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Viral tricks to grid-lock the type I interferon system

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Type I interferons (IFNs) play a crucial role in the innate immune avant-garde against viral infections. Virtually all viruses have developed means to counteract the induction, signaling, or antiviral actions of the IFN circuit. Over 170 different virus-encoded IFN antagonists from 93 distinct viruses have been described up to now, indicating that most viruses interfere with multiple stages of the IFN response. Although every viral IFN antagonist is unique in its own right, four main mechanisms are employed to circumvent innate immune responses: (i) general inhibition of cellular gene expression, (ii) sequestration of molecules in the IFN circuit, (iii) proteolytic cleavage, and (iv) proteasomal degradation of key components of the IFN system. The increasing understanding of how different viral IFN antagonists function has been translated to the generation of viruses with mutant IFN antagonists as potential live vaccine candidates. Moreover, IFN antagonists are attractive targets for inhibition by small-molecule compounds.

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Innate immunity during infection

The innate immune system forms the first line of defense against invading micro-organisms such as viruses. It dampens initial virus replication and ensures survival of the host until specialized adaptive responses are developed. Type I interferons (IFNs) are secreted key cytokines on the innate immune axis that protect uninfected cells and stimulate leukocytes residing at the interface of innate and adaptive immunity, such as macrophages and dendritic cells (DC) [1]. These cells prod the adaptive immune system to mount a full, specialized response against the invading microbe.

The ability to outrun innate immunity before adaptive immune responses are mounted is crucial for the survival of virtually all the mammalian viruses, regardless of their genome type and complexity. Relatively simple viruses such as RNA viruses from the *Picornaviridae* family, as well as DNA viruses with large genomes, such as members from the *Poxviridae* family, have been shown to inhibit the IFN system. This review covers the latest insights into how virus-encoded antagonists sidetrack the IFN machinery and how this knowledge is currently used to generate second generation live vaccines and antiviral compounds.

BOX 1: The IFN circuit

The IFN circuit consists of three distinct steps. The first step consists of recognition of pathogen-associated molecular patterns (PAMP), resulting in the synthesis and secretion of IFN-β (Figure 2). Subsequently, secreted IFN binds to the IFN-α receptor (IFNAR) on the same or surrounding cells, resulting in the transcription of hundreds of IFN-stimulated effector molecules (Figure 3).

Viral nucleic acid or proteins are recognized by Toll-like receptors on the plasma membrane or in endosomes of predominantly antigen presenting cells (APC). Moreover, most cells express cytoplasmic sensors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5) that recognize viral RNA [2]. Cytoplasmic microbial B-form DNA can be recognized by the DNA-sensors DAI and AIM2 [3–5] or cellular RNA polymerase III, which converts it into 5′-triphosphate containing RNAs that are recognized by RIG-I [6,7].

Upon activation, RIG-I and MDA-5 engage mitochondrial antiviral signaling adapter (MAVS) [8]. In turn, MAVS activates two kinase complexes that ultimately phosphorylate and activate the two key transcription factors for IFN-β induction: nuclear factor κB (NFκB) and IFN-regulatory factor 3 (IRF-3) [2]. The first kinase complex consists of TNF receptor associated factor 3 (TRAF-3), TRAF family member associated NF-κB activator (TANK), TANK-binding kinase 1 (TBK-1), and inhibitor of κB kinase (IκB) ε (IKKε) [8]. The second complex phosphorylates IκB and thereby activates NF-κB. It consists of TRAF-6, receptor interacting protein 1 (RIP-1), NF-κB essential modulator (NEMO), TGF-β activated kinase 1 (TAK-1), IKKα, and IKKβ [8]. Upon activation, NF-κB and IRF-3 translocate to the nucleus and drive IFN-β transcription (Figure 2).

Upon binding of extracellular IFN-β, the IFNAR recruits janus kinase 1 (JAK-1) and tyrosine kinase 2 (TYK-2) to

its cytoplasmic domain. These kinases phosphorylate the key transcription factors signal transducers and activators of transcription (STAT) 1 and 2, which together with IRF-9 form the IFN-stimulated gene factor 3 (ISGF3) complex [8]. This active transcription complex translocates to the nucleus, where it drives the expression of ISGs [9].

