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Parvovirus infection-induced DNA damage response

Review
Future Virology

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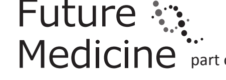
Parvoviruses are a group of small DNA viruses with ssDNA genomes flanked by two inverted terminal structures. Due to a limited genetic resource they require host cellular factors and sometimes a helper virus for efficient viral replication. Recent studies have shown that parvoviruses interact with the DNA damage machinery, which has a significant impact on the life cycle of the virus as well as the fate of infected cells. In addition, due to special DNA structures of the viral genomes, parvoviruses are useful tools for the study of the molecular mechanisms underlying viral infection-induced DNA damage response (DDR). This review aims to summarize recent advances in parvovirus-induced DDR, with a focus on the diverse DDR pathways triggered by different parvoviruses and the consequences of DDR on the viral life cycle as well as the fate of infected cells.

Parvoviruses are among the smallest DNA viruses and are widely spread in humans and many other species [1,2]. Parvovirus is nonenveloped with an icosahedral capsid of 18–26 nm in diameter. It contains a linear ssDNA genome of 5–6 kb, which is flanked by two terminal hairpin structures. The *Parvoviridae* family is composed of two subfamilies: *Parvovirinae*, which infects vertebrates, and *Densovirinae*, which infects only invertebrates. The subfamily Parvovirinae contains five genera: *Amdovirus*, *Bocavirus*, *Dependovirus*, *Erythrovirus* and *Parvovirus* [3]. Adeno-associated viruses (AAVs), in the genus *Dependovirus*, replicate only during coinfection with other helper viruses, such as adenovirus or herpes virus [4]. All other parvoviruses replicate autonomously in their respective host cells and are therefore called autonomous parvoviruses [5,6].

As intracellular parasites, parvoviruses modulate the host cellular environment through the control of the cell cycle as well as the regulation of cell signaling pathways. Due to their simplistic gene expression profiles [3,7], parvoviruses largely rely on host cellular factors for productive infections. In order to propagate their DNA genomes, most parvoviruses arrest host cells at S phase for access to the cellular DNA replication machinery [8–14]; however, observations of G2/M arrest of host cells are also widely reported, especially during the late stages of infection [14–19]. Moreover, parvovirus infections also induce cytopathic effects, which are characterized by their abilities to induce apoptosis, necrosis and autophagy [19–31]. The cell cycle arrest and cell death induced by parvovirus infections have been reviewed previously [32]. Although both *in vitro* and *in vivo* studies have suggested that basic cellular DNA replication factors are required for parvoviral DNA replication, accumulating evidence has suggested that parvovirus infection also induces a DNA damage response (DDR), and inhibition of DDR activation blocks viral DNA replication, suggesting that the cellular DDR plays an important role in parvovirus replication [33–35].

DDR was originally identified as a cellular safeguarding system that protects cellular genome integrity and stability [36,37]. Mammalian cells are constantly challenged by many kinds of stresses including intrinsic sources such as reactive oxygen species (ROS) produced from regular metabolism, and extrinsic sources such as UV, ionizing radiation and chemical treatment. These inducers create several types of damaged DNA structures, including ssDNA breaks (SSBs), dsDNA breaks (DSBs) and stalled replication forks [38,39]. DDR signaling is a complex signal transduction pathway that is transduced by three components: sensors, mediators and effectors. The signaling pathways are generally defined by which mediator is activated. The central mediators in the pathway are three PI3K-like kinases (PI3KKs): ATM, ATR and DNA-PKcs [39,40]. In response to different damaged DNA structures, different sets of DDR sensors are recruited. DSBs can be recognized by the Mre11–Rad50–NBS1 (MRN) complex, which further activates the ATM kinase [41]. The ATM kinase has hundreds of substrates, which are effectors involved in cell cycle checkpoint, DNA repair and apoptosis [42–45]. DSBs can also be bound by the Ku70 and Ku80 complex, which recruits DNA-PKcs, a critical player in

Keywords
▪ adeno-associated virus ▪ bocavirus ▪ DNA damage response ▪ minute virus of canines ▪ minute virus of mice ▪ parvovirus ▪ parvovirus B19 ▪ parvovirus H-1



DNA repair of nonhomologous end joining [46]. The ATR signaling pathway is in response to SSBs, stalled replication forks and DSB resection. In SSBs and stalled replication forks, the ssDNA regions are coated with replication protein A (RPA), which loads Rad17 and the Rad9–Rad1–Hus1 (9–1–1) complex, to recruit TopBP1 [47–50]. TopBP1 further sequesters ATR, which is transformed into a hyperphosphorylated state by autophosphorylation at multiple sites [51]. Meanwhile, during DSB resection, single-stranded overhangs are formed, which promote an ATM-to-ATR switch [51,52]. Following the binding of sensors and activation of these three PI3KKs, a number of downstream effectors, including DNA repair proteins and cell cycle checkpoint proteins, are recruited and phosphorylated. Depending on the extent of DNA damage, cells are arrested at different phases of the cell cycle, either for repairing the damage or triggering apoptosis if the damage is beyond repair [38].

