Functions of DNA damage machinery in the innate immune response to DNA virus infection

Ben J Trigg and Brian J Ferguson*

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP UK

*Corresponding Author: bf234@cam.ac.uk

Abstract

DNA is potently immunostimulatory, and self-DNA is packaged in the nucleus or mitochondria allowing it to remain silent to cell-intrinsic sensors. However, damaged or mislocalised self-DNA is sensed by our innate immune systems, resulting in the production of type I interferons (IFNI), chemokines and inflammatory cytokines. During DNA virus infection, the detection of viral DNA genomes by pattern recognition receptors (PRRs) is essential for the initiation of IFNI responses and host defence against these pathogens. It is intriguing that a number of molecular mechanisms have been found to be common to both of these DNA-induced stress responses and this has potentially important consequences for both sides of the host/pathogen arms race.

Introduction

The type 1 interferon (IFNI) response is critical for fighting viral infection and is initiated in vertebrates by an array of genome-encoded pattern recognition receptors (PRRs) that bind and respond to the presence of pathogen-associated molecular patterns (PAMPs). PRRs have evolved to sense PAMPs and/or damage-associated molecular patterns (DAMPs), the latter being mislocalised self-molecules indicative of cellular stress or damage [1,2]. In the context of virus infection, nucleic acid PAMPs are essential for initiating both IFNI production and for the inflammatory responses that attract leukocytes to assist in mounting a complete immune response [1]. Recent discoveries of intracellular PRRs that sense foreign or mislocalised self-DNA in the cytoplasm has led to a rapid expansion of the field, and have indicated that, in addition to viral infection, the immunostimulatory activity of DNA as a PAMP or a DAMP has significant consequences for bacterial and parasite infections, autoimmunity, vaccine development and carcinogenesis [1,3,4].

There is significant sharing of machinery between the innate immune system and the systems that regulate the cellular responses to damaged self-DNA. The output responses

following detection of any cellular stress are similar, including cytokine production, cell cycle regulation and programmed cell death, and both of these systems have evolved to sense and respond to specific stresses. It might be expected, therefore, that intracellular detection of infection or damaged self-DNA would activate several common downstream signalling responses. Indeed this is the case, since both DNA virus infection and genotoxic stress result in IFNI and cytokine production via nuclear factor kappa-B (NF-κB) and interferon regulatory factor (IRF) activation [5–8], and cell death induction [9–12]. On the other hand, new discoveries that several proteins that function to repair damaged self-DNA are also PRRs that sense viral DNA may have more surprising implications for the immune response to infection and the efficiency of, and signalling responses to, DNA damage.

There remain, however, many unanswered questions that bridge these two fields. 1) What are the precise DNA ligands for innate immune sensing during virus infection or damaged self-DNA and how does this relate to the PRRs? 2) How has viral inhibition of innate responses affected both DNA sensing and the response to damaged self-DNA? 3) Is there innate sensing of viral and self DNA in the nucleus by DNA damage response proteins and/or PRRs? This review aims to discuss these questions from the perspective of sensing DNA virus infection.

Intracellular viral DNA sensing mechanisms

Normally only small amounts of self-DNA are present outside of the nucleus and mitochondria, and DNA entering other compartments is broken down by DNases such as DNaseII in endosomes and 3' repair exonuclease 1 (TREX1) in the cytoplasm. Mutation of these nucleases in mice and humans leads to systemic autoinflammation and interferonopathy driven by a build-up of mislocalised DNA [2,13–15]. It has also been observed that direct transfection of pure, naked DNA into the cytoplasm of human cells can initiate an IFNI response via the adaptor protein stimulator of interferon genes (STING) [16], TANK-binding kinase-1 (TBK1) [17] and interferon regulatory factor 3 (IRF3) [18]. These

discoveries ultimately led to the identification of PRRs for cytoplasmic DNA. DNA-dependent protein kinase (DNA-PK) [19], interferon γ-inducible protein 16 (IFI16) [20], cyclic GMP-AMP synthase (cGAS) [21], RAD50 [22], DDX41 [23] and others [24] have been shown to function in this manner (Figure 1).

The importance of intracellular DNA sensing for the immune response to DNA virus infection has been demonstrated by many studies. Infection of cells by poxviruses, herpesviruses and adenoviruses is sensed by DNA PRRs (Figure 1), and is essential for host defence against these infections [19,21,22,25–29]. This is perhaps clearest for poxviruses, such as vaccinia virus (VACV), which replicate their large DNA genome exclusively in the cytoplasm. VACV DNA is released directly into the cytoplasm following entry and secondary virion uncoating [30]. Viral DNA replication then occurs at discrete cytoplasmic sites, or 'factories', that are devoid of cellular organelles. Thousands of copies of VACV genome can accumulate in viral factories in the first six hours following infection and, although it is not clear at what stage of the entry and replication process the viral genome is first sensed, this large accumulation of foreign DNA makes an excellent target for cytoplasmic DNA PRRs that detect its presence and respond by activating IRF3-dependent IFNI [19,31]. To counteract these responses, VACV has evolved inhibitors of PRR signalling, including a protein, C16, that binds directly to a subunit of DNA-PK to inhibit its DNA sensing activities [32], as well as many others that target downstream signalling pathways [12]. Hence the use of attenuated viruses, such as modified vaccinia Ankara (MVA) whose genome still replicates in human cells, but lacks many immunomodulatory proteins, can reveal these mechanisms in great detail [19,31].

