REVIEW

Manipulation of the nuclear factor-κB pathway and the innate immune response by viruses

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Viral and microbial constituents contain specific motifs or pathogen-associated molecular patterns (PAMPs) that are recognized by cell surface- and endosome-associated Toll-like receptors (TLRs). In addition, intracellular viral double-stranded RNA is detected by two recently characterized DExD/H box RNA helicases, RIG-I and Mda-5. Both TLR-dependent and -independent pathways engage the IκB kinase (IKK) complex and related kinases TBK-1 and IKKε. Activation of the nuclear factor κB (NF-κB) and interferon regulatory factor (IRF) transcription factor pathways are essential immediate early steps of immune activation; as a result, both pathways represent prime candidates for viral interference. Many viruses have developed strategies to manipulate NF-κB signaling through the use of multifunctional viral proteins that target the host innate immune response pathways. This review discusses three rapidly evolving areas of research on viral pathogenesis: the recognition and signaling in response to virus infection through TLR-dependent and -independent mechanisms, the involvement of NF-κB in the host innate immune response and the multitude of strategies used by different viruses to short circuit the NF-κB pathway.
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The NF-κB signaling network: regulating innate and adaptive immunity

Activation of the nuclear factor-κB (NF-κB) transcriptional program is a fundamental immediate early step of immune activation; as a result, NF-κB signaling represents a prime candidate for viral interference. Many viruses disrupt the innate immune responses and NF-κB through the use of multifunctional viral proteins that target specific aspects of the NF-κB pathway. On the other hand, certain viruses, including human immunodeficiency virus type I (HIV-I), human T-cell leukemia virus type 1 (HTLV-1), Human herpesvirus 8 (HHV8) and Epstein–Barr virus (EBV), have incorporated aspects of NF-κB signaling into their life cycle and pathogenicity, and thus induce NF-κB activation (Hiscott *et al.*, 2001). A convergence of knowledge about the host innate immune response to viral pathogens and the strategies used by viruses to short circuit the early host response, coupled with the identification of potential targets for therapeutic intervention in viral diseases, has created an energized period of research in this important area of molecular virology. This review discusses three rapidly evolving areas of research on viral pathogenesis: the recognition and innate immune response to virus infection, the involvement of NF-κB in the host response and the multitude of strategies used by different viruses to manipulate the NF-κB pathway. Because the organization of the mammalian NF-κB family of transcription factors as well as the knockout phenotypes of the NF-κB and IκB kinase (IKK)-deficient mice are described elsewhere in this issue (see Gerondakis *et al.*, 2006; Gilmore, 2006), this information will not be reiterated here.

Inducible activation of NF-κB signaling requires phosphorylation of IκB by the 700–900 kDa multiprotein IKK complex (see Scheidereit, 2006). The IKK complex contains two catalytic kinase components, IKKα and IKKβ, as well as a non-enzymatic regulatory subunit NEMO (NF-κB Essential MOdulator) (Hayden and Ghosh, 2004; Karin *et al.*, 2004). In addition, the chaperone molecule heat shock protein 90 (Hsp90) and cell division cycle 37 (Cdc37) protein are accessory molecules that directly interact with the IKK complex through the kinase domain of IKKα and IKKβ (Chen-Park *et al.*, 2002). Readers are referred to the description of the classical IKK complex in the issue (Scheidereit, 2006); herein, we focus on two virus-activated IKK-related kinases, TBK-1 and IKKε.

The virus activated, IKK-related kinases: TBK-1 and IKK

Two additional members of the family of IKK kinases – TBK-1/NAK/T2K and IKKε/IKKi – were identified based on limited sequence homology and the potential

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to stimulate NF-κB signaling (Peters and Maniatis, 2001). Inducible IKK (IKKε/IKKi) was isolated using a subtractive hybridization technique from lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages (Shimada *et al.*, 1999), as well as from an EST database search for IKK homologs (Peters *et al.*, 2000). The TBK-1/NAK/T2K kinase has been characterized as an IKK homolog involved in tumor necrosis factor (TNF)α-mediated NF-κB activation (Pomerantz and Baltimore, 1999; Bonnard *et al.*, 2000), acting upstream of IKKβ (Tojima *et al.*, 2000). Despite the homology and functional similarities between TBK-1 and IKKε, the expression patterns of these two IKK related kinases are considerably different. TBK-1 expression is ubiquitous and constitutive in a wide variety of cells, while IKKε expression is relegated to cells of the immune compartment (Shimada *et al.*, 1999; Peters and Maniatis, 2001), but is inducible in non-hematopoietic cells by stimulation with activating agents such as TNF, PMA, LPS and virus infection (Shimada *et al.*, 1999; Aupperle *et al.*, 2001; Kravchenko *et al.*, 2003; Hemmi *et al.*, 2004) through an NF-κB-dependent mechanism involving upregulation of IKKε expression by C/EBPδ and C/EBPβ (Kravchenko *et al.*, 2003).

A schematic representation of the IKK kinase family is presented in Figure 1. Like the classical IKK kinases IKKα and IKKβ, the IKK-related kinases (IKKε and TBK-1) contain a catalytic kinase domain, a leucine zipper domain and a helix-loop-helix domain that is involved in protein–protein interactions (Pomerantz and

Figure 1 Comparison of the IKK family: classical IKK kinases *vs* IKK-related kinases. The four IKK kinases are classified into two subgroups, based on sequence homology and substrate specificity. Numbers below the figure indicate the amino-acid residue number and important domains are indicated above (catalytic kinase domain; leucine zipper; helix–loop–helix). Classical IKK kinases – IKKα and IKKβ – share 52% overall homology to each other and the IKK-related kinases IKKε and TBK-1 share 61% overall homology to each other. Homology between the subgroups is limited, with 27% overall homology and approximately 33% homology within the catalytic kinase domain. The positions of critical residues involved in catalytic activity are represented. Mutation K44A (IKKα and IKKβ) or K38A (IKKε and TBK-1) within the ATP-binding pocket of the kinase domain generates a dominant-negative kinase; phosphomimetic mutations S176/180E (IKKα) or S177/181E (IKKβ) within the kinase activation loop generates a constitutively active kinase.

Baltimore, 1999; Shimada *et al.*, 1999; Tojima *et al.*, 2000; Peters and Maniatis, 2001). IKKε and TBK-1 are 64% homologous to each other, but exhibit limited homology to the classical IKKα and β kinases, with about 33% sequence identity within the kinase domain (Pomerantz and Baltimore, 1999; Shimada *et al.*, 1999; Peters *et al.*, 2000; Tojima *et al.*, 2000). IKKε and TBK-1 also possess a kinase activation loop located between kinase subdomains VII and VIII; serine phosphorylation within the activation loop is required for kinase autophosphorylation and induction of enzymatic activity (Shimada *et al.*, 1999; Peters *et al.*, 2000; Kishore *et al.*, 2002). However, different from IKKα and IKKβ, the primary amino-acid sequence of the IKKε and TBK-1 activation loop does not fit a canonical MEK consensus motif (Shimada *et al.*, 1999; Peters *et al.*, 2000; Kishore *et al.*, 2002); that is, the Ser-176 residue is replaced with glycine – resulting in an SXXXG sequence. A serine to alanine substitution at position 172 abolishes IKKε and TBK-1 catalytic activation. Differences within the activation loop sequence are consistent with the failure of MEKK1 or NIK expression to augment IKK-related kinase activity, as occurs with canonical IKKs (Shimada *et al.*, 1999). In contrast to IKKα and IKKβ, an S172E phosphomimetic mutation does not generate a constitutively active IKKε or TBK-1 kinase, but instead decreases catalytic activity (Shimada *et al.*, 1999; Peters *et al.*, 2000).

The distinct homologies between the canonical and the IKK-related kinases suggested, in retrospect, unique functions for TBK-1 and IKKε. Both of the IKK-related kinases were shown early on to phosphorylate *in vitro* only one of the two critical serine residues (Ser-36) within the signal response domain of IκBα, suggesting that IκBα was not a physiological substrate for these IKKs (Shimada *et al.*, 1999; Bonnard *et al.*, 2000; Peters *et al.*, 2000). Nevertheless, TBK-1/NAP1/T2K knockout mice (Bonnard *et al.*, 2000) showed striking phenotypic similarities to other mice disrupted in NF-κB signaling components (Peters and Maniatis, 2001). That is, TBK-1−/− mice are embryonic lethal, dying at day E14.5 of massive liver degeneration due to extensive hepatocyte apoptosis, a result that suggested that IKKε/TBK-1 are not redundant in the NF-κB signaling pathways but rather represent upstream components of the IKK complex (Peters and Maniatis, 2001).

Both TBK-1 and IKKε interact with the TNF-receptor-associated factor (TRAF)-interacting protein/TRAF family member-associated NF-κB activator (I-TRAF/TANK) protein, a modulator of TNFα-induced NF-κB activation (Cheng and Baltimore, 1996; Rothe *et al.*, 1996). Overexpression of IKKε or TBK-1 induces phosphorylation of I-TRAF/TANK, which results in its dissociation from TRAF2 and subsequent activation of NF-κB transcription through the canonical IKK pathway (Pomerantz and Baltimore, 1999; Nomura *et al.*, 2000). The NAK associated protein 1 (NAP1), a homolog of I-TRAF/TANK, has also been shown to directly interact with TBK-1 and IKKε (Fujita *et al.*, 2003). Overexpression of NAP1 specifically enhances cytokine induction of an
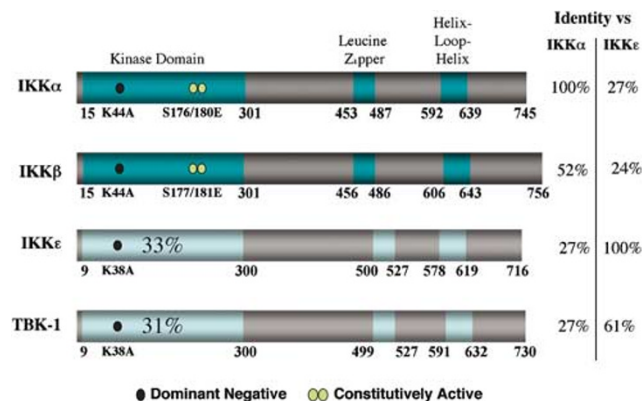
NF-κB-dependent reporter gene, while *in vivo* depletion of NAP1 reduces NF-κB-dependent reporter gene expression and sensitizes cells to TNFα-induced apoptosis (Fujita *et al.*, 2003). It is possible that I-TRAF/TANK or NAP1, or perhaps both, regulate a TBK-1 and IKKε complex through an essential adaptor function, much like the NEMO regulates the classical IKK complex (Rothwarf *et al.*, 1998; Yamaoka *et al.*, 1998; Makris *et al.*, 2000; Rudolph *et al.*, 2000). Physical association between TANK and NEMO suggest that the canonical IKK complex and the IKK-related kinases exist as a physically associated signaling complex responsible for phosphorylation of additional transcription factors (Chariot *et al.*, 2002). A recent study has also demonstrated a role for Hsp90 in the regulation of TBK-1 and interferon regulatory factor (IRF)-3 activity (Yang *et al.*, 2006).

Analysis of mouse embryonic fibroblasts derived from TBK-1 knockout mice showed that TBK-1 expression is dispensable for signal-responsive IκBα degradation and NF-κB DNA binding induction, but is required for NF-κB-dependent gene transcription in the nucleus (Pomerantz and Baltimore, 1999; Bonnard *et al.*, 2000). It was proposed that IKKε and TBK-1 regulate NF-κB activation at the level of C-terminal phosphorylation of the NF-κB DNA-binding subunits (Peters and Maniatis, 2001). Recent studies have in fact demonstrated that TBK-1 and IKKε directly phosphorylate the C-terminal transactivation domains of RelA and c-Rel, which modulates their subcellular localization and transactivation potential (Harris *et al.*, 2006; Mattioli *et al.*, 2006).

Triggering the interferon antiviral response through an IKK-related pathway

An important breakthrough in our understanding of the physiological function of TBK-1 and IKKε was the demonstration that the IRF-3 and IRF-7 transcription factors are the primary *in vivo* targets of the IKK-related kinases. C-terminal phosphorylation of IRF-3 and IRF-7 can induce nuclear localization, DNA binding and transactivation in cells overexpressing TBK-1 or IKKε (Sharma *et al.*, 2003; Fitzgerald *et al.*, 2003a). Both kinases directly phosphorylate IRF-3 and IRF-7 at key residues within their C-terminal signal-responsive domain (McWhirter *et al.*, 2004; tenOever *et al.*, 2004) and both kinases target identical serine residues (tenOever *et al.*, 2004; Paz *et al.*, 2006). Importantly, expression of the IKK-related kinases is essential to initiate IRF signaling in response to *de novo* Sendai, Vesicular Somatitis Virus (VSV) or measles virus infection, and treatment with RNAi directed against either IKKε or TBK-1 reduces VSV-inducible IRF-3 phosphorylation and IRF-dependent gene expression in human cells. Furthermore, expression of the IKK-related kinases generates an IRF-3-dependent antiviral state *in vivo* that can inhibit *de novo* VSV replication (Sharma *et al.*, 2003; tenOever *et al.*, 2004). Subsequent analysis of the response to virus infection in TBK1−/− and

IKKε−/− mice demonstrated that TBK-1 is principally involved in IRF-3 and IRF-7 phosphorylation and development of the antiviral response, with only an accessory role associated with IKKε (Hemmi *et al.*, 2004; Perry *et al.*, 2004). Ongoing studies however suggest that TBK-1 and IKKε are not redundant, but that IKKε selectively regulates a subset of interferon-responsive antiviral genes during influenza virus infection (tenOever and Maniatis, personal communication).

Activation of the transcription factors IRF-3 and IRF-7 is required for expression of type I IFN and numerous immunoregulatory genes in response to foreign pathogen (Sato *et al.*, 2000). IRF-3 acts in synergy with ATF/c-Jun and NF-κB as an immediate early activator of IFNβ transcription (Maniatis *et al.*, 1998). Early response genes are rapidly induced by virus infection and activation of latent, pre-existing IRF-3 in conjunction with ATF-2/c-jun and NF-κB occurs without the need for *de novo* protein synthesis. In non-hematopoietic cells, type I IFN signaling in a paracrine fashion results in *de novo* expression of IRF-7, which acts together with IRF-3 to increase the production of multiple IFNα subtypes, thus amplifying the antiviral response to paramyxovirus infection (Marie *et al.*, 1998; Lin *et al.*, 2000; Sato *et al.*, 2000).

Extensive mutagenesis of the IRF-3 C-terminal domains revealed a cluster of serine/threonine (S/T) residues that are targeted for phosphorylation during Sendai infection (Lin *et al.*, 1999), and these residues were subsequently shown to be targets of TBK-1 and IKKε. Significantly, mutation of five clustered residues (within aa 396–405) in IRF-3 to the phosphomimetic glutamic acid generates a constitutively active form of IRF-3 (IRF-3 5D) that can efficiently transactivate IRF-3-dependent promoters (Lin *et al.*, 1998, 1999) and can stimulate cellular apoptosis in the absence of virus infection (Heylbroeck *et al.*, 2000). Phosphorylation of the IRF-7 C terminus by the IKK-related kinases directly targets two serine residues, Ser-477 and Ser-479. Alignment of the primary sequence of the C-terminal domains of IRF-3 and IRF-7 revealed an extended SxSxxxS consensus motif that appears to be the target for phosphorylation by TBK-1 and IKKε (Paz *et al.*, 2006).

The three-dimensional crystal structure of the IRF-3 C-terminal domain has been reported by two groups (Qin *et al.*, 2003; Takahasi *et al.*, 2003). These studies describe a unique autoinhibitory mechanism for IRF-3 whereby N- and C-terminal autoinhibitory domains within the C-terminal IRF association domain (IAD) interact to form a highly condensed hydrophobic core. This interaction buries several key residues within the IAD involved in dimerization of the active protein, and therefore required for nuclear accumulation, DNA binding and transactivation by IRF-3. It is proposed that virus-inducible, C-terminal phosphorylation events abolish autoinhibitory interactions by introducing charge repulsions within this region that unmask the IAD active site and realign the DNA-binding domain to form the transcriptionally active IRF-3 protein with the

capacity to recruit the CBP histone acetyltransferase (Qin et al., 2003, 2005). Ser-386 in IRF-3 appears to be the primary target for virus-inducible, as well as IKKε- and TBK-1-mediated phosphorylation during the initial stages of activation, because of its accessibility in the three-dimensional structure (Takahasi et al., 2003). Interestingly, two groups noted that the C-terminal domain of IRF-3 exhibits sequence similarity to the Mad homology 2 domain of the Smad family of transcriptional regulator proteins, suggesting common molecular mechanisms of action among a superfamily of signaling mediators as well as a common evolutionary relationship between the IRF and Smad proteins (Qin et al., 2003; Takahasi et al., 2003).

Assembly of the interferon β enhanceosome

The type I IFNβ promoter represents an important paradigm of virus-activated transcriptional regulation requiring the coordinated activity of NF-κB and IRF transcription factors. Transcription of IFNβ requires the formation of a large, higher-order multiprotein complex called the enhanceosome, which consists of multiple promoter-specific transcription factors, associated structural components and basal transcription machinery bound to enhancer DNA (Thanos and Maniatis, 1995b; Kim and Maniatis, 1997; Agalioti et al., 2000; Merika and Thanos, 2001). The IFNβ promoter-enhancer region contains four positive (PRDI–IV) and one negative regulatory domains (NRDI): PRDI and III contain the binding sites for IRF-7 and IRF-3, respectively, as well as for other IRF members (Civas et al., 2006); PRDII is recognized by NF-κB heterodimers; and PRDIV by ATF-2 and c-Jun heterodimers (Hiscott et al., 1989; Lenardo and Baltimore, 1989; Lenardo et al., 1989; Visvanathan and Goodbourn, 1989; Thanos and Maniatis, 1995a; Chu et al., 1999a). Virus infection leads to the recruitment of histone acetyltransferase co-activators (GCN5 and CBP/p300), as well as the high mobility group protein (HMG 1(Y)), which binds to the minor groove of DNA at four sites within the IFNβ enhancer and contributes to the stability of the enhanceosome (Thanos and Maniatis, 1992, 1995a; Yie et al., 1997).

