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Design stars: how small DNA viruses remodel the host nucleus

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Abstract

Numerous host components are encountered by viruses during the infection process. While some of these host structures are left unchanged, others may go through dramatic remodeling processes. In this review, we summarize these host changes that occur during small DNA virus infections, with a focus on host nuclear components and pathways. Although these viruses differ significantly in their genome structures and infectious pathways, there are common nuclear targets that are altered by various viral factors. Accumulating evidence suggests that these nuclear remodeling processes are often essential for productive viral infections and/or viral-induced transformation. Understanding the complex interactions between viruses and these host structures and pathways will help to build a more integrated network of how the virus completes its life cycle and point toward the design of novel therapeutic regimens that either prevent harmful viral infections or employ viruses as nontraditional treatment options or molecular tools.

Keywords

adenovirus; chromatin remodeling; DNA damage response; nuclear bodies; nuclear envelope; nucleus; papillomavirus; parvovirus; polyomavirus; tumor suppressor protein

All viruses are obligate intracellular pathogens that rely on the machinery and metabolism of a host cell to replicate. There are intricate interactions between viral and host components or pathways on multiple molecular, cellular and organismal levels. In some cases, viruses simply take advantage of pre-existing host machinery for their reproduction; in other cases, however, viruses play architectural roles and remodel infected cells for their own benefit. The remodeling processes can be quite extensive, ranging from destroying or evading potential antiviral factors in the cells to creating novel structures or environments that host cells do not normally possess. The ultimate goals of these 'makeover operations' by viruses are to maximize their replication capacity under optimal conditions. Owing to the broad scope of cellular processes targeted by different viruses, we will focus on the remodeling of host nuclear components by small DNA viruses.

The viruses we will discuss include adenoviruses (Ads), papillomaviruses, polyomaviruses and parvoviruses. These viruses share some common features during their replication cycle, yet they all also possess unique characteristics. They are all nonenveloped and contain a DNA genome, albeit in different forms. Both papillomaviruses and polyomaviruses have a circular dsDNA genome and replicate episomally during the lytic life cycle [1,2]; Ads

contain a linear, dsDNA genome; and parvoviruses encapsidate a linear, ssDNA molecule. In order to be replicated, the viral DNA genomes need to be delivered into the host nucleus, where new genomes are synthesized and progeny viral particles are assembled. For papillomaviruses, productive infections are coupled to the differentiation stages of the skin or mucosal epithelium that they infect. Some parvoviruses, such as adeno-associated viruses (AAVs), require coinfection with a helper virus (Ad or herpesvirus) to complete the life cycle; when the helper viruses are absent, AAV can integrate into the host genome. Other parvoviruses are autonomous and are often associated with severe disease, their replication is dependent on the cell cycle stage of the host cell [3]. In addition, Ads, papillomaviruses and polyomaviruses all have oncogenic potential, and are therefore categorized as small DNA tumor viruses.

Over the past decade, many nuclear factors and subnuclear structures have been extensively studied since they are involved in both normal and disease biology. It is now well accepted that the nucleus is not only a place that holds the essential genetic material, but is also compartmentalized and functions as a harbor for many dynamic subnuclear components whose composition, location and interactions with each other can have significant impact on host-cell functions. Since all four families of viruses replicate in the nucleus, it is not difficult to envision that many host nuclear components will be altered during the course of infection. In this review, we will summarize some of the strategies used by these viruses to overcome replication barriers or potential antiviral entities present in the nucleus, and include some examples of viruses hijacking normal nuclear processes for the benefit of their own growth (see Table 1 for a list of viral proteins and their effects on the host nucleus).

Remodeling of the nuclear envelope during lytic infection

For all the DNA viruses discussed here, the viral genome needs to gain access into the nucleus for replication to occur. Once viral particles finish assembling, they need to be transported outside of the nucleus. The nuclear envelope (NE) acts as a first and final barrier for both nuclear entry and the egress of viruses, respectively; and viruses have coevolved different means to disrupt this hurdle.

Ad particles were observed decades ago to dissociate near nuclear pore complexes (NPCs) before viral DNA is imported into the nucleus [4,5]. It was later determined that the NPC-filament protein CAN/Nup214 serves as a docking site for incoming Ad capsids, and that subsequent capsid disassembly requires binding of nuclear histone H1 to the stably docked capsids [6]. Recently, researchers have found that Ad is able to recruit kinesin-1, a microtubule motor protein, to the docking site [7]. Moreover, the recruited kinesin-1 can bind to the nucleoporin Nup358. This interaction results in both capsid disassembly and partial disruption of the NPC to increase NE permeability, which allows for the nuclear import of viral genomes.