To date, over 500 publications have reported the identification and/or characterization of more than 170 different virus-encoded IFN antagonists by 93 distinct viruses (suppl. Table S1). Over the past four years, the number of reports describing new viral IFN antagonists has almost doubled, underpinning the speed with which the field is developing. Table S1 was compiled from all current literature and includes viral proteins that act as IFN antagonists and whether experimental proof comes from expression studies or infections with mutant viruses lacking the putative IFN antagonists. This latter issue is of great importance since single gene over expression may result in aberrant expression, localization and interactions of viral proteins compared to virus infected cells. Hence, proteins identified by overexpression may not retain the same phenotype in the context of infection. Figures 2 and 3 summarize the steps of the IFN system that different viruses interfere with. Although it lays beyond the scope of this review to discuss all antagonists in detail, the ones discussed exemplify the different strategies used by viruses to subvert the IFN response.

Main molecular mechanisms of viral antagonism

The majority of IFN antagonists exert their action by one of four different strategies: (i) general inhibition of cellular gene expression, (ii) sequestration of molecules in the IFN circuit, (iii) proteolytic cleavage of innate immune components, or (iv) targeting these components for proteasomal degradation (Table S1).

General block of gene expression is a feature seen in various RNA viruses (FluAV, VSV, PV, RVFV, and BunV) and seems to provide an efficient means to prevent synthesis of not only IFN, but also additional antiviral molecules detrimental to virus replication [10]. Whereas host shut-off in some large dsDNA viruses (such as herpes simplex virus) is partly related to its necessity to temporally regulate expression of its own genes [11], RNA viruses seem to mainly block viral gene expression to circumvent the expression of antiviral proteins [10].

Viruses interfere with all three steps of the IFN system

Evidently, inhibition of early steps in the cascade implicitly inhibits the induction of down-stream molecules and amplification of the IFN signal. Most viral IFN antagonists are exclusively produced in the infected cells, implying that surrounding uninfected cells can still be stimulated to establish an antiviral state by systemic IFN. For that reason some large dsDNA viruses express soluble IFN-binding proteins that compete with the cellular IFN receptor for its ligand (Table S1). These 'viroceptors' neutralize secreted IFN, thereby preventing autocrine amplification. Possibly, these strategies enable some of these viruses to persist in their host for longer times or disseminate to hard-to-infect cells.

The fact that nearly 50% of the viruses for which IFN antagonists have been identified interfere with multiple steps of the IFN response (Figure 1), exemplifies the necessity for viruses to successfully circumvent the IFN response. Moreover, approximately one third of the viruses encode an antagonist that interferes with more than one step of the IFN response system (Figures 2 and 3), underpinning the pleiotropic nature of viral proteins.

