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Review

New insights into the role of the subnuclear structure ND10 for viral infection

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ABSTRACT

Nuclear domains 10 (ND10), alternatively termed PML nuclear bodies (PML-NBs) or PML oncogenic domains (PODs), have been discovered approximately 15 years ago as a nuclear substructure that is targeted by a variety of viruses belonging to different viral families. This review will summarize the most important structural and functional characteristics of ND10 and its major protein constituents followed by a discussion of the current view regarding the role of this subnuclear structure for various DNA and RNA viruses with an emphasis on herpesviruses. It is concluded that accumulating evidence argues for an involvement of ND10 in host antiviral defenses either via mediating an intrinsic immune response against specific viruses or via acting as a component of the cellular interferon pathway.

1. Introduction

Viruses, even the most complex ones, rely on the cellular machinery in order to replicate efficiently. Consequently, viruses have evolved in tight association to the host cell in order to usurp the cellular apparatus that is necessary for their replication as well as to cope with the various antiviral defense mechanisms the cell has developed during co-evolution. Over a decade ago, a cellular subnuclear structure known as nuclear domain 10 (ND10) has been identified as a frequent target site for a variety of different viruses during the course of infection. Most of the work so far has mainly focused on nuclear-replicating DNA viruses but there is accumulating evidence that the replication of RNA viruses is also influenced by this nuclear substructure. However, there has always been a controversial debate about the functional role of ND10 for viral replication. The aim of this article is to summarize the current state of knowledge concerning the interplay of different viruses with ND10 during infection in light of what is presently known about the functional significance of these interactions. There will be an emphasis on herpesviruses, since these have been most extensively studied with respect to the functional consequences of ND10 association.

2. Structure and composition of ND10

Nuclear domains 10 (ND10), also referred to as nuclear dots, PML nuclear bodies (PML-NBs), or PML oncogenic domains (PODs), are dynamic, spherical, macromolecular structures which represent accumulations of multiple cellular proteins that assemble in distinct foci within the interchromosomal space of the nucleus [1]. Like many transcription or RNA processing factors, ND10 bodies are associated with the nuclear matrix, since treatment with RNase or DNase does not alter their morphology [2,3]. The apparent size of PML-NBs ranges from 0.2 to 1 μm, and their frequency depends on cell type and status, varying from 2 or 3 to as many as 30 per cell [3]. There is an ever-expanding list of proteins that have been reported to be present in or associated with ND10 structures. These proteins can be subdivided into two classes, based on whether they are constitutively present at ND10 or only under certain circumstances. Permanently localized at ND10 are the major components PML (promyelocytic leukemia protein), hDaxx, Sp100 (speckled protein of 100 kDa), SUMO-1 (small ubiquitin-related modifier 1), and the Bloom syndrome helicase BLM [1]. On the contrary, quite a variety of factors can be found at ND10 only under certain conditions (e.g. components of the DNA repair machinery) [4–6] or upon overexpression (e.g. BRCA1 — breast cancer protein 1) [1,7]. However, in the latter case, it has to be questioned, whether such polypeptides constitute natural ND10 components, as more and more data suggest a role of ND10 in the degradation of mis- or unfolded protein aggregates [8,9]. Thus, as the composition of ND10 varies both within and between cells, it is tempting to speculate that ND10 represent functionally heterogeneous protein accumulations.

The Sp100 protein was the first ND10 constituent identified using sera of patients suffering from the autoimmune disease primary biliary cirrhosis (PBC) [10]. Thereafter, PML was discovered as the defining factor of ND10. It functions as a kind of scaffold protein that is

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responsible for the assembly and maintenance of PML-NBs and recruits other ND10-associated proteins like hDaxx to this subnuclear structure [11,12]. Since PML constitutes the key component of ND10 integrity, loss of PML consequently leads to a dispersal of ND10-resident proteins as observed in mouse PML-null fibroblasts [11,12] or human PML-knockdown (PML-kd) fibroblasts [13,14].

3. The major ND10 constituents and their functions

3.1. PML

The PML protein was originally discovered in patients suffering from acute promyelocytic leukemia (APL), where a reciprocal chromosomal translocation resulting in a fusion of the PML protein to the retinoic acid receptor α turned out to be responsible for this hematopoietic malignancy [15]. PML, also known as TRIM19, belongs to the RBCC or tri-partite motif family (TRIM) of proteins that include a RING finger, one or two B-boxes (cysteine/histidine-rich motifs) and a predicted α-helical coiled-coil domain [16]. This motif, which allows PML to interact with other proteins as well as to homo-oligomerize, is essential for ND10 formation and for the function of PML as a growth- and transformation-suppressor [17]. Due to differential splicing of the PML gene transcript which consists of nine exons, at least seven different PML isoforms (I to VII) are expressed within cells, all sharing a common N-terminus but varying in their C-termini. The functions of the PML splice variants are not known. Alternative splicing could add new functional domains to the protein or may be an important mechanism for generating diverse PML-binding interfaces for a variety of factors. Moreover, all isoforms having molecular weights ranging from 48 to 97 kDa (kilo Dalton), are subject to posttranslational modifications like phosphorylation [18] or conjugation to the ubiquitin-homologous protein SUMO (SUMOylation) [19,20]. In addition to three SUMO modification sites at lysine residues 65 (in the RING finger domain), 160 (in the B1 box), and 490 (in the nuclear localization signal) [21], PML also contains a SUMO binding motif that enables it to interact noncovalently with SUMO [22]. Covalent as well as non-covalent SUMO modification is extremely important for the function of PML to orchestrate ND10 formation as clearly illustrated in case of the recruitment of hDaxx to this subnuclear structure [12,22,23].

3.2. hDaxx

HDaxx is a highly conserved nuclear protein that contains a serine/proline/threonine-rich domain, an acidic domain, a coiled-coil region, and two paired amphipathic helices [24–27]. It has been recognized as a regulator of both apoptosis and gene expression [28]. In gene regulation, hDaxx has been shown to function as a transcriptional corepressor. It negatively affects gene expression by suppressing the activity of several transcription factors, including Ets-1 (E twenty-six 1) [29], NF-κB (nuclear factor κB) [30], Pax3 [24], E2F-1 [31], Smad4 [32], p53 family members [33], and glucocorticoid, mineralocorticoid and androgen receptors [34]. Recent findings indicate that hDaxx associates via its newly identified SUMO-interacting motif (SIM) with SUMOylated DNA-binding transcription factors [23], thereby recruiting proteins involved in transcriptional repression, such as histone deacetylase 1 (HDAC1) [26], HDAC2 [35], DNA methyltransferase 1 (DNMT1) [36], or ATRX (α-thalassaemia/mental retardation syndrome X-linked) [37,38], a member of the SNF2 family of chromatin remodeling enzymes, to targeted promoters. The transrepressive effect of hDaxx, in turn, is modulated by its subnuclear compartmentalization. The SIM enables hDaxx to also non-covalently interact with SUMOylated PML, resulting in the sequestration of nucleoplasmic hDaxx to ND10 [22,23], which is accompanied by attenuation of its inhibitory function [26]. Beside ND10, hDaxx can also be targeted to the nucleolus, centromeres, or heterochromatin through its interactions with either the nucleolar protein MSP58 (microspherule protein of 58 kDa) [39], the centromeric protein CENP-C [27], or the heterochromatin-associated factor ATRX [37]. Taken together, these observations imply that, depending on its subnuclear localization, hDaxx seems to fulfill distinct functions.

3.3. Sp100

Like PML, the Sp100 protein represents a permanent ND10 constituent which is expressed via alternative splicing from a single gene transcript giving rise to four different isoforms designated as Sp100A [10], Sp100B [40], Sp100C [41], and Sp100-HMG [42,43]. All Sp100 splice variants share a common N-terminus harboring an HSR (homogeneously staining region) motif responsible for homo-oligomerization of Sp100 and its targeting to ND10 as well as a binding site for the non-histone chromosomal DNA-binding protein HP1 (heterochromatin protein 1). The larger splice variants Sp100B, -C, and -HMG encode additional functional motifs at the C-terminus such as a SAND domain (common to all three longer isoforms), a PHD finger-bromodomain (Sp100-C), or an HMG box (Sp100-HMG). All three motifs represent potential DNA-binding domains which can frequently be found in proteins affecting chromatin structure [44]. Together with the observation that Sp100 interacts with heterochromatin protein HP1 [42], which plays a central role in establishing a stable heterochromatic network, this suggests a role of Sp100 in transcriptional regulation. Indeed, the isoform Sp100B has been shown to function as a transcriptional repressor of both cellular and viral promoters in transient expression experiments [45]. In addition, as recently reported, Sp100B preferentially associates via its SAND domain with DNA sequences containing unmethylated CpG dinucleotides, since methylation of cytosines in the CpG context abrogates DNA binding of Sp100B [46]. This finding prompted the authors to speculate that the preference of Sp100B for non-methylated CpGs could provide a mechanism to specifically target this isoform to foreign DNA (including viral genomes), which is predominantly hypomethylated [46].

Like many ND10 constituents, including PML and hDaxx [20,47,48], Sp100 is also subject to posttranslational modification by SUMO [48]. While SUMOylation of Sp100 is not required for its ND10 localization [49], it has been shown to enhance the interaction with HP1 and thus to stabilize Sp100-HP1 complexes suggesting that the functional interplay of Sp100 between ND10 and heterochromatin could be regulated in this way [41]. Recent data indicate, that the SUMO modification status as well as the relative expression level of Sp100 are to some extent regulated, either directly or indirectly, by PML [13]. SiRNA-mediated knockdown of PML resulted in an apparent reduction in the abundance of Sp100-HMG and the SUMO-modified form of Sp100A, with a concomitant increase in unmodified Sp100A [13]. Thus, Sp100 metabolism seems to be closely linked to that of PML.

4. Functions of ND10

Unlike other, more specialized subnuclear structures such as Cajal and Polycomb group bodies, PML-NBs are functionally promiscuous and have been implicated in the regulation of diverse cellular processes. The research in a variety of fields has produced a substantial literature that links ND10 to oncogenesis [50], DNA damage repair [5], apoptosis [51,52], stress response [53], senescence [54], the ubiquitin pathway [55] as well as to the regulation of gene expression [56]. Despite these various cellular responses, the functions of ND10 are still not fully understood.

In the absence of a clear understanding of the biochemical function of ND10, the following, not mutually exclusive, models have been proposed to explain how this subnuclear structure could exert its biological functions [1,56–59]: (i) PML-NBs might operate as nuclear depots or storage sites for the accumulation of proteins both under

pathological conditions to sequester foreign or misfolded proteins as well as under normal conditions to accumulate proteins that can be released or relocated elsewhere as required in order to control their availability at nucleoplasmic sites other than ND10; (ii) PML-NBs may illustrate "catalytic surfaces" where the post-translational modification of proteins takes place (e.g. SUMOylation); (iii) ND10 domains could be active sites for defined nuclear functions such as transcriptional and chromatin regulation.

With respect to the latter activity, evidence continues to accumulate that ND10 play a role for transcriptional regulation since numerous transcription factors and transcriptional regulators dynamically localize to these domains [56] and nascent RNA has been detected in the immediate vicinity of PML-NBs [60]. Nevertheless, the exact molecular mechanisms of ND10-mediated transcriptional regulation remain elusive. Several studies indicate that PML-NBs might regulate transcription by modulating the nucleoplasmic availability or the activity status of transcription factors [56,61]. While not contradicting this model, another emerging idea is that ND10 could also control transcriptional activities indirectly on an epigenetic level by participating in chromatin-remodeling processes. For the ND10 components PML and hDaxx, an association with histone deacetylases (HDACs) [26,35,62] or DNA methyltransferases [63,64], which all exhibit a transcriptionally repressive function, has been demonstrated. Certain PML isoforms, in addition, have been shown to form complexes with multiple corepressors like c-Ski, N-CoR, mSin3A [65], or the novel KRAB-zinc finger repressor PAROT (PML-associated repressor of transcription) [66] as well as to silence transcription by recruiting the histone methyltransferase SUV39H1 [67] or the polycomb group (PcG) protein EZH2 [68], one of the core components of the Polycomb repressive complexes (PRC) 2/3/4. Similarly, Sp100 also behaves as a transcriptional repressor by interacting with the heterochromatin protein HP1 [42,45]. Thus, the fact that several ND10 components associate with potent repressors of gene expression gave rise to the idea of ND10 acting as sites of transcriptional repression. In contrast, however, the presence of transcriptional activators like the acetyltransferase CBP or p53 at ND10 has likewise been described [69,70], reflecting the still controversial debate about the role of this subnuclear structure in regulating gene expression.

Finally, as will be discussed in this review, ND10 have been implicated to play an important role during viral infection [4,59,71–73]. For instance, there are several indications that ND10 are linked to the interferon-mediated antiviral response of the cell [74,75]. Many ND10 proteins, including the major components PML and Sp100, are interferon (IFN) inducible [74,76,77]. Although PML and Sp100 are expressed in the absence of IFN, their expression is greatly increased and directly induced by type I (IFN-α and IFN-β) as well as type II (IFN-γ) interferons through an "IFN-stimulated response element" (ISRE) and an "IFN-gamma activation site" (GAS) which are located within the promoters of their genes [78,79]. As a consequence of this, in response to interferon treatment of cells, the number, size, and intensity of PML-NBs increases [80]. The study of ND10 structures in the context of viral infection provides further evidence to implicate ND10 in the IFN pathway. As specified in detail in the sections below, many viruses have evolved polypeptides in order to compromise ND10 integrity to variable extents. Such kind of structural modifications of ND10 have been shown to frequently correlate with the efficiency of viral infection and thus could be viewed as part of a viral strategy to evade an antiviral function of ND10 [75]. The following chapters will sum up the current state of knowledge concerning the potential role of ND10 for the replication of nuclear- and cytoplasmic-replicating DNA as well as RNA viruses.

5. DNA viruses and ND10

Interest in the interaction between DNA viruses and ND10 was first sparked by the observation that infection by herpes simplex virus type 1 (HSV-1) caused a rapid destruction of this subnuclear structure [81]. Subsequently, it was found that the parental genomes and replication complexes of HSV-1 and adenoviruses were preferentially located in close association with ND10 [82,83]. This observation has been extended to include the polyomavirus simian virus 40 (SV40) and papillomaviruses as well as members of all sub-families of the herpesviridae [71,73]. Thus, it appears to be a general feature of nuclear-replicating DNA viruses that their parental genomes preferentially become associated with ND10, and that their initial sites of transcription and the development of DNA replication centers are frequently juxtaposed to these domains or their remnants. The intimate spatial association between viruses and ND10 has raised important questions: What are the viral and host cell factors that are responsible for the association and what are the functional consequences of this tight interaction? Some progress has been made within the last years towards partially answering these questions.