For polyomaviruses, the NE is dramatically deformed during simian virus 40 (SV40) entry [8]. Lamin A/C, which is important for maintaining nuclear stability, is found to be dephosphorylated in a host caspase-6-dependent manner. The deformation, however, is transient, with the structure of the NE recovering soon after nuclear entry [8]. In addition to the alterations during entry process, changes in the NE integrity have also been observed during polyomavirus egress. For example, the agnoprotein of JC polyomavirus (JCPyV), a protein encoded in the late region of the genome, binds to HP1 and induces HP1 dissociation from the lamin B receptor [9]. This results in structural changes in the NE and an increased lateral mobility of the receptor. The authors proposed that these changes in the NE can aid in the nuclear egress of progeny virions [9]. Moreover, the minor capsid proteins VP2 and VP3 of mouse polyomavirus (MPyV) are potent inducers of apoptosis and are able to cause NE

damage [10]. Similarly, a recently discovered SV40 protein, VP4, possesses pore forming abilities in biological membranes [11]; and when expressed in mammalian cells, VP4 is seen mostly along the nuclear periphery and the NE is disrupted [12]. These data imply that VP4 can function to perforate the NE to allow for viral particle release.

Transient NE disruption and an altered lamin staining pattern have also been observed during the minute virus of mice (MVM) entry process [13]. Using both fluorescence and electron microscopy, the authors showed that MVM infection of host cells causes invagination of and large gaps to form in the nuclear lamins, and dramatic changes in the nuclear morphology. These changes are coincident with the peri-nuclear accumulation of input virus, suggesting that the disruption is caused by virus entry [13]. Recently, host caspase-3 was shown to be responsible for NE disruption and lamin cleavage [14]. Caspase-3 relocalized from the cytoplasm to the nucleus upon MVM infection, and a caspase-3 inhibitor blocked NE disruption and led to accumulation of viral capsids in the cytoplasm. The authors hypothesized that the alterations seen in the NE during MVM entry provides the virus with a NPC-independent nuclear entry mechanism.

Viral modulation of cell cycle, tumor suppressor proteins & the DNA damage response

Once in the nucleus, the viral genomes face a number of challenges to initiate and complete the replication process. First, most small DNA viruses do not encode their own DNA synthesis enzymes and, therefore, are dependent on host proteins to replicate. This process is usually closely associated with alterations in the normal cell cycle progression of the host cells. Second, the input viral genomes or replication intermediates may be recognized by host as 'aberrant DNA', and therefore encounter and need to overcome responses that are activated by the host to eliminate this potential damage. In this section, we will discuss some of the effects that viral infections have on nuclear tumor suppressor proteins and the cellular DNA damage response (DDR), both of which are closely related to host cell cycle regulation (see Figure 1 for a summary of the effects on tumor suppressor proteins and the DDR).

Virus interactions with tumor suppressor proteins

Viruses with dsDNA genomes are required to induce host cells to enter into S phase to take advantage of host DNA synthesis components. In order to achieve this S phase induction, viruses target tumor suppressor proteins not only to take control of the cell cycle, but also to inhibit the resulting apoptosis. pRb and p53 are the two most common targets of small DNA tumor viruses. By contrast, parvoviruses do not have the ability to activate host DNA synthesis in resting cells. Therefore, they either require the cell to go through S phase to replicate (autonomous parvoviruses) or rely on helper viruses to stimulate S phase entry (dependoviruses) [15]. Rather than try to cover all the details of the well-studied interactions between pRb, p53 and viral proteins, we will give a brief summary here and highlight some of the findings pertinent to the host remodeling processes.

During the normal cell cycle, phosphorylation of pRb by cyclin-dependent kinases releases the transcription factor E2F from pRb and allows E2F to activate genes that are necessary to drive cells into S phase and synthesize DNA [16]. Ad E1A, high-risk (strains that lead to cancer formation) HPV E7 and polyomavirus TAg all have the ability to disrupt the pRb–E2F association to facilitate S phase induction and the activation of the host DNA synthesis machinery [17]. A highly conserved LxCxE motif in all three viral proteins confers their ability to bind to the pRb protein [18]. For Ad E1A, the dissociation of E2F from Rb involves not only the high-affinity binding of E1A to pRb through LxCxE within conserved region 2 of E1A, but also competition between E1A CR1 with E2F for binding to pRb. For

TAg, the N-terminal J domain cooperates with the LxCxE motif to dissociate E2F from pRb [18]. A recent study using chromatin immunoprecipitation combined with microarrays revealed that the effect of E1A on pRb is not limited to relieving pRb inhibition of E2F [19]. Instead, there is a global relocalization of pRb family proteins (pRb, p130 and p107) onto target gene promoters, with a depletion of these pRb family proteins on promoters of cell cycle and growth genes, and an enrichment of these proteins on promoters of antiviral genes.

