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# Multifaceted Antiviral Actions of Interferon-stimulated Gene Products

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## Abstract

Interferons (IFNs) are extremely powerful cytokines for the host defence against viral infections. Binding of IFNs to their receptors activates the JAK/STAT signalling pathway with the Janus kinases JAK1, 2 and TYK2 and the signal transducer and activators of transcription (STAT) 1 and STAT2. Depending on the cellular setting, additional STATs (STAT3-6) and additional signalling pathways are activated. The actions of IFNs on infected cells and the surrounding tissue are mediated by the induction of several hundred IFN-stimulated genes (ISGs). Since the cloning of the first ISGs, considerable progress has been made in describing antiviral effector proteins and their many modes of action. Effector proteins individually target distinct steps in the viral life cycle, including blocking virus entry, inhibition of viral transcription and translation, modification of viral nucleic acids and proteins and, interference with virus assembly and budding. Novel pathways of viral inhibition are constantly being elucidated and, additionally, unanticipated functions of known antiviral effector proteins are discovered. Herein, we outline IFN-induced antiviral pathways and review recent developments in this fascinating area of research.

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## Abbreviations<sup>1</sup>

ADAR	Adenosine deaminase acting on RNA
APOBEC	Apolipoprotein B mRNA-editing catalytic polypeptide
BST-2	Bone marrow stromal antigen 2
ds	Double-stranded
eIF	Eukaryotic translation initiation factor
ER	Endoplasmatic reticulum
GAS	IFN $\gamma$ activated sequence
GBP	Guanylate binding protein
IFN	Interferon
IFNAR	IFN $\alpha/\beta$ receptor
IL	Interleukin
ISG	IFN-stimulated gene
IFIT	IFN-induced protein with tetratricopeptide repeats
IFITM	IFN-induced transmembrane protein
ISGF3	IFN-stimulated gene factor 3
IRF	IFN regulatory factor
ISRE	IFN-stimulated response element
JAK	Janus kinase
PML	Promyelocytic leukaemia
2-5A <sub>n</sub>	2'-5' oligoadenylate
OAS	2-5A <sub>n</sub> synthetase
MDA5	Melanoma differentiation-associated protein 5
PAMP	Pathogen-associated molecular pattern
PKR	dsRNA-dependent protein kinase
PRR	Pattern-recognition receptor
RIG-I	Retinoic acid-inducible protein I
RNase L	Latent ribonuclease
ss	Single-stranded
STAT	Signal transducer and activator of transcription
TRIM	Tripartite motif
TYK2	Tyrosine kinase 2

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## Introduction

Antiviral activity defines the bioactivity of interferons (IFNs). IFNs induce hundreds of IFN-stimulated genes (ISGs), whereby many are regulated by all IFNs, and others are induced more selectively (de Veer et al. 2001; Der et al. 1998). Even for several

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<sup>1</sup> Viruses mentioned in this article, see Table 2

long-known ISG-encoded proteins with direct or indirect antiviral activity, the mechanisms of action are often still poorly understood. Studies on antiviral activities of specific proteins became more complicated than anticipated, largely because of their high virus-specificity and their involvement in multiple pathways, including normal cellular physiology. Moreover, viral counteracting mechanisms can be very efficient and mechanistic discoveries often depended on mutant viruses. As another level of complexity, some of the antiviral effector proteins also have proviral effects.

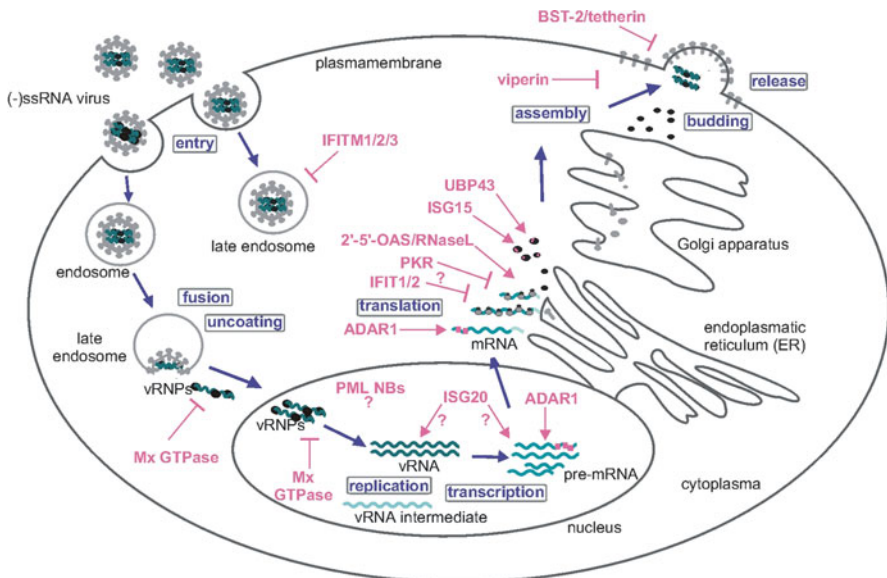
Recognition of virus infection is a central requirement for the initiation of an efficient host defence. Molecules and signalling cascades involved have been mainly characterized during the last decade and new players are still being identified. A number of germline-encoded pattern-recognition receptors (PRRs) sense the presence of so-called pathogen-associated molecular patterns (PAMPs). PRRs involved in host defence against viruses are localized in several cellular compartments and recognize distinct molecular structures, most prominently viral nucleic acids. Activation of distinct PAMP-induced signalling cascades converge in most cases at the induction of pro-inflammatory cytokines and type I IFNs (Kawai and Akira 2009; McCartney and Colonna 2009).

IFNs are grouped into three classes, called type I, type II and the more recently identified type III IFNs. Type I IFNs are the key cytokines for innate antiviral immunity, as they are rapidly induced upon virus recognition and act on presumably all cell types. Type I IFNs have been discovered more than 50 years ago and comprise a large group of cytokines. Among these, several distinct IFN $\alpha$  subtypes (13 in humans) and IFN $\beta$  are induced directly in response to viral infections. Type II IFN consists of only one member, IFN $\gamma$ , that is mainly produced by activated T cells and natural killer (NK) cells (Pestka et al. 2004). Type III IFNs are structurally more related to the interleukin- (IL-) 10 cytokine family, but have been classified as IFNs based on their similar biological activity as IFN $\alpha/\beta$ . In humans, this family has three members, IFN $\lambda$ 1–IFN $\lambda$ 3, originally designated as interleukin IL-29, IL-28A and IL-28B, respectively. IFN $\lambda$ s are also directly induced by viral infections by similar, but probably not identical, mechanisms. In contrast to IFN $\alpha/\beta$ , IFN $\lambda$ s act only on specific cell types due to cell type-restricted expression of their specific receptor chain. The latter appears to be mainly expressed on epithelial cells and thus IFN $\lambda$ s have a more restricted/specific role in the antiviral defence (Commins et al. 2008).

Signalling of type I IFNs is initiated by ligand binding to a common heterodimeric receptor complex consisting of the IFNAR1 and IFNAR2 chains. Ligand binding results in the activation of signal transducers and activators of transcription (STATs) by the receptor-associated Janus kinases (JAKs) TYK2 and JAK1. Mainly STAT1–STAT2 heterodimers, and to a lesser extent STAT1 homodimers, are activated in response to IFN $\alpha/\beta$ . Dependent on the cell type, other STAT family members can be activated, but their impact on cellular responses is often unclear. STAT1–STAT2 heterodimers, in association with IFN regulatory factor (IRF) 9, form the IFN-stimulated gene factor 3 (ISGF3) and induce transcription of genes containing IFN-stimulated response elements (ISREs) in their regulatory regions. Type III IFNs utilize the IL-10R2 and the IFN $\lambda$ R1 (IL-28R $\alpha$ ) receptor chains and activate TYK2, JAK1 and mainly ISGF3. Accordingly, responses appear very similar to those induced by type I IFNs, although

some differences have been reported. IFN $\gamma$  binds to IFNGR1 and IFNGR2 and activates JAK1, JAK2 and mainly STAT1 homodimers. STAT1 homodimers bind to IFN $\gamma$  activated sequences (GAS) in promoter regions of responsive genes. IFN $\gamma$  can also activate other STAT complexes but again, the contribution of these complexes to the overall response requires further characterization. In general, IFN $\alpha/\beta$  is mainly associated with antiviral and IFN $\gamma$  with antibacterial activity, but IFN $\gamma$  certainly also exhibits potent antiviral activity and IFN $\alpha/\beta$  impacts on the antimicrobial defence. It is important to note that in addition to the induction of an antiviral state in responsive cells, IFNs also exert important immunomodulatory activities, mainly by shaping adaptive immune responses (Borden et al. 2007; Schindler et al. 2007).

Several specific antiviral pathways have been reviewed recently (see below), so the emphasis herein will be on providing an overview about well-known IFN-induced antiviral pathways with a strong focus on recent developments. We present the specific antiviral effector proteins ordered according to their main effects on virus replication (Fig. 1). It has to be mentioned, however, that there are often multiple modes of action



**Fig. 1** Schematic overview over antiviral effector mechanisms of IFN-stimulated gene products. Viruses use substantially different replication strategies and we therefore use the life cycle of an enveloped (–)ssRNA virus as an example (depicted in blue letters). The virus binds to its cellular receptor at the plasma membrane, enters the cell *via* pH-dependent endocytosis and fusion, subsequently uncoated viral nucleoproteins are transported to the nucleus, where virus replication and transcription occurs. Viral proteins are translated by the cellular machinery, core proteins assemble with the viral genome, envelope proteins are transported via the ER/Golgi apparatus to the plasma membrane where virus assembly and budding occurs. ISG-encoded proteins targeting different steps during viral replication are depicted in pink letters. ISG products can either inhibit certain steps ( $\perp$ ), modify/degrade viral products ( $\downarrow$ ), or act *via* unknown/putative mechanisms (?). Only the major or best characterized pathways are shown, see text for details and additional functions of IFN-induced proteins and pathways

which are discussed in the respective subsections. Besides, other IFN-induced proteins not mentioned in this article might well have important functions in the cellular resistance against virus infections.