What is less clear is the location where foreign DNA is sensed during infection with nuclear replicating viruses, which encompass the majority of DNA viruses. As an example of this, herpes simplex virus 1 (HSV-1) DNA has been shown to be sensed in the cytoplasm by cGAS [21] and IFI16 (Figure 1) [20,33]. In the case of IFI16, there is also evidence that this sensing can occur in the nucleus [28,33] with IFI16 shuttling between the nucleus and

cytosol in an acetylation-dependent fashion to activate downstream signalling [34]. Since it is not currently clear as to where the exact initial detection of many viral DNAs takes place, further dissection of these responses will be required to fully understand the localisation-dependent responses of IFI16. For example, as with other nuclear replicating viruses, HSV-1 DNA accumulates in the nucleus during infection, but may also be exposed to the cytoplasmic machinery; inadvertent release of HSV-1 DNA may occur prior to capsids docking to the nuclear pore as a result of structural defects or cellular restriction factors [35]. Care must be taken with viral infection experiments as the presence of non-infectious virions in virus stocks may significantly alter immune responses in unpredictable and potentially artefactual ways. Non-infectious parainfluenza particles can contain replication-incompetent copyback genomes that are potent RNA PAMPs [36]. In the case of HSV-1, standard particle to plaque forming unit (pfu) ratios are around 10-20:1 [37], although may be higher depending on stock growth conditions. Hence non-infectious HSV-1 particles outnumber infectious virions by more than 10:1. In the case of another herpesvirus, varicella zoster virus, particle to pfu ratios of up to 40,000:1 have been measured [38]. Thus, it is possible that non-infectious DNA virus particles can provide a source of cytoplasmic DNA PAMP that acts as a PRR ligand following cell entry but in the absence of nuclear docking or DNA replication. Identification of the precise DNA ligands that activate intracellular DNA PRRs during virus infection will help to clarify this issue.

DNA damage response proteins in innate immunity

The maintenance of genomic integrity is crucial for the survival of all organisms, and so mechanisms have evolved to repair genotoxic damage. Mammalian DNA repair mechanisms consist of numerous proteins that can rapidly identify and fix a wide range of lesions, including double strand breaks (DSBs), base mismatches, and oxidative damage [39,40]. As well as recruiting the machinery that repairs specific lesions, the proteins that detect damaged self-DNA also activate signalling responses including pathways that lead to interferon production, cell cycle regulation, and programmed cell death [39]. As such there

are interesting parallels with the PRR signalling in the innate immune system and, indeed, shared machinery has been discovered between these two responses. Amongst the initial discoveries of such cross-talk was the observation that a kinase associated with co-ordination of DSB repair signalling, ataxia telangiectasia mutated (ATM), can activate inflammatory NF-κB signalling via the same kinase complex that functions downstream of PRR signalling (the inhibitor of kappa-B kinase (IKK) complex)) [7]. Other studies have confirmed the ability of genotoxic damage to activate IRF and IFN signalling, although the signalling mechanisms are not fully elucidated and may vary between cell types or DNA damaging agents [6,8] As outlined below, involvement of DSB repair proteins in the innate immune response to viral DNA is now widely appreciated.

DNA damage response proteins and cytosolic DNA-sensing

The sharing of a molecular toolbox between disparate cellular functions is relatively common [41,42]. Proteins with multiple functions are numerous, and in the main new functions evolve in existing proteins only when they are pre-disposed to the second task [43]. There is an obvious advantage for viruses to evolve multi-functional proteins in order to minimise genome sizes, but why this occurs in mammalian genomes where space is less restricted is not so clear. In any case, for DNA-binding proteins that have functions in repair of damaged self-DNA in the nucleus, the evolution of new functions in viral DNA sensing could involve adding cytoplasmic localisation or plugging into a new signalling pathway, either of which might only require a few amino-acid changes. The cytoplasmic viral DNA PRRs IFI16, DNA-PK, and RAD50, along with the negative regulator of innate DNA sensing, TREX1, all also function in DNA damage responses in the nucleus [40,44,45]. DNA-PK is a sensor and regulator of DSB repair by non-homologous end joining (NHEJ) [40]. IFI16 binds to BRCA1 and p53, and affects several signalling responses to DNA damage, including the homologous recombination repair pathway and cell-cycle checkpoint regulation [45]. Furthermore, another DNA repair protein, meiotic recombination 11 homolog A (MRE11) [46] is a putative cytoplasmic DNA PRRs, whilst X-box binding-1 protein (XBP1) is implicated in

endoplasmic reticulum (ER) stress responses, negatively regulating ER resident STING in the process, dampening DNA-induced IFNI production [47,48]. Whether MRE11 and XBP1 can also regulate innate responses to poxvirus or other DNA virus infections has not yet been addressed.

