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Review

Viruses as Hijackers of PML Nuclear Bodies

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Abstract. Promyelocytic leukemia nuclear bodies (PML-NBs) are discrete interchromosomal macromolecular structures. The integrity of this dynamic nuclear subcompartment critically depends on the presence of the name-giving PML protein. Among the permanent or transient residents of PML-NBs are various regulatory proteins, including Sp100, CBP, pRb, HIPK2, RAD51 and p53. PML-NBs are frequently targeted by viral infections, as a number of different RNA and DNA viruses, including herpesviruses, adenoviruses, papovaviruses, papillomaviruses and arenaviruses, cause changes in PML-NBs. Viruses interfere with PML-NB in two ways: 1) some viral proteins can associate with PML-NB proteins and/or lead to the destruction and lysis of this subnuclear compartment, thus aiding viral gene expression and disabling the host's innate immunity; 2) the parental genomes of some nuclear-replicating DNA viruses associate preferentially with PML-NBs, which presumably serves to assist in viral gene expression or replication. Here we feature the different viral strategies leading to the hijacking of PML-NBs and discuss the consequences for the immune response.

Key words: promyelocytic leukemia (PML); PML nuclear bodies; virus infection; SUMO; nuclear bodies.

Structure and Composition of PML Nuclear Bodies

Within the interphase nucleus, each chromosome is arranged within a certain chromosome territory, and active genes are thought to reside throughout the surface of the loosely packed territories[12]. The space between the chromosome territories, the interchromatin compartment, forms a spatially restricted fiber system consisting of a few loop-like arrays traversing the nucleus, presumably forming a continuous interconnected tunnel system[35]. The interchromatin space contains a large number of distinct so-called nuclear domains which are not surrounded by membranes, including Cajal bodies, the splicing factor compartment, and promyelocytic leukemia nuclear bodies (PML-NBs) (for a comprehensive list, see[24]).

PML-NBs (also known as nuclear dots, nuclear domain 10, PML oncogenic domains, Kremer bodies, or simply PML bodies)[7, 27, 40] were originally described 1960 by de Thé and colleagues and "rediscovered" many years later by using sera from autoimmune patients[4, 37]. PML-NBs typically appear as 10 to 30 small dots with sizes between 250 and 500 nm. The number, size and composition of the PML-NBs change during the cell cycle.

Constitutive PML-NB proteins are PML and Sp100[7]. Among the other, mostly transient to PML-NBs recruited proteins are transcription factor p53, the tumor suppressor Rb, the histone acetylase and co-activator CBP, heterochromatin protein 1 (HP1), Daxx, the Bloom syndrom helicase BLM, the serine/threonine kinase HIPK2, the deubiquitinating enzyme HAUSP(USP7), and DNA repair proteins such as RAD51 and RP-α[15, 21, 41].

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The ubiquitin-related protein small ubiquitin-like modifier-1 (SUMO-1), which covalently modifies several PML body proteins, also resides in these structures[22, 30, 43]. Depending on the target, SUMOlation of proteins can have in several consequences, including changes in localization, enzymatic activity, protein stability or alterations in protein/protein interactions[26]. The PML protein contains several important domains which form the tripartite motif. This is characterized by the presence of a zinc-binding domain that includes the really-interesting gene (RING) finger motif followed by two cysteine/histidine-rich B-boxes and a coiled-coil domain containing a nuclear localization signal[40]. PML can be SUMOlated at three sites: Lys65 in the RING finger domain, Lys160 in the B1 Box, and Lys490 in the nuclear localization signal[22].