Interactions between DNA viruses and the host DDR machinery have been widely documented [53–57]. Rather than triggering the protective effects of the DDR, viruses have evolved sophisticated strategies to redirect the DNA damage machinery. By selectively activating or suppressing components of the DDR, viruses are able to modulate the cellular environment for viral infection. Several reviews have summarized the relationship of different DNA virus species, especially dsDNA viruses, to the DDR machinery [53–57]. The interaction between viruses and the DNA damage machinery not only significantly affects the viral life cycle but also plays important roles in viral pathogenesis. Among the smallest DNA viruses, parvoviruses must exploit cellular machineries and signaling pathways, including DDR signaling pathways, for a productive viral infection. In addition, since parvoviruses contain ssDNA genomes with unique hairpin structures at the ends (Table 1) that are absent from cellular DNA, they are unique tools to study exogenous DNA-induced DDR. This review aims to summarize recent advances in parvovirus infection-induced DDR, with an emphasis on the diversity of signaling pathways utilized by different parvoviruses and the impact of the DDR machinery on the parvovirus life cycle as well as host cell fate decisions.

Genus *Dependovirus* (AAV2)

Replication of AAV2 requires coinfection with a helper virus such as adenovirus and herpes virus, through modulating the host cellular environment and activating viral gene expression [58]. In the absence of helper virus, the AAV2 genome can integrate into a specific locus on human chromosome 19 as latent infection [59]. AAV2 has not been associated with any human diseases and presents very low immunogenicity. Thus, it has been modified as one of the promising vectors in human gene therapy [60,61]. The AAV2 genome has identical inverted terminal repeats (ITRs), forming 'T' shaped hairpins at each end (Table 1) [62]. Studies on AAV2 infection-induced DDR have been performed under different conditions, such as infection with UV-inactivated AAV, transduction of recombinant AAV2 (rAAV2), coinfection with AAV2 and adenovirus, and coinfection with AAV2 and HSV1.

Both the AAV2 genome and the large nonstructural proteins Rep78/68 are able to induce a DDR. The structure of the AAV2 genome has a gap of approximately 4.4 kb between the two ITRs (Table 1). This structure could mimic a SSB, and is a perfect trigger for the activation of ATR signaling [47,63]. Indeed, studies on infection with UV-inactivated AAV2 proved that the AAV2 genome is able to induce a DDR, which arrests host cells at G2/M phase in the presence of p53, and results in apoptosis in the absence of p53 [64]. UV irradiation causes the formation of intra-strand cross-links in the AAV2 ssDNA genome, resulting in no expression of viral proteins. UV-inactivated AAV2 formed foci in infected cells, and DNA polymerase-δ, ATR, TopBP1, RPA and the 9–1–1 complex were found to colocalize within the foci, suggesting that the nonreplicative AAV2 genome, by mimicking a stalled replication fork, provokes ATR-Chk1 signaling [65]. Activated Chk1 is required for the G2/M arrest following UV-AAV2 infection [66,67]. In addition, ectopic expression of the Rep proteins was able to activate DDR signaling [10,68], possibly due to the endonuclease activity of the Rep proteins, which creates nonspecific nicks in chromosome DNA. However, during coinfection with adenovirus, the DDR signaling elicited by the Rep proteins was much weaker than that from AAV2 replication [68], suggesting that the majority of DDR signaling is generated from the replication event rather than from the Rep proteins or viral genome *per se*.

An early study using an ATM-defective cell line for rAAV2 transduction showed that knockout of ATM enhanced rAAV2 transduction efficiency [69], suggesting that the ATM signaling pathway blocks rAAV transduction.

Table 1. Summary of parvovirus infection-induced DNA damage responses.