5.1. Herpes simplex virus type 1 (HSV-1)

HSV-1, a member of the neurotropic alpha sub-family of herpesviruses, is able to attain a stable latent state in neurons after a primary infection of epithelial cells. It was the first virus to be shown to affect ND10 morphology during infection. The initial observation that HSV-1 disrupts ND10 but an ICP0-deficient mutant does not [81], rapidly led to the identification of ICP0 being necessary and sufficient for this effect [84,85]. ICP0 is a RING finger protein that is very important in certain cell types for initiating viral lytic infection and contributes to the reactivation of quiescent viral genomes in cultured cells as well as of latent virus from neurons in mouse models [86]. ICP0 initially precisely colocalizes with ND10 at early times upon infection and subsequently mediates the dis-aggregation of PML-NBs by inducing the degradation of the SUMO-1 modified forms of PML and Sp100 [87–90], leading to the release and dispersal of other ND10 proteins. This effect of ICP0 on ND10 is dependent upon ICP0 having an intact RING finger motif [84] and reflects its ability as an E3 ubiquitin ligase to target specific proteins for degradation by the proteasome [91]. However, it is likely that the apparent loss of various isoforms of Sp100 during wt HSV-1 infection is a consequence of ICP0-induced degradation of PML, rather than being a direct effect of ICP0 on Sp100 itself [13]. Disruption of ND10 by ICP0 correlates with its role in stimulating HSV-1 lytic infection and reactivation from quiescence or latency [86].

Especially by using an ICP0-deletion virus being defective in the ability to modify ND10, a preferential localization of parental HSV-1 DNA in close proximity to ND10 was demonstrated via in situ hybridization [82]. This observation could also be confirmed in wt HSV-1-infected cells when early time points prior to ICP0-mediated ND10 dispersal were analyzed [82]. Subsequent infection experiments with fluorescence-tagged viruses revealed that ND10 do not only serve as a nucleation site for incoming HSV-1 genomes but also for several viral regulatory proteins. Two virus particle-associated tegument proteins, VP13/14 and VP22 have been shown to accumulate in punctate nuclear dots which are distinct from but closely adjacent to ICP0 foci, and thus ND10 [92]. Similarly, by using the same technique, the IE transcriptional regulatory protein ICP4 was found to form dot-like accumulations at early times of infection many of which are juxtaposed to ND10 [93]. ICP4, which is essential for the activation of viral early and late gene expression, functions by binding to viral DNA and by interacting with components of the host transcriptional apparatus [94]. Indeed, as determined by in situ hybridization, ICP4 foci represent ICP4 molecules being recruited onto ND10-localized parental viral genomes as they directly colocalize with input HSV-1 DNA and later develop into replication compartments as infection progresses [93,95,96]. Since ICP4 accumulations only form during infection and are not detectable in transiently transfected cells, this implies that a viral factor, presumably the viral DNA, contributes to

their formation [92]. Finally, it was reported that ND10-associated ICP4 foci also contain the regulatory protein ICP27 [95], which plays a crucial role in the regulation of productive infection by functioning in the processing and efficient export of viral mRNAs [94]. The detection of ICP27 colocalizing with ICP4 led to the suggestion that these foci represent viral nucleoprotein complexes that are biologically active and engaged in viral transcription [95].

Infection studies using the ICP0 mutant virus provided additional evidence that viral replication compartments start to initiate in the periphery of PML-NBs [83] and recent experiments using time-lapse microscopy to study the fate of incoming HSV-1 amplicon genomes implied that ND10-associated DNA has an increased probability of being replicated [97]. This finding indicated that not all viral genomes in infected cells are functionally equivalent and that progression of HSV-1 artificial infectious replicons into active replication centers was favored by a close localization to ND10. In the same study, the frequency of ND10 association could be enhanced if active HSV-1 transcription units (e.g. a transcription cassette containing ICP4 binding sites) were included in the amplicon, suggesting that the formation of viral nucleoprotein complexes triggers ND10 localization. In agreement with this, Tang et al. [98] demonstrated that the viral origin of replication (oriS) together with the IE proteins ICP4 and ICP27 are the minimum HSV-1 components necessary to localize transcriptionally active DNA at ND10. However, these findings are in apparent conflict with earlier evidence showing that preventing protein or RNA synthesis at the time of infection did not inhibit the positioning of incoming viral genomes at ND10 [82]. Similarly, recent data based on an HSV-1 mutant with lesions that inactivate VP16, ICP0, and ICP4, which enables the establishment of cell cultures containing quiescent viral genomes revealed that transcriptionally inactive viral DNA still associates with ND10 [99]. This further strengthens the assumption that neither newly synthesized viral proteins nor extensive viral transcription are required for viral genome localization at ND10. Moreover, these observations illustrate that the intimate connections between HSV-1 DNA and ND10 that occur during lytic replication can also be extended to latent-like infections. Interestingly, in the latter case, abnormally enlarged ND10 are detectable, which appear as ring-like accumulations when analyzed by confocal microscopy but which actually represent spherical structures, with PML enclosing the viral DNA in the interior [99].

Nonetheless, also in the apparent absence of active transcription, incoming viral genomes are certainly never completely naked as transcription complexes containing the viral tegument protein VP16 as well as the host factors Oct-1 and HCF or other cellular chromatin or DNA binding proteins accumulate on them further encouraging the hypothesis that the formation of foreign nucleoprotein complexes or chromatin assembly mechanisms in general may cause their ND10 localization. ND10 association as a consequence of recognition of foreign nucleoprotein complexes has also been concluded by another study, in which chromosomal integration of a series of gene cassettes containing lacO and tetO binding sites resulted in an artificial tethering of the host chromosome to ND10 upon expression of EGFP–lacI or VP16–TetR fusion proteins [100]. Alternatively, since accumulating evidence suggests that ND10-like structures generally associate with sites of DNA damage, viral genome induced activation of the cellular DNA repair machinery could also be considered as a possible mechanism for ND10 sequestration [4].

The identification of cellular factors that are responsible for the localization of incoming viral genomes at ND10 likewise awaits further investigation. Recent studies conducted in cells being extensively depleted for PML demonstrated that Sp100 or hDaxx are efficiently recruited to HSV-1 as well as HCMV nucleoprotein complexes even in the absence of PML [13,14]. Therefore, it appears that PML is not necessarily required for the subnuclear positioning of other ND10 proteins in response to viral infection. Furthermore, simultaneous knockdown (kd) of PML and Sp100 does not prevent accumulation of hDaxx at input HSV-1 DNA [101]. Similarly, infection of PML and hDaxx double-kd cells with HCMV still induces the targeting of Sp100 to viral nucleoprotein complexes (N. Tavalai and T. Stamminger, unpublished observation). Taken together, these data support the notion that all three major ND10 constituents individually contain determinants that enable their localization to sites associated with viral nucleoprotein complexes.

Although the exact viral and cellular factors involved in virus genome association with ND10 still remain to be determined, several lines of evidence indicate that the interaction between viral nucleoprotein complexes and ND10 is due to a dynamic response of the cell. One argument in favor of this hypothesis is the observation that HSV-1 as well as HCMV infection of PML-kd cells, in which hDaxx and Sp100 are normally diffusely distributed, elicits the reorganization of ND10-like accumulations of both proteins [13,14]. Moreover, Everett and colleagues carried out experiments in which they examined cells at the edge of developing plaque. The consequential unidirectional entry of virions into neighboring cells resulted in an asymmetric distribution of not only the incoming HSV-1 genomes but also the adjacently located ND10 structures [95,96]. Given the apparently random distribution of PML-NBs in uninfected cells this finding underlines the notion that ND10 components actively relocate to sites of HSV-1 nucleoprotein complexes. Since at least a subgroup of ND10 is quite immobile [102] and individual ND10 constituents dynamically exchange between PML-NBs and the surrounding nucleoplasm [69,96], it is more likely that novel ND10 foci form in association with viral genomes rather than pre-existing domains move to these sites.

Taking into account that ND10 are IFN-inducible and actively target incoming DNA viruses, this makes it tempting to suggest an antiviral role for this nuclear substructure. In accordance with this assumption it has been shown that an ICP0-negative HSV-1, which fails to disrupt ND10 and thus to counteract the cellular response, is hypersensitive to the effects of IFN in certain cultured cell lines [103,104] and exhibits low pathogenicity in normal mice in vivo, while infection of mice that are unable to mount an interferon response restores its replication capacity [105]. Although neither infection with wt nor ICP0-deficient HSV-1 is enhanced in the absence of PML in $PML^{-/-}$ murine embryonic fibroblasts (MEFs) compared to control $PML^{+/+}$ MEFs, the replication of the ICP0-deletion virus is substantially compromised by IFN treatment of these cells in the presence but not absence of PML [106]. These results suggest that PML contributes to the cellular IFN-mediated restriction of HSV-1 infection and that one function of ICP0 is to efficiently counteract the repressive effect of PML. However, neither exogenous expression of PML isoforms III, IV, nor VI turned out to affect virus yield, even though PML overexpression blocked the dispersal of ND10 in response to HSV-1 infection [87,107,108]. In light of the increasing evidence that different PML isoforms possess distinct properties, one possible explanation could be that the PML variants used in those studies may play a minor role in the host IFN-mediated antiviral response. In addition, in contrast to the prior fixed cell analyses, live-cell imaging experiments clearly demonstrated that large ND10 aggregates as a consequence of high-level expression of PML are nonetheless subject to extensive modification in terms of both content and morphology during HSV-1 infection [107].

In principle, it was not until the recent generation of physiologically more relevant knockdown cells being derived from the natural human host, in which individual ND10 components were depleted, that substantial advances in understanding the biological relevance of ND10 for HSV-1 replication were made. Although infection of cells with an shRNA-mediated down-regulation of either PML or Sp100 had no effect on wt HSV-1 replication, it resulted in a significant increase in the efficacy of gene expression and plaque formation of an ICP0-null mutant virus [13,101]. On the same theme, Negorev et al. [109] previously reported that the restrictive activity of Sp100 is based on

isoforms B, C, and HMG having an intact SAND domain. In accordance with this, overexpression of Sp100B but not Sp100A suppressed HSV-1 gene expression [45,109]. Moreover, by specifically depleting the repressive Sp100 variants B, C, and HMG using RNA interference, these proteins could be confirmed as an essential part of the IFN-mediated restriction of HSV-1 replication [109]. Interestingly, simultaneous knockdown of both ND10 proteins, PML as well as Sp100, further stimulated gene expression and plaque forming ability of the ICP0-knockout virus but did not completely eliminate its replication defect indicating the involvement of additional repressive factors [101]. Nevertheless, this finding adds more weight to the concept of ND10 as an intrinsic antiviral defense mechanism of the cell as individual ND10 components contribute independently to the silencing of viral gene expression. Furthermore, a very recent publication suggested that not only nuclear PML but also a cytoplasmic PML isoform, lacking exons 5 and 6, could potentially contribute to an intrinsic cellular defense against HSV-1 by cytoplasmic sequestration of ICP0 [110].

5.2. Human cytomegalovirus (HCMV)

Also in case of HCMV, the prototype of the β-subgroup of herpesviruses, parental viral genomes associate with ND10 followed by the targeting of newly synthesized viral IE transactivators IE1 and IE2 to this subnuclear structure [111,112]. It was reported that both regulatory proteins, which are essential for initiating the lytic replication program of HCMV [94], become part of an immediate transcript environment (ITE) consisting of IE transcripts and the spliceosome assembly factor SC35, which evolves at ND10-associated viral genomes [112]. Detailed analysis revealed that there are subtle differences in the localization pattern of IE1 and IE2 at ND10 that may reflect on their respective functions. While IE1 perfectly colocalizes with all ND10 structures of the nucleus, and transfection experiments demonstrated that this localization is inherent in the protein, only transiently expressed IE2 illustrates a perfect colocalization with ND10 [111–113]. In the context of infection, the IE2 protein can also be found adjacent to these domains where it accumulates juxtaposed to the subpopulation of PML-NBs with viral transcripts attached [112–114]. While previous work with fixed cells suggested that some IE2 foci may colocalize, others may be adjacent to ND10, recent time-lapse experiments, instead, revealed an unexpectedly high dynamic of the relative locations of IE2 and ND10 foci, changing within minutes between precise colocalization and juxtaposition [115]. One important implication of this finding is that IE2 and ND10 foci represent separate structures which independently form in the context of viral infection. In fact, the same study provided evidence that it is the viral DNA, but not ND10, that acts as an important determinant for the formation of dot-like IE2 accumulations during infection [115]. In this regard, it should be noted that oligomerization of IE2 on HCMV-DNA early after infection is strikingly reminiscent of its functional analogous protein ICP4 of HSV-1 (see 5.1.), giving rise to the idea, that the formation of a viral nucleoprotein complex between the respective major transcriptional transactivator and viral DNA is likely to be a critical step common to the alpha- and betaherpesviruses.

The pronounced interplay between IE2 and ND10 structures, however, can only be observed within a narrow time window due to the action of the IE1 protein, which is responsible for the disruption of ND10 during infection with HCMV [111,113,116]. As a mechanism for this, it has been proposed that IE1 abrogates the SUMOylation of PML [117,118], but in contrast to ICP0 of HSV-1 (see 5.1.), IE1 does not require proteasome activity for this effect [119] nor does it possess any intrinsic desumoylation activity in vitro [120]. Thus, although the biochemical basis for ND10 disruption by IE1 remains unclear, structural modification of ND10 has been shown to correlate with the functional activities of IE1 in transcriptional regulation, resulting in increased efficacy of viral replication. In the same context, HCMV infection, on the contrary, progresses poorly in cells expressing high levels of exogenous PML due to a delay in IE1-mediated ND10 dispersal leading to impaired establishment of replication centers and reduced production of early and late proteins [121]. Consequently, these data already implied a repressive function of PML on HCMV replication which is counteracted by the IE1-induced reorganization of ND10. Conclusive evidence for this assumption was finally obtained from infection studies using cells being devoid of genuine ND10: extensive, siRNA-mediated depletion of PML in primary human fibroblasts significantly increased the plaque-forming efficiency of HCMV as a result of an augmented IE gene expression [14]. This effect was considerably enhanced after infection of PML-kd cells with an IE1-deficient HCMV, since loss of PML complemented the growth defect of this mutant virus [14]. Thus, the clear demonstration of an intrinsic antiviral activity of the major ND10 component PML was an important step forward in understanding the functional significance of the intimate relationship between HCMV and ND10.

The interaction of HCMV proteins with ND10 is not exclusive to the IE proteins IE1 and IE2. The viral tegument protein and transactivator pp71 likewise accumulates at ND10 immediately upon HCMV infection and before the production of IE proteins [122–124]. Direct binding to the ND10 component hDaxx has been shown to be responsible for the targeting of pp71 to PML-NBs [122,123]. Interestingly, abolishing pp71's ability to interact with hDaxx blocks pp71's localization at ND10 and inhibits pp71's ability to transactivate the major immediate early promoter (MIEP) of HCMV [122,123,125], indicating that the association of pp71 with hDaxx in ND10 is critical for its function as a facilitator of IE gene expression at the very start of a lytic infection. Although initial studies implied that pp71 and hDaxx associate at ND10 to cooperatively activate the MIEP [122], it is now clear from multiple subsequent work that hDaxx actually silences the MIEP, and that pp71 relieves this repression [126–130]. Consistent with this assumption, overexpression of the cellular restriction factor hDaxx abolishes HCMV infection while down-regulation of hDaxx by usage of small interfering RNA (siRNA) technology, on the contrary, results in increased gene expression and virus replication [126–130]. The hDaxx-mediated repression of viral IE gene expression correlates with changes of the chromatin structure around the MIEP as knockdown of hDaxx results in loss of transcriptionally-repressive and gain of transcriptionally active chromatin at the MIEP [126]. This regulation appears to involve histone deacetylases, since treatment of infected cells with HDAC-inhibitors relieves the repression of viral IE gene expression [126,127].