This virus inducible enhancer of IFNβ is silent in uninfected cells in part through the inhibitory effect of an NF-κB regulating factor (NRF) binding site that overlaps the PRDII site (Nourbakhsh and Hauser, 1997, 1999), the placement of p50 homo-dimers at the PRDII site, and the positioning of nucleosomes upstream of the IFNβ gene (Thanos and Maniatis, 1995b; Senger et al., 2000; Lomvardas and Thanos, 2001; Munshi et al., 2001). IFNβ transcription is quickly induced to high levels upon viral infection, with the recruitment of p50-RelA dimers to PRDII (Maniatis et al., 1998; Munshi et al., 1999) and hyperacetylation of histones H3 and H4 localized in the IFNβ promoter (Parekh and Maniatis, 1999). This hyperacetylation is known to play a crucial role in gene inducibility because enhanceosome assembly following infection requires precise spacing between the factor binding sites to ensure that each of the enhanceosome components simultaneously contact one another and DNA (Merika and Thanos, 2001).

More recently, Honda et al. (2005) generated IRF-7 knockout mice and demonstrated that IRF-7 is essential for the virus-mediated induction of type I IFN. The IRF-7 knockout mice develop normally with no overt differences in hematopoietic cell populations. However, IFNα mRNA induction is completely inhibited and IFNβ levels are greatly reduced in IRF7−/− cells. Also, serum IFN levels are significantly lower in IRF-7−/− mice. In IRF-3/IRF-7 double knockout mice, IFNβ levels are completely abrogated, thus reflecting the absolute requirement for these two factors in the type 1 IFN response to virus infection (Honda et al., 2005).

TLR-dependent signaling to NF-κB and the innate immune response

Innate immunity represents an ancient and evolutionarily conserved mechanism for detection and clearance of foreign pathogens (Janeway and Travera, 1997; Janeway and Medzhitov, 2002). In the plant and animal kingdoms, innate immune responses are triggered by a set of germline-encoded pathogen receptors called Toll-like receptors (TLRs) (Janeway and Medzhitov, 2002; Akira and Sato, 2003). Invading pathogens are recognized by specific motifs or pathogen-associated molecular patterns (PAMPs) through different TLRs (Iwasaki and Medzhitov, 2004; Kaisho and Akira, 2004; Takeda and Akira, 2005). The Toll receptor was originally identified in Drosophila as a receptor essential for the establishment of a dorsal-ventral pattern (Lemosy et al., 1998; Minakhina and Steward, 2006). Subsequently, multiple homologs of the Toll receptor were identified in mammals and the TLR family now consists of 13 members (10 in humans), which are expressed differentially among immune and non-immune cells and respond to different components of invading pathogens (Ulevitch, 2000). Of these, TLR3, hTLR7/mTLR8 and TLR9 recognize different nucleic acid motifs – dsRNA, ssRNA and CpG DNA, respectively.

The cytoplasmic intracellular tail of TLRs – which shows high homology with that of the (IL)-1 receptor family – mediates signal transduction, while the leucine-rich repeat (LRR) containing extracellular domains is responsible for PAMP recognition. The specificity of TLR signaling is conferred through unique protein–protein interactions and differential utilization of the TIR-containing adaptor molecules such as myeloid differentiation factor 88 (MyD88), TIRAP/Mal, TIR-containing adaptor molecule-1/Toll/IL-1 receptor domain-containing adaptor inducing IFNβ (TICAM-1/TRIF) (Hoebe et al., 2003; Oshiumi et al., 2003a; Yamamoto et al., 2004), TIR-containing adaptor molecule-2/TRIF-related adaptor molecule (TICAM-2/TRAM) (Oshiumi et al., 2003a; Fitzgerald et al., 2003b) and sterile alpha and HEAT/armadillo motif (SARM) (Mink et al., 2001). Over the past several years, it has become evident that TLR recognition of PAMPs and

downstream signaling is critical for the development of innate and adaptive immune responses to viruses and microbial pathogens through the induction of NF-κB, IRFs and TLR-responsive antiviral and inflammatory genes (Takeda and Akira, 2005; Akira et al., 2006; Kawai and Akira, 2006).

TLR-3 signaling

TLR3 is a 904 a.a. receptor for dsRNA – long considered a functional by-product of intracellular virus replication. TLR3 engagement transmits signals that activate IFN and inflammatory cytokines through NF-κB and IRF signaling pathways (Figure 2). TLR3 is expressed in intracellular vesicular compartments in DCs and on the cell surface in certain epithelial cells – where its expression is inducible by IFN – but not in monocytes, polymorphonuclear leukocytes, B, T, and NK cells (Muzio et al., 2000; Tissari et al., 2005; Cario and Podolsky, 2000; Schaefer et al., 2005; Hewson et al., 2005). At early times after infection, incoming virus particles or ribonucleoprotein complexes may be recognized within the endosomal compartment, while late after infection following replication and cell lysis, viral dsRNA is released into the extracellular space, where it is available to bind extracellular TLR3.

NF-κB and IRF-3 activation is mediated by the TLR-3-associated molecule TRIF/TICAM-1 and functions independently of the MyD88 pathway. TRIF consists of an N-terminal proline-rich domain, a TIR domain, and C-terminal proline-rich domain (Oshiumi et al., 2003b; Yamamoto et al., 2002b). The N-terminal region of

TRIF directly associates with TBK-1 (Fitzgerald et al., 2003a; Jiang et al., 2004) and TRAF6, a ubiquitin ligase (Sato et al., 2003). Following virus infection, the association with TRAF6 leads to activation of the canonical IKK complex (IKKα/IKKβ/NEMO) and NF-κB, which upregulates the transcription of pro-inflammatory genes such as IL-6, IL-1β and TNF-α. The recruitment of TBK-1 to the C-terminal region of TRIF initiates a signaling cascade that culminates in IRF-3 activation and the induction of IFNβ, RANTES and IP-10. In addition, the phosphatidyl-inositol-3 kinase pathway (PI3K) also contributes to dsRNA and TLR3-dependent IRF-3 phosphorylation. Specific mutations of tyrosine residue Tyr-759 and Tyr-858 inhibit the recruitment of PI3K to the receptor and TBK-1 activation, respectively (Sarkar et al., 2004). As a result, partial IRF-3 phosphorylation, dimerization and nuclear translocation occur, but activation of the IFNβ promoter is inhibited, suggesting that the PI3K–Akt pathway is essential for full dsRNA signaling to IRF-3 (Sarkar et al., 2004).

Both the C-terminal and N-terminal regions of TRIF can independently activate the NF-κB response. The N-terminal region of TRIF contains one functional TRAF6-binding motif that associates with the TRAF-C domain of TRAF6, leading to NF-κB induction (Ye et al., 2002; Sato et al., 2003). Mutation of the TRAF6 binding motifs of TRIF abolishes binding between TRIF and TRAF6, and partially reduces NF-κB promoter activity (Jiang et al., 2004). The C-terminal region of TRIF recruits the kinase receptor interacting
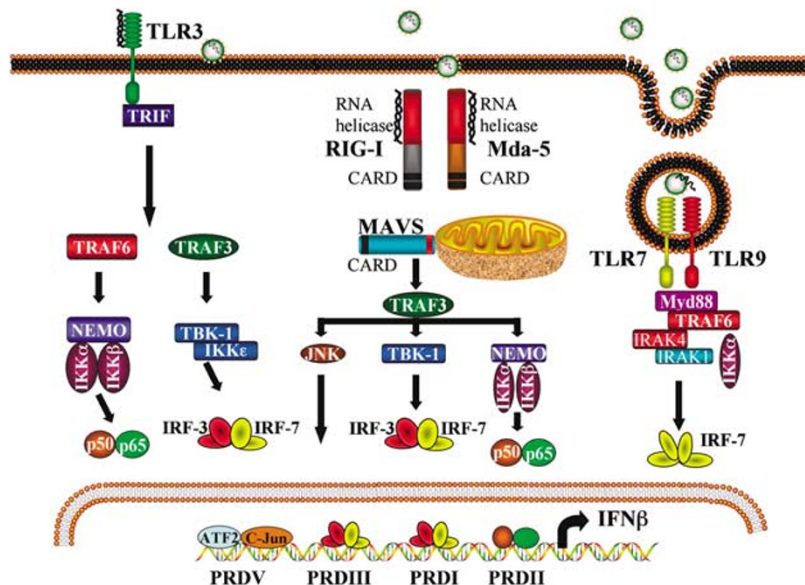

Figure 2 Summary of the signaling pathways that recognize virus infection. Virus replication results in the production of PAMPs such as single- and double-stranded RNA. Viral nucleic acids trigger multiple signaling cascades through Toll-like-receptor-dependent (TLR3, TLR7 and TLR9) and TLR-independent (RIG-I and Mda-5) pathways leading to kinase activation through TRAF family members. In pDCs, TLR7 or TLR9 engagement by ssRNA leads to direct activation of IRF-7 through MyD88/TRAF6/IRAK4/IRAK1 recruitment. TRIF and MyD88 are the adaptors linking TLRs to the TRAF proteins, whereas MAVS links RIG-1 and Mda-5 to TRAF3. TRAF-dependent induction of the kinases JNK, IKKα, IKKβ, IKKε, TBK-1 and IRAK-1 induce the binding of ATF2-cJun, NF-κB (p50-RelA), IRF-3 and IRF-7 to sequence-specific PRD located upstream of the IFNβ start site. Coordinated assembly of these factors forms the IFNβ enhanceosome, which is responsible for the transcriptional induction of this antiviral cytokine (modified from tenOever and Maniatis, 2006).

protein (RIP1) through its RIP homotypic interaction motif and also induces the NF-κB pathway, whereas RIP3 inhibits this pathway (Sun et al., 2002; Meylan et al., 2004).

TLR3 localizes to the intracellular vesicular compartment in dendritic cells and is not present on the cell surface (Matsumoto et al., 2003). Additionally, dendritic cell populations differentially express TLR3 along with hTLR7/mTLR8 and TLR9. TLR3 is not expressed in plasmacytoid dendritic cells (pDCs), but is highly expressed in human monocyte-derived dendritic cells. Upon TLR7 and TLR9 stimulation with their respective ligand, pDCs produce a high level of type I IFN, mainly IFNα, whereas myeloid DCs mainly produce IL-12 and IFNβ upon TLR3 stimulation, suggesting a differential response in distinct DC subtypes (Reis e Sousa, 2004; Degli-Esposti and Smyth, 2005). Furthermore, on synthetic dsRNA poly(I:C) stimulation of TLR3, DCs produce IFNβ and IL-12p70 and also upregulate co-stimulatory molecules such as CD80, CD83 and CD86 by a mechanism dependent on TRIF (Cella et al., 1999; Matsumoto et al., 2003). TLR3 signaling augments antigen cross-presentation by DC to trigger an anti-viral cytotoxic response mediated by CD8+ T cells (Schulz et al., 2005).

TLR-4 signaling
LPS is a major component of the outer membrane of Gram-negative bacteria. The host defense response to LPS includes production of pro-inflammatory cyto-kines, such as TNFα, IFNβ, as well as inducible NO (iNOS). TLR4, the receptor for LPS, was the first mammalian homolog of Drosophila Toll gene product to be discovered (Medzhitov and Janeway, 1997) and is a type I transmembrane protein. The TLR4 cytoplasmic domain contains a Toll-interleukin (IL-1) receptor (TIR) domain, which is common to all TLRs. Upon bacterial infection, lipid-binding protein (LBP), an acute phase protein that circulates in the liver, binds to the lipid A moiety of LPS (Schumann et al., 1990). Soluble CD14 binds and concentrates LPS present outside the cell. LBP-bound LPS forms a ternary complex with CD14, enabling the transfer of LPS to the TLR4–MD2 complex (Tobias et al., 1995). MD-2 is a secreted glycoprotein that acts as an extracellular adaptor protein that binds LPS and is essential for TLR4 signaling to occur (Visintin et al., 2003). Upon binding of LPS, the TLR4–MD2 complex homodimerizes and initiates the ensuing signaling cascade that bifurcates into two distinct pathways: MyD88-independent and MyD88-dependent pathways.

TLR4 signaling requires MyD88, an adaptor protein that contains a C-terminal TIR domain and an N-terminal death domain (Burns et al., 1998). A second adaptor protein, MyD88 adaptor-like protein (MAL; also known as TIRAP), was found to be indispensable along with MyD88 for TLR4 signaling leading NF-κB activation (Horng et al., 2001; Yamamoto et al., 2002b; Fitzgerald et al., 2003b). TIRAP-deficient mice showed defects in activation of the MyD88-dependent signaling pathway through TLR4, but not IL-1R,

indicating specificity of the TLR4-mediated MyD88-dependent pathway. However, in MyD88 knockout mice, LPS-induced activation of NF-κB and mitogen-activated protein kinase (MAPK) still occur, albeit in a delayed manner and – perhaps more striking – IRF-3 phosphorylation and IFNβ induction are unaffected, indicating that additional proteins beside MyD88 are involved in the early interferon response to infection (Fitzgerald et al., 2003b). The discovery of TRAM as a mediator of interactions between TRIF and TLR-4 and subsequent activation of IRF-3 unveiled the existence of a TLR-4 MyD88-independent pathway.

Two serine-threonine kinases, IRAK4 and IRAK1, are the principal mediators of the Myd88-dependent TLR4 signaling pathway. MyD88 recruitment to TLR4 is followed by the recruitment of IRAK4 and IRAK1 along with the adapter TRAF6 (Li et al., 2002), resulting in the formation of the receptor complex. During the formation of this complex IRAK4 is activated, leading to the hyperphosphorylation of IRAK1, which then leads to the dissociation of the negative regulator of IRAK1, Tollip. Hyperphosphorylated IRAK1 dissociates from the receptor complex to form a new complex with IRAK2 and TRAF6. Subsequently, TRAF6 physically interacts with the ubiquitin conjugating enzyme complex Ubc13/Uev1A to catalyse the formation of a unique Lys-63-linked polyubiquitin chain on IKK that positively regulates the NF-κB signaling pathway (Deng et al., 2000). TRAF6 then becomes activated, associates with TAK-1 binding protein (TAB2), which in turn activates the MAPK kinase TAK-1 (transforming growth factorβ-activated kinase), which is constitutively associated with its adaptor protein TAB1. This leads to the activation of MAPKs, such as extracellular signal-regulated kinases, p38 and c-Jun N-terminal kinase (JNK). In addition, TRAF6 activates the IKK complex and NF-κB (reviewed in Akira et al., 2006).

In the MyD88-independent pathway, TRAM together with TRIF recruits TBK-1/IKKε to activate IRF-3, leading to induction of IFNα/β (Yamamoto et al., 2002a). TRAM and TRIF are both required for TLR4 signaling to IRF3, as TRAM cannot restore IFNβ induction in response to LPS stimulation when overexpressed in TRIF−/− cells (Yamamoto et al., 2003). TRIF also binds to TRAF6 via an N-terminal TRAF6-binding domain leading to the activation of the signalosome, followed by ubiquitination and degradation of IκB, culminating in late phase NF-κB activation. Furthermore, TRIF directly recruits TBK-1, and possibly indirectly IKKε, leading to activation of the kinases (Sato et al., 2003; Fitzgerald et al., 2003a) that phosphorylate IRF-3. Activated IRF-3 dimerizes, translocates to the nucleus and binds the PRDI–PRDIII elements, inducing IFN-β production (reviewed in Akira et al., 2006).

TLR-7 signaling
Most cell types are able to produce type I IFN, yet pDCs are particularly adept at secreting very high IFN levels in response to virus infection (Colonna et al.,

2004). pDCs survey their environment for viruses by endocytic uptake and TLR7 is required for the recognition of ssRNA viruses such as VSV and influenza (Lund et al., 2004). Single-stranded oligoribonucleotides introduced into the endosomal but not the cytoplasmic compartment trigger TLR7 activation (Diebold et al., 2004; Heil et al., 2004). In TLR7 signaling, type I IFN secretion by pDCs is MyD88-dependent (Lund et al., 2004) and both MyD88 and TRAF6 are required to induce IFNα production (Figure 2).

IRAK-1 is a key regulator for TLR7- and TLR9-mediated IFNα production. On stimulation of TLR7 by ssRNA, IRAK-1 is recruited to the complex by MyD88, along with TRAF6, and IRAK-4. IRAK-4 phosphorylates IRAK-1, triggering autophosphorylation of IRAK-1 and increasing its affinity for TRAF6 (Uematsu et al., 2005). In vitro studies have shown that IRAK-1 can bind and phosphorylate IRF-7, although to date phosphorylation of endogenous IRF7 by IRAK-1 has not been demonstrated. In IRAK-1 knockout mice, both TLR7- and TLR9-mediated IFNα production is abolished. In TLR7- and TLR9-mediated responses, MyD88 interacts with and activates IRF-7 but fails to activate IRF-3. pDCs derived from IRF-7−/− mice are non-responsive for IFN induction upon TLR7 and TLR9 stimulation, whereas pDCs from IRF-3−/− mice show normal IFN induction. Hence, IRF-3 appears dispensable for the induction of type I IFN in pDCs (Honda et al., 2005). Furthermore, induction of CD8^{+} T-cell responses was completely dependent on IRF-7. Therefore, IRF-7 is not only important in the development of innate immunity but also clearly plays a central role in adaptive immunity (Honda et al., 2005).

Surprisingly, IKKα is critically involved in TLR7/9-induced IFNα production (Hoshino et al., 2006). TLR7/9-induced IFNα production is severely impaired in IKKα-deficient pDC, whereas inflammatory cytokine induction is decreased but still occurs. IKKα-deficiency in pDCs inhibits the ability of MyD88 to activate the IFNα promoter in synergy with IRF-7. Furthermore, IKKα can associate with and phosphorylate IRF-7, albeit weakly. These studies identify a role for IKKα in TLR7/9 signaling (Figure 2), and highlight the cross-talk between the canonical and the IKK-related kinases in regulating antiviral and inflammatory responses.