Inactivation of the tumor suppressor protein p53 is another important effect that many DNA viruses have on host cells preventing apoptosis resulting from abnormal S phase induction. Cell cycle deregulation by E1A results in the stabilization and accumulation of p53, but the Ad E1B and E4orf6 gene products have developed various mechanisms to inactivate p53 [20]. E1B55K inhibits p53 transcriptional activity [21]; moreover, in conjunction with E4orf6, E1B55K targets p53 to a CUL5-containing ubiquitin ligase complex for degradation [22]. These classic paradigms of p53 inactivation are not the only strategies used by Ad to inactivate p53. E4orf3 has been found to inactivate p53 via an E1B55K-independent manner by forming a novel nuclear structure that leads to *de novo* histone H3 lysine 9 trimethylation heterochromatin formation at p53 target promoters, thereby abolishing the ability of p53 to interact with these promoters [23].

The high-risk HPV oncoprotein E6 also inactivates p53 by blocking p53 transcription activity and degrading it via an E3 ubiquitin ligase complex composed of E6 and E6-AP [24–26]. During the normal life cycle of HPV, E6 and E7 levels are controlled by the replication protein E2, ensuring that they are expressed at moderate levels to induce the entry of cells into S phase for viral DNA replication, but not highly enough to induce carcinomas. Malignant progression of high-risk HPV-positive lesions, however, has been associated with HPV DNA integration into the host genome, as the integration can result in disruption of the *E2* gene, derepression of *E6* and *E7*, and cellular proliferation [27–29]. Unlike its human counterpart, the bovine papillomavirus E6 protein does not bind and degrade p53, and the underlying mechanism of how bovine papillomavirus E6 transforms cells remains to be revealed [1].

In contrast to both Ad and papillomavirus, SV40 TAg forms a complex with p53 and stabilizes it, simultaneously inactivating p53 transcriptional activity [30–32]. The relationship between MPyV T antigens and p53 is more complicated. During lytic infection of permissive mouse cells, an increase in p53 level is observed and TAg coimmunoprecipitates with phosphorylated p53 [33]; in nonpermissive cells, an interaction between MPyV TAg and p53 has not been detected [34]; however, MPyV TAg and/or tAg are able to block ARF–p53 signaling induced by the MPyV middle T antigen, thereby preventing p53 activation and allowing for cellular transformation [35]. More recently, it has been reported that the expression of either the Merkel cell polyomavirus (MCPyV) wild-type TAg or its truncated form (found in MCPyV-positive Merkel cell carcinomas) reduces p53 protein level in a MCPyV-negative Merkel cell carcinoma cell line [36]. These data suggest that the interactions between p53 and polyomavirus T antigens are different for specific viruses and thus require further investigation.

Viral modulation of the cellular DDR

The relationships between DNA viruses and the DDR have recently been intensive areas of research. In general, the DDR is a signaling cascade that cells mount when they sense the presence of various types of DNA damage to arrest cell cycle progression and allow for the repair of the damage. When the damage is too severe, the cells will initiate a p53-dependent apoptosis pathway to eliminate the damaged cell [37]. Failure to initiate the DDR can lead to genome instability and cancer development. There are two master PI3-kinase-like kinases that coordinate the damage response. The ATM kinase mainly responds to dsDNA breaks

(DSBs), whereas the ATR kinase reacts to other stresses including stalled replication forks and UV light-induced lesions [38]. When activated, these two kinases can phosphorylate numerous downstream effectors that are involved in signaling and repair processes. DSBs can be repaired by error-free homologous recombination carried out by Rad51 family proteins, or nonhomologous end joining (NHEJ) that requires DNA-PK, Ku70/80 proteins, DNA ligase IV and XRCC 4 protein [38]. The DDR is a double-edged sword during virus infections: while some viruses require the activation of a DDR and hijack this response to replicate, others need to completely eliminate or perturb the DDR to prevent its detrimental effects on viral replication.

Adenoviruses—Ads contain a linear, dsDNA genome that can be recognized by host cells as damaged DNA. Accordingly, these viruses have elegant methods to inactivate the DDR to prevent their genomes from being repaired into covalently linked concatemers. One approach used by Ad5 is to mislocalize DDR proteins through the function of the viral E4orf3 protein [39,40]. E4orf3 colocalizes with components of the Mre11–Rad50–NBS1 (MRN) complex, which functions as a DSB sensor, into nuclear tracks and also into large cytoplasmic aggresomes that are not found in uninfected cells [39,41]. The ability of E4orf3 to relocalize the MRN complex directly correlates with the establishment of viral replication centers during early infection [42], and the export of MRN complex to the aggresomes is believed to expedite MRN degradation [41]. The relocalization of MRN is thought not only to disrupt the ATM-mediated DDR, but also to prevent ATR activation during early Ad infection [43]. In contrast to wild-type Ad, an E4-deleted Ad is able to stimulate both the ATM- and ATR-mediated DDR and relocalize Mre11 to replication centers [40,44,45]. This Mre11 relocalization is dependent on NBS1 and is believed to be detrimental for viral replication [45,46].