In order to successfully antagonize hDaxx-mediated intrinsic immune defense, tegument-delivered pp71 induces the degradation of hDaxx at the start of a lytic infection [127,130], which has been postulated to occur in a proteasome-dependent [127] and ubiquitin-independent manner [131]. Inhibiting the proteasome at the time of infection, however, still suppresses viral IE gene expression even in the absence of hDaxx, suggesting the existence of additional antiviral targets for proteasomal degradation [132]. Consistent with a function of pp71 in counteracting the repressive effect of hDaxx, HCMV inefficiently enters productive infection in the absence of pp71 [125,133], unless hDaxx protein levels are depleted prior to infection, thus annihilating the impaired growth phenotype associated with a pp71-deficient mutant [128–130]. Failure of pp71 to overcome hDaxx repression blocks viral IE gene expression and may promote the establishment of latent HCMV infections [134]. However, whether hDaxx is a major factor contributing to the control of HCMV latency is still controversially discussed [134,135].

In summary, these data clearly identified hDaxx, like PML, as a cellular restriction factor being responsible for silencing of HCMV IE gene expression directly upon infection. Moreover, since knockdown of hDaxx in combination with PML led to a further increase in the replication efficacy of HCMV, this strongly argues for an independent involvement of both ND10 factors in the restriction of viral infection and gives rise to the following working model: Immediately after

infection incoming viral genomes are targeted by ND10 which are able to induce a repressive chromatin structure via epigenetic modifications resulting in transcriptionally inactive viral genomes (see Fig. 1A and B). As a first line of defense, the imported HCMV structural protein pp71 antagonizes hDaxx-mediated gene silencing by inducing the proteasomal degradation of hDaxx (Fig. 1B-1). The relief of hDaxx-mediated repression, then, allows the initiation of IE gene expression (Fig. 1B-2). The synthesis and accumulation of IE1 at ND10, in a next step, efficiently overcomes PML-mediated repression by inducing the disruption of this subnuclear structure thereby ensuring the initiation of an efficient lytic replication (Fig. 1B-3). In case pp71 is sequestered in the cytoplasm of infected cells, as postulated for cells in which latent-like HCMV infections are established [134], input viral genomes are likewise targeted by ND10 (Fig. 1A). However, the repressive effect of hDaxx on viral gene expression is not relieved as pp71 fails to enter the nucleus and to degrade hDaxx (Fig. 1A). As a consequence of this, the viral genomes remain in a transcriptionally repressed quiescent state (Fig. 1A).

Interestingly, recent unpublished observations of our group indicate that, comparable to the situation in PML- and hDaxx-kd cells, ablation of Sp100 also leads to a considerable increase in the number of IE protein-positive cells, suggesting that Sp100 likewise contributes to ND10-mediated viral repression. Overall, these data support a model in which, comparable to HSV-1 infection, individual ND10 components function in counteracting the initiation of an efficient productive viral replication. This further underlines the notion of ND10 as mediators of an intrinsic immune defense against herpesvirus infections in general.

5.3. Epstein–Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), and murine gamma-herpesvirus 68 (MHV-68)

In common with all herpesviruses, EBV from the γ-herpesvirus subfamily, possesses a biphasic life cycle consisting of a lytic and a latent phase. However, in contrast to HSV-1, its latent state is characterized by the expression of a set of proteins that are necessary for establishment and maintenance of latency [94]. While during latency, EBV genomes can be found in close association with interphase chromosomes, activation of the lytic replication cycle results in a spatial redistribution of viral DNA to ND10 [136]. Using in situ hybridization the authors also showed the development of replication centers starting at ND10 as replicating EBV genomes were frequently found beside this nuclear domain [136]. Subsequent studies identified the ori_{lyt} sequence as being required for the appearance of replicating episomes in association with PML-NBs [137]. However, the integrity of ND10 is no longer maintained as soon as productive EBV infection is initiated. The expression of lytic proteins is accompanied by a sequential redistribution of ND10 proteins starting with the dispersal of Sp100, hDaxx, and NDP55 which is then finally followed by PML relocalization [136–138]. Induction of productive EBV replication goes along with the expression of BZLF-1, the principal inducer of the lytic gene expression program [94]. Indeed, experiments carried out by Adamson and Kenney [138] illustrated, that BZLF-1 expression is sufficient to induce ND10 disruption by reducing the level of SUMOylated PML. BZLF-1, which is itself SUMO-1 modified, has been shown to compete with PML for limiting amounts of SUMO-1 [138]. In accordance with this, mutation of the SUMO-modification site of BZLF-1 abrogates desumoylation of PML [138]. However, this does not annihilate the capability of BZLF-1 to disperse ND10 after transient expression suggesting the existence of additional mechanisms for BZLF-1-mediated ND10 disruption. Further mutational analysis of BZLF-1 revealed a correlation between the capability of this protein to disperse Sp100 from ND10 and its efficiency to activate transcription via interaction with the ND10-located factor CBP [139].

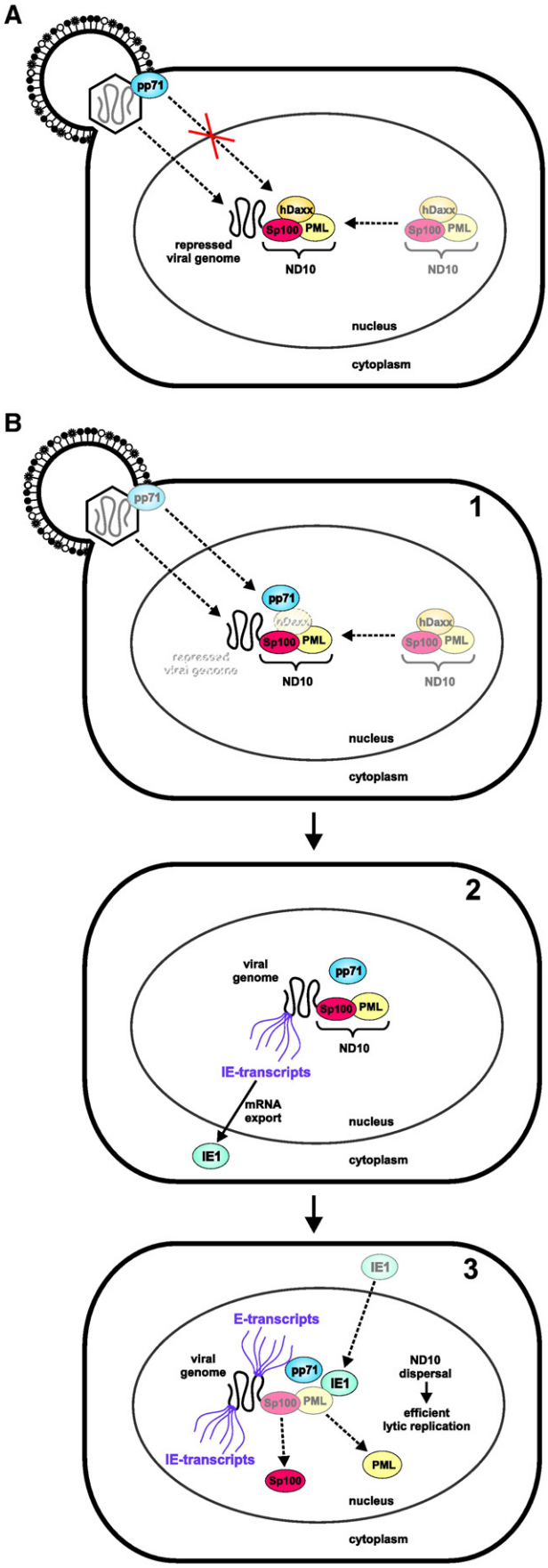
Although initial studies suggested that EBV infection has no influence on ND10 during latency [136], recent evidence indicates that the latently expressed protein EBNA-LP (also designated as EBNA5) is likewise modifying the composition of ND10 accumulations. Already over ten years ago, EBNA-LP, which is important for EBV-mediated B-cell immortalization by functioning as a potent gene-specific coactivator of the viral transcriptional activator EBNA2 [140], was reported to colocalize with ND10 in EBV-positive lymphoblastoid cell lines [141]. Novel findings imply that EBNA-LP coactivates EBNA2 through binding of the ND10 factor Sp100, thereby selectively displacing it together with HP1α from PML-NBs [142]. Since both HP1α and Sp100 have been shown to possess transcriptional repressor activity [41,42,143], and expression of a mutant form of Sp100, that was unable to associate with ND10, resulted in coactivation of EBNA2 even in the absence of EBNA-LP [142], it has been speculated that the EBNA-LP-induced ND10 rearrangements may mitigate transcriptional barriers that prevent efficient expression of viral latent genes important for establishing latency. In summary, these results were regarded as the first indication that modulation of ND10 might also be required for establishment of nonproductive or latent herpesvirus infections.

Human herpesvirus 8 (HHV8), a γ-herpesvirus also known as Kaposi's sarcoma-associated herpesvirus (KSHV), is a lymphotropic virus involved in the pathogenesis of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and a subset of multicentric Castleman's disease (MCDs). KSHV also encodes an early protein, K8, which is a distant evolutionary homologue of the EBV regulatory factor BZLF-1, that has been found to localize to ND10 and to establish replication compartments in association with this nuclear substructure [144,145]. However, unlike BZLF-1, K8 does not induce any changes to ND10 composition [145]. Consequently, the genome replication of KSHV occurs in close contact to intact PML-NBs [144]. Taken together, these data support the notion that it may be a general feature of herpesviruses to initiate their replication in the vicinity of ND10 structures.

MHV-68, which, like KSHV, belongs to the herpesvirus genus rhadinovirus, is emerging as a suitable model to study basic biological questions of gamma-herpesvirus host interactions due to the fact that it naturally infects mice. Intriguingly, data from our group as well as from others suggest that MHV-68 infection also affects ND10 (N. Tavalai, F. Full, A. Ensser and T. Stamminger, unpublished) [146]. As determined by Western blot (Fig. 2A) as well as immunofluorescence analysis (data not shown), MHV-68 completely eliminates PML upon infection of primary human fibroblasts in order to annihilate the antiviral activity of ND10. The complete loss of PML as a consequence of MHV-68 infection presumably accounts for the simultaneously observed disappearance of the SUMO-modified forms of Sp100 (Fig. 2B), comparable to the situation in HSV-1 infected cells (see 5.1.) or after depletion of the defining ND10 factor in PML-kd HFFs [13]. At the same time, MHV-68 also induces a significant reduction in hDaxx protein levels when compared to mock-infected cells (Fig. 2C). Although this effect on hDaxx is much less pronounced in comparison to MHV-68 mediated PML downregulation, it nevertheless mimics the findings for HCMV, where we were likewise never able to detect a total depletion of the transcriptional repressor hDaxx during the HCMV replicative cycle [130]. Thus, MHV-68 could constitute the first virus that negatively affects the abundance of all three major ND10 constituents directly upon infection in order to efficiently circumvent the intrinsic antiviral response instituted by ND10.

5.4. Adenoviruses

Adenoviruses (Ad) are a family of non-enveloped, nuclear-replicating DNA viruses that comprise more than 50 different human serotypes causing a variety of distinct pathologies ranging from respiratory disease and conjunctivitis to gastroenteritis.



Infection with human adenovirus type 5 (Ad5), the most extensively studied subtype, results in a redistribution of ND10 into characteristic elongated tracks during the early hours after virus inoculation [147,148]. By using a variety of Ad5 mutants, the early protein E4 ORF3 could be identified as being necessary to execute the rearrangement of ND10 from punctate bodies into these track-like structures [147,148]. Moreover, exogenous expression of E4 ORF3 alone was shown to be sufficient for this effect and the localization of E4 ORF3 in these structures [147,148]. The E4 ORF3-dependent tracks contain the known ND10 proteins, all of which, except for PML, are later recruited into viral replication compartments [82]. However, the physiological significance of the selective incorporation of ND10 factors like Sp100 or NDP55 into viral replication sites still remains unclear. Furthermore, infection studies using the E4 ORF3 deletion virus in order to retain intact PML-NBs demonstrated that Ad replication domains, like in case of herpesviruses, would normally be located directly adjacent to ND10 [82]. By monitoring the fate of input wt Ad genomes, a preferential association with this subnuclear structure prior to ND10 disruption and the start of viral DNA replication was observed similar to that seen for various other nuclear-replicating DNA viruses which has also been found to occur independent of viral gene expression comparable to the situation with HSV-1 [82].

Beside its role in redistributing ND10 components, E4 ORF3, in addition, functions in antagonizing the cellular Mre11-Rad50-Nbs1 (MRN)-dependent DNA damage response that is induced by adenoviral genomes during infection. Activation of the MRN DNA repair complex would otherwise lead to concatemerization of viral genomes and the efficient prevention of viral DNA replication [149]. To impede this host response, E4 ORF3 contributes to the inhibition of MRN function by sequestering the nucleoplasmic pool of MRN proteins into the E4 ORF3-containing track-like structures [150–153]. Mutational analyses have demonstrated that PML and MRN reorganization of E4 ORF3 are discrete activities of the protein [153]. While MRN relocalization by E4 ORF3 is specific to only a subset of Ads, the ability of E4 ORF3 to rearrange ND10 was found to be evolutionarily conserved among divergent serotypes, further underlining the significance of this phenomenon [151]. Only recently, the functional consequences of E4 ORF3-mediated ND10 track formation have been elucidated in greater detail: Ullman and colleagues [154,155] could show that E4 ORF3 successfully antagonizes an IFN-induced antiviral state mounted by ND10 via disrupting this subnuclear structure early during infection. While pretreatment of cells with INF-α or INF-γ does not prevent E4 ORF3 elicited ND10 rearrangement upon wt Ad infection despite the increased synthesis of individual ND10 components, replication of an E4 ORF3 deletion virus, on the contrary, is efficiently restricted under these conditions [154,155]. In accordance with this, reduction of either PML or hDaxx (but not Sp100) by shRNAs turned out to be sufficient to restore the replicative capacity of the E4 ORF3-deficient Ad following IFN stimulation of cells [155]. On the other hand, infection of cell lines, engineered to overexpress PML (isoform VI), block or severely delay adenovirus replication [148].

