Viral evasion and subversion of pattern-recognition receptor signalling

Andrew G. Bowie and Leonie Unterholzner

Abstract | The expression of pattern-recognition receptors (PRRs) by immune and tissue cells provides the host with the ability to detect and respond to infection by viruses and other microorganisms. Significant progress has been made from studying this area, including the identification of PRRs, such as Toll-like receptors and RIG-I-like receptors, and the description of the molecular basis of their signalling pathways, which lead to the production of interferons and other cytokines. In parallel, common mechanisms used by viruses to evade PRR-mediated responses or to actively subvert these pathways for their own benefit are emerging. Accumulating evidence on how viral infection and PRR signalling pathways intersect is providing further insights into the function of the pathways involved, their constituent proteins and ways in which they could be manipulated therapeutically.

Viruses are the most abundant, diverse and rapidly evolving pathogens that the host immune system is challenged by and they therefore represent a serious threat to human health. Paradoxically, viruses are obligate parasites that depend on host cells for survival and, throughout evolution, they have developed strategies to evade and subvert the immune response, and to use host proteins for their own life cycle. Viral infection of host cells leads to the initiation of antiviral innate immune responses, which results in the induction of expression of the type I interferons (IFNs) IFNα and IFNβ, and of pro-inflammatory cytokines. The importance of type I IFNs in directing the antiviral response has been characterized in detail and validated in vivo. For example, mice that are deficient in the type I IFN receptor have increased susceptibility to many viruses, and humans with genetic defects in IFN signalling components die of viral infections. Some of the important IFN antiviral effector pathways, which comprise host mechanisms to directly target the viral life cycle and to amplify host detection of viruses and IFN production, are shown in FIG. 1. In addition to influencing innate immune responses, type I IFNs direct the adaptive immune response by priming T helper cells and cytotoxic T cells (CTLs), thereby resulting in the induction of antigen-specific responses.

Eight years ago, almost nothing was known about the cellular mechanisms that are used to detect viruses and to subsequently produce IFNs and pro-inflammatory cytokines. It is now known that viruses, similar to bacteria and fungi, are initially recognized by host pattern-recognition receptors (PRRs), as was predicted by Janeway almost 20 years ago. In a short time, a large body of research has accumulated and now provides a detailed understanding of the molecular basis of PRR-mediated recognition of viral nucleic acids, and of the subsequent activation of signalling pathways that lead to the transcription of genes that encode pro-inflammatory cytokines and IFNs. Two families of PRRs that recognize viral nucleic acid have been characterized in detail. The first is a subfamily of Toll-like receptors (TLRs) that is made up of TLR3, TLR7, TLR8 and TLR9, which are mainly expressed in the endosomes of some cell types. The second family of receptors comprises the retinoic-acid-inducible gene 1 (RIG-I)-like receptors (RLRs), encompassing RIG-I, melanoma differentiation-associated gene 5 (MDA5; also known as IFIH1) and laboratory of genetics and physiology 2 (LGP2), which are ubiquitously expressed in the cytoplasm (FIG. 1). An additional family of intracellular PRRs that is probably important in the sensing of viral DNA in the cytoplasm is also emerging. Viral nucleic acid (both RNA and DNA) is the most important pathogen-associated molecular pattern (PAMP) that is recognized by the host (BOX 1), and there is now an excellent understanding of how viral nucleic acids bind to and activate PRRs at the molecular level (reviewed in REFs 6,7). In addition, the fundamental aspects of the PRR signalling pathways, and the components that are involved, have also been well characterized.

There has long been a substantial amount of literature on how the IFN effector phase of the antiviral response is antagonized by viruses, but with the more recent discoveries identifying how virus triggering of PRRs leads to...
IFN induction, the mechanisms by which viruses evade early events in PRR signalling pathways have become apparent. It is now clear that, following infection, viruses can induce complex intracellular pathways that affect many components of host signalling pathways. The effects of viruses on host defence not only inhibit viral detection, but also allow the virus to manipulate host responses. Owing to the fine-tuning of immune signalling by both virus and host, different viruses may activate the same PRR with either positive or negative outcomes on disease progression and immune response.

Elucidating the strategies that are used by viruses to inhibit and to manipulate host responses is important for two reasons. First, this knowledge will contribute to our understanding of viral pathogenesis. As viruses (especially RNA viruses) mutate very rapidly, the identification of mutations that increase or decrease the activity of a viral protein that targets the immune response may help to define the virulence factors of different strains or species of viruses that infect humans. For example, Ebola virus, which is usually highly virulent in humans and triggers lethal haemorrhagic fever, induces a minimal adaptive immune response (which requires innate immune detection of the virus by PRRs) in patients who succumb to infection, whereas those who survive infection mount an effective adaptive immune response.
Plasmacytoid dendritic cell (pDC). An immature DC that belongs to a DC subset with a morphology that resembles that of a plasmablast. Plasmacytoid DCs produce large amounts of type I interferons in response to viral infection.

Forward genetics
A classical genetic analysis approach that proceeds from phenotype to genotype by positional cloning or candidate-gene analysis.

Reverse genetics
A genetic analysis that proceeds from genotype to phenotype by gene-manipulation techniques, such as homologous recombination in embryonic stem cells.

Box 1 | Sensing of different classes of viruses

Virtually any double-stranded DNA (dsDNA) genome is detected by Toll-like receptor 9 (TLR9). Other dsDNA viruses include the poxviruses (such as vaccinia and variola virus), the retroviruses (such as herpes simplex virus, human herpesvirus 8, bovine herpesviruses and Kaposi’s sarcoma-associated herpesvirus) and African swine fever virus.

Role of TLRs in detecting viruses. The identification of TLR4 as the signalling receptor for bacterial lipopolysaccharide was a major breakthrough in the field of innate immune research. The first evidence that TLRs might also respond to viruses came with the finding that the fusion protein of respiratory syncytial virus (RSV) stimulated the secretion of interleukin-6 (IL-6) from macrophages, and this depended on TLR4 signalling.