Another way for Ad to eliminate the DDR is by viral E4orf6- and E1B55K-directed proteasome degradation of DDR components, which include the MRN complex [44], DNA ligase IV [47] and the Bloom helicase that is involved in processing DNA ends and initiating repair [48]. Various Ad types have different degradation substrates, suggesting that multiple mechanisms exist for Ad to disrupt the DDR [49]. For example, instead of relying on E4orf3 as observed with Ad5, Ad12 uses E4orf6 alone to degrade TopBP1, the activator protein for ATR [50], to inhibit ATR signaling. All Ad types examined so far degrade DNA ligase IV, implying the critical importance of eliminating NHEJ during Ad infections [49]. Degradation of DNA ligase IV is not the only means that Ad uses to disrupt the NHEJ. In the absence of E1B55k, E4orf6 is able to dissociate the DNA ligase IV–XRCC4 complex [51] and inhibit DNA-PK holoenzyme activity [52].

Papillomaviruses—During HPV infection, an activated ATM-mediated DDR facilitates viral genome replication in differentiating cells [53]. The authors postulate that HPV oncoprotein E7 binding to the ATM protein activates downstream Chk2 kinase, which results in caspase 3/7 activation, HPV replication protein E1 cleavage and genome amplification [53]. In addition to E7, E1 may also contribute to the full activation of an ATM-mediated DDR. E1 is a helicase recruited by the multifunctional E2 protein to the viral origin to initiate DNA replication [1]. Transient nuclear expression of E1 causes growth suppression and activates the ATM-mediated DDR, which requires both the origin-binding and ATPase functions of E1 [54,55]. E1 and E2 coexpression leads to the formation of E1–E2 nuclear foci that are also enriched in DDR proteins, and it is hypothesized that these foci represent viral replication centers and that ATM activation may facilitate replication [55]. During the genome maintenance phase in undifferentiated cells, however, the DDR is not required for HPV replication [53,54,56], and it is speculated that the nuclear export of E1, as well as E2 attenuation of the E1-induced DDR, can provide the cells with double safety mechanisms to prevent the premature activation of the DDR [54].

In contrast to the activation of certain aspects of the DDR for the benefit of viral replication, the HPV E6 and E7 oncoproteins interfere with other arms of the DDR, such as by abrogating growth arrest or disrupting DNA damage repair pathways, which may ultimately lead to genome instability and cancer formation in tissues persistently infected with HPV. For example, E6 inhibits the ability of XRCC1 to repair ssDNA [57], sensitizes cells to UV or carcinogen treatment by perturbing Chk1 activation [58], prevents recovery of stalled replication forks [59] and causes aberrant NHEJ DNA repair [60]. E7 is able to accelerate the degradation of claspin, a protein necessary for Chk1 activation [61]. Consistent with this, in a recent study comparing gene profiles between low- and high-risk HPV-infected vulvar tissues, the tissues that are infected with high-risk HPV appear to have more DNA damage and increased expression of DDR markers [62]. These data also suggest that the genome instability caused by HPV infection has multiple causes, and many DDR pathways may contribute to it. For instance, the Fanconi anemia (FA) pathway, which is involved in the repair process during replication stress, has recently been linked to the development of HPV-associated squamous cell carcinomas [63,64].

Polyomaviruses—For polyomaviruses, the DDR generally seems to be beneficial for viral replication. ATM is activated by MPyV infection, and the inhibition of ATM signaling reduces viral DNA replication [65]. SV40 TAg has been identified as an ATM substrate, and the phosphorylation is necessary for viral DNA replication [66]. TAg is also phosphorylated by DNA-PK, but a role for this modification during replication remains to be determined [67]. During SV40 infection, ATM, MRN complex, Rad51 and FancD2 (one of the FA proteins) are recruited into nuclear foci with TAg [68,69]. Inhibiting ATM signaling or knockdown of Rad51 or FancD2 can block viral infection [68,69]. Later in infection, MRN protein levels decrease, possibly due to the interaction between TAg and the ubiquitin ligase CUL7; however, the functional significance of this degradation also remains to be determined [68]. SV40 infection also activates the ATR–Deltap53–p21 pathway, which is responsible for an ATR-mediated intra-S checkpoint. The authors speculate that this activation can help the host cells stay in S phase and regulate host DNA polymerase [70].