Fig. 1. Schematic representation of the current model of HCMV-ND10 interplay during infection. (A) In cells in which quiescent HCMV infections are established, it is speculated that pp71 is somehow retained in the cytoplasm while incoming viral DNA is efficiently targeted by ND10. By this, pp71 is prevented from binding to and degrading the restriction factor hDaxx. As a consequence of this, hDaxx-induced epigenetic gene silencing is not abrogated resulting in transcriptionally repressed viral genomes that are unable to initiate the lytic gene expression program of HCMV. (B-1) In cells permitting productive HCMV infection, pp71 is capable of entering the cell nucleus and targeting hDaxx for proteasome-dependent destruction. (B-2) pp71-induced reduction of hDaxx protein levels annihilates the repressive effect of hDaxx on the viral chromatin leading to the initiation of viral IE gene expression and production of the regulatory protein IE1. (B-3) Upon entry of the nucleus, IE1 localizes to ND10 to finally induce the disruption of this subnuclear structure. ND10 dispersal by IE1 correlates with the induction of an efficient lytic replication.

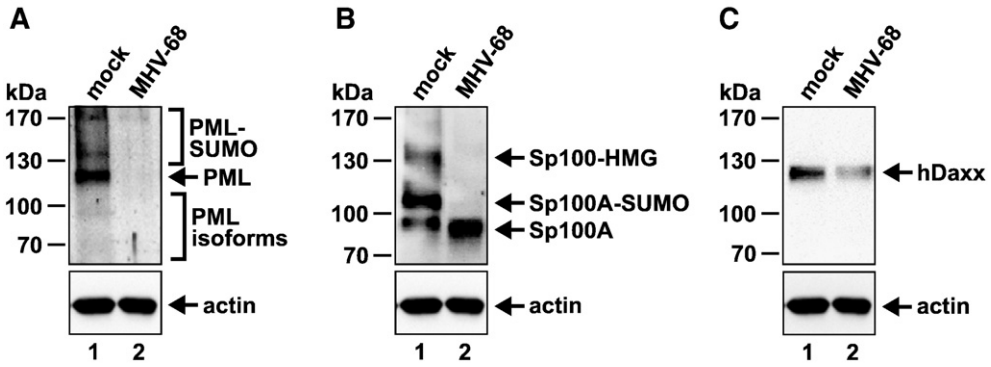


Fig. 2. Examination of the protein levels of the three major ND10 constituents PML, Sp100, and hDaxx upon MHV-68 infection. HFFs were either mock infected (lane 1) or infected with MHV-68 (lane 2) and lysates were harvested 24 h postinoculation to be subjected to Western blotting. (A) The various SUMOylated as well as non-SUMOylated isoforms of PML were detected with the anti-PML rabbit serum H-238 (Santa Cruz). The major PML isoform is indicated by an arrow. (B) Sp100 protein levels were assayed using the anti-Sp100 polyclonal antibody AB1380 (Chemicon). The different variants of Sp100 are specified by arrows. (C) Analysis of hDaxx abundance was achieved with an anti-hDaxx monoclonal rabbit antibody from Epitomics. Actin was included as an internal loading control.

Taken together, these results demonstrate a role for PML and hDaxx in the IFN-induced antiviral response against Ad which is normally counteracted by the E4 ORF3 protein. However, in contradiction to findings from herpesviruses like HSV-1 (see 5.1.) or HCMV (see 5.2.), the antiviral strategy of ND10 in case of Ad infection is not solely based on transcriptional repression since Ad early gene expression is only modestly affected to a degree that is not sufficient to compromise early protein function [155]. Instead, it was reported that PML-NBs mainly suppress Ad infection by negatively influencing viral DNA replication [154,155]. Therefore, the authors propose that, in addition to its abilities to repress gene expression, ND10 participate in additional innate immune defense activities.

In this regard, it is of note that the replication defect of the E4 ORF3 mutant virus upon interferon treatment could be restored by co-expression of either ICP0 or IE1 [155], the herpesvirus regulatory proteins known to successfully evade ND10 repression during HSV-1 (see 5.1.) and HCMV replication (see 5.2.), respectively. Thus, these data suggest that Ad E4 ORF3, HSV-1 ICP0, and HCMV IE1 behave in a functionally analogous manner with respect to antagonizing the antiviral role of ND10 as they share in common the ability to disrupt ND10 integrity albeit employing mechanistically distinct strategies to achieve this purpose.

The exact mechanism by which E4 ORF3 causes ND10 dispersal is still unclear. While transiently expressed E4 ORF3 has been shown to have no effect on the SUMOylation state of exogenous PML (isoform IV) [89], Ad infection results in a gradual loss of the high molecular weight SUMO-modified variants of PML [156]. In addition, the appearance of novel, infection-specific species of PML was observed, one of which became predominant later in the infectious course [156]. Since these effects on PML occur in an E4 ORF3-dependent manner [156], it is likely that they are related to the E4 ORF3-mediated changes in ND10 structure taking place during infection although direct evidence for this assumption is still missing. Consequently, in order to affect the PML modification status, E4 ORF3 either requires additional infection specific factors or a PML isoform other than PML-IV is the major target for the induction of ND10 track formation. Indeed, recent studies indicate that particularly the interaction of E4 ORF3 with PML isoform II seems to be required for ND10 disruption [157]. Moreover, the observation that the overall pattern of PML isoforms in Ad-infected cells is very reminiscent of the pattern seen in cells during mitosis when ND10 are temporarily dispersed [18], gave rise to following ideas: (i) The Ad-induced effect on ND10 could be an indirect consequence of changes to cell cycle regulation imposed upon infection. (ii) Ad simply utilizes related molecular triggers that are also used during cell cycle regulation to achieve similar changes of the PML modification pattern.

Of interest, during the late phase of the infection cycle an Ad structural protein, known as the minor capsid protein IX (pIX), has been implicated in maintaining the E4 ORF3-initiated neutralization of ND10. Rosa-Calatrava et al. [158] could demonstrate that pIX expression induces the formation of specific nuclear structures known as clear amorphous inclusions and the stable recruitment of ND10 components like PML and Sp100 into these structures at late times of infection in order to prevent ND10 re-formation [159]. These data imply that Ad has evolved a unique strategy to sustain the inactivation of ND10 throughout infection, thereby ensuring optimal viral replication. Intriguingly, infection with a mutant virus that lacks expression of the fibre protein, which normally builds up the spikes at the vertices of the virion, results in accumulation of other major capsid proteins like the viral hexon and penton base proteins, in these amorphous nuclear inclusions [160]. Although the concrete significance of this finding is uncertain, it could provide a first indication that Ad virion assembly might also be linked to ND10.

5.5. Papillomaviruses

Human papillomaviruses (HPV) comprise a heterogeneous group of non-enveloped, small DNA viruses that induce benign epithelial papillomas and have been implicated etiologically in human cervical carcinoma, as well as in a subset of other epithelial carcinomas [94]. HPV infections are characterized by a biphasic life cycle which is tightly linked to the differentiation process of the host squamous epithelia. Nonproductive infection initiates and is maintained in the basal cell layers of the epithelium. In these undifferentiated, mitotically active cells, a subset of viral genes called the early genes, are expressed and the viral DNA is replicated on average once per cell cycle and efficiently segregated to daughter cells. Upon terminal differentiation of keratinocytes the productive life cycle of HPV is induced, resulting in viral DNA amplification, structural protein expression, and progeny virus assembly [94].

The first report relating papillomavirus infection to ND10 came from Day et al. [161] showing that the minor capsid protein L2 from bovine papillomavirus type 1 (BPV1) colocalized with this subnuclear structure when exogenously expressed. This observation could subsequently be confirmed using the L2 protein from human papillomavirus type 16 (HPV16) [162]. In addition, L2 turned out to be responsible for the redistribution of the major capsid protein L1 as well as the nonstructural protein E2 to ND10 upon co-expression [161]. Analysis of L2 from HPV33, furthermore, revealed an L2-induced reorganization of ND10-asssociated proteins: while the major structural component PML remained unaffected, Sp100 protein levels were found to be significantly reduced upon L2 expression [163]. On the

other hand, hDaxx concentration at ND10 was enhanced in the presence of L2 and both proteins were shown to interact with each other, either directly or via an intermediate [163] which was suggested to be the driving force for L2 accumulation at ND10 [164]. Continuative experiments revealed that L2 aggregation at ND10 precedes that of L1, as the major capsid protein only arrives at these nuclear foci after L2-induced release of the ND10 factor Sp100 [165].

Given the fact that the E2 protein specifically binds to the viral DNA [166], this suggested that also viral genomes are deposited at ND10 through L2-mediated recruitment of E2 to these subnuclear sites, thus proposing a model in which ND10 serves as a place for encapsidation of the viral DNA. The changes to ND10 composition as a consequence of L2 expression could facilitate packaging of the newly synthesized virions at PML-NBs. However, recent findings indicate that ND10 structures are absent in the terminally differentiated layers of the stratified epithelium, the sites of papillomavirus progeny virion assembly, as determined in organotypic raft cultures of human keratinocytes harboring HPV genomes, which support the differentiation-dependent life cycle of HPV [167]. These data fit to the previous observation that L2 still assembles in distinct foci and recruits L1 to these sites to form virus-like particles even in the absence of genuine ND10 as assessed in PML-null fibroblasts [168]. Interestingly, similar to the results obtained for the hepresviruses HSV-1 (see 5.1.) and HCMV (see 5.2.), these L2 aggregates still attracted the transcriptional repressor hDaxx, which is normally diffusely distributed upon loss of the defining ND10 component [168]. Moreover, localization studies of L2 from HPV16 conducted under physiologically more relevant conditions demonstrated that the ability of L2 to accumulate at ND10 is dependent on the protein expression level as well as the presence of other heterologous viral factors [169]. In contrast to the former reports in which viral overexpression systems such as Semliki Forest virus or vaccinia virus were employed to dissect the intracellular distribution of L2 [161,163], the minor capsid protein only rarely localized at ND10 under moderate expression conditions if a codon-optimized version of L2 was used [169]. These results led the authors to conclude that L2 does not actively accumulate in ND10, but that ND10 location of L2 rather seems to be triggered by additional cellular factors which are induced, potentially as an antiviral defense mechanism.

In a transient replication assay the subnuclear topology of HPV11 ori DNA replication was characterized in more detail [170]. As judged by indirect immunofluorescence analysis, papillomaviral DNA amplification may also be coupled to ND10 [170]. The early proteins E1 and E2, which are involved in viral DNA replication, are known to interact and to bind together to the papillomavirus origin of replication [94]. While E2 was reported to be recruited to ND10 by L2 upon coexpression, E1 was not [161]. Both, E1 and E2 of HPV11, however, were found to accumulate at PML-NBs if expressed together in the presence of papillomavirus replication origin-containing plasmid DNA [170]. When the ori plasmid was omitted from the transfection, PML was still found to colocalize with E1 or E2, but at a much reduced frequency, suggesting that the degree of ND10 localization was influenced by active HPV DNA synthesis [170]. However, in a study conducted by Nakahara and Lambert et al. [167] it was demonstrated that PML and ND10 are not required for papillomavirus DNA replication in transfected cells. Nevertheless, these data imply that HPV replication compartments, as in case of other DNA viruses, are established in close proximity to ND10 but without having an impact on the integrity of this subnuclear structure.

In contrast to that, Roberts et al. [171] identified a late infection phase product, the E4 protein of HPV1, which is capable of modifying ND10 organization. Over-expression of the E4 protein results in the formation of nuclear inclusions [172]. Although the precise nature of the E4-derived inclusion bodies is not known, E4 induces the relocation of PML from ND10 to the periphery of these subnuclear structures [171]. Analysis of HPV1-induced warts demonstrated that similar nuclear PML-E4 inclusions were also present in productively infected keratinocytes [171]. Consequently, it was concluded that reorganization of PML occurs not only *in vitro* but also *in vivo*. Since E4 expression correlates with the onset of vegetative genome replication in naturally occurring lesions including those induced by HPV1, the authors suggested that the E4-induced reorganization of PML represents a switch in the HPV life cycle, from the nonproductive maintenance stage of replication in the basal cells to the virus-producing productive phase in differentiating cells. Given the emerging link between ND10 and viral genome replication, the E4-mediated redistribution of ND10 could be necessary for efficient replication of the virus during the productive phase.

Previous reports have also linked the early papillomavirus proteins E6 and E7 to ND10. Both viral proteins are known to possess oncogenic properties by predominantly targeting p53 (E6) and pRB (E7) for inactivation. While E6 and E7 from high-risk virus types (HPV16 or HPV18) display a diffuse distribution pattern throughout the nucleus, the equivalent oncoproteins expressed from a low-risk virus type (HPV11), on the contrary, were found to form punctate accumulations in association with PML [173]. In addition, the E7 protein was shown to inhibit PML isoform IV-induced senescence due to its ability to simultaneously disrupt pRb, p53/CBP, and PML functionality through direct interaction [174]. Normally, PML concomitantly activates the p53 as well as pRb tumor suppressor pathway to ensure complete drop out from the cell cycle. Similarly, also the E6 protein counteracts PML-mediated cellular senescence by specifically destabilizing PML isoform IV in a proteasome-dependent pathway [175]. Overall, it is tempting to speculate that the fundamental differences in ND10/PML interaction of the high- and low-risk E6 and E7 proteins contribute to the differences in oncogenicity and pathogenicity of the diverse papillomavirus subtypes.

Recent investigation of the trafficking of papillomavirus particles in infected cells revealed that DNA encapsidated in papillomavirus pseudovirions together with the minor capsid protein L2 associated with ND10 directly upon nuclear entry and prior to the start of DNA amplification [176]. Thus, in the meantime quite a series of data exist that connect ND10 with all stages of the papillomavirus life cycle. Initial functional studies conducted to address the biological relevance of ND10 for papillomavirus replication indicate that, in contradiction to the findings for HSV-1 (see 5.1.), HCMV (see 5.2.), or adenoviruses (see 5.4.), ND10 seem to positively affect papillomavirus infectivity [176]. The presence of PML and intact ND10 is associated with enhanced papillomaviral early gene expression [176]. However, it has to be emphasized that the efficiency of viral transcription as measured in the absence of PML and putative ND10 was compared to the transcriptional activity obtained after infection of PML-null MEFs which were stably transduced with PML isoform III only [176]. Given the accumulating evidence that PML cannot be considered as a single protein with the different variants having distinct properties and activities [16,177], and viruses specifically targeting particular isoforms for ND10 destruction only (e.g. see adenoviruses 5.4.) [157], it has to be questioned if reintroduction of other PML isoforms or usage of wt MEFs would yield similar results. Moreover, by performing a luciferase reporter gene assay another group found no difference in the efficacy of E2-mediated viral transcription in PML−/− versus PML+/+ cells [167]. Thus, the functional role of ND10 structures for papillomavirus infection is still controversially discussed and awaits further examination.