The integrity of PML-NBs depends on the presence of PML, as PML-NBs are not detectable in murine PML-deficient cells, where PML-NB-resident proteins occur as hundreds of aberrant tiny dots residing both in the nucleus and the cytoplasm, referred to as a "microspeckled" distribution. Reconstitution experiments in $PML^{-/-}$ cells showed that only the wild-type PML protein, but not a PML mutant that can no longer be modified by SUMO-1, allows the proper formation of PML-NBs[49]. PML-NBs are disrupted in acute promyelocytic leukemia (APL) cells carrying a t(15,17) translocation. This event creates a fusion between the PML protein and the retinoic acid receptor (RAR)-α, showing that PML is required for the structural integrity of PML-NBs in human cells[14]. The vast majority of APLs containing PML/RARα fusion proteins can be successfully treated with pharmacological doses of retinoic acid, which leads to the re-formation of PML-NBs and remission of the tumor[27, 50], implying that the structural integrity of the PML-NB is relevant for its cellular functions. PML-NBs were proposed to function as sites of transcriptional activation, repression, protein storage pools, post-translational protein modification platforms, or aggregates containing accumulations of misfolded proteins[7, 16, 33, 40, 49].

This enormously broad range of potential functions might be explained by the existence of multiple subclasses of PML-NBs. Experimental support for such a heterogeneity was provided by experiments analyzing their dynamic properties within living cells, which revealed stationary, slow-moving and metabolic-energy-dependent dynamics of PML-NBs[32]. Further evidence for the existence of PML subpopulations was added by a study showing that only the subset of PML-NBs containing nuclear DNA helicase II displays transcriptional activity[19]. While the function of PML-NBs does not yield a coherent picture, the physiological relevance of these subnuclear structures was revealed from the analysis of knockout mice and from human disease models. The importance of PML as a regulator of senescence, apoptosis, cell proliferation and transformation suppression was shown in numerous studies, and emerging evidence is uncovering a role for PML in innate immunity[7, 16, 33, 41].

The role of the PML-NB-resident Sp100 for the immune response was revealed by the occurence of anti-Sp100 autoantibodies in patients suffering from the autoimmune liver disease primary biliary cirrhosis (PBC). Epitope mapping has revealed a polyspecific response against the Sp100 protein, which suggests an autoantigen-driven immunization mechanism[45]. Besides Sp100, also anti-PML and anti-SUMO antibodies are frequently found in PBC patients[44]. One contribution of PML to the immune response might be its relevance to the expression of the MHC class I heavy chain[10], but this finding has been challenged[9]. Transcription of many PML-NB proteins, including PML and Sp100, is upregulated by interferons (IFNs), a family of secreted proteins with antiviral and immunomodulatory activity. Accordingly, IFN administration results in a significant increase in both the number and the size of the PML-NBs, which helps to fight the virus attack[3, 38].

Viral Counterattack to the IFN Response

Viruses have evolved an impressive variety of strategies which allow them to interfere with the host signaling pathways in order to ensure two goals: 1) viruses use the cellular machinery to replicate and propagate their genetic material and 2) they undermine the cellular defense mechanisms, such as the onset of the immune response or the induction of apoptosis. Since proteins contained in PML-NBs contribut to these biological processes, PML-NBs are frequent targets of viral proteins.

The involvement of PML-NBs in innate immunity relies at least to a substantial part, on their contribution to the IFN response. Type 1 (IFN-α and IFN-β) and type 2 (IFN-γ) interferons are cellular secreted cytokines which initiate an antiviral state in order to prevent virus spread and replication[3]. Their antiviral role has been demonstrated in mouse models, as animals lacking IFN receptors display a drastically increased susceptibility towards viral infections[31]. Any stage in virus replication is a target for the antiviral activity of IFNs, including virus entry, gene expression, maturation, ass-

embly and release. Accordingly, IFNs trigger several antiviral effector pathways, including the 2'5' A synthetase pathway, which leads to the activation of the endonuclease RNase L, the activation of RNA-dependent protein kinases, and increased expression of the antiviral matrix protein, which is found in association with or juxtaposed to PML-NBs[38]. IFNs also trigger transcription of the PML-NB proteins PML, Sp100 and its splice variants Sp110 and Sp140, as well as ISG20 and the proteasome activator PA28[43], which negatively interferes with viral gene expression and impairs virus protein stability. On the other hand, viruses have evolved a battery of different strategies, counterstriking all levels of the antiviral effects of IFNs, including ursupation of the PML-NBs, as will be discussed in this review.

