

Review

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DNA viruses and the cellular DNA-damage response

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It is clear that a number of host-cell factors facilitate virus replication and, conversely, a number of other factors possess inherent antiviral activity. Research, particularly over the last decade or so, has revealed that there is a complex inter-relationship between viral infection and the host-cell DNA-damage response and repair pathways. There is now a realization that viruses can selectively activate and/or repress specific components of these host-cell pathways in a temporally coordinated manner, in order to promote virus replication. Thus, some viruses, such as simian virus 40, require active DNA-repair pathways for optimal virus replication, whereas others, such as adenovirus, go to considerable lengths to inactivate some pathways. Although there is ever-increasing molecular insight into how viruses interact with host-cell damage pathways, the precise molecular roles of these pathways in virus life cycles is not well understood. The object of this review is to consider how DNA viruses have evolved to manage the function of three principal DNA damage-response pathways controlled by the three phosphoinositide 3-kinase (PI3K)-related protein kinases ATM, ATR and DNA-PK and to explore further how virus interactions with these pathways promote virus replication.

Introduction

The DNA-damage response (DDR) is a general term employed to describe a complex series of cellular pathways that detect DNA damage, initiate cell-cycle arrest so that mutated DNA is not duplicated, and then go on to repair the lesion or, if damage is too great, trigger apoptosis. Infection by many virus species is sufficient to initiate a DDR, activating some or all of the repair pathways. Simplistically, this has been seen as recognition by the host cell of viral DNA as its own damaged DNA, but it is now considered that this could, at least to some extent, be an antiviral response, aimed directly at combating infection. Viruses have a complex series of mechanisms that, in turn, have evolved to combat and inactivate the cellular damage-response pathways.

The aim of this review is to consider how and why different viruses affect the balance of these opposing pathways: for example, to what extent is an activated DDR directly required by particular virus species to facilitate or assist virus replication or, conversely, to what extent does the cellular damage response hinder replication. Additionally, it is often not clear whether activation of the DDR is a mere by-product of infection or a direct result of 'intentional' action of viral protein activity. If DDR activation is an unwanted result of infection, a further point of interest is to understand what measures viruses take to circumvent the deleterious effects of the cellular pathways. Past experience would suggest that viruses are so highly evolved and their primary structures so tightly constrained that the selective

activation of DDR pathways by some viruses might prove advantageous to them. Whether this is the case will only become evident after considerable further investigation.

The relationship of viruses to the DDR is now of particular and increasing scientific interest. There have been a number of recent excellent reviews that have addressed this subject (Lilley *et al.*, 2007; Weitzman *et al.*, 2010), although many have tended to concentrate on particular aspects of the cellular response such as the effect of the infecting virus on cell-cycle checkpoints (Chaurushiya & Weitzman, 2009) or ubiquitylation (Weitzman *et al.*, 2011). Here, we have adopted an alternative approach, dealing with the relationship of different DNA virus species to the DDR. For some of these, our knowledge is appreciable, such as human adenovirus and herpes simplex virus (HSV), but for other viruses, studies are at a very early stage. From these descriptions and a consideration of the aims mentioned above, we hope that common themes will emerge, both in the ways in which cells respond to viruses and the means by which viruses counter-attack cellular DDR pathways.

An historical perspective

Although almost all the research into the relationship between viruses and the DDR dates from the last two decades, relevant observations have been reported since the 1960s. With the growing sophistication of available technology during that decade, it became possible to visualize human chromosomes and to see that damage, in the form of

breaks and translocations, could be associated with agents such as chemicals, radiation and viruses. With relevance to this review, it became clear around this time that a large variety of virus species could damage cellular DNA. For example, adenovirus (Ad)12 induces chromosomal aberrations in human embryo kidney cells, causing breaks in chromosomes 1 and 17 (Zur Hausen, 1967; McDougall, 1970, 1971a). Numerous studies showed that other DNA viruses, such as simian virus 40 (SV40), HSV, hepatitis B virus (HBV) and Epstein–Barr virus (EBV), could also produce lesions in chromosomes of infected cells (Nichols *et al.*, 1978; Nichols, 1983). It had been considered that these chromosome breaks occurred at random in infected cells, but for three groups of DNA viruses it became clear that they cause non-random site-specific chromosome damage. These are the group A adenoviruses (Ad12, 18 and 31), HSV strains 1 and 2 and human cytomegalovirus (HCMV) (Fortunato & Spector, 2003).

At about the same time as reports of the effects of Ad12 were published, it was shown that infection of human cells with HSV resulted in limited chromosome breaks in some cells and/or complete severing of all the chromosomes (termed ‘pulverization’) in others (Stich *et al.*, 1964; Wahren *et al.*, 1972). Virus strain, cell type and time of infection all play a part in determining the outcome of HSV infection. Indeed, human epithelial tumour cell lines are much more susceptible to HSV than peripheral blood leukocytes (O’Neill & Miles, 1969; Mincheva *et al.*, 1984). The expression of HSV immediate-early (IE) and early (E) proteins is required to initiate the chromosomal damage. It has been suggested that ICP4 is required for chromosome uncoiling and the limited breaks seen at early times, whereas chromosome pulverization is caused by viral polymerases and nucleases (Peat & Stanley, 1986; Johnson *et al.*, 1992; Chenet-Monte *et al.*, 1986).

The initial report of site-specific HCMV-induced chromosome breaks was relatively recent compared with those for Ad12 and HSV (Fortunato *et al.*, 2000), although there had been earlier studies describing general damage and pulverization (Hartmann & Brunnemann, 1972; Lüleci *et al.*, 1980; Sakizli *et al.*, 1981). Of great importance for the observation of specific chromosomal breaks is the m.o.i., host-cell type and, most significantly, the stage of the cell cycle at which the virus is introduced (Fortunato & Spector, 2003). Virus entry into the cell is required to cause the breaks but, strangely, not the *de novo* expression of viral proteins. However, it is possible that incoming viral protein or DNA could be responsible (Fortunato *et al.*, 2000).

The recognition that viruses interact with the cellular DDR has become apparent as our understanding of the DDR itself has increased. It is interesting to consider, with hindsight, early reports that can now be seen to point to the involvement of the DDR in viral infection. These studies focussed on the viral infection of target cells from individuals with various inherited diseases that are now

known to be caused by mutations in DDR genes. For instance, infection of normal human skin fibroblasts with SV40 was shown to give rise to a limited number of transformed colonies. However, with cells from Fanconi’s anaemia (FA) patients there was a 10–20-fold increase in the number of transformants, whilst transformation of cells from ataxia telangiectasia (AT) and Bloom’s syndrome patients occurred at normal levels (Todaro *et al.*, 1966; Kersey *et al.*, 1972; Webb *et al.*, 1977; Webb & Harding, 1977).

Comparable experiments were carried out with Ad12, which induced similar chromosomal damage in both normal skin fibroblasts and in those from FA patients (most obviously breaks on chromosomes 1 and 17) (Zur Hausen, 1967; McDougall, 1970, 1971b). However, it seemed that Ad12 infection in the FA fibroblasts was somewhat delayed compared with normal and did not lead to cell lysis (at least at a low m.o.i.; McDougall, 1971b). Obviously the reports of the effects of SV40 and Ad12 on FA cells are not directly comparable, but both are early indications of an involvement of DDR pathways in transformation and infection by DNA tumour viruses.

DNA-damage pathways

DDR signalling and repair pathways are controlled principally by the PI3K-related protein kinases AT mutated (ATM), ATM and RAD3-related (ATR), and DNA-dependent protein kinase (DNA-PK). These Ser/Thr kinases regulate cell-cycle checkpoint control, DNA replication, DNA repair, and apoptosis in response to genotoxic stress (Fig. 1).

The ATM kinase is inactivated in AT, an autosomal recessive disorder characterized by progressive cerebellar ataxia, neurodegeneration and a predisposition to cancer (Derheimer & Kastan, 2010). It is activated in response to DNA double-strand breaks (DSBs) and coordinates DSB repair (DSBR). ATM normally exists as an inactive homodimer; autophosphorylation of S1981 in response to DSBs allows the formation of active ATM monomers, which are recruited with the MRE11–RAD50–NBS1 (MRN) complex to sites of DSBs (Derheimer & Kastan, 2010). MRN is not only a substrate for ATM kinase, but is required for full ATM activation. ATM also phosphorylates H2AX, which recruits another ATM substrate, MDC1, to DSBs which, in concert with phosphorylated H2AX (γ H2AX), recruits the histone-directed ubiquitin ligases RNF8 and RNF168 (Stewart *et al.*, 2009). ATM promotes the repair of DSBs through homologous recombination (HR) repair; ATM-dependent phosphorylation of TIF1 β /KAP1 facilitates the recruitment of 53BP1 and BRCA1 to repair foci in heterochromatin (Noon *et al.*, 2010). The role of ATM in p53-dependent G1/S cell-cycle checkpoint control, and intra-S phase and G2/M checkpoint control is also well-established (Derheimer & Kastan, 2010).

Hypomorphic mutations in ATR give rise to Seckel syndrome, an autosomal recessive disorder characterized

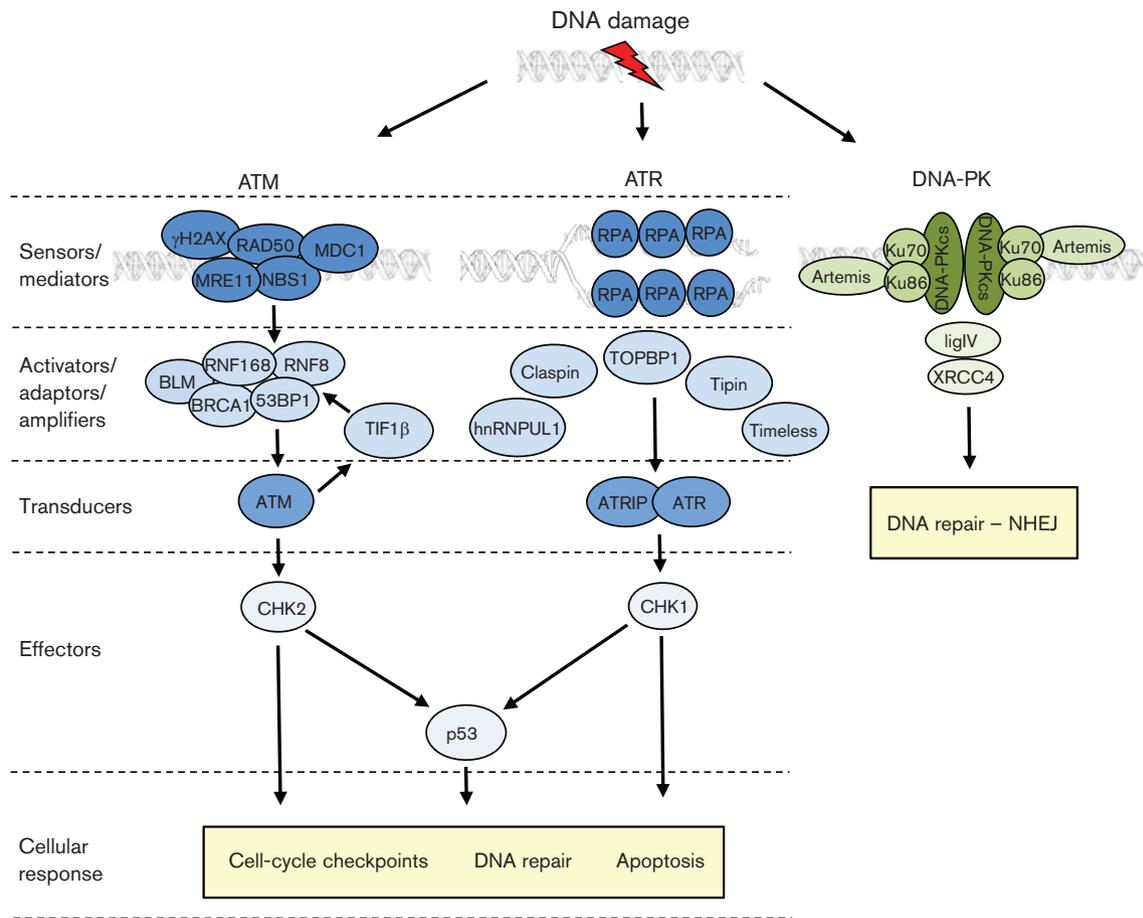


Fig. 1. Roles of protein complexes in ATM, ATR and DNA-PK pathways. Schematic representation of ATM, ATR and DNA-PK signalling pathways. ATM responds to DNA double-strand breaks; phosphorylated H2AX and MDC1 localize to sites of DNA damage, whereupon the MRN complex and ATM are recruited and ATM is activated. ATM activation promotes the recruitment of repair proteins to the site of damage and regulates cell-cycle checkpoints through the activation of CHK2 and p53. ATR is activated in response to ssDNA; ATR is recruited to RPA-coated ssDNA by its cognate binding partner ATRIP and the 9-1-1 complex. ATR is activated by a number of proteins such as TOPBP1, Claspin, Tipin and Timeless and promotes cell-cycle regulation through activation of CHK1. DNA-PK regulates NHEJ. Ku regulatory proteins recruit DNA-PK to double-strand breaks; two DNA-PKcs molecules in concert tether DNA ends together in a synaptic complex, and recruit the DNA ligase IV–XRCC4 complex to rejoin broken DNA ends. Arrows indicate the flow of the respective DDR pathways. See text for further details.

by microcephaly and mental and growth retardation. ATR coordinates the cellular response to ssDNA and regulates DNA replication during unperturbed S phase, at stalled replication forks and in response to genotoxic stress (Cimprich & Cortez, 2008). ATR, like ATM, is required for the activation of both DDR and repair pathways. It is recruited to replication sites or sites of damage through ATR-interacting protein (ATRIP), which binds directly to RPA70. The RAD9–RAD1–HUS1 (9-1-1) replicative sliding-clamp complex associates with dsDNA junctions adjacent to RPA-loaded ssDNA and recruits the BRCT-repeat protein TOPBP1 to ATR–ATRIP. The ATR-activation domain of TOPBP1 facilitates substrate binding and ATR kinase activation (Cimprich & Cortez, 2008). The best-characterized ATR effector is CHK1, which regulates the

G2/M checkpoint, principally by controlling the activity of the CDC25 phosphatases. Phosphorylation of CDC25 proteins inhibits CDC25 phosphatase activity against CDK1 and inhibits entry into mitosis and origin firing during S-phase in response to replication stress (Cimprich & Cortez, 2008).

DNA-PK plays a central role in DSB repair, regulating the process of non-homologous end-joining (NHEJ). DNA-PK comprises a large catalytic subunit, DNA-PKcs, and two regulatory subunits, Ku70 and Ku86. The Ku complex recognizes and binds to DSBs, and then recruits, and stabilizes, the interaction of DNA-PKcs with DNA. Two DNA-PKcs molecules in concert tether DNA ends together in a synaptic complex, and recruit the DNA ligase IV–XRCC4 complex to rejoin the broken DNA ends (Burma & Chen, 2004).

Adenoviruses

Adenoviruses are small DNA viruses with a linear dsDNA genome of approximately 35 kbp. Their ability to cause tumours in newborn rodents and to transform mammalian cells in culture has meant that they have been the subject of intense study for the past 40 years. Adenovirus early region 1A (AdE1A) initiates cell-cycle progression into S phase through interaction with the retinoblastoma (RB) family of proteins and/or CBP/p300 (reviewed by Berk, 2005; Frisch & Mymryk, 2002; Gallimore & Turnell, 2001). It also causes a dramatic increase in expression of p53 (Grand *et al.*, 1994). During the first few hours of infection, the host cell responds to the virus, perhaps in recognition of viral DNA or cellular stress, by activating a classical damage response, seen as phosphorylation of, for example, H2AX, SMC1 and RPA32 (much more prominently in the case of

Ad12 than of Ad5). In the case of various adenovirus early region mutants, a much more pronounced cellular response can be observed, with increased phosphorylation of DDR substrates and concatenation of viral DNA (Weiden & Ginsberg, 1994; Stracker *et al.*, 2002; Carson *et al.*, 2003; Blackford *et al.*, 2008). Importantly, after infection with wild-type (WT) group A and group C viruses, a number of cellular proteins such as p53, MRE11, DNA ligase IV and BLM are degraded (Querido *et al.*, 2001; Carson *et al.*, 2003; Stracker *et al.*, 2002; Baker *et al.*, 2007; Cheng *et al.*, 2011; Forrester *et al.*, 2011; Orazio *et al.*, 2011), ensuring that the HR and NHEJ pathways are incapacitated (Figs 2 and 3).