5.6. Polyomaviruses

In common with all other nuclear-replicating DNA viruses, input viral genomes of the simian polyomavirus SV40 have been found to localize preferentially to ND10 and start to replicate adjacent to these subnuclear sites but without noticeable modification of ND10 [82]. Subsequent experiments revealed that the replicative process also

initiates at ND10 if the small SV40 genome (5 kb) is transfected into cells, indicating that none of the capsid proteins is necessary for this activity [178]. However, plasmid replicons bearing a reporter gene only localized to or nearby to ND10 when they contained the SV40 origin of DNA replication in the presence of SV40 large T antigen, which is essential for SV40 DNA replication [178]. Detailed analysis indicated that the large T-antigen binding site II (the core origin) was sufficient for large T-induced DNA amplification at ND10 [178]. Furthermore, sequestration of DNA molecules to these structures appears to require active viral DNA synthesis [179]. Interestingly, a significant proportion of the viral DNA positioned at ND10 was found to be single-stranded implying that viral genome replication at ND10 could be induced by ND10-associated DNA repair mechanisms [179]. While depletion of PML had no significant effect on the overall efficacy of viral DNA replication, it completely abolished the accumulation of single-stranded DNA in nuclear foci [179].

In addition, reporter gene transcription was also detectable at ND10 but equivalent levels of expression were also observed from plasmid DNA which was not located at this subnuclear site and with transcripts being diffusely distributed throughout the nucleoplasm [178]. Although plasmid transcription can take place anywhere in the nucleus, T-antigen-directed replication is apparently restricted to ND10. These findings suggest that viral transcription at ND10 may only be a consequence of viral genome amplification at this domain. However, these data are in apparent conflict to results obtained by Bishop et al. [180], showing that plasmid DNA delivered by polyomavirus-like particles associated with ND10 even in the absence of SV40 large T-antigen and ori sequences. In the latter case, colocalization of virus like particle-delivered transgenes with PML-NBs was reported to be a consequence of transgene expression rather than the presence of specific viral replication factors as activation of transcription resulted in relocation of foreign DNA from transcriptionally inactive centromeric heterochromatin to ND10 and regions of transcriptionally active euchromatin [180]. The same study provided evidence that the association of foreign DNA with ND10 does not influence transgene expression per se, but that the PML protein is necessary for the α-IFN-mediated inhibition of foreign gene transcription [180]. This further strengthens the assumption that viral localization at ND10 is not for the benefit of the virus but rather represents a host-regulated process which is part of an inducible cellular defense mechanism.

Finally, comparable to adeno- (see 5.4.) or papillomavirus infection (see 5.5.), first evidence exists that also polyomavirus virion assembly is related to ND10. As reported for the human polyomavirus JC (JCV), transient co-expression of the major (VP1) as well as the minor capsid proteins (VP2 and VP3) along with a regulatory protein called agnoprotein resulted in cooperative accumulation of these viral components and assembly of virus-like particles in discrete nuclear domains which were identified as ND10 [181]. Similar nuclear foci were detected in oligodendrocytes isolated from human brain tissue of autopsied patients with progressive multifocal leukoencephalopathy what further underlines the physiological significance of this finding [181]. Intriguingly, recent data indicate that progression of viral progeny production at ND10 finally leads to the disruption of this subnuclear structure [182]. JCV-induced destruction of ND10 can presumably be regarded as the trigger for oligodendrocyte degeneration and the resulting demyelination, the hallmarks of progressive multifocal leukoencephalopathy [182].

6. RNA viruses and ND10

As specified in detail in the prior chapters, replication of quite a variety of DNA viruses that enter the nucleus as part of their replicative cycle has been found to be intimately connected to the subnuclear structure ND10. Even more surprisingly, evidence is accumulating that also infection of an increasing number of RNA viruses from several different families is affected by ND10. The current state of knowledge concerning RNA virus replication and ND10 is briefly discussed below.

6.1. Retroviruses

Retroviruses are characterized by their unique infection strategy, as their RNA genomes are initially reverse transcribed into DNA to be subsequently integrated into the host chromosome. Meanwhile, the infectious cycle of viruses from four different genera of the retrovirus family has been shown to be related to PML and ND10.

Exogenous expression of PML isoform III, for instance, was reported to confer resistance against human foamy virus (HFV), a complex retrovirus, as a substantial decrease in HFV mRNA expression levels, DNA and protein synthesis as well as reverse transcriptase activity is detectable [183]. The mechanism of this effect appears to involve the PML-induced complex formation with the viral transactivator Tas, thereby preventing Tas from binding to viral promoter sequences and activating viral gene expression [183]. Interestingly, HFV infection could similarly be compromised by treatment of cells with IFN. Since IFN application resulted in restriction of HFV replication in wt but not in PML−/− MEFs, this points to a role for PML in the IFN-mediated antiviral action against HFV [183]. However, PML seems not to be involved in the establishment of HFV latency [184]. Thus, the biological significance of PML-mediated suppression of HFV replication is still unclear.

The human T-cell leukemia virus type 1 (HTLV-1) encodes a transcriptional activator, the Tax protein, which promotes cellular transformation and is associated with the pathogenesis of adult T-cell leukemia [94]. The Tax oncoprotein has been demonstrated to alter the subcellular localization of an ND10-associated protein known as Int-6. Tax directly interacts with Int-6, as determined by yeast and mammalian two-hybrid analysis as well as co-immunoprecipitation and induces Int-6 redistribution from ND10 to the cytoplasm without affecting the localization of PML [185]. In addition, Tax was found to be a potent repressor of steroid and retinoid receptor transcription [186]. However, the repressive effect of Tax can be reversed by overexpression of PML [186], suggesting that the modulation of ND10 composition by Tax is of functional significance.

Also, infection with human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, was shown to affect ND10 morphology upon infection as incoming retroviral preintegration complexes trigger the cytoplasmic export of the key factor PML [187]. Nuclear redistribution of PML to ND10 as achieved via treatment with arsenic trioxide resulted in an increased transduction efficiency of HIV, suggesting that PML is acting as part of an antiviral response by opposing the establishment of infection. However, other groups failed to confirm these results as they were not able to observe a colocalization of viral preintegration complexes with PML [188,189]. In addition, further experiments revealed that PML is dispensable for the stimulatory effect of arsenic trioxide on HIV-1 replication [189]. Thus, the role of PML and ND10 for HIV-1 remains uncertain.

Finally, during avian sarcoma virus (ASV) infection an interaction of the ND10 constituent hDaxx with the retrovirus integrase (IN) as well as the reverse transcribed viral DNA is detectable [190]. The association of hDaxx with the retroviral DNA turned out to induce a silencing of ASV gene expression presumably via recruitment of HDACs to the viral genome [190]. By the usage of an siRNA-based approach, the involvement of hDaxx and HDAC in the epigenetic gene silencing of integrated retroviral DNA could be confirmed, as knockdown of both host factors annihilated transcriptional repression of a reporter gene construct [191]. These experiments furthermore indicated that hDaxx/HDAC-mediated gene silencing is largely independent of the integration site of the DNA or the type of promoter used for reporter gene expression [191], suggesting that hDaxx/HDAC functions in a fairly broad and unspecific manner against integrated

foreign DNA. Interestingly, expression of the HCMV structural protein pp71 (see 5.2.), for instance, with its well-established function in counteracting hDaxx antiviral activity, indeed, resulted in reactivation of reporter gene transcription [191]. In summary, these data further support the evolving concept of hDaxx as being part of an antiviral defense mechanism of the cell.

6.2. Other RNA viruses

Presumably the most convincing data in order to clarify the in vivo relevance of ND10 for viral infection have been obtained with RNA viruses like the arenavirus lymphocytic choriomeningitis virus (LCMV) or the rhabdovirus vesicular stomatitis virus (VSV), which are studied as model systems in mice. Examination of the functional role of ND10 structures in vivo revealed that PML deficiency renders mice more susceptible to LCMV and VSV infections [192]. These in vivo observations corroborate previous findings demonstrating that fibroblasts derived from PML−/− mice support enhanced LCMV growth [193] and are also more sensitive than wt MEFs to infection with rabies virus, another member of the rhabdovirus family [194]. Furthermore, increased expression of PML as a consequence of IFN treatment of cells [193] or stable transfection with a construct encoding PML isoform III [195], turned out to be capable of diminishing LCMV [193] and VSV replication [195], respectively, due to a decrease in viral mRNA and protein production. In addition, the capacity of IFN to guard against LCMV infection has been shown to be higher in wt MEFs compared to PML-null MEFs, although LCMV production is still diminished in IFN-treated PML−/− MEFs, indicating that the IFN sensitivity of LCMV is not entirely based on PML [193]. Thus, these results implicate PML in an intrinsic antiviral response of the cell that targets not only DNA viruses but also divers cytoplasmic replicating RNA viruses.

Interestingly, comparable to the situation with DNA viruses, some RNA viruses, for which the infectious cycle is inhibited by PML, have developed strategies to compromise ND10 composition to various extents. LCMV, for instance, was found to disrupt ND10 during infection due to the action of a small non-structural protein called Z [196]. The RING-finger protein Z colocalizes with PML both in transfected as well as infected cells and induces the redistribution of PML from nuclear ND10 bodies into the cytoplasm of cells [196], where both proteins directly interact with the elongation factor eIF4E, thereby reducing its affinity for the 5′ mRNA cap structure and inhibiting translation [197]. Similarly, the phosphoprotein P of rabies virus also binds to PML and causes its relocation into the cytoplasm [194]. Following rabies virus infection, however, PML delocalization could not be observed. Instead, PML-containing ND10 structures increase in size as likewise detected upon transfection of P3, an N-terminally truncated version of the phosphoprotein P, which is therefore believed to be responsible for this effect [194].

Finally, three viral proteins of the orthomyxovirus influenza A, have been shown to associate with PML and ND10 during infection, namely the matrix protein M1, as well as the nonstructural polypeptides NS1 and NS2 [198]. Although, the functional significance of this finding is still unclear, previous studies demonstrated that propagation of influenza A virus is likewise negatively influenced by PML as productive infection could be significantly suppressed by overexpression of PML isoforms III, IV as well as VI [195,199]. In accordance with this, downregulation of PML expression by RNAi, on the other hand, enhanced viral replication [199]. Thus, these data support the notion that PML also contributes to the countermeasures the cell has evolved against influenza A virus infection.

7. Concluding remarks

ND10 have been identified as preferential targets for viral modification by members of both DNA as well as RNA viral families. But why do viruses with such diverse replication strategies interact with and commonly alter ND10 accumulations during their replicative cycle? In principle, two theories have been established that try to explain the reason for the intimate virus interactions with ND10. Firstly, ND10 could harbor components or provide functions that are advantageous for the virus and support viral replication. Alternatively, ND10 structures could illustrate aggregations of proteins that compromise virus growth and which are therefore targeted by the virus for destruction to be inactivated.

In this review we summarized the rapidly growing body of information that mainly links ND10 to an antiviral defense mechanism of the cell that has evolved to efficiently counteract infection of quite a variety of distinct viruses. Evidence continues to accumulate that basically all three major ND10 components, PML, Sp100, as well as hDaxx constitute host factors with antiviral activities as supported by work on herpesviruses like HSV-1 (see 5.1.) or HCMV (see 5.2.), which show enhanced infectivity in the absence of these individual ND10 constituents.

The currently available data implicate ND10 as part of both the intrinsic as well as the IFN-mediated innate cellular response to viral infections. It was only recently that intrinsic immune mechanisms gained substantial interest as they form an antiviral front-line defense mediated by constitutively expressed proteins, designated as restriction factors, that are already present and active before a virus enters the cell [200]. The term “intrinsic” was originally used to define immunity that functions independent of cytokines and white blood cells. Thus, it refers to immune defenses that do not respond to viral infection like the innate immune system (e.g. NK cells and IFN) but which are ready to act even before the infecting agent is encountered [200]. Unlike other factors with IFN-dependent antiviral properties such as Mx proteins or 2′,5′-oligoadenylate synthetase, ND10 components are constitutively expressed which allows for immediate antiviral activity of these molecules. An effect, which is additionally potentiated through their IFN-mediated upregulation and by which ND10 components also contribute to the establishment of an antiviral state against certain viruses upon IFN induction.

Another characteristical aspect of cellular antiviral defense mechanisms is the fact that they are subject to viral countermeasures. Indeed, almost all viruses for which replication has been linked to ND10, have evolved regulatory proteins that are capable of inactivating single ND10 components or disturbing the integrity of the whole subnuclear structure. In many cases, these virus-induced alterations to ND10 composition have already been demonstrated to be important for the outcome of infection and correlate with the efficiency of viral propagation. Interestingly, however, each virus seems to have developed its own specific strategy to cope with the antiviral aspects of ND10.

Although there is considerable evidence in favor of the view that ND10 are involved in a cellular response aimed at restricting viral infection by actively targeting incoming viral components, this assumption obviously does not generally apply to all viruses as indicated in case of papillomavirus infection (see 5.5.). In addition, it cannot be excluded, that, upon initial disruption of ND10 and inactivation of their antiviral features, these subnuclear loci represent favorable sites for viral replication and the establishment of replication centers or progeny virion assembly sites. Nevertheless, the future challenge will be to get a detailed understanding of the underlying molecular mechanisms of ND10 as well as of the viral factors antagonizing its antiviral function since shifting the balance towards ND10-mediated viral repression could constitute a novel principle how to fight viral infections.