SV40 TAg is a major mediator of DDR activation: first, expression of SV40 TAg alone is sufficient to induce both the ATM and ATR DDR. The stimulation of the DDR requires TAg binding to Bub1, a protein important for the establishment of the mitotic spindle checkpoint, rather than the ability of TAg to bind to pRb [71]. Second, SV40 TAg activates the FA pathway and Rad51 foci: the induction of neither is dependent on Bub1 binding or pRb binding [69]. Third, SV40 TAg can also target NBS1, reversing the suppressive effect of NBS1 on DNA replication [72]. These properties of TAg, however, do not preclude contributions of other viral proteins or the viral genome to the modulation of DDR during infection.

JCPyV infection results in the activation of both ATM- and ATR-mediated signaling and cell cycle arrest in G2 phase, and it is believed that the association of TAg with cellular DNA contributes to this G2 arrest [73]. Although ATM signaling is required for JCPyV, NHEJ is inhibited through Ku70 mislocalization by the viral agnoprotein [74]. Furthermore, JCPyV TAg inhibits homologous recombination by translocating insulin receptor substrate 1 from the cytosol to the nucleus to inhibit Rad51 function [75]. Consistent with this, in human astrocytes, JCPyV infection results in deregulated DNA repair and an elevated level of Rad51 [76].

Parvoviruses—For parvoviruses, different branches of the DDR seem to play opposite roles depending on individual viruses. For example, knockdown of DNA-PK or inhibition of its activity can dramatically decrease AAV replication, indicating a positive role for DNA-PK in AAV infection [77]. On the other hand, the MRN complex and ATM prevent both

AAV transduction and replication [78–80]. The Ad helper proteins E1B55K and E4orf6 can overcome this barrier for AAV by targeting the MRN complex for degradation [79]. Unlike AAV, the ATM-mediated DDR is required for optimal viral replication during infections with both the autonomous parvoviruses MVM and the minute virus of canines [81,82].

In addition to the DDR activation induced by autonomous parvovirus infections or various viral components [83,84], the coinfection between helper-dependent parvoviruses and their helper viruses may also result in unique activation of DDR. For instance, a distinct DDR activation is observed during AAV and Ad coinfection, which is mainly mediated through DNA-PK and requires AAV genome amplification [85]. Coinfection of AAV2 and HSV-1 induces a different DDR compared with HSV-1 infection alone [86]. In particular, the degradation of DNA-PK by the HSV-1 ICP0 protein is dramatically delayed during coinfection.

Host chromatin remodeling

Some viral genomes are assembled into cell-like chromatin throughout the viral life cycle, whereas others can become chromatinized through histone deposition once entering the nucleus. Furthermore, many viruses modulate host chromatin through their interactions with both histones and chromatin-modifying enzymes. In this section, we will focus on the modifications on the host chromatin caused by viral infections.

In many cases, chromatin remodeling is associated with the DDR. One of the hallmarks of the activation of DDR is the phosphorylation of the histone variant H2AX, termed γ H2AX, which facilitates the recruitment of repair proteins to the sites of damage and amplifies the damage signals [87]. Many DNA viruses are known to induce γ H2AX, but the molecular trigger for this activation is unknown. In addition to the activation of γ H2AX by kinases, this activity is, in part, modulated by cellular phosphatases such as PP2A [88]. Besides phosphorylation, other forms of chromatin modifications important for regulating chromatin dynamics and the ability of the chromatin to be transcribed, such as acetylation and ubiquitination, are also affected by viruses [89].

Ad induces γ H2AX during different stages of the viral life cycle. Infection with wild-type Ad, which disrupts the DDR, results in an induction of pan-nuclear signal of γ H2AX during late infection [90]. This induction requires viral DNA replication, and the modification of H2AX is likely to occur on the host DNA [90]. Infection with AAV also leads to an increase in pan-nuclear γ H2AX that is similar to that seen in Ad-infected cells [85,91]. When host cells are infected with a mutant Ad that is E4-deleted and therefore defective in disrupting the DDR, γ H2AX is seen around viral replication centers during early infection. It is unclear, however, whether this modification of H2AX occurs on the host or viral DNA [44].

Ad E1A protein associates with multiple HAT complexes, and these associations have been proposed to alter both viral and cellular transcription programs [92]. Moreover, E1A induces a global relocalization of p300/CBP HAT to the promoter regions of a set of genes that facilitate cell growth [19]. The final outcome of the interactions between E1A and p300/CBP HAT proteins is a reduction in total cellular histone H3 lysine 18 (H3K18) acetylation [93]. Similar to Ad E1A, SV40 TAg also induces H3K18 hypoacetylation, leading to the speculation that global H3K18 hypoacetylation may be linked to oncogenic transformation [93].

