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MINIREVIEW

Virion Factors That Target Daxx To Overcome Intrinsic Immunity

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PML nuclear bodies and their associated functions are part of an intrinsic cellular mechanism aimed at maintaining transcriptional control over viral gene expression and preventing replication of invading viruses. To overcome these barriers, many viruses express early nonstructural, multifunctional proteins to support the viral replication cycle or modulate host immune responses. Virion proteins constituting the invading particle are traditionally investigated for their role in transport during entry or egress and in the assembly of new virions. The additional functions of virion proteins have largely been ignored, in contrast to those of their nonstructural counterparts. A number of recent reports suggest that several virion proteins may also play vital roles in gene activation processes, in particular by counteracting intrinsic immune mechanisms mediated by the PML nuclear body-associated cellular factors Daxx, ATRX, and Sp100. These virion proteins share several features with their more potent nonstructural counterparts, and they may serve to bridge the gap in the early phase of an infection until immediate early viral gene expression is established. In this review, we discuss how virion proteins are an integral part of gene regulation among several viral families and to what extent structural proteins of incoming virions may contribute to species barrier, latency, and oncogenesis.

When viral genetic information is transferred from cell to cell, it must be compacted and transcriptionally inactivated for storage in the viral capsid during transport. This process needs to be actively reversed upon infection against cellular transcriptional repression through intrinsic antiviral mechanisms. An important factor in the cellular control of viral gene expression is death domain-associated protein (Daxx). Daxx was initially described as a modulator of apoptotic signaling (1), but recent reports suggest that Daxx plays an active role in gene regulation by repressing or modulating transcription through chromatin remodeling (2, 3). Daxx mainly cooperates with alpha-thalassemia retardation syndrome x-linked (ATRX), a putative member of the SNF2 family of ATP-dependent chromatin-remodeling proteins. In this repressive complex, ATRX acts as the core ATPase subunit, while Daxx is the targeting factor, leading to histone deacetylase (HDAC) recruitment (4–6).

Daxx is found associated with the promyelocytic leukemia protein nuclear body (PML-NB) or chromatin. Association with PML-NB alleviates gene repression and activates apoptosis, while chromatin-bound Daxx represses transcription (7–9). It has been long established that PML-NBs are nuclear structures with antiviral activity, accumulating an expanding number of transient or constitutive cellular factors involved in transcriptional control, chromatin remodeling, genome integrity, apoptosis, and tumor suppression (10). Moreover, several PML-NB constituents can be induced by type I and II interferon (IFN), resulting in increased antiviral activity by attenuating viral gene expression and replication, notably through PML itself, Daxx, ATRX, and the PML-NB-associated factor Sp100 (11, 12).

Viruses counteract this repression by expressing nonstructural regulatory genes that target and functionally inhibit PML-NB-associated antiviral functions to ensure efficient viral replication, raising the question of how viruses overcome existing cellular transcriptional blocks and prevent apoptosis prior to transcriptional activation. Recent work showed that certain virion proteins of incoming viral particles possess functions similar to the early nonstructural gene products and, like their counterparts, target PML-NBs and Daxx/ATRX to minimize antiviral mechanisms. In this review, we focus on the function of these virion proteins as gene regulatory factors that enable the virus to initiate immediate early (IE) gene expression and synthesize large quantities of new viral proteins to gain full control of the host cell to either establish efficient viral replication or, alternatively, promote latency for prolonged survival. In addition, we discuss unresolved questions as to how virion proteins targeting Daxx- and PML-associated pathways may contribute to apoptosis prevention, oncogenesis, and transformation or help overcome species barriers.

DAXX IMPAIRMENT BY VIRION PROTEINS: A ROLE IN IMMEDIATE EARLY VIRAL GENE EXPRESSION?

An increasing number of viral families are reported to target Daxx using proteins from the incoming virion to favor viral gene expression and replication. This strategy is effective because it bypasses the immediate need for viral gene expression to combat cellular defense mechanisms. It also identifies Daxx as an important part of cellular intrinsic immunity against invading pathogens. Most studies of virion proteins involved in initiating IE viral gene expression have been done using herpesviruses, comprising important human pathogens and tumor-inducing viruses such as herpes simplex virus 1 (HSV-1), human cytomegalovirus (HCMV), human Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8 [HHV-8]). They share a common virion morphology, with icosahedral capsids containing the viral genome encased by a second protein layer, the tegument, and surrounded with a lipid bi-