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References

[1] D. Negorev, G.G. Maul, Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot, Oncogene 20 (2001) 7234–7242.
[2] N. Stuurman, G.A. de, A. Floore, A. Josso, B. Humbel, J.L. de, D.R. van, A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies, J.Cell Sci. 101 (1992) 773–784.
[3] C.A. Ascoli, G.G. Maul, Identification of a novel nuclear domain, J. Cell Biol. 112 (1991) 785–795.
[4] R.D. Everett, Interactions between DNA viruses, ND10 and the DNA damage response, Cell Microbiol. 8 (2006) 365–374.
[5] G. Dellaire, D.P. Bazett-Jones, PML nuclear bodies: dynamic sensors of DNA damage and cellular stress, Bioessays 26 (2004) 963–977.
[6] G. Dellaire, D.P. Bazett-Jones, Beyond repair foci: subnuclear domains and the cellular response to DNA damage, Cell Cycle 6 (2007) 1864–1872.
[7] G.G. Maul, D.E. Jensen, A.M. Ishov, M. Herlyn, F.J. Rauscher III, Nuclear redistribution of BRCA1 during viral infection, Cell Growth Differ. 9 (1998) 743–755.
[8] T. Wileman, Aggresomes and pericentriolar sites of virus assembly: cellular defense or viral design? Annu. Rev. Microbiol. 61 (2007) 149–167.
[9] L. Fu, Y.S. Gao, A. Tousson, A. Shah, T.L. Chen, B.M. Vertel, E. Sztul, Nuclear aggresomes form by fusion of PML-associated aggregates, Mol. Biol. Cell 16 (2005) 4905–4917.
[10] C. Szostecki, H.H. Guldner, H.J. Netter, H. Will, Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis, J. Immunol. 145 (1990) 4338–4347.
[11] S. Zhong, S. Muller, S. Ronchetti, P.S. Freemont, A. Dejean, P.P. Pandolfi, Role of SUMO-1-modified PML in nuclear body formation, Blood 95 (2000) 2748–2753.
[12] A.M. Ishov, A.G. Sotnikov, D. Negorev, O.V. Vladimirova, N. Neff, T. Kamitani, E.T.H. Yeh, J.F. Strauss, G.G. Maul, PML is critical for ND10 formation and recruits the PML-interacting protein Daxx to this nuclear structure when modified by SUMO-1, J. Cell Biol. 147 (1999) 221–233.
[13] R.D. Everett, S. Rechter, P. Papior, N. Tavalai, T. Stamminger, A. Orr, PML contributes to a cellular mechanism of repression of herpes simplex virus type 1 infection that is inactivated by ICP0, J.Virol. 80 (2006) 7995–8005.
[14] N. Tavalai, P. Papior, S. Rechter, M. Leis, T. Stamminger, Evidence for a role of the cellular ND10 protein PML in mediating intrinsic immunity against human cytomegalovirus infections, J. Virol. 80 (2006) 8006–8018.
[15] A. Kakizuka, W.H. Miller Jr., K. Umesono, R.P. Warrell Jr., S.R. Frankel, V.V. Murty, E. Dmitrovsky, R.M. Evans, Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML, Cell 66 (1991) 663–674.
[16] K. Jensen, C. Shiels, P.S. Freemont, PML protein isoforms and the RBCC/TRIM motif, Oncogene 20 (2001) 7223–7233.
[17] X.F. Le, P. Yang, K.S. Chang, Analysis of the growth and transformation suppressor domains of promyelocytic leukemia gene, PML, J. Biol. Chem. 271 (1996) 130–135.
[18] R.D. Everett, P. Lomonte, T. Sternsdorf, D.R. van, A. Orr, Cell cycle regulation of PML modification and ND10 composition, J.Cell Sci. 112 (1999) 4581–4588.
[19] T. Kamitani, H.P. Nguyen, K. Kito, T. Fukuda-Kamitani, E.T. Yeh, Covalent modification of PML by the sentrin family of ubiquitin-like proteins, J.Biol. Chem. 273 (1998) 3117–3120.
[20] S. Muller, M.J. Matunis, A. Dejean, Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus, EMBO. J. 17 (1998) 61–70.
[21] T. Kamitani, K. Kito, H.P. Nguyen, H. Wada, T. Fukuda-Kamitani, E.T. Yeh, Identification of three major sentrinization sites in PML, J. Biol.Chem. 273 (1998) 26675–26682.
[22] T.H. Shen, H.K. Lin, P.P. Scaglioni, T.M. Yung, P.P. Pandolfi, The mechanisms of PML-nuclear body formation, Mol.Cell 24 (2006) 331–339.
[23] D.Y. Lin, Y.S. Huang, J.C. Jeng, H.Y. Kuo, C.C. Chang, T.T. Chao, C.C. Ho, Y.C. Chen, T.P. Lin, H.I. Fang, C.C. Hung, C.S. Suen, M.J. Hwang, K.S. Chang, G.G. Maul, H.M. Shih, Role of SUMO-interacting motif in Daxx SUMO modification, subnuclear localization, and repression of sumoylated transcription factors, Mol. Cell 24 (2006) 341–354.
[24] A.D. Hollenbach, J.E. Sublett, C.J. McPherson, G. Grosveld, The Pax3-FKHR oncoprotein is unresponsive to the Pax3-associated repressor hDaxx, EMBO. J. 18 (1999) 3702–3711.
[25] M. Kiriakidou, D.A. Driscoll, J.M. Lopez-Guisa, J.F. Strauss III, Cloning and expression of primate Daxx cDNAs and mapping of the human gene to chromosome 6p21.3 in the MHC region, DNA Cell Biol. 16 (1997) 1289–1298.
[26] H. Li, C. Leo, J. Zhu, X. Wu, J. O'Neil, E.J. Park, J.D. Chen, Sequestration and inhibition of Daxx-mediated transcriptional repression by PML, Mol.Cell Biol. 20 (2000) 1784–1796.
[27] A.F. Pluta, W.C. Earnshaw, I.G. Goldberg, Interphase-specific association of intrinsic centromere protein CENP-C with HDaxx, a death domain-binding protein implicated in Fas-mediated cell death, J.Cell Sci. 111 (1998) 2029–2041.
[28] P. Salomoni, A.F. Khelifi, Daxx: death or survival protein? Trends. Cell. Biol. 16 (2006) 97–104.
[29] R. Li, H. Pei, D.K. Watson, T.S. Papas, EAP1/Daxx interacts with ETS1 and represses transcriptional activation of ETS1 target genes, Oncogene 19 (2000) 745–753.
[30] J. Park, J.H. Lee, M. La, M.J. Jang, G.W. Chae, S.B. Kim, H. Tak, Y. Jung, B. Byun, J.K. Ahn, C.O. Joe, Inhibition of NF-kappaB acetylation and its transcriptional activity by Daxx, J. Mol. Biol. 368 (2007) 388–397.
[31] J.S. Michaelson, P. Leder, RNAi reveals anti-apoptotic and transcriptionally repressive activities of DAXX, J.Cell Sci. 116 (2003) 345–352.
[32] C.C. Chang, D.Y. Lin, H.I. Fang, R.H. Chen, H.M. Shih, Daxx mediates the small ubiquitin-like modifier-dependent transcriptional repression of Smad4, J. Biol. Chem. 280 (2005) 10164–10173.
[33] E.J. Kim, J.S. Park, S.J. Um, Identification of Daxx interacting with p73, one of the p53 family, and its regulation of p53 activity by competitive interaction with PML, Nucleic. Acids. Res. 31 (2003) 5356–5367.
[34] D.Y. Lin, H.I. Fang, A.H. Ma, Y.S. Huang, Y.S. Pu, G. Jenster, H.J. Kung, H.M. Shih, Negative modulation of androgen receptor transcriptional activity by Daxx, Mol. Cell. Biol. 24 (2004) 10529–10541.
[35] A.D. Hollenbach, C.J. McPherson, E.J. Mientjes, R. Iyengar, G. Grosveld, Daxx and histone deacetylase II associate with chromatin through an interaction with core histones and the chromatin-associated protein Dek, J.Cell Sci. 115 (2002) 3319–3330.
[36] R. Muromoto, K. Sugiyama, A. Takachi, S. Imoto, N. Sato, T. Yamamoto, K. Oritani, K. Shimoda, T. Matsuda, Physical and functional interactions between Daxx and DNA methyltransferase 1-associated protein, DMAP1, J. Immunol. 172 (2004) 2985–2993.
[37] A.M. Ishov, O.V. Vladimirova, G.G. Maul, Heterochromatin and ND10 are cell-cycle regulated and phosphorylation-dependent alternate nuclear sites of the transcription repressor Daxx and SWI/SNF protein ATRX, J. Cell Sci. 117 (2004) 3807–3820.
[38] Y. Xue, R. Gibbons, Z. Yan, D. Yang, T.L. McDowell, S. Sechi, J. Qin, S. Zhou, D. Higgs, W. Wang, The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10635–10640.
[39] D.Y. Lin, H.M. Shih, Essential role of the 58-kDa microspherule protein in the modulation of Daxx-dependent transcriptional repression as revealed by nucleolar sequestration, J. Biol. Chem. 277 (2002) 25446–25456.
[40] A.L. Dent, J. Yewdell, F. Puvion-Dutilleul, M.H. Koken, H. de The, L.M. Staudt, LYSP100-associated nuclear domains (LANDs): description of a new class of subnuclear structures and their relationship to PML nuclear bodies, Blood 88 (1996) 1423–1426.
[41] J.S. Seeler, A. Marchio, R. Losson, J.M. Desterro, R.T. Hay, P. Chambon, A. Dejean, Common properties of nuclear body protein SP100 and TIF1alpha chromatin factor: role of SUMO modification, Mol. Cell Biol. 21 (2001) 3314–3324.
[42] J.S. Seeler, A. Marchio, D. Sitterlin, C. Transy, A. Dejean, Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 7316–7321.
[43] H.H. Guldner, C. Szostecki, P. Schroder, U. Matschl, K. Jensen, C. Luders, H. Will, T. Sternsdorf, Splice variants of the nuclear dot-associated Sp100 protein contain homologies to HMG-1 and a human nuclear phosphoprotein-box motif, J. Cell Sci. 112 (1999) 733–747.
[44] M.J. Bottomley, M.W. Collard, J.I. Huggenvik, Z. Liu, T.J. Gibson, M. Sattler, The SAND domain structure defines a novel DNA-binding fold in transcriptional regulation, Nat. Struct. Biol. 8 (2001) 626–633.
[45] K.W. Wilcox, S. Sheriff, A. Isaac, J.L. Taylor, SP100B is a repressor of gene expression, J. Cell Biochem. 95 (2005) 352–365.
[46] A. Isaac, K.W. Wilcox, J.L. Taylor, SP100B, a repressor of gene expression preferentially binds to DNA with unmethylated CpGs, J. Cell Biochem. 98 (2006) 1106–1122.
[47] M.S. Jang, S.W. Ryu, E. Kim, Modification of Daxx by small ubiquitin-related modifier-1, Biochem. Biophys. Res. Commun. 295 (2002) 495–500.
[48] T. Sternsdorf, K. Jensen, H. Will, Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1, J. Cell Biol. 139 (1997) 1621–1634.
[49] T. Sternsdorf, K. Jensen, B. Reich, H. Will, The nuclear dot protein sp100, characterization of domains necessary for dimerization, subcellular localization, and modification by small ubiquitin-like modifiers, J. Biol. Chem. 274 (1999) 12555–12566.
[50] P. Salomoni, P.P. Pandolfi, The role of PML in tumor suppression, Cell 108 (2002) 165–170.
[51] Y. Takahashi, V. Lallemand-Breitenbach, J. Zhu, T.H. de, PML nuclear bodies and apoptosis, Oncogene 23 (2004) 2819–2824.
[52] R. Bernardi, P.P. Pandolfi, Role of PML and the PML-nuclear body in the control of programmed cell death, Oncogene 22 (2003) 9048–9057.
[53] G.G. Maul, E. Yu, A.M. Ishov, A.L. Epstein, Nuclear domain 10 (ND10) associated proteins are also present in nuclear bodies and redistribute to hundreds of nuclear sites after stress, J. Cell Biochem. 59 (1995) 498–513.
[54] O. Bischof, O. Kirsh, M. Pearson, K. Itahana, P.G. Pelicci, A. Dejean, Deconstructing PML-induced premature senescence, EMBO J. 21 (2002) 3358–3369.
[55] V. Lallemand-Breitenbach, J. Zhu, F. Puvion, M. Koken, N. Honore, A. Doubeikovsky, E. Duprez, P.P. Pandolfi, E. Puvion, P. Freemont, T.H. de, Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11 S proteasome recruitment, and As2O3-induced PML or PML/retinoic acid receptor alpha degradation, J. Exp. Med. 193 (2001) 1361–1371.
[56] S. Zhong, P. Salomoni, P.P. Pandolfi, The transcriptional role of PML and the nuclear body, Nat. Cell Biol. 2 (2000) E85–E90.
[57] K.L. Borden, Pondering the promyelocytic leukemia protein (PML) puzzle: possible functions for PML nuclear bodies, Mol.Cell Biol. 22 (2002) 5259–5269.
[58] R. Bernardi, P.P. Pandolfi, Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies, Nat. Rev. Mol. Cell Biol. (2007).