Initial indication that there might be a relationship between adenoviruses and the cellular DDR dates from the observation that Ad12 can cause specific chromosomal breaks (Zur Hausen, 1967; Stich *et al.*, 1964; McDougall,

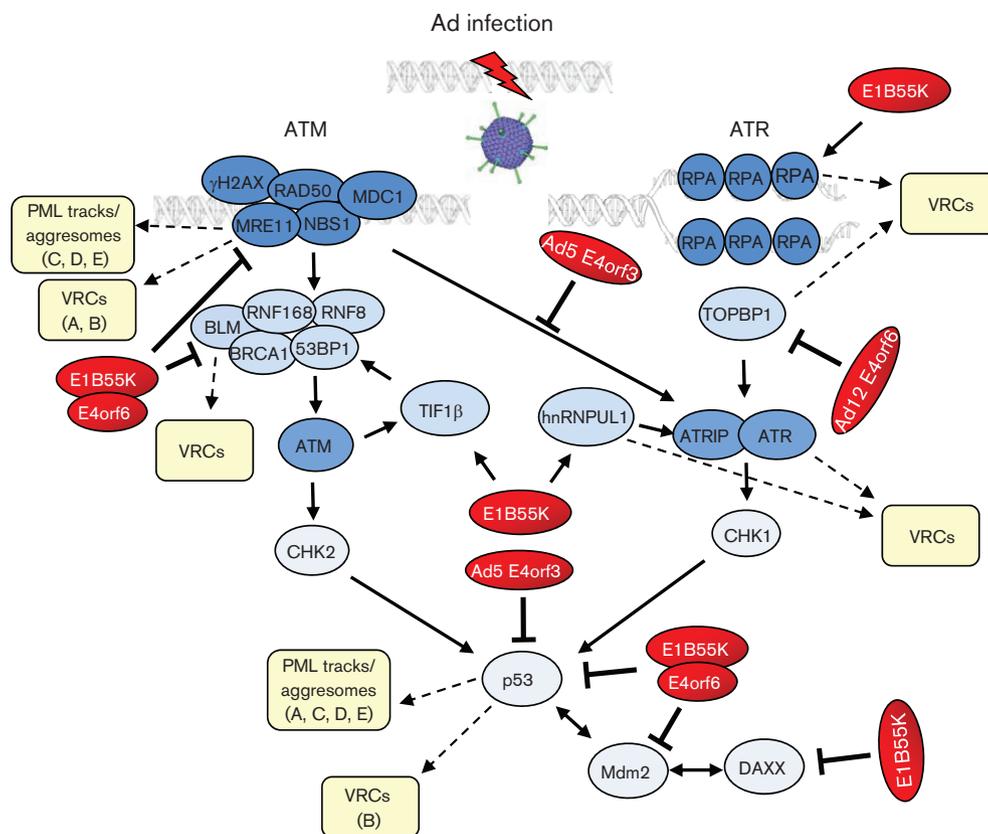


Fig. 2. Regulation of ATM and ATR signalling pathways by adenovirus. Schematic representation of ATM and ATR pathways depicting how Ad early region proteins E1B55K, E4orf3 and E4orf6 modulate both upstream and downstream signalling. E1B55K–E4orf6 complexes recruit cellular ubiquitin ligases to promote the degradation of p53, BLM and MRE11, whilst Ad12 E4orf6 recruits an E3 ligase to promote TOPBP1 degradation and E1B55K recruits an E3 ligase to promote the degradation of DAXX. Ad5 E4orf3 sequesters MRN to aggresomes to inhibit the MRN-dependent activation of ATR, and inhibits p53 epigenetically. Components of the ATM and ATR pathways are recruited to VRCs or PML-containing nuclear tracks and aggresomes during infection. MRE11 and p53 are regulated differentially by different Ad types (shown in parentheses). E1B-55K also associates with TIF1 β , hnRNPUL1 and the RPA complex, although the significance of these observations awaits clarification. Viral proteins are shown in red; cellular proteins are shown in blue. Broken arrows show cellular proteins that are recruited to PML-tracks, aggresomes or VRCs. Viral inactivation of cellular proteins is depicted by 'T'. Arrows linking viral proteins to cellular proteins indicate protein–protein interactions whose significance has yet to be fully established. See text for further details.

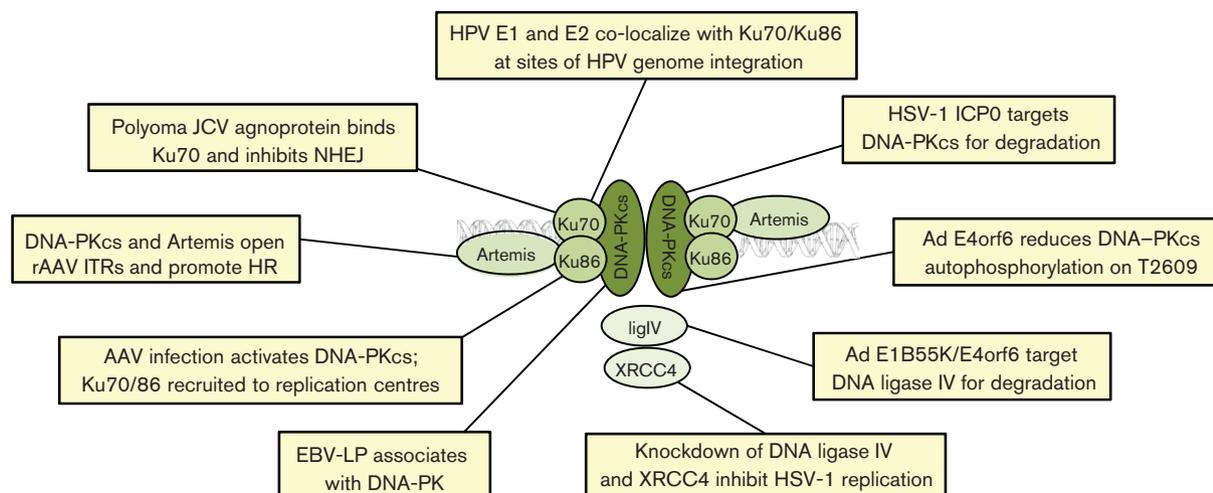


Fig. 3. Regulation of DNA-PK by human DNA viruses. Schematic representation of the role of DNA-PK and associated proteins in NHEJ. The schematic depicts how viral proteins from the DNA viruses considered in this review modulate DNA-PK function by targeting DNA-PK and other proteins involved in NHEJ. See text for further details.

1970, 1971a). Significantly, in later studies, p53 was shown to be degraded rapidly by the proteasome following Ad5 and Ad12 infection (Querido *et al.*, 1997, 2001; Steegenga *et al.*, 1998). This degradation requires the AdE1B55K and AdE4orf6 proteins acting in combination. After Ad5 infection the E1B55K–E4orf6 complex, together with cellular proteins Cullin 5 (Cul5), RING-box 1 (Rbx1) and elongins B and C, forms an E3 ligase that ubiquitylates p53 (Querido *et al.*, 2001; Harada *et al.*, 2002). Ad5E4orf6 contains at least two conserved BC box motifs that are similar to sequences in other elongin C-interacting proteins (Blanchette *et al.*, 2004; Luo *et al.*, 2007a). These sequences are necessary for the formation of the active Ad E3 ligase and p53 degradation (Blanchette *et al.*, 2004; Luo *et al.*, 2007a). In the case of Ad12, Cul2 rather than Cul5 is recruited to the E3 ligase (Blackford *et al.*, 2010; Forrester *et al.*, 2011; Cheng *et al.*, 2011).

Substrates amongst the DDR proteins vary with different adenovirus serotypes. Thus p53, BLM and MRE11 are not degraded by the group B and group D viruses (Forrester *et al.*, 2011; Cheng *et al.*, 2011; Fig. 2) and TOPBP1 appears to be degraded by only the group A viruses (Blackford *et al.*, 2010; Forrester *et al.*, 2011; Fig. 2). Although Cul5 has been identified as the ligase recruited by Ad5 for the degradation of p53 and Cul2 by Ad12 for the degradation of p53 and TOPBP1, there is some, as yet unresolved, dispute about the composition of the E3 ligase involved in the Ad5- and Ad12-mediated degradation of MRE11 (Forrester *et al.*, 2011; Blackford *et al.*, 2010; Cheng *et al.*, 2011; Carson *et al.*, 2003).

Proteins not involved directly in the DDR are also degraded during adenovirus infection (Schreiner *et al.*, 2012); the Ad-mediated degradation of the integrin $\alpha 3$ subunit and

transcriptional repressors DAXX and TIF1 γ facilitates the production of progeny virus (Dallaire *et al.*, 2009; Schreiner *et al.*, 2010; Forrester *et al.*, 2012). Interestingly, whilst degradation of p53, MRE11 and DNA ligase IV requires AdE1B55K and AdE4orf6, TOPBP1 degradation needs only the expression of Ad12E4orf6 (Blackford *et al.*, 2010), DAXX degradation requires only Ad5E1B55K (Schreiner *et al.*, 2010) and TIF1 γ requires neither E1B55K nor E4orf6, but only Ad E4orf3 (Forrester *et al.*, 2012). The TIF1 family member TIF1 β /KAP1 is also targeted by AdE1B55K during infection (Forrester *et al.*, 2012), although whether Ad modulates TIF1 β /KAP1 DDR function is not known. Mdm2 is also reduced after infection with all Ad serotypes examined, although this is probably due to the inactivation of p53 (Forrester *et al.*, 2011).

It is possible that adenoviruses can inhibit the DDR independently of protein degradation. Ad5E4orf6 and Ad5E4orf3 have been shown to bind directly to DNA-PK, although they did not appear to inhibit its kinase activity against heterologous substrates (Boyer *et al.*, 1999). However, autophosphorylation of DNA-PKcs (at T2609) was reduced during DSB repair (Hart *et al.*, 2005; Fig. 3). Ad5E4orf6 also enhances the DDR by inhibiting protein phosphatase 2A, which leads to prolonged phosphorylation of H2AX, activation of PARP and apoptosis (Hart *et al.*, 2007).

Although adenoviruses inhibit the DDR by promoting the rapid degradation of key components, they are also able to affect the localization of various DDR proteins. During infection, a number of DDR proteins are recruited to viral replication centres (VRCs; Fig. 2). RPA32 is recruited to these sites after infection with both WT and mutant viruses (Stracker *et al.*, 2005) and this has been used as a diagnostic marker for other adenoviruses. Other DDR proteins

recruited to VRCs after WT infection include ATR, ATRIP, Rad9, TOPBP1, Rad17 and hnRNPUL1 (Blackford *et al.*, 2008; Carson *et al.*, 2003, 2009). It has been suggested that relocalization to VRCs inhibits the functions of these DDR proteins, although this awaits confirmation.

As well as initiating protein degradation and localization to VRCs, adenoviruses can also promote relocalization to other sites. Promyelocytic leukaemia (PML) bodies play a role in sensing DNA damage, and a number of DDR proteins localize to them (Lombard & Guarente, 2000). Following Ad infection, PML bodies are disrupted and the PML protein is relocalized into 'track-like' structures (Fig. 2); this is dependent on the activity of the AdE4orf3 protein (Carvalho *et al.*, 1995; Doucas *et al.*, 1996; Leppard & Everett, 1999). AdE1B55K binds transiently to E4orf3 at PML-tracks and this interaction is necessary for AdE1B55K localization. In the absence of AdE1B55K and AdE4orf6, a mutant Ad5 virus is still able to inactivate the DDR by relocalizing the MRN complex to 'nuclear tracks' through the action of Ad5E4orf3, although this does not occur with Ad4 or Ad12E4orf3 (Stracker *et al.*, 2005). Relocalization of MRN by Ad5E4orf3 has been proposed to inhibit the activation of ATR (Carson *et al.*, 2009) and Ad5E4orf3 has also been shown to repress downstream DDR responses by promoting epigenetic silencing of p53 transcription (Soria *et al.*, 2010).

As well as the 'nuclear tracks', Ad infection (and Ad5E1-mediated transformation) results in the formation of large cytoplasmic bodies that have been shown to be aggresomes (Liu *et al.*, 2005; Zantema *et al.*, 1985; Araujo *et al.*, 2005). It has long been recognized that Ad5E1B55K localizes to the aggresomes, but it is now apparent that E4orf3, E4orf6, Cul5 and the MRN complex are also present (Liu *et al.*, 2005; Zantema *et al.*, 1985; Araujo *et al.*, 2005; Fig. 2). It has been suggested that, during Ad5 infection, the MRN complex is initially localized to 'nuclear tracks' by Ad5E4orf3, where it binds to the E1B55K protein. Protein complexes containing Ad5E1B55K, Ad5E4orf3, Ad5E4orf6 and MRN are then transported to the aggresomes, where MRE11 undergoes rapid degradation (Evans & Hearing, 2005; Liu *et al.*, 2005; Fig. 2). For some Ad types, p53 and the MRN complex are not recruited to nuclear tracks, but are instead recruited to VRCs (Forrester *et al.*, 2011; Fig. 2). The reasons for these differences await investigation.

ATR pathways are selectively regulated during Ad infection. Thus Ad12, and to a lesser extent Ad5, infection promotes the ATR-dependent phosphorylation of RPA32, whilst Ad12 promotes the Ad12 E4orf6-mediated degradation of TOPBP1, and prevents ATR activation of CHK1 (Blackford *et al.*, 2008, 2010; Fig. 2). The AdE1B55K associated protein hnRNPUL1 (E1B-AP5) is required for ATR activation during infection, and has recently been shown to interact with BLM and the MRN complex to promote 3' DNA end resection (Blackford *et al.*, 2008; Polo *et al.*, 2012).

It appears, therefore, that adenoviruses go to considerable lengths, seen as protein degradation and mislocalization, to

disable the DDR and yet still recruit DDR proteins to VRCs, suggesting that certain components might be required for viral replication.

The family *Polyomaviridae*

JC virus (JCV) and BK virus (BKV) are, together with the mouse polyomavirus and SV40, members of the family *Polyomaviridae*. They are non-enveloped DNA viruses with a small circular dsDNA genome. Polyomavirus appears to make use of the ATM pathway for optimal virus replication (Dahl *et al.*, 2005). Infection of mouse cells results in activation of the S and G2 checkpoints, presumably to allow the synthesis of viral DNA. Importantly, ATM is activated following infection, and SMC1, H2AX and p53 are all phosphorylated (Dahl *et al.*, 2005; Dey *et al.*, 2002). Inhibition of ATM or infection in ATM^{-/-} cells results in markedly reduced virus yields, as does substitution of a non-phosphorylatable form of SMC1 (Dahl *et al.*, 2005). It is considered that activation of ATM results from the interaction of polyomavirus large T antigen (LT) with RB, leading to E2F activation and the subsequent formation of viral initiation complexes (Dey *et al.*, 2002). As a result of infection, p53 levels are markedly increased, with a concomitant increase in the level of p53 downstream targets such as p21^{CIP1/WAF1} and Bax (Dey *et al.*, 2002). Deleterious effects of p53 are neutralized by the actions of polyomavirus LT and middle T antigens (Dahl *et al.*, 1998; Dey *et al.*, 2002; Doherty & Freund, 1997; Li *et al.*, 2004).

Studies of the closely related human virus JCV have shown phosphorylation of H2AX following infection and an increase in the levels of p53 and Rad51 (Darbinyan *et al.*, 2007). Significantly, increased expression of Rad51 has also been observed in astrocytes from patients suffering from progressive multifocal leukoencephalopathy, which is caused by the virus (Darbinyan *et al.*, 2007). Two JCV proteins have been shown to have an effect on DNA repair in transfected cells. LT antigen inhibits HR, resulting in the accumulation of mutations and restricted cell growth after DNA damage (Trojanek *et al.*, 2006; Reiss *et al.*, 2006). This appears to involve a novel mechanism in which LT expression causes the translocation of insulin receptor substrate-1 (IRS-1) from the cytoplasm to the nucleus, where it binds to Rad51 at the sites of damage (Trojanek *et al.*, 2003, 2006). A second JCV protein, agnoprotein, also expressed by SV40 and BKV, affects the DDR following transfection into NIH3T3 and human tumour cells. Expression of agnoprotein sensitizes cells to DNA-damaging agents, inhibiting DSB repair (Darbinyan *et al.*, 2004). The protein reduces expression of Ku70 and Ku86, but also binds to Ku70, relocalizing it to the perinuclear space (Darbinyan *et al.*, 2004; Fig. 3). In addition, agnoprotein causes G2/M arrest (Darbinyan *et al.*, 2002). Whether as a result of expression of LT, agnoprotein or other viral proteins, JCV is able to induce genome instability in human cells (Ricciardiello *et al.*, 2003; Theile & Grabowski, 1990; Neel *et al.*, 1996; Darbinyan *et al.*, 2007).

SV40 has a genome of 5.2 kbp. Its natural host is the rhesus macaque, but it is able to transform non-permissive cells, such as those of rodents, *in vitro*. The major early protein, LT antigen, is responsible for cellular transformation and is essential for virus replication (reviewed by Cheng *et al.*, 2009; Fanning & Zhao, 2009). Simplistically, SV40 LT duplicates the functions of the AdE1 region or HPV E6 and E7, inactivating p53 and the RB family of proteins. This has the effect of creating a favourable environment for virus replication as well as deregulating growth control, leading to proliferation and transformation.

There have been a number of studies examining the relationship of SV40 virus, and LT in isolation, to the DDR. During viral infection of CV1 cells and baby mouse kidney cells, ATM is activated and phosphorylates, in addition to its usual endogenous substrates, LT on S120 (Shi *et al.*, 2005; Dahl *et al.*, 2005). This phosphorylation is contemporaneous with, and essential for, optimal virus replication. Mutation of LT S120 or ablation of ATM function results in markedly reduced levels of infection (Shi *et al.*, 2005). The effects on ATM appear to be directly attributable to LT expression (Shi *et al.*, 2005; Dahl *et al.*, 2005; Hein *et al.*, 2009). Thus, LT is able to induce accumulation of γ H2AX and 53BP1 in damage foci as well as phosphorylation of CHK1, CHK2, ATM, RPA32 and p53 (Shi *et al.*, 2005; Hein *et al.*, 2009). It has been suggested that activation of the DDR is dependent on the binding of LT to Bub1; this interaction is necessary for LT-mediated p53 stabilization and the induction of tetraploidy seen following SV40 infection (Hein *et al.*, 2009).