Genus	Virus	Genome structure	Mediator of the DDR	Consequence of the DDR
Dependovirus	AAV2	3´ 5´	ATR (UV-AAV2)	ATR triggers G2/M arrest and apoptosis in UV-AAV2-infected cells
			DNA-PKcs and ATM (AAV2/adenovirus)	MRN complex limits AAV2 replication
			DNA-PKcs (AAV2/HSV-1)	Unknown
Erythrovirus	B19V	3´ 5´	ATR and DNA-PKcs	Facilitates B19V replication; dispensable for B19V infection-induced G2/M arrest
Parvovirus	MVM	3´ 5´	ATM	Facilitates MVM replication; partially mediates G2/M arrest
	H-1PV		Unknown	Unknown
Bocavirus	MVC	3´ 5´	ATM	Facilitates MVC replication and triggers p53-dependent apoptosis; partially mediates G2/M arrest
Amdovirus	AMDV	3´ 5´	Unknown	Unknown

Representative parvoviruses of each genus in the subfamily Parvovirinae *of the* Parvoviridae *family are listed with their genome structure shown in the form of a negative ssDNA genome. The mediators of the DDR induced during infection and the consequence of the DDR are summarized for each parvovirus. AAV2 and B19V contain equal amounts of positive and negative ssDNA genome* [136]*, whereas the other four parvoviruses contain over 90% negative ssDNA genome.*
AAV2: Adeno-associated virus type 2; AMDV: Aleutian mink disease virus; B19V: Human parvovirus B19; DDR: DNA damage response; H-1PV: Parvovirus H-1; MVC: Minute virus of canines; MVM: Minute virus of mice.

In keeping with this report, the MRN complex was shown as a barrier to rAAV2 transduction [70]. During processing of transduced rAAV2 or AAV2 replication, the MRN complex was found to relocalize into the foci with AAV2 genomes [70,71]. Mre11 physically associated with rAAV2 genomes [71], and Mre11 and NBS1 bound to AAV2 ITR hairpin structures [70]. Thus, the MRN complex has an intrinsic ability to bind to the hairpin structures of AAV2 genomes. Notably, the MRN complex is not required for the activation of DDR signaling during rAAV2 transduction and replication; conversely, it limits AAV2 replication and rAAV2 transduction efficiency. Silencing of NBS1 increased rAAV2 focus formation and rAAV2 transduction, while degradation of the MRN complex by E1b55K/E4orf6 created a more favorable environment for both wild-type AAV2 replication and rAAV2 transduction [70]. Therefore, the MRN complex may initially function as an antivirus cellular apparatus, while AAV2 requires the E1b55K/E4orf6 complex encoded from adenovirus to destroy this machinery. Adenovirus was the original identified helper virus for AAV2 productive infection [72]. The minimal set of adenovirus proteins required for AAV2 replication includes E1A, E1b55K and E4orf6, as well as *VA RNA* genes [73]. During adenovirus infection, E1b55K and E4orf6 proteins form a complex, which is important for export of viral mRNA [74,75]. The complex also causes the degradation of the MRN complex, promoting productive adenovirus infection by preventing the concatemerization of adenovirus genomes [76]. During coinfection of AAV2 and adenovirus, DNA-PKcs signaling is the major mediator of the induced DDR [68,77]. The DDR is represented by phosphorylation of a number of DDR factors such as SMC1, Chk1, Chk2, H2AX and RPA32 [68]. Interestingly, although the MRN complex was destroyed during coinfection, autophosphorylation of the ATM kinase was observed [68], even though it is not the mediator of the DDR. By contrast, DNA-PKcs is the primary mediator of the DDR in response to AAV2 and adenovirus coinfection. Along with its regulatory subunits, Ku70 and Ku86, DNA-PKcs localized to the AAV2 replication centers as well as the large nonstructural protein Rep-mediated AAV2 replication compartments that only contain the AAV2 replication origins (p5 promoter and the ITR) [68]. In addition, DNA-PKcs plays an important role in AAV2 DNA replication *in vitro* as well as rAAV2 replication in the presence of adenovirus or HSV1 [78]. DNA-PKcs also has been reported to be involved in the formation of circular rAAV2 episomes [79,80]. However, during coinfection with adenovirus, wild-type AAV2 DNA replication is not dramatically affected by DNA-PKcs inhibition [77]. Although it has not been shown whether ATR signaling is activated

during AAV2 and adenovirus coinfection, expression of a kinase-dead ATR did not affect the downstream signaling, indicating that ATR signaling is not involved [68]. Thus, during coinfection of AAV2 and adenovirus, both the ATM and DNA-PKcs signaling pathways are activated, although the DNA-PKcs is the major mediator of the signaling.

During coinfection with HSV1, AAV2 replication activates ATM and DNA-PKcs signaling [81]. The helper proteins provided by HSV1 include UL5, UL8, UL52 and the DNA binding protein ICP8, which play important roles in HSV1 replication [82]. HSV1 infection alone activates the ATM signaling and inhibits ATR signaling [83,84]. Moreover, DNA-PKcs is inhibited through ICP0-dependent proteasomal degradation [85]. During coinfection with HSV1, AAV2 infection induced phosphorylation of all three PI3KKs and delayed the degradation of DNA-PKcs [81]. DNA-PKcs and ATM phosphorylated several downstream substrates such as NBS1, p53, Chk2, H2AX and RPA. Although ATR phosphorylation and recruitment of its binding component ATR-interacting protein were observed, Chk1 was not phosphorylated, indicating that the ATR signaling is not activated [81]. However, whether phosphorylation of these kinases affects the AAV2 life cycle during the coinfection with HSV1 has not been studied.