The HPV oncoprotein E6 alters p53-dependent transcription activity through the inhibition of p300-mediated acetylation of p53 and nucleosomal core histones [94]. Both HPV E7 and SV40 TAg are capable of inducing γ H2AX [68,71,95]; E7 can also affect histone acetylation via interactions with pRb and histone deacetylase [96]. Additional interactions

between E7 and BRG-1, a component of the human SWI/SNF complex, confer alterations of chromatin structure to contribute to an optimal replication environment for HPV [97]. A quantitative histopathological analysis involving 2000 patients and 6000 biopsy samples suggests that HPV infection is correlated with condensation of chromatin [98]. It would be interesting to further investigate how HPV and other viruses affect the host chromatin on a more global scale, as has been demonstrated for Ad, and the implications for infections and disease progression.

Remodeling of promyelocytic leukemia nuclear bodies

A productive viral infection not only relies on the ability of viruses to activate cellular machinery that is beneficial to the infection, but also requires the disruption of pre-existing intrinsic antiviral entities such as promyelocytic leukemia nuclear bodies (PML-NBs). PML-NBs are ubiquitous subnuclear structures in which a wide range of proteins are assembled into distinct foci [99,100]. PML-NBs are quite dynamic: their protein composition can change rapidly under various conditions. Since their discovery, PML-NBs have been associated with many essential cellular processes, one of which is to function as an intrinsic antiviral entity [100,101]. Moreover, they have also been implicated in the IFN-mediated antiviral response [102]. Owing to the antiviral nature of PML-NBs, it is not surprising that many viruses have developed countermeasures to disrupt the PML-NB function using various mechanisms. The interplay between herpesviruses and PML-NBs is the best studied [101], but the relationships between PML-NBs and other nuclear-replicating viruses have also begun to be revealed. There are three proteins that are considered constitutively present within PML-NBs: PML protein, hDaxx and Sp100. PML protein is the nucleating factor to form PML-NBs, and both hDaxx and Sp100 are involved in transcription regulation [101,103].

Adenoviruses

Ad5 reorganizes PML-NBs from punctate nuclear foci into track-like structures during early infection, and this disruption of PML-NBs is believed to antagonize an IFN-induced antiviral state [104–107]. The reorganization is dependent on the expression of the viral protein E4orf3, and the ability of E4orf3 to form PML tracks is conserved among many Ad types [108]. An E4orf3 null mutant is defective for replication in the presence of IFN, but shRNA knockdown of either PML or hDaxx is sufficient to complement this defect [106,107]. It is not entirely known how E4orf3 reforms PML-NBs, but it seems to require a specific interaction between E4orf3 and PML isoform II [109]. During late infection, several PML-NB-related events occur. Other PML-NB proteins such as Sp100 and NDP55 move from PML-NBs to viral replication domains [105]. A structural Ad minor capsid protein, IX, induces specific nuclear structures that recruit PML and Sp100 to prevent PML-NB reformation [110]. In addition, Ad5 E1B55K mediates proteasome-dependent degradation of hDaxx, presumably to antagonize the intrinsic antiviral activity of hDaxx [111]. The degradation of hDaxx by E1B55K requires small ubiquitin-like modifier conjugation of E1B55K and may also contribute to Ad oncogenic transformation [112].

Papillomaviruses

The HPV minor capsid protein L2 also reorganizes PML-NB proteins, and is able to induce relocalization of the major capsid protein L1 and the early protein E2 to PML-NBs [113,114]. More specifically, Sp100 is released from PML-NBs, whereas the hDaxx concentration at PML-NBs is increased [114]. Moderate expression of HPV L2 alone is not sufficient to target L2 to PML-NBs, and it is thought that L2 localization to PML-NBs is a result of induction of some unknown cellular factors as part of an intrinsic antiviral response [115]. In productively infected keratinocytes, the HPV E4 protein causes PML protein to

shuttle from PML-NBs to E4-induced nuclear inclusions, and it is hypothesized that this reorganization of PML may be linked to efficient viral genome replication [116]. There are some contradictory reports with regard to the roles of PML-NBs in papillomavirus infection. In one report, PML isoform III was shown to play a positive role during early papillomavirus infection [117]. Another study indicated that PML is dispensable for E2-mediated transcription and viral DNA replication [118]. More detailed analyses are warranted to elucidate the function of PML-NBs and individual isoforms of PML protein during papillomavirus infection.