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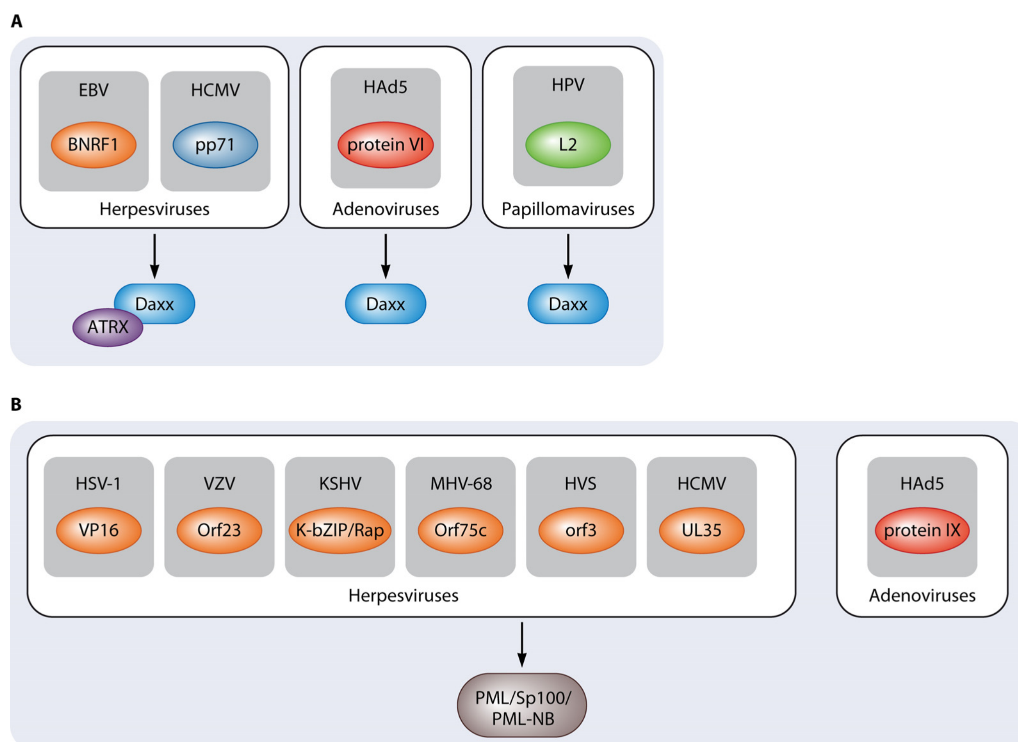

FIG 1 DNA viruses. Overview of DNA viruses modulating cellular factors. (A) DNA viruses known to modulate Daxx and/or ATRX regulatory proteins in the host cell (see the text for details). (B) Summary of DNA viruses known to modulate PML and/or PML-associated factors, e.g., Sp100. These are described in detail in the text. HAd5, human Ad5; VZV, varicella-zoster virus.

layer containing viral glycoproteins. Following receptor binding and fusion, virions are targeted to the nucleus, sequentially losing proteins of the tegument. Transcriptional activation and viral IE gene expression is imperative to activate downstream viral genes and establish a productive replication cycle (13). Daxx- and PML-NB-mediated intrinsic resistance works against the onset of IE viral gene expression. In the herpesvirus family, the best-characterized example of how intrinsic immunity can be overcome by incoming virions is that of HCMV. HCMV IE gene expression is stimulated by the virion tegument phosphoprotein pp71, encoded by open reading frame UL82 (Fig. 1A) (14–16). HCMV mutants lacking the UL82 coding region are impaired for viral gene expression, demonstrating that pp71 is important for virus replication in permissive cells (14, 17). Repression of viral IE gene expression in the absence of pp71 involves Daxx- and ATRX-mediated processes (Fig. 1A) (18–20). Pp71 is necessary to overcome Daxx and/or ATRX to stimulate IE viral gene expression (15, 21). There is still some debate as to how pp71 counteracts Daxx/ATRX. Some reports show pp71-dependent degradation of Daxx via a proteasome-dependent, ubiquitin-independent pathway (20) and dispersion of ATRX from PML-NBs early in infection (18). A different observation using inducible expression of pp71 and IE proteins suggests that pp71 plays a primary role in disrupting the Daxx/ATRX complex, while IE proteins themselves target PML and Sp100 (22). In summary, pp71 neutralizes repression by Daxx/ATRX, resulting in efficient transcription of HCMV IE genes (23–27). In cooperation with pp71, the HCMV UL35 tegument protein shares the capacity to also activate the major IE promoter (MIEP) in reporter assays (28). UL35 is produced late in infection, is packaged into progeny virions as a minor tegument component (29), and is therefore delivered to newly infected cells. HCMV pp71 enhances the association between HCMV UL35 and PML (Fig. 1B) (30), suggesting that UL35, like pp71, contributes to activation of IE viral gene expression by abrogating PML-dependent repression (31).