TLR-9 signaling
Bacterial or synthetic DNA containing unmethylated CpG – such as A/D type CpG oligodeoxynucleotides (ODN) – is highly immunostimulatory (Latz et al., 2004). Unmethylated CpG DNA binds to TLR9 in the endosomal compartment and activates IRF-7 in pDCs (Latz et al., 2004; Uematsu et al., 2005). TLR9 signals by recruiting MyD88 and IRAK family members through homophilic interactions between their death domains (Figure 2) (Dunne and O'Neill, 2003). The recruited IRAK-4 phosphorylates IRAK-1, which enables IRAK-1 to interact with TRAF6, leading to the phosphorylation of IRF-7 and subsequent production

of type I IFN (Uematsu et al., 2005). TBK-1−/− or IKKε−/− pDCs stimulated with CpG ODN have normal IFNα production, whereas in IRAK1-deficient mice TLR7- and TLR9-mediated IFNα production is abolished, suggesting that IRF-7 phosphorylation occurs independently of TBK/IKKε. Moreover, IRAK-1 physically interacts with and phosphorylates IRF-7 in vitro (Uematsu et al., 2005), providing further evidence that IRAK1 directly activates IRF-7. IRAK-1 is dispensable for TLR9-mediated induction of NF-κB and is therefore not involved in the induction of pro-inflammatory cytokines such as TNFα, IL-6 and IL-12p40. In contrast, IRAK-4 is essential for the activation of NF-κB and optimal induction of proinflammatory cytokines (Lye et al., 2004). In DCs, another IRF family member – IRF-8 – is essential for NF-κB activation in TLR9 signaling (Tsujimura et al., 2004); IRF-8−/− mice are completely unresponsive to unmethylated CpG and fail to induce NF-κB. However, this type of regulation is restricted to TLR9 signaling in DCs (Tsujimura et al., 2004).

TLR-independent signaling through RIG-I and Mda-5

Although viral and microbial pathogens are detected by the TLR family via the recognition of PAMPs, viral infection is also detected through TLR-independent mechanisms (Akira et al., 2006). Early viral replicative intermediates are detected by two recently characterized DExD/H box RNA helicases, RIG-I (Yoneyama et al., 2004) and Mda-5 (Andrejeva et al., 2004) that recognize viral double-stranded RNA and transmit signals to an independent downstream pathway (Figure 2). Structurally, RIG-I contains two caspase activation and recruitment domains (CARD) at its N-terminus and RNA helicase activity in the C-terminal portion of the molecule (Yoneyama et al., 2004). The helicase domain possesses ATPase activity and is responsible for dsRNA recognition and binding, which leads to dimerization and structural alterations that enable the CARD domain to interact with other downstream adapter protein(s). RIG-I signaling ultimately engages the canonical IKK kinase complex, the stress activated kinases, as well as the IKK-related kinases TBK-1 and IKKε, leading to phosphorylation and activation of NF-κB, ATF-2/c-jun and IRF-3 transcription factors, respectively. Coordinated activation of these transcription factors results in the formation of a transcriptionally competent enhanceosome that triggers IFNβ production (Maniatis et al., 1998).

The importance of the RIG-I pathway in antiviral immunity was confirmed with the generation of RIG-I-deficient mice (Kato et al., 2005), which revealed that RIG-I and not the TLR system played an essential role in the IFN antiviral response in most cell types – fibroblastic, epithelial and conventional dendritic cells. In contrast, pDCs utilize TLR-mediated signaling in preference to RIG-I (Kato et al., 2005). Furthermore, Mda-5 and RIG-I recognize different types of dsRNAs: Mda-5 recognizes poly(I:C), and RIG-I detects

in vitro-transcribed dsRNAs. RIG-I is essential for the production of IFN in response to RNA viruses including paramyxoviruses, influenza virus and Japanese encephalitis virus, whereas MDA-5 is critical for picornavirus detection. RIG-I−/− and MDA-5−/− mice are highly susceptible to infection with these respective RNA viruses compared to control mice, thus illustrating that these two important RNA sensors are not functionally redundant (Kato et al., 2006).

The adaptor molecule providing a link between RIG-I sensing of incoming viral RNA and downstream activation events was recently elucidated; four independent groups used high throughput screening and/or database search analyses to identify a new signaling component independently named IPS-1, MAVS, VISA or Cardif (Figure 2). IFNβ promoter stimulator 1 (IPS-1) was identified by Kawai et al. (2005), who demonstrated that overexpression of IPS-1 activates the IFNα, IFNβ, and NF-κB promoters in a TBK-1 dependent fashion. IPS-1 contains an N-terminal CARD domain like RIG-1 and a C-terminal effector domain that recruits the adaptor FADD and the kinase RIP1. The same molecule, but named mitochondrial antiviral signaling (MAVS), was identified by Chen's group who recognized, in addition to its essential role in RIG-I dependent signaling, that a C-terminal transmembrane domain localized MAVS to the mitochondrial membrane, thus suggesting a novel role for mitochondrial signaling in the cellular innate response (Seth et al., 2005). Xu et al. (2005) demonstrated that virus-induced signaling adaptor (VISA) was a critical component of IFNβ signaling, and suggested that VISA may mediate the bifurcation of the NF-κB and IRF-3 activation pathways in both TLR3 and RIG-I virus-triggered pathways. Finally, Meylan et al. (2005) described Cardif, which interacts with RIG-I via N-terminal CARD domain interactions and recruits IKKα, IKKβ and IKKε kinases through its C-terminal region. Overexpression of Cardif results in IFNβ and NF-κB promoter activation, and knockdown by siRNA inhibits RIG-I-dependent antiviral responses. Importantly, this latter study demonstrates that Cardif is cleaved at its C-terminal end – adjacent to the mitochondrial targeting domain – by the NS3–4A protease of Hepatitis C virus (see below).

The recent generation of MAVS-deficient mice demonstrated that loss of MAVS abolishes viral induction of IFN and prevents the activation of NF-κB and IRF-3 in multiple cell types, except pDCs (Sun et al., 2006). MAVS is critically required for the host response to RNA viruses but is not required for IFN production in response to cytosolic DNA (Ishii et al., 2006) or to Listeria monocytogenes. Mice lacking MAVS are viable and fertile, but fail to induce IFN in response to poly(I:C) stimulation and are severely compromised in their immune defense against viral infection. These results provide the in vivo evidence that the viral signaling pathway through mitochondrial bound MAVS is specifically required for innate immune responses against viral infection (Sun et al., 2006; tenOever and Maniatis, 2006).

Viral strategies to manipulate the NF-κB pathway and innate immunity

Just as tremendous advances have been made in our understanding of the host cell recognition of virus infection by TLR-dependent and TLR-independent pathways (Akira et al., 2006), important new knowledge about the mechanisms by which viruses manipulate, modify and evade the host response is also rapidly emerging (Meylan and Tschopp, 2006). Many viruses utilize multifunctional viral proteins to hijack and stimulate the NF-κB signaling pathway as part of their life cycle, diverting NF-κB immune regulatory functions to favor viral replication or to modulate cellular apoptosis and growth pathways (Table 1). Chronic activation of the NF-κB pathway due to persistent viral infection can promote inflammation and the progression to malignancy (reviewed in Karin, 2006). Below, we present a cross-section of the well-studied examples of viruses that modulate the NF-κB pathway and innate immune responses, and describe how these events contribute to viral pathogenesis and malignant transformation. Extensive recent reviews on these subjects are available (Gilmore and Mosialos, 2003; Conzelmann, 2005; Sun and Yamaoka, 2005; Brinkmann and Schulz, 2006; Karin, 2006).

Perhaps the most blatant example of viral 'abuse' of the NF-κB pathway is the incorporation and use of NF-κB DNA binding sites in the promoters of many different classes of viruses, including human pathogens such as HIV-1, cytomegalovirus, herpesvirus, human papillomavirus type 16, hepatitis B virus and EBV. A partial list of animal viruses that regulate their transcription through the use of NF-κB also includes avian and bovine leukosis viruses, the papovaviruses SV40, JC and BK, and adenoviruses (reviewed in Gilmore and Mosialos, 2003).

The NF-κB sites in the HIV-1 long terminal repeat (LTR) have undoubtedly received the most attention (reviewed in Hiscott et al., 2001) and in HIV-1-infected T cells, activation of NF-κB signaling promotes LTR-driven viral transcription. For most viral subtypes, the HIV-1 promoter has two NF-κB binding sites located approximately 100 bp from the start site of transcription (Hiscott et al., 2001), which act in synergy with nearby Sp1 binding sites to drive HIV-1 transcription. Unstimulated CD4$^+$ T cells have primarily p50–p50 DNA-binding activity, but T-cell activation leads to recruitment of p50–RelA complexes and enhanced expression from the HIV-1 LTR. The absolute requirement for these sites during the HIV-1 life cycle continues to be controversial, given that NF-κB sites are required for HIV transcription in some, but not all, cell types. Expression of the IκBα super-repressor reduces HIV-1 viral replication in T-cell cultures in vitro (Kwon et al., 1998; Quinto et al., 1999), but mutation of the NF-κB sites does not absolutely block virus growth (Chen et al., 1997). Furthermore, extensive heterogeneity in the architecture of the HIV-1 LTR with respect to functional NF-κB sites has been described, suggesting that functionality of these sites may be subject to

Table 1 Viral activators of NF-κB

Virus	Viral protein	Protein function
African swine fever virus	A224L	IAP-like activator of IKK
Bluetongue virus	VP2, VP5	Capsid proteins activates NF-κB
Epstein-Barr virus	LMP-1	CD40 receptor mimic
	EBNA-2	Transcription co-activator of NF-κB
Encephalomyocarditis virus	Capsid protein	Triggers Mda-5
Hepatitis B virus	HBx	Activation of Src, MAPK cascades and NF-κB
Hepatitis C virus	Core protein	Triggers IFN response
Herpesvirus Saimiri	Tip	Adaptor for LCK leading to NF-κB activation
Human Cytomegalovirus	IE1	Regulation of NF-κB induced genes
	US28	Constitutive transmembrane receptor signaling through the G protein q (Gq)/ phospholipase C pathway
	NS5A	Enhances full-length core protein-induced NF-κB activation
	NS5B	Regulates TNF signaling through effects on cellular IKK
	StpC	Interacts with TRAF2
Human herpesvirus-8	ORF74	G protein coupled chemokine receptor
	vFLIP	Associates with and activates IKK
	K7	Associates with PLIC1 to induce IκB degradation
	K15	Mediates TRAF2 induction of NF-κB
Human immunodeficiency virus 1	Tat	Enhances NF-κB mediated LTR activation
	Nef	Stimulates HIV-1 LTR via NF-κB activation
	Gp120	Engages CD4 receptor
Human T-cell leukemia virus-1	Tax	Adaptor for IKKgamma
Influenza A	HA, M and NP	Hemagglutinin, matrix and nucleorotein induces IKK activation
	NS1, NS2	Triggers RIG-I
Moloney and Feline LV	U3-LTR	Short RNA activates NF-κB via TLR-3
Murine cytomegalovirus	M33	Constitutive transmembrane receptor
Respiratory Syncytial Virus	M2-1	Associates with RelA
Rev-T	v-Rel	Activated c-Rel
Rotavirus	VP4 capsid protein	Activates IKK
SARS coronavirus	Nucleocapsid	Multiple functions (RIG-I signaling?)
VSV	Ribonucleoprotein	Activates TBK-1

Abbreviations: HIV-1, human immunodeficiency virus type I; IKK, IκB kinase; LMP, latent membrane protein; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; PLIC1, protein-linking integrin-associated protein and cytoskeleton 1; TRAF, tumor necrosis factor-receptor-associated factor; VSV, Vesicular Somatitis Virus.

mutational pressures (Roof *et al.*, 2002; van Opijnen *et al.*, 2004).

Involvement of the v-Rel protein in avian Rev-T-induced retroviral oncogenesis
The avian Rev-T retrovirus encodes the v-*rel* oncogene, an extensively mutated version of the avian c-*rel* proto-oncogene; transduction and extensive mutation of c-Rel represented the first demonstration that NF-κB transcription factors were associated with malignant transformation (reviewed in Gilmore, 1999). Rev-T, originally isolated from a turkey reticular malignancy, is a highly oncogenic virus that induces a fatal lymphoma/ leukemia in young birds and efficiently transforms and immortalizes chicken lymphoid cells *in vitro*. Over-expression of chicken, mouse or human c-Rel can also transform chicken lymphoid cells *in vitro*, although normal c-Rel is less efficient than v-Rel in transforming primary lymphoid cells (Gilmore *et al.*, 2001). In addition, T-cell-specific expression of v-*rel* in transgenic mice results in the development of T-cell lymphomas (Carrasco *et al.*, 1996). Four parameters determine the transforming activity of mutated v-Rel: (1) high-level expression; (2) homodimer formation; (3) DNA-binding activity; and (4) intact transcriptional activation potential (reviewed in Gilmore, 1999).

Extensive analysis of v-Rel has demonstrated that the increased oncogenicity of v-Rel is primarily due to the deletion of C-terminal c-Rel residues (Kamens *et al.*, 1990; Hrdlicková *et al.*, 1994); moreover, c-Rel proteins with C-terminal deletions often arise from transformation assays conducted with the full-length chicken c-Rel protein (Hrdlicková *et al.*, 1994; Gilmore *et al.*, 1995). The C-terminal deletion in v-Rel removes a strong c-Rel transactivation domain; thus, v-Rel is generally a weaker activator of transcription than c-Rel in many assays. However, inhibition of v-Rel by IκBα is also less effective than c-Rel, in part due to internal mutations in v-Rel that reduce its affinity for IκBα (Sachdev *et al.*, 1998). Thus, vRel transcriptional activity is less tightly regulated than its normal cellular counterpart. Many of the target genes that are affected by v-Rel in transformed lymphoid cells control growth or apoptosis, including genes encoding growth promoting transcription factors (c-Rel, c-Jun, STAT1 and IRF4), cytokine receptors (IL-2Rα), and anti-apoptotic proteins (IAP1) (reviewed in Gilmore, 1999). Thus, v-Rel impinges on various growth-promoting and survival pathways as part of its transforming process. Although v-Rel can transform a variety of cell types *in vitro*, its most potent oncogenic activity appears to be directed towards cells in the B-cell lineage. As discussed

previously, *REL* gene amplifications are common in human B-cell malignancies, especially in diffuse large B-cell lymphomas and Hodgkin's lymphomas (reviewed in Courtois and Gilmore, 2006), and it is likely that the mechanism by which v-Rel and overexpressed c-Rel proteins induce malignant transformation of avian lymphoid cells is similar to the mechanism by which *REL* gene amplification contributes to human B-cell malignancies.

Gamma-herpesviruses constitutively activate NF-κB and promote tumorigenesis

Two members of the *Gammaherpesvirinae* subfamily – EBV, a γ₁-herpesvirus, and HHV8 or Kaposi's Sarcoma herpesvirus ((HHV-8/KSHV), a γ₂-herpesvirus – show a natural tropism for human B cells. Like other herpesviruses, EBV and HHV-8 preferentially establish a latent mode of infection in the host cell with a very restricted pattern of viral gene expression. Only a few infected cells (<1% in the case of HHV-8) undergo productive infection where the virus expresses the full spectrum of viral proteins and generates new infectious particles. Both EBV and HHV-8 latent infections persistently activate the NF-κB pathway and this activation is associated with the ability of these viruses to induce cellular transformation and tumor formation (Brinkmann and Schulz, 2006).

EBV exhibits a tropism for epithelial cells and B lymphocytes and is associated with the development of several human malignancies, including Burkitt's lymphoma, Hodgkin's lymphoma (HL, in particular the classic subtype cHL), immunoblastic lymphomas, nasopharyngeal carcinomas and gastric carcinomas (Rickinson and Kieff, 2001). *In vitro* EBV induces the transformation of primary human B lymphocytes into proliferating lymphoblastoid long-term cultures that express nine EBV viral proteins – the integral membrane proteins Latent Membrane Protein (LMP)-1, -2A, -2B and six nuclear antigens EBNA1, 2, 3A, 3B, 3C and LP – and two small nuclear RNAs (Rickinson and Kieff, 2001). The primary *in vitro* transforming protein of EBV is the LMP-1 (Brinkmann and Schulz, 2006). Transgenic mice with LMP-1 under the control of the immunoglobulin promoter develop B-cell lymphoma at an increased frequency (Thornburg *et al.*, 2005). Moreover, LMP-1 is consistently expressed in all EBV-associated cHL cases.

LMP-1 is an integral membrane protein that localizes to lipid rafts (Hatzivassiliou and Mosialos, 2002) and functions like an activated CD40 receptor, promoting B lymphocyte survival, proliferation and expression of a highly specific spectrum of B-cell activation markers (reviewed in Mosialos, 2001; Brinkmann and Schulz, 2006). LMP-1 activates NF-κB (Herrero *et al.*, 1995; Cahir-McFarland *et al.*, 2000), mainly via effects on the NF-κB subunit c-Rel (Thornburg *et al.*, 2005). NF-κB activation by LMP-1 is essential for the survival of EBV-transformed cells (Cahir-McFarland *et al.*, 2000; He *et al.*, 2000) as activated NF-κB induces the expression of antiapoptotic molecules such as Bcl-2 (Henderson *et al.*, 1991; Wang *et al.*, 1996; Feuillard *et al.*, 2000),

Bfl1 (D'Souza *et al.*, 2000), Mcl1 and A20 (Laherty *et al.*, 1992) and prosurvival genes including IAPs, c-FLIP and IL-6 (Keller *et al.*, 2006). NF-κB activation also mediates many of the phenotypic effects of LMP-1, including the upregulation of ICAM-1, LFA-3, CD40, IL-6, Fas, TRAF1, EBI3 and cyclooxygenase-2, typical of EBV-induced transformation.

LMP-1 consists of three major domains: a short 24 amino-acid cytoplasmic N terminus that is largely dispensable for LMP-1-mediated transformation, six transmembrane domains that mediate protein oligomerization and localization to lipid rafts (Yasui *et al.*, 2004), and a 200 amino-acid cytoplasmic C terminus (CCT) that mediates transformation and NF-κB activation. Spontaneous oligomerization of LMP-1 is necessary for constitutive signaling by this 'pseudo-receptor', whereas transformation of B lymphocytes and activation of NF-κB have been mapped to two sequences within the CCT. These sequences have been termed transformation effector site (TES)-1 (aa 187–231) and TES-2 (aa 352–386) or C-terminal NF-κB activating regions (CTAR)-1 and CTAR-2. (Huen *et al.*, 1995; Mitchell and Sugden, 1995). The fact that the same regions in LMP-1 that mediate NF-κB activation (CTAR-1 and -2) are necessary for B-cell transformation (TES-1 and -2) provides strong evidence that these two processes are intimately linked.