Polyomaviruses

The relationship between polyomaviruses and PML-NBs seems to be unique for each virus. In the case of SV40, both viral DNA and TAg localize to PML-NBs, however, no disruption of PML-NBs is observed [119]. The presence of an origin of SV40 DNA replication and TAg is sufficient to target reporter transcripts to PML-NBs, suggesting that TAg-directed viral DNA replication is responsible for the recruitment to PML-NBs [120]. For JCPyV, no reorganization of PML-NBs is detected in the human glial cell line SVG-A upon infection, and shRNA knockdown of PML does not significantly affect JCPyV replication [121]. For another human polyomavirus, BK polyomavirus, lytic infection by the virus in its natural host cell, the renal proximal tubule epithelial cell, results in dramatic PML-NB reorganization [122]. The PML-NBs in these infected cells are larger in size and fewer in number, and hDaxx and Sp100 are dispersed from them. In addition, some PML-NBs appear as hollow ring-like structures as examined by confocal microscopy. It is hypothesized that this reorganization of PML-NBs represents an effort by BK polyomavirus to inactivate PML-NB antiviral functions [122].

Parvoviruses

Parvovirus MVM infection leads to the formation of large nuclear bodies that contain both autonomous parvovirus-associated replication bodies and PML-NBs [123]. The nuclear bodies are sites of active viral replication, and the expression of viral nonstructural proteins alone is not sufficient to drive this nuclear body reorganization [123]. Another parvovirus, H-1, also induces a nuclear structures called H-1 parvovirus-associated replication bodies, but these bodies do not seem to contain PML-NBs [124].

Remodeling of the nucleolus

The nucleolus, the site of ribosome biogenesis, also undergoes dramatic changes upon small DNA virus infection. Ad infection results in the relocalization of several nucleolar proteins from the nucleolus to other areas of the nucleus or even to the cytoplasm, as well as the targeting of several viral proteins to the nucleoli [125,126]. Some of the relocalization events are important for viral DNA replication, as in the case of nucleolar proteins B23 and UBV, whereas the functional significance of other relocalizations remain to be determined [126]. In addition to altering the components of nucleolus, Ad infection also affects the function of nucleolus by inhibiting ribosomal RNA processing and export [127].

HPV changes nucleolin distribution in HPV-positive cancerous tissues. Moreover, nucleolin itself has been implicated in the chromatin remodeling of HPV E6/E7 enhancer region, which contributes to E6/E7 transcription and cell proliferation [128]. For polyomavirus, SV40 DNA is able to relocalize telomerase from nucleoli to the nucleoplasm, which is believed to affect substrate access of the telomerase [129]. Both incoming and newly synthesized AAV capsid proteins are targeted to the nucleoli, and it is thought that nucleolus localization plays a role in both AAV input virion trafficking and uncoating, and virus assembly, respectively [125].

Conclusion & future perspective

In this review we summarized some of the effects that small DNA viruses have on host nuclear components and pathways. It is evident from these findings that, without exception, the viruses have evolved with their hosts and have developed elegant strategies to either utilize nuclear factors to create favorable replication environments or to evade and destroy cellular structures that are harmful to the viruses. It is not difficult to imagine that, with recent advances in the host-virus interaction field, additional host remodeling processes will be revealed. Future studies should aim at deciphering the functional significance and detailed molecular mechanism of these host remodeling events during viral infections. In addition, individual host remodeling procedures should not be perceived as independent maneuvers, but rather, an integrated network needs to be defined to assess the combinatorial effects of these host changes on virus life cycle, as many nuclear components and pathways are functionally intertwined.

Novel findings of how viruses remodel host components will also allow the design of more effective antiviral treatments by targeting previously unidentified host targets, either inhibiting host factors that are required for viral infection or preventing antiviral structures from being destroyed. Targeting host factors for combating viral infections has the advantage of reducing the risk of development of drug-resistant virus strains. Recent advances in high-throughput screening approaches will certainly facilitate the identification of such safer and more potent drugs. In the meantime, we can take advantage of the knowledge we gain studying these remodeling events and invent new tools for both basic science and translational research purposes. For example, understanding the nuclear remodeling processes caused by viruses will help in the creation of more efficient viral vectors for gene delivery or treatment purposes, as in the case of oncolytic Ads and herpesviruses. Moreover, the ability of certain viruses to inactivate the DDR has been used in conjunction with DNA-damaging agents to treat tumors that are otherwise chemoresistant [130]. Finally, investigating the effects of viruses on host components will undoubtedly also provide new insights into the cell biology of the host cell itself.

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Executive summary

Virus remodeling of the nuclear envelope

- Adenovirus (Ad) particle disassembly occurs near the nuclear pore complex and requires nucleoporins and kinesin-1.
- Multiple polyomavirus late proteins are involved in nuclear envelope disruption.
- Minute virus of mice input virus causes nuclear envelope disruption, which requires host caspase-3 activity.