Viral Strategies to Manipulate PML Functions

Disruption of PML-NBs

Very soon after the discovery of PML-NBs, the pioneering work of Gerd Maul and coworkers showed that infection with herpes simplex virus (HSV)-1 causes disruption of PML-NBs[25]. Furthermore they observed that HSV-1 infection leading to lysis of PML--NBs could not be observed with a HSV-1 mutant lacking the regulatory infected cell protein 0 (ICP0)[17]. Follow-up studies revealed that expression of the RING finger protein ICP0 alone (previously named Vmw110) is sufficient for the lysis of PML-NBs. Mapping experiments assigned this effect to the C-terminus of the viral protein. Here we will discuss the activities of ICP0 in further detail, since this viral protein exerts important biological functions and is one of the best-studied examples of a virus protein-induced disruption of PML-NBs. ICP0 activates the expression of many genes, stimulates lytic infection of the virus and participates in the reactivation of the latent virus in mouse models[34]. The good correlation between the ability of ICP0 mutants to disrupt PML-NBs and their capacity to promote viral growth and gene expression suggests that PML-NB disruption is of biological importance. Supporting evidence for this concept is being provided by an increasing number of studies reporting ursupation of PML-NBs by regulatory proteins from a variety of different viruses. ICP0 lyses PML-NBs upon proteasome-dependent degradation of Sp100 and several PML isoforms, dependent on the RING finger of ICP0[11]. The ICP0 protein specifically abrogates the SUMO-1 modification of PML and Sp100, and its potential to alter SUMO-1 modification directly correlates with its capacity to disassemble PML-NBs[29, 36]. A clue in understanding the biochemical function of ICP0 came from the finding that ICP0 possesses *in vitro* E3 ubiquitin ligase activity, activates the E2 ubiquitin-conjugating enzyme cdc34 and directly binds to the proteasome[47]. ICP0 contains an additional distinct E3 ligase activity specific to the UbcH5a- and UbcH6 E2-conjugating enzymes, an activity that maps to the RING finger domain[8, 20]. ICP0-deficient viruses are particularly sensitive to inhibition by IFN[28], suggesting that ICP0-mediated disruption of PML-NBs serves to bypass the antiviral effects of IFN and thus allows virus production. The experiments discussed so far show a clear correlation between the necessity of ICP0 for virus replication and its PML-NB disrupting activity. Further relevance for the necessity of PML-NB destruction could be obtained from experiments investigating HSV susceptibility in cells expressing a PML mutant not destructable by the ICP0 protein. Besides the well--studied ICP0 protein from herpes virus, ICP0-related proteins from other viruses, including varicella zoster virus, pseudorabies virus, and type 1 equine and bovine herpesviruses, also display the ability to disrupt PML--NBs[36]. Although the various ICP0-related proteins increase the concentration of PML-colocalizing ubiquitin, they do not directly contribute to PML ubiquitination and, therefore, their PML-NB-disrupting activity employs mechanisms distinct from that of HSV ICP0.

The recent decade has witnessed the identification of a steadily growing number of viral proteins which lead to the disruption of PML-NBs, but the molecular mechanisms underlying these activities are less well understood than that of HSV ICP0. These viral proteins include the human cytomegalovirus (HCMV)-encoded major immediate-early protein 1 (IE1)[2]. Expression of this viral protein abrogates the SUMO-1 modification of PML and Sp100[29], but the disruption of PML-NBs by HCMV IE1 does not involve proteasome-dependent degradation of PML[48]. The IE1 is also modified by SUMOlation, but its ability to disrupt PML-NBs is independent of this posttranslational modification. Another example of a viral protein disrupting PML-NBs is the Epstein-Barr virus immediate-early protein BZLF1, which is also subject to SUMO-1 modification[1].