NF-κB activation by LMP-1 is mediated by the recruitment of cellular adaptor proteins – TRAFs and TNFR-associated death domain (TRADD) – to the C-terminal domain (reviewed in Brinkmann and Schulz, 2006). Approximately 20–30% of the LMP1-induced NF-κB activation is mediated by CTAR1, whereas 70–80% is transduced by CTAR2. The principal mediators of NF-κB signaling from CTAR2 are TRAF6 (Wu *et al.*, 2006) and IRAK1, as well as TRADD (Izumi *et al.*, 1997) and RIP (Brinkmann and Schulz, 2006). LMP-1-mediated activation of the classical NF-κB requires IRAK1 and TRAF6, leading to the activation of IKKβ (Luftig *et al.*, 2004). Surprisingly, activation of NF-κB p65/p50 heterodimers occurs independently of IKKα and NEMO. IRAK1 acts as a scaffolding protein for the recruitment of TRAF6 and IRAK1 kinase activity is not required for the activation of the IKK complex or the phosphorylation of IκBα, but rather is necessary for the phosphorylation of p65 at Ser536 and NF-κB transcriptional activity. In addition, the MAP3K TAK1 is involved in CTAR2-mediated activation of NF-κB, possibly acting upstream of IKKβ (Wu *et al.*, 2006). CTAR1 directly recruits TRAF3 through a consensus PXQXT motif (Mosialos *et al.*, 1995; Devergne *et al.*, 1996, 1998) and indirectly interacts with TRAFs 1, 2 and 5 possibly due to oligomerization with TRAF3. Recruitment of TRAF3 leads to the activation of NIK and activation of the non-cannonical NF-κB pathway through IKKα-mediated phosphorylation of p100 followed by proteasome-mediated processing of the precursor and generation of p52 (Atkinson *et al.*, 2003; Luftig *et al.*, 2004).

Although major breakthroughs have been recently made in elucidating the pathways by which LMP-1

activates NF-κB, several questions remain unanswered. For example, previous reports using dominant-negative forms of NIK, IKKα and IKKβ all reduced activation of NF-κB by LMP-1 (Sylla et al., 1998); whether this demonstrates the existence of alternative pathways of NF-κB activation by LMP-1 remains to be established. As well, it has recently been shown that TRAF2 and TRAF6 can act as ubiquitin ligases leading to activation of the IKK complex (reviewed in Chen, 2005), but the role of TRAF ubiquitination with regard of LMP-1 activation of NF-κB remains controversial (Luftig et al., 2003). Nevertheless, it is clear that constitutive NF-κB activation by LMP-1 constitutes the most powerful transforming pathway used by EBV.

HHV-8 is associated with the development of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castelman's disease. Consistent with persistent activation of NF-κB being involved in HHV-8-associated disease, inhibition of NF-κB induces apoptosis in PEL cells (Keller et al., 2006), and as with EBV-infected cells, this appears to be due to down-regulation of NF-κB-dependent survival genes such as IAP, cFLIP and IL-6 (Keller et al., 2006). Moreover, inhibition of NF-κB by expression of the IκBα super-repressor blocks the production of infectious HHV-8 virions (Sgarbanti et al., 2004), demonstrating the importance of NF-κB activation in both latent and lytic infection.

The HHV-8 latent viral protein vFLIP is a viral homolog of FLICE inhibitory protein (cFLIP) that can prevent apoptotic cell death by inhibiting the activity of Caspase-8/FLICE (reviewed in Chaudhary et al., 1999). HHV-8-encoded vFLIP also binds to TRAF2 to interact with and activate the IKK complex, causing persistent activation of both the canonical (IKKα/β→IκBα→p50/RelA) and non-canonical (NIK→IKKα→p52/RelB) NF-κB pathways (Liu et al., 2002; Guasparri et al., 2006). The importance of this NF-κB activation is demonstrated by an increased incidence of lymphoma in vFLIP transgenic mice (Chugh et al., 2005), which correlates with constitutive activation of NF-κB but not with resistance to Fas-mediated apoptosis. In addition, vFLIP is able to transform certain established rodent cell lines, and this ability is blocked by inhibitors of NF-κB (Sun et al., 2003).

At the level of lytic proteins, HHV-8 ORF74 (also called vGPCR), a homolog of a G protein-coupled chemokine receptor, and K15 can also activate NF-κB. vGPCR can transform the morphology of human skin endothelial cells in culture (Pati et al., 2001) and cause KS-like tumors in transgenic mice (Yang et al., 2000). Expression of ORF74 can activate NF-κB and consequently induce the expression of several NF-κB target genes, including ones encoding cytokines, angiogenesis factors and cell-surface adhesion molecules (Pati et al., 2001; Schwarz and Murphy, 2001). Interestingly, co-expression of HIV-1 Tat enhances both ORF74-induced activation of NF-κB and ORF74-induced tumorigenesis (Guo et al., 2004). The K15 viral product of HHV-8 encodes a multipass transmembrane protein reminiscent of EBV's LMP-1 (reviewed in Brinkmann and Schulz, 2006). The full-length K15 protein localizes to lipid rafts and associates with TRAF1, -2 and -3 to activate the NF-κB pathway. In contrast to these viral activators, the HHV-8 K1 protein, a tyrosine kinase immuno-receptor-like protein, has been shown to inhibit activation of NF-κB; thus, HHV-8 may modulate NF-κB activity through both positive and negative pathways (Lee et al., 2002).

The transforming proteins Tip and StpC of Herpesvirus saimiri can induce fatal T-cell lymphoproliferation in primates and can transform human T-cell in vitro (reviewed in Brinkmann and Schulz, 2006). These transforming proteins of Herpesvirus saimiri also cooperatively induce the activity of NF-κB (Merlo and Tsygankov, 2001). StpC protein contains a TRAF-binding motif and its interactions with TRAF-2 and NIK are essential for NF-κB activation (Sorokina et al., 2004). NF-κB activation by StpC is crucial for the immortalization of human T lymphocytes but not for the transformation of monkey-derived lymphocytes (Lee et al., 1999).

The Tax oncoprotein of HTLV-1 targets multiple components of the NF-κB signaling pathway

One of the best-characterized examples of viral interference with NF-κB signaling is the appropriation of the pathway by the Human T-cell leukemia/lymphotropic virus type 1 (HTLV-1) (Jeang, 2001; Sun and Yamaoka, 2005). At present, between 20 and 30 million people worldwide are infected with HTLV-1, a delta retrovirus that is endemic to parts of South America and the Middle East, the Caribbean basin, sub-Saharan and central Africa, southern Japan and southeast Asia (Edlich et al., 2000; Eshima et al., 2003). Infection with HTLV-1 is etiologically associated with adult T-cell leukemia (ATL), an aggressive and often fatal malignancy of CD4+ T cells (Yoshida, 2005) as well as HTLV-1-associated myelopathy/tropical spastic paraperesis (HAM/TSP), a demyelinating syndrome of the central nervous system (Grindstaff and Gruener, 2005).

The HTLV-1 oncoprotein tax. HTLV-1-induced deregulation of the lymphocyte gene expression pattern, a key event in HTLV-1-induced transformation, is attributed to the activity of the virally encoded 40-kDa Tax oncoprotein. Tax is a regulator of both cellular and viral gene expression, and, as such, Tax is essential for both viral replication and pathogenesis. Tax is transcribed early in virus infection from a distal coding region of the HTLV-1 genome, which lies adjacent to the 3′ LTR. Although leukemic cells from ATL patients frequently have deleted HTLV-1 proviral genomes, the *tax* region is selectively retained in these cells (Grassmann et al., 2005). Ectopic expression of the *tax* gene is necessary and sufficient to immortalize primary human T-lymphocytes (Wano et al., 1988; Akagi et al., 1995). Mice stably expressing the *tax* transgene have been shown to develop soft tissue tumors (Hinrichs et al., 1987; Nerenberg et al., 1987) or large granular lymphocytic leukemia (Grossman et al., 1995).

To modulate both viral and cellular gene expression, phosphoprotein Tax (Bex et al., 1999) shuttles between the cytoplasmic and nuclear compartments of infected T cells (Smith and Greene, 1992; Alefantis et al., 2005). Although Tax lacks a classical DNA-binding domain, Tax activates transcription by functioning as an adaptor protein that interacts with cellular transcription factors, including the CREB/ATF and NF-κB (Bex and Gaynor, 1998; Sun and Ballard, 1999; Yoshida, 2001; Pise-Masison et al., 2005). Tax increases LTR-dependent transcription primarily by interaction of a Tax–CREB complex with the viral 21 bp Tax-responsive element (Yoshida, 2001). The N-terminal domain of Tax interacts with the basic leucine zipper (bZip) domain of the cellular CREB/ATF proteins (Bex and Gaynor, 1998) and guides CREB/ATF dimers to the proviral promoter (Lenzmeier et al., 1998, 1999). Acting as a molecular bridge, the C-terminal end of Tax recruits the transcriptional coactivator CBP to the CREB/ATF dimer, which drives proviral transcription (Kwok et al., 1996; Bex and Gaynor, 1998; Kashanchi and Brady, 2005). As CBP is present in limiting concentrations within a host cell, high Tax expression during the early stages of HTLV-1 infection alters the cellular gene expression profile, by favoring transcription driven by proteins which bind to Tax, such as CREB/ATF, serum response factor and NF-κB (Yoshida, 1994, 2001; Bex and Gaynor, 1998; Sun and Ballard, 1999).

Tax-mediated activation of NF-κB signaling. The oncogenic activity of Tax is primarily a result of its effects on the NF-κB pathway. Mutants of Tax that can no longer activate NF-κB, but can still activate CREB, do not immortalize human T cells (Robek and Ratner, 1999). HTLV-1-infected, Tax-expressing cells are characterized by constitutively nuclear, chronically activated NF-κB dimers that drive the expression of numerous genes (Bex and Gaynor, 1998; Sun and Ballard, 1999; Yoshida, 2001), including IL-6 (Mori et al., 1995), granulocyte–macrophage colony-stimulating factor (Himes et al., 1993) and c-Myc (Duyao et al., 1992b).

Tax affects NF-κB signaling in both the nucleus and the cytoplasm, and Tax has been demonstrated to interact directly with many components of the NF-κB pathway (Jeang, 2001; Kfoury et al., 2005), suggesting a direct role in the enhancement of nuclear transcriptional activity of NF-κB. More recent studies indicate that the primary action of Tax is to affect NF-κB signaling in the cytoplasm by targeting persistent activation of both canonical and non-canonical pathways through degradation of IκB or processing of p100, respectively (Sun and Yamaoka, 2005) (Figure 3).

Nuclear effects of Tax on NF-κB activity. In the nucleus, Tax appears to stimulate the activity of NF-κB complexes on DNA. In a manner similar to CREB/ATF, interaction of Tax with DNA-binding subunits of NF-κB favors gene expression by recruiting CBP/p300 (Gerritsen et al., 1997; Perkins et al., 1997). Consistent with this model, Tax mutants that are defective in nuclear translocation trap RelA–CBP complexes in the cytoplasm and do not allow them to enter the nucleus (Azran et al., 2005). Recently, the attachment of SUMO-1 to Tax has been shown to induce the recruitment of NEMO and RelA to nuclear structures called nuclear bodies (Lamsoul et al., 2005; Nasr et al., 2006), and the localization of these complexes within these subnuclear structures may be important for the induction of specific target genes through association with co-activator p300.

Tax-induced degradation of IκB proteins. The involvement of cellular signaling pathways in Tax activation of NF-κB was first suggested by the finding that IκBα undergoes constitutive phosphorylation and degradation in HTLV-1-infected T cells (Sun et al., 1994; Lacoste et al., 1995). Additional studies revealed that Tax induces the degradation of both IκBα and IκBβ (Good and Sun, 1996; McKinsey et al., 1996). It is now well established that the mechanism for Tax-mediated NF-κB activation involves direct activation of the classical IKK complex, which promotes constitutive IκB processing (Figure 3). Tax expression is associated with an elevated enzymatic activity of endogenous IKK complexes, which correlated with the phosphorylation and increased turnover of IκBα (Chu et al., 1998; Geleziunas et al., 1998). The non-catalytic NEMO subunit of IKK is the target for Tax-induced activation of NF-κB; genetic complementation in fibroblasts deficient for expression of NEMO showed that expression of the adaptor protein is essential for the formation of an active complex between Tax and the classical IKK complex (Yamaoka et al., 1998), and T-cell clones defective for Tax-mediated NF-κB-activation could be rescued by forced expression of wild-type NEMO (Harhaj et al., 2000). Similarly, interference with NEMO expression in T-cell lines using anti-sense oligonucleotides specifically abolishes Tax-mediated NF-κB activation, although CREB/ATF activation remains intact. Furthermore, a direct interaction between Tax and NEMO is required to activate the canonical IKK signaling pathway and this interaction requires leucine-rich sequences in both proteins (Harhaj and Sun, 1999; Chu et al., 1999b; Xiao and Sun, 2000; Xiao et al., 2000). In addition, ubiquitination of Tax promotes its association with NEMO (Lamsoul et al., 2005; Nasr et al., 2006). The Tax–NEMO interaction leads to activation of IKKβ (Harhaj and Sun, 1999; Chu et al., 1999b), probably because the Tax-NEMO complex promotes clustering of the IKK components and this induced proximity leads to persistent phosphorylation of the activation loop in IKKβ (Carter et al., 2003). Indeed, fusion of NEMO, IKKα or IKKβ to Tax is sufficient to activate IKK (Xiao and Sun, 2000). Tax-induced activation of IKK then targets IκBα and IκBβ for degradation, causing the chronic induction of NF-κB that is seen in Tax-transformed cells. Recently, the kinase AKT has also been shown to contribute to Tax-induced activation of IKKβ (Jeong et al., 2005a); moreover, AKT is also required for IKKβ-mediated phosphorylation of RelA, which leads to inhibition of p53-dependent transactivation (Jeong et al., 2005b).
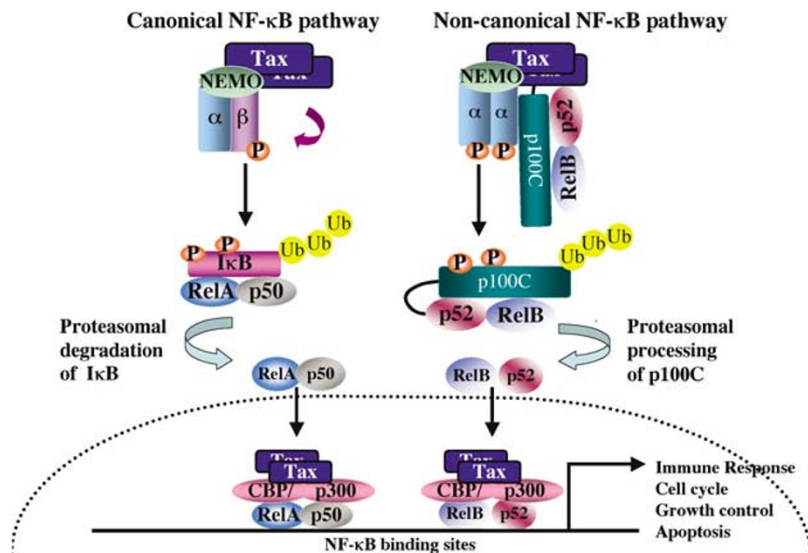
Figure 3 HTLV-1 Tax interactions with the canonical and non-canonical NF-κB pathways. Tax affects NF-κB signaling in both the nucleus and the cytoplasm. In the cytoplasm, Tax dimers interact with the non-catalytic IKK subunit NEMO, and facilitate Tax recruitment to the catalytic IKK subunits (α or β), leading to subsequent phosphorylation, ubiquitination and proteasomal degradation of IκB or processing of the C-terminal inhibitory region (p100C) of p100 in the canonical and non-canonical pathways, respectively. At the transcriptional level, Tax interacts with the NF-κB subunits and recruits the transcriptional coactivators CBP/p300, leading to the transcription of NF-κB-dependent cytokines, cell cycle regulators, genes modulating apoptosis and others (modified from Sun and Yamaoka, 2005).

Tax-induced processing of p100. In T cells transformed by HTLV-1, p100 processing is very active, resulting in a high-level expression of p52. In the case of p100, Tax binds to sequences within the Rel homology domain of p100 to bridge an interaction to the IKK complex through NEMO (Xiao *et al.*, 2001) (Figure 3). This interaction then activates the catalytic activity of IKKα, and leads to the phosphorylation, ubiquitination and proteasome-mediated processing of p100 to p52 (Qu *et al.*, 2004). Of note, Tax-induced processing of p100 to p52 appears to be distinct from normal processing of p100. In contrast to the cellular pathway, the Tax-stimulated p100 processing does not require NIK. Furthermore, this virus-specific pathway requires both NEMO and IKKα, whereas the cellular pathway requires IKKα but not NEMO (Pomerantz *et al.*, 2002; Hayden and Ghosh, 2004). These results imply that Tax-stimulated non-canonical NF-κB signaling bypasses NIK but goes through IKKα (Jeang, 2001; Sun and Yamaoka, 2005). More recent work suggests that Tax-induced deregulation of p100 processing involves both β-transducin repeat-containing protein-dependent and -independent mechanisms, further suggesting the involvement of different mechanisms in cellular and viral pathways of p100 processing (Qu *et al.*, 2004).

