs from viruses are illustrated along with the PRRs they bind to including TLRs, RLRs and DNA sensors. The PRR sensing ultimately leads to the activation of transcription factors that translocate to the nucleus and help initiate the transcription of type I interferons, e.g., IFNβ. Taken from: (66); edited.

3.2 Type I Interferons

Interferons are cytokines that cells produce in response to viruses and other pathogens. They initiate signalling cascades that repress viral replication and spread to surrounding cells. Based on their structure, receptor, and function, interferons are classified into three families: type I IFN (IFN-α, IFN-β), type II IFN (IFN-γ), and type III IFN (IFN-λ) (reviewed in (*67*)). Since this work focuses on type I INF signalling, type II and III IFNs will not be discussed.

The family of type I interferons comprises of IFN-α, -β, -κ, -ε and -ω. They all act by binding to the heterodimeric transmembrane receptor, the interferon-α receptor (IFNAR). The INF-signalling pathways are stimulated upon interferon molecule binding to the IFNAR. The receptor dimerises with the binding stimulus. The cytoplasmic domains of the receptor are associated with Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), which activate each other through phosphorylation and subsequently phosphorylate specific tyrosine residues on the IFNAR. Activated JAKs and TYKs then activate the canonical signalling pathways via signal transducers and activators of transcription (STATs), and the

non-canonical pathways like p38 cascade, MEK-ERK pathway or phosphatidylinositol-3 kinase (PI3K) pathway (reviewed in (1)).

The newly phosphorylated sites on IFNARs are binding sites for signal transducers and activators of transcription (STATs). For the type I IFN, STAT1 and STAT2 participate in the signal transduction. STATs dimerise upon phosphorylation, forming homodimers (STAT1) and heterodimers (STAT1-STAT2). Heterodimers interact with IFN-regulatory factor 9 (IRF9) and translocate into the nucleus as an IFN-stimulated gene factor 3 (ISGF3). The complex binds to a DNA sequence motif known as the IFN-stimulated response element (ISRE). Homodimers translocate into the nucleus without IRF9 and bind to a DNA sequence motif called gamma-activated sequence (GAS). ISRE and GAS are found in promoters of genes known as interferon-stimulated genes. The binding of ISGF3 and STAT1 homodimers to the ISRE and GAS motifs induces the expression of ISGs (Figure 4) (reviewed in(68)).
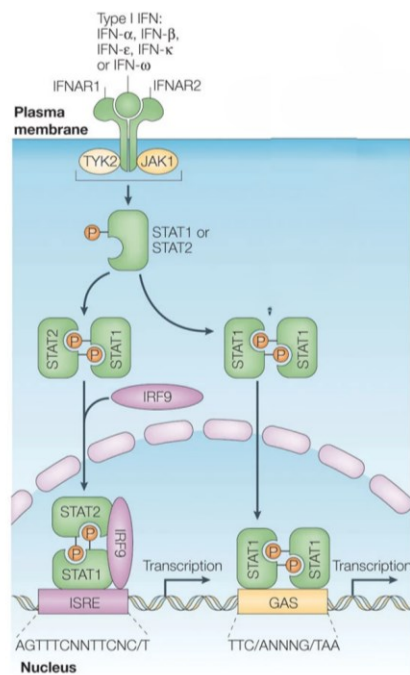

Figure 4. All type I interferons (IFNs) bind a common receptor at the surface of human cells, known as the type I IFN receptor. The type I IFN receptor comprises two subunits, IFNAR1 and IFNAR2, which are associated with the Janus activated kinases (JAKs) tyrosine kinase 2 (TYK2) and JAK1, respectively. Activation of the JAKs that are associated with the type I IFN receptor results in tyrosine phosphorylation of STAT2 (signal transducer and activator of transcription 2) and STAT1; this leads to the formation of STAT1–STAT2–IRF9 (IFN-regulatory factor 9) complexes, which are known as ISGF3 (IFN-stimulated gene (ISG) factor 3) complexes. These complexes translocate to the nucleus and bind IFN-stimulated response elements (ISREs) in DNA to initiate gene transcription. Type I IFNs also induce the formation of STAT1–STAT1 homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements that are present in the promoter of certain ISGs, thereby initiating the

3.3 Interferon-stimulated genes

Interferon-stimulated genes (ISGs) encode proteins that mediate the innate immune system's response. More than 300 ISGs are known (*69*). Among them are proteins that promote an antiviral state in the cell by interfering with viral replication at different stages or enhancing type I IFN signalling (*70*). However, several ISGs also contribute to viral infection (reviewed in (*71*)).

Besides the ISGF3, the expression of ISGs is activated by the NF-κB factor. The NF-kB translocation to the nucleus upon the PRRs signalling leads to stimulation of ISGs. This mechanism occurs simultaneously and independently of the interferon signalling (*72*). Some ISG products are expressed basally in the cell, like several PRRs (MDA5, RIG-I) or transcription factors (IRF3, IRF9), and the IFN stimulus upregulates their expression. At the same time, some ISGs are only IFN-inducible (*70*).