Activation of both ATM and ATR signalling pathways occurs following SV40 infection, with γ H2AX, ATM, MRN, Rad51 and FANCD2 co-localizing with LT to VRCs (Zhao *et al.*, 2008; Boichuk *et al.*, 2010; Shi *et al.*, 2005; Hein *et al.*, 2009). There is also evidence to suggest that, like adenovirus, SV40 promotes the proteasome-mediated degradation of MRN components (Zhao *et al.*, 2008; Stracker *et al.*, 2002; Carson *et al.*, 2003). LT is thought to promote MRN degradation through interaction with the Cul7/p185 component of the Rbx4 and F-box protein F-box E3 ligase (Ali *et al.*, 2004; Zhao *et al.*, 2008). Indeed, mutation of the Cul7-binding site on LT stabilizes the MRN complex and reduces the level of virus replication and LT-mediated transformation (Ali *et al.*, 2004; Zhao *et al.*, 2008). Interestingly, SV40 LT also binds directly to NBS1 (Wu *et al.*, 2004); whether this interaction is required for NBS1 degradation is not clear at present. It has been suggested, as a consequence of this interaction, that SV40 induces endoreduplication following infection (Wu *et al.*, 2004). Thus, LT may be responsible, through binding to NBS1, for inactivating the cellular control mechanisms that guarantee a single round of cellular DNA replication in S phase (Wu *et al.*, 2004). If inactivation of NBS1 occurs through LT binding, presumably the eventual degradation of the MRN complex would enhance these effects. It is now very well established that SV40 LT also binds to the RPA complex, interacting directly with RPA70 (Dornreiter *et al.*, 1992; Melendy & Stillman, 1993). This

association is necessary for the initiation of replication of the viral genome, but is probably quite distinct from the role of RPA in single-strand break repair (reviewed by Fanning *et al.*, 2006).

The relationship of viruses in the family *Polyomaviridae* to the damage response seems rather more complex than is the case for adenoviruses. Whilst the MRN complex is degraded during SV40 infection, it is not clear whether similar events occur with mouse polyomavirus, JCV or BKV. Activation of ATM is required for optimal replication of SV40 but probably not for JCV, which adopts the novel strategy of inhibiting HR by recruitment of Rad51 to IRS-1.

Human papillomavirus (HPV)

HPVs are members of the family *Papillomaviridae* of non-enveloped dsDNA viruses with a short circular genome of approximately 8 kbp that infect the epithelium of skin or mucosa; high-risk HPV types 16 and 18 are responsible for over two-thirds of cervical cancers (Chow *et al.*, 2010; Moody & Laimins, 2010). HPV infects undifferentiated cells in the basal layer of stratified epithelium, where the genome is maintained in episomal form. The HPV reproductive life cycle is intimately linked to the differentiation status of the epithelium, such that viral genome amplification, late gene expression and virus production occur in differentiated suprabasal epithelial cells, which are prevented from exiting the cell cycle by the concerted actions of E6 and E7. The ability of E6 and E7 to allow cell-cycle progression resides in their respective capacities to target p53 and RB for proteasome-dependent degradation, and to allow infected cells to bypass G1 checkpoint control and enter S phase (reviewed by Moody & Laimins, 2010). Recent evidence suggests that viral genome amplification in the suprabasal layer is dependent upon activation of the ATM pathway (Moody & Laimins, 2009).

The ATM pathway is constitutively activated in undifferentiated human keratinocyte cell lines that maintain high-risk HPV31 episomes (HFK-31), such that phosphorylated (p)-ATM-S1981 and p-CHK2-T68 can be detected in undifferentiated HFK-31 lines, but not in HFK cells (Moody & Laimins, 2009). Studies with ATM and CHK2 inhibitors suggest, however, that the constitutive activation of the ATM pathway is not required for HPV episomal genome maintenance in HPV31-positive cells. When HFK-31 cells are induced to differentiate in the presence of high calcium, the ATM pathway remains activated, despite an apparent reduction in the levels of the ATM protein. Interestingly, CHK2 is phosphorylated to a greater extent upon differentiation of HFK-31 cells, and NBS1 is phosphorylated in an exclusively HPV- and differentiation-dependent manner (Moody & Laimins, 2009). Consistent with ATM activation, MRN, p-ATM, p-CHK2 and γ H2AX all accumulate within nuclear foci that are reminiscent of sites of DNA damage in both undifferentiated and differentiated HFK-31 cells. Interestingly, ATM and CHK2 inhibitor studies indicate that HPV genome amplification in

differentiated cells is dependent on ATM and CHK2; inhibition of ATM impairs the formation of HPV replication foci in differentiated cells (Moody & Laimins, 2009). It has been determined that HPV E7 binds directly to ATM through its LXCXE motif and promotes the CHK2-regulated, caspase-dependent activation of the HPV E1 replication protein in differentiated cells (Moody & Laimins, 2009). Interestingly, it has previously been proposed that the switch from viral genome maintenance to viral genome amplification is accompanied by a change from bidirectional DNA replication to unidirectional, rolling-circle DNA replication and the formation of viral genome concatemers (Flores & Lambert, 1997). It will consequently be important in future to establish the molecular role of the ATM pathway in facilitating rolling-circle DNA replication and processing of viral genome concatemers.

The HPV E1 DNA helicase associates specifically with HPV origins of replication through an origin-binding domain. HPV E1 recruitment to viral origins of replication is dependent upon the HPV E2 DNA-binding protein (McBride, 2008). It has been determined that nuclear expression of a number of evolutionarily divergent HPV E1 types can induce cell-cycle arrest in early S phase through their ability to activate ATM and CHK2 (Sakakibara *et al.*, 2011; Fradet-Turcotte *et al.*, 2011). It is apparent that, for at least HPV16 and HPV31, E2 attenuates both CHK2 and H2AX phosphorylation, but retains its ability to induce cell-cycle arrest (Sakakibara *et al.*, 2011; Fradet-Turcotte *et al.*, 2011). Given these findings, it has been suggested that HPV16 and HPV31 E2 do not inhibit E1-induced H2AX and CHK2 phosphorylation fully, such that low-level phosphorylation might be sufficient to promote cell-cycle arrest. Interestingly, for HPV16 at least, co-expression studies have revealed that, in dividing cells, E1 and E2 partially co-localize at sites of viral DNA replication with γ H2AX, p-ATM and p-p53; E1 and E2 also co-localize with HPV genomes and the MRN component NBS1 (Sakakibara *et al.*, 2011). It is well-established that HPV16 E2 interacts with the ATR activator TOPBP1 (Donaldson *et al.*, 2007). E2 co-localizes at centrosomes with TOPBP1 in mitosis, suggesting that TOPBP1 might be the chromatin receptor for HPV16 E2 during HPV genome segregation at mitosis (Donaldson *et al.*, 2007). There is also evidence that TOPBP1 regulates E2 association with chromatin, but that it is not required for E2 transcription function (Donaldson *et al.*, 2007). Whether E2 interaction with TOPBP1 affects ATR damage signalling directly in either undifferentiated or differentiated cells infected with HPV has yet to be considered. Likewise, whether E2 interaction with TOPBP1 modulates E1-induced damage signalling pathways awaits investigation. It has been established, however, that HPV16 E1/E2-mediated DNA replication occurs in the presence of the topoisomerase inhibitor etoposide, which activates both the ATM and ATR pathways (King *et al.*, 2010).

The ability of the HPV E6 and E7 oncoproteins to induce cell-cycle re-entry, subvert cell-cycle checkpoints, promote genomic instability and cellular immortalization, inhibit

apoptosis and inactivate host interferon responses underlines their transforming potential and highlights their fundamental role in HPV oncogenesis (Chow *et al.*, 2010; Moody & Laimins, 2010). Early studies suggested that HPV16 E6 and E7 could promote genomic instability, such that misaligned chromosomes at metaphase and anaphase bridges are observed during mitosis in both HPV16 E6- and E7-expressing cells (Duensing & Münger, 2002). E6 and E7 expression also promotes centrosome amplification and the presence of giant metaphases with increased chromosomal material, supernumerary spindle poles and misaligned chromosomes (Duensing & Münger, 2002). Furthermore, E6 and E7 can also induce DNA damage and promote γ H2AX focus formation (Duensing & Münger, 2002). Consistent with these studies, there is a significant increase in γ H2AX foci in mitosis from biopsy samples from high-risk HPV-positive carcinomas relative to non-malignant tissue, and that an increase in γ H2AX focus formation is also observed upon expression of HPV16 E7 in primary human foreskin keratinocytes (Sparby *et al.*, 2009). HPV16 E7 appears to promote mitotic entry in the presence of DNA damage by causing accelerated proteolytic turnover of the ATR activator claspin and overriding recovery from the ATR-regulated G2/M checkpoint (Sparby *et al.*, 2009).

The ability of high-risk HPV18 E7 to activate DDR pathways has also been considered in suprabasal, differentiated cells (Banerjee *et al.*, 2011). Consistent with the earlier studies using HPV31, both HPV18 genomes and HPV18 E7 promote ATM and CHK2 phosphorylation; p-ATM can be detected in the majority of suprabasal cells transfected with HPV18 genomes or HPV18 E7 (Banerjee *et al.*, 2011). Perhaps surprisingly, there is no apparent positive correlation between the distribution of p-ATM and HPV18 viral DNA in the suprabasal layers, although the relative relationship of HPV18 genomes to p-CHK2 has not been considered. HPV18 genomes and HPV18 E7 also induce CHK1 S345 phosphorylation in differentiated cells; CHK1 activation is RB-independent (Banerjee *et al.*, 2011). Based on these data, it has been suggested that HPV18 E7 not only promotes S-phase re-entry in suprabasal cells, but also activates ATM and ATR DNA-damage cell-cycle checkpoints to prolong the G2 phase of the cell cycle and allow viral DNA replication. Consistent with the proposed requirement for ATM and ATR in promoting E7-mediated cell-cycle arrest in G2, the CDC25C phosphatase is inactivated by phosphorylation on S216 (Banerjee *et al.*, 2011). In contrast to HPV18 E7, expression of HPV16 E6 does not induce CHK1 phosphorylation (Chen *et al.*, 2009). It does, however, upregulate CHK1 (and CHK2) expression, and has also been shown to enhance CHK1 activation in response to UV irradiation or treatment with benzo[a]pyrene diol epoxide (BPDE; Chen *et al.*, 2009). E6 expression also sensitizes fibroblasts to increased DNA damage, as judged by the enhanced accumulation of γ H2AX. Consistent with these observations, E6 promotes a pronounced cell-cycle arrest in early S phase in response to

UV and BPDE treatment, whilst colony-survival assays indicate that E6-expressing fibroblasts are also hypersensitive to UV and BPDE treatment (Chen *et al.*, 2009).

It has been proposed that high-risk HPV types might activate the FA pathway, such that cervical carcinoma tissue exhibits enhanced nuclear FANCD2 focus formation (Spardy *et al.*, 2007). HPV16 E7 also promotes the formation of large nuclear FANCD2 foci in primary human keratinocytes, whilst low-risk HPV6 E7 does not; HPV16 E6 enhances the ability of E7 to promote FANCD2 focus formation (Spardy *et al.*, 2007). It has been suggested that HPV16 E7 activates the FA pathway by both RB-dependent and -independent mechanisms (Spardy *et al.*, 2007). In line with these findings, expression of HPV16 E7 accelerates chromosomal instability and promotes apoptosis in FA-deficient cells, whilst metaphase spreads reveal a significant increase in chromatid breaks and chromosome fusions in FA-deficient cells expressing HPV16 E7 (Spardy *et al.*, 2007). As FA patients have increased susceptibility to squamous cell carcinoma (SCC), a recent study has addressed whether there is a direct association between HPV, the FA pathway and SCC tumour susceptibility in a mouse model of FA (Park *et al.*, 2010). These studies showed that the FA pathway suppresses HPV-induced oncogenesis and that the FA pathway is normally activated in response to HPV16 E7-induced DNA damage in order to repair HPV-induced DNA damage (Park *et al.*, 2010); FANCA protein deficiency also stimulates HPV-associated hyperplastic growth in organotypic epithelial raft culture (Hoskins *et al.*, 2009).

As integration of the HPV genome into the host genome is a common event in HPV-mediated tumorigenesis, it could be reasoned that host-cell genomic instability and/or direct deregulation of DDR and DSBR pathways by the high-risk HPV E6 and E7 proteins might promote the generation, and/or repair, of DNA DSBs that would facilitate viral genome integration. Indeed, persistent knockdown of the Ku70 regulatory subunit of DNA-PK by RNA interference (RNAi) in the cervical keratinocyte cell line W12 leads to the loss of episomal HPV16 genomes and the integration of HPV16 into the host genome (Winder *et al.*, 2007; Fig. 3). Interestingly, the integration of HPV16 is not preceded by any alteration in genomic stability, suggesting that HPV-induced genomic instability might not be a prerequisite for integration (Winder *et al.*, 2007). Consistent with the model of cervical neoplastic progression, it is interesting that, during the long-term culture of W12 cells, there is a loss of episomally maintained HPV16 genomes and an increase in HPV16 genome integration (Pett *et al.*, 2006). Indeed, W12 transformants can be isolated that harbour both episomes and integrated DNA (Pett *et al.*, 2006). It has been proposed that, under such circumstances, episomally expressed E1 and E2 can drive the replication of integrated HPV genomes (Kadaja *et al.*, 2009). In support of this idea, expression of HPV18 E1 and E2 in HeLa cells drives the replication of integrated HPV18 genomes and results in the production of HPV replication

intermediates that activate DDR repair and recombination pathways. As such, E1 and E2 co-localize with ATM, ATRIP, MRN, Ku70/86, CHK2 and CHK1 at integrated HPV18 genome replication centres (Kadaja *et al.*, 2009).

As for other viruses, it therefore appears that HPV infection allows the selective activation or repression of DDR pathways to promote virus replication. Indeed, it could be reasoned that DDR proteins in the ATM and ATR pathways are activated selectively and specifically in response to the expression of HPV proteins and the production of viral DNA during infection, to facilitate both vegetative virus replication in the basal layer and viral genome amplification in the suprabasal layers, and are not merely unavoidable consequences of infection.

Adeno-associated virus (AAV)

AAV is an ssDNA virus that belongs to the family *Parvoviridae* and the genus *Dependovirus*. AAV possesses inverted terminal repeats (ITRs) that form double-stranded T-shaped hairpin structures at each end of the genome and these are required for replication, integration and packaging (Linden & Berns, 2000). AAV expresses four replication (Rep) proteins required for replication and packaging, and three structural, capsid (Cap) proteins required for virion assembly. AAV replication requires the cellular replication machinery and those supplied by helper viruses; AAV can also replicate when infected cells are treated with UV radiation or genotoxic drugs that facilitate integration. A minimal requirement for Ad5 early region proteins E1A, E1B55K, E4orf6 and E2-DNA-binding protein (DBP) in AAV replication has been established; E1A drives transcription of AAV genes and DBP plays a role in AAV replication (Geoffroy & Salvetti, 2005).

As Ad E1B55K and E4orf6 inactivate DNA-damage signalling pathways in Ad-infected cells through the degradation of MRE11 and p53, the requirement for their degradation in AAV replication has been considered (Schwartz *et al.*, 2007). Using Ad E1B55K mutants that selectively target p53 or MRE11, studies with recombinant (r)AAV have indicated that MRE11 degradation, but not p53 degradation, enhances rAAV transduction; MRE11 degradation also correlates positively with an increase in second-strand viral DNA synthesis following rAAV infection (Schwartz *et al.*, 2007). Furthermore, the Ad-induced degradation of MRE11 enhances the ability of WT AAV-1 to synthesize Rep proteins and undergo viral DNA replication. Interestingly, when an infectious AAV plasmid was transfected into cells with Ad DBP alone, MRE11 localized to AAV replication compartments (Schwartz *et al.*, 2007). These data suggest that the MRN complex suppresses AAV replication by recognizing AAV ITRs as DNA damage and initiating DDR signalling pathways. It is not surprising, therefore, that AAV has evolved to replicate with Ad types that promote the degradation of MRE11. It would be interesting to see whether Ad types that do not promote MRE11 degradation also support AAV replication.

Other studies have considered the individual contribution of damage kinases to AAV transduction and replication (Collaco *et al.*, 2009; Schwartz *et al.*, 2009). In the absence of a functional MRN complex, AAV co-infection with Ad5 activates ATM. AAV and Ad5 co-infection also promotes the phosphorylation of CHK2, NBS1, SMC1, RPA32, CHK1 and H2AX (Collaco *et al.*, 2009; Schwartz *et al.*, 2009). Studies with rAAV and an infectious AAV plasmid suggest that AAV replication is required to induce a DDR. Interestingly, DNA-PK is the kinase shown to be principally required for activating the DDR following AAV and Ad co-infection (Collaco *et al.*, 2009; Schwartz *et al.*, 2009). Indeed, DNA-PKcs and regulatory subunits Ku70 and Ku86 co-localize to AAV VRCs with Rep in AAV and Ad co-infected cells (Fig. 3). Furthermore, DNA-PK activation is detrimental to AAV replication, whilst knockdown of ATM or ATR reduces the ability of AAV to replicate under these circumstances (Collaco *et al.*, 2009; Schwartz *et al.*, 2009). It has also been determined that DNA-PKcs and the endonuclease Artemis cooperate to open rAAV ITR hairpin loops and promote genome recombination *in vivo* (Inagaki *et al.*, 2007; Fig. 3). Interestingly, however, DNA-PK also reduces the efficacy of rAAV persistence by preventing rAAV integration into host genomes, and rAAV transduction is also increased in Ku86-defective cells (Song *et al.*, 2004).