Target genes affected by Tax-mediated activation of NF-κB. ATL develops as a clonal expansion of leukemic CD4$^+$ T cells in HTLV-1-infected individuals only after a long latency, indicating that additional cellular alterations are required for T-cell leukemogenesis. Moreover, ATL cells from patients or T lymphocytes infected with HTLV-1 *in vitro* show chromosomal abnormalities (Jeang, 2001). Constitutive. activation of NF-κB by Tax contributes to the abnormal growth and survival of T cells during the early stages of ATL disease progression. In this regard, NF-κB is responsible for Tax-mediated inhibition of certain genes involved in DNA repair and cell cycle checkpoint regulation (Mamane *et al.*, 2005), especially those encoding β-polymerase and p53 (Marriott and Semmes, 2005; Sun and Yamaoka, 2005). Activation of NF-κB is also essential for Tax-induced IL-2-independent T-cell growth (Iwanaga *et al.*, 1999). ATL cells express high levels of both IL-2 and the IL2 receptor α-chain, both of which are induced by Tax and contain upstream NF-κB sites (Jeang, 2001; Sun and Yamaoka, 2005). Thus, Tax-driven, NF-κB-mediated upregulation of IL-2 and the IL2Rα stimulates an autocrine activation loop that drives T-cell proliferation in HTLV-1-infected cells. The progressive nature of the disease *in vivo* is in part mimicked in cell culture; HTLV-1-infected primary human T cells undergo an initial phase of proliferation that is dependent on the IL-2/IL-2 receptor mediated autocrine proliferation, followed by an IL-2 independent phase, wherein leukemic T-cell growth is no longer dependent on IL-2 and cells acquire chromosomal alterations (Jeang, 2001; Sun and Yamaoka, 2005).

Furthermore, Tax activates the expression of a number of other NF-κB-dependent cytokine genes, including several interleukins and TNF (Jeang, 2001; Sun and Yamaoka, 2005). The level of several NF-κB target genes/protein, including Bcl-2, Bcl-Xl, A1, cFLIP, and IAP, that promote the survival of lymphoid cells are increased in HTLV-1-transformed T cells and in cells from ATL patients (Harhaj *et al.*, 1999; Nicot *et al.*, 2000a, b; Okamoto *et al.*, 2006). Furthermore, cell cycle

genes and cellular oncogenes, such as cyclin D1, cyclin D2, c-*myc*, and c-*rel*, show increased expression due to the induction of NF-κB by Tax (Duyao *et al*., 1992a; Li *et al*., 1993; Harhaj *et al*., 1999; Huang *et al*., 2001; Mori *et al*., 2002).

Interestingly, one of the member of the IRF family, IRF-4, was shown to be highly expressed in cells derived from patients with ATL and in HTLV-1-infected cell lines (Yamagata *et al*., 1996; Mamane *et al*., 2000; Sharma *et al*., 2002). A detailed analysis of IRF-4 transcriptional regulation within the context of HTLV-1 infection has implicated the viral Tax protein in mediating chronic activation of the Sp1, NF-κB and NF-AT pathways leading to the overexpression of IRF-4 in ATL cells (Grumont and Gerondakis, 2000; Sharma *et al*., 2002). The role of IRF-4 *per se* in HTLV-1-induced leukemogenesis remains unclear. However, IRF-4 expression increases during the development of ATL, with IRF-4 expression levels highest during the late and ultimately fatal, acute phase of ATL (Imaizumi *et al*., 2001).

Using microarray analysis, constitutive IRF-4 expression was shown to result in the repression of multiple genes involved in the mitotic checkpoint, actin cytoskeletal rearrangement, DNA repair, apoptosis, metastasis and immune recognition. IRF-4 appears to repress several genes involved in DNA repair and chromosomal stability such as EB1, PCNA, RP-A, XRCC1 and SNF2b (Mamane *et al*., 2002, 2005). IRF-4 transcriptional downregulation of such genes would lead to an overall decrease in DNA repair and a subsequent increase in cellular mutations – as seen in HTLV-1-infected T cells – thus contributing to cellular transformation. IRF-4 also downregulates several genes involved in apoptosis and immune regulation (Mamane *et al*., 2002, 2005). Thus, the overall effect of IRF-4 in HTLV-1-infected cells is to contribute to the emergence of the transformed phenotype, to increase cell survival and promote ATL cell metastasis.

In summary, expression of vast network of genes affecting lymphoid cell growth and survival are modulated as a consequence of constitutive activation of NF-κB by Tax. Thus, inhibitors of NF-κB activity could potentially sensitize ATL cells to chemotherapeutic agents that induce apoptosis, as observed in other malignancies exhibiting chronic NF-κB activity (Karin, 2006). To date, such experiments/clinical trials have not been performed. However, primary ATL cells that are highly refractory to conventional chemotherapies are nevertheless sensitive *in vitro* to the oncolytic effects of VSV, in part, due to the constitutive activation of the NF-κB pathway in ATL cells (Cesaire *et al*., 2006).

Hepatitis C virus evasion of NF-κB- and IRF-mediated immune responses

The hepatitis virus C (HCV) is a major public health problem as an important cause of human chronic liver diseases (Choo *et al*., 1989; Kiyosawa *et al*., 1990). More than 170 million people worldwide are infected with HCV (Wasley and Alter, 2000). Of those individuals exposed to HCV, 80% become persistently infected (Hoofnagle, 2002), which is often associated with significant liver disease, including chronic active hepatitis, cirrhosis, and hepatocellular carcinoma (Alter and Seeff, 2000). HCV is an enveloped virus classified in the *Flaviviridae* family (Rosenberg, 2001). The positive-stranded viral RNA genome encodes a single polyprotein precursor that is processed into structural proteins (core, envelope protein 1 (E1) and 2 (E2), p7) and non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) by host and viral proteases (reviewed in Rehermann and Nascimbeni, 2005; Wieland and Chisari, 2005). The clinical outcome of HCV infection and the degree of liver damage is the result of complicated interactions between the virus and host immune responses (reviewed in Gale and Foy, 2005). The immune response is rarely effective in eradicating the virus and the majority of HCV-infected subjects develop chronic infection, demonstrating that HCV may have evolved strategies to overcome or evade the immune response of the host (reviewed in Chisari, 2005).

Since triggering the IFN antiviral response in HCV-infected hepatocytes would limit virus replication, HCV strategies to block the innate immune response are crucial for the establishment of a microenvironment conducive to HCV infection and replication (Gale and Foy, 2005). Several HCV structural and NS proteins, including E2, Core and NS5A proteins, have been shown to inhibit the innate immune response (Song *et al*., 1999; Taylor *et al*., 1999; Aizaki *et al*., 2000; Melen *et al*., 2004; Miller *et al*., 2004). Among these HCV immunsuppressive proteins, NS5A has the ability to modulate a number of cell-cycle regulatory genes (Ghosh *et al*., 1999, 2000), and has been implicated in the interference of IFN-mediated antiviral functions (Tan and Katze, 2001). E2 and NS5A have been shown to bind to the kinase domain of PKR and inhibit of IRF-1 activation (Gale *et al*., 1998; Taylor *et al*., 1999; Pflugheber *et al*., 2002).

Recent studies have demonstrated that the HCV gene product NS3–4A protease complex, a multifunctional serine protease, efficiently blocks the RIG-I signaling pathway and contributes to virus persistence by enabling HCV to escape the IFN antiviral response (Breiman *et al*., 2005; Foy *et al*., 2005). Nevertheless, RIG-I is not a direct target of NS3–4A and likewise the kinases TBK-1 and IKKε are not subject to proteolytic cleavage by NS3–4A (Breiman *et al*., 2005; Foy *et al*., 2005; Sumpter *et al*., 2005). Additional evidence for the importance of RIG-I comes from studies demonstrating that permissiveness for HCV RNA replication in Huh7.5 (Blight *et al*., 2003) cells is due to mutational inactivation of the CARD domain of RIG-I (Thr55 to Ile) (Sumpter *et al*., 2005). Furthermore, overexpression of IKKε results in strong inhibition of both negative and positive replicative strands of the HCV replicon, suggesting an important role for this kinase in the RIG-I pathway, as well as suppressing HCV replication (Breiman *et al*., 2005). Thus, RIG-I signaling appears to be an essential pathway regulating cellular permissiveness to HCV replication.

The demonstration that MAVS/Cardif was cleaved at its C-terminal end – adjacent to the mitochondrial

targeting domain – by the NS3–4A protease of HCV suggested an efficient mechanism by which HCV could interfere with NF-κB and IRF signaling to the antiviral response (Li et al., 2005b; Meylan et al., 2005; Lin et al., 2006; Loo et al., 2006). Interestingly, the NS3–4A protease also targets the TRIF/TICAM adapter of the TLR3 pathway and causes specific proteolytic cleavage of TRIF (Li et al., 2005a), thus targeting both the TLR-dependent and -independent arms of the host antiviral response (Figure 4).

Confocal microscopy and biochemical fractionation demonstrated that MAVS/Cardif is present in the outer mitochondrial membrane but moves into a detergent-resistant mitochondrial fraction upon viral infection (Seth et al., 2005). Deletion of the C-terminal trans-membrane domain, or importantly, cleavage by the NS3–4A protease adjacent at Cys-508 causes loss of MAVS signaling activity and relocalization of MAVS to the cytosol (Seth et al., 2005; Lin et al., 2006) (Figure 4). Furthermore, virus-induced recruitment of IKKε – but not TBK-1 – to the MAVS/Cardif complex and the entire mitochondrial-associated, IFN molecular signaling complex is disrupted by HCV NS3 protease (Lin et al., 2006).

The fact that MAVS functionality requires mitochondrial association suggests a linkage between recognition of viral infection, the development of innate immunity and mitochondrial function (Hiscott et al., 2006; Johnson and Gale, 2006). The localization of this CARD domain-containing adaptor to the mitochon-

drial membrane is highly strategic and may help the host cell sensing incoming viral challenge to coordinate an immune or apoptotic response, depending on the pathogen. HCV and many other viruses replicate in the membranous web that connects the ER to the mitochondria; dsRNA structures, possibly within repli-cating ribonucleoprotein complexes, may be recognized by RIG-I, resulting in downstream signaling through MAVS. In the case of HCV infection, cleavage of the MAVS-IKKε complex by the NS3 protease (Lin et al., 2006) results in disruption of both NF-κB and IRF pathways essential to the antiviral response, thus contributing to the establishment of chronic HCV persistence (Gale and Foy, 2005).

African swine fever virus encodes proteins with opposing effects on NF-κB signaling

African swine fever virus (ASFV) is a large double-stranded DNA virus recently re-classified into a newly created family of viruses called *Asfarviridae*. ASFV contains more than 150 genes, several of which encode viral homologs of host proteins with the potential to modulate the immune response to virus infection (Yanez et al., 1995). ASFV infects primarily macrophages and monocytes and induces a rapidly fatal hemorrhagic disease in pigs. Notably, ASFV infection is character-ized by the absence of an acute inflammatory response and immune response and many of the NF-κB-dependent cytokine genes are not induced upon infec-tion of macrophages by ASFV (Powell et al., 1996).
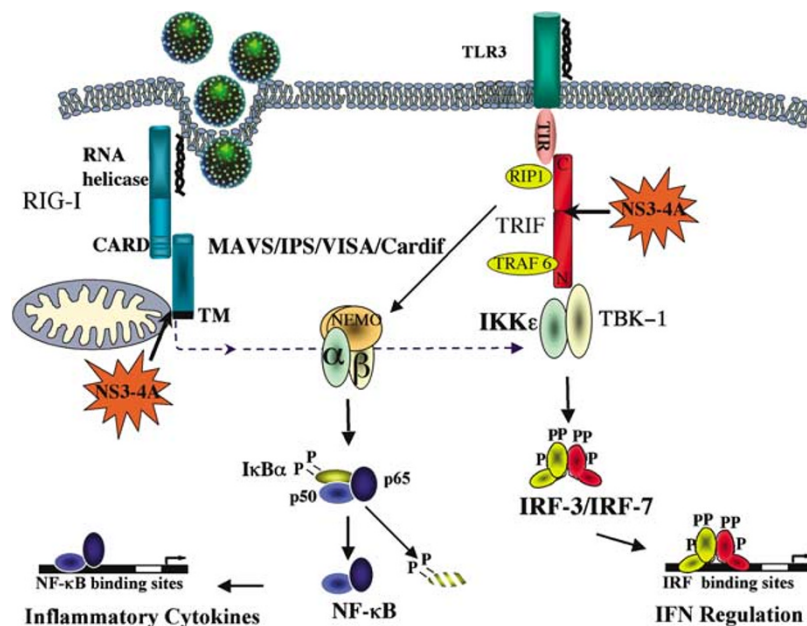

Figure 4 TLR3-dependent and RIG-I-dependent signaling to the innate immune response: specific cleavage of signaling adapters by HCV NS3–4A protease. Engagement of endosome-associated TLR3 by dsRNA recruits the TRIF adaptor, resulting in the activation of TBK-1 and IKKε kinases that phosphorylate IRF-3 and IRF-7. TRIF also signals NF-κB activation via the IKKα/β complex, which phosphorylates IκBα, resulting in the release of the NF-κB DNA-binding subunits. The RIG-1 pathway activates NF-κB and IRF-3/7, following the recognition of incoming viral ribonucleoprotein complexes. RIG-I, through C-terminal RNA helicase domain, interacts with viral dsRNA and through the CARD domains interacts with the MAVS/IPS/VISA/Cardif adaptor. MAVS contains a transmembrane domain (TM) that localizes this adaptor to the mitochondria. NS3–4A protease activity of HCV cleaves the C-terminal domain of MAVS at Cys-508, disrupts RIG-I signaling to IFN activation and establishes persistent infection. NS3–4A also targets the TRIF adaptor molecule in the TLR3-dependent pathway (modified from Hiscott et al., 2006).

This unusual porcine virus is included here as a unique example of the distinct strategies used by viruses to evade NF-κB and immune response signaling. Two viral proteins A238L and A224L appear to have direct effects on the NF-κB pathway. The A238L protein has extensive sequence similarity to IκB proteins and can bind to and inhibit NF-κB (Powell *et al.*, 1996; Revilla *et al.*, 1998). Interestingly, the A238L protein lacks the serine residues of IκBα required for signal-induced phosphorylation by IKK and for degradation. As such, A238L is resistant to signal-induced degradation (Tait *et al.*, 2000). Thus, while ASFV infection initially induces NF-κB, the degraded cellular IκBα is replaced by the viral IκB A238L, which stably inhibits the NF-κB complex (Tait *et al.*, 2000). Of note, A238L also inhibits calcineurin, a protein phosphatase that is required for the induction of the NFAT-dependent immune response genes (Miskin *et al.*, 1998), and the coactivator complex CBP/p300 (Granja *et al.*, 2006). The viral IκB homolog A238L, which is expressed early in ASFV infection, appears to block NF-κB- and NFAT- dependent immune and inflammatory responses.

In contrast, A224L protein is a viral homolog of the IAPs, cellular proteins that block apoptosis by inducing NF-κB activation and by blocking caspase activity. The ASFV IAP-like protein A224L can activate IKK and consequently NF-κB when stably expressed in Jurkat T cells (Rodriguez *et al.*, 2002). A224L is expressed late in infection and may prevent apoptosis (and thus prolong the viral infection state) both by blocking caspase activity and by inducing NF-κB activity. Recently, the insect virus Microplitis demolitor bracovirus has also been shown to encode several IκB-like proteins (Thoetkiattikul *et al.*, 2005), suggesting that interference with NF-κB signaling through the use of IκB-like homologs is a common evolutionary immune evasion strategy.

A distinct inhibitory strategy targeting IRF-3 was recently identified for classical swine fever virus (CSFV). Cells infected with CSFV fail to produce α/β interferon due to a block in IRF-3 activation and translocation to the nucleus, mediated by the CSFV N-terminal protease (Npro) (La Rocca *et al.*, 2005). The results demonstrate a novel viral evasion mechanism that specifically inactivates IRF-3 in CSFV-infected cells. It is likely that other strategies that target host immune responses will be identified with these and related viruses.

Vaccinia virus A46R inhibits TLR3-mediated signaling via its TIR domain
The dsDNA-containing poxvirus vaccinia virus, like all members of the poxvirus family, encodes many viral homologs of cellular immune regulatory proteins that function to dampen the host response to infection (Seet *et al.*, 2003). One virally encoded protein, A46R, was identified in a database search as a TIR domain-containing protein (Bowie *et al.*, 2000), the only virally encoded TIR domain-containing protein identified to date. Recent experiments have demonstrated that A46R inhibits TRIF-mediated IRF-3 activation through the TLR3 pathway and induction of the TRIF-dependent

gene RANTES (Stack *et al.*, 2005). Interestingly, A46R also binds three other TIR adaptors, MyD88, TIRAP/Mal and TRAM, and disrupts NF-κB and MAP kinase activation (Stack *et al.*, 2005), hinting at broader effects on the host immune response. Importantly, vaccinia virus lacking the A46R gene was tested in a murine model of intranasal infection, and was shown to have reduced vaccinia virus virulence *in vivo*.

The multifunctional NS1 protein of influenza virus
Influenza A and B viruses cause a highly contagious respiratory disease in humans and are responsible for periodic worldwide pandemics that cause high human mortality (Garcia-Sastre, 2004; Noah and Krug, 2005). The most devastating pandemic occurred in 1918, resulting in 30 million deaths worldwide (Tumpey *et al.*, 2005; Palese *et al.*, 2006). The current recommended trivalent influenza vaccine includes antigens from recently circulating strains of influenza A and B viruses. Despite the availability of these prophylactic and therapeutic measures, influenza viruses continue to be a significant cause of death. The avian influenza A H5N1 viruses that are currently circulating primarily in different regions of Asia are potential candidates for causing the next pandemic, should they acquire the capacity for efficient human-to-human transmission (Krug, 2006; Obenauer *et al.*, 2006). Understanding the mechanisms that contribute to influenza virus virulence is therefore of great importance for the design of novel therapeutics.

The non-structural NS1 protein is a candidate virulence factor of influenza A (A/NS1) and influenza B (B/NS1) that has been shown to act as an antagonist of the IFN response, although the mechanism(s) of its action is the subject of some controversy (Donelan *et al.*, 2004; Garcia-Sastre, 2004; Li *et al.*, 2006; Min and Krug, 2006). This IFN inhibitory effect of influenza is mediated in part by the ability of the A/NS1 protein to inhibit IRF-3 and NF-κB activation; the B/NS1 protein also functions as an IFN antagonist, in part through the inhibition of the IFN-inducible protein protein kinase R (PKR) *in vitro* (Li *et al.*, 2006). Elimination of the NS1 gene from the influenze genome results in enhanced IRF-3 translocation and activity, and correlates with higher levels of Type 1 IFN.