HSV-1 persists in neural ganglia. Activation of the lytic cycle and expression of IE proteins requires the virion component VP16, while its absence or cytoplasmic retention leads to latent infections with quiescent genomes (32). VP16 associates with PML-NBs, but no functional interaction with Daxx/ATRX has been described (33). VP16-activated HSV-1 genomes express the IE gene ICP0, which targets PML-NBs (34, 35). HSV-1 ICP0 combines the effect of HCMV pp71 and HCMV IE proteins (22). Similar to pp71, ICP0 displaces Daxx from ATRX, counteracting the repressive function (36, 37). It remains unclear whether some ICP0 activity is associated with incoming virions due to the presence of ICP0 in the tegument or if the antagonizing effect of ICP0 stems mainly from newly synthesized ICP0 as an IE protein (36). Discriminating between the two sources of ICP0 has been hampered because ICP0-defective viruses also show defects in nuclear transport, potentially linking entry and activation of IE gene expression (38).

Another herpesvirus with a virion tegument protein that counteracts Daxx/ATRX is the Epstein-Barr virus (EBV) (Fig. 1A). EBV major tegument protein BNRF1 (also termed Zta) was found to counteract cellular repression by binding Daxx at PML-NBs prior to BNRF1-mediated disassembly of the Daxx/ATRX chromatin-remodeling complex (39). BNRF1 is one of the most abundant

tegument proteins in the EBV virion, and it plays an essential role in establishing viral latent infection (40, 41). Interaction between BNRF1 and Daxx, as with their HCMV and HSV-1 counterparts, is required to promote expression of IE viral genes, suggesting that Daxx and ATRX are major components maintaining repression of EBV during latency (39).

Another family of DNA viruses are the adenoviruses (Ads). Ads are nonenveloped viruses with an icosahedral capsid and linear double-stranded DNA (42). The E1A region is the first transcription unit activated following Ad type 5 (Ad5) infection, analogous to the herpesvirus IE proteins (43, 44). E1A proteins force the infected cell to enter the S phase and are required for efficient transcription of other early viral transcription units. E1A binds and displaces the transcriptional repressor retinoblastoma tumor suppressor protein (pRb). This function of E1A is similar to that of HCMV pp71, which also displaces pRb (45). In contrast, proteasomal degradation of the transcriptional repressor Daxx is mediated through the early viral E1B-55K protein (46). As with HCMV, the onset of Ad genome transcription is linked to virus entry steps and involves the internal Ad capsid protein VI, which is released during endosomal passage, permitting endosomal escape (47, 48). Subsequently, some protein VI is imported into the nucleus, where it associates with PML-NBs (49). This virion protein activates the viral E1A promoter and thus promotes subsequent viral gene expression, presumably by a mechanism involving the displacement of Daxx from PML-NBs (49). Transactivating properties of protein VI include a conserved PPxY motif required for binding to ubiquitin ligases of the Nedd4 family of E3 ubiquitin ligases, although the exact role of this motif remains unclear (49). Ads with mutated PPxY in protein VI are subject to increased Daxx/ATRX-mediated transcriptional repression (50); transcription can be restored using wild-type protein VI in *trans* (49). A second capsid protein of the incoming Ad particle that targets PML-NBs and activates Ad gene expression is capsid protein IX (51, 52). When overexpressed, protein IX forms cages around PML-NBs, although it is currently unclear whether this also involves modulation of Daxx/ATRX and how it contributes to transcriptional control of Ads (52). As was the case for proteins VI and IX, the genome-associated protein VII was shown to stimulate Ad gene expression through an unknown mechanism (Fig. 1) (53, 54).

Like Ads, human papillomaviruses (HPVs) are nonenveloped, double-stranded DNA viruses. They infect epithelia such as skin and mucosa. Initial HPV infection is restricted to basal cells and requires cell differentiation to keratinocytes to complete the replication cycle (55). The viral particle comprises two structural proteins, the major external L1 capsid protein and the minor, more internal L2 capsid protein. After entry, L2 becomes exposed and escapes from the endosomal compartment, remaining associated with the viral genome upon separation from L1 and nuclear delivery (56–58). In the nucleus, L2 associates with and reorganizes PML-NBs in a concentration-dependent manner in cultured cells as well as in the upper part of cervical intraepithelial lesions caused by HPV infections (59). Daxx is enriched at PML-NBs concomitant with L2 expression, while Sp100 is removed from PML-NBs; both involve a NDLD peptide motif in L2 (59, 60). Daxx/L2 complexes can also form in the absence of PML, raising the question of how PML-NB association with the Daxx/L2 complex might be regulated (60). L2-mediated PML-NB association with HPV genomes appears to increase HPV transcriptional activation and replication, reminiscent of the IE expression in other viral systems described above (61, 62). However, as yet, no functional link accounting for transcriptional control has been made between L2 and any PML-NB constituent.

Despite all the above examples, no clear sequence homology or motif for Daxx interaction has been identified among the different viral capsid/tegument proteins targeting Daxx. This raises the question of whether Daxx binding is direct (e.g., through posttranslational modifications, such as SUMOylation) or indirect (e.g., through yet to be identified cellular factors) and if the consequences of Daxx binding by virion proteins are similar or different in their downstream effects. Similar effects could result in *trans*-complementation with possible synergistic effects on replication, or reactivation from latency, by nonrelated viruses if infections occur in the same cell.

