Viruses and the DNA Damage Response: Activation and Antagonism

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Abstract

Viruses must interact with their hosts in order to replicate; these interactions often provoke the evolutionarily conserved response to DNA damage, known as the DNA damage response (DDR). The DDR can be activated by incoming viral DNA, during the integration of retroviruses, or in response to the aberrant DNA structures generated upon replication of DNA viruses. Furthermore, DNA and RNA viral proteins can induce the DDR by promoting inappropriate S phase entry, by modifying cellular DDR factors directly, or by unintentionally targeting host DNA. The DDR may be antiviral, although viruses often require proximal DDR activation of repair and recombination factors to facilitate replication as well as downstream DDR signaling suppression to ensure cell survival. An unintended consequence of DDR attenuation during infection is the long-term survival and proliferation of precancerous cells. Therefore, the molecular basis for DDR activation and attenuation by viruses remains an important area of study that will likely provide key insights into how viruses have evolved with their hosts.

INTRODUCTION

Viruses are nucleic acid–based obligate intracellular microorganisms. They are small particles—on the order of less than a micrometer—but they contain sufficient genetic information to control their infected host cell. Regardless of whether viruses contain RNA or DNA genomes, their replication nearly uniformly requires host factors. Given that many activities at the virus-host interface involve DNA transactions, it is not surprising that viruses must contend with the host DNA damage machinery. DNA damage is sensed by an evolutionarily conserved signaling pathway, called the DNA damage response (DDR), that enables cells to halt DNA replication or the cell cycle in order to repair the damage. If the damage is too great or particularly difficult to repair, then the DDR makes the critical decision to commit the cell to programmed cell death. This decision serves to avert catastrophic levels of DNA damage that would otherwise compromise genomic integrity.

Viral genomes containing damaged DNA or aberrant DNA structures can be recognized by the DDR, leading to the recruitment and activation of repair proteins. Similarly, viral proteins involved in DNA replication, transcription, or cell cycle regulation may directly or indirectly prompt a DDR. In both scenarios, the DDR may be either beneficial or detrimental to the virus. Indeed, it is often the case that viruses must optimize the DDR. As such, viral proteins can tailor the DDR to various needs, such as replicating the virus, maintaining virus latency, or preventing the activation of the innate—and ultimately the adaptive—immune response. In this article, I discuss how viruses provoke the host DDR and how they harness this activity to promote replication or circumvent it to prevent untoward downstream consequences.

DNA damage response (DDR): the set of signaling pathways activated in response to damaged DNA, including sensors, mediators, and effectors

DSB: double-strand break

Ataxia telangiectasia mutated (ATM): the ATM pathway is the key pathway responding to cellular DSBs; it leads to cell cycle arrest, DNA repair, and apoptosis

ATM and Rad3-related (ATR): the ATR pathway is the key pathway responding to replicative stress through recognition of ssDNA-dsDNA junctions; it activates cell cycle checkpoints, DNA repair, and apoptosis

DNA-PK: DNA-dependent protein kinase

Homologous recombination: an error-free DNA repair pathway that is used when homologous template DNA is available

Checkpoint: the suppression of cell cycle progression, including at the G_1-S, intra-S, and G_2-M boundaries

THE EVOLUTIONARILY CONSERVED RESPONSE TO DNA DAMAGE

The response to DNA damage has been shaped through evolution as a mechanism to prevent loss of genetic and genomic integrity (1). DNA can be damaged by numerous factors, ranging from exogenous sources such as environmental toxins and ionizing radiation to endogenous sources such as DNA replication fork collapse and oxidative stress. The DDR can also sense aberrant DNA structures that form as a result of chromatin changes during nucleic acid transactions such as transcription and replication, as well as exposed telomeric DNA repeats at the ends of chromosomes. The types of damage elicited by these genotoxic and metabolic stressors include base and sugar residue modifications, oxidative DNA adducts, and DNA strand cross-links. Replicative stress can lead to exposed ssDNA, and the most lethal source of DNA damage is the double-strand break (DSB).

Damaged DNA triggers a response mediated by members of the PIKK (phosphatidylinositol 3-kinase-like protein kinase) family of serine/threonine kinases including ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related), and DNA-PK (DNA-dependent protein kinase) (2). The PIKKs then phosphorylate a host of downstream factors, leading to the recruitment of repair factors or, in the face of irreparable damage, to senescence or apoptosis. Repair of DNA damage occurs through two predominant mechanisms: homologous recombination and nonhomologous end joining. Homologous recombination uses a homologous DNA template during the S or G_2 phase of the cell cycle to repair DNA in an error-free manner, whereas nonhomologous end joining is active throughout the cell cycle and is error prone (1). DDR signaling to promote homologous recombination, nonhomologous end joining, cell cycle checkpoints, and apoptosis is controlled by specific PIKKs (1).

DNA DSBs are initially sensed by the ATM arm of the DDR (**Figure 1**) (reviewed in 1). First, the MRN (Mre11-Rad50-Nbs1) complex binds to the site of DNA damage; ATM is then recruited to this site and interacts with the MRN complex (3). ATM is activated by autophosphorylation