What is the ligand for DNA sensing during DNA virus infection?

There are many possible mechanisms for distinguishing self from non-self or damaged self-DNA; some clues might be gained from RNA virus-sensing, where the non-self RNA PAMPs are better defined. The intracellular RNA PRRs RIGI and MDA5 bind respectively to 3' di-or tri-phosphate dsRNA or long dsRNA species, which are generated in many RNA virus infections [49]. Hence it may be that unusual (or 'damaged') DNA structures, as well as mis-localised DNA, can be sensed by DNA PRRs.

Identifying the ligands sensed by PRRs during infection may help explain why there are multiple intracellular DNA PRRs [24]. It is possible that different sensors recognise different ligands, regulate each other's expression [49], or act together to present or process specific DNAs into appropriate ligand conformations or structures [50]. Different DDR proteins, for example, bind to different parts of DNA. The DNA-PK complex, comprising a catalytic subunit, DNA-PKcs, and the heterodimeric adaptor, Ku70/Ku80, binds the end 30 base pairs (bp) of DNA during DNA repair [51]. In comparison, IFI16 interacts along the length of DNA via its HIN domains in a sequence-independent manner [52].

The ligands of PRRs may also be identified by considering how viruses may have evolved to hide or remove them, and so it is interesting to discuss the different forms assumed by DNA viruses (Table 1). The VACV genome is linear and double-stranded, with hairpin ends and inverted terminal repeats that may attract specific DNA binding proteins, or mask the DNA ends from others. During replication VACV also forms structures such as holiday junctions, replication forks and concatenated genomes that might attract additional cytoplasmic DNA-

binding proteins [30]. The adenovirus genome is similar in structure to VACV, but smaller and nuclear [53]. In contrast, the HSV-1 genome is generated by rolling circle replication and contains a significant number of nicks, gaps and free ends that make it an attractive target for DNA repair proteins [54]. Modification of these genomic structures can result in increased or decreased immunogenicity [54] and may be a target for TREX1, which preferentially binds single-stranded DNA [55]. Viruses could also attempt to hide their DNA PAMPs from PRRs by various mechanisms. Adenoviruses coat their genome in proteins including the DNA-binding protein μ that may block PRR access [56]. VACV has an initial burst of transcription from inside its own virion allowing the DNA on incoming particles to stay hidden, at least until some immediate early PRR signalling inhibitors have been expressed [57]. Herpesvirus genomes, meanwhile, become coated in histones and perhaps use this as camouflage [58]. Although it is not fully understood how viruses evade being sensed by DNA PRRs, it is clear that they are effective at doing so.

How has viral inhibition of innate responses affected both DNA sensing and the DNA damage response?

Virus infection is a powerful driver of evolutionary selection and, in the case of proteins involved in PRR signalling pathways, selection may be driven by viral immunomodulators that inhibit these responses. Large DNA viruses make inhibitors of cytoplasmic DNA PRRs as part of the host/pathogen arms race; VACV C16 binds to Ku70/80 to inhibit its function in cytoplasmic VACV DNA sensing [32], and human cytomegalovirus (HCMV) UL83 protein binds and inhibits IFI16 [59]. It will be interesting to discover the identity of viral inhibitors of cGAS, Rad50 and other sensors that may exist in viruses or other DNA-containing pathogens. Such interactions can result in directional selection and there is evidence that DNA PRRs are being selected in this way [60,61]. There is, for example, a surprising directional selection of self-DNA repair proteins in bats [61]. The order Chiroptera are an important reservoir for numerous viruses, and so their immune system is of interest [62]. A recent study showed that many bat self-DNA repair proteins, including DNA-PK, Ku80 and

Rad50, are under high levels of directional selection. This correlates with directional selection observed in other immune proteins such as c-REL, a transcription factor of the NF-κB family [61], suggesting that viruses may be responsible for some of the selection pressures applied to proteins involved in repair of damaged self-DNA. Such pressure may influence the other functions of these PRRs, such as their ability to repair damaged host DNA. Hence it is possible that DNA virus infection over evolutionary time has influenced our ability to effectively repair DNA damage and has perhaps influenced cancer susceptibility [63].

Is there innate sensing of viral DNA in the nucleus?