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## Inhibition of Virus Entry or Uncoating

### Interferon-Induced Transmembrane (IFITM) Proteins

The IFITM proteins are relatively small (14–17 kDa) transmembrane proteins with cell type- and tissue-dependent subcellular localization. IFITM proteins have been found in proximity to the endoplasmic reticulum (ER), the Golgi apparatus, small vesicles, exosomes, or associated with the plasma membrane (Siegrist et al. 2011). They have been originally identified based on their IFN inducibility, although they are also expressed at low levels in untreated cells (Friedman et al. 1984). The antiviral potency of human IFITM1 (9–27, Ifi17, fragilis protein 2) has been first demonstrated by overexpression of *IFITM1*, which led to a marked decrease of vesicular stomatitis virus (VSV) but not influenza A virus (FLUAV) replication (Alber and Staeheli 1996). First mechanistic insights came from a quite recent study (Brass et al. 2009). Using viral pseudoparticles containing the murine leukaemia virus (MLV) genome and unique envelope proteins from different viruses, IFITM actions could be linked to viral surface proteins and to early steps in viral replication. Envelope proteins from several FLUAV strains, three different flaviviruses (i.e. West Nile virus (WNV), yellow fever virus (YFV), Omsk hemorrhagic fever virus (OMSK)), and VSV could confer IFITM1-, IFITM2- and IFITM3-sensitivity to pseudovirus particles, whereas no effect was observed with envelope proteins from three different arenaviruses (i.e. Machupo virus (MACV), Lassa virus (LASV), lymphocytic choriomeningitis virus (LCMV)), or MLV. Consistent with the pseudovirus particles, overexpression of *IFITM1*, *IFITM2* (fragilis protein 3, 1-8D) or *IFITM3* (1-8U, Ifi15, fragilis protein) in human cell lines results in strongly reduced replication of FLUAV. Moreover, siRNA-mediated knockdown of *IFITM3* enhances FLUAV replication and decreases IFN $\alpha$  or IFN $\gamma$ -mediated anti-FLUAV activity. The effect of *IFITM3* on WNV and Dengue virus (DV) replication was confirmed with overexpression and siRNA-mediated knockdown experiments. No effect was observed on hepatitis C virus (HCV) replication, supporting the notion that IFITM actions are virus-specific (Brass et al. 2009). Further evidence for the inhibition of flavivirus and of rhabdovirus entry and/or uncoating by IFITM1-3 has been provided using stably transfected human cell lines (Jiang et al. 2010; Weidner et al. 2010). Very recently, the IFITM-mediated inhibition *via* viral entry proteins was extended to filoviruses (i.e. Marburg virus (MARV) and Ebola virus (EBOV)), severe acute respiratory syndrome coronavirus (SARS-CoV) (Huang et al. 2011), and human immunodeficiency virus (HIV)-1 (Lu et al. 2011). Consistent with studies in human cells, increased FLUAV replication was observed in embryonic fibroblasts derived from mice lacking the entire *Ifitm* locus (Table 1) (Brass et al. 2009).

**Table 1** Gene-targeted mice of ISGs that encode proteins implicated in the IFN-induced antiviral defense: basic phenotype and virus susceptibility (in alphabetical order)

Gene-targeted mice <sup>a, b</sup>	Phenotype	Virus sensitivity in vivo <sup>c, d</sup>	References
<i>Adar1</i> ( <i>p110/p150</i> ) <sup>-/-</sup>	Embryonic lethal	–	Hartner et al. (2004, 2009), Wang et al. (2004), XuFeng et al. (2009)
<i>Adar1</i> ( <i>p150</i> ) <sup>-/-</sup>	Embryonic lethal	–	Ward et al. (2011)
<i>Apobec3</i> <sup>-/-</sup>	Viable, fertile	Increased levels of integrated MMTV DNA in LN DCs after MMTV infection ( <i>s.c.</i> ) (+/- LPS pre-treatment); increased MMTV DNA levels in LNs after MMTV infection ( <i>i.f.</i> )	Low et al. (2009), Okeoma et al. (2007, 2009a, b)
<i>Apobec3</i> <sup>-/-</sup> , <i>HIV-Tg</i>	Viable, fertile	Increased infection levels in bone marrow, spleen and thymus and earlier onset of leukaemias after neonatal infection ( <i>i.p.</i> ) with M-MuLV, no gross difference in M-MuLV-induced tumour types	
<i>Iftim1</i> <sup>-/-</sup>	Viable, fertile	Normal levels of core-associated HBV DNA in serum; normal dsRNA-induced inhibition of HBV DNA production	Turelli et al. (2008)
<i>Iftim1-6</i> <sup>-/-</sup> ( <i>IftimDel</i> )	Viable, fertile	Decreased survival after challenge with 2'-O-methyltransferase mutant WNV (WNV-E218A) ( <i>i.c.</i> )	Daffis et al. (2010), Pichlmair et al. (2011)
<i>Iftim3</i> <sup>-/-</sup>	Viable, fertile	Decreased survival after infection ( <i>i.n.</i> ) with VSV	
<i>Igp1</i> <sup>-/-</sup>	Viable, fertile	Normal survival after infection with EMCV ( <i>i.p.</i> )	
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	n.r.	Lange et al. (2008)
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	n.r.	Lange et al. (2008)
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Normal virus load in liver after MCMV infection ( <i>i.p.</i> )	Taylor et al. (2000)
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Increased lethality and increased virus load after FLUA/B virus ( <i>i.n.</i> ), HSV-1 ( <i>i.cor.</i> ) and $\gamma$ MHV68 ( <i>i.p.</i> ) infection	Kim et al. (2008a), Knobloch et al. (2005), Lai et al. (2009), Lenschow et al. (2007), Osiaik et al. (2005)
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Increased susceptibility to SINV infection ( <i>i.c.</i> )	
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Normal resistance to VSV infection ( <i>i.v.</i> )	
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Normal clearance of LCMV ( <i>i.v.</i> ) from spleens; no difference in survival after infection with LCMV ( <i>i.c.</i> )	
<i>Isg15</i> <sup>-/-</sup>	Viable, fertile	Normal clearance of injected ( <i>rv.</i> ) replication competent HBV DNA	

<i>PKR</i> <sup>-/-</sup>	Viable, fertile	Higher mortality after CV-B4 infection ( <i>i.p.</i> ) Decreased survival after VSV infection ( <i>i.n.</i> ) (+/- IFN $\alpha$ / $\beta$ pre-treatment) and higher viral titers in brain and lung Decreased survival after FLUAV strain A/WSN/33 infection ( <i>i.n.</i> ) and higher viral titer in lungs; decreased survival after delNS1 FLUAV infection ( <i>i.n.</i> ) No difference after SFV infection ( <i>i.p.</i> ) Higher viral titers after LCMV infection ( <i>i.p.</i> ) in lung, liver and spleen Higher mortality after HSV-2 infection ( <i>i.vag.</i> )	Barry et al. (2009), Nakayama et al. (2010), Sadler and Williams (2008)
<i>PKR</i> <sup>-/-</sup> , <i>RNase L</i> <sup>-/-</sup> <i>DKO</i>	Viable, fertile	Higher mortality after CV-B4 infection ( <i>i.p.</i> ) Higher mortality after WNV infection ( <i>i.f.</i> ) with higher viral load in the brain; no difference after WNV infection ( <i>i.c.</i> ) Higher mortality after EMCV infection ( <i>i.p.</i> ); higher mortality after HSV-2 infection ( <i>i.vag.</i> )	Nakayama et al. (2010), Silverman (2007)
<i>Pml</i> <sup>-/-</sup>	Viable, fertile; impaired terminal myeloid cell maturation	Increased footpad swelling after LCMV infection ( <i>i.f.</i> ); increased virus load in spleen after LCMV infection ( <i>i.v.</i> ); increased lethality after LCMV infection ( <i>i.cer.</i> )	Bonilla et al. (2002), Wang et al. (1998)
<i>RNase L</i> <sup>-/-</sup>	Viable, fertile	Increased neutralizing Ab production after VSV infection ( <i>i.v.</i> ) Increased mortality after EMCV infection ( <i>i.p.</i> ) (+/- IFN $\alpha$ pre-treatment) Higher mortality after CV-B4 infection ( <i>i.p.</i> ) Increased mortality after HSV-1 infection ( <i>i.cor.</i> ) Increased mortality after WNV infection ( <i>i.f.</i> ) with similar viral load in the brain; no difference after WNV infection ( <i>i.c.</i> ) Increased survival after HSV-2 infection ( <i>i.vag.</i> ); HSV-2 replication is similar in the vaginal epithelium, but lower in the nervous system	Silverman (2007)

(continued)

**Table 1** (continued)

Gene-targeted mice <sup>a, b</sup>	Phenotype	Virus sensitivity in vivo <sup>c, d</sup>	References
<i>Ube1l</i> <sup>-/-</sup>	Viable, fertile	Decreased survival and increased viral load in lungs after FLUBV virus infection ( <i>i.n.</i> )	Giannakopoulos et al. (2009), Kim et al. (2006), Lai et al. (2009)
<i>Ubp43</i> <sup>-/-</sup>	Brain injury, neurological disorders, reduced lifespan	Decreased survival after SINV infection ( <i>s.c.</i> , <i>i.cer.</i> ) No difference in survival after LCMV infection ( <i>i.cer.</i> ) Increased survival and reduced viral load in brain, liver and serum after infection with LCMV ( <i>i.cer.</i> ) No difference in survival after infection with LCMV ( <i>i.c.</i> ) Increased survival after infection with VSV ( <i>i.cer.</i> ) Increased clearance of injected ( <i>iv.</i> ) replication competent HBV DNA	Kim et al. (2008a), Knobeloch et al. (2005), Ritchie et al. (2002, 2004)
<i>Ubp43</i> <sup>-/-</sup> , <i>Isg15</i> <sup>-/-</sup> <i>DKO</i>	Brain injury, neurological disorders, reduced lifespan	No difference in survival after infection with LCMV ( <i>i.c.</i> )	Knobeloch et al. (2005)

<sup>a</sup>Note that most laboratory mouse strains are Mx1-negative, fail to upregulate *Gbp1* in response to IFNs and express a truncated *Oas1b* (see text for details)

<sup>b</sup>*DKO* double knockout

<sup>c</sup>*n.r.* not reported

<sup>d</sup>*i.c.* intracranial, *i.cer.* intracerebral, *i.cor.* intracorneal, *i.f.* intra-footpad, *i.n.* intranasal, *i.p.* intraperitoneal, *i.v.* intravenous, *i.vag.* intravaginal, *s.c.* subcutaneous, *iv.* tail vein, *LN* lymph node, *LPS* lipopolysaccharide