In addition, it was discovered that proteins from vaccinia virus (VACV) antagonized TLR signalling in cultured cells, further supporting a role for TLRs in sensing viruses. In 2001, TLR3 was identified as a receptor for viral double-stranded RNA (dsRNA) based on the observation that mice deficient in IFN-inducible dsRNA-dependent protein kinase (PKR; also known as EIF2AK2 and previously the only known dsRNA receptor) still responded to the synthetic dsRNA mimic polyinosinic–polycytidylic acid (polyI:C). As stimulation of cells with polyI:C was known to mimic many aspects of viral infection, TLR3 was expected to have a broad and crucial role in antiviral immunity. However, it soon became clear that this was not the case, as viral infection of hosts that were deficient in TLR3 did not alter viral pathogenesis or impair the host immune response to several viruses. It now seems that the absence of TLR3 is actually protective in the case of some viral infections (see later). Nonetheless, important and specific roles for TLR3 in the antiviral response have been shown, including host defence against certain viruses (reviewed in Refs 18–20).

TLR3 is part of a subset of TLRs that are present in endosomes and sense nucleic acids. Others include TLR7, TLR8 and TLR9. Human TLR8 and mouse TLR7 (TLR8 is non-functional in mice) detect single-stranded RNA (ssRNA) from RNA viruses, including HIV–1, vesicular stomatitis virus (VSV) and influenza A virus, and this leads to the induction of IFNα expression. TLR9 senses unmethylated CpG DNA in the genomes of DNA viruses, such as herpes simplex virus (HSV), and this induces the production of type I IFNs by plasmacytoid dendritic cells (pDCs). TLR7 and TLR9 seem to have a more important role than TLR3 in antiviral immunity, being responsible for the sensing of viruses by pDCs and their subsequent production of type I IFNs. Indeed, Akira and colleagues recently showed that TLR-mediated recognition of the ssRNA lymphocytic choriomeningitis virus (LCMV) in pDCs leads to the production of type I IFNs and the subsequent CTL response in vivo.

Viral evasion of TLR signalling pathways. Engagement of TLRs by viral PAMPs causes receptor dimerization followed by the initiation of intracellular signalling pathways that culminate in the activation of the transcription factors NFκB, IRF3 and IRF7 (for recent
reviews, see REFs 9,25). The cytoplasmic region of TLRs contains the conserved Toll/IL-1 receptor (IL-1R) (TIR) domain, which is common to the IL-1R family and is essential for signalling that is mediated by TLRs and IL-1Rs26. Receptor dimerization triggers homotypic interactions between the receptor TIR domain and TIR-domain-containing adaptor proteins. There are five TIR-domain-containing adaptor proteins that control signalling from activated TLRs, namely myeloid differentiation primary-response gene 88 (MyD88), MyD88-adaptor-like (MAL; also known as TIRAP), TIR-domain-containing adaptor protein inducing IFNβ (TRIF), TRIF-related adaptor molecule (TRAM) and sterile-α- and armadillo-motif-containing protein (SARM)25. MyD88 is required for signalling from all mouse TLRs except TLR3, as well as from signalling from the IL-1R26, although different TLRs signal through different repertoires of TIR-domain-containing adaptors. For example, TLR2 signalling requires MyD88 and MAL, TLR3 signalling needs TRIF, and TLR7 and TLR9 signalling requires only MyD88 (REF 25). The TLR-induced signalling pathways that lead to IRF3, IRF7 and NFκB activation are outlined in FIG 2. Some of the factors that are involved in TLR signalling are shared with other signalling pathways. More specifically, it has recently been shown that TRADD (tumour-necrosis factor receptor (TNFR)-associated via death domain) is required for the signalling pathways that are downstream of the TNFR, for TRIF-dependent TLR signalling and for both RIG-I and MDA5 signalling pathways26–28. This might provide the opportunity for crosstalk between the different innate immune signalling events.

Many viral antagonists of TLR signalling have been identified (FIG 2). One of the first characterized viral inhibitors of a TLR signalling pathway was the VACV protein A46R, which was found to directly target specific TIR-domain-containing adaptor proteins. VACV, a large dsDNA virus of the Poxviridae family, is now known to encode many inhibitors of innate immune signalling pathways, many of which also have a non-redundant, independent role in virulence29,30. A46R contains a TIR domain, which allows it to interact with TIR-domain-containing complexes and to bind directly to the TIR-domain-containing adaptors MyD88, MAL, TRIF and TRAM; it can therefore inhibit the activation of both NFκB and IRFs that is normally triggered by multiple TLR pathways31. The affinity of the interaction between A46R and TIR-domain-containing adaptors in an infected cell may be sufficient to prevent the recruitment of adaptors to TLRs and thereby inhibit host defence, although this theory has not yet been supported experimentally. When the gene that encodes A46R was deleted from VACV, the virus was attenuated32. It is worth noting that A46R does not interact with SARM, which is a negative regulator of TLR signalling33 and is therefore favourable in the context of VACV infection.

Although A46R is still the only known TIR-domain-containing viral protein, bacterial proteins containing TIR domains have been identified and have been found to have a role in bacterial virulence34,35, possibly by using mechanisms that are similar to those used by A46R.
Caspase-recruitment domain
A protein domain that is found in certain initiator caspases (for example, mammalian caspase-9) and their adaptor proteins (for example, apoptotic protease-activating factor 1). This domain mediates protein–protein interactions.

However, another non-TIR-domain-containing viral protein, namely the NS3–4A heterodimer from the ssRNA flavivirus hepatitis C virus (HCV), has also been shown to use a similar strategy to disable TIR adaptor proteins. NS3–4A has serine protease activity, is essential for the survival of the virus and has many functions in evading or subverting PRR signalling pathways (Table 1). One function is to cleave an HCV precursor polypeptide to generate mature structural proteins during viral replication. Further work showed that TRIF was also specifically cleaved by NS3–4A into two polypeptides that could no longer activate TLR-dependent transcription from the IFNβ promoter18. NS3–4A expression potently decreased the polyI:C-induced activity of the IFNβ promoter, the polyI:C-mediated activation of NFκB and IFR3, and the induction of IFR3-dependent genes18. Therefore, by targeting TRIF, HCV inhibits TLR3-mediated antiviral signalling. Although the importance of TLR3 in the host immune response to HCV infection is currently unclear, the targeting of TRIF by HCV indicated that TLR3 has an important role in the antiviral response to HCV. Furthermore, it has recently been shown that when four different HCV proteins (one of which was NS3–4A) were expressed individually in a mouse macrophage cell line, they inhibited TLR3-induced cytokine production. The target of the HCV protein NS5A was found to be MyD88 (Ref. 36).