The consequences of DDR activation on virus life cycle and the host cell fate varies from different conditions of viral infection. Infection of UV-inactivated AAV2 triggers G2/M arrest of host cells and activates p84N5, a proapoptotic protein that further activates caspase-6 and induces p53-independent apoptosis in several cancer cell lines [67,86]. Notably, AAV2 can replicate autonomously (to a limited extent) in UV-treated host cells [87], suggesting that DDR signaling may trigger replication of AAV2 DNA. Additionally, ectopic expression of the large Rep proteins induced ATM-dependent S phase arrest that facilitated AAV2 replication [10]. However, during coinfection with adenovirus or HSV1, the consequences of DDR on the AAV2 life cycle and host cell fate are still elusive, since these two helper viruses also interact with the cellular DNA damage machinery. Interfering with any DDR signaling alters the life cycle of these two viruses [88] and, therefore, indirectly affects AAV2 replication.

In conclusion, under different conditions of viral infection, AAV2 displays diverse patterns of interplay with the cellular DNA damage machinery. The MRN complex is a barrier to wild-type AAV2 replication and rAAV2 transduction. Although effects such as cell cycle arrest and apoptosis were observed during expression of the large Rep proteins and infection of UV-inactivated AAV, little is known about the impact of DDR signaling on the fate of host cells and the life cycle of AAV2 during coinfection with its helper viruses. Nevertheless, study of AAV2-induced DDR will help us to better understand the biology of AAVs for enhancing rAAV transduction and preventing the potential hazards of using AAV vectors in human gene therapy [89].

Genus *Erythrovirus*

Human parvovirus B19 (B19V) belongs to the genus *Erythrovirus* and naturally only infects human erythroid progenitor cells (EPCs) of the human bone marrow and fetal liver [90–94]. Most commonly, B19V infection causes a mild disease called 'fifth disease' [95]. In some conditions, B19V infection leads to more severe symptoms, for example, in pregnant women during the second trimester, B19V infection induces hydrops fetalis [96]; in immunocompromised patients, it causes chronic pure red cell aplasia [97–99]; and in sickle cell disease patients, it induces transient aplastic crisis [100,101].

The B19V genome contains two symmetric ITRs (Table 1). Under a single p6 promoter, it encodes three nonstructural proteins (NS1, 11 kDa and 7.5 kDa) and two capsid proteins (VP1 and VP2). The large nonstructural protein NS1 is a multifunctional protein with endonuclease, helicase and transactivation activities [102]. NS1 *per se* is able to trigger G2/M arrest and apoptosis [18,103]. The 11 kDa protein has been shown to induce apoptosis [104], while the function of the 7.5 kDa remains unknown. *Ex vivo*-expanded EPCs are highly permissive to B19V infection [105], which was greatly increased under hypoxic conditions [106,107]. In contrast to other autonomous parvoviruses, in which early infection induces S phase arrest [8–14], B19V infection induces G2/M arrest of infected UT7/Epo-S1 cells [15] and EPCs [18,108,109], a stage at which the arrested cells have a 4N DNA content as determined by 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) staining. However, a more careful examination of B19V-infected EPCs using a proliferation assay of BrdU incorporation combined with DAPI staining [110,111] showed that B19V-infected EPCs were actually arrested at late S phase, when they have a 4N DNA content [Luo Y, Qui J, Unpublished Data]. This

observation suggests that replication of B19V, as with other autonomous parvoviruses, requires cellular replication factors expressed in S phase.

B19V infection triggered a broad range of DDR, resulting in all three PI3KKs activated in infected EPCs, which localized to the B19V DNA replication centers [33]. Downstream effectors of these kinases, such as Chk1, Chk2 and Ku70/Ku86 proteins, also colocalized within the replication centers, indicating that B19V has a powerful ability to activate the DNA damage machinery. This phenomenon is somehow similar to that observed during AAV2 and HSV1 coinfection [81]. The difference is that ATR phosphorylates Chk1 during B19V infection, whereas phosphorylated ATR did not contribute to Chk1 activation during AAV2 and HSV1 coinfection.