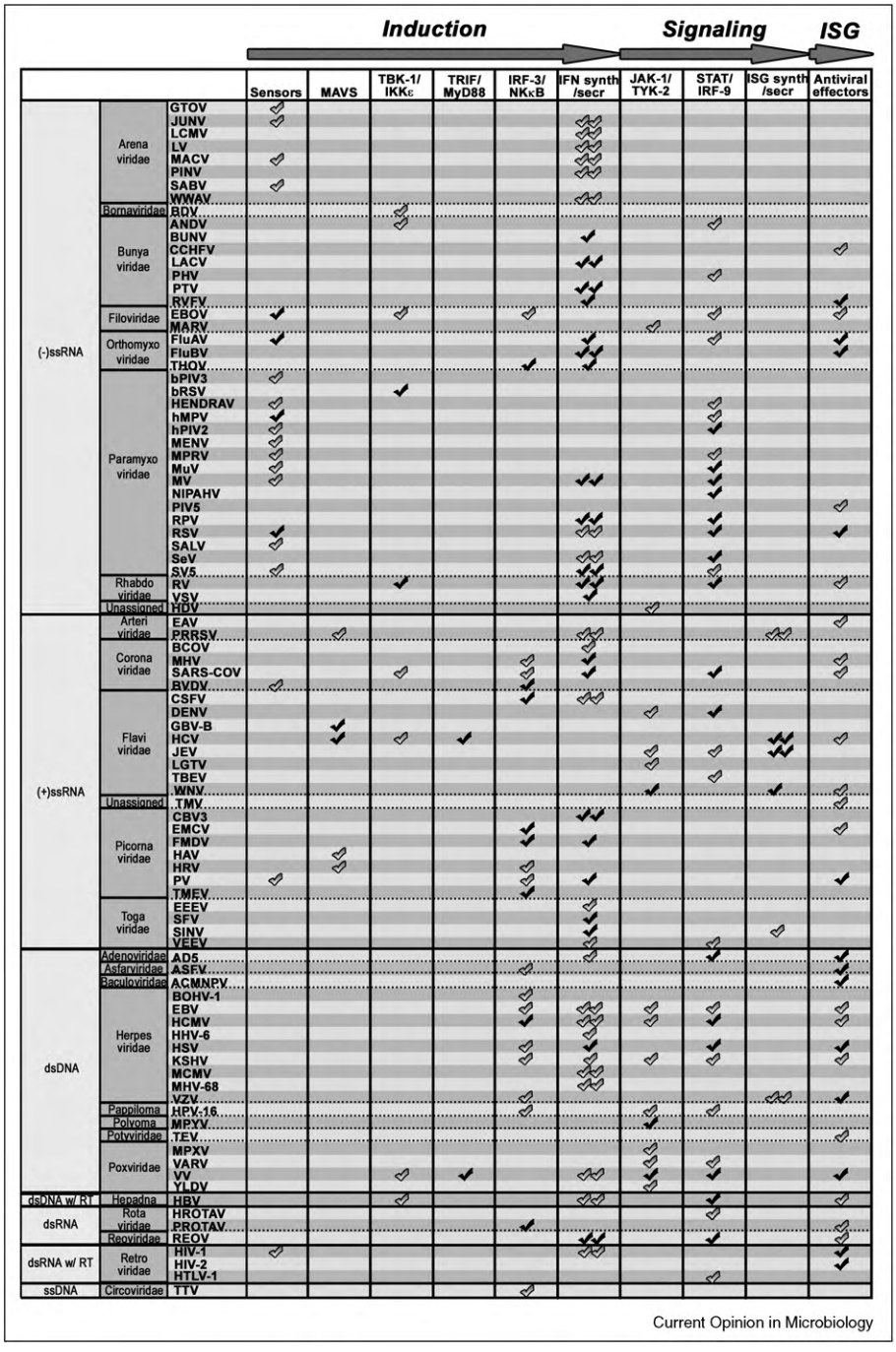
Conservation of IFN antagonists

Several viral IFN antagonists are conserved within distinct RNA virus families, whereas dsDNA viruses do not use common proteins to antagonize the IFN response (Table S1). This may partly be explained by bias in data available for different virus families. On the contrary, it may be explained by stricter genome restrictions for RNA viruses. Evolutionary pressure to encode maximum functionality by relatively limited genome capacity drives a high degree of multi-functionality in RNA virus proteins. Genome size restrictions make it such that they cannot derive new large genes encoding IFN antagonists from their hosts as is the case for large dsDNA viruses (e.g. soluble IFN receptor decoys). This considerably restricts the variability of accessory proteins found in RNA viruses and may explain why some related RNA viruses use the same viral protein to antagonize the IFN system, whereas this conservation is limited in large dsDNA viruses (Table S1). Nine common RNA virus antagonists stand out (Table S1).