Early studies established that Rep78 expression alone was able to induce a DDR and promote cell-cycle arrest (Berthet *et al.*, 2005). Rep78 promotes ATM, CHK2 and H2AX phosphorylation in the absence of other AAV genes or helper viruses and induces cell-cycle arrest in G1, S and G2/M. It is suspected that Rep78, and to a lesser extent Rep68, activate ATM-signalling pathways in AAV-infected cells by inducing DNA nicks in the host-cell chromatin that, through processing, result in DSBs (Berthet *et al.*, 2005). Rep78 also binds to CDC25A, inhibits its phosphatase activity and negates CDK activation and cell-cycle progression (Berthet *et al.*, 2005). It is thought that AAV DNA itself also contributes to activation of a DDR and G2/M arrest, which is mediated by the ATR/CHK1 pathway (Fragkos *et al.*, 2009). Studies using UV-inactivated AAV have demonstrated that infected cells display upregulated, pan-nuclear expression of γ H2AX; H2AX activation correlates positively with upregulation of p53 and p21^{CIP1/WAF1} (Fragkos *et al.*, 2009). Interestingly, in the absence of H2AX or p53, the DDR elicited by UV-AAV induces caspase-dependent mitotic catastrophe that is dependent upon ATR, CHK1 and CDK kinases (Fragkos *et al.*, 2009). Taken in their entirety, these data suggest that, like a number of viruses considered in this review, AAV can selectively activate or repress components of DDR pathways in order to promote viral DNA replication or viral DNA integration.

HSV

HSVs are large, linear dsDNA viruses from the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*; they replicate in the mucosal epithelium of the host cell

(Roizman & Knipe, 2007). Lytic infection necessitates circularization of the linear genome in the nucleus, where it is incorporated into euchromatin to allow viral gene expression and DNA replication in discrete HSV replication compartments. It is proposed that HSV replicates its DNA by a rolling-circle mechanism that results in large, linear dsDNA concatemers that are subsequently processed and packaged into nascent virions. Latency is associated with HSV spread and infection of sensory neuronal cells, whereupon the linear HSV genome is circularized in the nucleus of the ganglion, packaged into heterochromatin and maintained in episomal form (Knipe & Cliffe, 2008).

The relationship between HSV and the DDR pathway is complex (Fig. 4). A productive, lytic infection of primary human fibroblasts with HSV-1 or HSV-2 results in the activation of ATM and the subsequent activation of CHK2 and p53 (Shirata *et al.*, 2005). The viral DNA polymerase inhibitor phosphonoacetic acid inhibits the HSV-mediated activation of ATM, particularly at low m.o.i., suggesting a link between the initiation of viral DNA synthesis and ATM activation (Shirata *et al.*, 2005). Indeed, ATM, MRE11 and NBS1 accumulate at sites of virus replication with the viral replication protein UL42 during HSV-2 infection (Shirata *et al.*, 2005; Fig. 4). Interestingly, however, it appears that ATM recruitment to viral replication compartments is not dependent upon a functional MRN complex and, moreover, that NBS1 recruitment to viral replication compartments is not dependent upon ATM activation (Shirata *et al.*, 2005). These studies also determined that ATM knockdown has no effect upon the ability of HSV-2 to replicate (Shirata *et al.*, 2005). Unfortunately, these analyses were performed in 293T cells, where AdE1B55K or SV40LT might mask any requirements for the ATM pathway. A separate study has also established that HSV-1 infection activates ATM and promotes ATM and MRN recruitment to replication compartments (Lilley *et al.*, 2005). Using human cell lines that lack functional ATM and MRE11, these researchers also determined that the DDR to HSV-1 is mediated by ATM and the MRN complex and, in contrast to others, that an intact ATM pathway is required for efficient HSV-1 replication. Indeed, HSV-1 replication was severely impaired in AT and ATLD cell lines, relative to AT and ATLD cell lines where ATM or MRE11 expression was restored by complementation (Lilley *et al.*, 2005).

Roles for HSV proteins in the DDR have been established. UL29 associates with NBS1, RPA and RAD51 in pre-replicative microfoci at stage IIIb and in mature, stage IV replication compartments (Wilkinson & Weller, 2004; Fig. 4). It has been determined that the recruitment of these proteins to stage IIIb foci correlates positively with the HSV-1-dependent activation of the host-cell DDR. The HSV-1 UL12 gene product functions in concert with ICP8 as a recombinase and promotes viral recombination-dependent replication. UL12 associates strongly and directly with the MRN complex, independently of DNA, in HSV-1-infected Vero cells (Balasubramanian *et al.*, 2010). It has

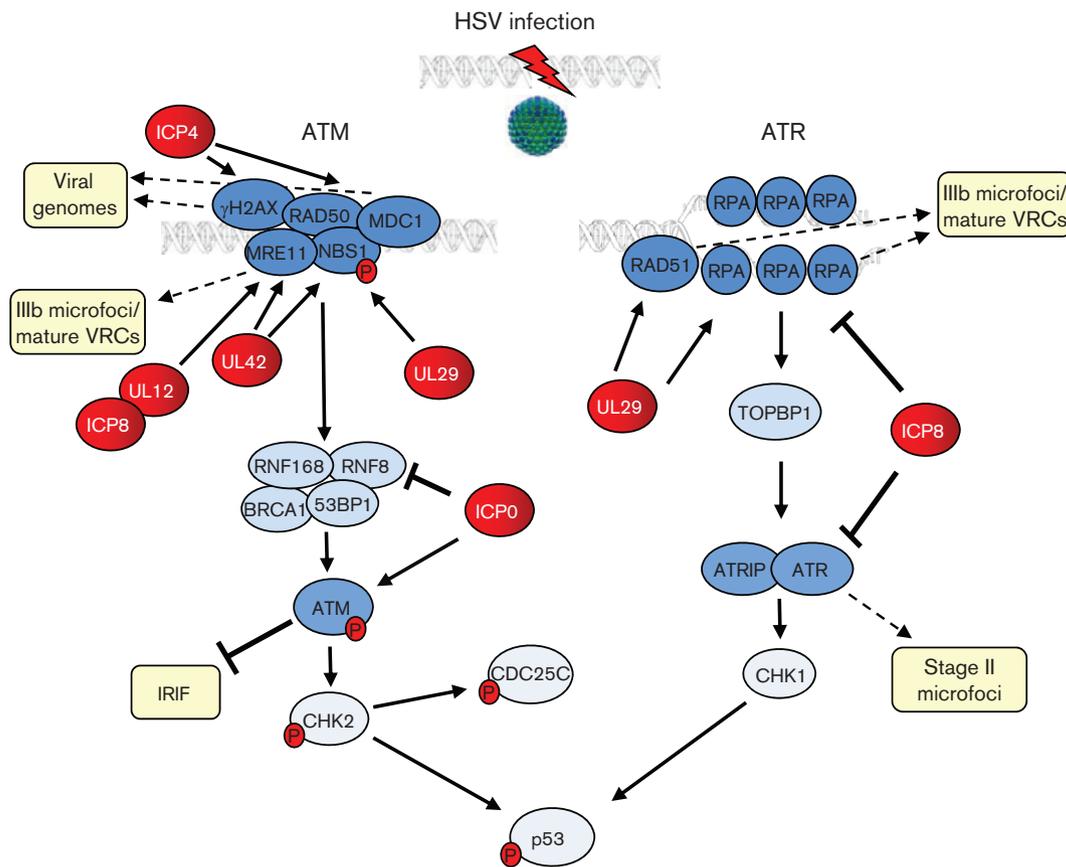


Fig. 4. Regulation of ATM and ATR signalling pathways by HSV. Schematic representation of how HSV proteins modulate both ATM and ATR signalling pathways. ICP4 co-localizes with γ H2AX and MDC1 at the nuclear periphery with incoming viral genomes. UL29 associates with NBS1, RPA and RAD51 in pre-replicative microfoci at stage IIIb and is associated with DDR activation. ATM, MRE11 and NBS1 all accumulate at sites of virus replication with the viral replication protein UL42. It has been proposed that UL12 and ICP8 cooperate with MRN to generate resected 3' viral DNA to promote the production of viral DNA concatemers, whilst ICP0 E3 ligase promotes the degradation of RNF8 to prevent full ATM activation. It has been suggested that ICP8 inhibits ATR signalling during infection, in part, by promoting the recruitment of ATRIP and RPA to pre-replicative, stage II microfoci. Viral proteins are shown in red; cellular proteins are shown in blue. Broken arrows show cellular proteins that are recruited to viral genomes, microfoci and viral replication compartments (VRCs), or are prevented from forming ionizing radiation-induced foci (IRIF). Viral inactivation of cellular proteins is depicted by 'T'. Arrows linking viral proteins to cellular proteins indicate protein-protein interactions whose significance has yet to be fully established. See text for further details.

been proposed that UL12 is required for production of viral DNA that can be packaged into virions (Balasubramanian *et al.*, 2010). HSV-1 viral DNA forms large concatemers prior to processing and packaging into virions; packaged HSV-1 genomes also possess nicks and gaps that could initiate DSBR (Balasubramanian *et al.*, 2010). Given these phenotypes, it has been suggested that UL12 and ICP8 cooperate with MRN to generate resected 3' viral DNA tails that participate in strand invasion or strand annealing and consequently promote HR and the production of viral DNA concatemers that can be subsequently processed and packaged (Balasubramanian *et al.*, 2010).

Although there is a general consensus that the ATM pathway is activated during HSV infection and that MRN is recruited to viral replication compartments, there is some

difference of opinion as to how MRE11 is regulated during infection. Most researchers suggest that MRE11 expression is not reduced during HSV-1 infection, although one report suggests that levels are reduced significantly (Gregory & Bachenheimer, 2008). Given that the ATM pathway is activated during HSV-1 infection and that the MRN complex is recruited to replication compartments to facilitate replication, it is not clear why HSV-1 would also promote the loss of the protein. In support of the notion that MRE11 expression might be reduced following HSV infection in some cell types, data from one of the original reports establishing ATM activation following HSV infection suggested that MRE11 levels are reduced to some extent following HSV-1 and HSV-2 infection, though this result was not discussed explicitly (Shirata *et al.*, 2005). Whether MRE11 degradation is a general phenomenon or reflects the

cell type used is not clear, but it is apparent that more work is needed to understand the role of MRE11 in HSV replication.

The IE gene product ICP0 is important for the switch from latency to lytic infection, and its deletion impairs lytic replication (Everett, 2000). ICP0 is a RING-finger-containing E3 ubiquitin ligase that promotes the proteasome-mediated degradation of PML isoforms during infection (Everett *et al.*, 1998). Recent work suggests that although HSV-1 infection promotes the activation of ATM and CHK2, ICP0 prevents full activation of the ATM pathway by promoting the degradation of the histone-directed ubiquitin ligases RNF8 and RNF168; ICP0 phosphorylated on T67 by casein kinase I binds to the FHA domain of RNF8 and promotes its targeted polyubiquitylation (Lilley *et al.*, 2010; Chaurushiya *et al.*, 2012; Fig. 4).

DDR proteins activated following HSV-1 infection accumulate at the sites of viral genome entry into the nucleus; sensors and mediators of DNA damage, γ H2AX and MDC1, co-localize with ICP4 in numerous foci at the nuclear periphery with incoming viral genomes (Lilley *et al.*, 2011; Fig. 4). Components of PML nuclear bodies that accumulate predominantly at sites adjacent to incoming HSV-1 genomes partially localize with γ H2AX and MDC1 at sites of viral genome entry, suggesting that DNA-damage proteins and PML bodies have discrete, separable roles during viral infection. Interestingly, RNF8 and RNF168 also accumulate at sites of incoming viral genomes when cells are infected with an ICP0-null virus. Downstream damage proteins such as 53BP1 are recruited to viral genomes in an RNF8/RNF168-dependent manner in the absence of ICP0 (Lilley *et al.*, 2011). Consistent with the beneficial role of ATM activation in HSV-1 replication, cells depleted of H2AX are severely defective in their ability to generate replication plaques during infection.

Early studies with ICP0 indicated that it targets DNA-PKcs for degradation in a RING-finger-dependent manner (Parkinson *et al.*, 1999). Further analysis revealed that ICP0 was solely responsible for promoting DNA-PKcs degradation, and that virus yield was enhanced 50- to 100-fold following infection of cells that lacked DNA-PKcs (Parkinson *et al.*, 1999). It is likely that the potent antiviral activity of DNA-PK resides in its ability to promote NHEJ and HR repair pathways and, as such, ICP0 inactivates these processes, in part by targeting DNA-PKcs for degradation. It is apparent that HSV, like many DNA viruses, targets the DNA-PK NHEJ pathway specifically during infection (Fig. 3).

In order to facilitate virus replication, HSV infection activates a G2/M checkpoint in the host cell. In a mode of action that is similar to that of HPV E1, HSV-1 ICP0 induces cell-cycle arrest at the G2/M border by promoting the ATM-dependent and CHK2-targeted phosphorylation of CDC25C-S216 (Fig. 4); inhibition of ATM or infection of cells lacking CHK2 severely limits virus yield (Li *et al.*, 2008). HSV-1 also disables ATR signalling during infection.

ATRIP and RPA are recruited, along with ICP8, to pre-replicative stage II microfoci during infection (Fig. 4), although RPA that is phosphorylated, and hence activated, by ATR in response to HU treatment is excluded from these sites (Mohni *et al.*, 2010). These data suggest that ATR signalling is inactivated specifically in these pre-replicative sites. Interestingly, this study also revealed that ATR recruitment to HSV-1 replication compartments is not dependent upon its kinase activity, suggesting that ATR recruitment is independent of DNA damage (Mohni *et al.*, 2010). Consistent with studies using adenovirus, HSV-1 infection attenuates the ATR-dependent activation of CHK1 in response to replication stress (Mohni *et al.*, 2010). Taken together, these data confirm that, although ATR/ATRIP are recruited to replication compartments during infection, ATR signalling is inactivated in the infected cell (Mohni *et al.*, 2010).

It appears that HSV, like adenovirus, inactivates DDR pathways during infection by ubiquitylation or protein sequestration. It is also apparent that, although it disables full ATM activation and the activation of ATM effector proteins, HSV does require ATM and the MRN complex for virus replication. Given these observations, it is clear that HSV has evolved to modulate components of the same pathway selectively and differentially in order to promote replication.

EBV and Kaposi's sarcoma-associated herpesvirus (KSHV)

EBV and KSHV are members of the subfamily *Gammaherpesvirinae* and each is considered to be a primary causative agent of human cancers. The γ -herpesviruses have large genomes, encoding up to 100 proteins as well as assorted microRNAs. The infectious cycles of EBV and KSHV are complex, involving latent and lytic infectious stages. Infection of B-cells by EBV is primarily latent; *in vivo*, resting B-cells express only the small non-coding RNA transcripts (EBERs). Infection of resting B-cells *in vitro* results in transformation into a lymphoblastic cell line (LCL) expressing the nuclear antigens EBNA1, 2, 3a, 3b, 3c and -LP, and latent membrane proteins LMP1, 2a and 2b. This expression pattern is only seen *in vivo* either during infectious mononucleosis or in post-transplant lymphoproliferative disease (PTLD). Furthermore, different variants of EBV latency are observed in other EBV-associated malignancies, including Burkitt's lymphoma (EBNA-1), Hodgkin's lymphoma (EBNA-1, LMP2a and b, LMP1) and in epithelial cell carcinomas (EBNA-1, LMP2a and b, LMP1). However, the virus can be reactivated periodically through the action of the IE lytic transactivator BLZF1 (Rooney *et al.*, 1989), leading to replication and virus propagation.

Despite the complexity of their genomes and patterns of protein expression, it is becoming apparent that both EBV and KSHV interact with the cellular DDR. In a manner analogous to that of certain adenoviruses, EBV causes the

rapid degradation of p53 during lytic infection, independently of Mdm2 (Sato *et al.*, 2009a, b); the IE lytic transactivator BZLF1 recruits functional Cul2- and/or Cul5- CRLs, through its N-terminal Cul box, to p53 and promotes its ubiquitylation and proteasome-mediated degradation (Sato *et al.*, 2009b).

Whilst it is not clear how certain viruses activate the DDR, there is now considerable evidence to show that EBV, through the actions of a number of viral proteins associated with latent infection, is able to induce genomic instability. This has the effect of activating DNA-repair pathways that are, in turn, inhibited by the virus (Kamranvar *et al.*, 2007; Gruhne *et al.*, 2009a, b; Lacoste *et al.*, 2010). For example, it has been shown that EBNA-1 promotes the generation of reactive oxygen species that cause DNA damage. DNA repair is inhibited by LMP1 through downregulation of ATM and subsequent inhibition of CHK2 phosphorylation and inactivation of the G2 checkpoint. Furthermore, LMP1 is able to inhibit repair by its ability to inactivate FOXO3a through activation of the PI3K/Akt signalling pathway (Chen *et al.*, 2008). In addition, EBNA-3C causes downregulation of BubR1 and inactivation of the mitotic spindle checkpoint (Gruhne *et al.*, 2009a, b). The early lytic protein EBV DNase BGLF5 is able to induce genomic instability in human epithelial cells by direct damage to the DNA and by inhibiting the expression of a range of DNA-repair genes (Wu *et al.*, 2010). This is probably a function of BGLF5's ability to shut off host mRNA in expressing cells (Rowe *et al.*, 2007). BGLF5 is also involved in the generation of linear viral genomes (Feederle *et al.*, 2009a, b) and so is likely to induce genomic instability in the host cell during lytic replication.