Another VACV protein, A52R, specifically targets IL-1R and TLR signalling and contributes to virulence37. A52R interacts with TNFR-associated factor 6 (TRAF6) and IL-1R-associated kinase 2 (IRAK2), two signalling molecules that are downstream of the TLR-domain-containing adaptors, and is an effective inhibitor of NFκB activation38. Although TRAF6 was known to have a central role in TLR signalling that leads to NFκB activation, the role of the pseudokinase IRAK2 was unknown. Surprisingly, it was shown that a mutant version of A52R, which could interact with IRAK2 but not TRAF6, induced maximal inhibition of NFκB38. This key observation led to a study which showed that IRAK2 is more important for NFκB activation than was previously appreciated, and certainly more important than IRAK1 for some TLR signalling pathways39. Because A52R, unlike A46R, had no effect on TLR-induced IRF activation, this suggested that IRAK2 was not involved in the TLR–IRF axis. Indeed, it is now appreciated that IRAK1 has a crucial role in this signalling pathway (Fig. 2), whereas IRAK2 is involved in NFκB activation, as confirmed by the study of IRAK2-deficient mice39. So, the study of A52R is a clear example of what we can learn about PRR signalling pathways by investigating viral evasion strategies (Box 2). Similar to NS3–4A, A52R also has many functions in evading or subverting PRR signalling (Table 1), and its ability to interact with TRAF6 might represent subversion (rather than evasion) of the TLR pathways by VACV (see later).

In addition to VACV and HCV, other viruses that target TLR signalling pathways include human T-cell leukaemia virus type 1 (HTLV-1) and West Nile virus (WNV). HTLV-1 downregulates the host cell-surface expression of TLR4 through the viral protein p30, which binds to and disables the transcription factor PU.1 (which is normally required for TLR4 expression)40. By contrast, the WNV protein non-structural protein 1 (NS1) inhibits TLR3-mediated induction of IFNβ expression by preventing the translocation of NFκB and IRF3 to the nucleus41. However, it is currently unclear whether inhibition that is mediated by NS1 is specific to the TLR3 signalling pathway or whether it also affects the induction of IFNβ expression downstream of other PRRs.

Important principles of viral evasion are illustrated by VACV and HCV targeting of TLR signalling pathways. Viral antagonists of innate immunity often have a similar sequence to the host proteins they target (as in the case of A46R), which can provide clues about their function, or they can be completely unrelated in sequence to host proteins (as in the case of NS3–4A). Furthermore, viral proteins with known functions (such as NS5A36) may have additional roles, which illustrates the high efficiency with which viruses use their limited protein-coding resources (Table 1).

Box 2 | Recent lessons about PRR signalling pathways learnt from viruses
As viruses target host proteins for the specific purpose of gaining an advantage over the host, newly discovered or ill-defined host proteins that interact with viral proteins can be implicated in the innate immune response. Recent examples show how understanding these virus–host interactions has clarified aspects of the pattern-recognition receptor (PRR) response. Vaccinia virus (VACV) protein A52R was found to be an inhibitor of Toll-like receptor (TLR)-mediated activation of nuclear factor-xB (NFκB), through an interaction with interleukin-1 receptor-associated kinase 2 (IRAK2)42. Prior to this it was unclear whether IRAK2 had a non-redundant role in PRR signalling, but further study of A52R showed that IRAK2 has a crucial role in human TLR signalling pathways43, and the study of IRAK2-deficient mice recently confirmed that it also has a role in mouse TLR signalling. Studies using another VACV PRR signalling inhibitor, K7R, showed that human DEAD-box protein 3 (DDX3) is involved in PRR signalling that leads to the expression of interferon-β (IFNβ). More specifically, DDX3 was found to be the host target of K7R, which explained the ability of K7R to inhibit PRR-induced activation of IFN-regulatory factors (IRFs)44. It is now clear that DDX3 is a component of the IRF-activating complex that contains TANK-binding kinase 1 (TBK1) and inhibitor of NFκB kinase-c (IKKc), which is essential for signalling by many PRRs. Additional aspects about PRR signalling were revealed by studies of the influenza A virus nonstructural protein 1 (NS1), this time clarifying the nature of the ligand for the PRR retinoic-acid-inducible gene I (RIG-I), which at that time was thought to be double-stranded RNA (dsRNA). NS1 was known to suppress the induction of type I IFN expression, and this was thought to be mediated through dsRNA binding. However, studies that aimed to elucidate the mechanism of action of NS1 revealed that influenza A virus infection produces very little dsRNA, and instead NS1 binds to and inhibits RIG-I directly45. This led the authors to conclude that RIG-I actually recognized influenza A virus genomic single-stranded RNA containing a 5′ triphosphate45.

RLRs and viruses
Recognition of viral RNA by RIG-I and MDA5. TLR7 and TLR9 are important for the recognition of viral nucleic acids in the endosomes of pDCs, but most other cell types recognize viral RNA through the RLRs RIG-I and MDA5 (REFS 4.5–45). RIG-I and MDA5 are closely related proteins that contain two amino (N)-terminal caspase-recruitment domains (CARDs), a central ATPase and helicase domain and a carboxy (C)-terminal regulatory
domain. The CARDs of RIG-I and MDA5 recruit the signalling adaptor protein IFNB-promoter stimulator 1 (IPS1; also known as CARDIF, VISA or MAVS)46–49. IPS1 resides at the outer mitochondrial membrane and interacts with RIG-I and MDA5 through its CARD. This interaction provides the link between the RLRs and the downstream kinases TANK-binding kinase 1 (TBK1) and IκB kinase-ε (IKKe), which phosphorylate and activate IRF3 and IRF7 [FIG. 3]. Recently, a protein known as stimulator of IFN genes (STING) has been identified as an additional factor in the RIG-I–IPS1 signalling complex, but not in the complex containing MDA5 (REF 50). STING is located in the endoplasmic reticulum, which suggests that this intracellular compartment is also involved in RIG-I-mediated signalling.