**Table 2** Viruses mentioned in this article: virus abbreviations, virus, virus family and genome organization

Abbreviation	Virus	Virus family	Genome organization <sup>a</sup>
ASFV	African swine fever virus	<i>Asfarviridae</i>	dsDNA
ASLV	Avian sarcoma leukosis virus	<i>Retroviridae</i>	ssRNA-RT
BRV	Bovine rotavirus	<i>Reoviridae</i>	dsRNA
BVDV	Bovine virus-diarrhoe virus	<i>Flaviviridae</i>	(+)ssRNA
CDV	Canine distemper virus	<i>Paramyxoviridae</i>	(-)ssRNA
CV	Coxsackie virus	<i>Picornaviridae</i>	(+)ssRNA
DV	Dengue virus	<i>Flaviviridae</i>	(+)ssRNA
EBOV	Ebola virus	<i>Filoviridae</i>	(-)ssRNA
EBV	Epstein-Barr virus	<i>Herpesviridae</i>	dsDNA
EMCV	Encephalomyocarditis virus	<i>Picornaviridae</i>	(+)ssRNA
FLUAV	Influenza A virus	<i>Orthomyxoviridae</i>	(-)ssRNA
FLUBV	Influenza B virus	<i>Orthomyxoviridae</i>	(-)ssRNA
FMLV	Friend-murine leukaemia virus	<i>Retroviridae</i>	ssRNA-RT
$\gamma$ MHV68	Murine $\gamma$ -herpesvirus 68	<i>Herpesviridae</i>	dsDNA
HAV	Hepatitis A virus	<i>Picornaviridae</i>	(+)ssRNA
HBV	Hepatitis B virus	<i>Hepadnaviridae</i>	dsDNA-RT
HCV	Hepatitis C virus	<i>Flaviviridae</i>	(+)ssRNA
HCMV	Human cytomegalovirus	<i>Herpesviridae</i>	dsDNA
HDV	Hepatitis delta virus	<i>Unassigned</i>	(-)ssRNA
HFV	Human foamy virus	<i>Retroviridae</i>	ssRNA-RT
HIV	Human immunodeficiency virus	<i>Retroviridae</i>	ssRNA-RT
HSV	Herpes simplex virus	<i>Herpesviridae</i>	dsDNA
KSHV, HHV8	Karposi's sarcoma-associated herpesvirus/human herpesvirus 8	<i>Herpesviridae</i>	dsDNA
LACV	La Crosse virus	<i>Bunyaviridae</i>	(-)ssRNA
LASV	Lassa virus	<i>Arenaviridae</i>	(-)ssRNA
LCMV	Lymphocytic choriomeningitis virus	<i>Arenaviridae</i>	(-)ssRNA
MACV	Machupo virus	<i>Arenaviridae</i>	(-)ssRNA
MARV	Marburg virus	<i>Filoviridae</i>	(-)ssRNA
MCMV	Murine cytomegalovirus	<i>Herpesviridae</i>	dsDNA
MeV	Measles virus	<i>Paramyxoviridae</i>	(-)ssRNA
MHV	Mouse hepatitis virus	<i>Coronaviridae</i>	(+)ssRNA
MLV	Murine leukaemia virus	<i>Retroviridae</i>	ssRNA-RT
MMTV	Mouse mammary tumor virus	<i>Retroviridae</i>	ssRNA-RT
M-MuLV	Moloney murine leukemia virus	<i>Retroviridae</i>	ssRNA-RT
MPV	Mouse polyoma virus	<i>Polyomaviridae</i>	dsDNA
NDV	Newcastle disease virus	<i>Paramyxoviridae</i>	(-)ssRNA
OMSK	Omsk hemorrhagic fever virus	<i>Flaviviridae</i>	(+)ssRNA
PV	Poliovirus	<i>Picornaviridae</i>	(+)ssRNA

(continued)

**Table 2** (continued)

Abbreviation	Virus	Virus family	Genome organization <sup>a</sup>
RSV	Respiratory syncytial virus	<i>Paramyxoviridae</i>	(–)ssRNA
RV	Rabies virus	<i>Rhabdoviridae</i>	(–)ssRNA
RVFV	Rift valley fever virus	<i>Bunyaviridae</i>	(–)ssRNA
SARS-CoV	Severe acute respiratory syndrome coronavirus	<i>Coronaviridae</i>	(+)ssRNA
SFV	Semliki Forest virus	<i>Togaviridae</i>	(+)ssRNA
SeV	Sendai virus	<i>Paramyxoviridae</i>	(–)ssRNA
SINV	Sindbis virus	<i>Togaviridae</i>	(+)ssRNA
TMEV	Theiler's murine encephalomyelitis virus	<i>Picornaviridae</i>	(+)ssRNA
THOV	Thogoto virus	<i>Orthomyxoviridae</i>	(–)ssRNA
VSV	Vesicular stomatitis virus	<i>Rhabdoviridae</i>	(–)ssRNA
VACV	Vaccinia virus	<i>Poxviridae</i>	dsDNA
WNV	West Nile virus	<i>Flaviviridae</i>	(+)ssRNA
YFV	Yellow fever virus	<i>Flaviviridae</i>	(+)ssRNA

<sup>a</sup>ssRNA single-stranded RNA, dsDNA double-stranded DNA, (+) positive-stranded, (–) negative-stranded, ssRNA-RT includes a reverse-transcription step in the life cycle

The precise mechanism of IFITM1-, 2- and 3-mediated antiviral activity is still unclear, but IFITM proteins might block virus-receptor interactions, prevent endocytosis or acidification, block fusion or induce signalling to other effector molecules. Recent evidence suggests that the inhibition occurs at late stages of the endocytic pathway. IFITM proteins do not decrease the primary attachment moieties for FLUAV (Brass et al. 2009) and do not affect SARS-CoV receptor expression (Huang et al. 2011). FLUAV still localizes to lysosomal compartments upon ectopic expression of *IFITM1*, 2 or 3. Moreover, the inhibitory effect of IFITMs on SARS-CoV could be circumvented by addition of trypsin, which promotes fusion at or near the plasma membrane (Huang et al. 2011). There is also evidence for different specificities of IFITM family members. Among the murine IFITMs, IFITM3 inhibits FLUAV entry more efficiently than SARS-CoV, MARV, or EBOV, whereas IFITM5 and IFITM6 are more efficient in inhibiting filoviruses (Huang et al. 2011). Interestingly, IFITM3 is posttranslationally modified by S-palmitoylation. Mutation of the palmitoylation site does not alter protein stability or trafficking, but prevents membrane clustering and abrogates its antiviral effect on FLUAV replication (Yount et al. 2010). Although there is accumulating in vitro evidence for an important role of IFITM proteins in the early antiviral defence against a broad range of enveloped viruses, the contribution of IFITM proteins to the antiviral defence in vivo remains to be determined.

## Block of Viral Trafficking

### Interferon-Inducible GTPases

IFNs induce the expression of the p47, p65, and Mx family of GTPases, and the very large GTPases (VLIG). They all share intrinsic GTPase activity and the capacity for self-assembly. Among these four families, only the Mx proteins have well-described antiviral activities (MacMicking 2004).

The Mx proteins are among the most potent antiviral effector proteins. The Mx family comprises Mx1 and Mx2 in mice, and MxA and MxB in humans. Mx proteins are 70–80 kDa in size, belong to the dynamin protein family and differ from the other IFN-inducible GTPases by their stronger induction by type I and type III IFNs as compared to IFN $\gamma$ . Mx1 localizes to the nucleus, whereas MxA and Mx2 are located near the smooth ER. MxB is found in the intranuclear and/or cytoplasmic face of nuclear pores and has no detectable antiviral activity. *Mx1* was identified as an important IFN-induced antiviral factor in early studies with mice exhibiting a genetically determined resistance against FLUAV infection (Haller and Kochs 2011; Haller et al. 2007). Importantly, many laboratory inbred mouse strains, including C57BL/6J, BALB/cJ and 129/J, carry non-functional *Mx1* alleles due to large deletions or nonsense mutations (Staeheli et al. 1988). Studies employing *Mx1*- and *MxA*-transgenic mice confirmed the importance of Mx proteins for the antiviral defence in vivo. For example, constitutive expression of *MxA* in *Ifnar1*<sup>-/-</sup> mice confers full resistance to Thogoto virus (THOV), La Crosse virus (LACV) and Semliki Forest virus (SFV) (Hefti et al. 1999). Mx proteins inhibit replication of a broad range of RNA viruses and some DNA viruses. The mechanism of Mx action has been extensively studied, but is still not entirely clear. Dependent on their localization Mx proteins can recognize and trap essential viral structures, the main targets appear to be viral nucleocapsids. Cytoplasmic MxA blocks the movement of FLUAV and THOV nucleocapsids into the nucleus, while nuclear Mx1 inhibits FLUAV replication prior to the onset of transcription. Mx1 and MxA associate with the viral nucleocapsids and block viral transcription. MxA-mediated changes in trafficking of viral components have also been shown for LACV and African swine fever virus (ASFV). Trapping of viral structures enables Mx proteins to inhibit viral replication already at early steps of infection and provides a very efficient antiviral strategy. It is unclear how exactly Mx proteins interact with viral constituents, but recent structural insights into the basis of Mx oligomerization might help to delineate the mechanism and molecular requirements. Besides, FLUAV strains appear to differ in their sensitivity to MxA actions and this appears to depend on their nucleoproteins (Haller and Kochs 2011; Haller et al. 2007). With respect to human populations, genetic polymorphisms in the *MxA* gene correlate with increased sensitivity to HCV, Hepatitis B virus (HBV) and measles virus (MeV) (Cao et al. 2009; Hijikata et al. 2000; Suzuki et al. 2004; Torisu et al. 2004).

The p65 GTPase family, also known as GBP family, consists of 11 members in mice (GBP1-11) and 7 in humans (GBP1-7). All murine GBPs and at least human

GBP1-5 are induced by IFN $\gamma$  and to a lesser extent, by IFN $\alpha/\beta$  (MacMicking 2004; Vestal and Jeyaratnam 2011). Note that similar numbered GBPs are not necessarily the most related ones. Many inbred mouse strains have a dysfunctional allele for *Gbp1* that cannot be induced by either type I or type II IFN (Staeli et al. 1984). The antiviral functions of GBPs are still undefined, but human GBP1 can form oligomers like Mx proteins. Overexpression of human *GBP1* and its putative murine homolog *Gbp2* inhibits VSV and encephalomyocarditis virus (EMCV) replication. Intriguingly, murine GBP2 GTP-binding activity is thereby essential for the inhibition of EMCV but not VSV. Furthermore, overexpression of human *GBP1* can inhibit replication of an HCV replicon (Vestal and Jeyaratnam 2011).

The p47 GTPase (IRG) family emerged as a crucial pathogen resistance system in mice that is absent in humans (Bekpen et al. 2005). Most characterized members are strongly induced by IFN $\gamma$ , localize along the phagocytic and secretory pathways and are crucially involved in the control of bacterial and protozoan infections. Antiviral activity has so far only been suggested by in vitro overexpression studies for *Tgtp* (*Irgb6*) and *Igtp* (*Irgm3*) against VSV and Coxsackie virus (CV), respectively (Bekpen et al. 2005; Howard 2008). So far, only normal resistance against murine cytomegalovirus (MCMV) has been reported for *Igtp*<sup>-/-</sup> mice (Taylor et al. 2000) (Table 1).