Replication of the B19V genome plays a key role in triggering a DDR [109]. Expression of individual viral proteins, including NS1, 11-kDa, 7.5-kDa, VP1 and VP2, in EPCs failed to induce a DDR [109]. Although studies in B19V-nonpermissive cells, for example the hepatocyte cell line HepG2, showed that B19V NS1 was able to nick cellular chromosome DNA and cause damage to cellular DNA [112,113], the DDR in response to such potential DNA damage was not obvious during ectopic expression of the NS1 in EPCs, as neither H2AX nor RPA32 was phosphorylated [109]. In UT7/Epo S1 cells, which are permissive to B19V infection [15], transfection of a B19V infectious DNA, but not a replication-deficient mutant that harbors mutation in the NS1 endonuclease motif, activated a DDR, indicating that the DDR induced by B19V is closely associated with the status of viral DNA replication [109]. The requirement of the viral replication process to trigger a robust DDR is similar to what was observed during AAV2 and adenovirus coinfection [68]. However, whether B19V ssDNA genome *per se* can induce a DDR warrants further investigation. The ATR-Chk1 signaling facilitates B19V DNA replication [33]. Either inhibition of ATR activation by the treatment of an ATM/ATR-specific pharmacological inhibitor or transient knockdown of ATR significantly blocked B19V DNA replication. During B19V infection, Chk1 was phosphorylated and localized into the viral DNA replication centers. Inactivation of Chk1 phosphorylation by the treatment of a Chk1-specific pharmacological inhibitor also reduced B19V replication. Additionally, the DNA-PKcs signaling contributes to enhance B19V DNA replication, but to a lesser extent. Interestingly, although the ATM signaling and its substrate Chk2 were activated during B19V infection, knockdown of ATM and inhibition of Chk2 phosphorylation did not affect B19V DNA replication significantly. Thus, the function of ATM signaling during B19V infection remains unknown.

An early study showed that G2 arrest of B19V-infected UT7-Epo/S1 cells was blocked by caffeine [114], a pan-inhibitor of both ATM and ATR signaling pathways [115], indicating that B19V infection-induced G2 arrest could be DDR dependent. However recent studies showed that B19V infection-induced DDR does not contribute to the G2/M arrest (a phase with a 4N DNA content) during infection [33,109]. Although both checkpoint kinases Chk1 and Chk2 have the ability to induce G2/M arrest [116,117], inhibition of either Chk1 or Chk2 activation in EPCs did not abolish G2/M arrest induced during B19V infection [33]. Additionally, knockdown of p53, which is phosphorylated at serine-15 by ATM, failed to abrogate B19V infection-induced G2/M arrest [109]. These results suggest that a p53- and Chk1/Chk2-independent pathway is involved in G2/M arrest induced during B19V infection. Indeed, the NS1 protein *per se* is able to induce G2/M arrest through deregulation of E2F family proteins [108]. A mutant infectious clone with a mutating putative transactivation domain of NS1 [109] replicated well in UT7/Epo S1 cells and induced a DDR, but not obvious G2/M arrest [109]. Thus, B19V infection-induced DDR is dispensable for the G2/M arrest induced during infection.

In conclusion, B19V infection not only triggers a broad range of DDR activation, but also hijacks ATR and DNA-PKcs for viral DNA replication. Viral DNA replication, but not individual viral protein, is required for B19V infection-induced DDR. The DDR has an unclear effect on the host cellular environment, since it is dispensable for the G2/M arrest of infected cells; however, it facilitates B19V DNA replication. It will be interesting to know whether the downstream effectors of ATR and DNA-PKcs signaling are involved in DDR-promoted B19V replication. Further study is warranted to examine the difference in the G2/M or late S (4N) phase arrest induced by B19V infection and by the NS1 protein using the BrdU-incorporation/DAPI staining proliferation assay. Such an examination will reveal the role of the DDR in the cell cycle arrest during B19V infection.

Genus *Parvovirus*

Minute virus of mice

Minute virus of mice (MVM) has been used as a classic model to study the replication mechanism of autonomous parvoviruses [118,119]. Together with parvovirus H-1 (H-1PV), they are oncolytic parvoviruses due to their ability to selectively infect and kill various human tumor cells and inhibit tumorigenesis in animal models [120]. In contrast to AAV2 and B19V, the terminal hairpins of the MVM and H-1PV genomes are asymmetric (Table 1). Replication of MVM is dependent on host cellular factors that are mainly expressed in S phase [9]. During infection, MVM replicates in viral replication centers called the autonomous parvovirus-associated replication (APAR) bodies, which include NS1, NS2 and several cellular DNA replication factors, such as polymerase-δ, polymerase-α, RPA and proliferating cell nuclear antigen [121].