3.4 Regulation of Type I interferon response

Precise regulations mediate an appropriate and balanced antiviral immune response. In particular, regulation of type I IFNs involves post-translational modifications (PTMs) – phosphorylation, polyubiquitylation, methylation, and ISGylation – of signalling molecules, transcription factors, histones, and DNA methylation.

Phosphorylation and dephosphorylation

Phosphorylation is the driving modification of the JAK/STAT pathway. Besides the JAK phosphorylation of STATs, the MAPK pathway and the PKC-δ kinases modify the STAT1s to maximise the IFN-mediated transcriptional activity (*73*, *74*). Another kinase, the inhibitor of nuclear factor κBkinase-related kinase (IKK-ε), phosphorylates STAT1s to encourage the DNA binding activity of the molecules (*75*).

Dephosphorylation mainly suppresses type I IFN signalling. The suppressor of cytokine signalling proteins (SOCSs) are known to negatively regulate the JAK/STAT signalling pathway. SOCSs bind to the IFNAR and suppress STAT phosphorylation (*76*). Phosphatases from the protein tyrosine phosphatases (PTP) family, specifically the SH2 domain-containing phosphatases SHP1 and SHP2, dephosphorylate STAT1s in the nucleus (*77*).

Ubiquitylation and ISGylation

The process of covalent binding of the ubiquitin to the substrate is done through the action of three enzymes: an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ligase (reviewed in (*78*)). Ubiquitylation regulates the type I IFN response from different levels, including IFNARs, the signal

transducers, and the ISGs (reviewed in (*79*)). The STAT-interacting LIM (SLIM) protein modifies STAT molecules in the nucleus, leading to their proteasome-mediated degradation (*80*).

ISGylation is a process in which a ubiquitin-like modifier, ISG15, is covalently bound to the substrate. Like ubiquitylation and SUMOylation, the covalent binding is done via an E3 ligase (*81*). ISGylation frequently modifies JAK1 and STAT1 molecules and positively regulates activation as well as STAT1 binding to DNA (*82*). The ubiquitin-specific peptidase 18 (USP18) is specific for deISGylation (*83*) and in USP18 knockdown cells, the modification promotes prolonged activation of JAK1 and STAT1 (*84*).

SUMOylation

SUMOylation has a dual regulatory effect on type I IFN response. Viral infection increases SUMOylation of both IRF3 and IRF7. The modification of transcription factors is mediated via TLR and RIG/MDA-5 signalling (*85*). Moreover, the decrease in SUMOylation of the *ifnb1* gene leads to an increase in the transcriptional activity of the *ifnb1* gene. The promoter accumulates the H3K4me3 and H3ac marks, prolonging the gene's transcription (*86*). SUMOylation of STAT1 via the E3 ligase protein inhibitors of activated STAT1 (PIAS1) downregulates the signalling and ISG expression (*87*).

RIG-I is also a SUMOylation target. However, in this case, the modification mediates positive regulation, enhancing IFN-β production and the resistance to viral infection (*88*).

A binding site for the SUMO modification target protein SETDB1, a methyltransferase, was found between the *ifnb1* gene promoter and enhancer. SETDB1 binding overlaps with H3K9me3 heterochromatin marks. Experiments with SUMOylation-defective mutants showed that SUMOylation is essential for SETDB1 binding at the heterochromatin region and maintaining H3K9me3 modifications. SETDB1 is associated with TRIM28, and their interaction is mediated via SUMO-SIM. Inhibition of SUMOylation disrupts the SETDB1-TRIM28 repressive complex, thus regulating *ifnb1* expression (*89*).
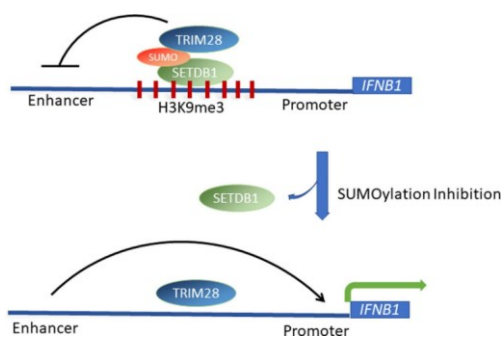

Figure 5. The SETDB1 and TRIM28 proteins interact via SUMO-SIM interactions and work together as a complex repressing gene expression. The complex binds to the heterochromatin region between the promoter and enhancer of the ifnb1 gene, overlapping with the H3K9me3 heterochromatin marks. SUMOylation inhibition affects the binding of SETDB1 to the

Acetylation and methylation

Acetylation and methylation are typically epigenetic markers, but both PTMs are also found to regulate type I IFN signalling on different levels. Histone deacetylase 9 (HDAC9) interacts with TANK-binding kinase 1 (TBK1) and activates it, which leads to enhanced induction of type I IFNs during viral infection (*90*). Phosphorylated IFNARs recruit the histone acetyltransferase CREB-binding protein (CBP), which acetylates the receptor. The acetyl group serves as a docking site for IRF9. Acetylation of STATs by CBP in their DNA-binding domain is essential for forming the ISGF3 complex. The IRF9 is also a target of acetylation by CBP to bind to STAT1 (*91*).