Cytoplasmic viral DNA, such as that generated by poxvirus replication [19,31] or released from non-replicative herpesvirus virions [35], is sensed by PRRs and activates IRF- and NF-κB-dependent IFNI production, even if the exact location and timing of the recognition event is unknown. Conventional thought postulated that the nucleus is an immunoprivileged site, mainly because the presence of undamaged self-DNA does not stimulate an immune response, but several lines of evidence suggest that this might be incorrect. Firstly, damage to self-DNA can initiate IRF and NF-κB activation, and STING-dependent IFNI production, indicating that these signalling pathways can be initiated from the nucleus via the generation of undesirable DNA structures or species [6–8,64]. Secondly, the fact that DNA damage response proteins can act as cytoplasmic DNA PRRs suggests they might carry out similar functions in the nucleus. IFI-16, for example, may also act as a nuclear viral DNA sensor [33,65]. Finally, the presence of unusual viral DNA structures or sequences might allow scope for distinguishing them from self-DNA or chromatin, allowing self/non-self discrimination to occur inside the nucleus. For example, if free self-DNA ends are sensed by DSB machinery following genotoxic damage, and can activate an IFNI response, it is feasible that free viral DNA ends could elicit a similar response. For this reason, it is interesting that many DNA virus genomes are either circular or circularise upon entry into the nucleus. Alternative theories have been proposed for this phenomenon, for example circular

replication, but none of these are conclusive. Further work is required to understand the role of nuclear DNA damage response proteins in the sensing of viral DNA.

Conclusions

The detection of and responses to viral DNA and damaged self-DNA share some common machinery. During DNA virus infection the sensing of the genome is a critical aspect of the innate immune response, but where and how this takes place, and how viruses have evolved to counteract these responses, is not clear, particularly for the majority of DNA viruses that replicate in the nucleus. To fully understand these mechanisms requires a greater consideration of the molecular biology of the pathogens and of the ligands that are presented to the DNA sensing PRRs. The role of DNA damage response proteins in innate immunity may reflect the shared aspects of sensing and signalling from foreign and damaged-self DNA. Research in this area is likely to reveal further interesting aspects of both host and virus biology.

Acknowledgements

The authors would like to thank Dr Joanna Shisler and Dr Daniel Mansur for critical reading of this manuscript.

References

1. Mansur DS, Smith GL, Ferguson BJ: **Intracellular sensing of viral DNA by the innate immune system.** *Microbes Infect.* 2014, **16**:1002–12.

2. Ablasser A, Hertrich C, Wassermann R, Hornung V: **Nucleic acid driven sterile inflammation.** *Clin. Immunol.* 2013, **147**:207–215.

3. Kobiyama K, Jounai N, Aoshi T, Tozuka M, Takeshita F, Coban C, Ishii K: **Innate Immune Signaling by, Genetic Adjuvants for, DNA Vaccination**. *Vaccines* 2013, **1**:278–292.

4. Woo S-R, Corrales L, Gajewski TF: **Innate Immune Recognition of Cancer**. *Annu. Rev. Immunol.* 2014, **33**:150126111626001.

5. Medzhitov R: **Pattern recognition theory and the launch of modern innate immunity.** *J. Immunol.* 2013, **191**:4473–4.

6. Kim T, Kim TY, Song Y-H, Min IM, Yim J, Kim TK: **Activation of Interferon Regulatory Factor 3 in Response to DNA-damaging Agents**. *J. Biol. Chem.* 1999, **274**:30686–30689.

7. Wu Z-H, Shi Y, Tibbetts RS, Miyamoto S: **Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli.** *Science* 2006, **311**:1141–6.

8. Brzostek-Racine S, Gordon C, Van Scoy S, Reich NC: **The DNA damage response induces IFN.** *J. Immunol.* 2011, **187**:5336–45.

9. Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, Greene WC: **IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV.** *Science* 2014, **343**:428–32.

10. Wenzel M, Wunderlich M, Besch R, Poeck H, Willms S, Schwantes A, Kremer M, Sutter G, Endres S, Schmidt A, et al.: **Cytosolic DNA triggers mitochondrial apoptosis via DNA damage signaling proteins independently of AIM2 and RNA polymerase III.** *J. Immunol.* 2012, **188**:394–403.

11. Creagh EM, O'Neill L a J: **TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity**. *Trends Immunol.* 2006, **27**:352–357.

12. Smith GL, Benfield CTO, Maluquer de Motes C, Mazzon M, Ember SWJ, Ferguson BJ, Sumner RP: **Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity.** *J. Gen. Virol.* 2013, **94**:2367–92.

13. Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S: **Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA.** *Nat. Immunol.* 2005, **6**:49–56.

14. Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, Ali M, Black DN, van Bokhoven H, Brunner HG, Hamel BC, et al.: **Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutières syndrome at the AGS1 locus.** *Nat. Genet.* 2006, **38**:917–20.

15. Stetson DB, Ko JS, Heidmann T, Medzhitov R: **Trex1 Prevents Cell-Intrinsic Initiation of Autoimmunity**. *Cell* 2008, **134**:587–598.

16. Ishikawa H, Barber GN: **STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling.** *Nature* 2008, **455**:674–8.

17. Ishii KJ, Coban C, Kato H, Takahashi K, Torii Y, Takeshita F, Ludwig H, Sutter G, Suzuki K, Hemmi H, et al.: **A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA.** *Nat. Immunol.* 2006, **7**:40–48.

18. Stetson DB, Medzhitov R: **Recognition of cytosolic DNA activates an IRF3-dependent innate immune response**. *Immunity* 2006, **24**:93–103.

19. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL: **DNA-PK is a DNA sensor for IRF-3-dependent innate immunity**. *Elife* 2012, **1**:e00047–e00047.

20. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S, Sirois CM, Jin T, Latz E, Xiao TS, et al.: **IFI16 is an innate immune sensor for intracellular DNA.** *Nat. Immunol.* 2010, **11**:997–1004.

* 21. Li X-D, Wu J, Gao D, Wang H, Sun L, Chen ZJ: **Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects.** *Science* 2013, **341**:1390–4.

* This study underlined the importance of cytoplasmic viral DNA PRRs in host defence against DNA virus infection.

** 22. Roth S, Rottach A, Lotz-Havla AS, Laux V, Muschaweckh A, Gersting SW, Muntau AC, Hopfner K-P, Jin L, Vanness K, et al.: **Rad50-CARD9 interactions link cytosolic DNA sensing to IL-1β production.** *Nat. Immunol.* 2014, **15**:538–45.

** Here, a function is described for RAD50, a protein previously only known for its ability to sense damaged self-DNA, in the cytolpasmic detection of viral DNA.

23. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu Y-J: **The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells.** *Nat. Immunol.* 2011, **12**:959–65.

24. Unterholzner L: **The interferon response to intracellular DNA: why so many receptors?** *Immunobiology* 2013, **218**:1312–21.

25. Ishikawa H, Ma Z, Barber GN: **STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity.** *Nature* 2009, **461**:788–792.

26. Paludan SR, Bowie AG: **Immune sensing of DNA.** *Immunity* 2013, **38**:870–80.

27. Stein SC, Falck-Pedersen E: **Sensing adenovirus infection: activation of interferon regulatory factor 3 in RAW 264.7 cells.** *J. Virol.* 2012, **86**:4527–37.

28. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, Chandran B: **IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection.** *Cell Host Microbe* 2011, **9**:363–75.

29. Conrady CD, Zheng M, Fitzgerald KA, Liu C, Carr DJJ: **Resistance to HSV-1 infection in the epithelium resides with the novel innate sensor, IFI-16.** *Mucosal Immunol.* 2012, **5**:173–83.

30. Traktman P: **Poxvirus DNA replication**. In *DNA Replication in Eukaryofic Cells*. . Cold Spring Harbor Laboratory Pres; 1996:775–798.

31. Dai P, Wang W, Cao H, Avogadri F, Dai L, Drexler I, Joyce JA, Li X-D, Chen Z, Merghoub T, et al.: **Modified vaccinia virus Ankara triggers type I IFN production in murine conventional dendritic cells via a cGAS/STING-mediated cytosolic DNA-sensing pathway.** *PLoS Pathog.* 2014, **10**:e1003989.

** 32. Peters NE, Ferguson BJ, Mazzon M, Fahy AS, Krysztofinska E, Arribas-Bosacoma R, Pearl LH, Ren H, Smith GL: **A Mechanism for the Inhibition of DNA-PK-Mediated DNA Sensing by a Virus**. *PLoS Pathog.* 2013, **9**:e1003649.

** This paper provides an example of viral antagonism of a cellular protein that has a function in both sensing foreign DNA and in repairing damaged-seld DNA

33. Orzalli MH, DeLuca NA, Knipe DM: **Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein.** *Proc. Natl. Acad. Sci. U. S. A.* 2012, **109**:E3008–17.

34. Li T, Diner BA, Chen J, Cristea IM: **Acetylation modulates cellular distribution and DNA sensing ability of interferon-inducible protein IFI16.** *Proc. Natl. Acad. Sci. U. S. A.* 2012, **109**:10558–63.

35. Horan K, Hansen K: **Proteasomal Degradation of Herpes Simplex Virus Capsids in Macrophages Releases DNA to the Cytosol for Recognition by DNA Sensors**. *J. …* 2013, **190**:2311–2319.

36. Killip MJ, Young DF, Gatherer D, Ross CS, Short JAL, Davison AJ, Goodbourn S, Randall RE: **Deep sequencing analysis of defective genomes of parainfluenza virus 5 and their role in interferon induction.** *J. Virol.* 2013, **87**:4798–807.

37. Watson DH, Russell WC, Wildy P: **Electron microscopic particle counts on herpes virus using the phosphotungstate negative staining technique**. *Virology* 1963, **19**:250–260.

38. Carpenter JE, Henderson EP, Grose C: **Enumeration of an extremely high particle-to-PFU ratio for Varicella-zoster virus.** *J. Virol.* 2009, **83**:6917–21.

39. Jackson S, Bartek J: **The DNA-damage response in human biology and disease.** *Nature* 2009, **461**:1071–1078.

40. Lieber MR, Ma Y, Pannicke U, Schwarz K: **Mechanism and regulation of human non-homologous DNA end-joining.** *Nat. Rev. Mol. Cell Biol.* 2003, **4**:712–720.