MVM infection induces a DDR that is predominantly regulated by ATM signaling [34,122]. In both MVM-infected murine and human cells DDR factors, for example H2AX, Nbs1, Chk2 and p53, were phosphorylated [34]. RPA32 was hyperphosphorylated at multiple sites, including serines 4, 8 and 33; however, the function of this phosphorylation remains unknown [122]. Although many of these phosphorylated proteins relocate into the APAR bodies, proteins such as γH2AX and phosphorylated MDC1 appeared to be diffused in some of the infected cells [122]. With treatment of an ATM-specific inhibitor, the majority of the phosphorylation events were reduced, indicating that ATM signaling is the major mediator of MVM infection-induced DDR [34]. By contrast, ATR signaling seems to be deregulated during MVM infection of asynchronous A9 cells, although it is not clear whether this is true in other cell lines [122]. In MVM-infected NB324K cells, the DNA-PKcs components Ku70 and Ku86 localized to the APAR bodies [34]. However, whether DNA-PKcs is phosphorylated during MVM infection was not examined.

Viral DNA replication is required for an MVM-induced DDR [34]. Ectopic expression of NS1, NS2 or NS1 plus NS2 did not generate significant levels of DDR signaling [34]. Infection with wild-type MVM and NS2-knockout mutant induced a similar DDR in asynchronous A9 cells, suggesting that NS2 is not required for DDR induction [122]. Expression of NS1 induced a slight increase in the level of γH2AX, which is likely due to the nonspecific nicking function of NS1, since NS1 was shown to nick chromosomal DNA through its endonuclease activity [34,123]. The finding that UV-inactivated MVM at low multiplicity of infection failed to induce an obvious DDR indicates that neither viral proteins nor viral genome alone is able to induce a DDR comparable to that observed during MVM replication [34].

ATM signaling contributes to MVM DNA replication [34]. Treatment of MVM-infected cells with an ATM-specific inhibitor significantly blocked MVM DNA replication [34]. However, Mre11, the sensor for ATM activation, was degraded during MVM infection [34]. DNA-PKcs signaling contributes minimally to MVM replication [34]. Whether the ATR signaling affects MVM DNA replication and host cell cycle arrest has not been studied. Inhibition of ATM signaling ameliorated MVM infection-induced G2/M arrest, indicating that ATM signaling partially contributes to MVM infection-induced cell cycle arrest [34]. Since Chk2 is phosphorylated during MVM infection [34], it is likely that Chk2 plays a role in MVM infection-induced G2/M arrest during late infection.

Taken together, MVM infection mainly hijacks ATM signaling for viral DNA replication. We are curious to know whether MVM replication in Mre11- and ATM-deficient cell lines is inhibited. ATM signaling also partially contributes to MVM infection-induced G2/M arrest. The viral DNA replication event is required for the induction of DDR. It is not clear how ATM signaling promotes viral DNA replication. Since MVM replication is limited in cells at S phase [11,12,124], it is currently unknown whether ATM signaling regulates S phase arrest during early infection. Recently, Adeyemi *et al.* showed that MVM infection depleted p21 of infected cells for viral replication [125], since p21 downregulation is an essential requirement for efficient restart of DNA synthesis after S phase arrest [126]. It would be interesting to know whether the ATM signaling contributes to the depletion of p21.

H-1PV

H-1PV infection triggers a DDR that is mediated by ROS production [19]. ROS are chemically generated in cells treated with xenobiotic agents such as peroxides and oxidants, or by-products of the oxygen metabolism. Accumulation of such molecules causes oxidative stress, which damages cellular structures through the initiation of apoptosis [127]. Recent studies also show that ROS production is a general source of DDR activation [128].

In contrast to the MVM NS1 protein, H-1PV NS1 *per se* induces apoptosis, ROS production and a DDR [19]. Either H-1PV infection or only NS1 expression in NS1-inducible stable cell line caused G2/M arrest and cytotoxic effects. NS1 expression-induced apoptosis was mediated through the activation of caspase-3 and -9. NS1 expression alone induced a high level of γH2AX, suggesting that the H-1PV NS1 protein has an intrinsic ability to induce a DDR. H-1PV infection increased ROS production. Treatment of H-1PV-infected or NS1-expressing cells with antioxidants blocked ROS production and decreased γH2AX expression, indicating that the NS1-induced ROS production contributes to DDR activation. In concert, antioxidant treatment blocked the NS1-induced apoptosis. These results demonstrated that ROS accumulation leading to DNA damage plays an important role in H-1PV-induced apoptosis. It is unknown yet which DDR pathway is activated during H-1PV infection or by NS1 expression alone. More importantly, whether the DDR signaling plays a role in the H-1PV replication remains to be determined.