Methylation is found to play a crucial role in the moment of STAT1 translocation from the cytoplasm to the nucleus; arginine methyltransferase 1 (PRMT1) modifies STAT1 and triggers the translocation so the PIAS1 cannot interact with it and cannot inhibit the signalling (*92*).

Histone modifications

Regulation via the histone modifications is mediated on different levels of the type I IFN response. The *ifnb1* gene expression is regulated through the histone acetyltransferase GCN5. The acetylation of the H3 histone at lysine 9 (H3K9ac) and lysine 14 (H3K14ac) and of the H4 histone at lysine 8 (H4K8ac) positioned at the promoter enhances the IFN expression (*93*). Di-methylation of the H3K9 by the lysine methyltransferase G9a is a repressive modification, and its deficiency leads to enhanced type I IFN and ISG expression (*94*).

The histone modifications of ISGs are mediated through type I IFN signalling pathway components. The STAT2 proteins recruit a histone acetyltransferase GCN5 to acetylate the H3 histone to induce the expression of ISGs (*95*). The STAT1 proteins bind the E3 ubiquitin ligase PARP9-DTX3L complex to catalyse the ubiquitylation of the H2B histone to enhance the expression of ISGs (*96*).

DNA methylation

DNA methylation of 5-cytosine by DNA methyltransferases is a repressive DNA modification. In terms of the *ifnb1* gene promoter, it was found to be regulated by single nucleotide methylation. Deficiency of the UHRF1 protein, which recruits the DNA methyltransferases to the modification sites, results in demethylation and enhanced interaction of the IRF3 complex with the promoter (*97*).

4 Role of PML NBs in antiviral innate immunity

Type I IFN stimulation leads to PML NBs juxtaposing the ISG loci (*98*, *99*). PML NBs regulate type I IFN signalling on various levels, and this chapter summarises how PML protein and PML NB-associated proteins regulate the type I IFN response.

4.1 PML

PML protein positively regulates type I IFN signalling in various stages. PML protein facilitates the transcription of both STAT1 and STAT2 proteins. It then interacts with the STAT1 and STAT2 proteins in the ISGF3 complex and the HDAC1 and HDAC2 to induce the ISG expression (*100*). Although HDAC activity is known for its transcriptional repression, interestingly HDACs play a positive role in the ISG transcription in response to type I IFN signalling (*101*, *102*). The HDAC1 interacts with STAT1 and STAT2 proteins and co-activates the expression of ISGF3-stimulated ISGs (*102*).

Besides the transcription regulation, the PML-ISGF3-HDAC complex also regulates expression epigenetically. After initial IFN stimulation, HDACs modify the ISGs at H3 histone with a tri-methylation on lysine 36 (H3K36me3) and by positioning the H3.3 histone onto the chromatin (*101*, *102*). Both modifications mark an IFN epigenetic memory, enabling faster recruitment of RNA polymerase II and other transcription factors to the promoters (*101*).

The PML-II isoform positively regulates the expression of IFN-β and interferon signalling. The isoform promotes the association of IRF3 and NF-κB transcription factors with the CBP co-activator to form transcriptional complexes at the IFN-β promoter. Moreover, PML-II regulates the downstream JAK/STAT signalling pathway as the activation of ISRE elements of ISGs depends on the presence of the isoform. PML-II also interacts with the transcriptional complex ISGF3 containing the STAT1 molecules. Overall, the PML-II isoform stabilises the transcription complexes and binds them to gene promoters, and the specific C-terminal domain was found to be behind the interactions (*103*).

The PML-IV isoform positively regulates the expression of IFN-β upon viral infection. The isoform requires SUMO modifications to successfully enhance the IFN synthesis. PML-IV enhances the IFN production by increasing the IRF3 activation. It is the only protein isoform that sequesters the PIN1, a prolyl isomerase that promotes the degradation of phosphorylated IRF3, to the PML NBs, thus suppressing the PIN1-induced degradation of IRF3 (*104*).

4.2 ATRX

The ATRX protein co-activates the innate immune response. Depleting ATRX in cells suppresses the cytosolic DNA sensing cGAS-STING pathway as the phosphorylation of IRF3 rapidly

decreases, reducing the mRNA levels of IFN-β. The same results were obtained for the RNA sensing RIG-I/MDA-5 pathway (*105*).