EBNA-3C binds directly to CHK2 and by this interaction is able to release cells from mitotic arrest induced by nocodazole (Choudhuri *et al.*, 2007). EBNA-3C is also able to disrupt other cell-cycle checkpoints, presumably helping to maintain the virus in its latent phase (Parker *et al.*, 2000; Wade & Allday, 2000). Following the switch from the latent to lytic phase of the EBV life cycle, an ATM-dependent DDR is induced (Kudoh *et al.*, 2005). The MRN complex, p-p53 and p-ATM are recruited to VRCs and it has been suggested that this is as a result of the recognition of linear viral DNA as damaged DNA by the cell (Kudoh *et al.*, 2005). By inactivation of p53, EBV is able to evade ATM-mediated checkpoints, leaving the host cell in S phase and thus facilitating virus replication (Kudoh *et al.*, 2005). Other studies suggest that activation of the DDR in EBV-infected B-cells is caused by a period of hyperproliferation and is quite independent of virus replication (Nikitin *et al.*, 2010). In complementary experiments, it has been shown that inhibition of ATM and CHK2 activity enhances EBV-induced B-cell transformation (Nikitin *et al.*, 2010). In further analysis, it has been shown that EBNA proteins, and in particular EBNA-3C, play a significant role in inhibition of DDR in normally proliferating lymphoblastoid cell lines (Nikitin *et al.*, 2010). Interestingly, it has recently been reported that EBV, through the actions of EBNA-3A and

EBNA-3C, can protect Burkitt's lymphoma-derived B-cells from apoptosis induced following DNA damage by reducing BIM and NOXA expression (Yee *et al.*, 2011). This might suggest that the virus requires some aspects of the cellular damage-response pathways, but not those associated with apoptosis. As well as the MRN complex and ATM, other proteins involved in HR locate to EBV VRCs. Thus RPA32, which is heavily phosphorylated, Rad51 and Rad52 co-localize with EBV BMRF1 and BALF2 in lytic replication compartments (Kudoh *et al.*, 2009). It has been suggested that the HR proteins are recruited to sites of DSBs in the viral DNA and may be utilized by the virus to facilitate viral genome production (Kudoh *et al.*, 2009). In support of this argument, it has been shown that depletion of RPA32 and Rad51 inhibits viral DNA synthesis. MRE11 and NBS1 have also been shown to be recruited to the EBV origin of plasmid replication (OriP) in mid-S phase during viral episome replication (Dheekollu *et al.*, 2007). It has been proposed that NBS1 and MRE11 are necessary for the formation of replication-associated recombination junctions, which are required for the maintenance of EBV episomes. It seems likely that the cellular proteins are not involved in a strict DNA damage role in this case, but contribute to replication initiation and/or reinitiation at sites of stalled replication forks (Maser & DePinho, 2004).

Two further interactions of EBV proteins with DDR components have been reported. DNA-PK and HA95 were identified as co-immunoprecipitating proteins with EBNA-LP (Han *et al.*, 2001). EBNA-LP appears to be a substrate for DNA-PK, although the biological significance of this association remains unclear (Fig. 3). More recently, EBV BZLF1 has been shown to bind directly to 53BP1, which is required for optimal virus replication (Bailey *et al.*, 2009). It is possible that 53BP1 is recruited to VRCs together with the MRN complex and ATM, although this has not been confirmed (Kudoh *et al.*, 2005). Probably more significantly, the EBV kinase BGLF4 interacts with, and activates, the histone acetyltransferase TIP60 (Li *et al.*, 2011). This is believed to be a major factor in triggering EBV-induced DDR. TIP60 is also necessary for the expression of EBV lytic genes (Li *et al.*, 2011). Extending the study to other herpesviruses, it was demonstrated that TIP60 co-immunoprecipitates with KSHV ORF36, HCMV UL97 and HSV1 UL13, suggesting strongly that it is a common cellular target for the viruses in the family *Herpesviridae* and that its activation may be responsible for effects on the DDR seen following herpesvirus infection. Interestingly, an appreciable number of DDR proteins are commonly phosphorylated by the conserved kinases from HSV, HCMV, EBV and KSHV (Li *et al.*, 2011).

KSHV also initiates a DDR during infection. Studies of the DDR in relation to KSHV have tended to concentrate on the effect of individual viral genes. For example, it has been shown that KSHV v-cyclin expression leads to activation of the DDR, seen as phosphorylation of H2AX, CHK2 and p53, and S-phase arrest (Koopal *et al.*, 2007). In contrast, the KSHV v-interferon regulatory factor 1 (v-IRF1) inhibits

ATM signalling by direct interaction with the kinase (Shin *et al.*, 2006). It has been proposed that inhibition of ATM by v-IRF1 reduces p53-S15 phosphorylation and promotes p53 ubiquitylation and proteasome-mediated degradation (Shin *et al.*, 2006). v-IRF1 also binds directly to p53, inhibiting its transcriptional activity and inhibiting apoptosis (Seo *et al.*, 2001; Nakamura *et al.*, 2001). Latency-associated nuclear antigen (LANA) interacts with p53 in co-transfection experiments and in primary effusion lymphomas (Friborg *et al.*, 1999; Chen *et al.*, 2010). LANA represses p53 transcriptional activity, inhibiting its ability to induce apoptosis. Interestingly, infection with the related murine γ -herpesvirus 68 (γ HV68) also results in phosphorylation of H2AX and this has been attributed to the activity of the orf36 kinase together with ATM (Tarakanova *et al.*, 2007). γ HV68 orf36 and the EBV homologue BGL4 can phosphorylate H2AX *in vitro* and it has been shown that ATM and H2AX, as well as orf36, are required for optimal γ HV68 replication in mouse macrophages.

EBV and KSHV both activate a DDR that is then inhibited by the virus. A number of DNA-damage proteins are recruited during EBV lytic infection to VRCs, where they appear to be required for viral genome production. Interactions between multiple EBV proteins and cellular DDR proteins have been reported, although the consequences of this are not clear at present.

HCMV

HCMV is the largest member of the subfamily *Betaherpesvirinae*, with a genome of approximately 230 kbp. It is a major cause of birth defects and is also a serious health risk in immunocompromised individuals. HCMV has a large linear dsDNA genome containing well over 200 ORFs. As with most viruses, HCMV is able to modulate the cycle of the infected cell to produce an environment favouring virus replication. This is achieved through degradation of the RB family of proteins by the action of HCMV pp71 (Kalejta *et al.*, 2003; Kalejta & Shenk, 2003) and inhibition of MCM loading onto host-cell chromatin, preventing replication licensing (Wiebusch *et al.*, 2003). HCMV replication occurs in a biphasic manner from the circularized genome. Initially, origin-specific single-copy replication takes place, followed by a rolling-circle mechanism that gives rise to DNA concatemers that may be branched, with multiple exposed double-strand ends. It has been suggested that these structures could be recognized by the infected cell as broken DNA, as appears to be the case during adenovirus infection. Thus, it seems that the DDR is activated following HCMV infection, but then is inhibited at later times by the virus (Shen *et al.*, 2004; Luo *et al.*, 2007b; Gaspar & Shenk, 2006; Li *et al.*, 2011). At early times post-infection, ATM is activated and phosphorylates p53 and NBS1 (Luo *et al.*, 2007b). There is an increase in p53 level and the protein colocalizes with HCMV UL112/113 (Jault *et al.*, 1995; Luo *et al.*, 2007b). At later times,

considerable phosphorylation of ATM and ATR substrates can be observed, as well as an apparent increase in overall level of a number of DDR proteins such as ATM, MRE11, RAD51 and ATRIP (Shen *et al.*, 2004; Gaspar & Shenk, 2006; Luo *et al.*, 2007b). Activation of ATM signalling and increase in p53 expression have been attributed to HCMV IE1-72 expression, although it should be borne in mind that ectopic expression of the viral protein itself has been reported to damage DNA (Castillo *et al.*, 2005; Shen *et al.*, 1997; Gaspar & Shenk, 2006). The recent observation that HCMV UL97 binds to TIP60 and phosphorylates a number of DDR proteins suggests that it may also be involved in ATM activation (Li *et al.*, 2011). However, the importance of ATM is confirmed by the fact that HCMV replication is markedly reduced by an ATM inhibitor (Li *et al.*, 2011). At later times of HCMV infection, the DDR is inhibited and ATM, CHK1, CHK2, the MRN complex and γ H2AX are relocalized to VRCs (Luo *et al.*, 2007b; Gaspar & Shenk, 2006). It is not clear which viral proteins are responsible for the relocalization of the DDR components, although it has been suggested that HCMV UL-84 could be a candidate (Gaspar & Shenk, 2006; Lischka *et al.*, 2003). Activation of the ATM signalling pathway by HCMV suggests that this activity is required by the virus. A recent study has suggested that ATM knockdown by RNAi results in reduced virus replication and numbers of mature VRCs, and reduced levels of expression of HCMV IE2, pp65 and gB55 but not IE1 (E *et al.*, 2011). These observations may, however, be contrasted with a previous study which suggested that HCMV replicated almost as well in ATM^{-/-} and MRE11^{-/-} fibroblasts as in WT cells (Luo *et al.*, 2007b). It has been suggested that IE1 and IE2 promote ATM-dependent activation through the activation of E2F1 (Ren *et al.*, 2002; Rogoff & Kowalik, 2004; Wade *et al.*, 1992; McElroy *et al.*, 2000; Poma *et al.*, 1996). Indeed, depletion of E2F1, but not of E2F2 or E2F3, before HCMV infection reduces the phosphorylation of H2AX, the expression of HCMV proteins and HCMV replication (E *et al.*, 2011). It has been determined that DNA-repair activity, more specifically HR, increases following HCMV infection; the increase in HR activity is stimulated by HCMV IE1-72 expression, and accompanied by elevated levels of RAD51C and FANCG (Kulkarni & Fortunato, 2011).

As with many viruses discussed here, HCMV initially activates the DDR and then inhibits it. However, an increase in expression of several DDR proteins and their relocalization to VRCs appears to be necessary for optimal virus replication, as is activity of the ATM kinase.

HBV

HBV has a partially dsDNA genome of 3.2 kbp. It is a member of the family *Hepadnaviridae*, predominantly infects hepatocytes and is associated with the development of hepatocellular carcinoma. Most of the effects of HBV on hepatocytes are attributable to the action of the HBX oncoprotein. The relationship of HBX to the DDR has been

reviewed quite recently (Matsuda & Ichida, 2009), so here we will give a brief overview of our current understanding.

It is now clear that HBV, probably through HBX, potentiates damage to DNA in infected cells, probably by inhibiting various aspects of the DDR. As with many other viruses, HBV inactivates p53 following infection. HBX binds directly to p53 and inhibits its transcriptional activation properties (Feitelson *et al.*, 1993; Lee & Rho, 2000; Wang *et al.*, 1994). It also inhibits the association of p53 with transcription factors such as ERCC3/XPD and ERCC2/XPB (Wang *et al.*, 1994, 1995; Qadri *et al.*, 1996) and, as a consequence, abrogates p53-induced apoptosis (Wang *et al.*, 1995).

HBV is involved directly in the inactivation of various DDRs. HBX interacts directly with DDB1 (ZAP-1/UVDDR), inhibiting nucleotide excision–repair (NER) (Lee *et al.*, 1995; Becker *et al.*, 1998; Sitterlin *et al.*, 1997; Jaitovich-Groisman *et al.*, 1999; Martin-Lluesma *et al.*, 2008). It also binds to DDB2 and these interactions have the effect of stabilizing the viral protein and inducing its nuclear accumulation (Nag *et al.*, 2001; Sitterlin *et al.*, 2000a; Bergametti *et al.*, 2002). Importantly, the ability of HBX to bind to DDB1 is essential for optimal virus replication and productive infection (Sitterlin *et al.*, 2000b). The site of interaction on HBX for DDB1 comprises a structurally conserved α -helical region lying between aa 88 and 100 (Li *et al.*, 2010). A similar motif has been identified in the paramyxovirus SV5 V protein and in the cellular DDB1–Cul4-associated factors (Li *et al.*, 2010). It is not clear in what way HBV utilizes the cullin-associated E3 ligase that is bound to HBX, but obviously understanding the implications of this interaction will provide exciting new lines of research. HBX also inhibits NER by its direct interaction with components of the TFIIH nucleotide basal excision repair complex (Jia *et al.*, 1999; Jaitovich-Groisman *et al.*, 2001; Qadri *et al.*, 1995, 2011; Haviv *et al.*, 1996). It has been suggested that this interaction may have the effect of specifically downregulating the level of expression of XPB and XPD (Jaitovich-Groisman *et al.*, 2001). As a result of attenuation of NER by HBX, cells expressing the viral protein become hypersensitive to UV irradiation (Capovilla *et al.*, 1997; Qadri *et al.*, 2011; Jia *et al.*, 1999). In addition to its effect on the DDR, it has been shown that HBV infection causes cell-cycle arrest in S phase, presumably to facilitate the production of viral DNA (Chen *et al.*, 2008; Martin-Lluesma *et al.*, 2008).

HBX is responsible for most of the reported cellular effects of HBV. With regard to this review, it inhibits p53 transcriptional activity and NER and both of these actions are required for virus replication. The relationship between HBX and components of the major DDR pathways is not clear at present.

Conclusions

Apart from some initial early reports that cellular DNA was subject to breaks and translocations following viral

infection, our knowledge of the relationship between viruses and the DDR has tended largely to parallel (although probably a step behind) our increasing understanding of the multiple ways in which cells respond to DNA damage. With an exciting, important and relatively new area of study such as this, it would have been gratifying to have been able to set out some broad conclusions that could apply to most, if not all, DNA viruses. Ideally these would contain some unifying principles which would summarize our knowledge to date and suggest novel approaches for future investigation. However, at present it is probably easier to list those aspects of the relationship of viruses to the DDR that we do not understand, rather than those that we do.

It is clear that different virus species adopt very different strategies to the damage response. In the case of adenoviruses, where our understanding is perhaps greatest, degradation of cellular DDR components is a priority and, presumably, this has the effect of disabling the cellular response [as seen by the absence of DNA concatenation (Carson *et al.*, 2003; Stracker *et al.*, 2002)]. Yet, even here, there are a large number of underlying observations that we do not understand, such as why numerous damage proteins are recruited to VRCs and why different adenovirus serotypes adopt very different approaches to DDR pathways (Cheng *et al.*, 2011; Forrester *et al.*, 2011). Other viruses also degrade various DDR proteins, presumably with the same objective of incapacitating the pathways and stopping ligation of viral DNA. Thus, SV40 causes the degradation of MRE11, whilst p53 levels are much reduced in HPV- and EBV-infected cells (Zhao *et al.*, 2008; Scheffner *et al.*, 1990; Sato *et al.*, 2009a, b). Protein degradation by HSV appears to be dependent on expression of the viral E3 ubiquitin ligase, ICP0. This is involved in degradation of DNA-PKcs, RNF8 and RNF168; the substrates for degradation seem to be quite distinct from those degraded by adenoviruses (Lilley *et al.*, 2010).

It is likely that one of the major determining factors in the relationship of viruses to the DDR is the structure of the viral DNA and, as a consequence, how the DNA is replicated. The linear dsDNA of adenovirus is more likely to elicit an immediate DDR response, by its mere presence, than a circular genome, such as SV40, which will probably not be seen as cellular DNA. However, the herpesviruses undergo replication by a rolling-circle mechanism that generates large head-to-tail concatemeric genomes that require processing, potentially by DDR proteins, prior to packaging. Obviously the infected cell will react differently in these cases, quite apart from any deliberate action of the virus in triggering activation of the pathways.

Activation of DDR pathways seems to be a very common response to viral infection, with phosphorylation of various downstream targets being observed after infection with all of the viruses discussed here. Because of cross-talk between the ATM, ATR and DNA-PK signalling pathways, it is difficult to know which particular one is activated or inhibited at any time during the response. It is also difficult to know whether transient activation, before subsequent

inhibition, is advantageous. Thus, activation of ATM and/or ATR signalling has been observed following infection with adenovirus, SV40, polyomavirus, HPV, AAV, HSV, EBV, KSHV and HCMV. This may be a result of virus-induced cellular stress, the recognition of the viral DNA as damaged cellular DNA or, in some cases, a deliberate activation of the pathways. Recognition of to what extent these alternatives apply to different virus species will, obviously, be essential for a full understanding of the complex relationship with the DDR. However, a recent report (Li *et al.*, 2011) demonstrating that kinases from α -, β - and γ -herpesviruses appear to have many substrates in common and these include an appreciable number of DDR proteins seems to be an excellent and exciting first step in providing a more general theory of the relationship.

A number of other features of the relationship seem to be common to many viruses. The ability to manipulate cell-cycle checkpoints, often to ensure that infected cells are in S phase, is shared by most species. This is an obvious requirement for virus replication and necessitates overriding damage checkpoints. In the case of the small DNA tumour viruses, progression into S phase for replication of viral DNA is most obviously achieved by the inactivation of the RB family, but presumably the damage-response checkpoint is activated in infected cells and this is required for virus replication.