Genus *Bocavirus*

Minute virus of canines (MVC) belongs to the genus *Bocavirus*, which also includes bovine parvovirus 1 and the newly identified human bocavirus [129–131]. MVC contains two asymmetric terminal hairpins (Table 1) [130]. Walter Reed/3873D cells were widely used for MVC infection [132]. MVC infection of Walter Reed/3873D cells triggered a gradual cell cycle change from S phase arrest in early infection to G2/M arrest in late infection as well as mitochondrion-mediated apoptosis [133]. MVC infection activated ATM signaling [35]. In contrast to MVM infection, ATR was phosphorylated during MVC infection. γH2AX was distributed in a pan-nuclear pattern, while phosphorylated RPA32 colocalized within the MVC replication centers. Inhibition of ATM signaling blocked H2AX phosphorylation, while inhibition of both ATM and ATR signaling by pan-inhibitors blocked RPA32 phosphorylation, indicating that both ATM and ATR signaling pathways are activated. Whether DNA-PKcs is phosphorylated has not been confirmed, due to the lack of a suitable antibody. However, neither knockdown of DNA-PKcs nor treatment of MVC-infected cells with a DNA-PKcs-specific inhibitor affected phosphorylation of H2AX and RPA32, strongly suggesting that DNA-PKcs signaling is not activated during MVC infection. The MRN complex colocalized with MVC NS1 at the early stage of infection, while at the late stage the Mre11 protein appeared to be degraded.

MVC DNA replication is required for MVC infection-induced DDR [35]. Individual transfection of an infectious clone and its derivatives generated positive DDR signaling as long as the derivative plasmid was able to replicate [35], which is similar to the situation in MVM- or B19V-induced DDR [34,113]. The authors speculate that MVC proteins may not cause a significant damage to cellular DNA, and that DDR signaling may come mainly from viral DNA replication events, such as specific nicking of viral DNA by the MVC NS1 protein, or production of viral DNA intermediates that are aberrant from any cellular DNA structure.

MVC hijacks ATM signaling for viral DNA replication [35]. Progeny virus production was reduced when ATM signaling was inactivated. Neither knockdown of ATR nor DNA-PKcs had a negative effect on MVC DNA replication. Ablation of Mre11 by its specific siRNA reduced MVC DNA replication, suggesting that the MRN complex functions as a mediator to activate ATM signaling in the early stage of MVC infection.

ATM signaling affects two aspects of host cells during MVC infection. On one hand, similar to MVM, inhibition of ATM signaling significantly reduced the G2/M arrest [35]; on the other hand, ATM phosphorylated p53 at serine-15, which contributed to MVC infection-induced cell death. Knockdown of p53 did not affect MVC infection-induced G2/M arrest, but rescued approximately 50% of cells from cell death in the late stage of infection [35]. Since p53 is a well-established linker between DDR and apoptosis [44,134,135], and MVC infection does trigger mitochondrion-mediated apoptosis [133], it was proposed that phosphorylation of p53 by the ATM signaling during MVC infection directly contributes to MVC infection-induced apoptosis, through which progeny virus can be quickly released from lysed host cells for another round of infection [35].

In summary, MVC hijacks ATM signaling to facilitate viral DNA replication and thereafter progeny virus production. Similar to MVM and B19V, MVC DNA replication is necessary for DDR induction. Activation of ATM signaling partially contributes to MVC infection-induced G2/M arrest, although it is not clear whether this is because of the direct activation of the G2/M checkpoint or a delay of S phase progression. Indeed, we observed that MVC infection

triggered an intra-S phase arrest that is dependent upon the ATM signaling pathway [Luo Y, Qiu J, Unpublished data]. The intra-S phase arrest not only blocked cellular DNA replication, but also delayed S phase progression. Therefore, the authors speculate that in the context of the intra-S phase arrest, MVC represses cellular DNA replication and continuously hijacks the cellular DNA replication machinery. These findings reveal the beneficial effects of ATM activation on autonomous parvovirus DNA replication, and suggest a novel model for parvovirus DNA replication.

Conclusion

Parvoviruses have evolved sophisticated strategies to coexist with their host species. Although their genomes are aberrant from any cellular DNA structure, and the proteins expressed from the viral genome are limited, they are able to propagate in various host cells of different species. Studies concerning DDR-induced during parvovirus infection have uncovered novel mechanisms underlying virus–host interactions. Dependovirus AAV2, DDR proteins, such as the MRN complex, function as a barrier for viral infection, and this barrier can be destroyed by the helper virus. By contrast, in autonomous parvoviruses, the DDR machinery is hijacked by viruses in order to create a cellular microenvironment conducive for viral DNA replication. Therefore, parvoviruses are able to utilize different strategies to adapt to their host cells and modulate the host cellular environment. The DDR induced by different parvoviruses is summarized in Table 1.