Early observations showed that HCMV virions could stimulate and reactivate attenuated HSV-1 mutants with impaired tegument protein VP16 or ICP0 upon coinfection (63, 64). This effect was later attributed to tegument protein pp71 from HCMV by showing stimulation of heterologous promoters located in the genome of an HSV-1 mutant lacking functional VP16, ICP0, and ICP4 (16). Subsequent analysis also showed that HCMV pp71 was capable of reactivating mutant HSV-1 in a sequence-independent but Daxx-dependent way (65). More recently, it was shown that an HSV-1 mutant lacking ICP0 could be fully reactivated from quiescence by the additive effects of HCMV proteins pp71 and IE1: pp71 prevented the formation of Daxx/ATRX complexes, while IE1 induced loss of PML and Sp100 SUMO modification (22). Consequently, none of the cellular factors accumulated at incoming HSV-1 genomes, suggesting that full compensation for loss of IE HSV-1 gene expression can be achieved and, in the case of HCMV, is divided between two proteins (22).

Examples of *trans*-complementation to counteract Daxx-mediated repression by virion proteins are not limited to single virus families. Early reports showed that HCMV infections could serve as a helper virus to promote efficient JC virus replication in otherwise nonpermissive fibroblasts, although no functional link to Daxx or PML-related mechanisms has been established (66). A more recent report showed that an Ad encoding a mutated capsid protein VI, which failed to efficiently counteract Daxx repression, could be successfully rescued by coexpressing the HCMV tegument protein pp71 or the HPV minor capsid protein L2 (49). In both cases, the *trans*-complementing effect was apparently driven by transcriptional activation of the IE Ad E1A promoter. In turn, Ad protein VI was capable of stimulating a virion-delivered MIEP of HCMV, showing interchangeability of the virion protein-associated IE promoter derepression function between nonrelated DNA viruses (49). Likewise, cell clones harboring retroviral integrates with silenced green fluorescent protein (GFP) expression cassettes could be reactivated by counteracting Daxx through the nonretroviral pp71 tegument protein from HCMV, but not when the Daxx-binding-deficient pp71 mutant was used (67).

DAXX IMPAIRMENT BY VIRION PROTEINS: A ROLE IN LATENCY AND SILENCING?

The above examples illustrate that Daxx-mediated antiviral activity is based on a common mechanism, and at least some viruses have found interchangeable measures to overcome the antiviral effect, which they incorporate into the incoming virion to permit

efficient IE gene expression. Conversely, if IE expression is efficiently suppressed, herpesviruses enter a state of latency. Latency is established when viral gene expression is silenced after initial infection but the viral genome is not eliminated by the host cell. As a result of external stimuli, viral gene expression can be resumed to enter lytic replication after prolonged periods of time. Recent work showed that HCMV latency can be established in $CD34^+$ cells infected *ex vivo* and that this state correlates with cytoplasmic retention of tegument-delivered pp71 (68). Moreover, HCMV pp71 inhibition of Daxx was shown to block heterochromatin being established on the HCMV MIEP (69), while HDAC inhibition or Daxx knockdown activated viral IE gene expression upon infection in $CD34^+$ cells (68). The data suggest that upon HCMV entry, Daxx (like ATRX) is restricted in the lytic infection stage by pp71, while cytoplasmic retention of pp71 during latency mediates transcriptional silencing, presumably by repressive chromatin assembling on the viral genome, mediated by Daxx-associated mechanisms (68). Unlike the case for pp71, with the EBV tegument protein BNFR1, Daxx remains prominently associated with PML-NBs (39). It remains to be shown whether this association helps to establish a chromatin structure supporting viral IE gene activity in support of latency or lytic infection.

Like EBV, KSHV (HHV-8) can enter the lytic or latent life cycle (70). The KSHV tegument protein K-bZIP protein (also called RAP) is a structural and positional analog of BNRF1. Like BNRF1, K-bZIP targets PML-NBs during virus infection (Fig. 1B) (71, 72), but unlike BNRF1, K-bZIP associates with K-Rta and exerts repressive rather than activating effects on some viral promoters (73), possibly as part of the latency-inducing complex (74, 75). However, no functional association with Daxx and/or ATRX has been reported.