For many viruses, there is a definite requirement for DDR proteins for efficient virus replication. For example, HSV replicates only very poorly in the absence of the ATM pathway, SV40 replication proceeds more efficiently in the presence of ATM-mediated phosphorylation of LT and, similarly, HCMV probably requires active ATM for optimal replication (Shi *et al.*, 2005; Lilley *et al.*, 2005; Gaspar & Shenk, 2006). In all of these cases, however, it is far from clear what precise function is served by the DDR proteins with regard to the virus. Recruitment of multiple DDR proteins to viral replication centres is very common although, again, it is not known what precise role they play there. Determination of the function of these cellular proteins during replication will be central to our understanding of the relationship between viruses and the DDR.

As we have tried to stress throughout this review, the relationship between viruses and the DDR is an exciting and rapidly expanding area of research. In spite of appreciable increases in our knowledge over the last decade, there are still many viruses for which our understanding is superficial at best. However, we believe that over the next few years this will be rectified, and concerted study by virologists on the one hand and cell biologists on the other will allow us to understand fully this very subtle relationship.

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References

- Ali, S. H., Kasper, J. S., Arai, T. & DeCaprio, J. A. (2004). Cul7/p185/p193 binding to simian virus 40 large T antigen has a role in cellular transformation. *J Virol* **78**, 2749–2757.
- Araujo, F. D., Stracker, T. H., Carson, C. T., Lee, D. V. & Weitzman, M. D. (2005). Adenovirus type 5 E4orf3 protein targets the Mre11 complex to cytoplasmic aggresomes. *J Virol* **79**, 11382–11391.
- Bailey, S. G., Verrall, E., Schelcher, C., Rhie, A., Doherty, A. J. & Sinclair, A. J. (2009). Functional interaction between Epstein-Barr virus replication protein Zta and host DNA damage response protein 53BP1. *J Virol* **83**, 11116–11122.
- Baker, A., Rohleder, K. J., Hanakahi, L. A. & Ketner, G. (2007). Adenovirus E4 34k and E1b 55k oncoproteins target host DNA ligase IV for proteasomal degradation. *J Virol* **81**, 7034–7040.
- Balasubramanian, N., Bai, P., Buchek, G., Korza, G. & Weller, S. K. (2010). Physical interaction between the herpes simplex virus type 1 exonuclease, UL12, and the DNA double-strand break-sensing MRN complex. *J Virol* **84**, 12504–12514.
- Banerjee, N. S., Wang, H. K., Broker, T. R. & Chow, L. T. (2011). Human papillomavirus (HPV) E7 induces prolonged G2 following S phase reentry in differentiated human keratinocytes. *J Biol Chem* **286**, 15473–15482.
- Becker, S. A., Lee, T. H., Butel, J. S. & Slagle, B. L. (1998). Hepatitis B virus X protein interferes with cellular DNA repair. *J Virol* **72**, 266–272.
- Bergametti, F., Sitterlin, D. & Transy, C. (2002). Turnover of hepatitis B virus X protein is regulated by damaged DNA-binding complex. *J Virol* **76**, 6495–6501.
- Berk, A. J. (2005). Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* **24**, 7673–7685.
- Berthet, C., Raj, K., Saudan, P. & Beard, P. (2005). How adeno-associated virus Rep78 protein arrests cells completely in S phase. *Proc Natl Acad Sci U S A* **102**, 13634–13639.
- Blackford, A. N., Bruton, R. K., Dirlik, O., Stewart, G. S., Taylor, A. M., Dobner, T., Grand, R. J. & Turnell, A. S. (2008). A role for E1B-AP5 in ATR signaling pathways during adenovirus infection. *J Virol* **82**, 7640–7652.
- Blackford, A. N., Patel, R. N., Forrester, N. A., Theil, K., Groitl, P., Stewart, G. S., Taylor, A. M. R., Morgan, I. M., Dobner, T. & other authors (2010). Adenovirus 12 E4orf6 inhibits ATR activation by promoting TOPBP1 degradation. *Proc Natl Acad Sci U S A* **107**, 12251–12256.
- Blanchette, P., Cheng, C. Y., Yan, Q., Ketner, G., Ornelles, D. A., Dobner, T., Conaway, R. C., Conaway, J. W. & Branton, P. E. (2004). Both BC-box motifs of adenovirus protein E4orf6 are required to efficiently assemble an E3 ligase complex that degrades p53. *Mol Cell Biol* **24**, 9619–9629.
- Boichuk, S., Hu, L., Hein, J. & Gjoerup, O. V. (2010). Multiple DNA damage signaling and repair pathways deregulated by simian virus 40 large T antigen. *J Virol* **84**, 8007–8020.
- Boyer, J., Rohleder, K. & Ketner, G. (1999). Adenovirus E4 34k and E4 11k inhibit double strand break repair and are physically associated with the cellular DNA-dependent protein kinase. *Virology* **263**, 307–312.
- Burma, S. & Chen, D. J. (2004). Role of DNA-PK in the cellular response to DNA double-strand breaks. *DNA Repair (Amst)* **3**, 909–918.
- Capovilla, A., Carmona, S. & Arbuthnot, P. (1997). Hepatitis B virus X-protein binds damaged DNA and sensitizes liver cells to ultraviolet irradiation. *Biochem Biophys Res Commun* **232**, 255–260.

- Carson, C. T., Schwartz, R. A., Stracker, T. H., Lilley, C. E., Lee, D. V. & Weitzman, M. D. (2003). The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J* **22**, 6610–6620.
- Carson, C. T., Orazio, N. I., Lee, D. V., Suh, J., Bekker-Jensen, S., Araujo, F. D., Lakdawala, S. S., Lilley, C. E., Bartek, J. & other authors (2009). Mislocalization of the MRN complex prevents ATR signaling during adenovirus infection. *EMBO J* **28**, 652–662.
- Carvalho, T., Seeler, J. S., Ohman, K., Jordan, P., Pettersson, U., Akusjärvi, G., Carmo-Fonseca, M. & Dejean, A. (1995). Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. *J Cell Biol* **131**, 45–56.
- Castillo, J. P., Frame, F. M., Rogoff, H. A., Pickering, M. T., Yurochko, A. D. & Kowalik, T. F. (2005). Human cytomegalovirus IE1-72 activates ataxia telangiectasia mutated kinase and a p53/p21-mediated growth arrest response. *J Virol* **79**, 11467–11475.
- Chaurushiya, M. S. & Weitzman, M. D. (2009). Viral manipulation of DNA repair and cell cycle checkpoints. *DNA Repair (Amst)* **8**, 1166–1176.
- Chaurushiya, M. S., Lilley, C. E., Aslanian, A., Meisenhelder, J., Scott, D. C., Landry, S., Ticau, S., Boutell, C., Yates, J. R., III & other authors (2012). Viral E3 ubiquitin ligase-mediated degradation of a cellular E3: viral mimicry of a cellular phosphorylation mark targets the RNF8 FHA domain. *Mol Cell* **46**, 79–90.
- Chen, Y. R., Liu, M. T., Chang, Y. T., Wu, C. C., Hu, C. Y. & Chen, J. Y. (2008). Epstein-Barr virus latent membrane protein 1 represses DNA repair through the PI3K/Akt/FOXO3a pathway in human epithelial cells. *J Virol* **82**, 8124–8137.
- Chen, B., Simpson, D. A., Zhou, Y., Mitra, A., Mitchell, D. L., Cordeiro-Stone, M. & Kaufmann, W. K. (2009). Human papilloma virus type16 E6 deregulates CHK1 and sensitizes human fibroblasts to environmental carcinogens independently of its effect on p53. *Cell Cycle* **8**, 1775–1787.
- Chen, W., Hilton, I. B., Staudt, M. R., Burd, C. E. & Dittmer, D. P. (2010). Distinct p53, p53: LANA, and LANA complexes in Kaposi's sarcoma-associated herpes virus lymphomas. *J Virol* **84**, 3898–3908.
- Chenet-Monte, C., Mohammad, F., Celluzzi, C. M., Schaffer, P. A. & Farber, F. E. (1986). Herpes simplex virus gene products involved in the induction of chromosomal aberrations. *Virus Res* **6**, 245–260.
- Cheng, J., DeCaprio, J. A., Fluck, M. M. & Schaffhausen, B. S. (2009). Cellular transformation by simian virus 40 and murine polyoma virus T antigens. *Semin Cancer Biol* **19**, 218–228.
- Cheng, C. Y., Gilson, T., Dallaire, F., Ketner, G., Branton, P. E. & Blanchette, P. (2011). The E4orf6/E1B55K E3 ubiquitin ligase complexes of human adenoviruses exhibit heterogeneity in composition and substrate specificity. *J Virol* **85**, 765–775.
- Choudhuri, T., Verma, S. C., Lan, K., Murakami, M. & Robertson, E. S. (2007). The ATM/ATR signaling effector Chk2 is targeted by Epstein-Barr virus nuclear antigen 3C to release the G2/M cell cycle block. *J Virol* **81**, 6718–6730.
- Chow, L. T., Broker, T. R. & Steinberg, B. M. (2010). The natural history of human papillomavirus infections of the mucosal epithelia. *APMIS* **118**, 422–449.
- Cimprich, K. A. & Cortez, D. (2008). ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* **9**, 616–627.
- Collaco, R. F., Bevington, J. M., Bhrigu, V., Kalman-Maltese, V. & Trempe, J. P. (2009). Adeno-associated virus and adenovirus coinfection induces a cellular DNA damage and repair response via redundant phosphatidylinositol 3-like kinase pathways. *Virology* **392**, 24–33.
- Dahl, J., Jurczak, A., Cheng, L. A., Baker, D. C. & Benjamin, T. L. (1998). Evidence of a role for phosphatidylinositol 3-kinase activation in the blocking of apoptosis by polyomavirus middle T antigen. *J Virol* **72**, 3221–3226.
- Dahl, J., You, J. & Benjamin, T. L. (2005). Induction and utilization of an ATM signaling pathway by polyomavirus. *J Virol* **79**, 13007–13017.
- Dallaire, F., Blanchette, P., Groitl, P., Dobner, T. & Branton, P. E. (2009). Identification of integrin alpha3 as a new substrate of the adenovirus E4orf6/E1B 55-kilodalton E3 ubiquitin ligase complex. *J Virol* **83**, 5329–5338.
- Darbinyan, A., Darbinian, N., Safak, M., Radhakrishnan, S., Giordano, A. & Khalili, K. (2002). Evidence for dysregulation of cell cycle by human polyomavirus, JCV, late auxiliary protein. *Oncogene* **21**, 5574–5581.
- Darbinyan, A., Siddiqui, K. M., Slonina, D., Darbinian, N., Amini, S., White, M. K. & Khalili, K. (2004). Role of JC virus agnoprotein in DNA repair. *J Virol* **78**, 8593–8600.
- Darbinyan, A., White, M. K., Akan, S., Radhakrishnan, S., Del Valle, L., Amini, S. & Khalili, K. (2007). Alterations of DNA damage repair pathways resulting from JCV infection. *Virology* **364**, 73–86.
- Derheimer, F. A. & Kastan, M. B. (2010). Multiple roles of ATM in monitoring and maintaining DNA integrity. *FEBS Lett* **584**, 3675–3681.
- Dey, D., Dahl, J. H., Cho, S. & Benjamin, T. L. (2002). Induction and bypass of p53 during productive infection by polyomavirus. *J Virol* **76**, 9526–9532.
- Dheekollu, J., Deng, Z., Wiedmer, A., Weitzman, M. D. & Lieberman, P. M. (2007). A role for MRE11, NBS1, and recombination junctions in replication and stable maintenance of EBV episomes. *PLoS ONE* **2**, e1257.
- Doherty, J. & Freund, R. (1997). Polyomavirus large T antigen overcomes p53 dependent growth arrest. *Oncogene* **14**, 1923–1931.
- Donaldson, M. M., Boner, W. & Morgan, I. M. (2007). TopBP1 regulates human papillomavirus type 16 E2 interaction with chromatin. *J Virol* **81**, 4338–4342.
- Dornreiter, I., Erdile, L. F., Gilbert, I. U., von Winkler, D., Kelly, T. J. & Fanning, E. (1992). Interaction of DNA polymerase alpha-primase with cellular replication protein A and SV40 T antigen. *EMBO J* **11**, 769–776.
- Doucas, V., Ishov, A. M., Romo, A., Juguilon, H., Weitzman, M. D., Evans, R. M. & Maul, G. G. (1996). Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. *Genes Dev* **10**, 196–207.
- Duensing, S. & Münger, K. (2002). The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability. *Cancer Res* **62**, 7075–7082.
- E, X., Pickering, M. T., Debatis, M., Castillo, J., Lagadinos, A., Wang, S., Lu, S. & Kowalik, T. F. (2011). An E2F1-mediated DNA damage response contributes to the replication of human cytomegalovirus. *PLoS Pathog* **7**, e1001342.
- Evans, J. D. & Hearing, P. (2005). Relocalization of the Mre11-Rad50-Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication. *J Virol* **79**, 6207–6215.
- Everett, R. D. (2000). ICP0, a regulator of herpes simplex virus during lytic and latent infection. *Bioessays* **22**, 761–770.
- Everett, R. D., Freemont, P., Saitoh, H., Dasso, M., Orr, A., Kathoria, M. & Parkinson, J. (1998). The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. *J Virol* **72**, 6581–6591.
- Fanning, E. & Zhao, K. (2009). SV40 DNA replication: from the A gene to a nanomachine. *Virology* **384**, 352–359.
- Fanning, E., Klimovich, V. & Nager, A. R. (2006). A dynamic model for replication protein A (RPA) function in DNA processing pathways. *Nucleic Acids Res* **34**, 4126–4137.