Both RIG-I and MDA5 were originally thought to recognize similar types of viral dsRNA. Recently, however, the exact nature of their nucleic acid ligands has been defined and shown to be distinct. Characterization of the mechanism by which the influenza virus A non-structural protein 1 (NS1) inhibited RIG-I-mediated detection of viral RNA revealed that the main ligand for RIG-I is ssRNA that has a 5′ triphosphate51–53, such as that generated by many viral RNA polymerases. Although the 5′ triphosphate is necessary, it is not sufficient for RIG-I activation44; the composition of the RNA sequence also has a role, as viral RNAs that contain a 5′ triphosphate and a sequence of poly-uridine or riboadenine are particularly potent activators of RIG-I45. RIG-I can also be activated by short fragments of dsRNA54, whereas MDA5 is the main cytoplasmic receptor for longer molecules of viral dsRNA and for polyLC55. On the one hand, viruses that produce large amounts of dsRNA during their life cycle, such as picornaviruses, are sensed mainly by MDA5 (REFS 56,57). On the other hand, negative-stranded RNA viruses, such as influenza virus, which produce barely detectable levels of dsRNA, are sensed by RIG-I, as confirmed by
Modification of viral RNAs and viral RNA-binding proteins. Viruses with an RNA genome mimic some of the strategies that are used by host cells to prevent the recognition of their RNA by RIG-I and MDA5. Many viruses either use the host mRNA processing machinery to cap their newly synthesized viral mRNAs, encode their own capping enzymes (such as poxviruses and rotaviruses) or even ‘snatch’ capped 5’ fragments from cellular mRNAs (a strategy used by influenza A virus68). Some RNA viruses, such as Bornavirus disease virus, express a phosphatase that converts the triphosphate into a monophosphate59, and picornaviruses protect the 5’ end of their genomic RNA with the covalently linked protein VPg60,61. By contrast, genomic RNA from many ssRNA viruses, such as influenza A and Rabies viruses, contains a 5’ triphosphate moiety and consequently activates RIG-I62.

Many viruses must produce dsRNA at some stage during their life cycle, which is why it is a useful target of host PRRs. For example, viruses with a dsRNA genome are susceptible to recognition by MDA5, as are positive-sense RNA viruses that use a dsRNA intermediate during their replication cycle. Even DNA viruses can produce considerable amounts of dsRNA owing to convergent transcription (that is, when two adjacent open reading frames are transcribed at opposite directions, resulting in a partial 3’ overlap between their transcripts)62. To avoid the antiviral responses that are initiated by RLRs (and by the dsRNA-activated effector proteins PKR and 2’-5’-oligoadenylate synthase; FIG. 1), some viruses encode cytoplasmic dsRNA-binding proteins, including VACV E3L, Ebola virus VP35 (REF 63) and HIV-1 Tat64, that shield the dsRNA species from recognition by PRRs. In addition to sequestering viral RNA, some of these proteins also bind to PKR and/or RIG-I directly, thereby inhibiting the antiviral response at many levels65. Furthermore, it is possible that these cytoplasmic proteins may also prevent the redistribution of dsRNA to endosomes, where it would be recognized by TLR3.

Interference with RIG-I and MDA5 signalling. Although the RLRs were discovered less than 5 years ago, many studies have already revealed that viruses can target these receptors and their adaptor IPS1 directly and inhibit their function; this validates the importance of these PRRs for the recognition of viral infections. Influenza A virus NS1 protein binds to the RIG-I–IP51 complex and blocks downstream signalling66. Analogously, the V proteins of many paramyxoviruses interact with MDA5 and inhibit its function67, and poliovirus induces the cleavage of MDA5 by caspases68.

Cleavage of the downstream adaptor IPS1 is also a common event that has been observed during infection with several viruses. More specifically, the protease precursor protein 3ABC of hepatitis A virus triggers the degradation of IPS1 at the mitochondrial membrane, which disrupts RIG-I– and MDA5-mediated activation of IRF3 (REF 68). In addition, as well as targeting TRIF (as discussed earlier), HCV uses the protease NS3–4A to cleave the short C-terminal

---

**Figure 3 | Viral evasion of retinoic-acid-inducible-gene-I-like receptor signalling.** RIG-I (retinoic-acid-inducible gene I) and MDA5 (melanoma differentiation-associated gene 5), termed RIG-I-like receptors (RLRs), are activated by cytoplasmic RNA during viral infection. Both signal using IFNβ-promoter stimulator 1 (IPS1), which is tethered to the mitochondrial membrane. When IPS1 is engaged by RLRs, it recruits downstream signalling complexes that lead to the activation of the IFN-regulatory factors (IRFs) and nuclear factor-κB (NFκB). In addition, signalling through RIG-I requires the adaptor STING (stimulator of IFN genes), which resides in the endoplasmic reticulum (ER), RLR signalling is inhibited by viral proteins that either bind RIG-I, MDA5 or IPS1 directly or cause their degradation. The IκB kinase (IKK) family members are also a common target for viral proteins. DDX3, DEAD-box protein 3; dsRNA, double-stranded RNA; FADD, FAS-associated via death domain; HAV, hepatitis A virus; HCV, hepatitis C virus; IFN, interferon; IκB, inhibitor of NFκB; LGP2, laboratory of genetics and physiology 2; NAP1, NFκB-activating kinase-associated protein 1; NS1, nonstructural protein 1; PPP, 5’- triphosphate; RIGP1, receptor-interacting protein 1; SINTBAD, similar to NAP1 TBK1 adaptor; ssRNA, single-stranded RNA; TANK, TRAF-family-member-associated NFκB activator; TRADD, TRAF2, TRAF3, TRAF6, TANK-binding kinase 1; TANK-binding kinase 2; NFκB, nuclear factor κB; B14R, vaccinia virus.
transmembrane domain of IPS1, causing it to dissociate from the mitochondrial membrane and rendering it incapable of RLR signal transduction. These studies confirmed the hypothesis that IPS1 must be tethered to the mitochondrial membrane to mediate its antiviral function.

**Intracellular DNA receptors**

The most recent addition to the expanding family of viral PRRs is the intracellular DNA receptor DNA-dependent activator of IFRs (DAI; previously known as Z-DNA binding protein). When dsDNA is introduced into the cytoplasm by transfection, by bacterial pore-forming proteins or by viral infection, it can elicit a TLR-independent innate immune response that involves the activation of IRF3 by TBK1 and STING. DAI is one such cytoplasmic DNA receptor, but its role in the in vivo recognition of cytoplasmic DNA has been controversial; additional cytoplasmic DNA receptors also probably have an important role.