Virion proteins of some RNA viruses, such as retroviruses, also target Daxx and PML-NBs, suggesting that the antiviral effect of PML-NB-associated mechanisms is not restricted to DNA viruses. Retroviruses, e.g., avian sarcoma virus (ASV), harbor RNA genomes that are reverse transcribed into DNA upon cell entry and targeted to the nucleus, where they integrate into the host cell genome by virus-encoded, virion-delivered integrases. Integrated retroviral genomes undergo frequent epigenetic silencing, resulting in a nontranscriptional state of the viral genome similar to the latency described for other virus families (see above). This host response may be advantageous to the virus, since in the absence of viral gene expression some infected cells escape the host immune response (76–80). Silencing is also commonly observed after transduction of therapeutic or reporter genes by using retrovirus-based vectors and occurs at various frequencies (81–85). Gene silencing is mediated mainly by DNA methylation and/or histone modifications (86). Daxx was reported to functionally associate with HDACs complexed with ASV DNA early after infection (87). The Daxx/HDAC complex is recruited to viral DNA through interaction with the ASV integrase protein (Fig. 2A) (87). In addition, Daxx also recruits Dmnt1 to the viral DNA, resulting in long terminal repeat (LTR) methylation and transcriptional repression (88). These findings suggest that Daxx/HDAC/Dmnt1 complexes play a role in initiating epigenetic silencing, possibly as part of an antiviral response against retroviruses (87, 89). Daxx knockdown was also shown to increase HIV-1-derived lentiviral reporter gene expression (90). Daxx associates with lentiviral DNA by interacting with the HIV-1 integrase, and like ASV HDACs, is recruited to viral DNA, resulting in lentiviral gene repression (90). Surprisingly, a bacteriophage-derived integrase also interacts *per se* with Daxx. Daxx knockdown increased recombination efficiency when the integrase was used in a eukaryotic cell system, showing that the antiviral silencing activity of Daxx may be much wider than anticipated (91). Taken together, these findings indicate that epigenetic silencing to control integration and/or expression of retrovirus-derived genetic material could be reversed by Daxx depletion, suggesting broad epigenetic control of pathogen DNA by Daxx-associated mechanisms (67). Meanwhile, it remains unclear why viral integrases interact with Daxx. Perhaps this interaction delays or accelerates epigenetic silencing of the integrated virus/transgene to promote an advantageous state of latency or replication for the virus.

DAXX IMPAIRMENT BY VIRION PROTEINS: HOW COULD IT WORK?

To understand such diverse viral strategies to target Daxx, one first needs to understand how Daxx exerts its antiviral activity. Several aspects of this phenomenon are still unanswered. Recently, Daxx was identified as an H3.3-specific chaperone, cooperating with ATRX in replication-independent chromatin assembly at telomeres (92). Recombinant Daxx assembles H3.3/H4 tetramers on DNA templates and aids in depositing and remodeling H3.3-containing nucleosomes as catalyzed by the Daxx/ATRX complex (92). Evidence suggests that in most cases, incoming viral genomes are not assembled as chromatin comprised of cellular nucleosomes (36, 93, 94). Nucleosome-free DNA may be subject to immediate assembly of repressive chromatin through Daxx/ATRX or other chromatin-assembly factors as part of a cellular surveillance mechanism or DNA damage response (95). It is clear that incoming virion proteins exist in limited numbers and thus can exert their effect only locally. Preventing the assembly of repressive chromatin on the viral genome by inactivating Daxx/ATRX or related complexes may be enough to permit initial IE gene expression. IE proteins subsequently expressed in larger numbers are far more potent as transactivators of transcription and can act individually or in cooperation with the virion proteins to sustain viral gene expression. In this scenario, transcriptional silencing is antiviral only in the sense that it inhibits viral replication. If the result of silencing is prolonged viral survival due to latency or immune evasion, transcriptional silencing may well be a proviral strategy. In addition, the observation that in some cells cytoplasmic retention of pp71 is responsible for latency indicates that cellular homeostasis could play a major role in the efficiency of Daxx-mediated antiviral effects and influence the fate of the viral genome (96).

DAXX IMPAIRMENT BY VIRION PROTEINS: A ROLE IN APOPTOSIS PREVENTION?

Daxx transcriptional control is thought to be a nuclear event. However, Daxx was initially described as an apoptosis-modulating factor that associates with the transmembrane death receptor FAS, enhancing apoptosis. The apoptosis-modulating effect of Daxx might involve nucleocytoplasmic translocation of Daxx and PML-NBs (3). There is little evidence of virion proteins that interfere with the proapoptotic role of Daxx. Hantaviruses are enveloped, spherical, negative-stranded RNA viruses encoding the nucleocapsid protein (N) (97–99). The nucleocapsid protein of Puumala virus (PUUV-N) was recently reported to bind Daxx (Fig. 2A) (100). Viral PUUV-N was found at perinuclear mem-

