The broad-spectrum antiviral functions of IFIT and IFITM proteins

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Abstract | Over the past few years, several groups have identified new genes that are transcriptionally induced downstream of type I interferon (IFN) signalling and that inhibit infection by individual or multiple families of viruses. Among these IFN-stimulated genes with antiviral activity are two genetically and functionally distinct families — the IFN-induced protein with tetratricopeptide repeats (IFIT) family and the IFN-induced transmembrane protein (IFITM) family. This Review focuses on recent advances in identifying the unique mechanisms of action of IFIT and IFITM proteins, which explain their broad-spectrum activity against the replication, spread and pathogenesis of a range of human viruses.

IFN-stimulated genes (ISGs). Genes that are induced by interferons or interferon-regulatory factors and have antiviral or immunomodulatory functions.

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To control infection by viruses, host cells must recognize invasion and develop a rapid and effective antiviral response. In mammalian cells, this response is initiated after the detection of non-self pathogen-associated molecular patterns (PAMPs), including single-stranded and double-stranded viral nucleic acids. These viral PAMPs are detected by specific host pattern-recognition receptors (PRRs) in endosomes and within the cytoplasm[1,2]. Such PRRs include Toll-like receptors (TLRs; specifically TLR3, TLR7, TLR8 and TLR9), RIG-I-like receptors (such as melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I)) and DNA sensors (namely DNA-dependent activator of IRFs (DAI; also known as ZBP1), IFNγ-inducible protein 16 (IFI16), DEAH box protein 9 (DHX9) and DHX36). The binding of viral PAMPs to these PRRs triggers signalling cascades that induce the expression of virus-responsive genes and pro-inflammatory cytokines (such as type I interferons (IFNs)), which restrict virus replication and modulate adaptive immunity (FIG. 1).

IFN signalling induces a broad and potent antiviral response against most viruses that infect vertebrate animals. Type I IFNs are a family of functionally and genetically related cytokines consisting of several members, with IFNα and IFNβ being the most extensively studied[3]. Type I IFN signalling is mediated through a common receptor, the IFNα/β receptor (IFNAR), which is a heterodimer of IFNAR1 and IFNAR2 (REF. 4). Signal transduction following the binding of a type I IFN to IFNAR occurs via Janus kinase (JAK) and signal transducer and activator of transcription (STAT) proteins and results in the translocation into the nucleus of the transcription factor complex IFN-stimulated gene factor 3 (ISGF3; which is comprised of IFN-regulatory factor 9 (IRF9) and phosphorylated STAT1 and STAT2). Nuclear ISGF3 induces the transcription of hundreds of different IFN-stimulated genes (ISGs); indeed, it is estimated that 500 to 1,000 genes are induced per cell or tissue type[5–7]. These ISGs encode distinct proteins with diverse biological effects that block multiple stages of the viral replication cycle, including entry into host cells, protein translation, replication, assembly of new virus particles and spread. They can also have immunomodulatory functions, including effects on leukocyte recruitment and the priming of adaptive immunity. Beyond this, a subset of ISGs is induced in an IFN-independent manner after viral infection through the actions of transcription factors (such as IRF3) that respond directly to signals downstream of PRRs.

Although the first antiviral ISGs were discovered decades ago (reviewed in REF. 8), until recently most experimental effort has been restricted to defining the mechanisms of action of a limited number of proteins, including RNA-activated protein kinase (PKR), ribonuclease L (RNase L), myxoma resistance protein 1 (MX1) and oligoadenylate synthases (OASs). More recent studies have expanded the analysis to several other ISGs, including those encoding APOBEC3 (REF. 9), BST2 (also known as tetherin)[10], ISG15 (REF. 11) and RSAD2 (also known as viperin)[12]. Moreover, progress has been made in understanding the IFN-mediated mechanisms that control particular