The NP proteins of *Arenaviridae* are conserved inhibitors of IFN induction, although their precise molecular mechanism of antagonism remains unclear [12–14]. Moreover, New World Arenaviruses express Z proteins that bind RIG-I and interfere with its association with MAVS [15]. Phleboviruses and orthobunyaviruses share an NSs protein to antagonize IFN transcription [16,17•,18,19], and influenza A and B viruses express an NS1 protein to inhibit the IFN-inducible antiviral effector protein PKR, as well as the viral sensor RIG-I [20,21,22•,23,24]. By far the largest virus family with conserved IFN antagonists is that of the *Paramyxoviridae*. Differences in mechanism exist between different family members, yet their V, P, and C proteins predominantly prevent MDA-5 dimerization and target STAT molecules for proteasomal degradation [25–28].

Several (+)RNA viruses share common antagonists within their virus family as well. Coronaviruses express the

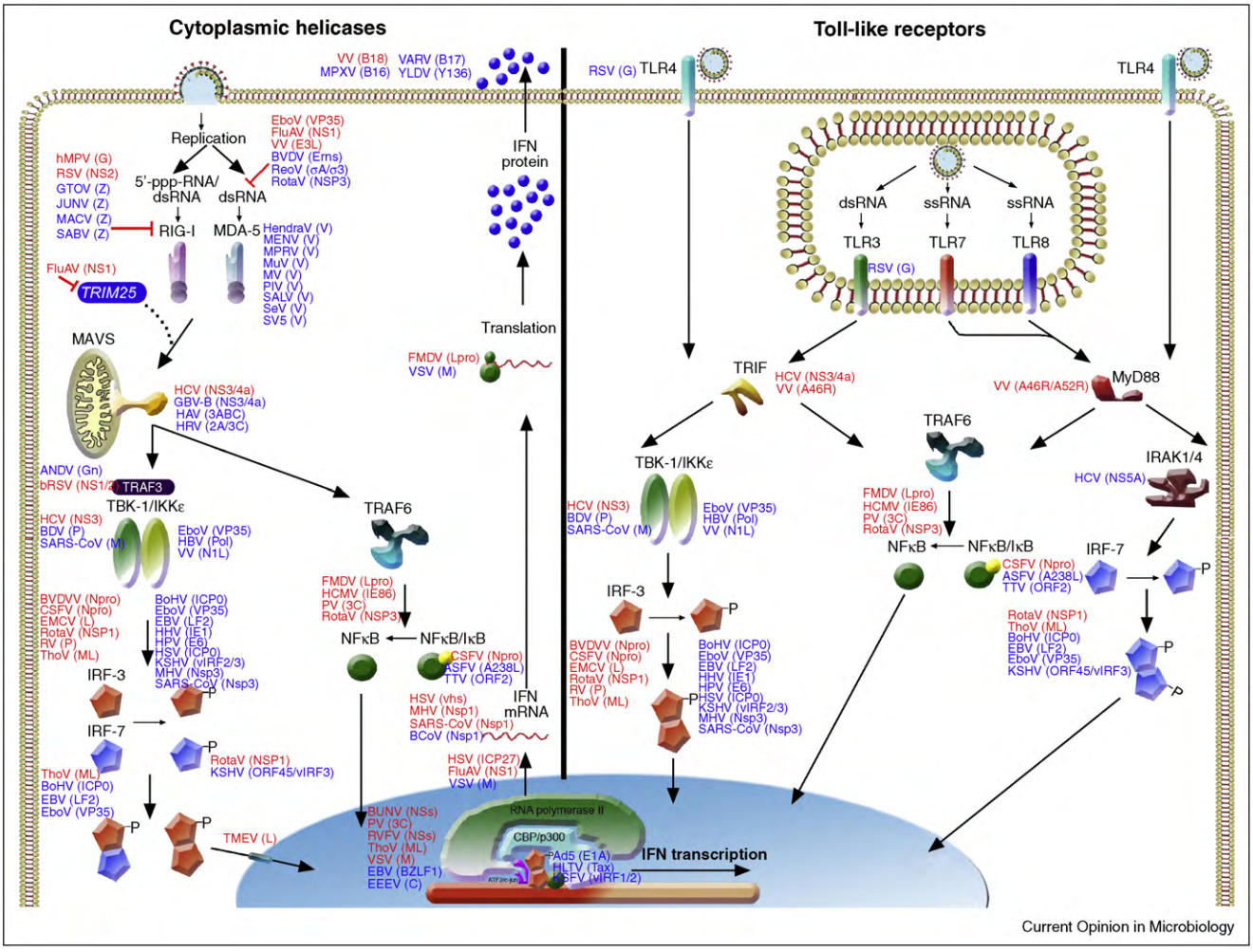
Figure 1



Viral antagonism with the IFN circuit. A check symbol is displayed at various steps of the IFN cascade (*X*-axis) that are impaired by a particular virus (*Y*-axis). The plot is organized by viral genome type. Double symbols indicate inhibition of IFN induction or signaling at yet