Interestingly, the VACV dsRNA-binding protein E3L also has a Z-DNA-binding domain, which can inhibit the DNA-induced expression of IFNβ and prevent the interaction of DAI with DNA. So, E3L may protect the VACV DNA genome from recognition by DNA receptors. As the DNA-responsive pathways are currently being investigated in more detail, additional virus-encoded inhibitors will probably be identified. Elucidating the mechanisms by which viruses disrupt DNA receptor signalling in host cells will probably be instrumental in defining the signalling mechanisms of these receptors.

**Viral evasion of downstream PRR signalling**

Many PRR signalling pathways converge at the level of IKKs. All of the PRRs described previously, namely the TLRs, RLRs and cytoplasmic DNA receptor (or receptors), activate signalling cascades that converge at the level of the IKK family of proteins. IKKα and IKKβ, which form a complex with the regulatory subunit IKKe (also known as NEMO), phosphorylate inhibitor of NFκB (IκB), which leads to its degradation and consequently the release and nuclear translocation of active NFκB. A second IKK complex, which is especially important for the induction of type I IFN expression, consists of IKKe, TBK1 and associated proteins. IKKe and TBK1 phosphorylate and activate IRF3 and IRF7, which homodimerize and translocate to the nucleus. In addition to activating the IKKs, PRR signalling also activates mitogen-activated protein kinase (MAPK) signalling cascades, thereby leading to the activation of activator protein 1 (AP1) family members. Together, NFκB, IRF3, IRF7 and AP1 form an active complex on the IFNB promoter that leads to transcriptional activation of this promoter. In the case of TLR7, TLR8 and TLR9, induction of IFNα expression is largely mediated through IRF7 activation, which involves IKKα.

Thus, the IKK family and the transcription factors they activate are an attractive target for inhibition by viral proteins, allowing viruses to disable the pathways that are triggered by many PRRs at the same time.

**Viral inhibition of IRF3 and IRF7 activation at the level of IKKs.** The interaction of the multifunctional HCV protein NS3 (REF 79), or of the VACV protein NL1,36, with TBK1 inhibits the activation of downstream transcription factors. TBK1 is also inhibited by the phosphoprotein of many negative-sense RNA viruses, such as rabies virus and Borna disease virus. Furthermore, the G1 protein of pathogenic hantaviruses can inhibit TBK1 function by disrupting the TRAF3–TBK1 interaction that is required for signalling. In cells in which the activity of TBK1 is inhibited, IRF3 is not phosphorylated and cannot dimerize or translocate to the nucleus. The dimerization and nuclear translocation of IRF3 is also inhibited by papain-like protease (PLpro) of severe acute respiratory syndrome (SARS)-associated coronavirus, which directly interacts with it.

A compelling example of how the study of viral evasion can reveal new host signalling mechanisms is a recent study of the VACV protein K7R. K7R was found to inhibit the PRR-mediated induction of IFNβ by preventing the interaction of the K1–IKKe-mediated activation of IRFs owing to its ability to target human DEAD-box protein 3 (DDX3). Further work confirmed that DDX3 is part of the complex downstream of both RLRs and cytoplasmic DNA receptors, showing for the first time that a DEAD-box helicase is involved in the K1–IKKe-mediated activation of IRFs. The role of DDX3 in the activation of the IFNB promoter downstream of PRR signalling was confirmed by another recent study.

**Direct targeting of IRFs.** Several viruses have viral IRF mimics that inhibit IRF3 and IRF7 signalling through various mechanisms. For example, the V proteins of paramyxoviruses, such as mumps virus and parainfluenza virus 5, act as IRF3 mimics that compete with cellular IRF3 for phosphorylation by the TBK1–IKKe complex. Human herpesvirus 8 also expresses several IRF homologues that act as dominant-negative inhibitors of host IRF3 and IRF7 signalling through various mechanisms. KSHV dimerizes with host IRF7 and inhibits its DNA-binding activity. Another KSHV protein, the transcription factor K-bZIP, competes with host IRF3 for binding sites in the IFNB promoter, thereby blocking promoter activation. Alternatively, HSV infected cell protein 0 (ICP0) binds to host IRF3 and sequesters it together with CBP and p300 in nuclear bodies away from its normal binding sites on host genes.

The related ICP0 protein from bovine herpesvirus also targets host IRF3, but it causes its degradation instead of temporarily sequestering it. Virus-induced IRF3 degradation is a commonly used mechanism of viral evasion — other IRF3-interacting viral proteins that mediate this effect include the HIV-1 proteins Vpr and Vif, and the Flaviviridae family protease. The rotavirus non-structural protein 1 (NSP1) targets IRF5 and IRF7, in addition to IRF3, for degradation. Therefore, during viral infection the expression levels of the IRFs are dynamically regulated by viral factors. In fact, it has been suggested that one crucial function of the IFN effector...
protein ISG15 (IFN-stimulated protein of 15 kDa) (Fig. 1) is to counteract virus-mediated proteolysis of IRF3, as ISG15 covalently binds to and stabilizes IRF3, and can prevent its ubiquitin-mediated degradation by Newcastle disease virus. Interestingly, VACV E3L protein has recently been shown to directly target and disable ISG15 function, which adds to the impressive repertoire of inhibitory strategies used by this viral protein.[1] Table 1.

Viral modulation of NFκB activity. The role of NFκB during viral infection is more complex than that of IRF3 and IRF7. Although NFκB is involved in the production of IFNβ and chemokines, both of which have antiviral effects, NFκB also inhibits apoptosis and promotes proliferation of host cells, which is beneficial to the virus as apoptosis is an important host defence strategy for the deletion of virus-infected cells. Therefore, some viruses activate NFκB to prevent the cell from undergoing apoptosis.[4] However, at early stages of infection viruses can temporarily block NFκB activity to delay the innate immune response until an infection is established and the virus is less vulnerable to elimination. An example of this biphasic regulation of NFκB activity is observed during infection with African swine fever virus (ASFV). The ASFV protein A238L is an early expressed degradation-resistant IκBα homologue that inhibits NFκB activity at early stages of infection by sequestering host NFκB in the cytoplasm.[5] However, once infection progresses, the late-stage protein A224L is expressed, which activates NFκB and inhibits caspasas.[6]

As in the case of IRFs, NFκB can also be regulated by viral proteins through the disruption of IKK function. Similarly to VACV N1L (described earlier), which targets multiple IκKs, VACV B14R specifically interacts with IκKβ and prevents its activation by inhibiting the phosphorylation of its activation loop.[7] Conversely, the KSHV protein K13 interacts with the IKKα–IKKβ complex such that NFκB is activated.[8]

The many roles of NFκB illustrate the dynamic nature of transcription factor activation, which is continually adjusted to respond to the precise needs of the cell or, as it may be, the virus.