The VLIG family members are around 280 kDa in size and are the largest GTPases described so far (MacMicking 2004). They are the last IFN-induced GTPases identified and appear to have emerged solely in vertebrates (Li et al. 2009a). To date their functional significance in IFN responses is unclear.

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## Editing of Viral Nucleic Acids

### Adenosine Deaminases Acting on RNA (ADARs)

ADARs catalyze the deamination of adenosine (A) to produce inosine (I) in RNAs with double-stranded (ds) character. A-to-I editing leads to an A- to guanosine (G) nucleotide exchange, since I is decoded as G during translation and RNA-dependent RNA replication. Among the three mammalian ADARs (ADAR1-3) described to date, only ADAR1 is IFN-inducible through an ISRE element in one of the alternative *ADAR1* gene promoters. In mice and humans, alternative splicing leads to the expression of the constitutively expressed p110 and the IFN-inducible p150 isoforms. ADAR1 p150 shuttles between the nucleus and the cytoplasm, ADAR1 p110 is predominantly and ADAR2 and ADAR3 are exclusively found in the nucleus. ADAR3 lacks catalytic activity, shows tissue-restricted expression and has been implicated in negative regulation of ADAR1 and ADAR2. RNA editing by ADAR1 can occur at multiple positions or at highly specific sites. Multiple A-to-G substitutions attributable to ADAR activity have been first described for MeV, followed by a large number of other viruses that mostly contain a negative-stranded RNA or an ambisense genome organization. More site-selective A-to-G exchanges have been reported for example for hepatitis delta virus (HDV), human herpesvirus 8 (HHV8) and Epstein-Barr (EBV) virus (Samuel 2011).

ADARs exert antiviral effects against viruses belonging to several families and using completely different replication strategies. A-to-I editing of viral RNAs can affect virus-host interactions at multiple levels and, importantly, can also have proviral effects. Nucleotide exchanges can lead to amino acid exchanges and altered protein products with different biological activities. This has for example been described for MeV, where extensive hypermutation of the matrix protein is associated with persistent infection of the central nervous system. A-to-I editing can also lead to frame-shift mutations or premature stop-codons and, consequently, to aberrant or reduced viral protein expression, as described for MeV, respiratory syncytial virus (RSV) and LCMV (Samuel 2011). A-to-I conversion can also induce structural changes in RNAs, as RNA duplex structures are less stable when A:uridine (U) base pairs are exchanged by I:U base pairs (Bass 2002; Serra et al. 2004). Reduced duplex-stability likely results in altered dsRNA-mediated activities. In support of this notion, shRNA-mediated downregulation of *ADAR1* leads to enhanced activation of dsRNA-dependent protein kinase (PKR) and IRF3 (Toth et al. 2009). Consistently, synthetic I:U-containing RNAs suppress dsRNA-mediated activation of IRF3 and ISG expression in HeLa cells (Vitali and Scadden 2010). A-to-I substitutions can also alter microRNA processing or silencing capacity, or target RNAs for degradation. These effects have been shown for cellular RNAs and are yet to be demonstrated for viral RNAs. It is also likely that RNA editing can lead to viral genome mutations in the case of single-stranded (ss) RNA viruses that use RNA-dependent RNA replication. Furthermore, RNA editing might also indirectly influence virus replication as it could affect cellular transcripts of proteins involved in the antiviral defence (Samuel 2011).

*Adar1(p150 or p110/p150)<sup>-/-</sup>* and *Adar2<sup>-/-</sup>* mice show severe phenotypes (Table 1). *Adar1*-deficiency leads to embryonic lethality (Hartner et al. 2004, 2009; Wang et al. 2004; Ward et al. 2011; XuFeng et al. 2009), whereas *Adar2<sup>-/-</sup>* mice are prone to seizures and die young (Higuchi et al. 2000). Thus, ADAR1 and ADAR2 are also crucially involved in cellular processes unrelated to host defence mechanisms. Nevertheless, studies with embryonic fibroblasts derived from these mice clearly established the selective functions of ADAR1 and ADAR2 in editing viral RNAs and in antiviral pathways. *Adar1(p150)<sup>-/-</sup>* cells ectopically expressing the receptor for MeV show dramatically increased MeV-induced cytopathic effects and markedly increased virus replication (Ward et al. 2011). This is consistent with the reported increased MeV-induced cytotoxicity in HeLa cells after shRNA-mediated *ADAR1* knockdown (Toth et al. 2009). Similarly, other members of the *Paramyxoviridae* (i.e. Newcastle disease virus (NDV), Sendai virus (SeV), canine distemper virus (CDV)) and FLUAV induce less pronounced cytopathic effects in *Adar1(p150)<sup>-/-</sup>* fibroblasts than in the respective wildtype cells. No effect of *Adar1(p150)* deficiency was found for LCMV and VSV replication (Ward et al. 2011).

Proviral effects of ADAR1 have been shown for VSV, HDV, HIV-1, Kaposi's sarcoma-associated herpesvirus (KSHV) and mouse polyoma virus (MPV), although the mechanisms seem to differ (Samuel 2011). For VSV this has been attributed to the interaction of ADAR1 with PKR, inhibition of PKR activity and consequent impairment of eukaryotic translation initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) phosphorylation (Nie et al. 2007). The proviral effect of ADAR1 for HDV is well established. HDV

requires site-specific A-to-I editing to switch from the short delta antigen protein, which is essential during early steps of replication, to the longer form, which is crucial for packaging of the viral genome and HDV particle formation (Samuel 2011). Inhibition of HDV occurs upon increased HDV RNA editing conditions, such as overexpression of *ADAR1* (*p110*) or *ADAR2*, or by IFN-mediated increased *ADAR1* (*p150*) expression (Hartwig et al. 2004; Jayan and Casey 2002). Proviral mechanisms in the case of HIV-1, KSHV and MPV are less well characterized, but they might involve site-specific editing of viral mRNA and the switch from early to late transcripts, respectively, for the latter two viruses (Samuel 2011).

## **Apolipoprotein B mRNA-Editing Catalytic Polypeptide (APOBEC) Family**

The APOBEC proteins are tissue-specific cytidine (C) deaminases that exhibit RNA editing and/or DNA mutator activity. In humans, the APOBEC family comprises 11 members that have cellular functions and inhibit the mobility of endogenous retroelements. Apart from that, the APOBEC3 subfamily exerts broad-spectrum anti-retroviral activity. However, retroviruses have evolved very efficient countermeasures and are therefore often insensitive to endogenous APOBEC3 actions unless they lack the respective antagonist (Goila-Gaur and Strebel 2008).

Most human APOBEC3 family members are expressed constitutively and expression is further enhanced by IFN $\alpha/\beta$  and IFN $\gamma$  (Goila-Gaur and Strebel 2008; Koning et al. 2009; Refsland et al. 2010; Stenglein et al. 2010; Trapp et al. 2009; Wang et al. 2009). APOBEC3 proteins are packaged into retroviral particles and cause extensive C-to-U mutations in the minus-strand of the viral DNA during reverse transcription. C-to-U editing can lead to mutations in viral structural and non-structural proteins causing replication defects at multiple levels. Degradation of uracilated viral cDNAs by cellular DNA glycosylases is believed to contribute to the APOBEC3-mediated antiviral activity (Goila-Gaur and Strebel 2008). Intriguingly, a very recent report implicates APOBEC3A catalytic activity and the cellular uracil DNA glycosylase UNG2 in the clearance of transfected plasmid DNAs, suggesting that APOBEC3s may act as a restriction factor for a broader range of foreign DNAs (Stenglein et al. 2010). In addition, APOBEC3s exert deaminase-independent antiviral functions and these include interference with tRNA-primed initiation of reverse transcription and reverse transcriptase-mediated DNA elongation (Goila-Gaur and Strebel 2008; Narvaiza et al. 2009). Apart from retroviruses, APOBEC3s can also interfere with the HBV life cycle, however, APOBEC3B, APOBEC3F and APOBEC3G are not required for the anti-HBV actions of IFN $\gamma$  in human cell lines (Goila-Gaur and Strebel 2008; Proto et al. 2008). In contrast, several reports suggest a contribution of APOBEC3s to the IFN $\alpha/\beta$ -induced antiviral activity against HIV-1 (Cheney and McKnight 2010; Goila-Gaur and Strebel 2008; Trapp et al. 2009).

Unlike humans, who have seven *APOBEC3* genes, mice only have one (*Apobec3*) and this is induced by type I and type II IFN (Okeoma et al. 2009a; Turelli et al. 2008). Murine APOBEC3 also induces hypermutations in retroviral cDNAs, but restriction

of its activity to specific viruses appears different from the human APOBEC3G (Browne and Littman 2008; Rulli et al. 2008). Murine *Apobec3* has been implicated in the in vivo control of Friend-murine leukaemia virus (FMLV) (Santiago et al. 2011; Takeda et al. 2008) and *Apobec3*<sup>-/-</sup> mice show increased sensitivity to mouse mammary tumour virus (MMTV) (Okeoma et al. 2007, 2009b). *Apobec3*<sup>-/-</sup> mice infected with Moloney murine leukaemia virus (M-MuLV) show higher virus titers and develop earlier leukaemia in comparison to control animals (Low et al. 2009). Importantly, pre-treatment of murine cells with IFN $\alpha$  results in enhanced *Apobec3* expression and resistance against MMTV. No effect of IFN $\alpha$  on MMTV replication was observed in *Apobec3*<sup>-/-</sup> cells, demonstrating that *Apobec3* crucially contributes to the anti-MMTV action of IFNs (Okeoma et al. 2009b). Consistent with data from the human system, dsRNA pretreatment inhibits HBV DNA production in HBV-transgenic and *Apobec3*-deficient mice to similar levels as in the control HBV-transgenic mice (Turelli et al. 2008) (Table 1).