41. Jeffery CJ: **Moonlighting proteins**. *Trends Biochem. Sci.* 1999, **24**:8–11.

42. Huberts DHEW, van der Klei IJ: **Moonlighting proteins: an intriguing mode of multitasking.** *Biochim. Biophys. Acta* 2010, **1803**:520–5.

43. Aharoni A, Gaidukov L, Khersonsky O, McQ Gould S, Roodveldt C, Tawfik DS: **The “evolvability” of promiscuous protein functions.** *Nat. Genet.* 2005, **37**:73–6.

44. Miyazaki T, Kim Y-S, Yoon J, Wang H, Suzuki T, Morse HC: **The 3’-5' DNA exonuclease TREX1 directly interacts with poly(ADP-ribose) polymerase-1 (PARP1) during the DNA damage response.** *J. Biol. Chem.* 2014, **289**:32548–58.

45. Ouchi M, Ouchi T: **Role of IFI16 in DNA damage and checkpoint.** *Front. Biosci.* 2008, **13**:236–9.

46. Kondo T, Kobayashi J, Saitoh T, Maruyama K, Ishii KJ, Barber GN, Komatsu K, Akira S, Kawai T: **DNA damage sensor MRE11 recognizes cytosolic double-stranded**

DNA and induces type I interferon by regulating STING trafficking. *Proc. Natl. Acad. Sci. U. S. A.* 2013, **110**:2969–74.

47. Mitzel DN, Lowry V, Shirali AC, Liu Y, Stout-Delgado HW: **Age-enhanced endoplasmic reticulum stress contributes to increased Atg9A inhibition of STING-mediated IFN-β production during Streptococcus pneumoniae infection.** *J. Immunol.* 2014, **192**:4273–83.

48. Chowdhury D, Beresford PJ, Zhu P, Zhang D, Sung J-S, Demple B, Perrino FW, Lieberman J: **The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death.** *Mol. Cell* 2006, **23**:133–42.

49. Goubau D, Deddouche S, Reis e Sousa C: **Cytosolic sensing of viruses.** *Immunity* 2013, **38**:855–69.

50. Yoh SM, Schneider M, Seifried J, Soonthornvacharin S, Akleh RE, Olivieri KC, De Jesus PD, Ruan C, de Castro E, Ruiz PA, et al.: **PQBP1 Is a Proximal Sensor of the cGAS-Dependent Innate Response to HIV-1.** *Cell* 2015, **161**:1293–1305.

51. Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, Phipps BM, Rambo RP, Hura GL, Pelikan M, So S, et al.: **Ku and DNA-dependent protein kinase dynamic conformations and assembly regulate DNA binding and the initial non-homologous end joining complex.** *J. Biol. Chem.* 2010, **285**:1414–23.

52. Jin T, Perry A, Jiang J, Smith P, Curry JA, Unterholzner L, Jiang Z, Horvath G, Rathinam VA, Johnstone RW, et al.: **Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor.** *Immunity* 2012, **36**:561–71.

53. Hoeben RC, Uil TG: **Adenovirus DNA replication.** *Cold Spring Harb. Perspect. Biol.* 2013, **5**:a013003.

** 54. Smith S, Reuven N, Mohni KN, Schumacher AJ, Weller SK: **Structure of the herpes simplex virus 1 genome: manipulation of nicks and gaps can abrogate infectivity and alter the cellular DNA damage response.** *J. Virol.* 2014, **88**:10146–56.

** Analysis of the strucuture of HSV-1 DNA in this study showed the presence of gaps in the genome that, when modified, altered cellular responses to the virus

55. Yang Y-G, Lindahl T, Barnes DE: **Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease.** *Cell* 2007, **131**:873–86.

56. Anderson CW, Young ME, Flint SJ: **Characterization of the adenovirus 2 virion protein, mu.** *Virology* 1989, **172**:506–12.

57. Broyles SS: **Vaccinia virus transcription.** *J. Gen. Virol.* 2003, **84**:2293–303.

58. Kent JR, Zeng P-Y, Atanasiu D, Gardner J, Fraser NW, Berger SL: **During lytic infection herpes simplex virus type 1 is associated with histones bearing modifications that correlate with active transcription.** *J. Virol.* 2004, **78**:10178–86.

59. Li T, Chen J, Cristea IM: **Human cytomegalovirus tegument protein pUL83 inhibits IFI16-mediated DNA sensing for immune evasion**. *Cell Host Microbe* 2013, **14**:591–599.

60. Cagliani R, Forni D, Biasin M, Comabella M, Guerini FR, Riva S, Pozzoli U, Agliardi C, Caputo D, Malhotra S, et al.: **Ancient and recent selective pressures shaped genetic diversity at AIM2-like nucleic acid sensors.** *Genome Biol. Evol.* 2014, **6**:830–45.

** 61. Zhang G, Cowled C, Shi Z, Huang Z, Bishop-Lilly K a, Fang X, Wynne JW, Xiong Z, Baker ML, Zhao W, et al.: **Comparative analysis of bat genomes provides insight into the evolution of flight and immunity.** *Science* 2013, **339**:456–60.

