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Viral Modulation of the DNA Damage Response and Innate Immunity: Two Sides of the Same Coin

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Abstract

The DDR consists of multiple pathways that sense, signal, and respond to anomalous DNA. To promote efficient replication, viruses have evolved to engage and even modulate the DDR. In this review, we will discuss a select set of diverse viruses and the range of mechanisms they evolved to interact with the DDR and some of the subsequent cellular consequences. There is a dichotomy in that the DDR can be both beneficial for viruses yet antiviral. We will also review the connection between the DDR and innate immunity. Previously believed to be disparate cellular functions, more recent research is emerging that links these processes. Furthermore, we will discuss some discrepancies in the literature that we propose can be remedied by utilizing more consistent DDR-focused assays. By doing so, we hope to obtain a much clearer understanding of how broadly these mechanisms and phenotypes are conserved among all viruses. This is crucial for human health since understanding how viruses manipulate the DDR presents an important and tractable target for antiviral therapies.

Keywords

DNA damage response; innate immunity; viral-host interactions; viral replication; antiviral response

Introduction

To maintain genomic integrity, cells possess various mechanisms to repair, protect, and replicate genetic material. At the heart of this is the DNA damage response (DDR), a

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Andrew Lopez: Conceptualization, Writing – original draft, Visualization. **Randilea Nichols Doyle:** Conceptualization, Writing – original draft. **Carina Sandoval:** Conceptualization, Writing – original draft. **Karly Nisson:** Writing – original draft. **Vivian Yang:** Writing – original draft. **Oliver I. Fregoso:** Conceptualization, Writing – review & editing.

signaling cascade that functions to sense, signal, and respond to aberrant nucleic acid. However, in addition to maintaining genomic integrity, the DDR is exquisitely poised to regulate viral infection, since to the cell, viruses are essentially aberrant nucleic acids. In support of this, the connection between viral replication and the DDR has emerged in two primary roles: (1) viruses modulate the DDR proteins and pathways required for viral replication; (2) there is significant crosstalk between the DDR and the innate immune response against viruses. These connections have been observed extensively across viral classifications and are relevant to a variety of both DNA and RNA viruses, including single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) viruses, positive (+) and negative (−) stranded RNA viruses, and retroviruses (a (+) RNA virus that relies on a dsDNA intermediate), which we separately classify here according to the Baltimore Classification.[1]

In this review, we will break down the interaction of viruses with the DDR into four primary sections (Figure 1). First, we will discuss how viruses induce DNA damage and antagonize the sensing of this DNA damage. Second, we will discuss how viruses modulate the DDR signaling cascade – from mediators, to transducers, to effectors – with a focus on specific DNA, RNA, and retroviruses. Third, we will highlight the many cellular consequences of viral-induced modulation of the DDR. Finally, we will connect DDR signaling to innate immunity to emphasize how they are two sides of the same coin, since both are signaling cascades that sense, signal, and respond to aberrant nucleic acids. Each “side of the coin” (cellular function) has been traditionally thought to be separate, but more recent work has shown them to be more interconnected. We will not be able to cite all the work that has gone into understanding the connections between viral replication and the DDR (for additional specialized topic reviews, see 2,3 and others highlighted throughout the text). Additionally, some of the data we cite is limited and has yet to be corroborated. We understand the limitations this brings, yet we have included the work to demonstrate that examples exist across diverse viruses to substantiate the larger themes and concepts we discuss. We aim for this review to serve not only as an additional resource but to stimulate the fields of virology, DDR, and innate immunity to look for potential crosstalk in these interconnected fields that are essential for human health.

Overview of the DNA damage response (DDR)

The first step in activation of the DDR is induction of DNA damage. DNA damage occurs through endogenous pathways, such as DNA replication errors and reactive oxygen species generated during cellular metabolism, as well as exogenous factors, such as ultraviolet and ionizing irradiation, chemical mutagens, and viral replication. These genotoxic stresses can result in double-strand DNA breaks (DSBs), single-strand DNA breaks (SSBs), and single-base modifications such as mismatched bases, DNA adducts, or intra-strand crosslinks. Depending upon the type of damage, specific protein sensors are responsible for recognizing damaged DNA and initiating the DDR signaling. For the purpose of this review, we will focus on sensing and signaling associated with DSBs and SSBs (Figure 2). DSBs are primarily recognized by the MRE11, Rad50, NBS1 (MRN) complex, leading to ATM activation and recruitment to sites of genotoxic stress. Active ATM phosphorylates various downstream effector proteins including histone variant γH2AX, CHK2, and 53PB1.[4–6]

Alternatively, DSBs can be recognized by the DNA-PK holoenzyme (Ku70, Ku80, and DNA-PKcs) to be repaired by non-homologous end joining (NHEJ), a more error-prone process than homologous recombination (HR) repair and primarily occurs in the G1 phase of the cell cycle.[4–6] SSBs are sensed by RPA; when bound to ssDNA, RPA activates ATR, which stimulates downstream signaling proteins such as CHK1.[4–6] Depending on the type and severity of the lesion, activation of the DDR results in various cellular outcomes such as DNA repair, cell cycle arrest, chromatin dynamics, and transcriptional changes.

I Viral manipulation of the DDR

In the following section, we will discuss examples of how diverse viruses modulate all steps of the DDR, including induction of DNA damage, recognition by damage sensors, signaling via mediator, transducer, and effector proteins, and cellular consequences of the DDR (Figure 2 and Table 1). We have focused on specific examples which we hope will convey three main points: (1) modulation of the DDR is conserved by diverse viruses, regardless of viral genome type or location of replication; (2) the DDR both enhances and inhibits viral replication; (3) specific proteins as well as DDR signaling pathways play important roles in viral replication. In addition, while not explicit to this section, we will begin to highlight how the DDR and innate immunity are directly linked, which is further explored in the second part of this review.

Induction and recognition of host DNA damage

Viruses induce host DNA damage

Many examples exist that demonstrate induction of DNA damage during viral replication. Simian Virus 40 (SV40), a dsDNA virus, induces DNA damage via the large T antigen,[7] and Human adenovirus type 12 (Ad12) induces chromosomal aberrations in human embryonic kidney cells (HEK).[8] Influenza A (IAV) subtype H3N2, a segmented (−) RNA virus that replicates in the cytoplasm and the nucleus, causes DNA damage in leukocytes early during infection.[9] Human T-lymphotropic virus type 1 (HTLV-1), a retrovirus, induces DSBs during DNA replication through Tax.[10] While retroviruses may induce DNA damage through the process of integration,[11,12] it is becoming more apparent that retroviruses also induce DNA damage independently of integration, which we will describe throughout this review. However, what remains unclear for many of these viruses is how DNA damage occurs, whether viral-induced DNA damage is sensed and signaled by canonical cellular DDR pathways, and what role induction of DNA damage plays in viral replication and disease pathogenesis.