[59] C.H. Eskiw, D.P. Bazett-Jones, The promyelocytic leukemia nuclear body: sites of activity? Biochem. Cell Biol. 80 (2002) 301–310.
[60] F.M. Boisvert, M.J. Hendzel, D.P. Bazett-Jones, Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA, J. Cell Biol. 148 (2000) 283–292.
[61] H.M. Shih, C.C. Chang, H.Y. Kuo, D.Y. Lin, Daxx mediates SUMO-dependent transcriptional control and subnuclear compartmentalization, Biochem. Soc. Trans. 35 (2007) 1397–1400.
[62] W.S. Wu, S. Vallian, E. Seto, W.M. Yang, D. Edmondson, S. Roth, K.S. Chang, The growth suppressor PML represses transcription by functionally and physically interacting with histone deacetylases, Mol. Cell Biol. 21 (2001) 2259–2268.
[63] L.A. Puto, J.C. Reed, Daxx represses RelB target promoters via DNA methyltransferase recruitment and DNA hypermethylation, Genes. Dev. 22 (2008) 998–1010.
[64] L. Di Croce, V.A. Raker, M. Corsaro, F. Fazi, M. Fanelli, M. Faretta, F. Fuks, C.F. Lo, T. Kouzarides, C. Nervi, S. Minucci, P.G. Pelicci, Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor, Science 295 (2002) 1079–1082.
[65] M.M. Khan, T. Nomura, H. Kim, S.C. Kaul, R. Wadhwa, T. Shinagawa, E. Ichikawa-Iwata, S. Zhong, P.P. Pandolfi, S. Ishii, Role of PML and PML-RARalpha in Mad-mediated transcriptional repression, Mol. Cell 7 (2001) 1233–1243.
[66] S. Fleischer, S. Wiemann, H. Will, T.G. Hofmann, PML-associated repressor of transcription (PAROT), a novel KRAB-zinc finger repressor, is regulated through association with PML nuclear bodies, Exp. Cell Res. 312 (2006) 901–912.
[67] R. Carbone, O.A. Botrugno, S. Ronzoni, A. Insinga, L. Di Croce, P.G. Pelicci, S. Minucci, Recruitment of the histone methyltransferase SUV39H1 and its role in the oncogenic properties of the leukemia-associated PML-retinoic acid receptor fusion protein, Mol. Cell. Biol. 26 (2006) 1288–1296.
[68] R. Villa, D. Pasini, A. Gutierrez, L. Morey, M. Occhionorelli, E. Vire, J.F. Nomdedeu, T. Jenuwein, P.G. Pelicci, S. Minucci, F. Fuks, K. Helin, L. Di Croce, Role of the polycomb repressive complex 2 in acute promyelocytic leukemia, Cancer Cell 11 (2007) 513–525.
[69] F.M. Boisvert, M.J. Kruhlak, A.K. Box, M.J. Hendzel, D.P. Bazett-Jones, The transcription coactivator CBP is a dynamic component of the promyelocytic leukemia nuclear body, J. Cell Biol. 152 (2001) 1099–1106.
[70] V.J. LaMorte, J.A. Dyck, R.L. Ochs, R.M. Evans, Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4991–4996.
[71] R.D. Everett, DNA viruses and viral proteins that interact with PML nuclear bodies, Oncogene 20 (2001) 7266–7273.
[72] A. Moller, M.L. Schmitz, Viruses as hijackers of PML nuclear bodies, Arch. Immunol. Ther. Exp. (Warsz.) 51 (2003) 295–300.
[73] G.G. Maul, Nuclear domain 10, the site of DNA virus transcription and replication, Bioessays 20 (1998) 660–667.
[74] T. Regad, M.K. Chelbi-Alix, Role and fate of PML nuclear bodies in response to interferon and viral infections, Oncogene 20 (2001) 7274–7286.
[75] R.D. Everett, M.K. Chelbi-Alix, PML and PML nuclear bodies: implications in antiviral defence, Biochimie 89 (2007) 819–830.
[76] M.K. Chelbi-Alix, L. Pelicano, F. Quignon, M.H. Koken, L. Venturini, M. Stadler, J. Pavlovic, L. Degos, T.H. de, Induction of the PML protein by interferons in normal and APL cells, Leukemia 9 (1995) 2027–2033.
[77] H.H. Guldner, C. Szostecki, T. Grotzinger, H. Will, IFN enhance expression of Sp100, an autoantigen in primary biliary cirrhosis, J. Immunol. 149 (1992) 4067–4073.
[78] M. Stadler, M.K. Chelbi-Alix, M.H. Koken, L. Venturini, C. Lee, A. Saib, F. Quignon, L. Pelicano, M.C. Guillemin, C. Schindler, Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element, Oncogene 11 (1995) 2565–2573.
[79] T. Grotzinger, K. Jensen, H. Will, The interferon (IFN)-stimulated gene Sp100 promoter contains an IFN-gamma activation site and an imperfect IFN-stimulated response element which mediate type I IFN inducibility, J. Biol. Chem. 271 (1996) 25253–25260.
[80] T. Grotzinger, T. Sternsdorf, K. Jensen, H. Will, Interferon-modulated expression of genes encoding the nuclear-dot-associated proteins Sp100 and promyelocytic leukemia protein (PML), Eur. J. Biochem. 238 (1996) 554–560.
[81] G.G. Maul, H.H. Guldner, J.G. Spivack, Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0), J. Gen. Virol. 74 (Pt 12) (1993) 2679–2690.
[82] A.M. Ishov, G.G. Maul, The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition, J. Cell. Biol. 134 (1996) 815–826.
[83] G.G. Maul, A.M. Ishov, R.D. Everett, Nuclear domain 10 as preexisting potential replication start sites of herpes simplex virus type-1, Virology 217 (1996) 67–75.
[84] R.D. Everett, G.G. Maul, HSV-1 IE protein Vmw110 causes redistribution of PML, EMBO. J. 13 (1994) 5062–5069.
[85] G.G. Maul, R.D. Everett, The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICP0, J. Gen. Virol. 75 (Pt 6) (1994) 1223–1233.
[86] R.D. Everett, ICP0, a regulator of herpes simplex virus during lytic and latent infection, Bioessays 22 (2000) 761–770.
[87] M.K. Chelbi-Alix, H. de The, Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins, Oncogene 18 (1999) 935–941.
[88] R.D. Everett, P. Freemont, H. Saitoh, M. Dasso, A. Orr, M. Kathoria, J. Parkinson, The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms, J. Virol. 72 (1998) 6581–6591.
[89] S. Muller, A. Dejean, Viral immediate-early proteins abrogate the modification by SUMO-1 of PML and Sp100 proteins, correlating with nuclear body disruption, J. Virol. 73 (1999) 5137–5143.
[90] J. Parkinson, R.D. Everett, Alphaherpesvirus proteins related to herpes simplex virus type 1 ICP0 affect cellular structures and proteins, J. Virol. 74 (2000) 10006–10017.
[91] C. Boutell, S. Sadis, R.D. Everett, Herpes simplex virus type 1 immediate-early protein ICP0 and is isolated RING finger domain act as ubiquitin E3 ligases in vitro, J. Virol. 76 (2002) 841–850.
[92] I. Hutchinson, A. Whiteley, H. Browne, G. Elliott, Sequential localization of two herpes simplex virus tegument proteins to punctate nuclear dots adjacent to ICP0 domains, J. Virol. 76 (2002) 10365–10373.
[93] R.D. Everett, G. Sourvinos, A. Orr, Recruitment of herpes simplex virus type 1 transcriptional regulatory protein ICP4 into foci juxtaposed to ND10 in live, infected cells, J. Virol. 77 (2003) 3680–3689.
[94] D.M. Knipe, P. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman, S.E. Straus, Fields Virology, 5th EdLippincott-Williams and Wilkins, Philadelphia, Pa, 2007.
[95] R.D. Everett, G. Sourvinos, C. Leiper, J.B. Clements, A. Orr, Formation of nuclear foci of the herpes simplex virus type 1 regulatory protein ICP4 at early times of infection: localization, dynamics, recruitment of ICP27, and evidence for the de novo induction of ND10-like complexes, J. Virol. 78 (2004) 1903–1917.
[96] R.D. Everett, J. Murray, ND10 components relocate to sites associated with herpes simplex virus type 1 nucleoprotein complexes during virus infection, J. Virol. 79 (2005) 5078–5089.
[97] G. Sourvinos, R.D. Everett, Visualization of parental HSV-1 genomes and replication compartments in association with ND10 in live infected cells, EMBO. J. 21 (2002) 4989–4997.
[98] Q. Tang, L. Li, A.M. Ishov, V. Revol, A.L. Epstein, G.G. Maul, Determination of minimum herpes simplex virus type 1 components necessary to localize transcriptionally active DNA to ND10, J. Virol. 77 (2003) 5821–5828.
[99] R.D. Everett, J. Murray, A. Orr, C.M. Preston, Herpes simplex virus type 1 genomes are associated with ND10 nuclear substructures in quiescently infected human fibroblasts, J. Virol. 81 (2007) 10991–11004.
[100] T. Tsukamoto, N. Hashiguchi, S.M. Janicki, T. Tumbar, A.S. Belmont, D.L. Spector, Visualization of gene activity in living cells, Nat. Cell Biol. 2 (2000) 871–878.
[101] R.D. Everett, C. Parada, P. Gripon, H. Sirma, A. Orr, Replication of ICP0 null mutant herpes simplex virus type 1 is restricted by both PML and Sp100, J. Virol. (2007).
[102] M. Muratani, D. Gerlich, S.M. Janicki, M. Gebhard, R. Eils, D.L. Spector, Metabolic-energy-dependent movement of PML bodies within the mammalian cell nucleus, Nat. Cell. Biol. 4 (2002) 106–110.
[103] P. Harle, B. Sainz Jr., D.J. Carr, W.P. Halford, The immediate-early protein, ICP0, is essential for the resistance of herpes simplex virus to interferon-alpha/beta, Virology 293 (2002) 295–304.
[104] K.L. Mossman, H.A. Saffran, J.R. Smiley, Herpes simplex virus ICP0 mutants are hypersensitive to interferon, J. Virol. 74 (2000) 2052–2056.
[105] W.P. Halford, C. Weisend, J. Grace, M. Soboleski, D.J. Carr, J.W. Balliet, Y. Imai, T.P. Margolis, B.M. Gebhardt, ICP0 antagonizes Stat 1-dependent repression of herpes simplex virus: implications for the regulation of viral latency, Virol. J. 3 (2006) 44.
[106] A.V. Chee, P. Lopez, P.P. Pandolfi, B. Roizman, Promyelocytic leukemia protein mediates interferon-based anti-herpes simplex virus 1 effects, J. Virol. 77 (2003) 7101–7105.
[107] R.D. Everett, A. Zafiropoulos, Visualization by live-cell microscopy of disruption of ND10 during herpes simplex virus type 1 infection, J. Virol. 78 (2004) 11411–11415.
[108] P. Lopez, R.J. Jacob, B. Roizman, Overexpression of promyelocytic leukemia protein precludes the dispersal of ND10 structures and has no effect on accumulation of infectious herpes simplex virus 1 or its proteins, J. Virol. 76 (2002) 9355–9367.
[109] D.G. Negorev, O.V. Vladimirova, A. Ivanov, F. Rauscher III, G.G. Maul, Differential role of Sp100 isoforms in interferon-mediated repression of herpes simplex virus type 1 immediate-early protein expression, J. Virol. 80 (2006) 8019–8029.
[110] B.A. McNally, J. Trgovcich, G.G. Maul, Y. Liu, P. Zheng, A role for cytoplasmic PML in cellular resistance to viral infection, PLoS ONE 3 (2008) e2277.
[111] J.H. Ahn, G.S. Hayward, The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells, J. Virol. 71 (1997) 4599–4613.
[112] A.M. Ishov, R.M. Stenberg, G.G. Maul, Human cytomegalovirus immediate early interaction with host nuclear structures: definition of an immediate transcript environment, J. Cell Biol. 138 (1997) 5–16.
[113] F. Korioth, G.G. Maul, B. Plachter, T. Stamminger, J. Frey, The nuclear domain 10 (ND10) is disrupted by the human cytomegalovirus gene product IE1, Exp. Cell Res. 229 (1996) 155–158.
[114] J.H. Ahn, W.J. Jang, G.S. Hayward, The human cytomegalovirus IE2 and UL112–113 proteins accumulate in viral DNA replication compartments that initiate from the periphery of promyelocytic leukemia protein-associated nuclear bodies (PODs or ND10), J. Virol. 73 (1999) 10458–10471.
[115] G. Sourvinos, N. Tavalai, A. Berndt, D.A. Spandidos, T. Stamminger, Recruitment of human cytomegalovirus immediate-early 2 protein onto parental viral genomes in association with ND10 in live-infected cells, J. Virol. 81 (2007) 10123–10136.
[116] G.W. Wilkinson, C. Kelly, J.H. Sinclair, C. Rickards, Disruption of PML-associated nuclear bodies mediated by the human cytomegalovirus major immediate early gene product, J. Gen. Virol. 79 (Pt 5) (1998) 1233–1245.