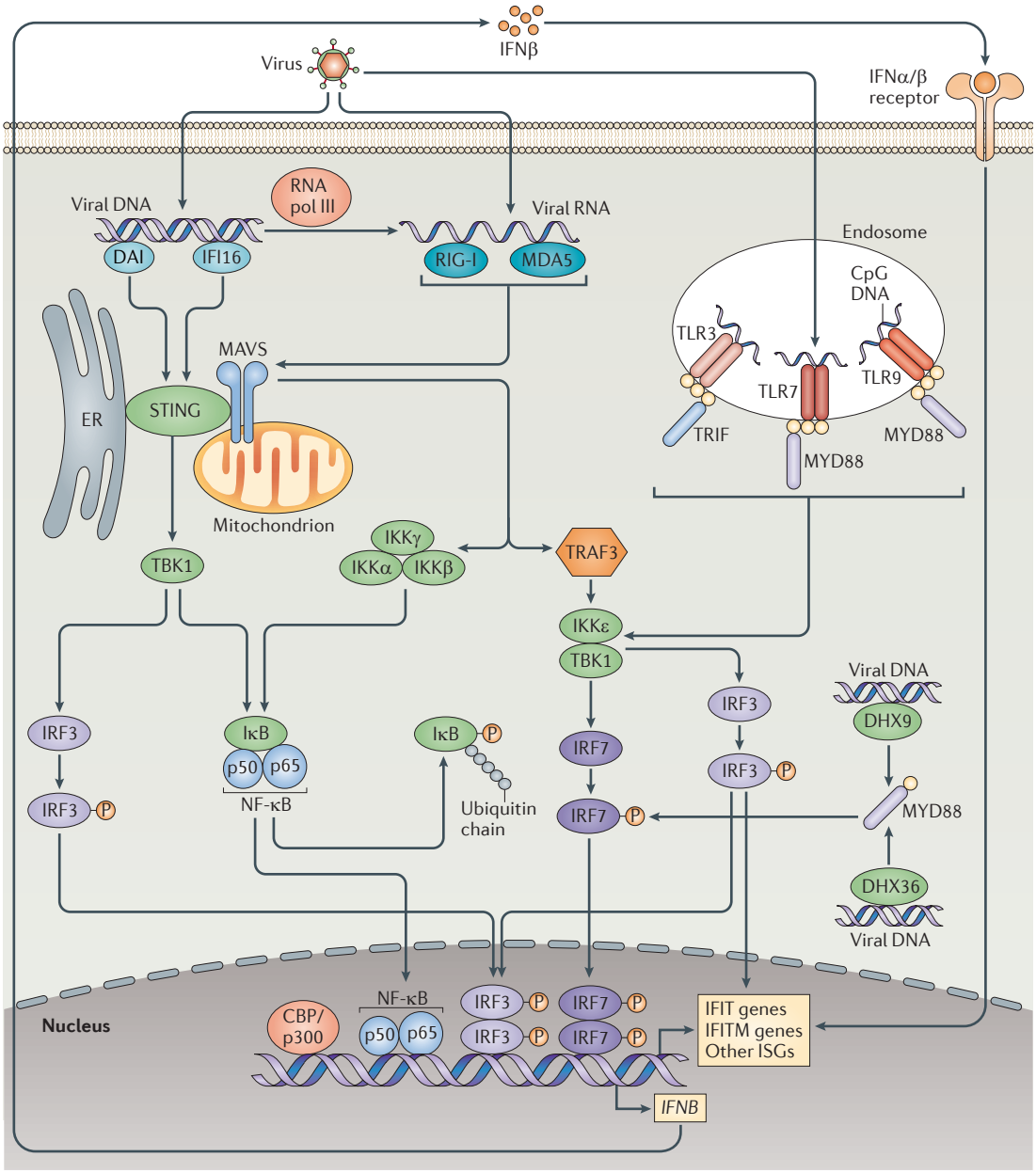


Figure 1 | **Detection of pathogen RNA and DNA in the cytoplasm and activation of *IFNB* and ISGs.** IFN-induced protein with tetratricopeptide repeats (IFIT) genes and IFN-induced transmembrane protein (IFITM) genes are induced by host innate immune defences after pathogen infection. The figure shows a scheme of innate immune signalling triggered by viral infection. Viral RNA and DNA is detected by: cytosolic RIG-I-like receptors (RLRs), such as melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I); cytosolic DNA sensors, such as DNA-dependent activator of IRFs (DAI), IFNγ-inducible protein 16 (IFI16), DEAH box protein 9 (DHX9) and DHX36; and endosomal Toll-like receptors (TLRs), including TLR3, TLR7 and TLR9. Infection by RNA viruses produces RNA intermediates that are recognized as non-self by RIG-I and MDA5 in the cytosol and by TLR3 and TLR7 in endosomes. The RLRs interact with mitochondrial antiviral signalling protein (MAVS), leading to the recruitment of TNFR-associated factor 3 (TRAF3), TANK-binding kinase 1 (TBK1) and IκB kinase-ε (IKKε), or of IKKγ (also known as NEMO), IKKα and IKKβ, which results in the activation and nuclear translocation of IFN-regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB), respectively. TLRs interact with the adaptor proteins TRIF and MYD88, leading to the activation of IRF3 or IRF7. IRF3, IRF7 and NF-κB bind to the interferon-β (*IFNB*) gene promoter and induce transcription. Secretion of IFNβ by the infected cells results in paracrine type I IFN signalling through the IFNα/β receptor, which induces hundreds of IFN-stimulated genes (ISGs). Phosphorylated IRF3 also can activate the expression of ISGs (such as IFIT and IFITM genes) independently of IFN signalling. DNA can be present in the cytoplasm and in endosomes during viral or bacterial infection and following the phagocytosis of dead cells. TLR9 recognizes CpG DNA in endosomes and activates MYD88. The binding of DNA by DAI or IFI16 results in stimulator of IFN genes (STING)-dependent activation of IRF3 and NF-κB. RNA polymerase III transcribes this DNA to produce short RNAs containing a 5′-ppp motif, which are ligands for RIG-I. DHX9 and DHX36 bind to DNA ligands (such as CpG-A and CpG-B DNA) in the cytosol and induce MYD88- and IRF7-dependent responses. ER, endoplasmic reticulum; IκB, NF-κB inhibitor.