One example of a viral protein that induces DNA damage is the lentiviral protein Vpr. Human Immunodeficiency Virus 1 (HIV-1) Vpr induces both SSBs and DSBs, independent of other lentiviral proteins.[13–15] While Vpr localizes to chromatin and is reported to bind DNA,[16–18] it does not display any nuclease activity, suggesting Vpr may induce DNA damage through an indirect mechanism.[14] One possibility is that Vpr induces DNA damage indirectly by binding to chromatin and inhibiting DNA replication,[19] leading to DNA damage following replication fork collapse. However, Li et al. showed that Vpr causes DNA damage independently of DNA replication stalling.[15] Another leading hypothesis is that Vpr

induces DNA damage as a consequence of degradation of a DNA repair protein. Vpr recruits the host $Cul4A^{DCAF1}$ ubiquitin ligase complex and interacts with many host DDR proteins – including UNG2,[20,21] HLTF,[22,23] SLX4 complex proteins MUS81 and EME1,[24,25] EXO1,[26] TET2,[27] MCM10,[28] hHR23A,[29] and SAMHD1[30,31] – yet degradation of most of these proteins has not been shown to be required for induction of DNA damage likely because the function of Vpr is complex and induces proteomic changes across the entire cellular landscape.[15,32,33] Thus, like many other viral proteins, it remains unclear exactly how DNA damage is induced by Vpr, and further exploration will better inform us why viruses induce DNA damage.

One of the problems we face in the viral DDR field is that many of the central phenotypes of viral DDR modulation have not been tested directly or with methods that are easily reproducible. For example, induction of DNA damage has often been identified through detection of γH2AX activation rather than probing for DNA damage directly. Utilizing γH2AX in lieu of detecting DSBs or SSBs is problematic because activation of γH2AX is not necessarily a direct indicator of DSBs or SSBs, and γH2AX could potentially be activated by viruses in the absence of DNA damage. To ameliorate this, we recommend that the virologists move toward directly testing for DNA damage through more specific DNA damage assays (Box 1).

Viruses modulate DDR sensors

Subsequent to induction of DNA damage, viruses also modulate the primary sensors of this damage, including the MRN complex, RPA, and Ku70/80 (Figure 2). The MRN complex, which is a major sensor of DSBs, has been shown to inhibit replication of many diverse viruses.[34–36] Thus, many viruses inhibit MRN. For DNA viruses such as adenoviruses, the MRN complex inhibits viral replication primarily by impairing viral DNA replication. To overcome this inhibition, Ad5 employs multiple E proteins to both relocalize and degrade components of the MRN complex.[35,37,38] Interestingly, not all Ad serotypes can overcome the MRN complex,[39–41] indicating differences in the evolution of MRN antagonism. Another dsDNA virus, the herpesvirus Kaposi's Sarcoma-associated Herpesvirus (KSHV), antagonizes MRN through the viral LANA protein to block innate immune inhibition of viral replication and to support lytic reactivation.[42] Similar to adenoviral E proteins, LANA facilitates the relocation of the MRN complex to the cytoplasm. Additionally, RNA viruses such as rotavirus antagonize MRN by relocalizing the complex to the cytoplasm via viral proteins NSP2 and NSP5,[36] and the retrovirus HTLV-1 p30 directly binds to MRN components Rad50 and NBS1 to sequester and inhibit MRN complex formation.[43] MRN antagonism through sequestration and/or relocalization is conserved among diverse viruses, suggesting that evading damage detection by MRN is a strategy beneficial for productive infection.

Viruses also modulate the heterodimeric SSB sensor RPA (composed of RPA70, RPA32, and RPA14). However, unlike MRN antagonism, viruses primarily activate RPA – indicating that RPA enhances viral replication. For example, the Ad5 and Ad12 E1B-55K protein directly interacts with the host E1B-AP5 protein, which binds to the RPA component RPA32 in adenovirus replication centers. This is essential for ATR-dependent phosphorylation of

RPA32, suggesting Ad5 and 12 regulate the ATR pathway through direct modulation of RPA phosphorylation.[44] For HIV-1, Zimmerman *et al.* showed that Vpr is responsible for inducing activated RPA foci in primary human CD4+ lymphocytes.[19] However, Vpr does not colocalize with RPA32 foci, suggesting Vpr may indirectly modulate RPA activity.[15] Thus, while less well defined than MRN antagonism, some viruses have evolved to activate RPA through direct and indirect mechanisms.

Finally, viruses antagonize the DSB sensor Ku70/80 complex, which is required for NHEJ-mediated DNA repair. For example, HTLV-1 transcriptionally silences Ku80 expression, inhibiting DNA-PK and innate immune activation.[45] Antagonism of Ku70/80 is important to the viral lifecycle as Ku70 directly recognizes a HTLV-1 reverse transcription intermediate (ssDNA90) and stimulates type 1 IFN and cytokine production, which together limit HTLV-1 infection before retroviral integration.[46] Therefore, Ku70/80 complex antagonism may be necessary to overcome innate immune sensing and to promote viral replication.

DDR signaling

Downstream of sensing DNA damage, there is a vast signaling cascade consisting of mediator, transducer, and effector proteins (Figure 2). Despite differences in genome type and where they replicate in the cell, DNA, RNA, and retroviruses have evolved several mechanisms to modulate the DDR effectively. Although virus-induced DDR signaling is broadly conserved across viruses, the viral classification does not necessarily correlate with the proteins and pathways modulated. Here we will discuss and exemplify the three primary mechanisms that viruses use to engage DDR signaling: activation, inhibition, and degradation (Figure 2).

Human Papilloma Virus (HPV) strains modulate many aspects of the DDR associated with ATR and ATM, predominantly through the early viral proteins E1, E2, E6, and E7. Most notable is the capacity of E6 and E7 to directly interact with p53 and Rb, two important regulator proteins in DDR signalling,[47–49] respectively.[50,51] By inhibiting p53, E6 directly affects the ATR and ATM pathways, which in turn alters cellular processes such as cell cycle, DNA repair, and transcription. In addition to Rb binding, Moody and Laimins showed that high-risk HPV-37 E7 directly interacts with ATM leading to phosphorylation of Ser1981, causing activation and further downstream phosphorylation of CHK2. Moreover, they observed phosphorylation of CHK1 by E7, which is typically associated with ATR signaling, further suggesting a potential role for ATR in HPV replication.[52] Many of these signaling pathways are activated directly by viruses through alternative mechanisms and are important for viral replication. However, whether ATM and ATR are both activated by diverse HPV subtypes, whether this activation occurs in conjunction with inhibition of either p53 or Rb, and whether this is dependent on antagonism of MRN remains to be studied.

As previously discussed, adenoviruses impair ATM signaling through sequestration of the MRN complex. However, adenoviruses also directly impair DNA-PK signaling, highlighting the necessity to target multiple arms of the DDR. Because all adenoviruses encode a linear double-stranded genome, they are particularly vulnerable to DNA-PK, which functions by re-ligating DSBs with exposed ends. As a response, adenoviruses disable the DNA-PK

pathway by proteasome mediated degradation of DNA ligase IV via the interaction of E4 and E1b proteins with the host Cul5 ubiquitin ligase complex.[53,54] Consequently, this allows the virus to replicate efficiently without being antagonized by host repair machinery, which acts as an antiviral defense mechanism and highlights the capacity of canonical DDR associated proteins to exhibit antiviral innate immune functions (Figure 3).