The ATRX protein also interacts with the IRF3 complex and positively regulates IRF3-mediated ISG expression, independently of type I IFN signalling. In addition to the indirect regulation of ISG expression, ATRX directly regulates a distinct set of ISGs, like *CCL8* and *TNFSF10*, upon IFN-β stimuli. Interestingly, the regulation via ATRX is independent of the DAXX protein, as the expression of studied ISGs did not change in DAXX knockdown cells (*105*).

Although ATRX is known to repress gene expression via heterochromatinisation, upon IFN-β treatment, the protein positively affects the chromatin accessibility of the *ifnb1* gene and ISGs. ATRX, thus, most likely modifies the binding of transcription factors. Moreover, the protein binds directly to the IFN-β promoter region, mediating the gene transcription as well (*105*).

4.3 SP100A

The SP100A protein, another PML NB-associated protein regulating the type I IFN signalling, is the only isoform that localises in both cytosol and nucleus. The type I IFN response initiates an active translocation of SP100A to the nucleus soon after the cell is exposed to the IFN-β. Moreover, the protein can be transported back to the cytosol (*106*).

The SP100A protein interacts directly with the pyruvate kinase 2 (PKM2), and experiments showed that SP100A translocation to the nucleus utilises the ERK1/2-PKM2-PIN1-importin axis in response to the type I IFN. The interaction and subsequent translocation of SP100A are regulated via the phosphorylation of serine 188 on SP100A and the PI3K pathway. IFN signalling also activates inhibitors, including GDC0941, which blocks the PI3K pathway and thus both positively and negatively regulates the translocation of SP100A to the nucleus (*106*).

In the nucleus, SP100A protein binds to the promoter regions of antiviral ISGs, like *OAS2*, *ISG15* and *RIG-I*, with higher affinity in response to the IFN-β and increases the mRNA levels of these genes. Surprisingly, in the absence of a DNA binding domain in the SP100A protein, the isoform is associated with the promoter regions independently of the PML proteins and the PML NBs. Similar findings were obtained from cells infected with the Vesicular stomatitis Indiana virus (VSV) (*106*). Figure 6 shows a proposed model of the SP100A translocation to the nucleus in response to the type I IFN stimulation and the SP100A regulation of ISGs expression.
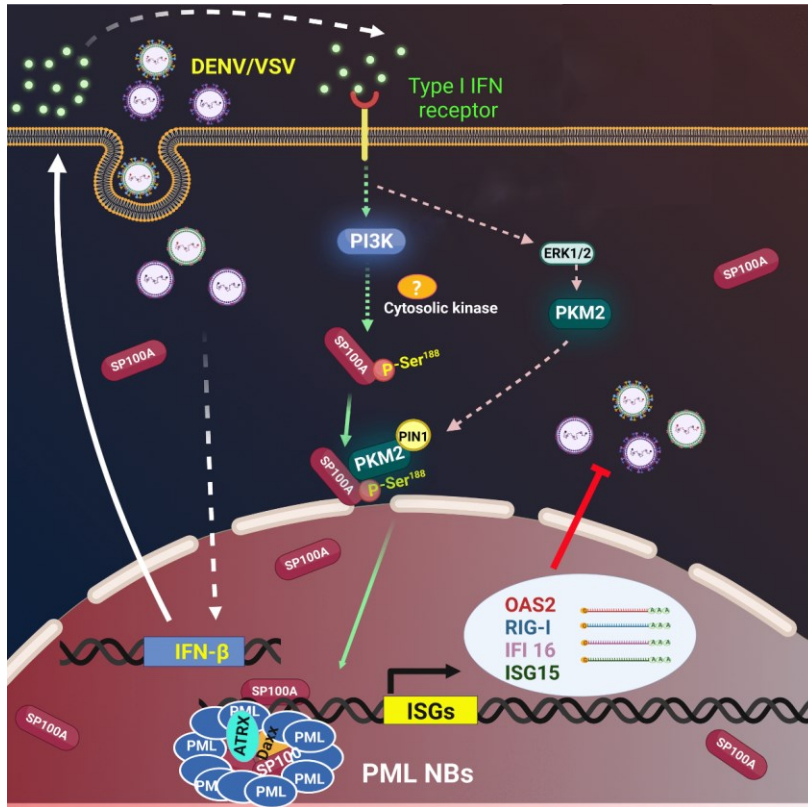
Figure 6. Proposed model of the SP100A translocation to the nucleus in response to the type I IFN stimulation and the SP100A regulation of ISGs expression. The infection with the VSV virus triggers the IFN-β production and secretion. IFN-β binds to the type I IFN receptor and stimulates the PI3K signalling pathway and the ERK1/2, activating the PKM2. SP100A is phosphorylated on serin 188 by an unknown cytosolic kinase, and the modified protein is translocated to the nucleus via the ERK1/2-PKM2-PIN1-importin axis. SP100A then interacts with a distinct set of antiviral ISGs at their promoter regions and upregulates their transcription. Taken from (106); edited.