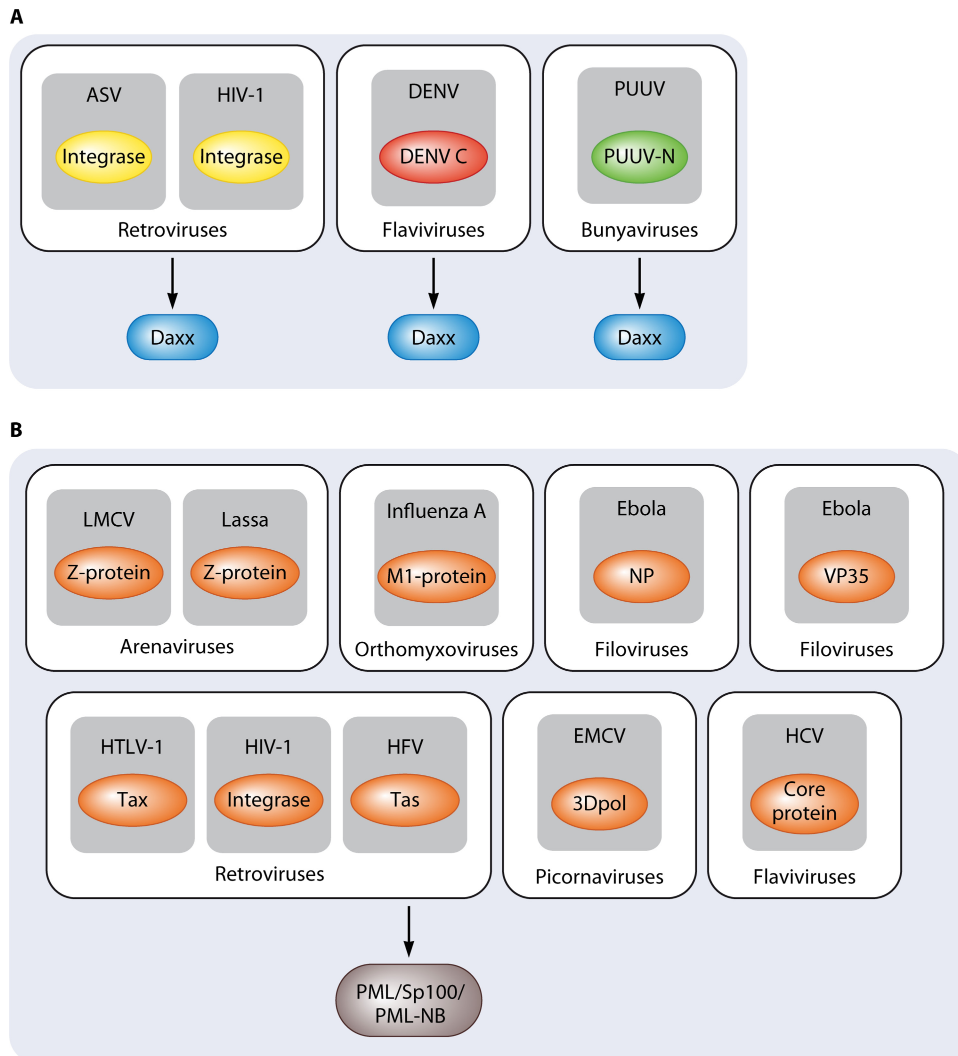

FIG 2 RNA viruses. Overview of RNA viruses modulating cellular factors. (A) RNA viruses known to modulate Daxx regulatory proteins in the host cell (see the text for details). (B) Summary of RNA viruses known to modulate PML and/or PML-associated factors, e.g., Sp100. LCMV, lymphocytic choriomeningitis virus; HTLV-1, human T-lymphotropic virus type 1; HFV, human foamy retrovirus; EMCV, encephalomyocarditis virus.

branes in infected cells (101), although Daxx overexpression promoted accumulation of PUUV-N in PML-NB-associated nuclear structures (100). PUUV-N interacts with the nuclear localization signal of Daxx and could affect nucleocytoplasmic shuttling of Daxx, such as its role in apoptosis. The interaction domain with Daxx also interferes with PUUV-N auto-oligomerization, which in turn might help sequester and inactivate Daxx prior to virion assembly (100, 102). Several questions remain unresolved, but it is possible that hantavirus interferes with the apoptotic pathway by targeting Daxx independently of ATRX and transcriptional control. This would be the first example of a virion component targeting Daxx in the cytoplasm to prevent apoptosis rather than to stimulate viral gene expression. Another example of a virus that replicates in the cytoplasm and that might target Daxx to prevent apoptosis is dengue virus (DENV), containing a single positive-stranded RNA genome with a single polypeptide precursor. One of the structural proteins, DENV capsid protein (DENV C), is required for the maturation of viral particles and assembly of the nucleocapsid (103). This small protein shuttles between the cytoplasm and nucleus of the infected cells (104). DENV C associates with Daxx, and loss of DENV C nuclear localization due to functional inhibition of its nuclear localization signal (NLS) impairs interaction with Daxx (Fig. 2A). Moreover, disrupting the Daxx/DENV C complex results in pronounced apoptosis in infected cells (105). DENV C interacts with the C-terminal region of Daxx, which contains the SUMO-interacting motif (SIM) domain and mediates the interaction with PML (106). It was suggested that competitive binding between DENV C, PML, and Daxx might modulate Daxx/PML cooperation, thereby affecting Daxx-mediated apoptosis (105). Similarly, a recent report suggested that reovirus-infected cells in the central nervous system undergo differential apoptosis dependent on the subcellular localization and expression levels of Daxx, but so far, no link to virion proteins has been established (107). Another flavivirus is hepatitis C virus (HCV), which primarily infects the liver and is often asymptomatic. Recently, HCV core protein was identified to target PML-NBs in the host cell nucleus, where it cooperates with the specific PML-IV isoform, a key regulator of p53