The viral lifecycle of many RNA viruses is primarily cytosolic. Despite this, RNA viruses still take advantage of the DDR machinery that largely reside and function in the nucleus. Rotavirus, a double-stranded RNA virus, is an interesting example of an RNA virus that modulates the DDR. The viral proteins NSP2 and NSP5 noncanonically activate ATM signaling independent of DNA damage and γH2AX activation and further relocalize ATM, CHK2, and the MRN complex from the nucleus to the cytoplasm.[36] Strikingly, ATM and CHK2 only interact with NSP5 in the presence of replicating viral genomes and inhibition of the ATM pathway reduces viral replication, suggesting that activation of these signaling pathways is important for viral genome replication.[55] Orthomyxoviruses are also of particular interest as they consist of a segmented (−) RNA genome that, unlike many RNA viruses, is shuttled to the nucleus for genome replication and transcription. As such, these viruses also encode nuclear viral proteins to modulate the host DDR effectively. Specifically, the IAV viral protein NS1 suppresses RhoA and pRb signaling, directly activating the ATM signaling cascade.[56] In addition to these pathways, IAV infection modulates the protein abundance of various fundamental DDR proteins, such as Ku70, Ku80, Rad51, γH2AX, and PCNA, all of which are critical for ATM, ATR, and DNA-PK signaling.[57] Altogether, this exemplifies the evolutionary importance of modulating nuclear DDR factors for all viruses and could suggest that engagement of the DDR drove nuclear replication of some RNA viruses such as orthomyxoviruses and retroviruses. Despite cellular localization of viral replication, viruses require host DDR factors that they do not encode to replicate and thus evolving to activate the DDR through both canonical and noncanonical mechanisms is crucial for viral replication.

Cellular consequences of DDR

Depending on the type and severity of the genomic lesion, activation of the DDR results in a myriad of cellular consequences, including but not limited to DNA repair, cell cycle arrest, chromatin dynamics, and transcriptional changes (Figure 2). In this section, we will highlight how diverse viruses utilize common mechanisms to alter the cellular consequences of the DDR. We will specifically focus on how DNA, RNA, and retroviruses dysregulate DNA repair, promote cell cycle arrest, confer changes in chromatin organization, and induce transcriptional changes.

Repair

One of the major consequences of modulating the DDR is the disruption of the five primary repair pathways: BER, NER, and MMR, which repair single-strand lesions, and HR and NHEJ, which repair DSBs. Disruption of DNA repair has been observed for many of the viruses that we have discussed thus far. Despite how much is known about viral modulation of DDR signaling, repair as a cellular consequence remains poorly understood. HR is one

of the major repair pathways a cell utilizes extensively during late S and G2 phases of the cell cycle and can be activated in response to DSBs, primarily via ATM signaling. Fittingly, many viruses that regulate ATM signaling also regulate HR. For example, HPV represses HR efficiency by 50–60% through the recruitment of a variety of DNA repair host factors such as Rad51, RPA70, BRCA1, and BRCA2[58] away from chromatin and relocalization to the viral genome.[59] Consequently, these cells are more sensitive to exogenous genotoxic stress.[60] The role of DNA repair pathways in HIV infection is not well understood. In one system, HT1080 cells were transfected with pBHRF, a plasmid vector used to measure HR, in the presence or absence of transfected Vpr to assess HR repair of truncated GFP. In the presence of Vpr, GFP expression increased, suggesting Vpr enhances HR.[61] Using the DR-GFP assay to assess repair efficiency (described in Box 1), HR and NHEJ have been shown to be repressed in U2OS cells expressing HIV-1 or HIV-2 Vpr.[15] Since discrepancies remain for the effects of Vpr on HR, it is crucial that the field uses similar systems and assays, such as the DR-GFP assay, to create reproducible and comparable data as discussed further in Box 1. This will also help to determine whether functions such as repression or activation of HR are directly beneficial to viral replication or a consequence of redistributing host DDR factors.

In addition to HR, other repair mechanisms can also play an important role in viral replication. As previously discussed, adenoviruses broadly inhibit the DNA-PK pathway by disrupting DNA ligase IV activity via proteasomal degradation, leading to the downregulation of NHEJ, which affects processes such as V(D)J recombination.[54] Though the role of DNA repair in RNA viruses remains understudied, it has been proposed that DNA repair is exploited during RNA virus infections. For example, IAV modulates and exploits MMR to promote cell survival during infection. An MMR activity assay, which utilizes a mismatch start codon on a luciferase expression plasmid, revealed that maintaining MMR activity is important for the IAV viral life cycle.[62] Strikingly, unlike HR or NHEJ, MMR activity leads to decreased transcription of antiviral innate immune factors, suggesting that affecting this particular DNA repair pathway has the additional cellular effect of dampening the innate immune response that would otherwise inhibit viral replication.

Cell cycle

Many viruses utilize the DDR to inhibit or activate cell cycle progression to facilitate an environment conducive to viral replication. Our current understanding is that certain cell cycle phases, such as S-phase, can promote viral replication, whereas the roles of others, such as G1 or G2/M, are still less clear. Here we will highlight different strategies viruses have evolved to both inhibit and promote cell cycle progression to benefit viral replication.

dsDNA viruses are the textbook example of cell cycle control by a virus, as they require a cell to be in S-phase in order to replicate their genomes.[63] This is because dsDNA viruses utilize much of the same machinery as the host to replicate DNA, including cellular DNA replication proteins and dNTPs. To achieve this, almost all dsDNA viruses encode early proteins that directly inhibit the master cell cycle regulators Rb and p53[47–49] through degradation, relocalization, and/or sequestration.[64,65] Some examples include HPV E6 and E7 proteins, adenovirus E1A and E1B proteins, and SV40 large T antigen, and

has been extensively reviewed elsewhere.[3,66–68] By studying how dsDNA viruses regulate S-phase, we have not only learned about mechanisms of viral replication but have also uncovered many molecular mechanisms underlying cell cycle regulation and the cellular consequences of dysregulation, such as cancer. Thus, viruses have been an instrumental tool in understanding viral and host biology.

Unlike the aforementioned DNA viruses that primarily push cells into a single cell cycle stage, coronaviruses represent a single family of RNA viruses that have all evolved to differentially regulate the cell cycle and display a range of cell cycle phenotypes. This is accomplished through an assortment of viral proteins, such as CoV-N, nsp13, p28, and ORF3/M, which converge on inhibiting cyclin-CDK complexes or upstream signaling cascades, such as p53, to induce cell cycle arrest. Consistent with the different viral proteins, the type of arrest induced varies between G0/G1, S, and G2/M.[69–72] Despite these differences, induction of cell cycle arrest is conserved among coronaviruses and allows viruses to exploit host resources, such as translation and replication factors, that are essential for viral replication but are not encoded by the viral genome.

Many retroviruses also alter cell cycle; though the effects on cell cycle progression and viral replication seem to be distinct. For example, HTLV-1 infection allows cells to bypass the G1/S checkpoint despite DNA damage.[73] This is regulated by the interaction of HTLV-1 Tax with the cellular phosphatase Wip1, which dephosphorylates γH2AX and RPA to bypass the DDR-initiated G1/S checkpoint.[73] Interestingly, Tax has more than one function and also mediates G2 accumulation through the direct binding and activation of CHK2 independent of ATM.[74,75] Primate lentiviruses induce arrest, with at least three HIV-1 proteins implicated in a G1 (Tat) or G2/M (Vif and Vpr) arrest.[76–79] The primary role of cell cycle arrest in HIV-1 replication is unclear, but G2/M arrest has been proposed to promote viral expression[80] and/or prevent nuclear breakdown to exploit nuclear factors in cycling T cells. Lentiviruses also have the ability to infect non-dividing cells, such as macrophages and dendritic cells. While at least one study has shown that prevention of cell cycle progression into mitosis in monocyte-derived dendritic cells is important for LTR-mediated viral transcription,[81] it will be important for the field to directly address the role of activating cell cycle-associated pathways in noncycling cells.