4.4 HIRA

PML NBs and HIRA chaperone complex regulate the expression of ISGs via H3.3 histone deposition. HIRA complex accumulates in PML nuclear bodies via the SUMO-SIM interactions upon the type I IFN stimuli. Whether HIRA is sequestered into PML NBs depends on the SP100 and DAXX protein levels in the PML NBs and the H3.3 levels in the nucleoplasm. The SP100 protein serves as a positive regulator of the PML NBs-HIRA interaction. In contrast, the DAXX protein and the H3.3 histone in the nucleoplasm are negative regulators of the HIRA accumulation in PML NBs (*99*).

Upon the type I IFN stimuli, the PML NBs are juxtaposed to the interferon-stimulated genomic loci. Interestingly, the existing PML NBs do not seem to be involved in the regulation; instead, the PML protein molecules initially involved in the transcription regulation of ISGs form new PML NBs and the

HIRA complex is accumulated in them. The H3.3 histone deposition via the HIRA complex then happens in the transcription end sites region of ISGs. Moreover, the deposition of H3.3 persists for up to 48 hours after the type I IFN stimulus, extending well beyond the peak of ISGs transcription, and is concomitant with an H3 histone modification, the memory marker H3K36me3, mediated by the methyltransferase SETD2 (*99*).

Although the HIRA complex mediates the deposition of H3.3 histone onto the ISG loci, its accumulation in juxtaposed PML NBs is unnecessary for the histone loading onto the chromatin. Figure 7 presents a possible model describing the role of PML NBs as HIRA depots and regulators of ISG expression, and HIRA complex upon the type I IFN stimuli (*99*).
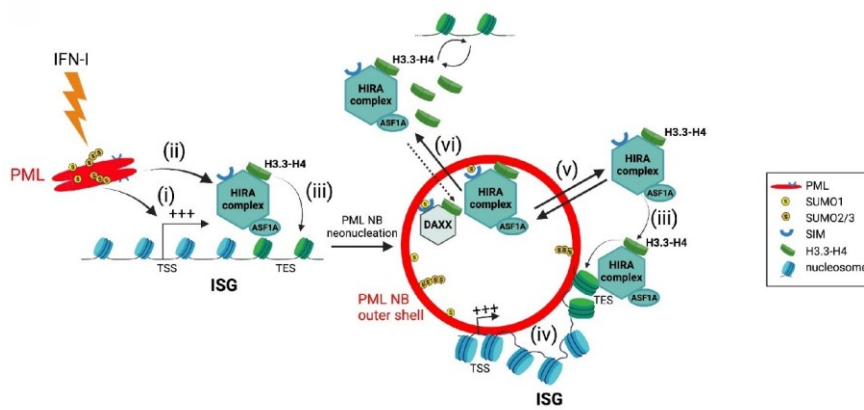

*Figure 7. Model for the dual role of PML/PML NBs in type I interferon response. At early time points after an initial type I IFN stimulus, (i) PML is required for ISGs transcription and (ii) this could indirectly help to load HIRA on ISGs participating in H3.3 dynamics. (iii) While HIRA depletion does not affect ISGs transcription per se, it could participate in H3.3 deposition at ISGs, a function which does not seem to require its accumulation in PML NBs. (iv) PML neonucleation would mediate juxtaposition of PML NBs with ISGs at late times after type I IFN treatment which could help to keep a memory of the physiological state of the cell. (v) In addition, PML NBs play a second independent role by buffering the extra pool of HIRA complex available in the nucleus. (vi) Increase of DAXX protein levels could modulate the amount of available binding sites for HIRA within PML NBs or overexpression of the HIRA substrate H3.3 as a pool of free soluble H3.3 in the nucleoplasm could force HIRA out of PML NBs. Taken from (*99*).*

5 Modulation of PML NBs by viruses

PML protein induces resistance to viral infection (*7*). Experiments with PML knock-out cells showed that the interferon response was diminished in the absence of PML protein (*107*). This observation is evidence of the importance of PML protein and the PML NBs in the antiviral state. It explains why viruses target PML NBs and disrupt or modulate them upon entering the nucleus. This chapter describes how DNA viruses interfere with the innate immune response by modifying PML NBs during infection. The chapter does not discuss the molecular mechanisms of viral infection but only mentions viral proteins that interact with PML NBs.

5.1 Herpesviruses

The *Herpesviridae* family is a group of viruses with a double-stranded DNA genome. This work will focus on the herpes simplex virus 1 (HSV-1) and the human cytomegalovirus (HCMV). The role of PML nuclear bodies during infection has been studied the most with these two viruses from the *Herpesviridae* family.

5.1.1 Herpes Simplex Virus 1

When the HSV-1 genome enters the nucleus, immediate early genes are expressed. The immediate early infected cell protein 0 (ICP0) is an E3 ubiquitin ligase (*108*). ICP0 positively regulates viral and cellular gene transcription and enhances viral replication (*109–111*). The protein also induces the lytic programme from latency (*109*). During the latent programme, only latency-associated transcripts (LATs), non-coding RNAs that establish the latency and reactivation, are expressed (*112*).