[117] J.H. Ahn, E.J. Brignole III, G.S. Hayward, Disruption of PML subnuclear domains by the acidic IE1 protein of human cytomegalovirus is mediated through interaction with PML and may modulate a RING finger-dependent cryptic transactivator function of PML, Mol. Cell Biol. 18 (1998) 4899–4913.
[118] H.R. Lee, D.J. Kim, J.M. Lee, C.Y. Choi, B.Y. Ahn, G.S. Hayward, J.H. Ahn, Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells, J. Virol. 78 (2004) 6527–6542.
[119] Y. Xu, J.H. Ahn, M. Cheng, C.M. apRhys, C.J. Chiou, J. Zong, M.J. Matunis, G.S. Hayward, Proteasome-independent disruption of PML oncogenic domains (PODs), but not covalent modification by SUMO-1, is required for human cytomegalovirus immediate-early protein IE1 to inhibit PML-mediated transcriptional repression, J. Virol. 75 (2001) 10683–10695.
[120] H. Kang, E.T. Kim, H.R. Lee, J.J. Park, Y.Y. Go, C.Y. Choi, J.H. Ahn, Inhibition of SUMO-independent PML oligomerization by the human cytomegalovirus IE1 protein, J. Gen. Virol. 87 (2006) 2181–2190.
[121] J.H. Ahn, G.S. Hayward, Disruption of PML-associated nuclear bodies by IE1 correlates with efficient early stages of viral gene expression and DNA replication in human cytomegalovirus infection, Virology 274 (2000) 39–55.
[122] H. Hofmann, H. Sindre, T. Stamminger, Functional interaction between the pp71 protein of human cytomegalovirus and the PML-interacting protein human Daxx, J. Virol. 76 (2002) 5769–5783.
[123] A.M. Ishov, O.V. Vladimirova, G.G. Maul, Daxx-mediated accumulation of human cytomegalovirus tegument protein pp71 at ND10 facilitates initiation of viral infection at these nuclear domains, J. Virol. 76 (2002) 7705–7712.
[124] K.R. Marshall, K.V. Rowley, A. Rinaldi, I.P. Nicholson, A.M. Ishov, G.G. Maul, C.M. Preston, Activity and intracellular localization of the human cytomegalovirus protein pp71, J. Gen. Virol. 83 (2002) 1601–1612.
[125] S.R. Cantrell, W.A. Bresnahan, Interaction between the human cytomegalovirus UL82 gene product (pp71) and hDaxx regulates immediate-early gene expression and viral replication, J. Virol. 79 (2005) 7792–7802.
[126] D.L. Woodhall, I.J. Groves, M.B. Reeves, G. Wilkinson, J.H. Sinclair, Human Daxx-mediated repression of human cytomegalovirus gene expression correlates with a repressive chromatin structure around the major immediate early promoter, J. Biol. Chem. 281 (2006) 37652–37660.
[127] R.T. Saffert, R.F. Kalejta, Inactivating a cellular intrinsic immune defense mediated by Daxx is the mechanism through which the human cytomegalovirus pp71 protein stimulates viral immediate-early gene expression, J. Virol. 80 (2006) 3863–3871.
[128] C.M. Preston, M.J. Nicholl, Role of the cellular protein hDaxx in human cytomegalovirus immediate-early gene expression, J. Gen. Virol. 87 (2006) 1113–1121.
[129] S.R. Cantrell, W.A. Bresnahan, Human cytomegalovirus (HCMV) UL82 gene product (pp71) relieves hDaxx-mediated repression of HCMV replication, J. Virol. 80 (2006) 6188–6191.
[130] N. Tavalai, P. Papior, S. Rechter, T. Stamminger, Nuclear domain 10 components promyelocytic leukemia protein and hDaxx independently contribute to an intrinsic antiviral defense against human cytomegalovirus infection, J. Virol. 82 (2008) 126–137.
[131] J. Hwang, R.F. Kalejta, Proteasome-dependent, ubiquitin-independent degradation of Daxx by the viral pp71 protein in human cytomegalovirus-infected cells, Virology 367 (2007) 334–338.
[132] M. Kaspari, N. Tavalai, T. Stamminger, A. Zimmermann, R. Schilf, E. Bogner, Proteasome inhibitor MG132 blocks viral DNA replication and assembly of human cytomegalovirus, FEBS Lett. 582 (2008) 666–672.
[133] W.A. Bresnahan, T.E. Shenk, UL82 virion protein activates expression of immediate early viral genes in human cytomegalovirus-infected cells, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 14506–14511.
[134] R.T. Saffert, R.F. Kalejta, Human cytomegalovirus gene expression is silenced by Daxx-mediated intrinsic immune defense in model latent infections established in vitro, J. Virol. 81 (2007) 9109–9120.
[135] I.J. Groves, J.H. Sinclair, Knockdown of hDaxx in normally non-permissive undifferentiated cells does not permit human cytomegalovirus immediate-early gene expression, J. Gen. Virol. 88 (2007) 2935–2940.
[136] P. Bell, P.M. Lieberman, G.G. Maul, Lytic but not latent replication of epstein-barr virus is associated with PML and induces sequential release of nuclear domain 10 proteins, J. Virol. 74 (2000) 11800–11810.
[137] W. Amon, R.E. White, P.J. Farrell, Epstein-Barr virus origin of lytic replication mediates association of replicating episomes with promyelocytic leukaemia protein nuclear bodies and replication compartments, J. Gen. Virol. 87 (2006) 1133–1137.
[138] A.L. Adamson, S. Kenney, Epstein-barr virus immediate-early protein BZLF1 is SUMO-1 modified and disrupts promyelocytic leukemia bodies, J. Virol. 75 (2001) 2388–2399.
[139] Z. Deng, C.J. Chen, D. Zerby, H.J. Delecluse, P.M. Lieberman, Identification of acidic and aromatic residues in the Zta activation domain essential for Epstein-Barr virus reactivation, J. Virol. 75 (2001) 10334–10347.
[140] R. Peng, S.C. Moses, J. Tan, E. Kremmer, P.D. Ling, The Epstein-Barr virus EBNA-LP protein preferentially coactivates EBNA2-mediated stimulation of latent membrane proteins expressed from the viral divergent promoter, J. Virol. 79 (2005) 4492–4505.
[141] L. Szekely, K. Pokrovskaja, W.Q. Jiang, H. de The, N. Ringertz, G. Klein, The Epstein-Barr virus-encoded nuclear antigen EBNA-5 accumulates in PML-containing bodies, J. Virol. 70 (1996) 2562–2568.
[142] P.D. Ling, R.S. Peng, A. Nakajima, J.H. Yu, J. Tan, S.M. Moses, W.H. Yang, B. Zhao, E. Kieff, K.D. Bloch, D.B. Bloch, Mediation of Epstein-Barr virus EBNA-LP transcriptional coactivation by Sp100, EMBO. J. 24 (2005) 3565–3575.
[143] N. Lehming, S.A. Le, J. Schuller, M. Ptashne, Chromatin components as part of a putative transcriptional repressing complex, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 7322–7326.
[144] F.Y. Wu, J.H. Ahn, D.J. Alcendor, W.J. Jang, J. Xiao, S.D. Hayward, G.S. Hayward, Origin-independent assembly of Kaposi's sarcoma-associated herpesvirus DNA replication compartments in transient cotransfection assays and association with the ORF-K8 protein and cellular PML, J. Virol. 75 (2001) 1487–1506.
[145] H. Katano, K. Ogawa-Goto, H. Hasegawa, T. Kurata, T. Sata, Human-herpesvirus-8-encoded K8 protein colocalizes with the promyelocytic leukemia protein (PML) bodies and recruits p53 to the PML bodies, Virology 286 (2001) 446–455.
[146] P.D. Ling, J. Tan, J. Sewatanon, R. Peng, The murine gammaherpesvirus 68 open reading frame 75c tegument protein induces the degradation of PML and is essential for production of infectious virus, J. Virol. (2008).
[147] T. Carvalho, J.S. Seeler, K. Ohman, P. Jordan, U. Pettersson, G. Akusjarvi, M. Carmo-Fonseca, A. Dejean, Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies, J. Cell Biol. 131 (1995) 45–56.
[148] V. Doucas, A.M. Ishov, A. Romo, H. Juguilon, M.D. Weitzman, R.M. Evans, G.G. Maul, Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure, Genes Dev. 10 (1996) 196–207.
[149] M.D. Weiden, H.S. Ginsberg, Deletion of the E4 region of the genome produces adenovirus DNA concatemers, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 153–157.
[150] Y. Liu, A. Shevchenko, A. Shevchenko, A.J. Berk, Adenovirus exploits the cellular aggresome response to accelerate inactivation of the MRN complex, J. Virol. 79 (2005) 14004–14016.
[151] T.H. Stracker, D.V. Lee, C.T. Carson, F.D. Araujo, D.A. Ornelles, M.D. Weitzman, Serotype-specific reorganization of the Mre11 complex by adenoviral E4orf3 proteins, J. Virol. 79 (2005) 6664–6673.
[152] T.H. Stracker, C.T. Carson, M.D. Weitzman, Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex, Nature 418 (2002) 348–352.
[153] J.D. Evans, P. Hearing, Relocalization of the Mre11-Rad50-Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication, J. Virol. 79 (2005) 6207–6215.
[154] A.J. Ullman, N.C. Reich, P. Hearing, Adenovirus E4 ORF3 protein inhibits the interferon-mediated antiviral response, J. Virol. 81 (2007) 4744–4752.
[155] A.J. Ullman, P. Hearing, The cellular proteins PML and Daxx mediate an innate antiviral defense antagonized by the adenovirus E4 ORF3 protein, J. Virol. (2008).
[156] K.N. Leppard, R.D. Everett, The adenovirus type 5 E1b 55 K and E4 Orf3 proteins associate in infected cells and affect ND10 components, J. Gen. Virol. 80 (Pt 4) (1999) 997–1008.
[157] A. Hoppe, S.J. Beech, J. Dimmock, K.N. Leppard, Interaction of the adenovirus type 5 E4 Orf3 protein with promyelocytic leukemia protein isoform II is required for ND10 disruption, J. Virol. 80 (2006) 3042–3049.
[158] M. Rosa-Calatrava, L. Grave, F. Puvion-Dutilleul, B. Chatton, C. Kedinger, Functional analysis of adenovirus protein IX identifies domains involved in capsid stability, transcriptional activity, and nuclear reorganization, J. Virol. 75 (2001) 7131–7141.
[159] M. Rosa-Calatrava, F. Puvion-Dutilleul, P. Lutz, D. Dreyer, H. de The, B. Chatton, C. Kedinger, Adenovirus protein IX sequesters host-cell promyelocytic leukaemia protein and contributes to efficient viral proliferation, EMBO Rep. 4 (2003) 969–975.
[160] F. Puvion-Dutilleul, V. Legrand, M. Mehtali, M.K. Chelbi-Alix, H. de The, E. Puvion, Deletion of the fiber gene induces the storage of hexon and penton base proteins in PML/Sp100-containing inclusions during adenovirus infection, Biol. Cell 91 (1999) 617–628.
[161] P.M. Day, R.B. Roden, D.R. Lowy, J.T. Schiller, The papillomavirus minor capsid protein, L2, induces localization of the major capsid protein, L1, and the viral transcription/replication protein, E2, to PML oncogenic domains, J. Virol. 72 (1998) 142–150.
[162] P. Heino, J. Zhou, P.F. Lambert, Interaction of the papillomavirus transcription/replication factor, E2, and the viral capsid protein, L2, Virology 276 (2000) 304–314.
[163] L. Florin, F. Schafer, K. Sotlar, R.E. Streeck, M. Sapp, Reorganization of nuclear domain 10 induced by papillomavirus capsid protein l2, Virology 295 (2002) 97–107.
[164] K.A. Becker, L. Florin, C. Sapp, M. Sapp, Dissection of human papillomavirus type 33 L2 domains involved in nuclear domains (ND) 10 homing and reorganization, Virology 314 (2003) 161–167.
[165] L. Florin, C. Sapp, R.E. Streeck, M. Sapp, Assembly and translocation of papillomavirus capsid proteins, J. Virol. 76 (2002) 10009–10014.
[166] E.J. Androphy, D.R. Lowy, J.T. Schiller, Bovine papillomavirus E2 *trans*-activating gene product binds to specific sites in papillomavirus DNA, Nature 325 (1987) 70–73.
[167] T. Nakahara, P.F. Lambert, Induction of promyelocytic leukemia (PML) oncogenic domains (PODs) by papillomavirus, Virology 366 (2007) 316–329.
[168] K.A. Becker, L. Florin, C. Sapp, G.G. Maul, M. Sapp, Nuclear localization but not PML protein is required for incorporation of the papillomavirus minor capsid protein L2 into virus-like particles, J. Virol. 78 (2004) 1121–1128.
[169] E. Kieback, M. Muller, Factors influencing subcellular localization of the human papillomavirus L2 minor structural protein, Virology 345 (2006) 199–208.
[170] C.S. Swindle, N. Zou, B.A. Van Tine, G.M. Shaw, J.A. Engler, L.T. Chow, Human papillomavirus DNA replication compartments in a transient DNA replication system, J. Virol. 73 (1999) 1001–1009.
[171] S. Roberts, M.L. Hillman, G.L. Knight, P.H. Gallimore, The ND10 component promyelocytic leukemia protein relocates to human papillomavirus type 1 E4 intranuclear inclusion bodies in cultured keratinocytes and in warts, J. Virol. 77 (2003) 673–684.

[172] C. Rogel-Gaillard, G. Pehau-Arnaudet, F. Breitburd, G. Orth, Cytopathic effect in human papillomavirus type 1-induced inclusion warts: in vitro analysis of the contribution of two forms of the viral E4 protein, J. Invest Dermatol. 101 (1993) 843–851.
[173] E. Guccione, P. Massimi, A. Bernat, L. Banks, Comparative analysis of the intracellular location of the high- and low-risk human papillomavirus oncoproteins, Virology 293 (2002) 20–25.
[174] O. Bischof, K. Nacerddine, A. Dejean, Human papillomavirus oncoprotein E7 targets the promyelocytic leukemia protein and circumvents cellular senescence via the Rb and p53 tumor suppressor pathways, Mol. Cell. Biol. 25 (2005) 1013–1024.
[175] E. Guccione, K.J. Lethbridge, N. Killick, K.N. Leppard, L. Banks, HPV E6 proteins interact with specific PML isoforms and allow distinctions to be made between different POD structures, Oncogene 23 (2004) 4662–4672.
[176] P.M. Day, C.C. Baker, D.R. Lowy, J.T. Schiller, Establishment of papillomavirus infection is enhanced by promyelocytic leukemia protein (PML) expression, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 14252–14257.
[177] S.J. Beech, K.J. Lethbridge, N. Killick, N. McGlincy, K.N. Leppard, Isoforms of the promyelocytic leukemia protein differ in their effects on ND10 organization, Exp. Cell. Res. 307 (2005) 109–117.
[178] Q. Tang, P. Bell, P. Tegtmeyer, G.G. Maul, Replication but not transcription of simian virus 40 DNA is dependent on nuclear domain 10, J. Virol. 74 (2000) 9694–9700.
[179] A. Jul-Larsen, T. Visted, B.O. Karlsen, C.H. Rinaldo, R. Bjerkvig, P.E. Lonning, S.O. Boe, PML-nuclear bodies accumulate DNA in response to polyomavirus BK and simian virus 40 replication, Exp. Cell Res. 298 (2004) 58–73.
[180] C.L. Bishop, M. Ramalho, N. Nadkarni, K.W. May, C.F. Higgins, N. Krauzewicz, Role for centromeric heterochromatin and PML nuclear bodies in the cellular response to foreign DNA, Mol. Cell. Biol. 26 (2006) 2583–2594.
[181] Y. Shishido-Hara, S. Ichinose, K. Higuchi, Y. Hara, K. Yasui, Major and minor capsid proteins of human polyomavirus JC cooperatively accumulate to nuclear domain 10 for assembly into virions, J. Virol. 78 (2004) 9890–9903.
[182] Y. Shishido-Hara, K. Higuchi, S. Ohara, C. Duyckaerts, J.J. Hauw, T. Uchihara, Promyelocytic leukemia nuclear bodies provide a scaffold for human polyomavirus JC replication and are disrupted after development of viral inclusions in progressive multifocal leukoencephalopathy, J. Neuropathol. Exp. Neurol. 67 (2008) 299–308.
[183] T. Regad, A. Saib, V. Lallemand-Breitenbach, P.P. Pandolfi, H. de The, M.K. Chelbi-Alix, PML mediates the interferon-induced antiviral state against a complex retrovirus via its association with the viral transactivator, EMBO. J. 20 (2001) 3495–3505.
[184] C.D. Meiering, M.L. Linial, The promyelocytic leukemia protein does not mediate foamy virus latency in vitro, J. Virol. 77 (2003) 2207–2213.
[185] C. Desbois, R. Rousset, F. Bantignies, P. Jalinot, Exclusion of Int-6 from PML nuclear bodies by binding to the HTLV-I Tax oncoprotein, Science 273 (1996) 951–953.
[186] V. Doucas, R.M. Evans, Human T-cell leukemia retrovirus-Tax protein is a repressor of nuclear receptor signaling, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2633–2638.
[187] P. Turelli, V. Doucas, E. Craig, B. Mangeat, N. Klages, R. Evans, G. Kalpana, D. Trono, Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication, Mol. Cell 7 (2001) 1245–1254.
[188] P. Bell, L.J. Montaner, G.G. Maul, Accumulation and intranuclear distribution of unintegrated human immunodeficiency virus type 1 DNA, J. Virol. 75 (2001) 7683–7691.
[189] L. Berthoux, G.J. Towers, C. Gurer, P. Salomoni, P.P. Pandolfi, J. Luban, As(2)O(3) enhances retroviral reverse transcription and counteracts Ref1 antiviral activity, J. Virol. 77 (2003) 3167–3180.
[190] J.G. Greger, R.A. Katz, A.M. Ishov, G.G. Maul, A.M. Skalka, The cellular protein daxx interacts with avian sarcoma virus integrase and viral DNA to repress viral transcription, J. Virol. 79 (2005) 4610–4618.
[191] A. Poleshko, I. Palagin, R. Zhang, P. Boimel, C. Castagna, P.D. Adams, A.M. Skalka, R.A. Katz, Identification of cellular proteins that maintain retroviral epigenetic silencing: evidence for an antiviral response, J. Virol. 82 (2008) 2313–2323.
[192] W.V. Bonilla, D.D. Pinschewer, P. Klenerman, V. Rousson, M. Gaboli, P.P. Pandolfi, R.M. Zinkernagel, M.S. Salvato, H. Hengartner, Effects of promyelocytic leukemia protein on virus-host balance, J. Virol. 76 (2002) 3810–3818.
[193] M. Djavani, J. Rodas, I.S. Lukashevich, D. Horejsh, P.P. Pandolfi, K.L. Borden, M.S. Salvato, Role of the promyelocytic leukemia protein PML in the interferon sensitivity of lymphocytic choriomeningitis virus, J. Virol. 75 (2001) 6204–6208.
[194] D. Blondel, T. Regad, N. Poisson, B. Pavie, F. Harper, P.P. Pandolfi, H. de The, M.K. Chelbi-Alix, Rabies virus P and small P products interact directly with PML and reorganize PML nuclear bodies, Oncogene 21 (2002) 7957–7970.
[195] M.K. Chelbi-Alix, F. Quignon, L. Pelicano, M.H. Koken, H. de The, Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein, J. Virol. 72 (1998) 1043–1051.
[196] K.L. Borden, E.J. Campbell Dwyer, M.S. Salvato, An arenavirus RING (zinc-binding) protein binds the oncoprotein promyelocyte leukemia protein (PML) and relocates PML nuclear bodies to the cytoplasm, J. Virol. 72 (1998) 758–766.
[197] A. Kentsis, E.C. Dwyer, J.M. Perez, M. Sharma, A. Chen, Z.Q. Pan, K.L. Borden, The RING domains of the promyelocytic leukemia protein PML and the arenaviral protein Z repress translation by directly inhibiting translation initiation factor eIF4E, J. Mol. Biol. 312 (2001) 609–623.
[198] Y. Sato, K. Yoshioka, C. Suzuki, S. Awashima, Y. Hosaka, J. Yewdell, K. Kuroda, Localization of influenza virus proteins to nuclear dot 10 structures in influenza virus-infected cells, Virology 310 (2003) 29–40.
[199] S. Iki, S. Yokota, T. Okabayashi, N. Yokosawa, K. Nagata, N. Fujii, Serum-dependent expression of promyelocytic leukemia protein suppresses propagation of influenza virus, Virology 343 (2005) 106–115.
[200] P.D. Bieniasz, Intrinsic immunity: a front-line defense against viral attack, Nat. Immunol. 5 (2004) 1109–1115.