Beyond inhibition: viral subversion of PRRs In contrast to viral evasion of host detection, it is now clear that viruses can subvert aspects of PRR-mediated signalling in addition to NFκB activation for their own benefit. There are now examples of viruses that use and manipulate TLR signalling pathways to create a host cytokine environment that favours the viral life cycle. Interestingly, RLRs seem less amenable to subversion, probably because they have a more important role in viral detection than TLRs.

Studies of TLR3-deficient mice that had been infected with WNV, influenza A virus and VACV showed that the absence of TLR3 actually favoured the host, suggesting that these viruses might use TLR3-mediated inflammation to disseminate and establish an infection.[9–10] For example, infection with WNV led to the TLR3-dependent production of pro-inflammatory cytokines and the subsequent disruption of the blood–brain barrier, which facilitated entry of the virus into the brain. By contrast, infection in the absence of TLR3 led to a reduced viral load and dampened inflammatory responses in the brain.[10] However, this may have been a consequence of mouse experimental models of viral infection in which the high viral titres that are used may lead to an overpronounced inflammatory response compared with the more protective TLR3-mediated host response that occurs during natural infections.[11] Evidence for a protective role for TLR3 in natural infections comes from recent studies showing that human patients that bear mutations in TLR3 are predisposed to HSV-associated encephalitis.[12]

Another potential effect of viral manipulation of TLRs involves the selective stimulation of TLR pathways that lead to the production of IL-10, a cytokine that is required for viral persistence.[13,14] For example, the VACV protein A52R inhibits the activation of NFκB by interacting with IRAK2 (as discussed earlier), but it also interacts with TRAF6 in a way that leads to the activation of the MAPK p38 and the subsequent activation of the IL10 promoter.[15] Thus, under conditions of TLR stimulation (as may occur during VACV infection), A52R inhibits the expression of NFκB-dependent genes, such as IL8, and instead enhances the production of IL-10.[16] A52R also inhibits the activation of NFκB-dependent genes, such as IL8, and instead enhances the production of IL-10.[16] This, in turn, can lead to an overpronounced inflammatory response compared with the more protective TLR3-mediated host response that occurs during natural infections.[11] Evidence for a protective role for TLR3 in natural infections comes from recent studies showing that human patients that bear mutations in TLR3 are predisposed to HSV-associated encephalitis.[12]
stimulation of TLR4 in B cells, which was necessary for viral persistence in the host\(^\text{107}\). In mice lacking functional TLR4, the virus was eliminated by the cytotoxic immune response\(^\text{107}\).

Viruses can also use signalling proteins to support their life cycle. Although VACV K7R binds to DDX3 to inhibit its ability to enhance IFNβ expression\(^\text{81}\), HIV-1 and HCV go a step further as they manipulate host DDX3 for different purposes. Both viruses have proteins that are known to interact with DDX3: HCV requires DDX3 for replication\(^\text{108}\) and HIV-1 uses DDX3 to shuttle viral mRNAs out of the host-cell nucleus\(^\text{109}\). Therefore, for these viruses, targeting DDX3 may provide the dual benefit of suppressing the host type I IFN response in parallel to facilitating the viral life cycle.

Conclusions and future perspectives

In this Review, we have described how advances in the past 5 years of research have increased our understanding of how host cells sense viruses through PRRs and how viruses evade and subvert their detection. For some aspects of PRR-mediated signalling there is now unprecedented molecular detail, provided in part by ‘seeing’ the host immune response from the perspective of the virus. In the near future, we can expect a more complete description of a new family of PRRs, the cytoplasmic DNA receptors, and it will be of interest to decipher how large DNA viruses, such as poxviruses and herpesviruses, interact with these receptors.

Stepping back from what we now know about virus-host interactions, some common themes become apparent. Viruses adopt many different strategies that inhibit PRR signalling; viral RNA can be ‘hidden’ from PRRs, RLRs can be directly inhibited, TIR-domain-containing adaptors can be targeted by both DNA (VACV) and RNA (HCV) viruses, TLR signalling pathways can be actively subverted to manipulate the cytokine environment and benefit viruses, IKK family members can be disabled to prevent the activation of transcription factors at common points of convergence of PRR pathways and IFNs can be degraded.

A key challenge in future studies will be to harness the information from virus evasion studies for the benefit of human health. It will be important to identify the in vitro and mouse in vivo studies that are directly relevant to humans, which is a difficult task given the host-range specificities of most viruses. However, there are already some clues, as the effect of polymorphisms in genes that encode PRRs and signalling components on the susceptibility to viral disease in humans is being uncovered\(^\text{106,107}\).

In addition, it is now clear that the molecular basis of viral pathogenesis in humans can be defined by understanding how viral PRR inhibitors act\(^\text{108,109}\).

Further studies that aim to understand PRR evasion and subversion will have several implications in human health. First, the molecular mechanisms that are revealed by such studies are relevant to other pathogens and diseases, as the signalling components are shared among PRRs that sense other types of pathogen. For example, in addition to its recently described role in RLR-mediated IRF3 and IRF7 activation\(^\text{81}\), DDX3 was also found to be important for the induction of IFNβ expression by Listeria monocytogenes\(^\text{82}\). Second, PRR signalling discoveries are also relevant to non-pathogen-induced disease, as many PRR pathways can contribute to autoimmunity and inflammatory disease if they inappropriately sense host molecules. So, some of the identified targets of viral proteins (such as IRAK2 and DDX3) might turn out to be new drug targets for treating a range of diseases. Third, the viral proteins themselves, or derivatives of them, may have therapeutic uses in suppressing inappropriate PRR signalling; viruses have been ‘examining’ the host immune machinery for millions of years, so their proteins are optimized to specifically and maximally inhibit their targets, which is analogous to a naturally occurring drug-development programme. Finally, targeting viral inhibitors therapeutically may be beneficial for restoring innate immune defences during chronic viral infections, as has been shown in in vitro studies of HCV in which NS3–4A protease inhibitors restored RIG-I signalling during infection\(^\text{111}\).