NS1 participates in both protein–RNA and protein–protein interactions during infection. The N-terminal 73 amino acids of A/NS1 binds to dsRNA with low affinity (Noah and Krug, 2005), but because the A/NS1 dsRNA-binding domain (dsRBD) has a lower affinity for dsRNA than cellular dsRBD (Min and Krug, 2006), it is unclear that A/NS1 can compete with cellular dsRBDs during virus infection. The role of the dsRNA-binding activity of the NS1A protein during influenza A virus infection has not been identified. Latent protein kinase PKR is not activated in cells infected with a recombinant influenza A virus expressing an NS1A that lacks dsRNA-binding activity (Noah and Krug, 2005); therefore, dsRNA sequestration by NS1A does not appear to be the mechanism by which PKR activation is inhibited during influenza A virus infection.

The A/NS1 protein has been shown independently to bind and inhibit the function of two cellular proteins that are required for the 3′ end processing of cellular pre-mRNAs: the 30-kDa subunit of the cleavage and polyadenylation specificity factor (CPSF) and poly(A)-binding protein II (Noah and Krug, 2005; Min and Krug, 2006). Consequently, the production of IFN mRNA – and hence the antiviral response – is reduced, although not eliminated (Noah and Krug, 2005; Min and Krug, 2006). A recent study argues that the dsRNA-binding activity of the A/NS1 does not inhibit the production of IFN mRNA but rather protects influenza A against the antiviral state induced by type 1 IFN; A/NS1 dsRNA-binding activity protects primarily by inhibiting the IFN-induced 2′-5′-oligo (A) synthetase (OAS)/RNase L pathway (Min and Krug, 2006).

The molecular basis of the virulence of the avian H5N1 viruses in humans is likely dependent on several virus-encoded proteins (Garcia-Sastre, 2006), including NS1, hemagglutinin and neuraminidase. Recent sequence analysis of a large number of H5N1 viral particles has revealed that the NS1 protein of the majority of avian H5N1 contains a novel sequence motif – Glu-Ser-Glu-Val (ESEV) – that is predicted to mediate protein interactions with PDZ domain-containing proteins which are involved in protein trafficking, cell morphology and organization. Virulent H5N1 viruses possess a different PDZ-binding sequence – Glu, Pro, Glu, Val (EPEV) – at the C-terminus of the NS1 protein whereas NS1 protein from low virulence influenza A viruses do not have a PDZ-binding motif (Obenauer *et al.*, 2006). The presence of these functional C-terminal PDZ-binding domains in NS1 of H5N1 viruses correlates with human virulence. These observations indicate

that the PDZ-interaction domains of NS1 may contribute to virulence by binding cellular PDZ-containing proteins and disrupting their participation in important cellular processes (Krug, 2006).

Negative-stranded RNA viruses inhibit the antiviral response

The group of enveloped, non-segmented negative-strand RNA viruses has been extensively studied and the mechanisms by which these viruses interfere with the antiviral response are beginning to emerge (Figure 5 and Table 2). Recent reviews describe in detail the strategies used by these viruses to interfere with the innate response (Conzelmann, 2005; Hengel *et al.*, 2005).

Rhabdoviruses
Rhabdoviruses, such as VSV and rabies virus (RV), encode five structural proteins, the nucleoprotein (N), the phosphoprotein (P), the inner matrix protein (M), the transmembrane glycoprotein (G) and the RNA polymerase (L). VSV is highly sensitive to IFN and infection of susceptible cells leads to a highly cytopathic infection, with rapid and robust activation of NF-κB and IRF-3 (tenOever *et al.*, 2004). Nevertheless, VSV has established an effective mechanism to limit IFN expression through the inhibition of host cell gene expression. VSV shutdown and inhibition of IFN are largely due to the multifunctional matrix M protein, which complexes at the nuclear pore with the nucleoporin Nup98 and blocks nuclear to cytoplasmic export of cellular RNA (Black and Lyles, 1992; Ferran and Lucas-Lenard, 1997; Her *et al.*, 1997). Although this
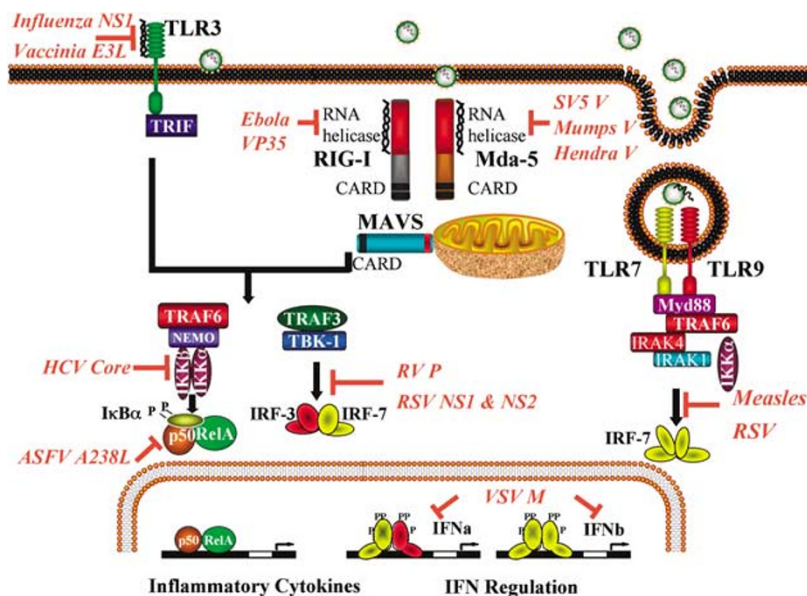

Figure 5 Viruses inhibit distinct aspects of the antiviral response. Influenza virus NS1 protein, as well as Vaccinia E3L prevents viral dsRNA from being recognized. The V proteins of paramyxoviruses, including SV5, Mumps and Hendra viruses, bind to Mda-5 and block downstream signaling. Ebola virus VP35 bids to RIG-I and blocks downstream signaling. RSV and RV interfere with activation of IRF-3 in virus-infected cells. The RV P protein inhibits TBK-1-mediated phosphorylation of IRF-3. VSV M protein blocks nuclear to cytoplasmic export of IFN and cellular mRNA (modified from Conzelmann, 2005). HCV core protein and ASFV block NF-κB activation. Measles virus and RSV block IFNα and IFNβ production.

Table 2 Viral Inhibitors of NF-κB

Virus	Viral protein	Protein function
Adenovirus	E1A	Suppresses IKK activity and IκB degradation
	E1B	Suppresses NF-κB activity
	E3-14.7K	Binds to IKK and p50
	E3-10.4/14.5K	Regulator of Fas, inhibits TNF-α− induced NF-κB activation
African swine fever virus	A238L	Blocks IκB degradation
Hepatitis C virus	NS5A	Inhibits NF-κB activation signaled by TRAF2
	Core protein	Inhibits IKK complex
	NS3-4A	Protease cleaves MAVS from the mitochondria and disrupts RIG-I signaling
Human herpesvirus-8	K1	Tyrosine kinase immunoreceptor mediates constitutive Lyn kinase activation
Human immunodeficiency virus 1	Nef	Induces IκBα
Human papillomavirus	E7	Disrupts NF-κB activation by targeting the IKK complex
Influenza A	NS1	Binds dsRNA and blocks IFN effects
Microplitis demolitor bracovirus	H4, N5	IκB-like proteins
Molluscum contagiosum virus	MC159	Inhibits IκB degradation
	MC160	Destabilizes IKKα
Mumps virus	SH protein	Blocks TNF-mediated NF-κB activation
Myxoma virus	MNF	IκB-like protein inhibits NF-κB
Poliovirus	3C protease	Cleaves RelA
Sendai virus	V, C proteins	Blocks NF-κB activation
Vaccinia Virus	K1L	Inhibits IκB degradation
	A46R	Inhibits TLR3 signaling through TIR-like domain

Abbreviations: IKK, IκB kinase; NF-κB, nuclear factor κB; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α.

mechanism is not particularly specific, it appears that the general shutdown of host gene expression obviates the need for specific NF-κB or IRF antagonists with this cytopathic virus.

Compared to VSV, RV grows more slowly and the generalized inhibition of host gene expression is not observed. The RV P protein – a polymerase cofactor involved in assembly of RNPs, and a chaperone for specific and proper encapsidation of viral RNA by the N protein – has been shown to interfere with IRF-3 activation. IRF-3 induction was blocked at the level of phosphorylation, dimer formation, nuclear import, and transcriptional activity (Brzozka et al., 2005), suggesting that P interferes with the function of TBK-1. Understanding the relationship between rhabdoviruses and the antiviral reponse will be critical in the development of strategies for live vaccines and oncolytic virus vectors (Stojdl et al., 2003; Lichty et al., 2004a, b).

Paramyxoviridae
The phosphoprotein P gene of most members of the *Paramyxovirinae* subfamily encode additional, non-essential proteins, including the V proteins, which are translated from 'edited' P gene transcripts, a mechanism that involves the cotranscriptional introduction of one or more G residues at defined sites by viral RNA polymerase (Kolakofsky et al., 1998). The V and P proteins therefore have identical N-terminal moieties and specific C-terminal domains. V proteins of most paramyxoviruses are responsible for the pronounced resistance to IFN of these viruses. The V proteins associate with STAT1/STAT2 complexes and abolish IFN JAK/STAT signaling by targeting either STAT1 or STAT2 for proteasomal degradation or by interfering with STAT phosphorylation. In addition to V proteins, Sendai virus and measles virus encode one or more C

proteins that are expressed from the P mRNAs by alternative translation initiation. These proteins do not share any sequences with the P or V proteins. In Sendai virus, multiple C proteins rather than the V protein abolish IFN STAT signaling; with measles virus, JAK/STAT interference has been attributed to both C (Shaffer et al., 2003) and V proteins (Gotoh et al., 2002; Palosaari et al., 2003; Garcia-Sastre, 2004; Nagai and Kato, 2004; Ohno et al., 2004).

Paramyxoviruses can in addition directly block IFN-β induction. A simian virus 5 (SV5) in which expression of the V protein-specific C-terminal domain was abolished lost the ability to degrade STAT1, as well as the ability to suppress nuclear import and activation of IRF-3 and activation of NF-κB, leading to the induction of IFNβ expression (He et al., 2002). Ectopic expression of the SV5 V protein blocked induction of IFNβ by dsRNA and virus infection. Moreover, the C-terminal Cys-rich domain of V alone was sufficient to counteract IFNβ expression, whereas this domain is not sufficient to induce STAT degradation (Poole et al., 2002).

The DExD/H RNA helicase Mda-5 is a direct target of the SV5 V protein, as well as the V proteins from human parainfluenza virus type 2, Sendai virus, mumps virus and Hendra virus (Andrejeva et al., 2004). In cells overexpressing Mda-5, the activation of IRF-3 and NF-κB activity as well as IFN expression was inhibited by co-expression of full-length V or the C-terminal domain of V protein. SV5 V, however, does not bind RIG-I and therefore appears to represent a distinct mechanism of inhibition of the antiviral response through Mda-5 (Andrejeva et al., 2004).

Human respiratory syncytial virus
Human respiratory syncytial virus (HRSV) and the bovine counterpart (BRSV) represent a subfamily of the

paramyxoviruses – *Pneumovirinae* – that do not edit RNA and do not encode a V protein, but rather encode additional NS proteins, NS1 and NS2. HRSV and BRSV are highly resistant to treatment with IFNα/β (Andrejeva *et al.*, 2004), with the IFN resistance phenotype mapping to the NS genes (Schlender *et al.*, 2000). Viruses lacking either NS1 or NS2 lose their ability to replicate in IFN-treated cells, suggesting a cooperative function of the two proteins in counteracting antiviral IFN effects (Bossert and Conzelmann, 2002). Further analyses of BRSV and HRSV NS gene deletion virus mutants revealed that the NS proteins are also critical for preventing induction of IFNβ in RSV-infected cells (Bossert *et al.*, 2003; Valarcher *et al.*, 2003; Spann *et al.*, 2004). Recombinant viruses lacking either NS1 or NS2 displayed increased IRF-3 phosphorylation and activity compared to wild-type RSV. NF-κB activities remained high in cells infected with wild-type and NS deletion mutant viruses, indicating that RSV NS proteins specifically targeted the IRF-3 pathway (Bossert *et al.*, 2003), in contrast to the V proteins of paramyxoviruses, which inhibit both IRF-3 and NF-κB activation by blocking Mda-5.

Ebola virus
The VP35 protein of Ebola virus was identified as an IFN antagonist on the basis of its ability to rescue the growth of an NS1-deficient influenza A virus in IFN-competent cells (Basler *et al.*, 2000) and blocks the virus-induced activation of IRF-3 (Basler *et al.*, 2003). Recent evidence indicates that VP35 possesses dsRNA-binding activity, and two VP35 point mutants, R312A and K309A, were significantly impaired in their dsRNA-binding activity (Cardenas *et al.*, 2006). VP35 blocked activation of IRF-3 in cells overexpressing RIG-I, and the VP35 mutants impaired for dsRNA binding had decreased IFN antagonist activity. Wild-type VP35 and the R312A and K309A mutants were also able to inhibit IFNβ promoter activity induced by the MAVS/Cardif adapter. These studies argue that dsRNA binding may contribute to VP35 antagonism of IFN signaling

(Figure 5). However, additional mechanisms of inhibition, at a point proximal to the IRF-3 kinases, may also exist (Cardenas *et al.*, 2006).

Conclusions

Significant advances in our understanding of the sensing, recognition and response to viral pathogens have been made in the past decade; at pace with these discoveries has been the increase in our understanding of the mechanisms used by viruses to interfere with and manipulate the host immune response (Akira *et al.*, 2006; Garcia-Sastre and Biron, 2006; Meylan and Tschopp, 2006). The NF-κB and IRF pathways are prime targets for viral evasion and most viruses have evolved strategies to impact on these 'early response systems'. Viruses have been probing the immune response for millions of years and this 'investigation' of the immune response is yielding important clues about which pathways must be compromised in order for virus infection to perpetuate. Utilization of this knowledge will be a cornerstone in the understanding of the molecular aspects of viral pathogenesis and the improvement of strategies for the development of vaccines and antiviral agents.

References

Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D. (2000). *Cell* **103**: 667–678.

Aizaki H, Saito S, Ogino T, Miyajima N, Harada T, Matsuura Y *et al.* (2000). *J Interferon Cytokine Res* **20**: 1111–1120.

Akagi T, Ono H, Shinotohno K. (1995). *Blood* **86**: 4243–4249.

Akira S, Sato S. (2003). *Scand J Infect Dis* **35**: 555–562.

Akira S, Uematsu S, Takeuchi O. (2006). *Cell* **124**: 783–801.

Alefantis T, Jain P, Ahuja J, Mostoller K, Wigdahl B. (2005). *J Biomed Sci* **12**: 961–974.

Alter HJ, Seeff LB. (2000). *Semin Liver Dis* **20**: 17–35.

Andrejeva J, Childs KS, Young DF, Carlos TS, Stock N, Goodbourn S *et al.* (2004). *Proc Natl Acad Sci USA* **101**: 17264–17269.

Atkinson PG, Coope HJ, Rowe M, Ley SC. (2003). *J Biol Chem* **278**: 51134–51142.

Aupperle KR, Yamanishi Y, Bennett BL, Mercurio F, Boyle DL, Firestein GS. (2001). *Cell Immunol* **214**: 54–59.

Azran I, Jeang KT, Aboud M. (2005). *Oncogene* **24**: 4521–4530.

Basler CF, Mikulasova A, Martinez-Sobrido L, Paragas J, Muhlberger E, Bray M *et al.* (2003). *J Virol* **77**: 7945–7956.

Basler CF, Wang X, Muhlberger E, Volchkov V, Paragas J, Klenk HD *et al.* (2000). *Proc Natl Acad Sci USA* **97**: 12289–12294.

Bex F, Gaynor RB. (1998). *Methods* **16**: 83–94.

Bex F, Murphy K, Wattiez R, Burny A, Gaynor RB. (1999). *J Virol* **73**: 738–745.

Black BL, Lyles DS. (1992). *J Virol* **66**: 4058–4064.

Blight KJ, McKeating JA, Marcotrigiano J, Rice CM. (2003). *J Virol* **77**: 3181–3190.

Bonnard M, Mirtsos C, Suzuki S, Graham K, Huang J, Ng M *et al.* (2000). *EMBO J* **19**: 4976–4985.

Bossert B, Conzelmann KK. (2002). *J Virol* **76**: 4287–4293.

Bossert B, Marozin S, Conzelmann KK. (2003). *J Virol* **77**: 8661–8668.

Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O'Neill LA. (2000). *Proc Natl Acad Sci USA* **97**: 10162–10167.

Breiman A, Grandvaux N, Lin R, Ottone C, Akira S, Yoneyama M *et al.* (2005). *J Virol* **79**: 3969–3978.

Brinkmann MM, Schulz TF. (2006). *J Gen Virol* **87**: 1047–1074.

Brzozka K, Finke S, Conzelmann KK. (2005). *J Virol* **79**: 7673–7681.

Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer JL *et al.* (1998). *J Biol Chem* **273**: 12203–12209.

Cahir-McFarland ED, Davidson DM, Schauer SL, Duong J, Kieff E. (2000). *Proc Natl Acad Sci USA* **97**: 6055–6060.

Cardenas WB, Loo YM, Gale Jr M, Hartman AL, Kimberlin CR, Martinez-Sobrido L *et al.* (2006). *J Virol* **80**: 5168–5178.

Cario E, Podolsky D. (2000). *Infection Immun* **68**: 7010–7017.

Carrasco D, Rizzo CA, Dorfman K, Bravo R. (1996). *EMBO J* **15**: 3640–3650.

Carter RS, Pennington KN, Ungurait BJ, Ballard DW. (2003). *J Biol Chem* **278**: 19642–19648.

Cella M, Salio M, Sakakibara Y, Langen H, Julkunen I, Lanzavecchia A. (1999). *J Exp Med* **189**: 821–829.

