Emerging Role of PML Nuclear Bodies in Innate Immune Signaling

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Research in the last 2 decades has demonstrated that a specific organelle of the cell nucleus, termed PML nuclear body (PML-NB) or nuclear domain 10 (ND10), is frequently modified during viral infection. This correlates with antagonization of a direct repressive function of individual PML-NB components, such as the PML, hDaxx, Sp100, or ATRX protein, that are able to act as cellular restriction factors. Recent studies now reveal an emerging role of PML-NBs as coregulatory structures of both type I and type II interferon responses. This emphasizes that targeting of PML-NBs by viral regulatory proteins has evolved as a strategy to compromise intrinsic antiviral defense and innate immune responses.

PML-NBs and Intrinsic Immunity

The role of PML-NBs in intrinsic immunity, which represents the first line of intracellular defense against invading pathogens, has been discovered and extensively characterized in the context of herpesviral infections (3, 6). As observed for many nuclear-replicating viruses, the genomes of herpesviruses like HSV-1 or HCMV become associated with PML-NBs as soon as they enter the nucleus. This association results in epigenetic silencing of viral genomes and, thus, affects one of the first steps of the herpesviral life cycle (Fig. 1). Employment of the small interfering RNA (siRNA) technology in numerous studies by our and other groups has convincingly demonstrated that several NB proteins, including PML, hDaxx, Sp100, and ATRX, act as cellular restriction factors and contribute to this repression process in a cooperative manner (6). A different restriction mechanism, acting on a later stage of viral infection, has been found to affect the herpesvirus varicella-zoster virus (VZV). During VZV infection, enlarged PML-NBs entrap newly assembled VZV nucleocapsids, based on the interaction of one specific PML isoform with the open reading frame 23 (ORF23) capsid protein, and prevent their nuclear egress (7). This indicates that PML-NBs can inhibit viral replication by mechanistically different modes of action ranging from chromatin modification to physical entrapment.

Evidence continues to accumulate that the restricting activity of NB proteins not only extends to further DNA viruses, including adeno-, papilloma- and paroviruses, but also affects specific processes in the life cycle of cytoplasmically replicating RNA viruses (8–11). While in vivo experiments with PML knockout mice demonstrated restriction of the arenavirus lymphocytic choriomeningitis virus (LCMV) and the rabdovirus vesicular stomatitis virus (VSV) more than a decade ago, convincing data for an involvement of PML in HIV-1 restriction were only recently provided by two publications (4, 5). They report that PML-NBs undergo a rapid relocalization into cytoplasmic bodies after infection with HIV-1 and other retroviruses, thus enabling an interference with early viral events in the cytoplasm. Both studies agree that PML restricts HIV-1 at the level of reverse transcription, but the underlying mechanism remains unclear. Dutrieux et al. describes a PML-mediated stabilization of the reverse transcription inhibitor hDaxx, while Kahle et al. detected no involvement of hDaxx in HIV-1 restriction (4, 5). Nevertheless, both studies indicate an...
unconventional mobility of PML-NBs, which appear to sense viral nucleoprotein complexes or replication intermediates, resulting in movement of the respective proteins even to the cytoplasm. Additionally, PML and hDaxx were implicated as contributing to the control of retroviral latency via epigenetic repression (12, 13).

**VIRAL PML-NB ANTAGONISTS**

In order to overcome the restricting activities of different PML-NB components, viruses have evolved antagonistic effector proteins. Indeed, almost all viruses for which replication has been linked to PML-NBs encode regulatory proteins that employ different strategies to inactivate single NB components or disrupt the integrity of the whole structure (Fig. 1). Two well-studied examples are the ICP0 protein of HSV-1 and the immediate early protein IE1 of HCMV. ICP0 has been shown to act as a SUMO-targeted ubiquitin ligase (STUbL), inducing an immediate proteasomal degradation of SUMO-conjugated proteins, including NB components (14). This results in a rapid and very efficient antagonization of PML-NB-mediated silencing of viral gene expression. In contrast, IE1 uses a more gentle way to disarm PML-NBs. It specifically affects the SUMOylation of the restriction factors PML and Sp100 in a proteasome-independent manner (15). Since SUMOylation of PML is essential for the integrity of PML-NBs, this results in a dispersal and inactivation of PML-NB accumulations. The recent structural characterization of IE1 has revealed that the globular core domain of IE1 (IE1CORE) shares secondary structure features of the conserved coiled-coil domain of tripartite motif (TRIM) proteins (16). Furthermore, our group has demonstrated that IE1CORE binds efficiently to the coiled-coil domain of PML, alternatively termed TRIM19. Consequently, via coiled-coil interactions, IE1 may not only inhibit the SUMOylation of PML but possibly affects other members of the TRIM E3 ligase family of innate immune regulators (16). In addition to IE1, HCMV encodes a second PML-NB antagonistic protein, the tegument protein pp71. This protein displaces the chromatin-associated factor, ATRX, from PML-NBs and degrades hDaxx in a...
ubiquitin-independent but proteasome-dependent manner immediately after infection; however, the exact mechanism of this degradation is still unclear (6).