Chromatin dynamics

Chromatin bound to damaged DNA must reorganize to allow DDR proteins to access damaged DNA and facilitate repair. Histone proteins bound to damaged DNA undergo post-translational modifications (PTMs), such as methylation, acetylation, phosphorylation, and ubiquitylation. This alters chromatin structure, DNA repair, and the local transcriptional environment. Thus, many viruses directly target histone modifying proteins to influence the availability and abundance of nuclear factors and ultimately enhance viral replication. Some of the more widely conserved viral targets, which we will specifically discuss here, are the ubiquitin ligase proteins RNF8 and RNF168 and the acetyltransferase Tip60.

RNF8 and RNF168 are ubiquitin-protein ligases that play key roles in DNA damage signaling by catalyzing and amplifying ubiquitylation of histones H2A and H2AX to promote the recruitment of DNA repair proteins at DSBs.[82] Viruses inhibit RNF8 and

RNF168 through several diverse mechanisms, including degradation or relocation of RNF8 and limiting the recruitment of DDR proteins to sites of damage. For example, HSV-1 ICP0 degrades RNF8 and RNF168, causing the loss of H2A ubiquitylation and DNA repair factor recruitment to DNA damage sites; thus, inhibiting DDR signaling.[83] Similarly, HPV E7 directly binds to and inhibits RNF8, which again limits the recruitment of 53PB1 to radiation-induced damage sites and increases repair by HR.[84] The EBV immediate-early protein BZLF1/ZEBRA similarly antagonizes RNF8 by relocating RNF8 and 53BP1 away from sites of DNA damage and consequently inhibiting DNA damage repair.[85] HTLV-1 Tax relocalizing RNF8 from the nucleus to the cytoplasm stimulates the DDR and induces assembly of K63-pUb chains that also activate NF-κB.[86] Together, this exemplifies the central role ubiquitylation plays in viral modulation of the host DDR to alter the availability of DDR factors.

Viruses also alter the chromatin environment by modulating the host acetyltransferase Tip60, which is a component of the NuA4 complex that acetylates histones to regulate gene expression and DNA repair.[87] Tip60 also directly regulates DNA repair by acetylation and activation of ATM, independent of NuA4.[88] Tip60 was first identified through its interaction with the HIV-1 transcriptional activator Tat.[89] While the precise role of the Tat-Tip60 interaction in HIV-1 replication remains unclear,[90–92] binding of HTLV-1 $p30^{II}$ to Tip60 promotes acetylation, chromatin remodeling, and transcription of c-Myc target genes,[93] suggesting this could be a conserved and important retroviral-host interaction.

In addition to altering the chromatin environment, Tip60 inhibits gene expression of several dsDNA viruses, including adenoviruses,[94] herpesviruses,[95–97] papillomaviruses,[98–101] and the hepadnavirus HBV,[102] and thus, DNA viruses have evolved diverse mechanisms to antagonize Tip60. For example, adenovirus antagonizes Tip60 through the viral EIB55K and E4orf6. Both EIB55K and E4orf6 bind to Tip60 during infection and target it for proteasome-mediated degradation, causing cellular chromatin inaccessibility and promoting viral early gene transcription.[94] Recently, Tip60 was indicated to be upregulated in response to IAV infection and to activate type I IFN.[103] It remains to be seen whether Tip60 and other histone modifying proteins may have additional roles in response to viral infection.

Transcriptional changes

Another major consequence of DNA damage is modulation of the cellular transcriptome. Specifically, DNA damage limits global transcription by inhibiting RNA polymerase II[104] and promoting activation of specific transcriptional pathways, such as NF-κB. Activation of NF-κB by DNA damage is dependent on the ATM-NEMO pathway and upregulates NF-κB-regulated genes important for facilitating cell survival by inhibiting apoptosis and mediating DNA repair[105–107] (reviewed in 108).

Many viruses induce DNA damage causing transcriptional changes that benefit viral replication and are often linked to NF-κB.[42,109–112] For example, HPV regulates transcription initiation by recruiting NF-κB through the viral helicase E1. Activation of NF-κB leads to the destabilization of E1, establishing a negative feedback loop to regulate E1-dependent genome amplification and NF-κB transcriptional changes.[113] Similarly, during HTLV-1 and HIV-1 co-infection, HTLV-1 Tax can regulate transcription initiation by

facilitating the recruitment of NF-κB to the unintegrated HIV-1 LTR. Mechanistically, HTLV-1 Tax promotes the recruitment of the NF-κB subunits RelA and RelB to the HIV-1 LTR, which induces viral gene expression and facilitates viral replication.[114] HTLV-1 can also activate NF-κB through Tax-independent mechanisms, which promote survival and proliferation of HTLV-1 infected cells by upregulating genes responsible for proliferation and clonal expansion.[115] Interestingly, transcriptional changes induced by DNA damage enhance viral gene expression and promote viral replication. For example, DNA damage via ultraviolet light or mitomycin C enhances transcription of the HIV-1 LTR,[116] further suggesting that DNA damage can also alter viral transcription (see Box 2 for more information on the DDR and reactivation of latent viral gene expression).

II Crosstalk between the DDR and innate immunity

Both the DDR and the innate immune system are signaling cascades that function to sense, signal, and respond to aberrant nucleic acids. Once considered to be independent cellular functions, evidence now suggests that they may not be mutually exclusive processes, and there is more crosstalk than previously considered (Figure 3). For example, recent research indicates that the major innate immune DNA sensor cGAS can also sense perturbations and disruptions of genome maintenance and DNA repair and potentially even inhibit HR (Figure 3).[117–121] In this section, we examine the interplay between innate immunity and the DDR and the “role reversal” of a few proteins canonically believed to either play roles in innate immunity or the DDR. We will highlight recent data supporting the crosstalk between the DDR and innate immunity to make explicit the connections between the two systems. Since we will not be discussing the innate immune response to viral infection, please see the suggested reviews for more information.[122,123]

SAMHD1

SAMHD1 sits at the intersection of innate immunity and the DDR. SAMHD1 is a dNTP hydrolase that depletes intracellular dNTP pools and is an important antiviral restriction factor against primate lentiviruses such as HIV-1 (Figure 3).[124,125] SAMHD1 also shows evolutionary signatures of long-standing genetic conflict, called positive selection, consistent with an important conserved role in antagonizing viral replication (see Box 3 for more information on the rapid evolution of DDR genes).[30,31,126]

SAMHD1 plays an important role in the DDR to maintain genome integrity. Patients with either Aicardi-Goutières syndrome, a severe autoimmune disorder, or chronic lymphoid leukemia that also have SAMHD1 loss-of-function mutations have increased levels of dNTPs and DNA damage, suggesting that SAMHD1 engages the DDR. This is consistent with previous research demonstrating dysregulated dNTP levels can activate the DDR through replication stress.[127–129] Overexpressed HA-tagged SAMHD1 has been shown to colocalize with 53BP1 even at steady state; though the number and size of the foci increase after treatment with the DNA damaging agent camptothecin.[128] SAMHD1 can mediate HR repair through recruiting and interacting with CtIP at sites of DNA damage and promoting DNA end resection.[130] Coquel et al. showed SAMHD1 physically interacting with MRE11 at stalled replication forks and stimulating the exonuclease activity of MRE11 to degrade

nascent DNA. ATR–CHK1 is activated, restarting the replication forks. Without SAMHD1 acting at stalled replication forks, ssDNA fragments are released into the cytosol, where they activate the cGAS-STING pathway.[131] Taken together, SAMHD1 is recruited to sites of DNA damage, and loss of SAMHD1 leads to replication fork stalling, demonstrating SAMHD1 has a direct role in genome maintenance.