families of viruses (such as retroviruses[13]) and the ways in which these viruses can evade such control. In addition, systematic investigations of the antiviral functions of large groups of ISGs using ectopic gene screens[14,15] have identified genes that coordinately control infection by several families of RNA and DNA viruses. There has been a resurgence of interest in defining ISGs with broad-spectrum antiviral activity, possibly as a means to identify new classes of drugs that activate these genes directly. Indeed, antiviral therapies that target host proteins rather than viral proteins could in theory minimize the emergence of resistance and the collateral effects associated with type I IFN therapy that limit its current clinical use. This Review describes recent advances in understanding the antiviral activity and mechanisms of action of two particular ISG families with broad-spectrum antiviral activity: the IFN-induced protein with tetratricopeptide repeats (IFIT) and IFN-induced transmembrane protein (IFITM) families. Although these families are genetically and functionally distinct, a combined analysis of IFIT and IFITM proteins clarifies more generally how specific ISGs inhibit the replication, spread and pathogenesis of a range of human viruses.

The IFIT family

The gene and protein family. IFIT genes encode a family of proteins that are induced after IFN treatment, viral infection or PAMP recognition[16] (FIG. 2a). IFIT genes have a similar genomic structure, in that most of these genes are composed of two exons, with the second exon containing almost all of the coding sequence. IFIT gene homologues have been reported in several mammalian species, as well as in birds, fish and amphibians (reviewed in REF. 17). Four family members have been characterized in humans: *IFIT1* (also known as *ISG56*), *IFIT2* (also known as *ISG54*), *IFIT3* (also known as *ISG60*) and *IFIT5* (also known as *ISG58*). All four of these genes are located on chromosome 10q23. By contrast, three members are expressed in mice — *Ifit1* (also known as *Isg56*), *Ifit2* (also known as *Isg54*) and *Ifit3* (also known as *Isg49*) — and they are located on chromosome 19qC1. Additional uncharacterized but highly related IFIT genes (namely *IFIT1B* in humans and *Ifit1b*, *Ifit1c* and *Ifit3b* in mice) exist in the same chromosomal regions as the known IFIT genes, although their functional significance and expression patterns remain undefined. Moreover, a non-transcribed *IFIT1*-related pseudogene is present on human chromosome 13 (REF. 18).