** Genomic sequencing of multiple bat species revealed the unexpeditdly rapid selection of genes inolved in repaing damaged DNA

62. Mühldorfer K, Speck S, Kurth A, Lesnik R, Freuling C, Müller T, Kramer-Schadt S, Wibbelt G: **Diseases and causes of death in European bats: dynamics in disease susceptibility and infection rates.** *PLoS One* 2011, **6**:e29773.

63. Lou DI, McBee RM, Le UQ, Stone AC, Wilkerson GK, Demogines AM, Sawyer SL: **Rapid evolution of BRCA1 and BRCA2 in humans and other primates.** *BMC Evol. Biol.* 2014, **14**:155.

* 64. Härtlova A, Erttmann SF, Raffi FA, Schmalz AM, Resch U, Anugula S, Lienenklaus S, Nilsson LM, Kröger A, Nilsson JA, et al.: **DNA Damage Primes the Type I Interferon System via the Cytosolic DNA Sensor STING to Promote Anti-Microbial Innate Immunity.** *Immunity* 2015, **42**:332–343.

* This study provides evidence that damaged self-DNA can leak into the cytoplasm, accumulating to high enough levels to activate STING-dependent IFNI production.

65. Veeranki S, Choubey D: **Interferon-inducible p200-family protein IFI16, an innate immune sensor for cytosolic and nuclear double-stranded DNA: regulation of subcellular localization.** *Mol. Immunol.* 2012, **49**:567–71.

66. Soloski MJ, Holowczak JA: **Characterization of supercoiled nucleoprotein complexes released from detergent-treated vaccinia virions.** *J. Virol.* 1981, **37**:770–83.

67. Klemperer N, Ward J, Evans E, Traktman P: **The vaccinia virus I1 protein is essential for the assembly of mature virions.** *J. Virol.* 1997, **71**:9285–9294.

68. Graham FL: **Covalently closed circles of human adenovirus DNA are infectious.** *EMBO J.* 1984, **3**:2917–22.

69. Knipe DM, Lieberman PM, Jung JU, McBride AA, Morris K V, Ott M, Margolis D, Nieto A, Nevels M, Parks RJ, et al.: **Snapshots: chromatin control of viral infection.** *Virology* 2013, **435**:141–56.

70. Wodrich H, Cassany A, D'Angelo MA, Guan T, Nemerow G, Gerace L: **Adenovirus core protein pVII is translocated into the nucleus by multiple import receptor pathways.** *J. Virol.* 2006, **80**:9608–18.

71. Mocarski ES, Roizman B: **Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA**. *Cell* 1982, **31**:89–97.

72. Tamashiro JC, Spector DH: **Terminal structure and heterogeneity in human cytomegalovirus strain AD169.** *J. Virol.* 1986, **59**:591–604.

73. Dyson PJ, Farrell PJ: **Chromatin Structure of Epstein-Barr Virus**. *J. Gen. Virol.* 1985, **66**:1931–1940.

74. Pfister H, zur Hausen H: **Characterization of proteins of human papilloma viruses (HPV) and antibody response to HPV 1.** *Med. Microbiol. Immunol.* 1978, **166**:13–9.

75. Friedmann T, David D: **Structural Roles of Polyoma Virus Proteins**. *J. Virol.* 1972, **10**:776–782.

76. Ponder BAJ, Crawford LV: **The arrangement of nucleosomes in nucleoprotein complexes from polyoma virus and SV40**. *Cell* 1977, **11**:35–49.

77. Marcus-Sekura CJ, Carter BJ: **Chromatin-like structure of adeno-associated virus DNA in infected cells.** *J. Virol.* 1983, **48**:79–87.

78. Cotmore SF, Tattersall P: **Parvovirus diversity and DNA damage responses.** *Cold Spring Harb. Perspect. Biol.* 2013, **5**.

Figure Legend

Figure 1. The induction of interferon by foreign and damaged self-DNA. DNA virus genomes stimulate intracellular DNA sensing PRRs, most of which act via the STING-TBK1-IRF3 pathway. Cytosolic DNA sensors recognise cytosolic-replicating vaccinia, or the DNA from defective or degraded herpesvirus or adenovirus virions. IFI16 has been shown to be a nuclear sensor of HSV-1 DNA that can shuttle out of the nucleus to activate STING. DNA damage is repaired in the nucleus by several proteins that function in the innate immune response and, at the same time, can induce interferon production via ATM, the IKK complex, NF-κB and IRF3. DNA sensors are shown in orange, transcription factors in green, and kinases in blue. ROS, reactive oxygen species.