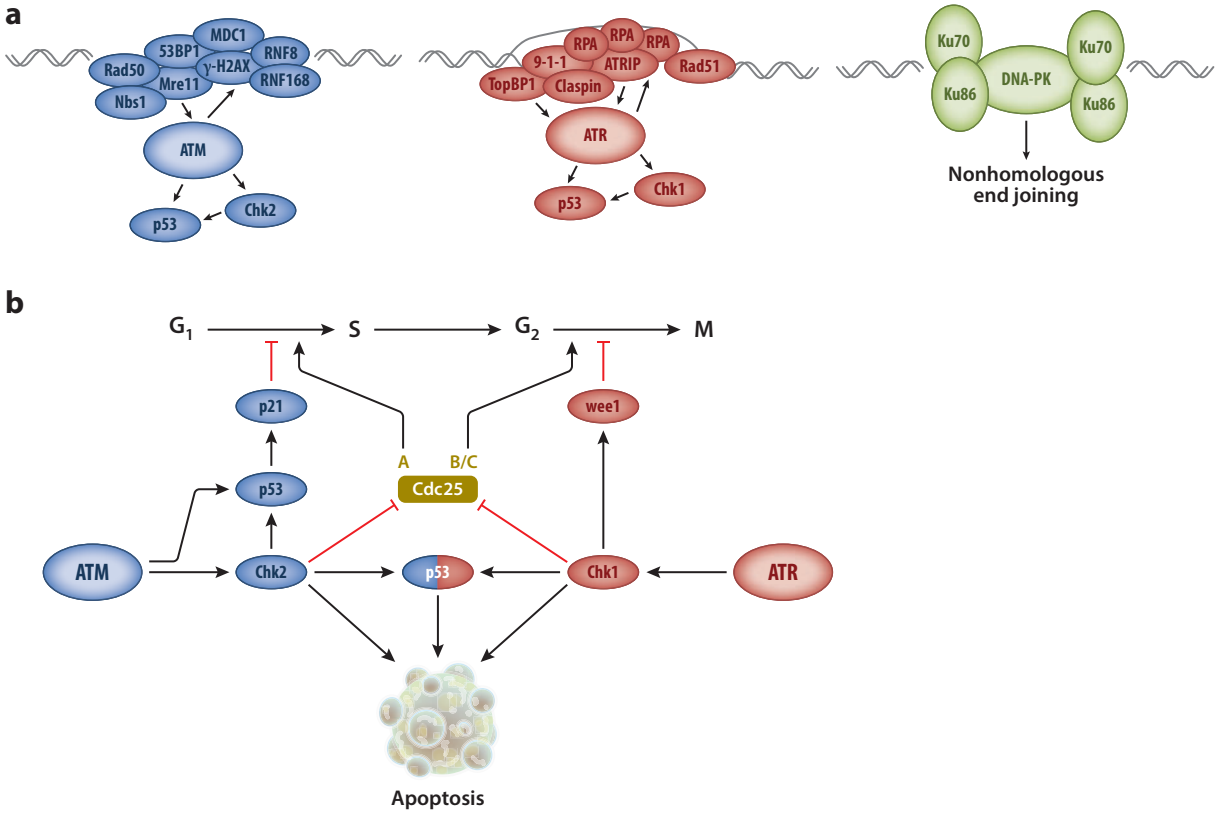

Figure 1

DNA damage response signaling pathways. (*a*) The ATM pathway (*left*, *blue*) recognizes double-strand breaks. The MRN complex recruits ATM, H2AX is phosphorylated to form γ-H2AX, and a series of factors assemble to stabilize the break and recruit repair proteins. ATM also phosphorylates downstream effectors to regulate cell cycle checkpoints and apoptosis. The ATR pathway (*middle*, *red*) recognizes ssDNA exposure—for example, following replicative stress or the formation of UV-induced thymidine dimers. The complex of proteins assembled at the exposed ssDNA includes proteins that are important for activating ATR such that it can phosphorylate downstream effectors that regulate DNA replication, repair, cell cycle progression, and apoptosis. The DNA-PK pathway (*right*, *green*) is activated by double-strand breaks and is stabilized by the Ku70-Ku86 heterodimer. Full DNA-PK activation promotes nonhomologous end joining repair of DNA damage. (*b*) The ATM and ATR pathways control cell cycle progression and apoptosis through phosphorylation of checkpoint kinases, key transcription factors, and Cdc25 phosphatases, among other effectors. The apoptotic signal due to excessive or irreparable damage is mediated primarily through p53.

at Ser1981, promoting monomerization (4). Full activation of ATM further depends on lysine acetylation by Tip60 (5). ATM then phosphorylates the histone H2AX on Ser139; the resulting γ-H2AX (6) recruits the essential adaptor MDC1 (7). The ubiquitin ligase proteins RNF8 and RNF168 specifically recognize ATM-phosphorylated MDC1. This recognition promotes non-degradative ubiquitination of γ-H2AX, which then scaffolds interactions with 53BP1 and Brca1 (8–11). These initiating events nucleate and retain additional DNA repair factors at sites of DNA damage and facilitate the phosphorylation of hundreds of downstream ATM targets—including Chk2, Cdc25, and p53—that mediate cell cycle arrest and apoptosis (2, 12).

ATR uniquely responds to ssDNA exposure at ssDNA-dsDNA junctions. As such, ATR is required for DNA replication as a sensor of DNA replication fork collapse and replication complex uncoupling (**Figure 1**) (reviewed in 13). RPA (replication protein A) coats the exposed

ssDNA and recruits ATR through its partner, ATRIP (ATR-interacting protein) (14). TopBP1 (topoisomerase-binding protein 1) is then recruited to these sites through the 9-1-1 (Rad9-Rad1-Hus1) complex; this complex stimulates ATR kinase activity, leading to the phosphorylation of downstream effectors (12, 15). The critical mediator Claspin is an early target that recruits the kinase Chk1 for ATR phosphorylation and activation, promoting checkpoint activation (2, 16, 17).

The final PIKK, DNA-PK, is the central regulator of nonhomologous end joining–mediated repair of DSBs. DNA-PK is recruited to DSBs and is stabilized by the Ku70-Ku86 heterodimer (**Figure 1**) (reviewed in 18). Following Artemis-mediated resection of the DSB, the Ku–DNA-PK complex recruits the XRCC4 adaptor and DNA ligase IV to promote nonhomologous end joining (19, 20).

Downstream of PIKKs, Chk1 is the primary effector of the intra-S and G_2-M checkpoints, whereas Chk2 primarily establishes the G_1-S and intra-S checkpoints (**Figure 1*b***) (21, 22). Specificity is established during the cell cycle by Chk-mediated phosphorylation of Cdc25 phosphatases, which suppresses their ability to activate specific CDKs (cyclin-dependent kinases) (2). Furthermore, both Chk1 and Chk2 can phosphorylate p53, with downstream consequences on cell cycle progression and apoptosis. Finally, Chk1 and Chk2 both play an additional role in DNA repair through the phosphorylation and activation of homologous recombination pathway components (22).

HOW DOES VIRUS INFECTION ACTIVATE THE HOST DNA DAMAGE RESPONSE?

Viruses, by definition, depend on the infected host cell for their replication. As such, most viruses will inevitably engage host cell DNA, whether through modulating gene expression or cellular DNA replication or as a consequence of viral genome integration. As Matthew Weitzman elegantly noted (23), this sets up a conflict between two genomes: The viral genome must persist and replicate within the cell without perturbing the host cell genome. Immediately upon infection, viral nucleic acids can be sensed by the intrinsic and innate immune response and, if the virus contains a DNA genome, by components of the DDR (24–27). Typically, the consequences of this interaction are the inhibition of viral gene expression through repressive chromatin marks and the activation of innate immune effectors. Escape of intrinsic sensing allows viral replication and gene expression; however, both of these processes can also generate aberrant DNA structures that may activate the DDR (28). Furthermore, viral proteins that modulate host transcription or cell cycle checkpoints, or otherwise engage DNA, can provoke a host DDR as well. Therefore, the early phase of an infectious cycle, from initial genome deposition through gene expression and nucleic acid replication, is rife with DDR-activating potential.