- Feederle, R., Mehl-Lautscham, A. M., Bannert, H. & Delecluse, H. J. (2009a). The Epstein-Barr virus protein kinase BGLF4 and the exonuclease BGLF5 have opposite effects on the regulation of viral protein production. *J Virol* **83**, 10877–10891.
- Feederle, R., Bannert, H., Lips, H., Müller-Lantzsch, N. & Delecluse, H. J. (2009b). The Epstein-Barr virus alkaline exonuclease BGLF5 serves pleiotropic functions in virus replication. *J Virol* **83**, 4952–4962.
- Feitelson, M. A., Zhu, M., Duan, L. X. & London, W. T. (1993). Hepatitis B x antigen and p53 are associated *in vitro* and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene* **8**, 1109–1117.
- Flores, E. R. & Lambert, P. F. (1997). Evidence for a switch in the mode of human papillomavirus type 16 DNA replication during the viral life cycle. *J Virol* **71**, 7167–7179.
- Forrester, N. A., Sedgwick, G. G., Thomas, A., Blackford, A. N., Speiseder, T., Dobner, T., Byrd, P. J., Stewart, G. S., Turnell, A. S. & Grand, R. J. (2011). Serotype-specific inactivation of the cellular DNA damage response during adenovirus infection. *J Virol* **85**, 2201–2211.
- Forrester, N. A., Patel, R. N., Speiseder, T., Groitl, P., Sedgwick, G. G., Shimwell, N. J., Seed, R. I., Catnaigh, P. Ó., McCabe, C. J. & other authors (2012). Adenovirus E4orf3 targets transcriptional intermediary factor 1 γ for proteasome-dependent degradation during infection. *J Virol* **86**, 3167–3179.
- Fortunato, E. A. & Spector, D. H. (2003). Viral induction of site-specific chromosome damage. *Rev Med Virol* **13**, 21–37.
- Fortunato, E. A., McElroy, A. K., Sanchez, I. & Spector, D. H. (2000). Exploitation of cellular signaling and regulatory pathways by human cytomegalovirus. *Trends Microbiol* **8**, 111–119.
- Fradet-Turcotte, A., Bergeron-Labrecque, F., Moody, C. A., Lehoux, M., Laimins, L. A. & Archambault, J. (2011). Nuclear accumulation of the papillomavirus E1 helicase blocks S-phase progression and triggers an ATM-dependent DNA damage response. *J Virol* **85**, 8996–9012.
- Fragkos, M., Jurvansuu, J. & Beard, P. (2009). H2AX is required for cell cycle arrest via the p53/p21 pathway. *Mol Cell Biol* **29**, 2828–2840.
- Friborg, J., Jr, Kong, W., Hottiger, M. O. & Nabel, G. J. (1999). p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature* **402**, 889–894.
- Frisch, S. M. & Mymryk, J. S. (2002). Adenovirus-5 E1A: paradox and paradigm. *Nat Rev Mol Cell Biol* **3**, 441–452.
- Gallimore, P. H. & Turnell, A. S. (2001). Adenovirus E1A: remodelling the host cell, a life or death experience. *Oncogene* **20**, 7824–7835.
- Gaspar, M. & Shenk, T. (2006). Human cytomegalovirus inhibits a DNA damage response by mislocalizing checkpoint proteins. *Proc Natl Acad Sci U S A* **103**, 2821–2826.
- Geoffroy, M. C. & Salvetti, A. (2005). Helper functions required for wild type and recombinant adeno-associated virus growth. *Curr Gene Ther* **5**, 265–271.
- Grand, R. J., Grant, M. L. & Gallimore, P. H. (1994). Enhanced expression of p53 in human cells infected with mutant adenoviruses. *Virology* **203**, 229–240.
- Gregory, D. A. & Bachenheimer, S. L. (2008). Characterization of mre11 loss following HSV-1 infection. *Virology* **373**, 124–136.
- Gruhne, B., Sompallae, R. & Masucci, M. G. (2009a). Three Epstein-Barr virus latency proteins independently promote genomic instability by inducing DNA damage, inhibiting DNA repair and inactivating cell cycle checkpoints. *Oncogene* **28**, 3997–4008.
- Gruhne, B., Kamranvar, S. A., Masucci, M. G. & Sompallae, R. (2009b). EBV and genomic instability – a new look at the role of the virus in the pathogenesis of Burkitt's lymphoma. *Semin Cancer Biol* **19**, 394–400.
- Han, I., Harada, S., Weaver, D., Xue, Y., Lane, W., Orstavik, S., Skalhegg, B. & Kieff, E. (2001). EBNA-LP associates with cellular proteins including DNA-PK and HA95. *J Virol* **75**, 2475–2481.
- Harada, J. N., Shevchenko, A., Shevchenko, A., Pallas, D. C. & Berk, A. J. (2002). Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. *J Virol* **76**, 9194–9206.
- Hart, L. S., Yannone, S. M., Naczki, C., Orlando, J. S., Waters, S. B., Akman, S. A., Chen, D. J., Ornelles, D. & Koumenis, C. (2005). The adenovirus E4orf6 protein inhibits DNA double strand break repair and radiosensitizes human tumor cells in an E1B-55K-independent manner. *J Biol Chem* **280**, 1474–1481.
- Hart, L. S., Ornelles, D. & Koumenis, C. (2007). The adenoviral E4orf6 protein induces atypical apoptosis in response to DNA damage. *J Biol Chem* **282**, 6061–6067.
- Hartmann, M. & Brunnemann, H. (1972). Chromosome aberrations in cytomegalovirus-infected human diploid cell culture. *Acta Virol* **16**, 176.
- Haviv, I., Vaizel, D. & Shaul, Y. (1996). pX, the HBV-encoded coactivator, interacts with components of the transcription machinery and stimulates transcription in a TAF-independent manner. *EMBO J* **15**, 3413–3420.
- Hein, J., Boichuk, S., Wu, J., Cheng, Y., Freire, R., Jat, P. S., Roberts, T. M. & Gjoerup, O. V. (2009). Simian virus 40 large T antigen disrupts genome integrity and activates a DNA damage response via Bub1 binding. *J Virol* **83**, 117–127.
- Hoskins, E. E., Morris, T. A., Higginbotham, J. M., Spardy, N., Cha, E., Kelly, P., Williams, D. A., Wikenheiser-Brokamp, K. A., Duensing, S. & Wells, S. I. (2009). Fanconi anemia deficiency stimulates HPV-associated hyperplastic growth in organotypic epithelial raft culture. *Oncogene* **28**, 674–685.
- Inagaki, K., Ma, C., Storm, T. A., Kay, M. A. & Nakai, H. (2007). The role of DNA-PKcs and artemis in opening viral DNA hairpin termini in various tissues in mice. *J Virol* **81**, 11304–11321.
- Jault, F. M., Jault, J.-M., Ruchti, F., Fortunato, E. A., Clark, C., Corbeil, J., Richman, D. D. & Spector, D. H. (1995). Cytomegalovirus infection induces high levels of cyclins, phosphorylated Rb, and p53, leading to cell cycle arrest. *J Virol* **69**, 6697–6704.
- Jaitovich-Groisman, I. J., Koshy, R., Henkler, F., Groopman, J. D. & Alaoui-Jamali, M. A. (1999). Downregulation of DNA excision repair by the hepatitis B virus-x protein occurs in p53-proficient and p53-deficient cells. *Carcinogenesis* **20**, 479–483.
- Jaitovich-Groisman, I., Benlimame, N., Slagle, B. L., Perez, M. H., Alpert, L., Song, D. J., Fotouhi-Ardakani, N., Galipeau, J. & Alaoui-Jamali, M. A. (2001). Transcriptional regulation of the TFIIF transcription repair components XPB and XPD by the hepatitis B virus x protein in liver cells and transgenic liver tissue. *J Biol Chem* **276**, 14124–14132.
- Jia, L., Wang, X. W. & Harris, C. C. (1999). Hepatitis B virus X protein inhibits nucleotide excision repair. *Int J Cancer* **80**, 875–879.
- Johnson, P. A., Miyahara, A., Levine, F., Cahill, T. & Friedmann, T. (1992). Cytotoxicity of a replication-defective mutant of herpes simplex virus type 1. *J Virol* **66**, 2952–2965.
- Kadaja, M., Isok-Paas, H., Laos, T., Ustav, E. & Ustav, M. (2009). Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. *PLoS Pathog* **5**, e1000397.
- Kalejta, R. F. & Shenk, T. (2003). The human cytomegalovirus UL82 gene product (pp71) accelerates progression through the G1 phase of the cell cycle. *J Virol* **77**, 3451–3459.
- Kalejta, R. F., Bechtel, J. T. & Shenk, T. (2003). Human cytomegalovirus pp71 stimulates cell cycle progression by inducing the proteasome-dependent degradation of the retinoblastoma family of tumor suppressors. *Mol Cell Biol* **23**, 1885–1895.

- Kamranvar, S. A., Gruhne, B., Szeles, A. & Masucci, M. G. (2007).** Epstein-Barr virus promotes genomic instability in Burkitt's lymphoma. *Oncogene* **26**, 5115–5123.
- Kersey, J. H., Gatti, R. A., Good, R. A., Aaronson, S. A. & Todaro, G. J. (1972).** Susceptibility of cells from patients with primary immunodeficiency diseases to transformation by simian virus 40. *Proc Natl Acad Sci U S A* **69**, 980–982.
- King, L. E., Fisk, J. C., Dornan, E. S., Donaldson, M. M., Melendy, T. & Morgan, I. M. (2010).** Human papillomavirus E1 and E2 mediated DNA replication is not arrested by DNA damage signalling. *Virology* **406**, 95–102.
- Knipe, D. M. & Cliffe, A. (2008).** Chromatin control of herpes simplex virus lytic and latent infection. *Nat Rev Microbiol* **6**, 211–221.
- Koopal, S., Furuhejelm, J. H., Järviuoma, A., Jäämaa, S., Pyakurel, P., Pussinen, C., Wirzenius, M., Biberfeld, P., Alitalo, K. & other authors (2007).** Viral oncogene-induced DNA damage response is activated in Kaposi sarcoma tumorigenesis. *PLoS Pathog* **3**, 1348–1360.
- Kudoh, A., Fujita, M., Zhang, L., Shirata, N., Daikoku, T., Sugaya, Y., Isomura, H., Nishiyama, Y. & Tsurumi, T. (2005).** Epstein-Barr virus lytic replication elicits ATM checkpoint signal transduction while providing an S-phase-like cellular environment. *J Biol Chem* **280**, 8156–8163.
- Kudoh, A., Iwahori, S., Sato, Y., Nakayama, S., Isomura, H., Murata, T. & Tsurumi, T. (2009).** Homologous recombinational repair factors are recruited and loaded onto the viral DNA genome in Epstein-Barr virus replication compartments. *J Virol* **83**, 6641–6651.
- Kulkarni, A. S. & Fortunato, E. A. (2011).** Stimulation of homology-directed repair at I-SceI-induced DNA breaks during the permissive life cycle of human cytomegalovirus. *J Virol* **85**, 6049–6054.
- Lacoste, S., Wiechec, E., Dos Santos Silva, A. G., Guffei, A., Williams, G., Lowbeer, M., Benedek, K., Henriksson, M., Klein, G. & Mai, S. (2010).** Chromosomal rearrangements after ex vivo Epstein-Barr virus (EBV) infection of human B cells. *Oncogene* **29**, 503–515.
- Lee, S. G. & Rho, H. M. (2000).** Transcriptional repression of the human p53 gene by hepatitis B viral X protein. *Oncogene* **19**, 468–471.
- Lee, T. H., Elledge, S. J. & Butel, J. S. (1995).** Hepatitis B virus X protein interacts with a probable cellular DNA repair protein. *J Virol* **69**, 1107–1114.
- Leppard, K. N. & Everett, R. D. (1999).** The adenovirus type 5 E1b 55K and E4 Orf3 proteins associate in infected cells and affect ND10 components. *J Gen Virol* **80**, 997–1008.
- Li, D., Tian, Y., Ma, Y. & Benjamin, T. (2004).** p150(Sal2) is a p53-independent regulator of p21(WAF1/CIP). *Mol Cell Biol* **24**, 3885–3893.
- Li, H., Baskaran, R., Krisky, D. M., Bein, K., Grandi, P., Cohen, J. B. & Glorioso, J. C. (2008).** Chk2 is required for HSV-1 ICP0-mediated G2/M arrest and enhancement of virus growth. *Virology* **375**, 13–23.
- Li, T., Robert, E. I., van Breugel, P. C., Strubin, M. & Zheng, N. (2010).** A promiscuous α -helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. *Nat Struct Mol Biol* **17**, 105–111.
- Li, R., Zhu, J., Xie, Z., Liao, G., Liu, J., Chen, M. R., Hu, S., Woodard, C., Lin, J. & other authors (2011).** Conserved herpesvirus kinases target the DNA damage response pathway and TIP60 histone acetyltransferase to promote virus replication. *Cell Host Microbe* **10**, 390–400.
- Lilley, C. E., Carson, C. T., Muotri, A. R., Gage, F. H. & Weitzman, M. D. (2005).** DNA repair proteins affect the lifecycle of herpes simplex virus 1. *Proc Natl Acad Sci U S A* **102**, 5844–5849.
- Lilley, C. E., Schwartz, R. A. & Weitzman, M. D. (2007).** Using or abusing: viruses and the cellular DNA damage response. *Trends Microbiol* **15**, 119–126.
- Lilley, C. E., Chaurushiya, M. S., Boutell, C., Landry, S., Suh, J., Panier, S., Everett, R. D., Stewart, G. S., Durocher, D. & Weitzman, M. D. (2010).** A viral E3 ligase targets RNF8 and RNF168 to control histone ubiquitination and DNA damage responses. *EMBO J* **29**, 943–955.
- Lilley, C. E., Chaurushiya, M. S., Boutell, C., Everett, R. D. & Weitzman, M. D. (2011).** The intrinsic antiviral defense to incoming HSV-1 genomes includes specific DNA repair proteins and is counteracted by the viral protein ICP0. *PLoS Pathog* **7**, e1002084.
- Linden, R. M. & Berns, K. I. (2000).** Molecular biology of adeno-associated viruses. *Contrib Microbiol* **4**, 68–84.
- Lischka, P., Sorg, G., Kann, M., Winkler, M. & Stamminger, T. (2003).** A nonconventional nuclear localization signal within the UL84 protein of human cytomegalovirus mediates nuclear import via the importin α/β pathway. *J Virol* **77**, 3734–3748.
- Liu, Y., Shevchenko, A., Shevchenko, A. & Berk, A. J. (2005).** Adenovirus exploits the cellular aggresome response to accelerate inactivation of the MRN complex. *J Virol* **79**, 14004–14016.
- Lombard, D. B. & Guarente, L. (2000).** Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres. *Cancer Res* **60**, 2331–2334.
- Lüleci, G., Sakizli, M. & Günalp, A. (1980).** Selective chromosomal damage caused by human cytomegalovirus. *Acta Virol* **24**, 341–345.
- Luo, K., Ehrlich, E., Xiao, Z., Zhang, W., Ketner, G. & Yu, X. F. (2007a).** Adenovirus E4orf6 assembles with Cullin5-ElonginB-ElonginC E3 ubiquitin ligase through an HIV/SIV Vif-like BC-box to regulate p53. *FASEB J* **21**, 1742–1750.
- Luo, M. H., Rosenke, K., Czornak, K. & Fortunato, E. A. (2007b).** Human cytomegalovirus disrupts both ataxia telangiectasia mutated protein (ATM)- and ATM-Rad3-related kinase-mediated DNA damage responses during lytic infection. *J Virol* **81**, 1934–1950.
- Martin-Lluesma, S., Schaeffer, C., Robert, E. I., van Breugel, P. C., Leupin, O., Hantz, O. & Strubin, M. (2008).** Hepatitis B virus X protein affects S phase progression leading to chromosome segregation defects by binding to damaged DNA binding protein 1. *Hepatology* **48**, 1467–1476.
- Maser, R. S. & DePinho, R. A. (2004).** Telomeres and the DNA damage response: why the fox is guarding the henhouse. *DNA Repair (Amst)* **3**, 979–988.
- Matsuda, Y. & Ichida, T. (2009).** Impact of hepatitis B virus X protein on the DNA damage response during hepatocarcinogenesis. *Med Mol Morphol* **42**, 138–142.
- McBride, A. A. (2008).** Replication and partitioning of papillomavirus genomes. *Adv Virus Res* **72**, 155–205.
- McDougall, J. K. (1970).** Effects of adenoviruses on the chromosomes of normal human cells and cells trisomic for an E chromosome. *Nature* **225**, 456–458.
- McDougall, J. K. (1971a).** Adenovirus-induced chromosome aberrations in human cells. *J Gen Virol* **12**, 43–51.
- McDougall, J. K. (1971b).** Spontaneous and adenovirus type 12-induced chromosome aberrations in Fanconi's anaemia fibroblasts. *Int J Cancer* **7**, 526–534.
- McElroy, A. K., Dwarakanath, R. S. & Spector, D. H. (2000).** Dysregulation of cyclin E gene expression in human cytomegalovirus-infected cells requires viral early gene expression and is associated with changes in the Rb-related protein p130. *J Virol* **74**, 4192–4206.
- Melendy, T. & Stillman, B. (1993).** An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication. *J Biol Chem* **268**, 3389–3395.