The high incidence of viral targeting of PML-NBs suggests that the demolition of these subnuclear structures serves to destroy their antiviral properties. Evidence for such a function was obtained in experiments using $PML^{-/-}$ cells. Mouse embryonic fibroblasts (MEFs) from $PML^{-/-}$ mice produced 20 times more

rabies virus and expressed viral proteins at a higher level compared with wild-type cells[5]. A more systematic analysis elucidating the role of PML *in vivo* compared the immune responses and virus loads of PML--deficient and control mice infected with lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus[6]. $PML^{-/-}$ animals show accelerated primary footpad swelling reactions in response to low doses of LCMV and more swelling upon high-dose inoculation. Also, viral loads are higher in $PML^{-/-}$ animals compared with wild-type control animals. Infection with a hepatotropic LCMV requires 10- to 100-times lower inocula in $PML^{-/-}$ mice in order to trigger T cell-mediated hepatitis. PML-deficient mice are 10 times more susceptible to lethal immunopathology upon intracerebral LCMV inoculation[6], thus revealing PML as an important contributor to innate immunity during defense against viral infections.

The mechanistic basis for the antiviral activity of PML against human foamy virus (HFV) was revealed in a study in which overexpression of the PML isoform PML-IV conferred resistance to HFV infection similar to that mediated by the antiviral cytokine IFN-α[39]. It was shown that this process is mediated by the interference of PML with the HFV Tas transactivator protein. PML thereby represses the transactivating potential of Tas, which is essential for viral replication. This repression depends on PML and cannot be observed in IFN-α-treated $PML^{-/-}$ MEFs, demonstrating the importance of PML for this antiviral effect[39].

Dislocation of PML-NBs

Some viruses do not destroy PML-NBs, but rather displace them to remote positions in order to interfere with their physiological functions. An example is provided by the cytoplasmatically replicating rabies virus and the virus-encoded phosphoprotein P, which causes reorganization of the PML-NBs as they become larger and appear as dense aggregates when analyzed by confocal or electron microscopy, respectively[5]. The papilloma virus minor capsid protein L2 promotes the translocation of the major capsid protein L1 and the regulatory E2 protein to PML-NBs. This L2-dependent recruitment mechanism probably serves to promote the assembly of papilloma viruses either by providing the physical architecture necessary for efficient packaging or by increasing the local concentrations of virion constituents and regulatory proteins needed for virus expression [13]. The latter mechanism is relevant for replication of human-herpesvirus-8. Here, the virus-encoded K8 protein localizes to PML-NBs, depending on an intact leucine zipper domain. The K8 protein binds to p53 and high expression of the K8 protein causes recruitment of p53 to PML-NBs[23]. However, it remains to be seen whether this protein/protein interaction serves to activate p53-dependent viral gene expression or whether it inhibits cellular p53-mediated growth arrest or apoptosis. On the other hand, virus-induced redistribution must not necessarily promote viral survival, but rather represents an antiviral response. For instance, the incoming human immunodeficiency virus retroviral preintegration complexes trigger the exportin-mediated cytoplasmic translocation of the PML protein, which serves as a cellular defense mechanism[46].

Virus integration and replication in the vicinity of PML-NBs

Some viruses have the tendency to associate with the periphery of PML-NBs, as exemplified by HSV-1. While ICP0 exactly colocalizes with these structures (see above), the parental viral genome at early stages of HSV-1 infections preferentially integrates at the periphery of PML-NBs. Direct visualization of parental and replicated HSV-1 genomes in living cells shows that the frequency of the juxtaposition of viral genomes to PML-NBs is substantially increased by an active HSV-1 early gene transcription unit[42]. These data also suggest that the association is neither random nor passive. Tagging the HSV proteins ICP4 and ICP0 with cyan fluorescent protein and yellow fluorescent protein, respectively, allowed study of their subnuclear distribution during infection. At the onset of infection, ICP4 localizes to discrete foci which are in the direct vicinity of PML-NBs. Some of the ICP4 foci develop into replication compartments[18], suggesting that they contain parental viral genomes. However, the biological relevance for PML-NB-association and the molecular mechanisms aiding in virus replication remain to be worked out. A detailed understanding of these processes will be the prerequisite for the development of antiviral drugs that target viruses by preventing their successful hijacking of PML-NBs.

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