TABLE 1 DNA viruses: virion factors targeted by host-cell Daxx, ATRX, Sp100, or PML

Virus family	Type	Viral protein	Major function	Reference(s)
Adenoviridae	Ad5	Protein VI	Virus entry Virion assembly Transcriptional activity	49
		Protein IX	Virion stability Transcriptional activity	52
		Protein VII	Transcriptional activity	53, 54
Papillomaviridae	HPV16	L2	Genome encapsidation Virus entry	59
Herpesviridae	HSV-1	VP16	Transcriptional activity, latency	33, 129
	HCMV	pp71	Transcriptional activity	14, 17, 49
		UL35	Transcriptional activity	29–31
	VZV	Orf23	Virion stability	130
	EBV	BNRF1/Zta	Transcriptional activity	39–41
	KSHV (HHV-8)	K-bZIP/Rap	Transcriptional activity	71–73
	MHV-68	Orf75c	Transcriptional activity	126, 127
	HVS	Orf3	Transcriptional activity	27

[a] HPV16, human papillomavirus type 16; VZV, varicella-zoster virus.

transactivating potential (Fig. 2A). HCV core protein interferes with and inhibits the proapoptotic capacity of PML-IV prior to modulation of p53 posttranslational modifications. These findings indicate that HCV core-mediated inactivation of distinct PML isoforms is largely connected with the establishment of HCV-associated apoptosis prevention and oncogenesis (108).

DAXX IMPAIRMENT BY VIRION PROTEINS: A ROLE IN VIRUS-MEDIATED TRANSFORMATION?

Incoming DNA tumor virus genomes preferentially associate with PML-NBs to initiate transcription and replication. As highlighted in this review, transcriptional activation of the genomes and the process of overcoming repressive effects of PML, Daxx, and/or ATRX are initiated by virion proteins, at least in several cases. However, PML-NBs have also been shown to be sites of oncogenic processes. The basic principles of virus-induced tumorigenesis involve initiating unscheduled cell cycle progression and reversing antiproliferative states by modulating tumor suppressor molecules, such as pRb or p53. The mechanisms by which viral oncoproteins act are complex and involve altering large protein networks associated with transcription, apoptosis, cell cycle control, DNA repair, cell signaling, posttranslational modification, and the integrity of PML-NBs (35, 60, 109, 110). For instance, PML has been intensively investigated in the context of leukemogenesis, where it was initially described as the causative agent of acute promyelocytic leukemia (111). Unfortunately, most of the molecular mechanisms explaining how PML contributes to transformation of different human tumor types, especially the involvement of Daxx and/or other PML-associated factors, are unknown. Daxx was shown to bind directly to the tumor suppressor protein p53, promoting p53-dependent apoptosis (7, 112, 113). At the same time, Daxx enhances the protein stability of the RING domain-containing E3 ubiquitin ligase mouse double minute 2 (Mdm2), therefore affecting Mdm2-dependent proteasomal degradation of p53 (114–118). These findings show that Daxx is directly involved in regulating the tumor suppressor p53. Thus, intriguingly, virion proteins targeting Daxx during entry may contribute (in part) to viral transformation processes.

DAXX IMPAIRMENT BY VIRION PROTEINS: OVERCOMING THE SPECIES BARRIER

Some viruses exhibit very strong species specificity, and replication is restricted to a specific host. This viral adaptation is thought to have coevolved with the host. It is not clear what mechanisms retain a species barrier and prevent human pathogens from replicating in nonhuman cells or *vice versa*. Daxx possesses interspecies-conserved domains as well as species-specific variations, including differences between murine and human Daxx (96). For murine cytomegalovirus (MCMV), whose replication is restricted to murine cells, coexpression of a subset of HCMV tegument proteins, including pp71, could overcome the replication block in human cells (119). While additional factors could play a role (120), these data provide *a priori* evidence that mechanisms such as Daxx/ATRX-dependent transcriptional control and adaptation of early viral gene expression are important contributors to species restrictions, at least in the case of some herpesviruses (121). In line with this, viruses undergo the full productive replication cycle in permissive cells, inevitably leading to efficient progeny production, whereas infection of nonpermissive host cells results in an abortive process or can lead to immortalization and/or partial transformation (122).