Another interesting example for the evolution of PML-NB antagonistic proteins can be found within the subfamily of gammaherpesviruses. All known gammaherpesviruses encode at least one conserved tegument protein that contains sequence homology to the cellular purine biosynthesis enzyme phosphoribosylformylglycinamidine amidotransferase (FGARAT). While no enzymatic activities have been detected on these viral FGARAT homologous proteins (vFGARAT), various members of this family modify PML-NBs; however, they do so in different ways. For instance, the ORF75c protein encoded by murine gammaherpesvirus 68 (MHV-68) specifically induces the proteasomal degradation of PML (17). In contrast, ORF3 protein from herpesvirus saimiri (MHV-68) specifically induces the proteasomal degradation of PML-NBs and innate immunity was first discovered with the observation that interferon (IFN) treatment induces an upregulation of several NB proteins, including PML and Sp100, and enhances their antiviral activity. In addition, PML depletion reduces the capacity of IFNs to protect from viral infections, indicating an important contribution of PML-NBs to the establishment of an IFN-induced antiviral state (24, 25). Further results by our and other groups suggest an even closer cross talk, since they implicate PML as a direct, positive regulator of IFN signaling (26–28). Type I (alpha interferon [IFN-α] and IFN-β) and type II (IFN-γ) IFNs induce the expression of interferon-stimulated genes (ISGs) through intracellular signaling cascades that eventually lead to the association of activated signal transducer and activator of transcription (STAT) complexes with ISG promoter regions. Evidence exists that PML has the capacity to modulate different stages of this signaling pathway, since it can enhance the expression of IFN-β and, additionally, is required for efficient IFN-induced transcription of ISGs (Fig. 1) (29). This holds true for numerous ISGs regulated by type I IFNs, as well as for IFN-γ-induced major histocompatibility complex (MHC) class II genes (26–29), while controversial results are available concerning MHC class I gene expression (30, 31). Interestingly, IFN-γ treatment induces an increased spatial proximity between PML-NBs and the MHC class II gene cluster. This topology is maintained long after IFN stimulation and correlates with a sustained transcription-permissive epigenetic state of the MHC class II gene DRA (32). Thus, PML-NBs may generate a transcriptional memory that facilitates rapid gene expression upon IFN-γ restimulation.

The molecular mechanisms by which PML stimulates the IFN pathway are far from being fully understood, but they appear to depend on the modulation of downstream signaling events taking place in the cell nucleus. In particular, PML has been found to associate with transcription factor complexes that control IFN and ISG expression, resulting in stabilization of their components and in enhanced promoter occupancy (26, 27, 29). Intriguingly, these activities correlate with specific interactions of the individual PML isoforms, which can be attributed to their unique C-terminal domains. The PML isoform II (PML II) seems to be of particular importance, as it undergoes interactions with different components of type I and type II IFN signaling pathways. PML II but no other PML isoform binds and recruits the MHC class II transactivator CIITA to PML-NBs, thus leading to stabilization and prolonged activation of the transactivator (26). Furthermore, PML II directly associates with transcription factors like interferon regulatory factor 3 (IRF3) and STAT1 and promotes their recruitment to IFN-β and ISG promoters, respectively (29). PML isoform IV has also been reported to enhance the activity of IRF3, thereby participating in IFN-β production during VSV infection (33). However, this is achieved through a different strategy that involves recruitment of the peptidyl-prolyl isomerase Pin1 to PML-NBs and prevention of Pin1-mediated IRF3 degradation, thus highlighting the complex role of PML in the regulation of IFN signaling.

Finally, there is evidence that the coregulatory function of PML in innate immune signaling not only affects IFNs but targets an extended spectrum of cytokines. For instance, the production of the proinflammatory cytokines interleukin 1β (IL-1β) and IL-6 has been reported to be markedly decreased in PML-deficient cells (34, 35). In accordance with a deregulation of IL-6 that has a prominent role in the acute-phase response, PML knockout mice display an aberrant immune response to bacterial infections and
are resistant to acute lipopolysaccharide (LPS)-mediated lethality (34). These data encourage the hypothesis that the coregulatory role of PML in innate immune signaling may be even broader than anticipated from previous studies and may include thus-far- unrecognized biological functions, such as the regulation of acute inflammatory responses during viral infections.

CONCLUSION

Consistent with the recently recognized importance of many TRIM family members for innate immune signaling, PML emerges as a significant coregulator of the IFN pathway. This further accentuates an as-yet- enigmatic functional dichotomy of PML-NBs during viral infection. On one hand, PML-NBs act as powerful repressors that induce a silencing of viral gene expression. On the other hand, these structures serve as coactivators of cellular genes that have antiviral activity (Fig. 1). Further studies will be necessary to mechanistically understand how PML-NBs mediate these complementary effects via at-first-glance contradictory mechanisms. However, as a consequence, viral antagonists of PML-NBs may have evolved not only to overcome the intrinsic restriction of PML-NBs but also to specifically inactivate an IFN-mediating function of PML. This has already been demonstrated for the HCMV IE1 protein, which blocks IFN signaling during HCMV infection via binding PML (27, 28). In conclusion, targeting PML-NBs likely represents a common viral strategy to antagonize both intrinsic and innate immune mechanisms.

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