Detection of Viral Nucleic Acids

DNA viruses contain genomes that can directly activate the DDR. This activation may be triggered by the initial incoming viral DNA or by replicating genomes (**Figure 2**). Furthermore, viral DNA genomes are subject to the same environmental and metabolic threats as cellular genomes. Therefore, viral DNA can accumulate base modifications, cross-links, ssDNA nicks, and DSBs, all of which can activate the DDR. RNA viruses that depend on a DNA intermediate to replicate can also activate the DDR. Indeed, the integration step of retroviral replication induces a DDR following the formation of a cellular DSB (29).

Structures in viral genomes that can provoke the DDR include linear dsDNA molecules (adenoviruses and herpesviruses), ssDNA molecules with dsDNA hairpin termini (parvoviruses), circular dsDNA molecules (polyomaviruses and papillomaviruses), and RNA genomes that are reverse

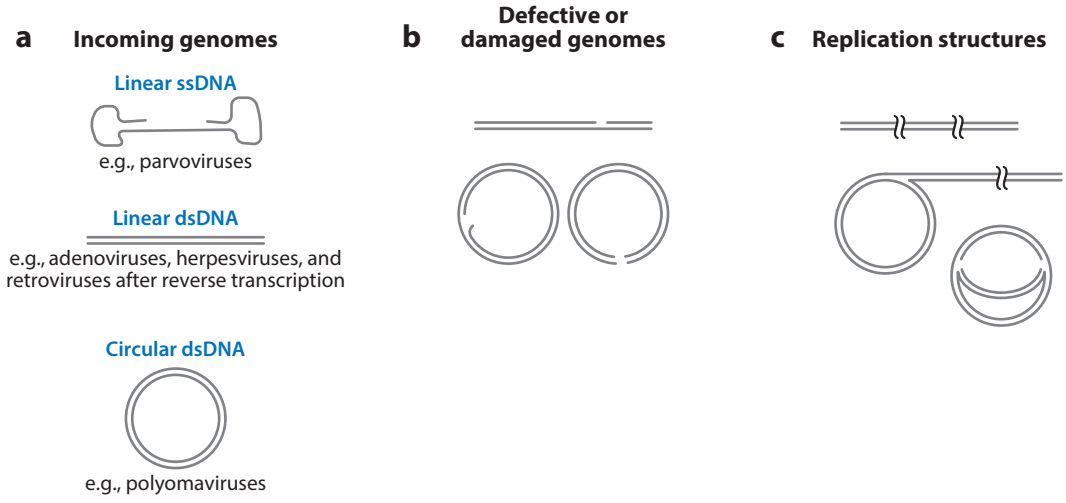

Figure 2

Schematic diagram of viral nucleic acid structures that provoke the DNA damage response. (*a*) DNA genomes, including reverse-transcribed retroviruses. (*b*) Aberrant structures that may form following DNA damage to viral genomes. (*c*) Structures of viral DNA replication intermediates.

transcribed to linear dsDNA (retroviruses) (**Figure 2**). Infection can activate the DDR as an incoming viral genome reaches the nucleus, even prior to and independent of genome replication. For example, herpes simplex virus (HSV) activates the DDR within hours after infection, as characterized by the accumulation of mediators such as MDC1 and γ-H2AX along with the viral protein ICP4 and viral DNA (25).

Following the initial deposition of viral genomes into the nucleus, viral DNA replication typically occurs in well-defined microorganelles termed replication centers. DDR effectors, including repair and recombination proteins, are often recruited to replication centers (**Figure 3*a***) (recently reviewed in 30). Most DNA viruses recruit such factors to their replication centers, including adenoviruses (31–33); parvoviruses (34–37); herpesviruses such as HSV (38–41), cytomegalovirus (CMV) (42), and Epstein–Barr virus (EBV) (43, 44); papillomavirus (45, 46); and polyomaviruses such as simian virus 40 (SV40) (47, 48) and murine polyomavirus (49). The reverse transcription and integration steps of retrovirus replication can also be sensed by the DDR, as characterized by activation of DNA-PK and ATM and phosphorylation of H2AX and other DDR effectors (29, 50). The core DDR sensing machinery—including the MRN complex, ATM, RPA, ATRIP, and ATR—is often detected within replication centers, whereas γ-H2AX is often found at the periphery of replication centers. Additional factors involved in homologous recombination, such as Rad51 and Brca1, are often recruited to replication centers as well. Although the precise molecular architecture of the interaction of DDR components with viral proteins and nucleic acid remains to be determined, the formation of replication centers that are adjacent to depots of DNA repair factors may be advantageous to viruses as they proofread and resolve their genomes prior to packaging.

Replication center: a microorganelle (typically nuclear for DDR-inducing viruses) where viruses replicate their genomic material and form new virion particles

Viral Proteins Provoke the DNA Damage Response

Viral genomes and viral nucleic acid replication are not the only triggers for DDR activation during infection. Several mechanisms exist whereby the expression of viral proteins can provoke