1. Sadler, A. J. & Williams, B. R. Interferon-inducible antiviral effectors. Nature Rev. Immunol. 8, 559–568 (2008).
2. Janeway, C. A. Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54, 1–13 (1989).
3. Ishii, K. J. et al. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nature Immunol. 7, 60–68 (2006).
4. Takaoka, A. et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 448, 501–505 (2007).
5. Wang, Z. et al. Regulation of innate immune responses by DAI (DLM-1/ZBP1) and other DNA-sensing molecules. Proc. Natl Acad. Sci. USA 105, 5477–5482 (2008).
6. Jin, M. S. & Lee, J. O. Structures of TLR-ligand complexes. Curr. Opin. Immunol. 29, 182–191 (2008).
7. Saito, T. & Gale, M. Jr. Differential recognition of double-stranded RNA by RIG-I-like receptors in antiviral immunity. J. Exp. Med. 205, 1525–1527 (2008).
8. Takeuchi, O. & Akira, S. MDA5/RIG-I and virus recognition. Curr. Opin. Immunol. 20, 17–22 (2008).
9. O’Neill, L. A. When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity 29, 12–20 (2008).
10. Zampieri, C. A., Sullivan, N. J. & Nabel, G. J. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nature Immunol. 8, 1159–1164 (2007).
11. Hartman, A. L., Ling, L., Nichol, S. T. & Hibberd, M. L. Whole-genome expression profiling reveals that inhibition of host innate immune response pathways by Ebola virus can be reversed by a single amino acid substitution in the VP35 protein. J. Virol. 82, 5548–5558 (2008).
12. Beutler, B. et al. Genetic analysis of resistance to viral infection. Nature Rev. Immunol. 7, 753–766 (2007).
13. Kurt-Jones, E. A. et al. Pattern recognition receptors TLRs and CD14 mediate response to respiratory syncytial virus. Nature Immunol. 1, 598–601 (2000).
14. Bowie, A. et al. A6RR and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proc. Natl Acad. Sci. USA 97, 10162–10167 (2000).
15. Janeway, C. J. & Medzhitov, R. Viral interference with IL-1 and toll signaling. Proc. Natl Acad. Sci. USA 97, 10682–10683 (2000).
16. Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. Nature 413, 732–738 (2001).
17. Edelman, K. H. et al. Does Toll-like receptor 3 play a biological role in virus infections? Virology 322, 231–238 (2004).
18. Schroder, M. & Bowie, A. C. TLRs in antiviral immunity: key player or bystander? Trends Immunol. 26, 462–468 (2005).
19. Vercammen, E., Staal, J. & Beyaert, R. Sensing of viral infection and activation of innate immunity by Toll-like receptor 3. Clin. Microbiol. Rev. 21, 13–25 (2008).
20. Quintana-Muriel, L., Alcais, A., Abel, L. & Casanova, J.-L. Immunology in natura: clinical, epidemiological and evolutionary genetics of infectious diseases. Nature Immunol. 8, 1165–1171 (2007).
21. Bowie, A. C. Translational minireview series on Toll-like receptors: recent advances in understanding the role of Toll-like receptors in anti-viral immunity. Clin. Exp. Immunol. 147, 217–226 (2007).
22. Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. Toll-like receptor 9-mediated recognition of herpes simplex virus 2 by plasmacytoid dendritic cells. J. Exp. Med. 198, 515–520 (2005).
This study shows that targeting of TLR-promoting adaptor proteins by a VACV protein contributes to virulence, which predicts that TLRs have a role in host responses against poxviruses.

The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. Nature Immunol. 10, 1074–1081 (2009).

Li, K. et al. Immune evasion by hepatitis C virus NS5A–4A peptide vaccines. J. Virol. 82, 5677–5684 (2008).

Yamagishi, T. et al. Hepatitis C virus NS5A–4A cleaves from the mitochondrial membrane, which defined the mechanism of action of NS5A–4A and showed that NS1 needs to be attached to mitochondria to mediate antiviral responses. J. Virol. 81, 104–112 (2007).

Kato, H. et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene I–inducible gene I IFN-β producing cells. J. Virol. 82, 7570–7578 (2008).

Miyashita, M. et al. Inhibition of retinoic acid-inducible gene I–inducible gene I IFN-β producing cells by the NS5A protein of hepatitis C virus. J. Virol. 81, 514–526 (2007).

Barre, P. M. et al. NS5A is cleaved in poliovirus-infected cells. J. Virol. 81, 5677–5684 (2007).

Yang, Y. et al. Disruption of innate immune due to mitochondrial targeting of a picornaviral protease precursor. Proc. Natl Acad. Sci. USA 104, 7255–7258 (2007).

Loo, Y. M. et al. Viral and therapeutic control of IFN-γ promoter stimulator 1 gene expression in hepatitis C virus infection. Proc. Natl Acad. Sci. USA 103, 6001–6006 (2006).

Lin, R. et al. Disassociation of a MAVS–IPS-1/VISA/CARD若 IKRF molecular complex from the mitochondria outer membrane by hepatitis C virus NS5A–4A proteolytic cleavage. J. Virol. 80, 6072–6083 (2006).

References 68–70 showed that NS5A–4A cleaves from the mitochondrial membrane, which defined the mechanism of action of NS5A–4A and showed that NS1 needs to be attached to mitochondria to mediate antiviral responses.
REVIEWS

85. Joo, C. H. et al. Inhibition of interferon regulatory factor 7 (IRF7)-mediated interferon signal transduction by the Kaposi’s sarcoma-associated herpesvirus viral IFN homolog vIRF5. J. Virol. 81, 8282–8292 (2007).

86. Lefort, S., Soucy-Faulkner, A., Grandvaux, N. & Flamand, L. Binding of Kaposi’s sarcoma-associated herpesvirus K12IFN to interferon-responsive factor 3 elements modulates antiviral gene expression. J. Virol. 81, 10950–10960 (2007).

87. Metroe, G. T., Silva, L., Schaffer, P. A. & Kriple, D. M. Recruitment of activated IRF-3 and CBP/p50 to herpes simplex virus ICP0 nuclear foci: potential role in blocking IFN-β induction. Virology 360, 305–321 (2007).