SAMHD1 is activated in response to specific DNA damage and inhibits HIV-1 infectivity. Induction of DSBs through the use of neocarzinostatin, etoposide, or camptothecin activates SAMHD1 in monocyte-derived macrophages and blocks HIV-1 infection.[132,133] However, stimulating the DDR with ultraviolet light did not activate SAMHD1, suggesting it is DSBs that are important for SAMHD1 activation.[133] Interestingly, treatment with camptothecin greatly reduced infectivity, and cells had better survival in comparison to etoposide-treated cells. This suggests DNA damaging agents block HIV-1 infectivity with different efficiencies, perhaps due to the level of DNA damage induced. The authors proposed that DNA damage activates SAMHD1 causing a post-RT block and inhibition of HIV-1 infectivity.[132] Neocarzinostatin, etoposide, camptothecin and ultraviolet light all stimulate γH2AX, suggesting activation of γH2AX is not sufficient to activate SAMHD1.[133]

ATM

Ataxia–Telangiectasia (AT) syndrome patients display worsening movement coordination, a weakened immune system, and an increased risk of cancer. ATM is also a key protein in the canonical NF-κB response to genotoxic stress (Figure 3),[134–136] and recent work has expanded upon the role ATM plays in innate immunity. In irradiated mouse macrophages, ATM stimulates an IFN response in a STING-independent, IRF1-dependent manner, in addition to activating NF-κB.[137] ATM has also been shown to play a role in an IFI16-STING-IRF3 signaling cascade in response to etoposide treatment. In that model, ATM phosphorylates p53 which then associates with IFI16 and STING in the cytoplasm.[138]

The expanding role of ATM in innate immunity during microbial infections is under active investigation. Currently, the evidence is murky, with most of the work focusing on antibacterial responses. Though this does not specifically inform the role of ATM during antiviral immune responses, by understanding the role of ATM during the antibacterial immune response, we can learn how ATM generally interacts with and within innate immunity. Conflicting publications have shown that the loss of ATM can either hinder or enhance antimicrobial responses. Härtlova et al. saw enhanced antiviral and antibacterial responses in cells from patients with AT and ATM knockout mice. Unrepaired DNA lesions in ATM deficient cells activates STING and the production of type I IFNs.[139] However, in their follow-up paper, ATM knockout mice were more susceptible to pulmonary bacterial infections. They concluded the loss of ATM leads to ROS production, impairing some innate immune sensors.[140] In another publication, murine macrophages deficient in both ATM and DNA-PK had reduced cytokine production when infected with Listeria monocytogenes. However, they only included data for macrophages deficient in both ATM and DNA-PK or only DNA-PK, so the individual role of ATM in this system is unclear.[141] Conversely, Härtlova et al. infected ATM knockout mice with *L. monocytogenes* and still saw an increase in type I IFNs.[139] The differences between all of these publications could be due to

using assays specific for certain innate immune pathways versus others, or the experimental system such as *in vitro* macrophages vs. *in vivo* mouse models. Not only does more work need to be done to clarify what role ATM might play in an innate immune response, but also specifically the importance of ATM in the response to viral pathogens.

MRE11, MUS81, RAD51, and RPA

Within the DDR there are sensors, mediators, transducers, and effectors that play a role in activating or repressing innate immunity. MRE11 has been shown to localize to the cytoplasm to detect dsDNA and activate STING dependent type 1 IFN expression. HEK293 cells treated with IFN stimulatory DNA or ultraviolet light accumulate activated ATM, suggesting the DDR is activated in response to extracellular DNA and DNA damage. Activation of type I IFN genes were detected in response to treatment with the extracellular DNA and decreased in a MRE11 knock-down. The MRE11 binding partners RAD50 and NSB1 are required to sense DSBs, yet neither were required to respond to HSV-1 or the bacterial pathogen *L. monocytogenes*. Thus, the working model suggests that MRE11 alone may initiate a type I IFN response downstream of cell-intrinsic damage.[142]

MUS81 is a DNA repair endonuclease that forms a complex with EME1, SLX4 and SLX1 and is important for DNA replication through its regulation of replication fork progression.[143,144] HIV-1 downregulates Mus81 through recruitment of the $Cul4A^{DCAF1}$ ubiquitin ligase complex,[24,25] suggesting a potential role for this host DDR protein in innate immunity. Additionally, it has been shown that MUS81 activity leads to the accumulation of cytosolic dsDNA fragments in prostate cancer cells. These fragments are recognized by the STING pathway leading to type I IFN production, but they did not investigate which specific dsDNA sensor is required. Some data indicate that the immune response stimulated by MUS81-STING is required for rejection of the tumor cells when injected into mice. This is very preliminary and needs to be followed-up on more thoroughly.[145] However, the role STING plays in both the development of but also the potential treatment of cancer is a growing area of research.[146]

While some DDR factors sense and activate an antiviral response, other DDR factors can inhibit aberrant activation independent of viral infection. RAD51 and RPA are involved in DNA repair following damage. Two publications have shown them to be important for protecting against leakage of ssDNA into the cytosol, which could stimulate an innate immune response in the absence of viral infection. Depleting just one is sufficient to cause increased levels of ssDNA fragments in the cytosol and IFNb production via the cGAS-STING pathway. Thus, implying that RAD51 and RPA protect a cell from initiating an inflammatory response to self-DNA.[147,148]

DNA-PK

As previously discussed, DNA-PK is a heterotrimeric protein complex that is an essential mediator in the cellular response to DNA damage and is targeted by viruses. Using pulldowns in HEK293 and HEK293T cells, two groups identified that DNA-PK can bind DNA in the cytoplasm, and depending upon the cell type used, initiate type I or III IFN immune responses. Zhang et al. identified Ku70 specifically as the DNA sensor and showed

IRF1-IRF7 activation and expression of type III IFNs.[149] Ferguson et al. determined the type I IFN response was independent of DNA-PK kinase activity, agreeing with the previously mentioned publication indicating Ku70 is the specific sensor. The authors were able to show DNA-PK contributing to the type I IFN immune response both in vitro and in vivo with a DNA-PK knockout mouse. They hypothesized DNA-PK signals through the STING-TBK1 pathway and showed activation of TBK1. Neither group adequately addressed the contribution of STING.[150] However, recent work expanded upon the mechanism of Ku70 recognizing DNA in the cytosol and the requirement of STING-IRF1-IRF7 to stimulate a type III IFN response.[151,152]