unknown step of the pathway. Black symbols indicate proof for viral antagonism of the indicated step in the pathway by recombinant viruses lacking the IFN antagonist. Grey symbols indicate proof by over expression and/or wild-type virus infection.

largest known viral RNA genome (>30 000 nt). Their Nsp1 protein is the first protein expressed in infected cells and is an important virulence factor *in vivo* [29,30••]. It degrades cellular mRNA, thereby limiting the expression of antiviral genes [30••,31]. Interestingly, SARS-CoV recently transmitted from bats to humans and expresses several accessory proteins that are not expressed by related coronaviruses such as MHV. Two of these accessory gene products (ORF3b and ORF6) have reported IFN antagonistic activity [32,33]. An ORF6 deletion virus

Figure 2



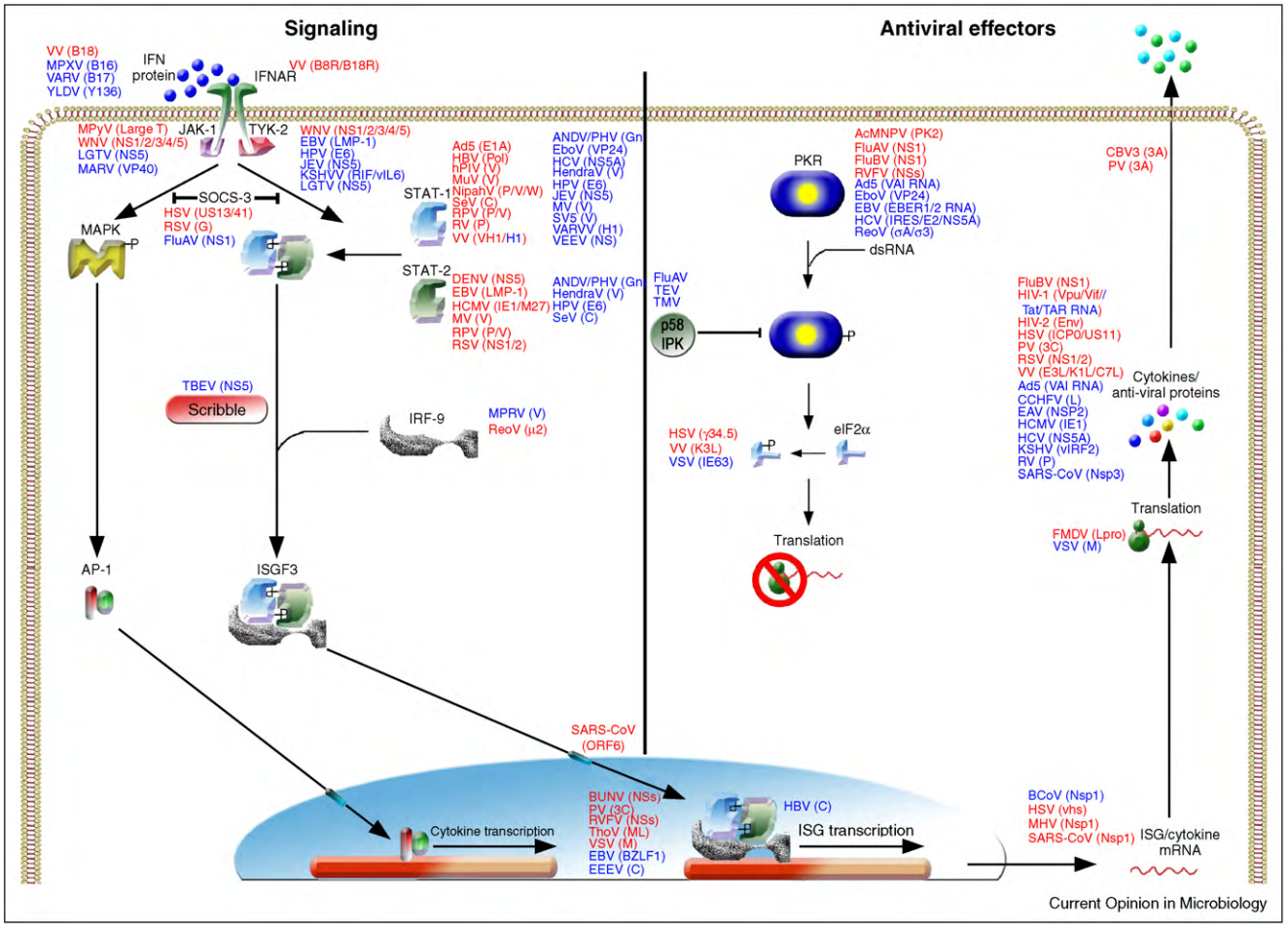
Schematic representation of type I IFN induction through RLRs and TLRs. Viruses and their antagonistic proteins are indicated at the steps of the IFN pathway they affect. Antagonistic proteins are shown adjacent to their targets in alphabetical order. Antagonists in red indicate proof for IFN antagonist by recombinant viruses lacking the IFN antagonist. Antagonists in blue indicate proof by over expression and/or wild-type virus infection.