Virus interactions with tumor suppressor proteins

- Many DNA viruses target host tumor suppressor proteins to take control of the cell cycle.
- Ad E1A, HPV E7 and polyomavirus TAg all disrupt pRb–E2F association.
- Ad E1A causes a global relocation of pRb family proteins.
- Ad, HPV and polyomaviruses use various mechanisms to inhibit p53.

Viral modulation of the DNA damage response

- The DNA damage response (DDR) has important roles during a number of small DNA virus infections.
- Ad has various means to inactivate the DDR to prevent its linear genome from being concatemered.
- The ATM-mediated DDR is beneficial for HPV replication in differentiated cells, whereas the oncoproteins E6 and E7 interfere with the DDR, resulting in host genome instability.
- The ATM- and ATR-mediated DDRs are, in general, beneficial for polyomavirus infections.
- Unique DDR activations are observed with helper-dependent parvovirus infections.

Host chromatin remodeling

- Many DNA viruses induce γ H2AX, one of the hallmarks of the DDR.
- Small DNA viruses interact with host chromatin remodeling enzymes to alter the host transcription program.

Remodeling of promyelocytic leukemia nuclear bodies

- Promyelocytic leukemia nuclear body (PML-NB) reorganization/degradation is seen in several small DNA virus infections and is associated with the disruption of the PML-NB antiviral function.
- PML-NB proteins may also play positive roles during certain DNA virus infections.

Figure 1. Viral modulation of tumor suppressor proteins and the DNA damage response
 The effects of small DNA virus infections on tumor suppressor proteins **(A)** and the DNA damage response components **(B)** covered in this review. Host targets are highlighted, and the virus/viral components and their corresponding effects on host targets are labeled. Ad: Adenovirus; FA: Fanconi anemia; JCPyV: JC polyomavirus; MPyV: Mouse polyomavirus; MRN: Mre11–Rad50–NBS1 complex; NHEJ: Nonhomologous end joining; SV40: Simian virus 40.

Table 1

Viral proteins and their nuclear targets covered in this review.

Virus	Viral protein	Host target	Effect on host target
<i>Adenovirus</i>			
Ad 5	E1A	Rb	Dissociation of Rb from E2F; global relocalization
		p53	Stabilization
		p300/CBP	Global relocalization
		H3	Histone 3 lysine 18 hypoacetylation
	E1B55K	p53	Inhibition of transcription
		hDaxx	Degradation
	E1B55K/E4orf6	p53	Degradation
		MRN	Degradation
		DNA ligase IV	Degradation
		Bloom helicase	Degradation
	E4orf6	DNA ligase IV	Dissociation of DNA ligase IV/XRCC4 complex
		DNA-PK	Inhibition
	E4orf3	p53	Inhibition of p53 target promoters through histone 3 lysine 9 trimethylation heterochromatin formation
		MRN	Mislocalization
		PML	Reorganization into tracks
	IX	PML, Sp100	Recruitment into nuclear structures
Ad12	E4orf6	TopBP1	Degradation
<i>Papillomavirus</i>			
HPV	E7	Rb	Dissociation of Rb from E2F
		ATM	Activation
		Claspin	Degradation
		γ H2AX	Induction
		Histone deacetylase	Alteration of histone acetylation
	E6	p53	Inhibition of transcription; degradation
		XRCC1	Inhibition
		Chk1	Inhibition
	E1	ATM	Activation
	L2	PML	Reorganization
	E4	PML	Relocalization
<i>Polyomavirus</i>			
JCPyV	Agnoprotein	HP1	Dissociation of HP1 from lamin B receptor
		Ku70	Mislocalization
	TAg	ATM	Activation
		ATR	Activation
		Rad51	Inhibition
MPyV	VP2/3	NE	Disruption
	TAg	p53	Block ARF-p53 signaling

Virus	Viral protein	Host target	Effect on host target
SV40	tAg	p53	Block ARF-p53 signaling
	VP4	NE	Disruption
	TAg	Rb	Dissociation of Rb from E2F
		p53	Stabilization; inhibition of transcription
		ATM	Activation
		MRN	Degradation
		ATR	Activation
		FA pathway	Activation
		NBSI	Inhibition
		H3	Histone 3 lysine 18 hypoacetylation
		γ H2AX	Induction
MCPyV	TAg	p53	Decrease of protein level

Ad: Adenovirus; FA: Fanconi anemia; JCPyV: JC polyomavirus; MCPyV: Merkel cell polyomavirus; MPyV: Mouse polyomavirus; MRN: Mre11-Rad50-NBSI complex; NE: Nuclear envelope; PML: Promyelocytic leukemia; SV40: Simian virus 40.