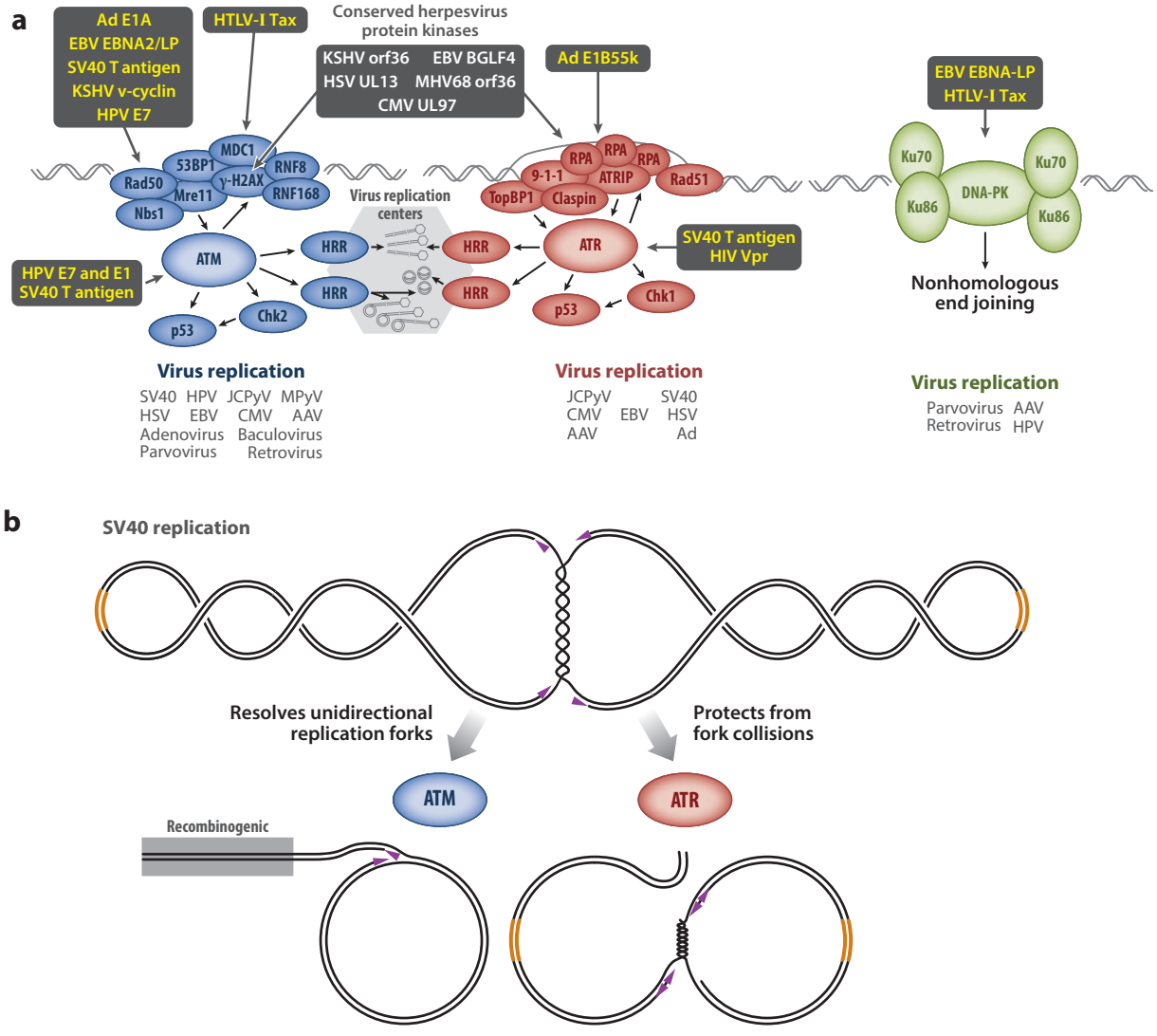

Figure 3

The activation of the DNA damage response by virus replication and specific viral proteins. (*a*) The viruses listed below each pathway are those in which the pathway was reported to be activated either upon sensing the genome or during replication. The individual viral proteins listed have been shown to activate the specific arm of the DNA damage response either upon expression in the absence of replication or in the context of virus infection. A schematic illustrates the viral replication centers where viral DNA is replicated adjacent to activated ATM and ATR. These sites likely serve as depots for homologous recombination and repair factors that contribute to virus replication. (*b*) The replication of SV40 simultaneously activates the ATM and ATR pathways with unique structures formed during replication (90). Both pathways are important for efficient completion of SV40 DNA replication by T antigen. Purple arrowheads indicate replication forks, and orange lines represent origins of replication. Abbreviations: AAV, adeno-associated virus; Ad, adenovirus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HRR, homologous recombination and repair factors; HSV, herpes simplex virus; HTLV-I, human T lymphotropic virus type I; JCPyV, JC polyomavirus; KSHV, Kaposi sarcoma–associated herpesvirus; MHV68, murine gammaherpesvirus 68; MPyV, murine polyomavirus; SV40, simian virus 40.

the response independent of viral nucleic acid (**Figure 3*a***). One class of proteins with this capability is the DNA and RNA tumor virus transforming oncoproteins, which drive aberrant proliferation. These proteins can inappropriately drive cells into S phase to provide a milieu for virus replication. Viral proteins can also directly bind to and activate DDR components. Additionally, viral enzymes that affect DNA metabolism can trigger a DDR, particularly when aberrantly targeting host DNA. Finally, viral proteins can trigger DDR activation by promoting the accumulation of genotoxic stressors such as reactive oxygen species.

DNA viruses often infect quiescent cells and therefore must promote entry into the cell cycle to generate the nucleotide pools and other building blocks required for virus replication. Viral proteins that promote S phase can activate the DDR as an oncogenic stress response. The small DNA tumor virus–encoded proteins SV40 T antigen, human papillomavirus (HPV) E7 protein, and adenovirus E1A all disrupt the interaction between Rb and E2F proteins, leading to S phase entry (51–53). However, heightened E2F levels can lead to hyperproliferation and consequently to DNA replicative stress due to an increase in fork collapses (54–56). Therefore, the early stage of infection with these viruses triggers a DDR as a consequence of cellular DNA replicative stress (42, 57). The establishment of latency by large DNA tumor viruses also promotes cell proliferation as a means to facilitate viral genome replication and persistence. Indeed, Kaposi sarcoma–associated herpesvirus (KSHV) v-cyclin activates the DDR in endothelial cells, and EBV early latent proteins activate the DDR during primary infection of B cells (58, 59). Viral oncoproteins also mitigate other cell cycle checkpoints to produce an activated DDR. For example, HPV E7, SV40 T antigen, and EBV EBNA3C all perturb mitotic signaling. Both T antigen and EBNA3C interfere with the mitotic spindle checkpoint to promote ATM activation (60–62), whereas E7 triggers centrosome duplication (63, 64).

Viral proteins also directly bind to and activate cellular DDR factors. HPV E7 and murine gammaherpesvirus 68 (MHV68) M2 bind directly to ATM, leading to the activation of a specific subset of downstream targets (45, 65). The human T lymphotropic virus type I (HTLV-I) Tax oncoprotein forms pseudo-DDR foci in cells by tethering MDC1 to chromatin and recruiting specific factors including Brca1, γ-H2AX, and activated DNA-PK (66, 67). SV40 T antigen can also directly aggregate the MRN complex through Nbs1 to activate ATM and provoke DNA hyperreplication (68). The human immunodeficiency virus (HIV) Vpr protein also induces a robust ATR-mediated DDR due to perturbation of the G_2-M checkpoint through interactions with the DDB1 protein (69).

Viral enzymes that are expressed during replication can also induce a DDR, either through direct action on viral DNA or through off-target effects on host DNA. For example, the HPV E1 helicase facilitates viral DNA replication and recruits DDR proteins to viral replication centers (70, 71). However, E1 also targets cellular DNA and induces damage that promotes the formation of DDR foci and the activation of checkpoints (71, 72). During the lytic phase of EBV replication, the major tegument protein, BPLF1, acts as a deubiquitinating and deneddylating enzyme—controlling Cdt1 levels and PCNA ubiquitination—leading to a robust DDR, activated as a consequence of constitutive pseudo–S phase induction (73, 74).