Cesaire R, Oliere S, Sharif-Askari E, Loignon M, Lezin A, Olindo S *et al.* (2006). *Oncogene* **25**: 349–358.

Chariot A, Leonardi A, Muller J, Bonif M, Brown K, Siebenlist U. (2002). *J Biol Chem* **277**: 37029–37036.

Chaudhary PM, Jasmin A, Eby MT, Hood L. (1999). *Oncogene* **18**: 5738–5746.

Chen BJ, Feinberg MB, Baltimore D. (1997). *J Virol* **71**: 5495–5504.

Chen ZJ. (2005). *Nat Cell Biol* **7**: 758–765.

Cheng G, Baltimore D. (1996). *Genes Dev* **10**: 963–973.

Chen-Park FE, Huang DB, Noro B, Thanos D, Ghosh G. (2002). *J Biol Chem* **277**: 24701–24708.

Chisari FV. (2005). *Nature* **436**: 930–932.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. (1989). *Science* **244**: 359–362.

Chu WM, Ostertag D, Li ZW, Chang L, Chen Y, Hu Y *et al.* (1999a). *Immunity* **11**: 721–731.

Chu Z-L, Didonato J, Hawiger J, Ballard DW. (1998). *J Biol Chem* **273**: 15891–15894.

Chu Z-L, Shin YA, Yang JM, DiDonato JA, Ballard DW. (1999b). *J Biol Chem* **274**: 15297–15300.

Chugh P, Matta H, Schamus S, Zachariah S, Kumar A, Richardson JA *et al.* (2005). *Proc Natl Acad Sci USA* **102**: 12885–12890.

Civas A, Genin P, Morin P, Lin R, Hiscott J. (2006). *J Biol Chem* **281**: 4856–4866.

Colonna M, Trinchieri G, Liu YJ. (2004). *Nat Immunol* **5**: 1219–1226.

Conzelmann KK. (2005). *J Virol* **79**: 5241–5248.

Courtois G, Gilmore TD. (2006). *Oncogene* **25**: 6831–6843.

D'Souza B, Rowe M, Walls D. (2000). *J Virol* **74**: 6652–6658.

Degli-Esposti MA, Smyth MJ. (2005). *Nat Rev Immunol* **5**: 112–124.

Deng L, Wang C, Spencer E, Yang L, Braun A, You J *et al.* (2000). *Cell* **103**: 351–361.

Devergne O, Cahir McFarland ED, Mosialos G, Izumi KM, Ware CF, Kieff E. (1998). *J Virol* **72**: 7900–7908.

Devergne O, Hatzivassiliou E, Izumi KM, Kaye KM, Kleijnen MF, Kieff E *et al.* (1996). *Mol Cell Biol* **16**: 7098–7108.

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. (2004). *Science* **303**: 1529–1531.

Donelan NR, Dauber B, Wang X, Basler CF, Wolff T, Garcia-Sastre A. (2004). *J Virol* **78**: 11574–11582.

Dunne A, O'Neill LA. (2003). *Sci STKE* **2003**: re3.

Duyao MP, Kessler DJ, Spicer DB, Bartholomew C, Cleveland JL, Siekevitz M *et al.* (1992a). *J Biol Chem* **267**: 16288–16291.

Duyao MP, Kessler DJ, Spicer DB, Sonenshein GE. (1992b). *Curr Top Microbiol Immunol* **182**: 421–424.

Edlich RF, Arnette JA, Williams FM. (2000). *J Emerg Med* **18**: 109–119.

Eshima N, Tabata M, Okada T, Karukaya S. (2003). *Math Med Biol* **20**: 29–45.

Ferran MC, Lucas-Lenard JM. (1997). *J Virol* **71**: 371–377.

Feuillard J, Schuhmacher M, Kohanna S, Asso-Bonnet M, Ledeur F, Joubert-Caron R *et al.* (2000). *Blood* **95**: 2068–2075.

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT *et al.* (2003a). *Nat Immunol* **4**: 491–496.

Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E *et al.* (2003b). *J Exp Med* **198**: 1043–1055.

Foy E, Li K, Sumpter Jr R, Loo YM, Johnson CL, Wang C *et al.* (2005). *Proc Natl Acad Sci USA* **79**: 2689–2699.

Fujita F, Taniguchi Y, Kato T, Narita Y, Furuya A, Ogawa T *et al.* (2003). *Mol Cell Biol* **23**: 7780–7793.

Gale Jr M, Foy EM. (2005). *Nature* **436**: 939–945.

Gale Jr M, Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM *et al.* (1998). *Mol Cell Biol* **18**: 5208–5218.

Garcia-Sastre A. (2004). *Curr Top Microbiol Immunol* **283**: 249–280.

Garcia-Sastre A, Biron CA. (2006). *Science* **312**: 879–882.

Garcia-Sastre A. (2006). *Emerg Infect Dis* **12**: 44–47.

Geleziunas R, Ferrell S, Lin X, Mu Y, Cunningham Jr ET, Grant M *et al.* (1998). *Mol Cell Biol* **18**: 5157–5165.

Gerondakis S, Grumont R, Gugasyan R, Wong L, Isomura I, Ho W *et al.* (2006). *Oncogene* **25**: 6781–6799.

Gerritsen ME, Williams AJ, Neish AS, Moore S, Shi Y, Collins T. (1997). *Proc Natl Acad, Sci USA* **7**: 2927–2932.

Ghosh AK, Majumder M, Steele R, Yaciuk P, Chrivia J, Ray R *et al.* (2000). *J Biol Chem* **275**: 7184–7188.

Ghosh AK, Steele R, Meyer K, Ray R, Ray RB. (1999). *J Gen Virol* **80**: 1179–1183.

Gilmore TD. (2006). *Oncogene* **25**: 6680–6684.

Gilmore TD, Cormier C, Jean-Jacques J, Gapuzan M-E. (2001). *Oncogene* **20**: 7098–7103.

Gilmore TD, Mosialos G. (2003). *Nuclear Factor-kB: Regulation and Role in Disease*. In: Beyaert R (ed). Kluwer Academic Publishers: The Netherlands, pp 91–115.

Gilmore TD, White DW, Sarkar S, Sif S. (1995). *Howard Temin's Scientific Legacy*. In: Cooper GM, Greenberg Temin R, Sugden B (eds). American Society for Microbiology: Washington, DC, pp 109–128.

Gilmore TD. (1999). *Oncogene* **18**: 6925–6937.

Good L, Sun SC. (1996). *J Virol* **70**: 2730–2735.

Gotoh B, Komatsu T, Takeuchi K, Yokoo J. (2002). *Rev Med Virol* **12**: 337–357.

Granja AG, Nogal ML, Hurtado C, Del Aguila C, Carrascosa AL, Salas ML *et al.* (2006). *J Immunol* **176**: 451–462.

Grassmann R, Aboud M, Jeang KT. (2005). *Oncogene* **24**: 5976–5985.

Grindstaff P, Gruener G. (2005). *Semin Neurol* **25**: 315–327.

Grossman WJ, Kimata JT, Wong F-H, Zutter M, Ley TJ. (1995). *Proc Natl Acad Sci USA* **92**: 1057–1061.

Grumont RJ, Gerondakis S. (2000). *J Exp Med* **191**: 1281–1292.

Guasparri I, Wu H, Cesarman E. (2006). *EMBO Rep* **7**: 114–119.

6864

Guo HG, Pati S, Sadowska M, Charurat M, Reitz M. (2004). *J Virol* **78**: 9336–9342.

Harhaj EW, Sun S-C. (1999). *J Biol Chem* **274**: 22911–22914.

Harhaj EW, Good L, Xiao G, Sun S-C. (1999). *Oncogene* **18**: 1341–1349.

Harhaj EW, Good L, Xiao G, Uhlik M, Cvijic ME, Rivera-Walsh I et al. (2000). *Oncogene* **19**: 1448–1456.

Harris J, Oliere S, Sharma S, Sun Q, Lin R, Hiscott J et al. (2006). *J Immunol* **177**: 2527–2535.

Hatzivassiliou E, Mosialos G. (2002). *Front Biosci* **7**: d319–d329.

Hayden MS, Ghosh S. (2004). *Genes Dev* **18**: 2195–2224.

He B, Paterson RG, Stock N, Durbin JE, Durbin RK, Goodbourn S et al. (2002). *Virology* **303**: 15–32.

He Z, Xin B, Yang X, Chan C, Cao L. (2000). *Cancer Res* **60**: 1845–1848.

Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S et al. (2004). *Science* **303**: 1526–1529.

Hemmi H, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H et al. (2004). *J Exp Med* **199**: 1641–1650.

Henderson S, Rowe M, Gregory C, Croom-Carter D, Wang F, Longnecker R et al. (1991). *Cell* **65**: 1107–1115.

Hengel H, Koszinowski UH, Conzelmann KK. (2005). *Trends Immunol* **26**: 396–401.

Her LS, Lund E, Dahlberg JE. (1997). *Science* **276**: 1845–1848.

Herrero JA, Mathew P, Paya CV. (1995). *J Virol* **69**: 2168–2174.

Hewson CA, Jardine A, Edwards MR, Laza-Stanca V, Johnston SL. (2005). *J Virol* **79**: 12273–12279.

Heylbroeck C, Balachandran S, Servant MJ, DeLuca C, Barber GN, Lin R et al. (2000). *J Virol* **74**: 3781–3792.

Himes SR, Coles LS, Katsikeros R, Lang RK, Shannon MF. (1993). *Oncogene* **8**: 3189–3197.

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G. (1987). *Science* **237**: 1340–1343.

Hiscott J, Alper D, Cohen L, Leblanc J-F, Sportza L, Wong A et al. (1989). *J Virol* **63**: 2557–2566.

Hiscott J, Kwon H, Genin P. (2001). *J Clin Invest* **107**: 143–151.

Hiscott J, Lin R, Nakhaei P, Paz S. (2006). *Trends Mol Med* **12**: 53–56.

Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim SO et al. (2003). *Nature* **424**: 743–748.

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T et al. (2005). *Nature* **434**: 772–777.

Hoofnagle JH. (2002). *Hepatology* **36**: S21–S29.

Horng T, Barton GM, Medzhitov R. (2001). *Nat Immunol* **2**: 835–841.

Hoshino K, Sugiyama T, Matsumoto M, Tanaka T, Saito M, Hemmi H et al. (2006). *Nature* **440**: 949–953.

Hrdlicková R, Nehyba J, Humphries EH. (1994). *J Virol* **68**: 2371–2382.

Huang Y, Ohtani K, Iwanaga R, Matsumura Y, Nakamura M. (2001). *Oncogene* **20**: 1094–1102.

Huen DS, Henderson SA, Croom-Carter D, Rowe M. (1995). *Oncogene* **10**: 549–560.

Imaizumi Y, Kohno T, Yamada Y, Ikeda S, Tanaka Y, Tomonaga M et al. (2001). *Jpn J Cancer Res* **92**: 1284–1292.

Ishii KJ, Coban C, Kato H, Takahashi K, Torii Y, Takeshita F et al. (2006). *Nat Immunol* **7**: 40–48.

Iwanaga Y, Tsukahara T, Ohashi T, Tanaka Y, Arai M, Nakamura M et al. (1999). *J Virol* **73**: 1271–1277.

Iwasaki A, Medzhitov R. (2004). *Nat Immunol* **5**: 987–995.

Izumi KM, Kaye KM, Kieff ED. (1997). *Proc Natl Acad Sci USA* **94**: 1447–1452.

Janeway CA, Travers P, Walport M, Capra JD. (1999). *Immunobiology: The Immune System in Health and Disease*, 4th edn. Garland Publishing: New York.

Janeway Jr CA, Medzhitov R. (2002). *Annu Rev Immunol* **20**: 197–216.

Jeang KT. (2001). *Cytokine Growth Factor Rev* **12**: 207–217.

Jeong SJ, Pise-Masison CA, Radonovich MF, Park HU, Brady JN. (2005a). *Oncogene* **24**: 6719–6728.

Jeong SJ, Pise-Masison CA, Radonovich MF, Park HU, Brady JN. (2005b). *J Biol Chem* **280**: 10326–10332.

Jiang Z, Mak TW, Sen G, Li X. (2004). *Proc Natl Acad Sci USA* **101**: 3533–3538.

Johnson CL, Gale JR. (2006). *Trends Immunol* **27**: 1–4.

Kaisho T, Akira S. (2004). *Microbes Infect* **6**: 1388–1394.

Kamens J, Richardson P, Mosialos G, Brent R, Gilmore TD. (1990). *Mol Cell Biol* **10**: 2840–2847.

Karin M, Yamamoto Y, Wang QM. (2004). *Nat Rev Drug Discov* **3**: 17–26.

Karin M. (2006). *Nature* **441**: 431–436.

Kashanchi F, Brady JN. (2005). *Oncogene* **24**: 5938–5951.

Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K et al. (2005). *Immunity* **23**: 19–28.

Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K et al. (2006). *Nature* **441**: 101–105.

Kawai T, Akira S. (2006). *Nat Immunol* **7**: 131–137.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H et al. (2005). *Nat Immunol* **6**: 981–988.

Keller SA, Hernandez-Hopkins D, Vider J, Ponomarev V, Hyjek E, Schattner EJ et al. (2006). *Blood* **107**: 3295–3302.

Kfoury Y, Nasr R, Hermine O, de The H, Bazarbachi A. (2005). *Cell Death Differ* **12**: s871–s877.

Kim TK, Maniatis T. (1997). *Mol Cell* **1**: 119–129.

Kishore N, Huynh QK, Mathialagan S, Hall T, Rouw S, Creely D et al. (2002). *J Biol Chem* **277**: 13840–13847.

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y et al. (1990). *Hepatology* **12**: 671–675.

Kolakofsky D, Pelet T, Garcin D, Hausmann S, Curran J, Roux L. (1998). *J Virol* **72**: 891–899.

Kravchenko VV, Mathison JC, Schwamborn K, Mercurio F, Ulevitch RJ. (2003). *J Biol Chem* **278**: 26612–26619.

Krug RM. (2006). *Science* **311**: 1562–1563.

Kwok RPS, Laurance ME, Lundblad RJ, Goldman PS, Shih H-M, Connor LM et al. (1996). *Nature* **380**: 642–646.

Kwon H, Pelletier N, De Luca C, Genin P, Cisternas S, Lin R et al. (1998). *J Biol Chem* **273**: 7431–7440.

La Rocca SA, Herbert RJ, Crooke H, Drew TW, Wileman TE, Powell PP. (2005). *J Virol* **79**: 7239–7247.

Lacoste J, Petropoulos L, Pepin N, Hiscott J. (1995). *J Virol* **69**: 564–569.

Laherty CD, Hu HM, Opipari AW, Wang F, Dixit VM. (1992). *J Biol Chem* **267**: 24157–24160.

Lamsoul I, Lodewick J, Lebrun S, Brasseur R, Burny A, Gaynor RB et al. (2005). *Mol Cell Biol* **25**: 10391–10406.

Latz E, Visintin A, Espevik T, Golenbock DT. (2004). *J Endotoxin Res* **10**: 406–412.

Lee BS, Paulose-Murphy M, Chung YH, Connlole M, Zeichner S, Jung JU. (2002). *J Virol* **76**: 11299–12185.

Lee H, Choi JK, Li M, Kaye K, Kieff E, Jung JU. (1999). *J Virol* **73**: 3913–3919.

LeMosy EK, Kemler D, Hashimoto C. (1998). *Development* **125**: 4045–4053.

Lenardo MJ, Baltimore D. (1989). *Cell* **58**: 227–229.

Lenardo MJ, Fan C-M, Maniatis T, Baltimore D. (1989). *Cell* **57**: 287–294.

Lenzmeier BA, Baird EE, Dervan PB, Nyborg JK. (1999). *J Mol Biol* **291**: 731–744.

Lenzmeier BA, Giebler HA, Nyborg JK. (1998). *Mol Cell Biol* **18**: 721–731.

Li C-CH, Ruscetti FW, Rice NR, Chen E, Yang N-S, Mikovits J *et al*. (1993). *J Virol* **67**: 4205–4213.

Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M *et al*. (2005a). *Proc Natl Acad Sci USA* **102**: 2992–2997.

Li S, Min JY, Krug RM, Sen GC. (2006). *Virology* **349**: 13–21.

Li S, Strelow A, Fontana EJ, Wesche H. (2002). *Proc Natl Acad Sci USA* **99**: 5567–5572.

Li XD, Sun L, Seth RB, Pineda G, Chen ZJ. (2005b). *Proc Natl Acad Sci USA* **102**: 17717–17722.

Lichty BD, Power AT, Stojdl DF, Bell JC. (2004a). *Trends Mol Med* **10**: 210–216.

Lichty BD, Stojdl DF, Taylor RA, Miller L, Frenkel I, Atkins H *et al*. (2004b). *Hum Gene Ther* **15**: 821–831.

Lin R, Genin P, Mamane Y, Hiscott J. (2000). *Mol Cell Biol* **20**: 6342–6353.

Lin R, Heylbroeck C, Pitha PM, Hiscott J. (1998). *Mol Cell Biol* **18**: 2986–2996.

Lin R, Lacoste J, Nakhaei P, Sun Q, Yang L, Paz S *et al*. (2006). *J Virol* **80**: 6072–6083.

Lin R, Mamane Y, Hiscott J. (1999). *Mol Cell Biol* **19**: 2465–2474.

Liu L, Eby MT, Rathore N, Sinha SK, Kumar A, Chaudhary PM. (2002). *J Biol Chem* **277**: 13745–13751.

Lomvardas S, Thanos D. (2001). *Cell* **106**: 685–696.

Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM *et al*. (2006). *Proc Natl Acad Sci USA* **103**: 6001–6006.

Luftig M, Prinarakis E, Yasui T, Tsichritzis T, Cahir-McFarland E, Inoue J *et al*. (2003). *Proc Natl Acad Sci USA* **100**: 15595–155600.

Luftig M, Yasui T, Soni V, Kang MS, Jacobson N, Cahir-McFarland E *et al*. (2004). *Proc Natl Acad Sci USA* **101**: 141–146.

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW *et al*. (2004). *Proc Natl Acad Sci USA* **101**: 5598–5603.