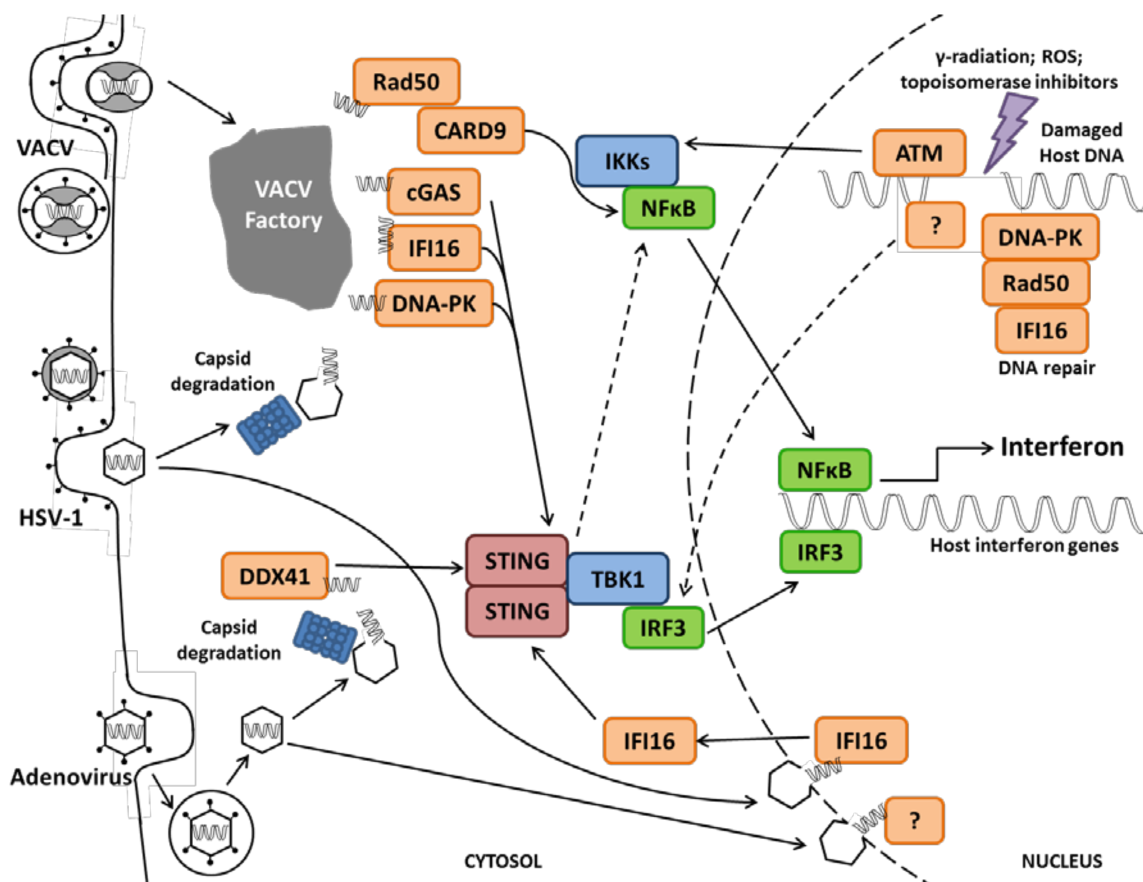

Table 1: Genomic structures of DNA viruses with human tropism.

Virus	Structure	Associated Proteins	Condensation	Site of Replication
VACV (Poxvirus)	Linear, dsDNA, 192 kb, AT rich ends, hairpin ends	Proteins such as I1 keep it supercoiled DNA in virion, but naked upon release [66,67]	Supercoiled in virion [66]	Cytoplasm
Human Adenovirus V (Adenovirus)	Linear, dsDNA, 36 kb. Circularises upon nuclear entry [68]	Protein µ, protein V, protein VII, terminal proteins. Protein VII replaced by cellular histones after infection [56,69,70]	Tightly in virion, and as nucleosomes after infection [69]	Nucleus
HSV-1 (α-herpesvirus)	Linear, dsDNA with gaps and nicks. 152 kb. Circularises after entering the nucleus. 3’ overhang of one nucleotide [71]	Cellular histone proteins after nuclear entry and especially during latency (but not in the virion) [69]	As nucleosomes after infection [69]	Nucleus
HCMV (β-herpesvirus)	Linear, dsDNA, 235 kb. 3’ overhang of one nucleotide [72]	Cellular histone proteins after nuclear entry and especially during latency (but not in the virion) [69]	As nucleosomes after infection [69]	Nucleus
EBV (γ-herpesvirus)	Linear, dsDNA. Circularises upon latency. 172 kb.	Cellular histone proteins after nuclear entry and especially during latency (but not in the virion) [73]	As nucleosomes after infection [73]	Nucleus
HPV (Papillomavirus)	Circularised, dsDNA, 8 kb	Cellular histone proteins in the virion and during infection [69,74]	As nucleosomes [69]	Nucleus
BK-virus (Polyomavirus)	Circularised, dsDNA, 5 kb	Cellular histone proteins in the virion and during infection [75]	A Nucleosomes [76]	Nucleus
Adeno-associated virus (Parvovirus)	Linear, ssDNA with duplex hairpin ends. Circularises for replication. 5 kb [77,78]	Cellular histone proteins during infection, but not in the virion [77]	As nucleosomes after infection [77]	Nucleus