Another recently characterized set of viral enzymes capable of activating a host DDR is the CHPKs (conserved herpesvirus protein kinases). While these enzymes are capable of influencing cell cycle progression (75, 76), the first direct link between the CHPKs and the DDR was an elegant study by Tarakanova et al. (77) that demonstrated that MHV68 orf36 could directly phosphorylate H2AX, thus mimicking ATM function at DSBs. Subsequent studies using protein microarrays identified enrichment of DDR-related proteins, including Tip60, RPA, Chk1, and Rad51, as substrates for CHPKs (78). These enzymes play a key role in virus replication, which implicates DDR activation in this process.

A number of viral proteins trigger the production of reactive oxygen species that can lead to the activation of a DDR signaling pathway. Indeed, a variety of mechanisms have been described for the increased levels of reactive oxygen species during infection. For example, the EBV episome maintenance protein, EBNA1, also *trans*-activates cellular genes including the catalytic subunit of the NADPH oxidase gene, NOX2, which promotes reactive oxygen species accumulation and DDR activation in Burkitt lymphoma cells (79). Similarly, the HTLV-I Tax protein promotes increased levels of reactive oxygen species, which can activate the DDR independent of viral nucleic acid (80).

THE ROLE OF THE DNA DAMAGE RESPONSE IN VIRUS REPLICATION

Although many viruses and viral gene products activate the DDR, it is not uniformly clear whether this is beneficial or detrimental to virus infection. The role of the DDR in replication often depends on the structures formed by the replicating DNA, the type of infection (e.g., latent or productive), and the cell type and cell cycle stage in which replication occurs. The characterization of specific DDR proteins that are recruited to, or excluded from, viral replication centers is beginning to shed light on this important question. Indeed, understanding the role of this key cellular signaling pathway may provide clues to pathogenesis across a wide range of viruses and may suggest novel therapeutic targets.

The DNA Damage Response as an Antiviral Mechanism

Early studies on adenovirus-mediated activation of the DDR indicated a strongly antiviral response. In fact, the molecular phenotype associated with adenovirus serotype 5 (Ad5) early gene region loss (E1B or E4) is a potent DDR and long concatenated genomic DNA (31, 81). Thus, viral genomes are "repaired" by the host DDR to prevent proper packaging, thereby crippling replication. The genetic loss of DDR factors, including MRN components and ATM, rescues Ad5 early region gene loss, confirming that the DDR plays an inhibitory role in adenovirus replication (31).

The activation of the DDR as a consequence of cellular hyperproliferation driven by latent herpesvirus infection is also antiviral (58, 59). In the case of EBV, viral transcriptional activators promote very rapid entry into S phase, and this hyperproliferation leads to an ATM/Chk2-dependent DDR (59). The consequences of ATM activation in this setting are suppression of EBV-induced B cell growth and, as a result, suppression of latent infection and genome replication. Interestingly, recent studies indicate that the DDR lies at the balance between latent infection and lytic replication in herpesviruses (82).

The Benefits of DNA Damage Response Activation for Virus Replication

The lytic replication of DNA viruses nearly uniformly requires an activated DDR. Recruitment of DDR factors to replication centers is a common theme of DNA virus replication. Although the processing of adenoviral DNA into concatemers precludes packaging, the key functions of the cellular DDR—including processing aberrant DNA structures, cleaving and resecting damaged sites, and repairing mismatches and bulky adducts—and the robustness of homologous recombination–mediated repair collectively provide a wealth of tools for DNA viruses to perform quality control on their genomes prior to assembly. Therefore, a common theme has emerged for DNA virus infection: the need for DDR activation, proximal signaling through a PIKK, and specific subsets of DNA repair factors. Concurrently, the downstream signaling pathway that leads to apoptosis or senescence is often mitigated by specific viral proteins to ensure cell survival during replication.

Human herpesviruses, including HSV (40), CMV (42), EBV (78, 83), and MHV68 (77, 84), require ATM signaling for efficient lytic viral DNA replication. Similarly, the vegetative episome amplification of papillomaviruses (45) and the replication of polyomaviruses (47–49) also require ATM kinase activity. Adeno-associated virus (AAV) robustly activates the DNA-PK arm of the DDR during coinfection with adenovirus as a helper (34, 36), which is important for its replication (85). However, the ATM pathway is uniquely important for the replication of murine and canine parvoviruses (37, 86). Finally, evidence exists for (50, 87) and against (88) a role for ATM activation by HIV-1 during integration. Further, DNA-PK activation also potentially plays an important role in retroviral integration (29), despite promoting apoptosis during primary T cell infection by HIV-1 (89). Therefore, it is clear that many diverse viruses require DDR signaling for their replication.

Despite the strong genetic and pharmacological evidence for the role of DDR components during virus infection, the molecular mechanisms by which these factors act during virus replication remain poorly characterized. Recent work from Ellen Fanning's laboratory (90) on the replication of polyomaviruses suggests that ATM and ATR both play a key role in the resolution of viral replication intermediates through homologous recombination. Specifically, ATM prevents the accumulation of unidirectional replication products, whereas ATR protects the integrity of functional replication forks if they engage stalled forks (**Figure 3*b***) (90). During HSV replication, the ATR signaling pathway is subverted such that specific components are available for recombination-dependent repair to facilitate replication (91). The efficient replication of latent EBV episomes also requires DDR activity to promote the resolution of Holliday junctions (92). In the absence of ATM or Nbs1, EBV episomes are forced to integrate into host chromosomes. The activities of ATM and ATR during virus replication are similar to those required during cellular DNA replication (1); virus-recruited ATM and ATR activities are selective, ensuring the proper maintenance and resolution of replication products while mitigating downstream untoward consequences of DDR activation, such as apoptosis.

DDR signaling can also play a key role in viral gene expression. The activation of DDR proteins, including the histone acetyltransferase Tip60, by the CHPKs plays an important role in the replication of herpesviruses such as EBV, CMV, and HSV. In addition to its role in facilitating viral genome replication, ATM/Tip60 also directly impacts lytic viral gene expression (78). Specifically, in the case of EBV, Tip60 is phosphorylated by the CHPK BGLF4 and is recruited to late lytic genes, where it promotes the acetylation of histones to induce late lytic gene expression (78).