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## Viral RNA Degradation and Translational Inhibition

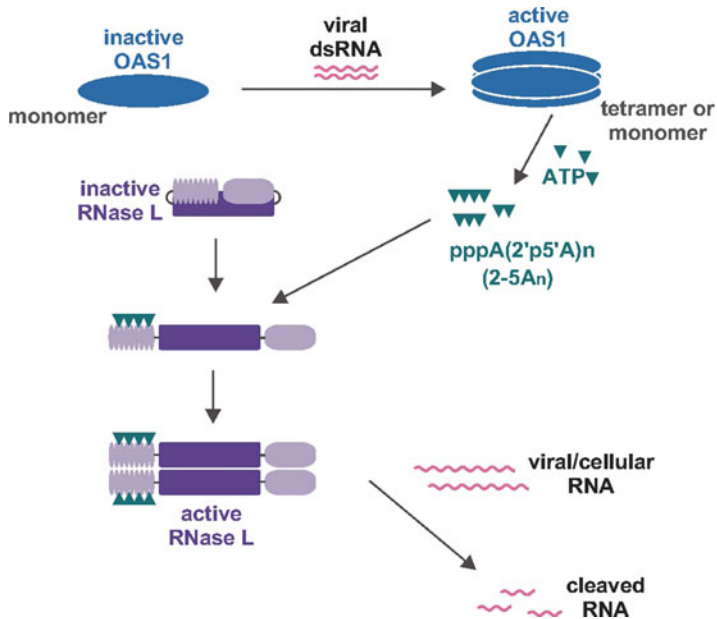
### 2'-5' Oligoadenylate Synthetases (OAS) and Latent Ribonuclease (RNase L)

The OAS/RNase L pathway belongs to the best characterized antiviral pathways to date. It results in the degradation of viral and cellular RNAs and blocks replication of a number of RNA and DNA viruses (Chakrabarti et al. 2011; Kristiansen et al. 2011).

In humans the OAS family consists of four genes (*OAS1*, 2, 3 and *L*). As a result of gene duplication, mice have eight different *Oas1* (*Oas1a-h*), one *Oas2*, one *Oas3*, and two *OasL* (*OasL1* and *OasL2*) genes. *OAS* genes are expressed at low levels in resident cells and are induced by type I IFN and upon virus infections. OAS1 proteins contain one unit of the OAS domain, OAS2 and OAS3 contain two and three copies of the catalytic units, respectively. Among the murine OAS1 proteins, only OAS1a and OAS1g are catalytically active. OASL proteins contain one OAS unit which is, with the exception of murine OASL2, without catalytic activity (Kristiansen et al. 2011). Mice and humans harbour one *RNase L* gene, which is widely expressed in most, if not all mammalian tissues and further upregulated by type I IFN exposure in murine cells, but only barely in human cells. RNase L is a latent endoribonuclease that consists of a regulatory ankyrin repeat domain (ARD), a protein kinase (PK)-like domain and the RNase domain (Chakrabarti et al. 2011).

Activation of OAS family members occurs through recognition and binding of viral dsRNA followed by a conformational change. Therefore, OASs are not only antiviral proteins but also considered as PRRs. Activated OAS polymerizes ATP into 2'-5' oligoadenylates (2-5A<sub>n</sub>). Binding of these oligomers to the ankyrin domain of monomeric RNase L leads to dimerization and activation of RNase L. Subsequently, the endoribonuclease degrades viral and cellular RNAs with ssRNA loops and thus prevents viral protein synthesis (Fig. 2). Each of the active human OAS family





**Fig. 2** The OAS1/RNase L pathway. Latent OAS1 is activated by viral dsRNA and, subsequently, oligomerizes ATP into 2'-5' oligoadenylates (2-5A<sub>n</sub>). Binding of 2-5A<sub>n</sub> to the ankyrin repeat domain of RNase L leads to activation and dimerization of RNase L through their kinase-like domains. Activated RNase L dimers in turn cleave cellular and/or viral RNAs

members appears to have some unique biological features. For example, catalytically active OAS1 is a monomer or tetramer, OAS2 a dimer and OAS3 functions as a monomer. OAS3 synthesizes dimeric 2-5A<sub>n</sub>, whereas OAS1 and OAS2 synthesize trimeric and tetrameric oligomers. As dimeric 2-5A<sub>n</sub> do not efficiently activate RNase L, OAS3 might have different functions. Several overexpression studies demonstrated antiviral capacity for human OAS family members. OAS1 and OAS2 inhibit replication of EMCV, but not VSV, OAS3 is effective against alphaviruses, i.e. SFV and Sinbis virus (SINV) (Chakrabarti et al. 2011; Kristiansen et al. 2011; Sadler and Williams 2008). Little is known about the characteristics of dsRNA recognition and activation of OAS family members. Human OAS1 appears to also bind ssRNA, but activation only occurs upon dsRNA binding, with a preference for longer dsRNAs (Kristiansen et al. 2011). There also might be differential specificities in dsRNA recognition among OAS family members, as recently suggested for murine OAS1a and OAS1b (Elbahesh et al. 2011).

RNase L, once activated, can degrade viral and cellular (including ribosomal) RNAs. RNase L cleavage products are small, mostly structured ssRNAs with 5'-hydroxyl and 3'-monophosphate at their termini. Interestingly, these cleavage products can act as PAMPs and induce IFN $\beta$  production *via* recognition by retinoic acid-inducible protein I (RIG-I) and/or melanoma differentiation-associated protein 5 (MDA5). The 3'-phosphate of RNase L cleavage products appears to be required at



least for the activation of RIG-I (Luthra et al. 2011; Malathi et al. 2007, 2010). Knockout mice for *RNase L* are susceptible to EMCV, CV-B4, herpes simplex virus (HSV)-1 and WNV (Table 1), although the RNase L-specific effects seem to be strongly dependent on the cell type and the virus strain. Several reports showed that RNase L can also negatively influence host survival upon virus infection and promote viral replication as shown for HSV-2, SINV, SFV and some reovirus strains (Silverman 2007). On the other hand, RNase L appears to be protective against virus-induced demyelination. Infection of *RNase L*<sup>-/-</sup> mice with the neurotropic mouse hepatitis virus (MHV)-JHM strain results in higher susceptibility, but does neither affect virus control in the CNS nor IFN $\alpha$ / $\beta$  expression (Ireland et al. 2009).

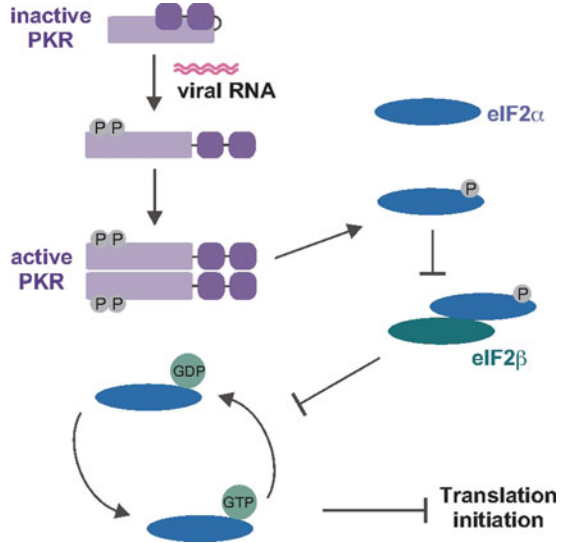
Several studies suggest RNase L-independent antiviral activities of OAS family members. The enzymatically inactive human OAS1L and murine OAS1b can inhibit EMCV and WNV replication, respectively, when expressed in cell culture. Furthermore, OAS1b is required for resistance of mice against flavivirus infection. Dependent on the mouse strain, the OAS1b protein exists in two forms, a full-length OAS1b and a truncated form as a result of a point mutation in the *OAS1b* gene generating a premature stop codon. Only the full-length protein leads to resistance against WNV infection. Notably, most laboratory inbred mouse strains express the truncated version of *OAS1b* (Kristiansen et al. 2011). Recently, lack of enzymatic activity of OAS1b was confirmed and, additionally, full-length OAS1b was shown to inhibit synthetic dsRNA-induced 2-5A<sub>n</sub> production both in vivo and in vitro (Elbahesh et al. 2011).

Of potential clinical relevance, exogenously applied OAS1 can induce an antiviral state. Exogenous recombinant porcine OAS1 protects HepG2 cells from the cytopathic effects of EMCV and VSV in a dose-dependent manner and inhibits virus replication in Vero cells. These effects are again independent of both, enzymatic activity of OAS1 and the presence of RNase L. Moreover, injection of OAS1 into mice results in tenfold reduced viral titers in organs upon subsequent EMCV infections (Kristiansen et al. 2010). It will be of interest to determine if and to what extent assumable autocrine/paracrine actions of OAS1 contribute to the in vivo antiviral defence.

## Double Stranded RNA-Dependent Protein Kinase (PKR)

Similar to Mx and OAS/RNase L, the antiviral activities of PKR are long-known and have been extensively characterized. PKR is encoded by the *Eif2ak2* gene and is one out of four members of a serine-threonine kinase family that regulates protein synthesis upon diverse stress signals mainly through phosphorylation of eIF2 $\alpha$ . PKR consists of two N-terminal RNA binding motifs (RBMs) and a C-terminal catalytic kinase domain (KD). PKR is constitutively expressed in all differentiated cells at low levels and is upregulated upon type I and III IFN through activation of an ISRE binding site in the promoter. In uninfected cells, PKR exists as an inactive monomer by autoinhibition of the kinase domain. DsRNA and other ligands like heparin, ceramide and the PKR-associated factor PACT induce the release of the

**Fig. 3** Scheme of PKR activation and action. In resident cells PKR exists as inactive monomer through intramolecular autoinhibition. Sensing of viral RNA activates PKR by autophosphorylation. Dimerized PKR molecules phosphorylate eIF2 $\alpha$  on serine 51, which inhibits the nucleotide exchange factor eIF2 $\beta$ . This disables the exchange of inactive eIF2 $\alpha$ -GDP with eIF2 $\alpha$ -GTP and leads to a block of translation initiation



inactive molecule and lead to autophosphorylation and dimerization of PKR. Activation of PKR can also occur through binding of ssRNA containing duplex regions and a 5'-triphosphate. Interestingly, endogenous transcripts also efficiently activate PKR, as known for the IFN $\gamma$  mRNA. Once PKR is activated, it phosphorylates the serine residue 51 of eIF2 $\alpha$  leading to inhibition of the guanine nucleotide exchange factor eIF2 $\beta$ . This prevents exchange of GDP to GTP, thus blocking translation initiation (Fig. 3). Intriguingly, PKR regulates its own activity through inhibition of its mRNA translation initiation in the presence of high PKR protein levels (Pindel and Sadler 2011; Sadler 2010).

Two independent knockout mouse models and a transgenic mouse expressing a dominant-negative, kinase-defective PKR gave further insight into PKR functions in antiviral responses (Barry et al. 2009; Nakayama et al. 2010; Sadler and Williams 2008). PKR-deficient mice are susceptible to VSV, FLUA mutant virus, and LCMV (Table 1). Protection against several viruses like HCV, EMCV, WNV, HIV-1, HDV, SINV and HSV-1 have been shown to be dependent on PKR in several in vitro systems (Nakayama et al. 2010; Sadler and Williams 2008).