PML NBs and ICP0

Upon entry to the nucleus, the HSV-1 genome associates with the PML NBs (*113*). The viral ICP0 disrupts the PML NBs by degrading PML, SP100, and SUMO proteins through its SUMO-targeted E3 ubiquitin ligase (STUbL) activity (*114–116*). The ICP0-mediated PML degradation reduces the effect of type I IFN treatment on viral replication (*117*).

The ICP0 interacts with SUMO proteins via SIM motifs and ubiquitinates SUMO to initiate protein degradation. The viral protein also degrades the PML-I isoform independently of SUMO modifications (*114*). On the other hand, the ICP0 protein does not disrupt the DAXX/ATRX complex (*118*).

In an ICP0-null HSV-1 mutant, the PML and SP100 proteins increase viral replication repression (*119*, *120*). Proteins are translocated to the sites of early replication compartments of the viral genomes, forming PML NB-like complexes (*119–121*). Other PML NB-associated proteins, like the

Okomentoval(a): [PM1]: Here is the info about PML-mediated IFN response. Is it okay if it's here or is it too ‚hidden'?

DAXX/ATRX complex, also translocate to the viral genomes. The presence of ATRX at the early replication compartments depends on DAXX (*118*).

Cells depleted of PML protein and infected with the ICP0-null mutant showed that the PML-I and PML-II isoforms could partially reverse the increased viral replication. The PML-I isoform restrictive activity is implemented via SUMO modifications and SIM domains (*122*). Depletion of SP100, DAXX and ATRX also increases viral replication (*118*, *120*).

DAXX/ATRX and HIRA chaperon complexes repress the HSV-1 genome expression

The DAXX/ATRX complex in the early replication compartments interacts with promoters and restricts viral gene transcription by facilitating heterochromatin. The deposited histones H3 are modified on lysine 9 and 27 by three methyl groups (H3K9me3; H3K27me3), resulting in expression silencing. ATRX maintains the viral heterochromatin during the infection with the help of PML protein. However, neither the DAXX/ATRX chaperon complex nor PML protein is uniquely required to form heterochromatin on the viral genome (*123*).

The HIRA complex is also involved in the chromatinisation of HSV-1 genomes. The presence of the HSV-1 genome in the nucleoplasm and the type I IFN response trigger the HIRA complex co-localisation in PML NBs. The HIRA complex then binds to the naked HSV-1 and depositions the H3.3 histone on the genome, resulting in gene expression repression (*56*).

PML nuclear bodies during the latent cycle of HSV-1

During the latent cycle, PML NBs and HSV-1 genome molecules interact and make a structure called viral DNA-containing PML NBs (vDCP NBs) (*124*, *125*). The PML protein outer shell wraps around the viral DNA. There are multiple copies of the viral genome in the nucleus during the latency, and the copies are distributed in two patterns: a "single" (S) pattern and a "multiple latency" (ML) pattern. The S pattern showed one spot where the viral genomes were accumulated in the nucleus, and these copies were part of vDCP NBs. The ML pattern detected up to 30 spots with accumulated genome copies; only some were formed vDCP NBs (*124*).

The formation of vDCP NBs leads to transcriptional suppression of LATs. LATs are expressed only in ML pattern neurons from genomes not trapped in the vDCP NBs (*124*). The suppression of HSV-1 is caused by chromatinisation of the genomes via the DAXX/ATRX and HIRA complexes found in vDCP NBs. The H3.3 histone co-localizes with the chaperon complexes in vDCP NBs and is deposited onto the HSV-1 genome. H3.3 histone is post-translationally modified on lysine 9 with three methyl groups (H3.3K9me3), resulting in transcriptional silencing of HSV-1 (*126*). The depletion of either one or both histone chaperone complexes leads to a decrease of H3.3 association with the HSV-1 genome and destabilisation of the vDCP NB (*126*).

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The transcriptional repression in vDCP NBs is not permanent, and the expression can be recovered via ICP0. The expression of ICP0 destabilises the vDCP NBs, the HSV-1 genome is de-repressed, and the transcription of viral genes is recovered together with HSV-1 replication (*126*).

5.1.2 Human Cytomegalovirus

During the lytic cycle, upon the entry of the HCMV genome into the nucleus, the expression of genes encoding the immediate early (IE) proteins begins. IE proteins regulate the transcription of viral and cellular genes.

PML NBs and the IEs

Within the first hours of the infection, the IE1 and IE2 proteins colocalise with PML NBs and cause their disruption (*127*, *128*). The IE1 protein interacts directly with the PML and SP100 protein (*129*, *130*), abrogating the PML SUMOylation (*130*, *131*).