- Mincheva, A., Dundarov, S. & Bradvarova, I. (1984).** Effects of herpes simplex virus strains on human fibroblast and lymphocyte chromosomes and the localization of chromosomal aberrations. *Acta Virol* **28**, 97–106.
- Mohni, K. N., Livingston, C. M., Cortez, D. & Weller, S. K. (2010).** ATR and ATRIP are recruited to herpes simplex virus type 1 replication compartments even though ATR signaling is disabled. *J Virol* **84**, 12152–12164.
- Moody, C. A. & Laimins, L. A. (2009).** Human papillomaviruses activate the ATM DNA damage pathway for viral genome amplification upon differentiation. *PLoS Pathog* **5**, e1000605.
- Moody, C. A. & Laimins, L. A. (2010).** Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer* **10**, 550–560.
- Nag, A., Datta, A., Yoo, K., Bhattacharyya, D., Chakraborty, A., Wang, X., Slagle, B. L., Costa, R. H. & Raychaudhuri, P. (2001).** DDB2 induces nuclear accumulation of the hepatitis B virus X protein independently of binding to DDB1. *J Virol* **75**, 10383–10392.
- Nakamura, H., Li, M., Zarycki, J. & Jung, J. U. (2001).** Inhibition of p53 tumor suppressor by viral interferon regulatory factor. *J Virol* **75**, 7572–7582.
- Neel, J. V., Major, E. O., Awa, A. A., Glover, T., Burgess, A., Traub, R., Curfman, B. & Satoh, C. (1996).** Hypothesis: “Rogue cell”-type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncogenesis. *Proc Natl Acad Sci U S A* **93**, 2690–2695.
- Nichols, W. W. (1983).** Viral interactions with the mammalian genome relevant to neoplasia. In *Chromosome Mutation and Neoplasia*, pp. 317–332. Edited by J. German. New York: Alan R. Liss.
- Nichols, W. W., Bradt, C. I., Toji, L. H., Godley, M. & Segawa, M. (1978).** Induction of sister chromatid exchanges by transformation with simian virus 40. *Cancer Res* **38**, 960–964.
- Nikitin, P. A., Yan, C. M., Forte, E., Bocedi, A., Tourigny, J. P., White, R. E., Allday, M. J., Patel, A., Dave, S. S. & other authors (2010).** An ATM/Chk2-mediated DNA damage-responsive signaling pathway suppresses Epstein-Barr virus transformation of primary human B cells. *Cell Host Microbe* **8**, 510–522.
- Noon, A. T., Shibata, A., Rief, N., Löbrich, M., Stewart, G. S., Jeggo, P. A. & Goodarzi, A. A. (2010).** 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. *Nat Cell Biol* **12**, 177–184.
- O’Neill, F. J. & Miles, C. P. (1969).** Chromosome changes in human cells induced by herpes simplex, types 1 and 2. *Nature* **223**, 851–852.
- Orazio, N. I., Naeger, C. M., Karlseeder, J. & Weitzman, M. D. (2011).** The adenovirus E1b55K/E4orf6 complex induces degradation of the Bloom helicase during infection. *J Virol* **85**, 1887–1892.
- Park, J. W., Pitot, H. C., Strati, K., Spardy, N., Duensing, S., Grompe, M. & Lambert, P. F. (2010).** Deficiencies in the Fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer. *Cancer Res* **70**, 9959–9968.
- Parker, G. A., Toutou, R. & Allday, M. J. (2000).** Epstein-Barr virus EBNA3C can disrupt multiple cell cycle checkpoints and induce nuclear division divorced from cytokinesis. *Oncogene* **19**, 700–709.
- Parkinson, J., Lees-Miller, S. P. & Everett, R. D. (1999).** Herpes simplex virus type 1 immediate-early protein vmw110 induces the proteasome-dependent degradation of the catalytic subunit of DNA-dependent protein kinase. *J Virol* **73**, 650–657.
- Peat, D. S. & Stanley, M. A. (1986).** Chromosome damage induced by herpes simplex virus type 1 in early infection. *J Gen Virol* **67**, 2273–2277.
- Pett, M. R., Herdman, M. T., Palmer, R. D., Yeo, G. S., Shivji, M. K., Stanley, M. A. & Coleman, N. (2006).** Selection of cervical keratinocytes containing integrated HPV16 associates with episome loss and an endogenous antiviral response. *Proc Natl Acad Sci U S A* **103**, 3822–3827.
- Polo, S. E., Blackford, A. N., Chapman, J. R., Baskcomb, L., Gravel, S., Rusch, A., Thomas, A., Blundred, R., Smith, P. & other authors (2012).** Regulation of DNA-end resection by hnRNPU-like proteins promotes DNA double-strand break signaling and repair. *Mol Cell* **45**, 505–516.
- Poma, E. E., Kowalik, T. F., Zhu, L., Sinclair, J. H. & Huang, E. S. (1996).** The human cytomegalovirus IE1-72 protein interacts with the cellular p107 protein and relieves p107-mediated transcriptional repression of an E2F-responsive promoter. *J Virol* **70**, 7867–7877.
- Qadri, I., Maguire, H. F. & Siddiqui, A. (1995).** Hepatitis B virus transactivator protein X interacts with the TATA-binding protein. *Proc Natl Acad Sci U S A* **92**, 1003–1007.
- Qadri, I., Conaway, J. W., Conaway, R. C., Schaack, J. & Siddiqui, A. (1996).** Hepatitis B virus transactivator protein, HBx, associates with the components of TFIIF and stimulates the DNA helicase activity of TFIIF. *Proc Natl Acad Sci U S A* **93**, 10578–10583.
- Qadri, I., Fatima, K. & Abdel-Hafiz, H. (2011).** Hepatitis B virus X protein impedes the DNA repair via its association with transcription factor, TFIIF. *BMC Microbiol* **11**, 48.
- Querido, E., Marcellus, R. C., Lai, A., Charbonneau, R., Teodoro, J. G., Ketner, G. & Branton, P. E. (1997).** Regulation of p53 levels by the E1B 55-kilodalton protein and E4orf6 in adenovirus-infected cells. *J Virol* **71**, 3788–3798.
- Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W. G., Conaway, R. C., Conaway, J. W. & Branton, P. E. (2001).** Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes Dev* **15**, 3104–3117.
- Reiss, K., Khalili, K., Giordano, A. & Trojanek, J. (2006).** JC virus large T-antigen and IGF-I signaling system merge to affect DNA repair and genomic integrity. *J Cell Physiol* **206**, 295–300.
- Ren, B., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A. & Dynlacht, B. D. (2002).** E2F integrates cell cycle progression with DNA repair, replication, and G₂/M checkpoints. *Genes Dev* **16**, 245–256.
- Ricciardiello, L., Baglioni, M., Giovannini, C., Pariali, M., Cenacchi, G., Ripalti, A., Landini, M. P., Sawa, H., Nagashima, K. & other authors (2003).** Induction of chromosomal instability in colonic cells by the human polyomavirus JC virus. *Cancer Res* **63**, 7256–7262.
- Rogoff, H. A. & Kowalik, T. F. (2004).** Life, death and E2F: linking proliferation control and DNA damage signaling via E2F1. *Cell Cycle* **3**, 845–846.
- Roizman, B., Knipe, D. M. & Whitley, R. J. (2007).** Herpes simplex viruses. In *Fields Virology*, 5th edn, pp. 2501–2602. Edited by D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott Williams & Wilkins.
- Rooney, C. M., Rowe, D. T., Ragot, T. & Farrell, P. J. (1989).** The spliced BZLF1 gene of Epstein-Barr virus (EBV) transactivates an early EBV promoter and induces the virus productive cycle. *J Virol* **63**, 3109–3116.
- Rowe, M., Glaunsinger, B., van Leeuwen, D., Zuo, J., Sweetman, D., Ganem, D., Middeldorp, J., Wiertz, E. J. & Rensing, M. E. (2007).** Host shutoff during productive Epstein-Barr virus infection is mediated by BGLF5 and may contribute to immune evasion. *Proc Natl Acad Sci U S A* **104**, 3366–3371.
- Sakakibara, N., Mitra, R. & McBride, A. A. (2011).** The papillomavirus E1 helicase activates a cellular DNA damage response in viral replication foci. *J Virol* **85**, 8981–8995.
- Sakizli, M., Lüleci, G. & Günalp, A. (1981).** Effect of cytomegalovirus on adult human chromosomes. *Acta Virol* **25**, 248–250.

- Sato, Y., Kamura, T., Shirata, N., Murata, T., Kudoh, A., Iwahori, S., Nakayama, S., Isomura, H., Nishiyama, Y. & Tsurumi, T. (2009a). Degradation of phosphorylated p53 by viral protein-ECS E3 ligase complex. *PLoS Pathog* 5, e1000530.
- Sato, Y., Shirata, N., Kudoh, A., Iwahori, S., Nakayama, S., Murata, T., Isomura, H., Nishiyama, Y. & Tsurumi, T. (2009b). Expression of Epstein-Barr virus BZLF1 immediate-early protein induces p53 degradation independent of MDM2, leading to repression of p53-mediated transcription. *Virology* 388, 204–211.
- Scheffner, M., Werness, B. A., Huijbregtse, J. M., Levine, A. J. & Howley, P. M. (1990). The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63, 1129–1136.
- Schreiner, S., Wimmer, P., Sirma, H., Everett, R. D., Blanchette, P., Groitt, P. & Dobner, T. (2010). Proteasome-dependent degradation of Daxx by the viral E1B-55K protein in human adenovirus-infected cells. *J Virol* 84, 7029–7038.
- Schreiner, S., Wimmer, P. & Dobner, T. (2012). Adenovirus degradation of cellular proteins. *Future Microbiol* 7, 211–225.
- Schwartz, R. A., Palacios, J. A., Cassell, G. D., Adam, S., Giacca, M. & Weitzman, M. D. (2007). The Mre11/Rad50/Nbs1 complex limits adeno-associated virus transduction and replication. *J Virol* 81, 12936–12945.
- Schwartz, R. A., Carson, C. T., Schubert, C. & Weitzman, M. D. (2009). Adeno-associated virus replication induces a DNA damage response coordinated by DNA-dependent protein kinase. *J Virol* 83, 6269–6278.
- Seo, T., Park, J., Lee, D., Hwang, S. G. & Choe, J. (2001). Viral interferon regulatory factor 1 of Kaposi's sarcoma-associated herpesvirus binds to p53 and represses p53-dependent transcription and apoptosis. *J Virol* 75, 6193–6198.
- Shen, Y., Zhu, H. & Shen, T. (1997). Human cytomegalovirus IE1 and IE2 proteins are mutagenic and mediate "hit-and-run" oncogenic transformation in cooperation with the adenovirus E1A proteins. *Proc Natl Acad Sci U S A* 94, 3341–3345.
- Shen, Y. H., Utama, B., Wang, J., Raveendran, M., Senthil, D., Waldman, W. J., Belcher, J. D., Vercellotti, G., Martin, D. & other authors (2004). Human cytomegalovirus causes endothelial injury through the ataxia telangiectasia mutant and p53 DNA damage signaling pathways. *Circ Res* 94, 1310–1317.
- Shi, Y., Dodson, G. E., Shaikh, S., Rundell, K. & Tibbetts, R. S. (2005). Ataxia-telangiectasia-mutated (ATM) is a T-antigen kinase that controls SV40 viral replication *in vivo*. *J Biol Chem* 280, 40195–40200.
- Shin, Y. C., Nakamura, H., Liang, X., Feng, P., Chang, H., Kowalik, T. F. & Jung, J. U. (2006). Inhibition of the ATM/p53 signal transduction pathway by Kaposi's sarcoma-associated herpesvirus interferon regulatory factor 1. *J Virol* 80, 2257–2266.
- Shirata, N., Kudoh, A., Daikoku, T., Tatsumi, Y., Fujita, M., Kiyono, T., Sugaya, Y., Isomura, H., Ishizaki, K. & Tsurumi, T. (2005). Activation of ataxia telangiectasia-mutated DNA damage checkpoint signal transduction elicited by herpes simplex virus infection. *J Biol Chem* 280, 30336–30341.
- Sitterlin, D., Lee, T. H., Prigent, S., Tiollais, P., Butel, J. S. & Transy, C. (1997). Interaction of the UV-damaged DNA-binding protein with hepatitis B virus X protein is conserved among mammalian hepadnaviruses and restricted to transactivation-proficient X-insertion mutants. *J Virol* 71, 6194–6199.
- Sitterlin, D., Bergametti, F. & Transy, C. (2000a). UVDDDB p127-binding modulates activities and intracellular distribution of hepatitis B virus X protein. *Oncogene* 19, 4417–4426.
- Sitterlin, D., Bergametti, F., Tiollais, P., Tennant, B. C. & Transy, C. (2000b). Correct binding of viral X protein to UVDDDB-p127 cellular protein is critical for efficient infection by hepatitis B viruses. *Oncogene* 19, 4427–4431.
- Song, S., Lu, Y., Choi, Y. K., Han, Y., Tang, Q., Zhao, G., Berns, K. I. & Flotte, T. R. (2004). DNA-dependent PK inhibits adeno-associated virus DNA integration. *Proc Natl Acad Sci U S A* 101, 2112–2116.
- Soria, C., Estermann, F. E., Espantman, K. C. & O'Shea, C. C. (2010). Heterochromatin silencing of p53 target genes by a small viral protein. *Nature* 466, 1076–1081.
- Sparby, N., Duensing, A., Charles, D., Haines, N., Nakahara, T., Lambert, P. F. & Duensing, S. (2007). The human papillomavirus type 16 E7 oncoprotein activates the Fanconi anemia (FA) pathway and causes accelerated chromosomal instability in FA cells. *J Virol* 81, 13265–13270.
- Sparby, N., Covella, K., Cha, E., Hoskins, E. E., Wells, S. I., Duensing, A. & Duensing, S. (2009). Human papillomavirus 16 E7 oncoprotein attenuates DNA damage checkpoint control by increasing the proteolytic turnover of claspin. *Cancer Res* 69, 7022–7029.
- Steegenga, W. T., Riteco, N., Jochemsen, A. G., Fallaux, F. J. & Bos, J. L. (1998). The large E1B protein together with the E4orf6 protein target p53 for active degradation in adenovirus infected cells. *Oncogene* 16, 349–357.
- Stewart, G. S., Panier, S., Townsend, K., Al-Hakim, A. K., Kolas, N. K., Miller, E. S., Nakada, S., Ylanko, J., Olivarius, S. & other authors (2009). The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 136, 420–434.
- Stich, H. F., Hsu, T. C. & Rapp, F. (1964). Viruses and mammalian chromosomes. I. Localization of chromosome aberrations after infection with herpes simplex virus. *Virology* 22, 439–445.
- Stracker, T. H., Carson, C. T. & Weitzman, M. D. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* 418, 348–352.
- Stracker, T. H., Lee, D. V., Carson, C. T., Araujo, F. D., Ornelles, D. A. & Weitzman, M. D. (2005). Serotype-specific reorganization of the Mre11 complex by adenoviral E4orf3 proteins. *J Virol* 79, 6664–6673.
- Tarakanova, V. L., Leung-Pineda, V., Hwang, S., Yang, C. W., Matatal, K., Basson, M., Sun, R., Piwnicka-Worms, H., Sleckman, B. P. & Virgin, H. W., IV (2007). γ -Herpesvirus kinase actively initiates a DNA damage response by inducing phosphorylation of H2AX to foster viral replication. *Cell Host Microbe* 1, 275–286.
- Theile, M. & Grabowski, G. (1990). Mutagenic activity of BKV and JCV in human and other mammalian cells. *Arch Virol* 113, 221–233.
- Todaro, G. J., Green, H. & Swift, M. R. (1966). Susceptibility of human diploid fibroblast strains to transformation by SV40 virus. *Science* 153, 1252–1254.
- Trojanek, J., Ho, T., Del Valle, L., Nowicki, M., Wang, J. Y., Lassak, A., Peruzzi, F., Khalili, K., Skorski, T. & Reiss, K. (2003). Role of the insulin-like growth factor I/insulin receptor substrate 1 axis in Rad51 trafficking and DNA repair by homologous recombination. *Mol Cell Biol* 23, 7510–7524.
- Trojanek, J., Croul, S., Ho, T., Wang, J. Y., Darbinyan, A., Nowicki, M., Del Valle, L., Skorski, T., Khalili, K. & Reiss, K. (2006). T-antigen of the human polyomavirus JC attenuates faithful DNA repair by forcing nuclear interaction between IRS-1 and Rad51. *J Cell Physiol* 206, 35–46.
- Wade, M. & Ailday, M. J. (2000). Epstein-Barr virus suppresses a G₂/M checkpoint activated by genotoxins. *Mol Cell Biol* 20, 1344–1360.
- Wade, M., Kowalik, T. F., Mudryj, M., Huang, E. S. & Azizkhan, J. C. (1992). E2F mediates dihydrofolate reductase promoter activation and multiprotein complex formation in human cytomegalovirus infection. *Mol Cell Biol* 12, 4364–4374.

- Wahren, B., Lampert, F. & Goetz, O. (1972).** Herpes viruses and chromosomal alterations seen with light and whole-mount electron microscopy. *Exp Cell Res* **75**, 271–274.
- Wang, X. W., Forrester, K., Yeh, H., Feitelson, M. A., Gu, J.-R. & Harris, C. C. (1994).** Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci U S A* **91**, 2230–2234.
- Wang, X. W., Gibson, M. K., Vermeulen, W., Yeh, H., Forrester, K., Stürzbecher, H. W., Hoeijmakers, J. H. & Harris, C. C. (1995).** Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. *Cancer Res* **55**, 6012–6016.
- Webb, T. & Harding, M. (1977).** Chromosome complement and SV40 transformation of cells from patients susceptible to malignant disease. *Br J Cancer* **36**, 583–591.
- Webb, T., Harnden, D. G. & Harding, M. (1977).** The chromosome analysis and susceptibility to transformation by Simian Virus 40 of fibroblasts from ataxia-telangiectasia. *Cancer Res* **37**, 997–1002.
- Weiden, M. D. & Ginsberg, H. S. (1994).** Deletion of the E4 region of the genome produces adenovirus DNA concatemers. *Proc Natl Acad Sci U S A* **91**, 153–157.
- Weitzman, M. D., Lilley, C. E. & Chaurushiya, M. S. (2010).** Genomes in conflict: maintaining genome integrity during virus infection. *Annu Rev Microbiol* **64**, 61–81.
- Weitzman, M. D., Lilley, C. E. & Chaurushiya, M. S. (2011).** Changing the ubiquitin landscape during viral manipulation of the DNA damage response. *FEBS Lett* **585**, 2897–2906.
- Wiebusch, L., Uecker, R. & Hagemeyer, C. (2003).** Human cytomegalovirus prevents replication licensing by inhibiting MCM loading onto chromatin. *EMBO Rep* **4**, 42–46.
- Wilkinson, D. E. & Weller, S. K. (2004).** Recruitment of cellular recombination and repair proteins to sites of herpes simplex virus type 1 DNA replication is dependent on the composition of viral proteins within prereplicative sites and correlates with the induction of the DNA damage response. *J Virol* **78**, 4783–4796.
- Winder, D. M., Pett, M. R., Foster, N., Shivji, M. K., Herdman, M. T., Stanley, M. A., Venkitaraman, A. R. & Coleman, N. (2007).** An increase in DNA double-strand breaks, induced by Ku70 depletion, is associated with human papillomavirus 16 episome loss and de novo viral integration events. *J Pathol* **213**, 27–34.
- Wu, X., Avni, D., Chiba, T., Yan, F., Zhao, Q., Lin, Y., Heng, H. & Livingston, D. (2004).** SV40 T antigen interacts with Nbs1 to disrupt DNA replication control. *Genes Dev* **18**, 1305–1316.
- Wu, C. C., Liu, M. T., Chang, Y. T., Fang, C. Y., Chou, S. P., Liao, H. W., Kuo, K. L., Hsu, S. L., Chen, Y. R. & other authors (2010).** Epstein-Barr virus DNase (BGLF5) induces genomic instability in human epithelial cells. *Nucleic Acids Res* **38**, 1932–1949.
- Yee, J., White, R. E., Anderton, E. & Allday, M. J. (2011).** Latent Epstein-Barr virus can inhibit apoptosis in B cells by blocking the induction of NOXA expression. *PLoS ONE* **6**, e28506.
- Zantema, A., Schrier, P. I., Davis-Olivier, A., van Laar, T., Vaessen, R. T. & van der EB, A. J. (1985).** Adenovirus serotype determines association and localization of the large E1B tumor antigen with cellular tumor antigen p53 in transformed cells. *Mol Cell Biol* **5**, 3084–3091.
- Zhao, X., Madden-Fuentes, R. J., Lou, B. X., Pipas, J. M., Gerhardt, J., Rigell, C. J. & Fanning, E. (2008).** Ataxia telangiectasia-mutated damage-signaling kinase- and proteasome-dependent destruction of Mre11-Rad50-Nbs1 subunits in Simian virus 40-infected primate cells. *J Virol* **82**, 5316–5328.
- Zur Hausen, H. (1967).** Induction of specific chromosomal aberrations by adenovirus type 12 in human embryonic kidney cells. *J Virol* **1**, 1174–1185.