VIRAL ANTAGONISM OF THE DNA DAMAGE RESPONSE

Most DNA viruses activate the DDR to enable the actions of specific factors important for replication. However, the triggering of ATM- and ATR-dependent signaling cascades can promote deleterious effects, including p53-mediated apoptosis. Therefore, viruses have evolved elegant mechanisms to optimize the DDR in order to prevent such effectors from suppressing virus replication (**Figure 4*a***). These mechanisms include virus-encoded direct and indirect antagonists of DDR signaling. Viral proteins can serve as ubiquitin ligases to trigger the degradation of unwanted effectors. Similarly, viral proteins can directly bind to and antagonize the function of various downstream signaling components. Finally, indirect, broad-scale attenuation of DDR function can be elicited in the context of specific viral infections that promote an antiviral DDR.

Viral ubiquitin ligase: a protein that facilitates the assembly of ubiquitin on effector substrates, typically with the purpose of degrading the target

Viral Ubiquitin Ligases Directly Target DNA Damage Response Proteins

The direct targeting of DDR components by a viral ubiquitin ligase was first described in a landmark paper that examined restriction of adenovirus replication (31). Infection of cells with a mutant

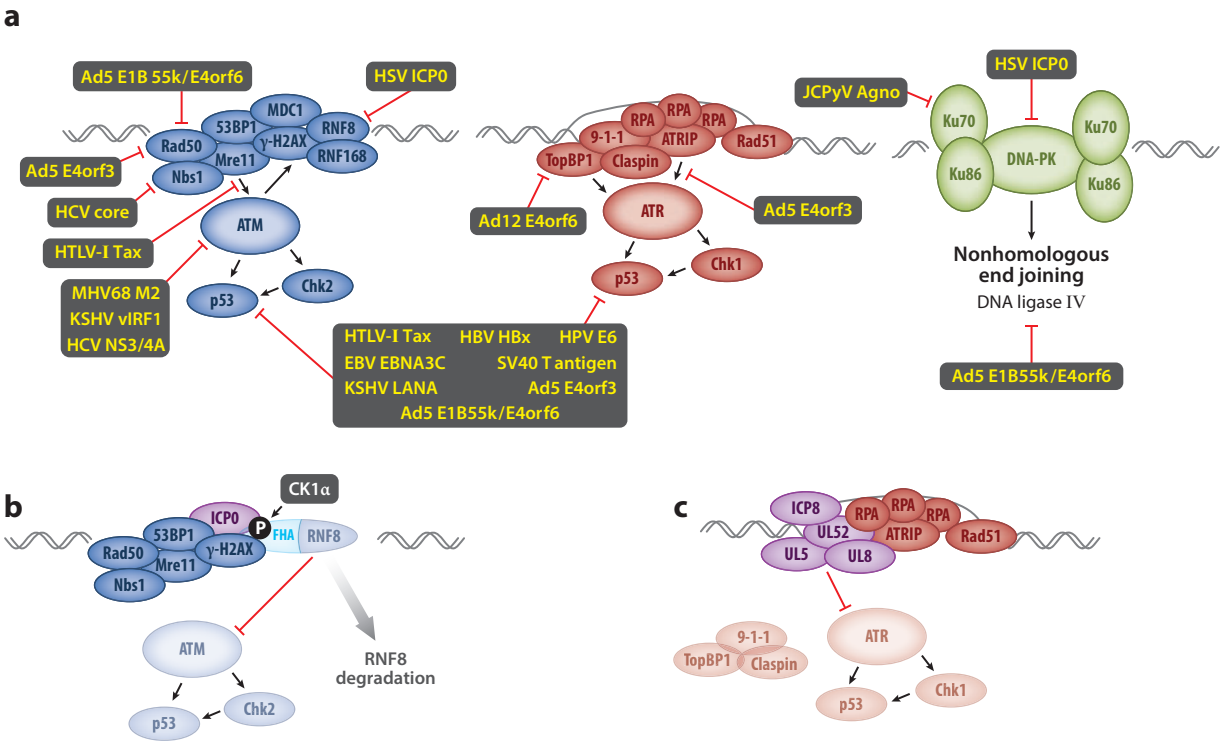

Figure 4

The suppression of DNA damage response signaling by viral proteins. (*a*) Specific viral proteins have been shown to antagonize the DNA damage response at multiple points. The majority of these interactions have been confirmed biochemically and prevent downstream signaling in the respective DNA damage response pathway. (*b*) Schematic diagram of the molecular mimicry by which HSV ICP0 targets the RNF8 protein for degradation; adapted with permission from M. Weitzman (97). The cellular kinase CK1α phosphorylates ICP0, which mimics MDC1 phosphorylation by ATM. When ICP0 is phosphorylated, it binds with high affinity to RNF8, thereby promoting its degradation and inhibiting ATM signaling. (*c*) The HSV helicase/primase complex (UL5-UL8-UL52) and ICP8 bind to the ssDNA-dsDNA junction adjacent to RPA and ATRIP, allowing ATR recruitment to HSV DNA replication sites and cellular damage sites but preventing the 9-1-1–TopBP1–Claspin complex from being recruited (101). The net result of this molecular mimicry is strongly attenuated ATR signaling during HSV infection. Abbreviations: Ad5, adenovirus serotype 5; Ad12, adenovirus serotype 12; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; HSV, herpes simplex virus; HTLV-I, human T lymphotropic virus type I; JCPyV, JC polyomavirus; KSHV, Kaposi sarcoma–associated herpesvirus; MHV68, murine gammaherpesvirus 68; SV40, simian virus 40.

Ad5 lacking the E4 or E1B region led to the accumulation of concatenated genomes as well as an activated DDR. Fine mapping of this phenotype indicated a role for both the E4orf6 and E4orf3 proteins in suppression of the DDR and concatemerization of Ad5 DNA. As an E1B55k/E4orf6 ubiquitin ligase activity had been noted, expression of this complex was found to be sufficient to trigger the degradation of the Mre11 component of the MRN complex (31). Further studies have identified additional substrates of this ubiquitin ligase complex, including enzymes important in DNA repair, such as DNA ligase IV (93)—which is essential for nonhomologous end joining—and the Bloom syndrome helicase (94). Surprisingly, screening these functions across different adenovirus serotypes identified unique mechanisms to mitigate the DDR. For example, adenovirus serotype 12 (Ad12) uses its E4orf6 ubiquitin ligase to uniquely degrade TopBP1, thereby blocking the ATR signaling pathway (95). Taken together, these results are consistent with a critical need for adenovirus to prevent its linear dsDNA ends from being recognized as a DSB and therefore

being processed by nonhomologous end joining to a form that cannot be packaged into virions. Interestingly, the dependoviruses, such as AAV, require Ad5-mediated degradation of Mre11 and suppression of ATM signaling to promote their replication (36). Indeed, the E1B55k/E4orf6 complex is sufficient to promote AAV replication, and MRN and ATM associate with AAV genomes in their absence.