To start the expression of IE genes, the viral protein PP71 interacts with the DAXX protein and induces its degradation. This way, DAXX cannot repress the synthesis of the IE proteins through the HDAC (*132*).

The IE1 protein inhibits IFN-β-induced ISG expression, and it was found that IE1 requires the PML protein to repress the ISG expression efficiently. The IE1 interacts with the PML protein, the ISGF3 complex and the HDAC1 and HDAC2, repressing the proteins from binding to the ISG promoters. The type I IFN induced expression of ISGs is PML dependent (*100*).

HCMV gene repression depends on PML NBs, specifically the PML and SP100 proteins. The PML and SP100 protein production was upregulated with the IFN-beta treatment. The ability to inhibit the HCMV infection is abrogated when the IFN production is blocked, leading to the level of PML and SP100 proteins not being upregulated in cells. The protein levels of DAXX did not increase during the IFN-beta treatment (*133*).

5.2 Adenoviruses

The *Adenoviridae* family comprises medium-sized viruses with double-stranded DNA genomes. The viral proteins are expressed at different phases of the viral replication cycle and thus are separated into three groups: early, intermediate, and late. In this chapter, only human adenoviruses (HAdV) will be described.

The early viral protein E1A (early region 1A) activates the transcription of other early viral genes (*134*). Two isoforms of the early viral E1A protein, E1A-13S and E1A-12S, are produced from the mRNA transcripts via alternative splicing. The E1A-13S isoform contains a unique conserved region 3 (CR3), an interaction site for a wide range of transcription factors to activate transcription (*135*).

Other early viral proteins are E2A (early region 2A), E1B-55K (early region 1B-55K), E4 ORF3 and ORF6 (early region 4 open reading frame 3 and 6). The early viral protein E2A localises at the replication centres of HAdV (*136*).

PML NBs and E4 ORFs

The E4 ORF3 viral protein colocalises with PML NBs upon viral infection (*137*). E4 ORF3 quickly disrupts the PML NBs into PML fibrous-like tracks (*128*, *129*) by directly interacting with the PML-II isoform (*138*). PML protein mediates the type I IFN-induced antiviral state (*139*), and the E4 ORF3 protein antagonises the type I IFN response to the infection (*140*) via the interaction with PML-II isoform. Overexpression of the PML protein blocks the PML NBs disruption (*137*). Interestingly, the disruption of PML NBs and reduction of DAXX, but not SP100, restores the viral replication in both IFN-unstimulated and stimulated cells transfected with mutated E4 ORF3 protein (*139*).

PML and E1A-13S

Upon early viral protein expression, the E1A-13S isoform co-localises to the PML NBs, explicitly interacting with the PML-II isoform through the CR3. Besides stimulating the viral gene expression, the E1A-13S-PML complex also interacts with cellular transcriptional coactivators, like p300, and enhances their stimulating activity when inducing the expression of cellular genes (*141*).

Moreover, the PML-II isoform positively regulates the replication of HAdV, as the experiment with PML knockdown cells showed a modest effect on the viral replication. Interestingly, PML-II isoforms containing mutant SIM domains stimulated the E1A-13S-dependent transcription of viral proteins more efficiently than the wild-type isoform (*141*).

PML, SP100A and E2A

A recent study found three SUMO conjugating motives in the E2A protein sequence. The E2A interacts with the UBC9 conjugating enzyme but not with the PIAS4 ligase. The SUMOylation of E2A, a novel finding, controls the binding to PML protein and thus participates in the recruitment of PML to replication centres. The modification of E2A does not affect the DNA binding capacity of the viral protein. Experiments showed that viral progeny production depends on SUMOylation of E2A as the late protein expression was lower than the wild-type infection, resulting in less viral progeny production. However, mutations in SUMO conjugating motives do not impair the E2A expression. The E2A SUMOylation also promotes the interaction of E2A with SP100A protein that is localised in PML tracks. The SP100A protein level was higher during wild-type infection than during the infection of HAdV with mutant SUMO conjugating motives. Based on the gathered information, a model describing the role of E2A SUMOylation mediating the localisation of PML tracks by the viral replication centres has been proposed (Figure 8) (*142*).
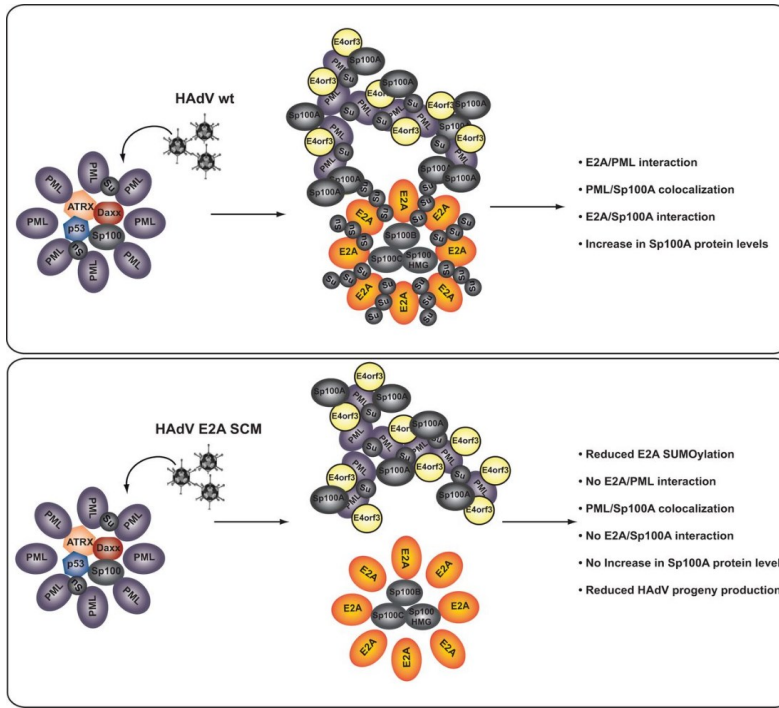
Figure 8. Model of E2A SUMO modification-mediated localisation of PML tracks next to viral replication centres during infection. Shown is a schematic representation illustrating a proposed model linking E2A SUMOylation during infection with PML-NB subcellular localisation. The model shows the influence of E2A SUMO PTM on PML-NB localisation adjacent to HAdV RCs by E2A SUMOylation-dependent E2A/PML and E2A/Sp100A interactions. E2A SCM mutations prevent the virus from using beneficial components from PML-NBs, leading to defects in virus gene expression and progeny production. Taken from (142).