Although phosphorylation of eIF2 $\alpha$  and consequent translational regulation was considered as the major function of PKR, several reports have suggested involvement of PKR in different signalling networks. For example, PKR has been shown to regulate the transcription factors IRF1, STAT1, STAT3, nuclear factor NF $\kappa$ B, c-Jun, cyclic AMP-dependent transcription factor (ATF) 3 and ATF4 (Pindel and Sadler 2011). Two recent reports suggest that PKR is an important regulator of IFN $\alpha$ / $\beta$  synthesis/secretion. This has been shown in response to infection with a subset of RNA viruses, like EMCV, SFV and Theiler's murine encephalomyelitis virus (TMEV). Mechanistically, absence of PKR results in strongly reduced levels of polyadenylated IFN $\beta$  mRNAs. Thus, PKR maintains the integrity of IFN $\beta$

mRNA thereby promoting its translation. PKR action in this case seems to be independent of eIF2 $\alpha$  phosphorylation. Consistently, serum levels of IFN $\beta$  are reduced in EMCV infected *Pkr*<sup>-/-</sup> mice (Schulz et al. 2010). These data were supported by a later study with in vitro bovine rotavirus (BRV) infections. Virus infection leads to a strong RIG-I-, MDA5- and IRF3-dependent IFN $\beta$  mRNA induction. IFN $\beta$  mRNA levels are normal in fibroblasts derived from *Pkr*<sup>-/-</sup> mice, but IFN $\beta$  protein secretion is dramatically reduced (Sen et al. 2011). It is unclear, how PKR prevents mRNA de-adenylation and whether other mRNAs are similarly regulated by PKR-dependent stabilizing mechanism.

## Interferon-Stimulated Gene Product of 20 kDa (ISG20)

ISG20 (HEM45) was identified independently based on its induction by type I and type II IFNs and by estrogen (Gongora et al. 1997; Mattei et al. 1997; Pentecost 1998). ISG20 is a nuclear 3'-5' exoribonuclease associated with nuclear bodies that acts on ssRNA and, to a lesser extent, on ssDNA (Degols et al. 2007).

Several overexpression studies of *ISG20* in human cell lines demonstrated inhibition of RNA virus replication. Antiviral activity appears virus-specific and ectopic expression of *ISG20* inhibits VSV, EMCV, FLUAV, DV-2, DV- and WNV-like particles, HCV, Bovine virus-diarrhoea virus (BVDV), hepatitis A virus (HAV) and YFV, but not SARS-CoV replication. For HCV, DV- and WNV-like particles antiviral activity of ISG20 is dependent on its enzymatic activity and catalytically inactive ISG20 reduces IFN-mediated antiviral activity against VSV, but surprisingly not against EMCV or FLUAV. In murine embryonic fibroblast cell lines, overexpression of *Isg20* inhibits SINV replication and siRNA-mediated knockdown of *Isg20* results in enhanced virus replication. Increased survival rates are found in neonatal mice after subcutaneous inoculation with SINV RNA encoding *Isg20* as compared to the respective controls (Zhou et al. 2011). Besides, an HIV-1-derived virus expressing *ISG20* shows strongly delayed replication in a human T-lymphoblastoid cell line and peripheral blood mononuclear cells (Espert et al. 2005).

With respect to DNA viruses, inhibitory effects of *ISG20* on HBV protein synthesis have been suggested in transfected HepG2 cells (Hao and Yang 2008), whereas *ISG20* overexpression in HeLa cells was reported to not affect adenovirus replication (Espert et al. 2003).

It is still unclear how exactly ISG20 inhibits virus replication. The studies outlined above show a requirement for its exoribonuclease activity, thus it seems likely that ISG20 directly degrades viral RNA and/or DNA, however, evidence remains to be provided. ISG20 does not degrade transfected replication-incompetent HCV RNA, suggesting that either viral RNA associated with replication complexes or replication intermediates are targets for ISG20-mediated degradation. It is notable in that context, that ISG20 specifically degrades ssRNA but not RNA containing stem-loop structures at the 3'-end (Nguyen et al. 2001). Alternatively, ISG20 might act indirectly by e.g. targeting other (cellular) factors that are required for viral replication. The contribution of endogenous

ISG20 to IFN-induced antiviral activity and its role in the in vivo defence against specific viruses needs further delineation.

## Interferon-Induced Proteins with Tetratricopeptide Repeats (IFITs)

Members of the IFIT gene family were among the first IFN-inducible genes cloned (Chebath et al. 1983; Levy et al. 1986; Wathelet et al. 1986). Four members have been described in humans and three in mice. All IFIT proteins contain multiple tetratricopeptide repeats known to mediate protein-protein interactions. IFIT1 (ISG56) and IFIT2 (ISG54) have well established inhibitory effects on protein synthesis by targeting the translation initiation complex. Although this globally affects protein synthesis, IFIT1 might more specifically block translation of viral RNAs. For example, HCV IRES-driven reporter expression shows increased sensitivity to IFIT1-mediated translational inhibition in comparison to 5'-cap-driven reporter expression in human cell lines (Fensterl and Sen 2011).

A few reports suggested inhibitory functions of IFITs on SeV, WNV, LCMV, VSV, EMCV and HCV (Schmeisser et al. 2010; Wachter et al. 2007; Wang et al. 2003; Zhang et al. 2007), although these were not further characterized. Only very recently, extensive analyses including gene-targeted mice and mutant viruses established that the antiviral functions of IFITs depend on the 5'-structures of viral RNAs (Daffis et al. 2010; Pichlmair et al. 2011). In higher eukaryotes, 2'-O-methylation of cellular mRNA 5'-caps occurs, in addition to the well-known essential methylation at the N-7 position of the cap guanosine residue, at the ribose-2'-O-position of one or two adjoining nucleotides (Langberg and Moss 1981). Several RNA and DNA viruses also have 2'-O-methylated 5'-capped mRNAs (Fechter and Brownlee 2005; Wei and Moss 1975) and mutant WNV, MHV and vaccinia (VACV) viruses lacking their respective 2'-O-methyltransferase activity are sensitive to IFIT1 and/or IFIT2 actions (Table 1) (Daffis et al. 2010; Zust et al. 2011). A mechanistic explanation for the 5'-nucleic acid structure-specific IFIT functions came from the finding that IFITs can form multi-protein complexes that bind to "non-self" 5'-triphosphorylated RNA (PPP-RNA) in human cell lines (Pichlmair et al. 2011). IFIT1 and IFIT5 (ISG58), the latter existing in humans but not in mice, directly bind to PPP-RNA, whereas IFIT2 and IFIT3 (ISG60) can associate with PPP-RNA through interaction with IFIT1. Consistently, siRNA mediated downregulation of *IFIT1* inhibits replication of VSV and Rift Valley fever virus (RVFV), both viruses known to generate PPP-mRNAs, and *Ifit1*<sup>-/-</sup> mice and fibroblasts show increased sensitivity to VSV infection (Table 1). Proofing the specificity of IFIT1 action, absence of IFIT1 does not affect replication of EMCV, a virus that does not generate PPP-mRNA. Interestingly, sequestration of viral mRNA rather than direct inhibition of translational initiation appeared as the main antiviral effector function in this study, although the fate of the bound viral RNA remained undefined (Pichlmair et al. 2011).

Apart from translational inhibition and viral RNA sequestration, two further and unrelated antiviral effector functions have been ascribed to human IFIT1. Firstly,

IFIT1 can directly inhibit human papillomavirus (HPV) DNA replication by binding to and blocking HPV E1 protein helicase activity (Saikia et al. 2010; Terenzi et al. 2008) and, secondly, IFIT1 reportedly inhibits SeV-induced IFN $\beta$  production through interaction with stimulator of IFN genes (STING) (Li et al. 2009b). However, virus-induced IFN $\beta$  production was similar in cells derived from *Ifit1*<sup>-/-</sup> and wildtype mice (Pichlmair et al. 2011).

In summary, IFIT proteins emerged as both virus-sensing and antiviral effector proteins. Their function within “versatile” multi-protein complexes (Pichlmair et al. 2011) might explain some of the inconsistencies in antiviral activities observed between overexpression and siRNA-mediated or genetic knockdown of specific IFIT family members. Stoichiometry of IFITs, association with other RNA-binding or accessory proteins and viral countermeasures likely determine their mode of action and virus specificity. Several IFIT-like genes have been found both in mice and humans (Fensterl and Sen 2011) and their putative protein products might add to the complexity. Future work will be required in order to define the function of the distinct IFIT family members/complexes, the exact nature and fate of their target RNAs and, potential species specificities.

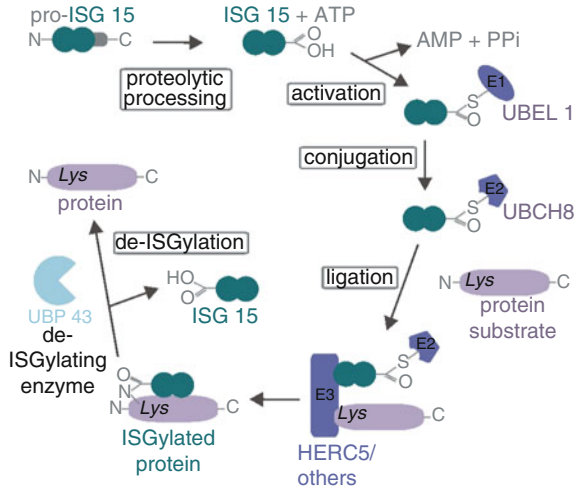
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## Posttranslational Modification of Viral and Cellular Proteins

### Interferon-Stimulated Gene Product of 15 kDa (ISG15)

ISG15 is among the most prominently induced genes during virus infection and in response to type I IFNs. ISG15 is an ubiquitin-like protein that is conjugated to target proteins. The so-called ISGylation of proteins has many common features with protein-ubiquitination. Both involve a series of stepwise enzymatic reactions that result in covalent protein modifications (Fig. 4). ISG15 is synthesised as a precursor protein that is processed to expose a C-terminal LRLRGG motif. The GG motif is adenylated in the presence of ATP and then conjugated sequentially to cysteine residues of three enzymes (E1–E3) and, finally, ISG15 is transferred to lysine residues within its target substrates. The E1 enzyme (ISG15-activating enzyme, UBE1L) is specific for the ISGylation pathway, whereas there is an overlap with enzymes involved in ubiquitination for the E2 (ISG15/ubiquitin-conjugating enzyme) and E3 (ISG15/ubiquitin ligase) enzymes. Both ubiquitination and ISGylation are reversible processes and several de-ubiquitinating and de-ISGylating enzymes can cleave off the respective protein modification. Similar to ISG15, many enzymes involved in the ISGylation pathway are induced by IFNs, e.g. the E1 enzyme UBE1L, the E2 enzyme UBCH8, the E3 enzymes HERC5 and tripartite motif protein 25 (TRIM25), and the de-ISGylating enzyme UBP43 (USP18) (Harty et al. 2009; Zhang and Zhang 2011).