Another salient example of a viral ubiquitin ligase that promotes the degradation of DDR factors is the HSV ICP0 protein. ICP0 is a RING-finger ubiquitin ligase that targets several DDR components. Early studies determined that the critical nonhomologous end joining mediator DNA-PK is an ICP0 substrate and is thereby prevented from processing HSV DNA ends (96). Recent work has identified a new ICP0 substrate, the DDR mediator and ubiquitin ligase RNF8 (97). During the acute response to DSBs, RNF8 amplifies the signal through nondegradative K63-linked ubiquitination of H2AX (98). During HSV infection, ICP0 is constitutively phosphorylated by CK1α, leading to RNF8 recognition of a phosphopeptide similar to that found in the DDR adapter MDC1 (97). As a consequence, ICP0 subverts the amplification of the ATM response in HSV-infected cells, allowing selective substrates and activities to be retained for virus DNA replication (**Figure 4*b***).

Viral Proteins Relocalize DNA Damage Response Components

In a mechanism complementary to protein degradation, several viruses alter the localization of DDR components and effectors as a strategy to optimize DDR signaling to benefit virus replication. Early studies on HSV indicated that the ATR signaling pathway components RPA, ATRIP, and ATR are recruited to HSV replication centers during replication, whereas downstream ATR signaling is inhibited (39, 99, 100). Recent work indicates that the mechanism of ATR signaling at both viral and cellular sites of ssDNA exposure is as follows: The viral helicase/primase complex (UL8-UL5-UL52), together with the origin-binding protein (UL9) and the ssDNA-binding protein (ICP8), binds to ssDNA-dsDNA junctions and prevents recruitment of the ATR-activating 9-1-1–TopBP1–Claspin complex (101). This elegant mechanism of molecular mimicry ensures efficient viral DNA processing during replication (**Figure 4*c***). These findings are consistent with reports in HCMV-infected cells wherein UV-induced DNA damage is enriched outside of replication centers, which suggests preferential repair of these lesions within viral DNA and impaired ATR responses at cellular sites of damage (102, 103).

Another example of viral protein mislocalization of DDR proteins is provided by the adenovirus E4orf3 protein, which promotes the assembly of nuclear tracks of tumor-suppressor proteins to facilitate replication (104, 105). Among the relocalized proteins are the MRN complex components (31). The recently described three-dimensional structure of E4orf3, together with high-resolution microscopy, indicates that E4orf3 has a propensity to coassemble as mixed dimers that can form large polymers in the cell (106). Interestingly, unique E4orf3 polymers promote interaction with and mislocalization specifically of MRN as opposed to other antiviral factors during adenovirus infection (106). Following nuclear track formation, Ad5 E4orf3 further promotes cytoplasmic aggregation of MRN, leading to inhibition of ATM and ATR signaling (107–109).

Targeting the proximal mediators of the ATM pathway is a common theme among viruses. The MHV68 protein M2 directly binds to ATM and thereby suppresses MRN and γ-H2AX formation in response to DNA damage (65). KSHV vIRF1 uses a similar strategy in targeting ATM, as does the hepatitis C virus (HCV) NS3/4A enzyme (110, 111). HCV core protein directly interacts with Nbs1, inhibiting MRN assembly and thereby preventing ATM activation (112). Finally, as noted above, the HTLV-I Tax protein directly binds MDC1 and prevents the proper activation of ATM complexes upon irradiation (66).

Downstream Attenuation of DNA Damage Response Signaling Pathways

Activation of DDR signaling pathways at the level of PIKKs can promote recombination or repair complexes that viruses take advantage of for their replication. However, downstream signaling to cell cycle checkpoints and apoptosis can negatively impact virus replication. Therefore, in addition to direct inactivation of proximal signal components, viruses have developed strategies to target those downstream components with the most profound consequences. The p53 transcription factor is a robust activator of genes that promote cell cycle arrest and apoptosis. Therefore, many viruses have evolved mechanisms to prevent p53 activation from killing the infected cell prior to the completion of virus replication (**Figure 4*a***).

The activation by small DNA tumor viruses of oncogenic stress due to hyperproliferation is mitigated by the attenuation of p53 by both degradation and inhibition of *trans*-activation. The adenovirus E1B55k/E4orf6 ubiquitin ligase (113), the HPV E6/E6AP ubiquitin ligase (114), and the EBV BZLF1 protein (115) can all promote p53 degradation. The adenovirus E1B55k and E4orf3 proteins can also suppress p53 transcriptional activity (116–118). Several additional viral proteins can complex with p53 and potentially alter target gene activation as well, including KSHV LANA (119, 120), EBV EBNA3C (121), HBV HBx (122), and HTLV-I Tax (123, 124).

CONSEQUENCES OF VIRAL PERTURBATION OF THE DNA DAMAGE RESPONSE

Attenuation and optimization of the DDR may be critical for virus replication but can also prove dangerous if the infected cell persists. Oncogenic viruses impact cell cycle regulatory nodes to drive proliferation. The expected outcome is viral genome replication, particle formation, and, typically, cell death. However, in the event of an aberrant infectious cycle, such as an illegitimate integration event in the case of papillomaviruses, the cell may be subject to overexpression of oncoproteins (e.g., HPV E6 and E7) that drive proliferation and activate the DDR. The attenuation of the DDR in this setting, independent of viral genome replication, may cause untoward consequences for the cell and the organism. Indeed, the uncontrolled proliferation of such infected cells promotes tumorigenesis. The DDR can be attenuated by a number of mechanisms to promote malignancy, including the mechanisms described above that involve direct antagonism of downstream components of the signaling pathway such as checkpoint kinases or p53. Alternatively, the selection pressure to lose an allele of a DDR component will be great in the context of strong proliferative signals such as viral oncoprotein expression. Not surprisingly, DDR components such as Nbs1, ATM, and Chk2 are tumor suppressors found to be mutated across a wide variety of cancers (125–128).