DAXX and E1B-55K

The E1B-55K viral protein also colocalises with PML NBs (*137*). Experiments showed that the DAXX protein negatively regulates the HAdV replication, specifically in the early phase of the infection, as cell lines with reduced DAXX produced more mRNA for early viral proteins. The DAXX protein level is reduced at late times of the infection as the protein is a target of proteasomal degradation, which depends on the presence of E1B-55K protein but not E4 ORF6 (*143*).

5.3 Polyomavirus

Polyomaviruses are small viruses with a circular double-stranded DNA genome. The genomes encode early gene products, like large T protein, which participates in gene expression and interferes with the host immune system response, and late gene products, like VPs capsid proteins and agnoprotein (reviewed in (*144*)). In this chapter, only the JC virus will be described.

Experiments with arsenite showed that the JC infection is not affected by IFN-β when the PML protein is not present in the cell. The IFN-β response to JC infection is PML-dependent. The early viral protein large T is accumulated in microdomains near PML NBs. The depletion of PML protein does not affect the localisation of viral protein. However, the type I IFN treatment caused the viral microdomains to associate more closely with the PML NBs (*145*).

During the late phase of the viral replication cycle, the VP1 protein accumulates in PML NBs only in the presence of VP2/VP3 and agnoprotein. PML NBs are where the capsid proteins assemble into virions (*146*).

Conclusion

Intensive research on antiviral innate immune response outlined the complicated mechanisms of regulation. It is maintained through various types of post-translational regulations of the components of signalling cascades as well as through epigenetic modifications of chromatin on the levels of post-translational modifications of histones and DNA methylation.

PML nuclear bodies are positive regulators of type I interferon response and maintain their function by different ways. They promote targeting of the transcription complexes on the promoters of ISGs and histone modifications and prevent the degradation of transcription factors.

Interestingly, the research provided information about proteins that are known to repress gene expression, like ATRX and HDACs, positively modifying the transcription of type I IFN and ISGs. Recently discovered participation of the HIRA chaperone complex in regulating ISGs expression explains the mechanism behind the upregulation and suggests a mechanism of memorising the cell's physiological state upon type I IFN signalling.

Despite significant progress, there are still unresolved aspects of PML nuclear bodies. The granular structures in the core of PML NBs have been a subject of ongoing debate and research due to conflicting results. Initially, the granular inside was detected as RNA molecules (*147*). However, subsequent studies have contradicted this, stating that PML NBs do not contain nucleic acid molecules in their core (*148*). As of now, there is no consensus on the components of the granular structures in the core of PML NBs, highlighting the need for further investigation in this area.

SUMO modification regulates interferon signalling. In the nucleus, the main factories for SUMOylation are PML NBs. However, the relationship between PML NBs and SUMOylation of signal mediators and/or transcription factors involved in the interferon response is not well established and requires future studies.

DNA viruses, such as herpesviruses, adenoviruses or polyomaviruses, place their entering genomes in the vicinity of the PML NB and establish replication centres there. One of the consequences is the modulation of the PML NBs leading to abrogation or inhibition of the function of the bodies in the regulation of the interferon response and is beneficial for viruses. The exact molecular mechanism of this process is well understood for herpesviruses, which are examples of large DNA viruses. For small DNA viruses (e.g. polyomaviruses) this mechanism needs to be elucidated.

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