convincingly demonstrated that the ORF6 protein is involved in preventing nuclear translocation of STAT1 by binding to the nuclear transport protein karyopherin α2 and contributes to pathogenicity. In addition to *Coronaviridae*, three genera within the *Flaviviridae* family also share common antagonists within their genus. Pestiviruses BVDV and CSFV Npro proteins target IRF-3 for proteasomal degradation [34,35]. Moreover, their Erns protein was reported to sequester dsRNA [36–38]. By contrast, all flaviviruses modulate JAK–STAT signaling, albeit by slightly different mechanisms [39–43]. The hepaciviruses HCV and GBV-B both cleave MAVS with their main protease NS3/4a, thereby rendering it unable to confer the signal from RIG-I to TBK-1/IKKε [44,45]. Using a similar strategy, enteroviruses commonly cleave MAVS and other innate immune molecules with their main protease 3C [46–48]. By contrast, non-enteroviruses within the *Picornaviridae* family express an L protein to target IRF-3 and NFκB [49–51].

Virus-host coevolution

Despite all the sophisticated mechanisms used by viruses to inhibit the IFN response, a hallmark of virus infection is the induction of IFN in infected hosts. Thus, viral IFN antagonism is not complete, which probably underscores the need for viruses to economize resources to assure optimal replication and transmission. Moreover, hosts have also been under selection pressure to overcome viral antagonism of IFN, and this is exemplified by examples of positive selection in some of the key components of the IFN system targeted by viruses, such as PKR, which appears to have been rapidly evolving in mammalian hosts to escape from antagonism by K3L, a poxvirus PKR inhibitor [52••].

Figure 3



Schematic representation of type I IFN signaling. Viruses and their antagonistic proteins are indicated at the steps of the IFN pathway they affect. Antagonistic proteins are shown adjacent to their targets in alphabetical order. Antagonists in red indicate proof for IFN antagonist by recombinant viruses lacking the IFN antagonist. Antagonists in blue indicate proof by over expression and/or wild-type virus infection.