Other nonhuman primate CMVs encode homologs of HCMV pp71, which efficiently initiate IE viral gene expression (123). However, unlike human pp71, the simian UL82 proteins did not support long-term expression from quiescent HSV-1 genomes, and kinetics were significantly different in both the intranuclear localization of the simian homologs and the effects on Daxx and/or ATRX relocalization (123). Recently, a UL82 homolog encoded by guinea pig cytomegalovirus (GPCMV) was found to stimulate the transfection efficiency of GPCMV DNA and to complement replication of a HSV-1 VP16 mutant. These data support the fact that, like pp71, the GPCMV UL82 gene product is an activator of viral IE gene

TABLE 2 RNA viruses: virion factors targeted by host-cell Daxx, ATRX, Sp100, or PML[a]

Virus family	Type	Viral protein	Major function	Reference(s)
Picornaviridae	EMCV	EMCV 3Dpol	Transcriptional activity	131
Arenaviridae	LCMV	Z protein	mRNA synthesis and replication	132
	Lassa virus	Z protein	mRNA synthesis and replication	132
Flaviviridae	DENV	DENV C	Virion maturation Virion assembly	105
	HCV	HCV core protein	Genome encapsidation Cellular transformation	108, 133, 134
Orthomyxoviridae	Influenza A virus	M1 protein	Virion stability	135
Bunyaviridae	Hantavirus/PUUV	PUUV-N	Transcriptional activity	100
Retroviridae	HTLV-1	Tax	Transcriptional activity	136
	HIV-1	Integrase	Genome integration	90
	ASV	Integrase	Genome integration	67
	HFV	Tas	Genome transactivation	137
Filoviridae	Ebola virus	NP VP35	Virion stability	138

[a] EMCV, encephalomyocarditis virus; LCMV, lymphocytic choriomeningitis virus; HTLV-1, human T-lymphotropic virus type 1; HFV, human foamy retrovirus; 3Dpol, 3D polymerase.

expression, although it remains to be shown whether this involves counteracting Daxx in a species-specific way (124, 125). Other members of the herpesvirus family also harbor virion proteins that target PML-NBs after infection. Murine herpesvirus 68 (MHV-68) is a member of the *Gammaherpesvirus* subfamily. The EBV BNRF1 homolog in MHV-68 is Orf75c, which has been shown to mediate the rapid degradation of PML through a proteasome-dependent mechanism (Fig. 1B) (126, 127). Another gammaherpesvirus, herpesvirus saimiri (HVS), is closely related to human KSHV and was isolated from squirrel monkeys (*Saimiri sciureus*) (128). A variety of human cell types can be efficiently infected with HVS, mostly resulting in abortive infections. As humans are not the natural host of HVS, it is suggested that the lytic replication cycle is blocked due to cellular intrinsic restriction factors that cannot be counteracted by HVS (27). The HVS genome encodes Orf3 and Orf75, two putative tegument proteins (27). Recently, the Orf3 tegument protein of HVS was reported to induce the proteasomal degradation of Sp100 with possible effects on latency (Fig. 1B) (27). This observation suggests that the homologs BNRF1 (from EBV) targeting Daxx, Orf75c (from MHV-68) targeting PML, and Orf3 (from HVS) targeting Sp100 may have diverged during evolution and may have adapted a species-specific activity to overcome PML-NB-associated intrinsic immunity.

DAXX IMPAIRMENT BY VIRION PROTEINS: OPEN QUESTIONS?

Taken together, the accumulating evidence indicates that the virion proteins comprising the incoming viral particle have more to offer to the viral replication cycle than simply structure or transport functions. As several examples in this review suggest, they may play essential roles in the transcriptional control of (IE) viral gene expression, at least until viruses can access their full genetic repertoire. Depending on the context, they may also be essential factors in establishing latency by preventing viral gene expression for the long-term survival of the viral genome.

The examples in this review are not exhaustive, and many more examples of virion proteins targeting PML-NBs can be found (summarized in Tables 1 and 2). Nevertheless, we aim here to provide an expansive framework for future investigations in this exciting and rapidly expanding field. Several virus families have not been investigated for virion proteins that target Daxx/ATRX. No detailed common mechanism has been identified, and the full cellular target range of PML-NB-associated factors for incoming virion proteins remains unexplored. Future studies need to address the interplay between virion proteins and intrinsic immunity mechanisms during the onset of lytic and latent infections, as well as identify any oncogenic capacity or contribution to virus-induced tumorigenesis. Equally important is investigating any species differences in factors of the PML-NB-associated intrinsic immunity and how they relate to the outcome of a virus infection. This is particularly relevant since nonhuman viruses can cross species barriers to become health threats. Meanwhile, as the species diversity of viruses is increasingly exploited as therapeutic vehicles, one needs to revise the assumption that the virion itself is biologically inert. On the positive side, understanding how virion proteins affect cellular pathways might reveal new unanticipated drug targets to help improve therapeutic antiviral tools.

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