88. Saira, K., Zhou, Y. & Jones, C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) induces degradation of interferon response factor 3 and, consequently, inhibits interferon promoter activity. J. Virol. 81, 3077–3086 (2007).

89. Okumura, A. et al. HIV-1 accessory proteins Vpr and Vif modulate antiviral response by targeting IRF-3 for degradation. Virology 373, 85–97 (2008).

90. Bautofer, O. et al. Classical swine fever virus NS1 interacts with interferon regulatory factor 3 and induces its proteasomal degradation. J. Virol. 81, 3087–3096 (2007).

91. Barro, M. & Patton, J. T. Rotavirus NSP1 inhibits expression of type I interferon by antagonizing the function of interferon regulatory factors IRF3, IRF5, and IRF7. J. Virol. 81, 4475–4481 (2007).

92. Lu, G. et al. ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation. Cell. Mol. Biol. (Noisy-le-grand) 52, 29–41 (2006).

93. Guerra, S., Caceres, A., Knobil, C. P., Horak, I. & Esteban, M. Vaccinia virus E3 protein prevents the antiviral action of ISG15. PLoS Pathog. 4, e1000096 (2008).

94. Hiscott, J., Nguyen, T. L., Arguello, M., Nakhaei, P. & Paz, S. Manipulation of the nuclear factor-kB pathway and the innate immune response by viruses. Oncogene 25, 6844–6867 (2006).

95. Tait, S. W., Reid, E. B., Greaves, D. R., Wileman, T. E. & Powell, P. P. Mechanism of inactivation of NF-kB by a viral homologue of IxBu. Signal-induced release of IxBu results in binding of the viral homologue to NF-kB. J. Biol. Chem. 275, 34656–34664 (2000).

96. Rodriguez, C. I. et al. African swine fever virus IAP-like protein induces the activation of nuclear factor-kB. J. Virol. 76, 3596–3592 (2002).

97. Chen, R. A. J., Ryzhakov, G., Corray, S., Randov, F. & Smith, G. L. Inhibition of IxB kinase by vaccinia virus virulence factor B14. PLoS Pathog. 4, e22 (2008).

98. Matta, H. et al. Kaposi’s sarcoma-associated herpesvirus (KSHV) oncoprotein K13 bypasses TRAFs and directly interacts with the IxB kinase complex to selectively activate NF-κB without JNK activation. J. Biol. Chem. 282, 24858–24865 (2007).

99. Wang, T. et al. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nature Med. 10, 1356–13573 (2004).

100. Le Corff, R. et al. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog. 2, e53 (2006).

101. Hutchens, M. et al. TRIF increases disease morbidity and mortality from vaccinia infection. J. Immunol. 180, 485–491 (2008).

102. References 99–101 show that for three distinct viruses the absence of TLR3 benefits the host, suggesting that some viruses actually use TLR3 to facilitate viral dissemination.

103. Gowen, B. B. et al. TLR3 deletion limits mortality and disease severity due to Phlebovirus infection. J. Immunol. 177, 6501–6507 (2006).

104. Zhang, S. Y. et al. TLR3 deficiency in patients with herpes simplex encephalitis. Science 317, 1522–1527 (2007). This is a rare demonstration of the importance of a PRR in human viral disease.

105. Brooks, D. G. et al. Interleukin-10 determines viral clearance or persistence in vivo. Nature Med. 12, 1301–1309 (2006).

106. Eijnas, M. et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J. Exp. Med. 203, 2461–2472 (2006).

107. Maloney, G., Schroder, M. & Bowie, A. G. Vaccinia virus protein A52R activates p38 mitogen-activated protein kinase and potentiates lipopolysaccharide-induced interleukin-10. J. Biol. Chem. 280, 30838–30844 (2005).

108. Jade, B. A. et al. Subversion of the innate immune system by a retrovirus. Nature Immunol. 5, 575–578 (2003). This study shows that viruses can subvert TLRs to complete their life cycle.

109. Arumugam, Y. et al. DDX5 DEAD-box RNA helicase is required for hepatitis C virus RNA replication. J. Virol. 81, 15922–15926 (2007).

110. Vedavalli, V. S. R. K., Neuvru, C., Chi, Y.-H., Kleiman, L. & Jeang, K.-T. Requirement of DDX5 DEAD Box RNA helicase for HIV-1 Rev-RRE export function. Cell 119, 381–392 (2004).

111. Awomoyi, A. A. et al. Association of TLR4 polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children. J. Immunol. 179, 5171–5177 (2007).

112. Johnson, C. L., Owen, D. M. & Gale, M. J. Functional and therapeutic analysis of hepatitis C virus NS3 4a protease control of antiviral immune defense. J. Biol. Chem. 282, 10792–10803 (2007).

113. Cardenas, W. B. et al. Ebola virus VP55 protein binds double-stranded RNA and inhibits α/β interferon production induced by RIG-I signaling. J. Virol. 80, 5168–5176 (2006).

114. Gale, M. J. Jr et al. Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PRK protein kinase by the nonstructural SA protein. Virology 250, 217–227 (1997).

115. Taguchi, T. et al. Hepatitis C virus NS5A protein interacts with 2’-O-methyladenosine synthetase and inhibits antiviral activity of IFN in an IFN sensitivity-determining region-independent manner. J. Gen. Virol. 85, 959–969 (2004).

116. Chang, H. W., Watson, J. C. & Jacobs, B. L. The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc. Natl Acad. Sci. USA 89, 4825–4829 (1992).

117. Romano, P. R. et al. Inhibition of double-stranded RNA-dependent protein kinase PKR by vaccinia virus E3: role of complex formation and the E3 N-terminal domain. Mol. Cell. Biol. 18, 7304–7316 (1998).

118. Hatada, E. & Fukuda, R. Binding of influenza A virus NS1 protein to dsRNA in vitro. J. Gen. Virol. 73, 3325–3329 (1992).

Acknowledgements
The work in our laboratory is supported by Science Foundation Ireland and the Health Research Board.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

FURTHER INFORMATION
Andrew G. Bowie’s homepage: http://www.tcd.ie/Biochemistry/research/agbowie.php

ALL LINKS ARE IN THE ONLINE PDF