Differential prevalence of antagonistic strategies among different virus classes

Some inhibitory strategies seem to have a higher prevalence in certain virus classes. For example, nearly all viruses with (−)ssRNA genomes interfere with sensory molecules, whereas this is much less so for viruses with other genomes (Figure 1). By contrast, (+)ssRNA and dsDNA viruses preferably target IRFs as means to interfere with IFN induction. Finally, (+)ssRNA viruses target the IFN signaling step to a lesser extent than other viruses (Figure 1). Notable exceptions are members of the *Flaviviridae*. Although these patterns may be explained by selection bias for studied viruses, they may also indicate that different virus families may utilize certain dominant strategies to antagonize the IFN response. These strategies not necessarily provide the broadest possible antagonism (e.g. block of general gene expression), but may be strongly influenced by the type of infection (e.g. acute, chronic, and persistent), vector/host species, and infected cell types.

Upregulation of cellular factors that downregulate the IFN response

Recently, several viruses have been reported to partly circumvent the IFN circuit by activation or upregulation of cellular inhibitors of the JAK–STAT pathway and PKR. The cellular PKR inhibitor p58(IPK) is activated during FluAV, TMV, and TEV infection [53,54] and contributes to negative regulation of PKR by direct protein–protein interaction [55,56]. By contrast, FluAV, RSV, HCV, and HSV induce SOCS-1/3 expression, which negatively regulates the JAK–STAT pathway [57–61]. It remains unclear if inhibition by cellular components is a general cellular stress-induced negative feedback loop that is activated during virus infection to resolve or fine-tune the IFN response, or if these

viruses specifically modulate cells to use their own proteins against them.

IFN antagonists are promising vaccines and antiviral targets

Better understanding of molecular mechanisms by which IFN antagonists influence viral replication and pathogenesis has indicated that IFN antagonist knockout viruses are promising candidates for live virus vaccines. Several groups have shown that viruses lacking fully functional IFN antagonists are rapidly cleared *in vivo* as the result of a potent IFN response, while their replication competent nature ensures the establishment of long-lasting immune memory. Moreover, these viruses can be grown in large quantities in IFN-deficient hosts for potential vaccine stocks. So far, Influenza A/B virus NS1 deletion mutants have been tested in mice and showed that they could protect against challenge with wild-type virus [62–64,65•]. Also, recent evidence from studies with SARS-CoV nsp-1, JEV E, and RSV NS2 mutant viruses suggests that these viruses likewise represent good vaccine candidates [29,65•,66•]. The generation of attenuated viruses is aimed at achieving an optimal balance between minimal clinical symptoms and maximum replication in the nasal mucosa. Mucosal immunity is especially effective against respiratory viruses such as influenza viruses, RSV, and coronaviruses [67].

Vaccination is the most cost-effective method for preventing virus infection and disease. However, under certain circumstances, such as in immune compromised patients, antiviral drugs are crucial to treat acute life-threatening cases. Viruses lacking IFN antagonists are severely attenuated. This makes viral IFN antagonist proteins prime drug targets. Screens with large libraries of small-molecule inhibitors have already identified potential new lead compounds against various clinically relevant viruses. Several studies in tissue culture indicate that this is a viable strategy for the development of antiviral drugs [68–72], although the potential of such compounds to inhibit virus replication *in vivo* remains unclear.

Concluding remarks

The recent surge in publications on viral IFN antagonists underpins the necessity of every virus to interfere with the innate immune system and provides potential targets for antiviral drugs and rational vaccine design. However, only a limited number of these described antagonists have been extensively validated in animal models by infection studies with recombinant viruses lacking their IFN antagonists. Nevertheless, recent studies with recombinant Ebola virus and SARS-CoV mutants lacking IFN antagonists, initially identified by over expression studies, prove the potential of these approaches [30••,31,73–76]. In depth characterization of IFN antagonists *in vivo* will be crucial to the development of new vaccine candidates and identification of lead compounds that diminish IFN antagonist function during viral infection.

Since various IFN antagonists target multiple proteins, drugs against these viral factors may prove to work as a double edged sword. The benefit of such a drug would not only be that it inhibits two distinct modes of antagonism, but also that resistant virus mutants are less likely to arise since they would have to facilitate both functions. In particular viral antagonists that also have a direct role in virus replication would be good drug targets. Obtaining crystal structures of viral antagonists in complex with their different viral and cellular ligands will be crucial for the rational design of such drugs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mib.2010.05.009.

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