These publications either showed or assumed a requirement of STING. Burleigh et al., however, revealed DNA-PK induction of type I IFNs that is both STING- and TBK1-independent. They discovered this pathway after still seeing IRF3 phosphorylation in STING or TBK1 knockout cells treated with calf thymus DNA. Contrary to the other publications, their results suggest that all three subunits of DNA-PK are required (Figure 3).[153] It has been previously shown that DNA-PK can directly phosphorylate IRF3, but at different amino acid sites than those in Burleigh et al.[154] More recently, another group found the Ku complex recognizes accumulated cytoplasmic DNA in aged human and mouse CD4+ T cells in a cGAS-STING independent manner, leading to T cell activation and an autoimmune pathology in aged mice. However, the data indicates a different mechanism than Burleigh et al. DNA-PK sensing of DNA leads to the phosphorylation of the ZAK kinase, which then activates AKT and the mTOR pathways.[155]

Taken altogether, the DNA-PK complex appears to be able to act as a cytosolic innate immune DNA sensor. Though, it is likely to be secondary to the cGAS-STING DNA sensing pathway. However, there are many discrepancies and contradictions in the published literature, such as the role of STING downstream of DNA-PK and which IFNs are produced. These differences could be due to species specificity, different cell types, or even different assays. These issues, though, are not unique to DNA-PK. Most of the other DDR factors discussed in this section face these same issues. Therefore, more work must be done to understand whether these differences are due to experimental set-up, choice of assays, data interpretation, or something more biologically important. Delving deeper will also help us understand how DDR factors switch from acting in the DDR to acting in innate immunity, and vice versa.

Perspective

Engagement and modulation of the DDR are central to the life cycles of a range of diverse viruses and are a phenotype that is broadly conserved beyond the viruses which we have discussed in this review. Despite the diversity in viral genomes, mechanisms of replication, and subcellular localization, the commonality of DDR engagement collectively highlights the importance of engaging ATM, ATR, and DNA-PK signaling pathways as well as the individual proteins in these pathways to promote the viral lifecycle. Converse to the benefits of utilizing the DDR, many of these factors themselves have antiviral activity as well as connections to innate immunity. As such, it is evolutionarily imperative for viruses to overcome this, and further demonstrates the convergence of these two important cellular

functions which reflect two sides of the same coin that functions to sense, signal, and respond to aberrant nucleic acid – whether this be self or non-self.

Many questions remain as we are only just beginning to scratch the surface of the role the DDR plays in viral replication and innate immunity. For example, it is still unclear for many viruses whether steps such as induction of DNA damage or cell cycle arrest are active processes required for viral replication or consequences of other steps in viral replication. One way we propose to tackle this is to look for evolutionary conservation within viral genera. While conserved "byproducts" may exist, like spandrels in cathedrals,[156] conservation of function is a strong indicator of significance in viral replication. It will also be important to directly address the causal relationships between the many ways a specific virus engages the DDR. Many steps in DDR signaling are intertwined, and most viruses we discussed activate and/or repress multiple aspects of the DDR. Thus, how one phenotype may influence another, such as how viruses induce transcriptional changes as a cellular consequence of DNA damage or whether there is a correlation between repression of DNA repair and changes in chromatin dynamics, will be essential to identify important DDR-associated drivers of viral replication. Moreover, determining what viral proteins overcome DDR proteins acting as innate immune proteins, and what additional DDR factors have important roles in innate immunity, is paramount to uncovering the connection between these two interconnected signaling pathways. One area of innate immunity where the DDR may be especially poised is in nuclear surveillance. It is clear from the literature that the cell contains a vast array of cytosolic innate immune sensors; however, nuclear innate immune sensors, such as cGAS, are only now just beginning to emerge. The DDR is perfectly situated to function in this role, and by systematically assessing potential roles of DDR proteins in both the nuclear and cytoplasmic innate immune response, we can further expand on these emerging fields. At the technical level, one aspect that must be addressed to help answer many of these questions is the use of consistent and reproducible assays that have been pioneered by the DDR field but are often overlooked by virologists.

We should consider how we can leverage the interconnection with the DDR to establish new therapeutics to treat viral infection. As the DDR is a major therapeutic target for cancer therapy, many drugs already exist that could be screened for antiviral roles, such as those found to inhibit SARS-CoV-2 replication.[157] Viral dysregulation of the DDR could also be used to selectively deplete infected cells. For example, a direct outcome of DNA damage is that infected cells are hypersensitive to additional DNA damage. In both HTLV-1 and HIV infected cells, induction of DNA damage or repression of DNA repair makes infected cells hypersensitive to additional low levels of exogenous DSBs.[10,15] Moreover, patients with HPV+ head and neck tumors have increased sensitivity and long-term survival when treated with chemotherapy that induces DNA damage.[158] This concept of "synthetic lethality" has been proposed to treat many types of cancer that are deficient in DNA repair.[159] For example, BRCA1/2-deficient tumors are highly susceptible to inhibition of the DNA repair protein PARP1 due to the inability to repair double-strand breaks by HR or NHEJ.[160,161] Given the ability of diverse viruses to induce DNA damage and/or antagonize DDR signaling and repair, we propose that a synthetic lethality approach may be feasible to selectively deplete infected cells. Based on the breadth of drugs available to induce low levels of genotoxic stress (including orphaned drugs and others that never made it to clinical

trials), it will be important to thoroughly assess the efficacy of a synthetic lethality approach to killing infected cells *in vitro* and *in vivo*.

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Abbreviations:

Ad
adenovirus

ATR
ataxia telangiectasia and Rad3related protein

ATM
ataxia-telangiectasia mutated

cGAS
cyclic GMP–AMP synthase

DDR
DNA damage response

DNA-PK
Ku70, Ku80, and DNA-PKcs

DSB
double-stranded DNA break

dsDNA
double-stranded DNA

E
early

EBV
Epstein-Barr virus

HSV
herpes simplex virus

HR
homologous recombination

HEK

human embryonic kidney

HIV
human immunodeficiency virus

HPV
human papillomavirus

HTLV
human T-lymphotropic virus

IBV
infectious bronchitis virus

IAV
influenza A

IFI16
nterferon gamma inducible protein 16

IRF
interferon regulatory factor

IFN
interferon

KSHV
Kaposi’s sarcoma-associated herpesvirus

LANA
latency-associated nuclear antigen

MMR
mismatch repair

MRE11 meiotic recombination 11
MRN, MRE11, Rad50, NBS1

NEHJ
nonhomologous end joining

NF-κB
nuclear factor kappa B

PARP1
poly(ADP) ribose polymerase

PS
positive selection

PTM
post-translational modification

PFGE
pulsed-field gel electrophoresis

RPA
replication protein A

SAMHD1
SAM domain and HD domain-containing protein 1

SV40
simian virus 40

SSB
single-strand DNA break

ssDNA
single-stranded DNA

STING
stimulator of interferon genes

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Box 1

Methods

Using assays that directly test for DNA damage or repair and are easily reproduced is a crucial step towards synthesizing the mechanisms by which viruses modulate the DDR. For example, γH2AX is often used as a proxy of DNA damage. This is problematic, since γH2AX activation can occur in the absence of DSBs or SSBs. Furthermore, γH2AX activation fails to distinguish between induction of DSBs or SSBs, leaving the type of DNA damage induced by the virus unknown. We recommend using assays that directly test for DNA damage, for example, the comet assay and pulsed-field gel electrophoresis (PFGE) that directly detect DSBs and SSBs. The comet assay quantifies DNA damage at single-cell resolution. Cells are embedded in an agarose-coated glass slide, permeabilized, and gel electrophoresed. Fragments of DNA that contain SSBs or DSBs are separated from the core of the cell and create a "comet tail." The amount of DNA damage per cell is quantified by measuring the percent of DNA in the comet tail.[162] Similarly, the PFGE assay is an electrophoresis-based DNA fractionation method to detect DSBs. Cells are embedded in an agarose gel, lysed, and undergo PFGE, leading small DNA fragments to migrate in the agarose gel. The total quantity of damaged DNA is determined along with the molecular sizes of the fragmented DNA.[163] To directly test for DNA repair, we recommend using I-Sce1-based reporter assays, such as the DR-GFP assay for HR that are amendable to various cell types. Two incomplete GFP cassettes are stably integrated into the genome of cell lines such as U2OS. The first GFP gene contains an I-SceI endonuclease cut site, and the second contains a truncated *GFP* sequence. Transient expression of I-SceI in HR- or NEHJ-proficient cells induces a DSB in the first *GFP* gene that once repaired results in GFP+ cells, which are then detected and quantified using flow cytometry. Additional GFP reporter systems have been developed to assay single strand annealing and alternative end joining DNA repair.[164–167] While CRISPR-based assays are starting to make headway due to their flexibility in different cellular systems,[168–170] assays such as the DR-GFP are conventional assays in the field.

Box 2

Latency and the DDR

Viral latency is a reversible state characterized by a long-term infection where no new viral particle is formed. As latent viruses are often dormant and sometimes silent, these reservoirs make it difficult to clear an infection, allowing for latency reversal and viral persistence. The two viral families most often associated with latency are herpesviruses and retroviruses. As with active infections, latent viral infections can also cause DNA damage. One well studied example is EBV, which still expresses low levels of some viral proteins during latency, including nuclear antigen (EBNA) and membrane protein (LMP).[171] Combined expression of these proteins can lead to an increase of reactive oxygen species that can damage DNA.[172,173] Latently expressed EBNA and LMP also impair the DNA damage response through modulating the autophagic response, leading to accumulation of DNA damage without cell death[173] and contributing to the oncogenic potential of EBV. Cells infected with latent HIV are more sensitive to DNA damaging agents,[174,175] which may provide a novel approach to selectively purge latently infected cells. Another key mechanism linking latency and DNA damage is the potential for latency reversal through DNA damage. For example, ultraviolet irradiation enhances reactivation of many viruses, including HIV-1 in cell culture and animal models,[116,176] most probably by exposing transcriptionally repressed regions to host transcription machinery through changes in epigenetic status of the latent viral genome.[177,178] This suggests that modulating DNA damage can regulate viral replication in latent cells. By concentrating more on the ways DNA damage and latency are related, we could further understand both the effects of a latent infection and how to clear a latent reservoir.

Box 3

DDR genes under positive selection

DNA damage signaling and repair pathways critically maintain genome integrity and exhibit high evolutionary conservation across eukaryotes. Despite this, the sequences of many genes that comprise these pathways are marked by signatures of rapid evolution, called positive selection (PS). PS is often the consequence of long-standing evolutionary conflict and can be indicative of an important role in viral replication.[179] These signatures can be found in genes encoding proteins involved in specific DDR pathways, including HR and NHEJ, which have been shaped by recurrent PS.[180] Crystal structures of primate NHEJ factors XRCC4, Nbs1 and Polk reveal PS sites located exclusively on exposed protein surfaces, supporting the idea that binding of viral proteins is driving the rapid evolution of residues at virus-host interfaces. Yet, due to the critical nature of NHEJ factors in cell survival, the functions of these genes are evolutionarily constrained, and sites of PS don't appear to alter their primary roles in DNA repair. In some cases, however, recurrent PS may lead to altered functions of host genes, even in genes in which mutations often lead to cancer. For instance, BRCA1 and BRCA2 show signatures of PS,[181] though whether they have been driven by viruses has yet to be determined. Because BRCA1/2 are responsible for most hereditary forms of breast and ovarian cancer,[182] one would expect high conservation in the sequences of these essential genes. Thus far, these signatures have resulted in nonsynonymous point mutations in BRCA1/2 rather than the premature stop codons and frameshifts immediately associated with cancers. Yet, it is possible that the subtle effects of these mutations on BRCA1/2 function and cancer-risk may take years to be realized. Eventually, this recurrent PS may result in antagonistic pleiotropy, where positively-selected-for BRCA1/2 residues would favor viral resistance at the expense of an elevated risk of cancer.[181]

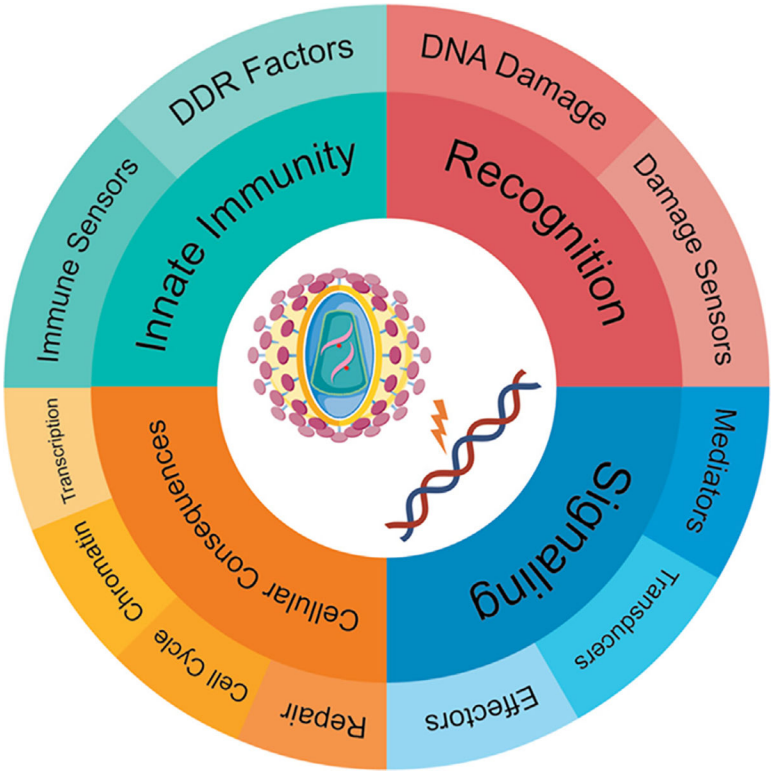

Figure 1. Four major features of interplay between viruses and the DDR.

Viral engagement of the DDR embodies four major characteristics: Recognition, Signaling, Cellular Consequences, and Innate Immunity. Each of these four pillars can be further broken down into specific components that make up the larger characteristics that viruses have evolved to modulate either through a precise mechanism or as a consequence of infection. (1) Recognition initiates the DDR and is activated by DNA damage. Sensors recognize this DNA damage to activate ATM, ATR, or DNA-PK signaling. Viruses have evolved to modulate this step by inducing DNA damage and antagonizing damage sensors. (2) Signaling then occurs through a variety of mediator, transducer, and effector proteins. Viruses modulate downstream signaling by activating or inhibiting mediators, transducers, and effectors in the DDR. (3) Cellular consequences occur in response to recognition and signaling in the form of DNA repair, cell cycle checkpoints, chromatin remodeling, and transcriptional changes. Viruses can modify repair pathways, elicit specific cell cycle arrest, alter chromatin organization, and induce transcriptional changes (4) Innate immunity is directly tied to these processes as DDR factors can elicit antiviral activity. Together, these four pillars represent the interplay between viruses and the DDR and exemplify how the DDR and innate immunity are directly interconnected and central to viral replication.