Unlike ubiquitination, ISGylation does not target proteins for degradation but rather resembles mono-ubiquitination and mainly affects protein function. ISGylation can also increase protein stability by protecting proteins from degradation. ISGylation has a very broad specificity and over 150 putative cellular ISG15 target



**Fig. 4** Schematic diagram of the protein ISGylation system. ISG15 is proteolytically processed by an ISG15-specific protease to expose a C-terminal LRLRGG motif. In the presence of ATP, UBE1L catalyzes adenylation and forms a thioester bond with the C-terminal end of ISG15. ISG15 is then transferred to UBCH8, which is also covalently linked via a thioester bond. Catalyzed by HERC5 or other E3 enzymes, the C-terminus of ISG15 is then linked *via* an isopeptide bond to a lysine residue within the target protein. The de-ISGylation enzyme UBP43 can remove the ISG from the substrate. E1, ISG15-activating enzyme; E2, ISG15-conjugating enzyme; E3, ISG15 ligase

proteins have been identified so far. ISG15 has a broad antiviral activity against both DNA and RNA viruses. *Isg15*<sup>-/-</sup> mice are highly susceptible to FLUAV and influenza B virus (FLUBV), SINV, HSV, and murine  $\gamma$ -herpesvirus 68 ( $\gamma$ MHV68) infections (Harty et al. 2009). Consistent with the involvement of ISGylation, *Ube1l*<sup>-/-</sup> mice show increased susceptibility to FLUBV and SINV infection. In contrast, ISG15 and UBE1L are redundant for the *in vivo* defence against LCMV (see also Table 1). Very recently, the FLUAV NS1A protein was identified as ISG15 target protein in two independent studies (Tang et al. 2010; Zhao et al. 2010). FLUAV NS1A protein was shown to directly interact with the major human E3 ISG15 ligase HERC5. Overexpression of *HERC5* in human cell lines enhances the anti-FLUAV activity of IFN $\beta$  and, consistently, downregulation of *ISG15*, *UBE1L*, *UBCH8* or *HERC5* by siRNA increases viral protein synthesis and replication (Hsiang et al. 2009; Tang et al. 2010; Zhao et al. 2010). Multiple lysine (K) residues within the NS1A can be modified by ISGylation (Tang et al. 2010; Zhao et al. 2010). *In vitro* ISGylated NS1A is unable to bind to PKR and to dsRNA and fails to dimerize *via* its RNA-binding domains (Zhao et al. 2010). ISGylated truncated NS1A protein, lacking its second C-terminal nuclear-localization signal, shows impaired interaction with importin- $\alpha$  (Tang et al. 2010). Although the major ISGylated lysine residues identified within the NS1A protein differ among the two studies, FLUAV expressing NS1A mutant for specific lysine residue are more

virulent in vivo (Tang et al. 2010) or show enhanced replication in IFN $\beta$  treated cell lines, respectively (Zhao et al. 2010).

ISG15 can also inhibit budding of retroviruses, EBOV and VSV. The mechanisms are unclear, but inhibition of ubiquitination might at least be partially involved. ISG15 blocks ubiquitination of HIV-1 Gag protein and the EBOV VP40 matrix protein, both of which have been linked to budding ability (Harty et al. 2009). Besides, ISG15 was reported to inhibit the association of the HIV-1 and the avian sarcoma leukosis virus (ASLV) budding complex with cellular proteins involved in endosome sorting (Pincetic et al. 2010).

Proviral effects of ISG15 have been described for HCV. Several studies using overexpression and/or siRNA-mediated downregulation of *ISG15* or *UEB1L* revealed a positive effect of the ISG15 system for HCV RNA production in HCV replicon cell lines (Broering et al. 2010; Chen et al. 2010b; Chua et al. 2009). In line with this, high ISG15 levels correlate with high HCV load and low responsiveness of patients to IFN $\alpha$  therapy (Broering et al. 2010; Chen et al. 2010a). However, negative effects of ISGylation on HCV replication have also been reported and were attributed to ISGylation of the HCV NS5A protein and decreased NS5A protein stability (Kim and Yoo 2010).

Among the cellular ISG15 target proteins identified to date are many proteins involved in IFN signal transduction (e.g. JAK1, STAT1), virus recognition and downstream signalling (e.g. RIG-I, MDA5, and IRF3), and in the antiviral effector pathways (e.g. Mx, PKR and RNase L) (Malakhov et al. 2003; Zhao et al. 2005). ISGylation of these target proteins can have diverse effects, whereby it generally results in increased antiviral activity. For example, ISGylation has been reported to prevent NDV-induced degradation of IRF3 resulting in enhanced IFN $\beta$  production in human fibrosarcoma cells (Lu et al. 2006). This notion was confirmed by a later study showing direct interaction of IRF3 with HERC5 in HEK293 cells (Shi et al. 2010). Ectopic expression of HERC5 potentiates IRF3 transcriptional activity and siRNA-mediated downregulation of HERC5 or ISG15 reduces the expression of IRF3-responsive genes upon SeV infection. Notably, a HERC5 mutant protein that lacks its ligase activity does not affect IRF3 target gene-activation. Downregulation of *HERC5* by siRNA results in increased replication of SeV, VSV and NDV. SeV-induced IRF3 poly-ubiquitination and proteasomal degradation is reduced upon ectopic expression of the ISGylation system. IRF3 was shown to be ISGylated predominantly at three lysine residues and, as expected, the triple lysine mutant does not show a change in ubiquitination upon HERC5 downregulation and SeV infection. Furthermore, the triple lysine IRF3 mutant cannot fully rescue IFN $\beta$  reporter gene activation in response to SeV infection in *Irf3*<sup>-/-</sup> mouse embryo fibroblasts and displays accelerated degradation in comparison to wildtype IRF3 (Shi et al. 2010).

ISGylation of protein phosphatase 1B (PPM1, PP2Cb) reduces its activity and results in enhanced I $\kappa$ B $\alpha$  degradation and increased NF $\kappa$ B signalling (Takeuchi et al. 2006). ISGylated eIF4E family member 2 (eIF4E2, 4EHP) has increased affinity to 5'-capped RNAs compared to the non-ISGylated form (Okumura et al. 2007). ISGylation of cellular proteins has been recently also shown to be



responsible for the inhibition of early steps of the FLUAV replication cycle (Hsiang et al. 2009), although proteins involved remained undefined. Negative regulation by ISGylation has been shown for RIG-I. ISGylation of RIG-I leads to reduced basal and virus-induced IFN production and cells derived from *Ube1l*-deficient mice show significantly lower levels of RIG-I mRNA and protein. Thus, a negative feedback mechanism acting on RIG-I was postulated which might be required to balance cellular innate immune responses (Kim et al. 2008b).

## Ubiquitin Carboxyl-terminal Hydrolase 43 (UBP43, USP18)

As mentioned above, UBP43 is an IFN-induced de-ISGylating enzyme. *Ubp43*<sup>-/-</sup> mice show increased resistance against LCMV and VSV and display strongly increased clearance of injected replication competent HBV DNA (Table 1). However, *Ubp43*<sup>-/-</sup> mice develop brain injury, accompanied by hydrocephalus and early death and thus in vivo virus challenges have their limitations (Knobeloch et al. 2005; Ritchie et al. 2002, 2004). Fibroblasts derived from *Ubp43*<sup>-/-</sup> mice exhibit enhanced type I IFN-mediated protection from cytopathic effects caused by VSV and SINV infection (Ritchie et al. 2004). *Ubp43*<sup>-/-</sup> cells show dramatically increased levels of ISGylated proteins after IFN treatment which is also associated with increased JAK/STAT signalling and hyper-responsiveness to type I IFN (Malakhova et al. 2003). Curiously, the phenotype of *Ubp43*<sup>-/-</sup> cells is not rescued in fibroblasts derived from *Ubp43*<sup>-/-</sup>/*Isg15*<sup>-/-</sup> or *Ubp43*<sup>-/-</sup>/*Ube1l*<sup>-/-</sup> double knockout mice (Kim et al. 2006; Knobeloch et al. 2005), suggesting that the IFN hyper-responsiveness and the virus-resistant phenotype of *Ubp43*<sup>-/-</sup> cells is not associated with de-ISGylating activity. A possible explanation is provided by studies demonstrating that UBP43 also negatively regulates type I IFN signalling (Malakhova et al. 2003, 2006). UBP43 was shown to directly interact with IFNAR2 and, consequently, inhibit JAK/STAT signalling. This effect is independent of UBP43 isopeptidase activity as complementation of *Ubp43*<sup>-/-</sup> cells with an enzymatically inactive mutant UBP43 inhibits IFN-induced STAT1 phosphorylation to similar levels as observed in wildtype cells (Malakhova et al. 2006). In line with this, STAT1 phosphorylation is barely affected despite the downregulation of total ISGylation levels in fibroblasts derived from *Ube1l*<sup>-/-</sup> mice (Kim et al. 2006).

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## Inhibition of Virus Assembly, Budding and Release

### Viperin (RSDA2, CIG5)

*Viperin* was cloned as human cytomegalovirus (HCMV)-induced gene in human fibroblasts (Zhu et al. 1997) and as IFN $\gamma$ -activated gene in human macrophages (Chin and Cresswell 2001). The former study defined viperin as an ER-associated protein with anti-HCMV activity in human fibroblasts. Antiviral activity of viperin was subsequently shown for HCV (Helbig et al. 2005; Jiang et al.



2008), HIV-1 (Rivieccio et al. 2006), FLUAV (Wang et al. 2007), SINV (Zhang et al. 2007), WNV and DV (Jiang et al. 2010). Concerning the mechanism of action, detailed studies are only available for FLUAV infections (Wang et al. 2007). Viperin strongly impairs FLUAV release and, consequently, reduces virus replication in stably transfected HeLa cells. The authors demonstrate that inhibition of virus release occurs *via* disruption of plasma membrane lipid rafts. Viperin expression results in higher fluidity of membranes and, correspondingly, in an increased mobility of the viral haemagglutinin protein. No effect of viperin was observed in the course of VSV infection, a virus whose replication is believed to be lipid raft-independent. Additionally, farnesyl diphosphate synthase (FPPS) was identified as viperin-interacting protein and overexpression of FPPS reversed the effects of viperin on FLUAV replication. Consistently, siRNA-mediated knockdown of FPPS reduces virus release. Thus viperin exerts antiviral activity by sequestering FPPS to the ER and inhibiting its enzymatic activity (Wang et al. 2007). FPPS has important roles in isoprenoid biosynthesis by catalyzing the formation of farnesyl diphosphate, the precursor of sterols, dolichols, carotenoids, and ubiquinones (Szkopinska and Plochocka 2005). It remains to be determined which of the FPPS-regulated pathways is essentially involved in facilitating viral release.