Lye E, Mirtsos C, Suzuki N, Suzuki S, Yeh WC. (2004). *J Biol Chem* **279**: 40653–40658.

Makris C, Godfrey VL, Krahn-Senftleben G, Takahashi T, Roberts JL, Schwarz T *et al*. (2000). *Mol Cell* **5**: 969–979.

Mamane Y, Loignon M, Palmer J, Hernandez E, Cesaire R, Alaoui-Jamali M *et al*. (2005). *J Interferon Cytokine Res* **25**: 43–51.

Mamane Y, Sharma S, Grandvaux N, Hernandez E, Hiscott J. (2002). *J Interferon Cytokine Res* **22**: 135–143.

Mamane Y, Sharman S, Petropoulos L, Lin R, Hiscott J. (2000). *Immunity* **12**: 129–140.

Maniatis T, Falvo JV, Kim TH, Kim TK, Lin CH, Parekh BS *et al*. (1998). *Cold Spring Harb Symp Quant Biol* **63**: 609–620.

Marie I, Durbin JE, Levy DE. (1998). *EMBO J* **17**: 6660–6669.

Marriott SJ, Semmes OJ. (2005). *Oncogene* **24**: 5986–5995.

Matsumoto M, Funami K, Tanabe M, Oshiumi H, Shingai M, Seto Y *et al*. (2003). *J Immunol* **171**: 3154–3162.

Mattioli I, Geng H, Sebald A, Hodel M, Bucher C, Kracht M *et al*. (2006). *J Biol Chem* **281**: 6175–6183.

McKinsey TA, Brockman JA, Scherer DC, Al-Murrani SW, Green PL, Ballard DW. (1996). *Mol Cell Biol* **16**: 2083–2090.

McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. (2004). *Proc Natl Acad Sci USA* **101**: 233–238.

Medzhitov R, Janeway Jr CA. (1997). *Cell* **91**: 295–298.

Melen K, Fagerlund R, Nyqvist M, Keskinen P, Julkunen I. (2004). *J Med Virol* **73**: 536–547.

Merika M, Thanos D. (2001). *Curr Opin Genet Dev* **11**: 205–208.

Merlo JJ, Tsygankov AY. (2001). *Virology* **279**: 325–338.

Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M *et al*. (2004). *Nat Immunol* **5**: 503–507.

Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R *et al*. (2005). *Nature* **437**: 1167–1172.

Meylan E, Tschopp J. (2006). *Mol Cell* **22**: 561–569.

Miller K, McArdle S, Gale Jr MJ, Geller DA, Tenoever B, Hiscott J *et al*. (2004). *J Interferon Cytokine Res* **24**: 391–402.

Min JY, Krug RM. (2006). *Proc Natl Acad Sci USA* **103**: 7100–7105.

Minakhina S, Steward R. (2006). *Oncogene* **25**: 6749–6757.

Mink M, Fogelgren B, Olszewski K, Maroy P, Csiszar K. (2001). *Genomics* **74**: 234–244.

Miskin JE, Abrams CC, Goatley LC, Dixon LK. (1998). *Science* **281**: 562–565.

Mitchell T, Sugden B. (1995). *J Virol* **69**: 2968–2976.

Mori N, Fujii M, Hinz M, Nakayama K, Yamada Y, Ikeda S *et al*. (2002). *Int J Cancer* **99**: 378–385.

Mori N, Shirakawa F, Abe M, Kamo Y, Koyama Y, Murakami S *et al*. (1995). *J Rheumatol* **22**: 2049–2054.

Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. (1995). *Cell* **80**: 389–399.

Mosialos G. (2001). *Cytokine Growth Factor Rev* **12**: 259–270.

Munshi N, Agalioti T, Lomvardas S, Merika M, Chen G, Thanos D. (2001). *Science* **293**: 1133–1136.

Munshi N, Yie J, Merika M, Senger K, Lomvardas S, Agalioti T *et al*. (1999). *Cold Spring Harb Symp Quant Biol* **64**: 149–159.

Muzio M, Polentarutti N, Bosisio D, Prahladan MK, Mantovani A. (2000). *J Leukoc Biol* **67**: 450–456.

Nagai Y, Kato A. (2004). *Curr Top Microbiol Immunol* **283**: 197–248.

Nasr R, Chiari E, El-Sabban M, Mahieux R, Kfoury Y, Abdulhay M *et al*. (2006). *Blood* **107**: 4021–4029.

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G. (1987). *Science* **237**: 1324–1329.

Nicot C, Mahieux R, Takemoto S, Franchini G. (2000a). *Blood* **96**: 275–281.

Nicot C, Opavsky R, Mahieux R, Johnson JM, Brady JN, Wolff L *et al*. (2000b). *AIDS Res Hum Retroviruses* **16**: 1629–1632.

Noah DL, Krug RM. (2005). *Adv Virus Res* **65**: 121–145.

Nomura F, Kawai T, Nakanishi K, Akira S. (2000). *Genes Cells* **5**: 191–202.

Nourbakhsh M, Hauser H. (1997). *Immunobiology* **198**: 65–72.

Nourbakhsh M, Hauser H. (1999). *EMBO J* **18**: 6415–6425.

Obenauer JC, Denson J, Mehta PK, Su X, Mukatira S, Finkelstein DB *et al*. (2006). *Science* **311**: 1576–1580.

Ohno S, Ono N, Takeda M, Takeuchi K, Yanagi Y. (2004). *J Gen Virol* **85**: 2991–2999.

Okamoto K, Fujisawa J, Reth M, Yonehara S. (2006). *Genes Cells* **11**: 177–191.

Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M, Seya T. (2003a). *J Biol Chem* **278**: 49751–49762.

Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. (2003b). *Nat Immunol* **4**: 161–167.

Palese P, Tumpey TM, Garcia-Sastre A. (2006). *Immunity* **24**: 121–124.

Palosaari H, Parisien JP, Rodriguez JJ, Ulane CM, Horvath CM. (2003). *J Virol* **77**: 7635–7644.

Parekh BS, Maniatis T. (1999). *Mol Cell* **3**: 125–129.

Pati S, Cavrois M, Guo HG, Foulke Jr JS, Kim J, Feldman RA *et al*. (2001). *J Virol* **75**: 8660–8673.

Paz S, Sun Q, Nakhaei P, Romieu-Mourez R, Goubau D, Julkunen I et al. (2006). Cell Mol Biol 52, in press.

Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ. (1997). Science 275: 523–527.

Perry AK, Chow EK, Goodnough JB, Yeh WC, Cheng G. (2004). J Exp Med 199: 1651–1658.

Peters RT, Maniatis T. (2001). Biochim Biophys Acta 1471: M57–M62.

Peters RT, Liao SM, Maniatis T. (2000). Mol Cell 5: 513–522.

Pflugheber J, Fredericksen B, Sumpter Jr R, Wang C, Ware F, Sodora DL et al. (2002). Proc Natl Acad Sci USA 99: 4650–4655.

Pise-Masison CA, Jeong SJ, Brady JN. (2005). Arch Immunol Ther Exp 53: 283–296.

Pomerantz JL, Baltimore D. (1999). EMBO J 18: 6694–6704.

Pomerantz JL, Denny EM, Baltimore D. (2002). EMBO J 21: 5184–5194.

Poole E, He B, Lamb RA, Randall RE, Goodbourn S. (2002). Virology 303: 33–46.

Powell PP, Dixon LK, Parkhouse RM. (1996). J Virol 70: 8527–8533.

Qin BY, Liu C, Lam SS, Srinath H, Delston R, Correia JJ et al. (2003). Nat Struct Biol 10: 913–921.

Qin BY, Liu C, Srinath H, Lam SS, Correia JJ, Derynck R et al. (2005). Structure 13: 1269–1277.

Qu Z, Qing G, Rabson A, Xiao G. (2004). J Biol Chem 279: 44563–44572.

Quinto I, Mallardo M, Baldassarre F, Scala G, Englund G, Jeang KT. (1999). J Biol Chem 274: 17567–17572.

Rehermann B, Nascimbeni M. (2005). Nat Rev Immunol 5: 215–229.

Reis e Sousa C. (2004). Semin Immunol 16: 27–34.

Revilla Y, Callejo M, Rodriguez JM, Culebras E, Nogal ML, Salas ML et al. (1998). J Biol Chem 273: 5405–5411.

Rickinson AB, Kieff E. (2001). Fields Virology. In: Knipe DM, Howley PM (eds). Lippincott Williams and Wilkins: Philadelphia, pp 2575–2628.

Robek MD, Ratner L. (1999). J Virol 73: 4856–4865.

Rodriguez CI, Nogal ML, Carrascosa AL, Salas ML, Fresno M, Revilla Y. (2002). J Virol 76: 3936–3942.

Roof P, Ricci M, Genin P, Montano MA, Essex M, Wainberg MA et al. (2002). Virology 296: 77–83.

Rosenberg S. (2001). J Mol Biol 313: 451–464.

Rothe M, Xiong J, Shu HB, Williamson K, Goddard A, Goeddel DV. (1996). Proc Natl Acad Sci USA 93: 8241–8246.

Rothwarf DM, Zandi E, Natoli G, Karin M. (1998). Nature 395: 297–300.

Rudolph D, Yeh WC, Wakeham A, Rudolph B, Nallainathan D, Potter J et al. (2000). Genes Dev 14: 854–862.

Sachdev S, Hoffmann A, Hannink M. (1998). Mol Cell Biol 18: 2524–2534.

Sarkar SN, Peters KL, Elco CP, Sakamoto S, Pal S, Sen GC. (2004). Nat Struct Mol Biol 11: 1060–1067.

Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, Nakao K et al. (2000). Immunity 13: 539–548.

Sato S, Sugiyama M, Yamamoto M, Watanabe Y, Kawai T, Takeda K et al. (2003). J Immunol 171: 4304–4310.

Schaefer TM, Fahey JV, Wright JA, Wira CR. (2005). J Immunol 174: 992–1002.

Scheidereit C. (2006). Oncogene 25: 6685–6705.

Schlender J, Bossert B, Buchholz U, Conzelmann KK. (2000). J Virol 74: 8234–8242.

Schulz O, Diebold SS, Chen M, Naslund TI, Nolte MA, Alexopoulou L et al. (2005). Nature 433: 887–892.

Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC et al. (1990). Science 249: 1429–1431.

Schwarz M, Murphy PM. (2001). J Immunol 167: 505–513.

Seet BT, Johnston JB, Brunetti CR, Barrett JW, Everett H, Cameron C et al. (2003). Annu Rev Immunol 21: 377–423.

Senger K, Merikia M, Agalioti T, Yie J, Escalante CR, Chen G et al. (2000). Mol Cell 6: 931–937.

Seth RB, Sun L, Ea CK, Chen ZJ. (2005). Cell 122: 669–682.

Sgarbanti M, Arguello M, tenOever BR, Battistini A, Lin R, Hiscott J. (2004). Oncogene 23: 5770–5780.

Shaffer JA, Bellini WJ, Rota PA. (2003). Virology 315: 389–397.

Sharma S, Grandvaux N, Mamane Y, Genin P, Azimi N, Waldmann T et al. (2002). J Immunol 169: 3120–3130.

Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. (2003). Science 300: 1148–1151.

Shimada T, Kawai T, Takeda K, Matsumoto M, Inoue J, Tatsumi Y et al. (1999). Int Immunol 11: 1357–1362.

Smith MR, Greene WC. (1992). Virology 187: 316–320.

Song J, Fujii M, Wang F, Itoh M, Hotta H. (1999). J Gen Virol 80: 879–886.

Sorokina EM, Merlo Jr JJ, Tsygankov AY. (2004). J Biol Chem 279: 13469–13477.

Spann KM, Tran KC, Chi B, Rabin RL, Collins PL. (2004). J Virol 78: 4363–4369.

Stack J, Haga IR, Schroder M, Bartlett NW, Maloney G, Reading PC et al. (2005). J Exp Med 201: 1007–1018.

Stojdl DF, Lichty BD, tenOever BR, Paterson JM, Power AT, Knowles S et al. (2003). Cancer Cell 4: 263–275.

Sumpter Jr R, Loo YM, Foy E, Li K, Yoneyama M, Fujita T et al. (2005). J Virol 79: 2689–2699.

Sun Q, Sun L, Liu HH, Chen X, Seth RB, Forman J et al. (2006). Immunity 24: 633–642.

Sun Q, Zachariah S, Chaudhary PM. (2003). J Biol Chem 278: 52437–52445.

Sun S-C, Ballard DW. (1999). Oncogene 18: 6948–6958.

Sun S-C, Yamaoka S. (2005). Oncogene 24: 5952–5964.

Sun S-C, Elwood J, Beraud C, Greene WC. (1994). Mol Cell Biol 14: 7377–7384.

Sun X, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM. (2002). J Biol Chem 277: 9505–9511.

Sylla BS, Hung SC, Davidson DM, Hatzivassiliou E, Malinin NL, Wallach D et al. (1998). Proc Natl Acad Sci USA 95: 10106–10111.

Tait SW, Reid EB, Greaves DR, Wileman TE, Powell PP. (2000). J Biol Chem 275: 34656–34664.

Takahasi K, Suzuki NN, Horiuchi M, Mori M, Suhara W, Okabe Y et al. (2003). Nat Struct Biol 10: 922–927.

Takeda K, Akira S. (2005). Int Immunol 17: 1–14.

Tan SL, Katze MG. (2001). Virology 284: 1–12.

Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. (1999). Science 285: 107–110.

tenOever BR, Maniatis T. (2006). Immunity 24: 510–512.

tenOever BR, Sharma S, Zou W, Sun Q, Grandvaux N, Julkunen I et al. (2004). J Virol 78: 10636–10649.

Thanos D, Maniatis T. (1992). Cell 71: 777–789.

Thanos D, Maniatis T. (1995a). Mol Cell Biol 15: 152–164.

Thanos D, Maniatis T. (1995b). Cell 83: 1091–1100.

Thoetkiattikul H, Beck MH, Strand MR. (2005). Proc Natl Acad Sci USA 102: 11426–11431.

Thornburg NJ, Kulwichit W, Edwards RH, Shair KHY, Bendt KM, Raab-Traub N. (2005). Oncogene 25: 288–297.

Tissari J, Siren J, Meri S, Julkunen I, Matikainen S. (2005). J Immunol 174: 4289–4294.

Tobias PS, Soldau K, Gegner JA, Mintz D, Ulevitch RJ. (1995). J Biol Chem 270: 10482–10488.

Tojima Y, Fujimoto A, Delhase M, Chen Y, Hatakeyama S, Nakayama K *et al.* (2000). *Nature* **404**: 778–782.

Tsujimura H, Tamura T, Kong HJ, Nishiyama A, Ishii KJ, Klinman DM *et al.* (2004). *J Immunol* **172**: 6820–6827.

Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE *et al.* (2005). *Science* **310**: 77–80.

Uematsu S, Sato S, Yamamoto M, Hirotani T, Kato H, Takeshita F *et al.* (2005). *J Exp Med* **201**: 915–923.

Ulevitch RJ. (2000). *Immunol Res* **21**: 49–54.

Valarcher JF, Furze J, Wyld S, Cook R, Conzelmann KK, Taylor G. (2003). *J Virol* **77**: 8426–8439.

van Opijnen T, Jeeninga RE, Boerlijst MC, Pollakis GP, Zetterberg V, Salminen M *et al.* (2004). *J Virol* **78**: 3675–3683.

Visintin A, Latz E, Monks BG, Espevik T, Golenbock DT. (2003). *J Biol Chem* **278**: 48313–48320.

Visvanathan KV, Goodbourn S. (1989). *EMBO J* **8**: 1129–1138.

Wang S, Rowe M, Lundgren E. (1996). *Cancer Res* **56**: 4610–4613.

Wano Y, Feinberg M, Hosking JB, Bogerd H, Greene WC. (1988). *Proc Natl Acad Sci USA* **85**: 9733–9737.

Wasley A, Alter MJ. (2000). *Semin Liver Dis* **20**: 1–16.

Wieland SF, Chisari FV. (2005). *J Virol* **79**: 9369–9380.

Wu L, Nakano H, Wu Z. (2006). *J Biol Chem* **281**: 2162–2169.

Xiao G, Sun S-C. (2000). *Oncogene* **19**: 5198–5203.

Xiao G, Harhaj EW, Sun S-C. (2000). *J Biol Chem* **275**: 34060–34067.

Xiao G, Harhaj EW, Sun S-C. (2001). *Mol Cell* **7**: 401–409.

Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. (2005). *Mol Cell* **19**: 727–740.

Yamagata T, Nishida J, Tanaka T, Sakai R, Mitani K, Yoshida M *et al.* (1996). *Mol Cell Biol* **16**: 1283–1294.

Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T *et al.* (2003). *Nat Immunol* **4**: 1144–1150.

Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K *et al.* (2002). *J Immunol* **169**: 6668–6672.

Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T *et al.* (2002b). *Nature* **420**: 324–329.

Yamamoto M, Takeda K, Akira S. (2004). *Mol Immunol* **40**: 861–868.

Yamaoka S, Courtois G, Bessia C, Whiteside ST, Weil R, Agou F *et al.* (1998). *Cell* **93**: 1231–1240.

Yanez RJ, Rodriguez JM, Nogal ML, Yuste L, Enriquez C, Rodriguez JF *et al.* (1995). *Virology* **208**: 249–278.

Yang K, Shi H, Qi R, Sun S, Tang Y, Zhang B *et al.* (2006). *Mol Biol Cell* **17**: 1461–1471.

Yang TY, Chen SC, Leach MW, Manfra D, Homey B, Wiekowski M *et al.* (2000). *J Exp Med* **191**: 445–454.

Yasui T, Luftig M, Soni V, Kieff E. (2004). *Proc Natl Acad Sci USA* **101**: 278–283.

Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK *et al.* (2002). *Nature* **418**: 443–447.

Yie J, Liang S, Merika M, Thanos D. (1997). *Mol Cell Biol* **17**: 3649–3662.

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M *et al.* (2004). *Nat Immunol* **5**: 730–737.

Yoshida M. (1994). *Leukemia* **8**: S51–S53.

Yoshida M. (2001). *Annu Rev Immunol* **19**: 475–496.

Yoshida M. (2005). *Oncogene* **24**: 5931–5937.