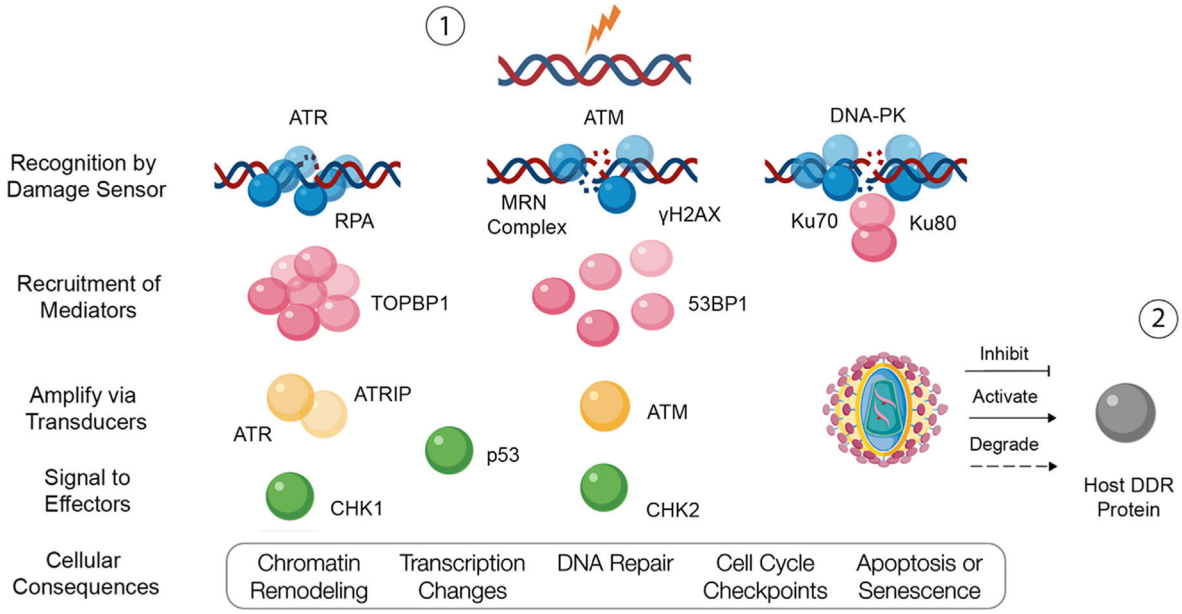

Figure 2. The DDR senses, signals, and responds to aberrant DNA through three primary pathways, ATM, ATR, and DNA-PK, that are modulated by viruses.

(1) The DDR is a protein signaling cascade that maintains genome integrity. The DDR consists of sensors, which recognize specific DNA lesions, mediators and transducers that transmit this signal of damaged DNA, and effectors, which directly execute a cellular response. While many of these pathways are interconnected, in general, the ATR pathway is activated in response to SSBs, and ATM and DNA-PK pathways are activated in response to DSBs. DDR signaling induces cellular consequences, including DNA repair, cell cycle arrest, chromatin dynamics, and transcriptional changes. Shown are representative DDR proteins, pathways, and cellular consequences that are highlighted in this review with the exception of apoptosis and senescence. (2) Viruses have developed several mechanisms to activate, inhibit, or degrade various parts of these core signaling pathways. Modulation of these pathways ultimately leads to several cellular consequences which are beneficial to the viral lifecycle.

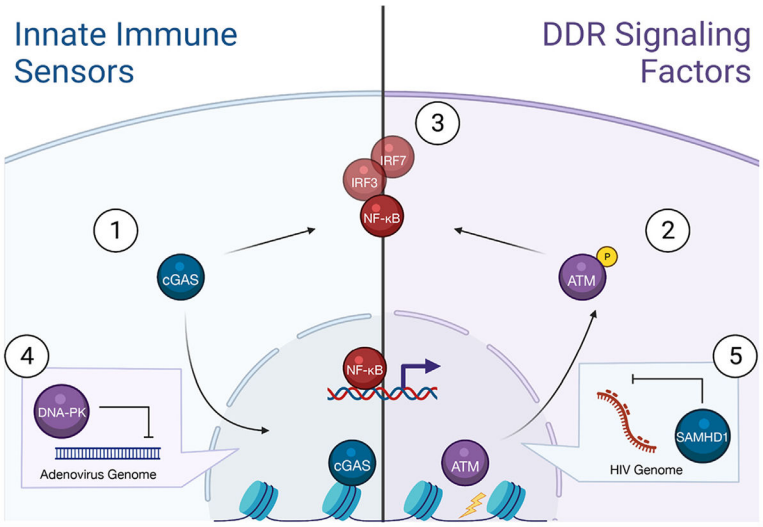

Figure 3. Innate Immune sensors and DDR signaling factors are two sides of the same coin.

Innate immune sensors and DDR proteins and pathways have been canonically attributed to either innate immunity or DDR signaling, but there are multiple examples of how proteins being multifunctional and involved in both processes. (1) cGAS can function in the nucleus and interacts directly with chromatin, and (2) ATM is not limited to the nucleus and can relocalize to the cytosol. (3) Both cGAS and ATM can activate NF-κB signaling. (4) DDR factors involved in NHEJ repair, such as DNA-PK, can have direct antiviral activity and inhibit adenovirus, (5) and innate immune factors such as SAMHD1, which inhibit HIV, are also involved in nuclear DDR signaling.

Table 1

A summary of various viruses and the cellular consequences caused by viral modulation of the DDR.

Genome Type	Virus	Viral Proteins	Damage	Signaling	Repair	Cell Cycle	References
(+) RNA	CoV	CoV-N, nsp13, p125	Stalled Forks	ATR	N/S	G0/G1, G2/M	69–72,183
(−) RNA	IAV	NS1	DSB	ATM, DNA-PK	Modulate MMR	G0/G1	56,57
dsRNA	Rotavirus	NSP2, NSP5	N/S	ATM	N/S	G2	36,55
Retro	HIV	Tat, Vif, Vpr	SSB, DSB	ATR ATM	Repress HR	G1, G2/M	13–15,61,76,77,79
Retro	HTLV	Tax, p30	DSB	ATM	Repress HR, Promote NHEJ	G1, G2	10,73–75
DNA	HPV	E1, E2, E6, E7	DSB, Stalled forks	ATM, ATR	Repress HR	G2	50–52,58
DNA	EBV	LMP-1, EBNA3C, ZEBRA	DSB	ATM	N/S	G2/M	85,95,173,184–189
DNA	Adenovirus	E4, E1a, E1b, EIB55K, E4orf6	N/S	ATM, ATR, DNA-PK	Repress NHEJ	Dysregulation	44,53,54,65,94

Damage, signaling, cell cycle, and DNA repair are four major phenotypes central to characterizing the ability of viruses to modulate the DDR that we recommend the field focus on moving forward. N/S indicates not shown.