Latent herpesviral infections promote proliferation and attenuate the DDR. Both EBV and KSHV drive cell proliferation upon infection through the regulation of S phase entry (58, 59). Following expression of the viral EBNA2/LP proteins and KSHV v-cyclin, a DDR-induced cell cycle arrest ensues that can be mitigated by EBNA3C and v-FLIP, respectively (59, 129). Whereas EBNA3C overcomes viral oncogene-induced senescence by suppressing expression of the potent CDK inhibitor p16, KSHV v-FLIP directly antagonizes ATG3 and the autophagy pathway to mitigate oncogene-induced senescence (129, 130). Therefore, oncogenic gammaherpesviruses stimulate uncontrolled cell proliferation and bypass a growth-suppressive DDR, which could serve as an early lesion that is critical to promote tumorigenesis.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The activation of the DDR can be provoked by both DNA and RNA viruses. The associated recruitment of host DNA repair and recombination factors to viral replication centers can be

deleterious, though it often leads to a productive milieu for virus replication. Concomitantly, virus-specified antagonism of downstream DDR signaling effectors can prevent untoward consequences of pathway activation such as death of the host cell or inhibition of viral replication. However, one such unintended consequence of DDR signaling attenuation is DNA mutagenesis coupled with increased survival, which could promote tumorigenesis. Overall, the molecular sources of DDR activation and the precise mechanistic role of the DDR during infection remain exciting areas for discovery. Three emerging themes are (*i*) dissecting the interplay between the DDR and other intrinsic immune defenses, (*ii*) defining the breadth of molecular mimicry viruses use to subvert the DDR, and (*iii*) determining the role of the DDR in promoting tumorigenesis in the presence or absence of ongoing viral replication.

The interface between the DDR and the intrinsic mechanisms for sensing foreign DNA is emerging as an important area of study that may illuminate how the DDR fits into the larger network of host defenses. Viral DNA genomes may be detected by intrinsic immune sensors such as PML nuclear bodies, nuclear and cytosolic DNA sensors, or pattern-recognition receptors, depending on the mechanism and route of entry into cells. In the case of HSV, Matthew Weitzman's and Roger Everett's groups have elegantly characterized the relationship between the DDR and intrinsic sensing by PML (28). The PML nuclear body constituents PML, Daxx, and Sp100 are redistributed early after infection to what appear to be de novo PML-like bodies adjacent to incoming HSV genomes (131–133). These PML-containing structures partially overlap with activated DDR components (28). However, PML sensing of HSV does not require the DDR proteins Mre11 or ATM, and DDR activation does not require PML (25). Although these pathways are genetically distinct, several key connections between PML sensing and the DDR suggest intriguing hypotheses for their cooperation. DNA damage can promote PML body formation, which can persist at sites of irreparable damage (134, 135). Furthermore, sumoylation and SUMO interaction are critical components of both PML activation and the DDR (136, 137). As such, these pathways are likely to intersect during viral infection, and they have been implicated in the recognition of and defense against several DNA viruses (138, 139). However, the mechanisms of cooperation remain to be fully articulated. One point of intersection between these pathways is the mechanism by which HSV counteracts them—namely, ICP0 degradation of PML and RNF8 (98, 140).

The mimicry that ICP0 uses to target RNF8 provides a salient example of how a virus directly antagonizes the DDR at the molecular level. Whereas ICP0 promotes the degradation of PML through its RING domain, it uses a phosphopeptide to mimic ATM-phosphorylated MDC1 in targeting RNF8 for degradation (97). ICP0 is constitutively phosphorylated by CK1α during HSV infection and engages the BRCT domain of RNF8. This interaction allows ICP0 to promote RNF8 degradation, thereby blocking downstream DDR signaling (**Figure 4*b***). Viral ubiquitin ligase–mediated degradation of DDR factors remains an exciting area of discovery (141). Another elegant mechanism of DDR antagonism through molecular mimicry involves the ability of the HSV helicase/primase complex (UL8-UL5-UL52) and ICP8 to recognize ssDNA-dsDNA junctions that normally activate ATR (101). By doing so, these complexes are able to prevent the recruitment of the 9-1-1–TopBP1–Claspin complex to these ssDNA-dsDNA junctions, thereby inhibiting downstream ATR signaling (**Figure 4*c***). The consequence of HSV proteins mimicking 9-1-1–TopBP1–Claspin recruitment at these sites within cellular DNA is the loss of checkpoint control and apoptosis upon DNA damage. Indirectly, ATR inhibition during HSV infection could impact cell growth control and promote tumorigenesis if the infected cell were to survive infection.

Another link between intrinsic defenses and the DDR that could promote tumorigenesis is the activation of the APOBEC family of cytidine deaminases (142). These factors often respond to viral infection by directly mutagenizing viral nucleic acid, thereby decreasing replication efficiency

(143–145). However, an unintended consequence of APOBEC activation during viral infection is the mutation of cellular DNA. The failure to properly resolve virus-stimulated, APOBEC-dependent cellular DNA damage may be a mechanism by which viruses, even those not considered oncogenic, could promote cancer. This type of hit-and-run mechanism has been proposed in the context of breast cancer, where there are hallmarks of tumorigenesis driven by APOBEC3B-induced mutation (146, 147). Other cancers also likely arise as a consequence of aberrant DDR activation and attenuation of downstream signals. The extent to which cellular DNA damage during viral infection and viral countermeasures to suppress the DDR contribute to tumorigenesis remains a highly important area for future investigation.

SUMMARY POINTS

1. Viruses must contend with the DNA damage response, as aberrant nucleic acid structures that are present in their genomes and that arise during their replication are sensed by this evolutionarily conserved response.
2. Virus-induced DNA damage response activation can be broad, including ATM, ATR, or DNA-PK.
3. The aggregation of DNA damage response factors at sites of viral replication may serve to facilitate recombination or repair of genomes, which could have deleterious or beneficial consequences.
4. Many viruses have a counterstrategy to DNA damage response activation that optimizes downstream signaling to the benefit of replication.
5. The precise molecular mechanisms for virus infection–induced DNA damage response activation remain poorly characterized.
6. Molecular mimicry, typically using viral ubiquitin ligase proteins, is a common theme for viral subversion of the DNA damage response.
7. Activation and subsequent attenuation of the DNA damage response during virus infection could promote tumorigenesis due to increased mutation or decreased repair of cellular DNA, both of which lead to genomic instability, as well as resistance to apoptosis.

FUTURE ISSUES

1. The molecular signals for DNA damage response activation in viral replication centers need to be defined.
2. The substrates of viral ubiquitin ligases that perturb the DNA damage response should be broadly assessed.
3. An understanding is needed of the interplay between the DNA damage response and intrinsic sensors of viral infection, including PML, APOBEC, TLR, and inflammasome pathways.
4. The contribution of viral infection–induced DNA damage to promoting tumorigenesis should be assessed.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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