Viperin might inhibit other viruses by different mechanisms, however, data are still scarce. Retrovirally expressed viperin inhibits late, but not early, HCMV protein accumulation and strongly reduces HCMV replication in human fibroblasts (Chin and Cresswell 2001). Viperin relocates from the ER to the Golgi and to vacuoles, the sites of viral glycoprotein maturation and viral assembly, respectively. It is unclear if relocation of viperin is beneficial or detrimental for viral replication. In the case of HCV and WNV, ER-association of viperin is important, but not absolutely required, for its antiviral activity (Jiang et al. 2008, 2010). Recently, viperin has been structurally characterized in more detail and its proposed S-adenosyl-L-methionine (SAM) enzyme activity catalyzing the formation of 5'-deoxyadenosyl radicals was confirmed (Shaveta et al. 2010). This is of particular importance, as enzymatic activity is required for the effect against HCV (Jiang et al. 2008), WNV and DV (Jiang et al. 2010).

### **Bone Marrow Stromal Antigen 2 (BST-2, Tetherin, mPDCA-1, CD137)**

BST-2 is a small type II transmembrane protein that associates with lipid rafts at the cell surface or with internal membranes (Andrew and Strebel 2011). The *BST-2* promoter region contains GAS and ISRE consensus sites and is thus likely induced by type I and type II IFN (Ohtomo et al. 1999). BST-2 antiviral function was first suggested for HIV-1. HIV-1 requires its Vpu gene product for virus release in a cell type-specific manner. BST-2 has been identified as a virus restriction factor by its ability to induce a Vpu-restricted phenotype in cells that otherwise show Vpu-independent HIV-1 release (Andrew and Strebel 2011). BST-2 was also found to

inhibit virus release of all retroviruses tested, but also of arenavirus (i.e. LASV), filovirus (i.e. EBOV, MARV) and rhabdovirus (i.e. VSV) family members (Jouvenet et al. 2009; Sakuma et al. 2009; Weidner et al. 2010). The exact mechanism of how BST-2 inhibits virus release is still unclear, but BST-2 tethers virions to the producer cell. BST-2 might contribute to IFN-mediated antiviral activity against a wider range of enveloped viruses that bud from the host cell plasma membrane, however, this remains to be shown. On the other hand, BST-2 might also be proviral in the sense that it enables a shift of the mode of viral transmission, i.e. cell-to-cell versus cell-free spread (Andrew and Strebel 2011).

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## Additional Pathways of Viral Inhibition

### Tripartite Motif (TRIM) Proteins

The TRIM family has been originally defined as proteins that contain a so-called tripartite motif consisting of a RING domain, one or two B-boxes, and a coiled-coil domain. The RING domain of many TRIM proteins has an E3 ubiquitin ligase activity, whereas the other two domains may be involved in mediating protein-protein interactions. TRIM proteins form high-molecular-mass complexes and localize to specific subcellular compartments either in the nucleus or the cytoplasm. TRIM proteins are involved in diverse cellular processes like cell growth, apoptosis and innate immunity (Ozato et al. 2008). The TRIM family consists of 72 genes in humans, whereby 16 *TRIM* genes are induced by type I and/or type II IFN (Carthagen et al. 2009). Among the TRIM proteins, TRIM5, TRIM19 and TRIM22 are strongest upregulated by type I and type II IFN. No homologs of TRIM5 and TRIM22 have been found in mice (Carthagen et al. 2009). Several members have reported antiviral activity that is exerted at multiple levels (Kajaste-Rudnitski et al. 2010), but their contribution to the IFN-mediated antiviral response is largely unknown.

TRIM19, better known as promyelocytic leukaemia (PML) protein, is a constitutive component of PML nuclear bodies (PML NBs), which are small nuclear substructures. PML, Sp100 and small ubiquitin-like modifier (SUMO) are constitutively present in PML NBs and many other proteins are transiently or permanently associated with PML NBs. PML NBs are potentially highly dynamic and the proteins associated can vary between cell types. PML is expressed constitutively, but type I and type II IFNs lead to a strong increase in the size and number of PML NBs. PML exists as many different isoforms that are generated by alternative splicing and grouped into seven classes (PML I-VII). PML isoforms likely have different functions, which still remain to be defined. In addition, Sp100, Daxx and other PML NB proteins are IFN-inducible. PML NBs are involved in the regulation of chromatin structure, transcription and DNA repair, unfolded protein responses and, in the regulation of apoptosis. Since PML NBs are constitutively present, numerous reports described their contribution to the intrinsic antiviral defence against a broad range of DNA and RNA viruses. The exact mechanisms of viral inhibition are still largely unknown, but they involve inhibition of viral mRNA and protein synthesis (Geoffroy and Chelbi-Alix 2011).

Despite the constitutive expression of PML NB constituents, the IFN-mediated induction of PML and Sp100 has in several cases been linked to the antiviral function of PML NBs. For example, the IFN-mediated antiviral effect against an HSV-1 ICP10 mutant virus that lacks the ability to disrupt PML NBs was strongly reduced in *Pml*<sup>-/-</sup> cells (Chee et al. 2003). Reduced IFN-induced protection against LCMV (Djavani et al. 2001), human foamy virus (HFV) (Regad et al. 2001) and rabies virus (RV) (Blondel et al. 2010) was reported in *Pml*<sup>-/-</sup> fibroblasts and *Pml*<sup>-/-</sup> mice are more susceptible to LCMV and VSV infection (Table 1) (Bonilla et al. 2002).

PML and PML NBs can also affect p53 activity. IFN-induced p53 has been shown to be required for the induction of apoptosis and the antiviral defence (Porta et al. 2005; Takaoka et al. 2003). In the case of poliovirus (PV) infections, p53 activation and target gene expression is dependent on the presence of PML. Induction of apoptosis and the resulting inhibition of virus replication is abolished upon siRNA-mediated downregulation of PML and enhanced by PML III overexpression. PV infection induces PML phosphorylation and SUMOylation leading to enhanced recruitment and activation of p53 (Pampin et al. 2006).

In contrast to the direct antiviral activity of TRIM19, TRIM21 and TRIM25 have emerged as crucial components of IFN $\alpha/\beta$ -inducing pathways (see below). Using ectopic expression and siRNA knockdown experiments, it has just recently been shown that TRIM56 inhibits BVDV replication. The mechanism is unclear, but the antiviral activity was dependent on its ubiquitin ligase activity and not effective against either VSV or HCV (Wang et al. 2011).

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## Virus Recognition and Amplification of Type I IFN Production

Several proteins involved in virus recognition and IFN $\alpha/\beta$  production are long-known IFN-inducible proteins whose functions have only recently been identified. Although these do not directly impact on virus replication, their proper function is essential for sensing virus infections and initiating antiviral responses, i.e. by directly or indirectly inducing genes with antiviral effector function and/or by inducing autocrine/paracrine IFN $\alpha/\beta$  signalling (Kawai and Akira 2009; McCartney and Colonna 2009). Proteins involved in virus recognition, including RNA sensors like RIG-I (DDX58) and MDA5 (IFIH1), as well as proteins implicated in the recognition of cytoplasmic DNA, e.g. DNA-dependent activator of IFN-regulatory factors (DAI, ZBP1, DLM-1) and IFN $\gamma$ -inducible protein 16 (IFI16, p204) are induced by IFNs (Cui et al. 2004; Fu et al. 1999; Imaizumi et al. 2004; Kang et al. 2002; Trapani et al. 1992). The IFN-inducible protein absent in melanoma 2 (AIM2, IFI210) has also been identified as a cytoplasmic DNA-sensor, but associates with the inflammasome and does not lead to IFN $\alpha/\beta$  induction upon activation. IFI202 belongs to the same HIN200 family of IFN-inducible proteins as IFI16 and AIM2, but is a negative regulator of AIM2 at least in murine cells. Notably, there are also indications that MCMV and HCMV require IFI16 for replication (Gariglio et al. 2011).

Type I IFN also mediates its own amplification *via* the induction of IRF7. Similar to IRF3, IRF7 gets activated by several PRRs and transcriptionally induces IFN $\alpha/\beta$ . Although IRF3 and IRF7 recognize similar consensus sequences in promoter regions, IRF3 mainly regulates IFN $\alpha 4$  and IFN $\beta$ , whereby IRF7 is required for the induction of other IFN $\alpha$  subtypes. Different from the constitutively expressed IRF3, IRF7 is normally not expressed (except for specialized cell types) unless it is induced by IFNs. Hence, IRF7 mediates a switch from low-level to high-level of IFN $\alpha/\beta$  production and broadens the range of IFN $\alpha$  subtype expression in response to virus infections (Levy et al. 2002).

TRIM21 was found to interact with IRF3 and to increase IRF3 stability and downstream target gene expression upon SeV infection (Yang et al. 2009). TRIM21 can also negatively impact on IFN $\alpha$  production. Together with FAS-associated death domain (FADD) protein, TRIM21 directly ubiquitinates IRF7 and reduces its phosphorylation, thereby limiting IFN $\alpha/\beta$  production in response to virus infection (Young et al. 2010). TRIM25 has emerged as central component of the RIG-I pathway. TRIM25 directly interacts and ubiquitinates RIG-I which is required for its binding to the mitochondrial antiviral-signaling protein (MAVS, IPS1, VISA, Cardiff) and the induction of IFN $\alpha/\beta$  (Gack et al. 2007).

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## Concluding Remarks and Future Directions

The complexity of host-virus interactions remains a challenging field for future research on innate immune responses. While specificity and molecular mechanisms of viral recognition are important issues, further characterization of antiviral functions of specific ISGs seems equally essential. Many studies have so far only been performed *in vitro* and/or in overexpression studies and it will be of particular interest how these ISGs impact on antiviral responses under physiological conditions and *in vivo*. This might not be trivial, as there is most likely a high redundancy of pathways and ISGs can affect viral life cycles at multiple levels. Moreover, several ISGs also exert important, but not fully characterized, cellular functions. Genome-wide association studies in human populations provide another important ongoing future direction. Genetic polymorphisms in any of the ISG loci might reveal association with virus susceptibility or other potential novel immune regulatory functions. Besides, a lot of information has been gained by studies of virus mutants. Viruses have evolved very efficient mechanisms to counteract the host immune system and further analysis from this perspective will help to decipher the multiple levels of virus-host interactions. The more detailed understanding of the exact interplay between viral and cellular factors might help to better control virus infections and to more specifically direct antiviral responses.

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