Both viral proteins and the ssDNA genome of parvoviruses are able to elicit a DDR. The nonstructural protein of parvoviruses, such as the NS1 protein of H-1PV and MVM and the large Rep proteins of AAV2, are able to induce a DDR without the presence of viral genomes [10,19,34,68,123], suggesting that they may create damaged cellular DNA though their endonuclease activity. The UV-inactivated AAV2 genome triggered ATR-Chk1 signaling [67,86]. However, during infection of MVM, MVC, B19V and coinfection of AAV2 and adenovirus, the DDR signaling is majorly activated from the DNA replication events [33–35,68], likely due to the specific nicking of viral DNA or generation of viral DNA intermediates that mimic damaged DNA.

During infection of autonomous parvoviruses, the DDR machinery is hijacked to facilitate viral DNA replication [33–35]. However, the MRN complex is a barrier to AAV2 replication and rAAV transduction when adenovirus is coinfected or its helper genes are expressed [70]. It is still unknown whether the three DDR pathways have an impact on the life cycle of AAV2 during coinfection, because both adenovirus and HSV1 interfere with the DDR machinery as well. The DDR machinery regulates cell cycle arrest and apoptosis induced during parvovirus infection.

Executive summary

Adeno-associated virus type 2

- In the presence of helper virus, DNA-PKcs activation mediates Adeno-associated virus type 2 (AAV2) infection-induced DNA damage response (DDR).
- The Mre11–Rad50–NBS1 complex limits AAV2 DNA replication.
- UV-inactivated AAV2 infection induces ATR-Chk1 activation and cell cycle arrest at G2/M.

Human parvovirus B19

- Human parvovirus B19 (B19V) infection induces an ATR-mediated DDR.
- B19V DNA replication induces a DDR, but expression of viral proteins only does not.
- Both ATR and DNA-PKcs activation facilitate B19V DNA replication.
- The DDR is dispensable for the G2/M arrest induced during B19V infection.

Minute virus of mice & minute virus of canines

- Infection of both minute virus of mice (MVM) and minute virus of canines (MVC) induces an ATM-mediated DDR.
- Expression of MVM and MVC proteins does not induce a DDR.
- ATM activation promotes DNA replication of both MVM and MVC.
- The DDR is responsible in part for the infection-induced G2/M arrest.
- MVC DNA replication induces a DDR.

Parvovirus H1

- Parvovirus H1 infection induces a DDR.
- Expression of parvovirus H1 NS1 alone induces a DDR.
- The induced DDR is accompanied by an increase in reactive oxygen species production.

The DDR triggered either by expression of the largest nonstructural viral proteins of parvoviruses [10,17,19,123] or by infection with autonomous parvoviruses [33–35] is able to arrest infected cells at S phase or G2/M phase. UV-inactivated AAV2-induced DDR not only arrests cells at G2/M [67], but also triggers apoptosis in tumor cells [86]. Viral infection-induced apoptosis was also observed following DDR activation during MVC infection, which activates the ATM-p53 signaling pathway [35]. Hence, parvovirus infection-induced DDR influences the host cell fate through cell cycle regulation and apoptosis induction. The mechanisms by which viral infection-induced DDR coordinates host cell cycle change and apoptosis require further investigation.

Future perspective

Studies on parvovirus-induced DDR have greatly facilitated the understanding of parvovirus replication. Parvoviruses have evolved various strategies to interact with the DDR machinery under different conditions of infection. Viral proteins, genomes and replication events all contribute to parvovirus infection-induced DDR. Some DDR proteins limit parvovirus infection and are destroyed, while in most cases the DDR machinery is hijacked by parvoviruses to modify the host cellular microenvironment into being conducive to viral DNA replication during early infection, and to cause cell cycle arrest and apoptosis during late infection. Other autonomous parvoviruses such as canine parvovirus also showed activation of DDR [20], while the mechanism and the consequence of the DDR require further investigation. Since autonomous parvovirus replicates in cells at S phase and also induces a DDR, we speculate that DDR may play a critical role in inducing S phase arrest to promote parvovirus DNA replication. On the other hand, localization of the DDR signaling factors in the APAR bodies or viral DNA replication centers leads us to hypothesize that parvovirus infection-induced DDR signals DNA replication or repair factors to the APAR bodies, which replicate viral genome. The mechanisms by which parvoviruses induce S phase arrest by the DNA damage machinery and how DDR signaling facilitates viral DNA replication are topics for future investigation.

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▪ of interest
▪▪ of considerable interest

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