IFIT proteins are localized within the cytoplasm and ostensibly lack any enzymatic domains or activity. However, they contain multiple tetratricopeptide repeats (TPRs). The TPR motif is present in various host proteins and is composed of 34 amino acids that adopt a helix–turn–helix structure and mediate protein–protein interactions. Proteins containing TPR motifs regulate the cell cycle, transcription, protein transport and protein folding[19]. The sequence identity between human and mouse IFIT orthologues ranges from 52% to 62%, but there is less similarity (~40–45%) between orthologues in other species[16], suggesting that mouse and human IFIT proteins were generated by the duplication of a common ancestral gene. However, different IFIT family members have been predicted by sequence analysis to have distinct numbers of TPR motifs, which may dictate specific functions. For example, IFIT1 and IFIT2 were predicted to have six and four TPR motifs, respectively[20].

Structure. A recent paper published the first X-ray crystallographic structure of an IFIT family member — that of human IFIT2 (REF. 21) (FIG. 2b). By determining the structure with a resolution of 2.8 Å, the authors showed that IFIT2 monomers actually have nine TPR motifs and form domain-swapped dimers. Moreover, IFIT2 has a positively charged carboxy-terminal region that supports RNA binding, and the mutation or deletion of charged residues in this region altered viral RNA binding and negatively affected antiviral activity against Newcastle disease virus. This study also suggested that IFIT2 can bind to RNA containing AU-rich elements, which are sometimes found in mRNAs encoding cytokines or apoptotic factors, indicating a potential mechanism by which IFIT proteins might regulate inflammatory responses (see below).

Expression. Most cell types do not express IFIT proteins under basal conditions, with the possible exception of some myeloid cell subsets[22]. However, the transcription of IFIT genes is induced rapidly to high levels in many cells after virus infection[20]. This expression pattern is determined in part by the upstream promoter regions of IFIT genes, which contain IFN-stimulated response elements (ISREs)[23–25]. Accordingly, *Ifit1* and *Ifit2* are induced within 2 hours of exogenous IFNα treatment[24], but less so after exposure to IFNγ[5]. Moreover, the expression kinetics of individual IFIT genes have been reported to be cell type and tissue specific[26–29]. IFIT mRNA levels after IFN stimulation can be sustained or transient depending on the cell type. In some cells, subsets of IFIT genes are induced selectively after stimulation with type I IFNs or following viral infection[30]. The differential expression of individual IFIT genes in a given cell or tissue is hypothesized to confer non-redundant antiviral functions against particular viral infections[28,29].

IFIT gene expression can also be triggered independently of type I IFNs, through signals generated after the ligation of PRRs (such as TLR3, TLR4, MDA5 and RIG-I) by PAMPs (such as double-stranded RNA and lipopolysaccharide (LPS)). Indeed, IFIT genes have been described as viral stress-inducible genes[20] and are induced at the transcriptional level directly by IRF3 (REFS 31,32), which is activated soon after viral infection, often before the induction of type I IFNs. Other IRF proteins (such as IRF1, IRF5 and IRF7) also can induce the expression of IFIT genes directly[33,34], presumably after the stimulation of host defence signalling cascades, although these pathways remain less well defined. Human IFIT genes are also induced by retinoic acid[35], although this mechanism is slower than PAMP-dependent induction and might be regulated in part by IFNα induction[34].

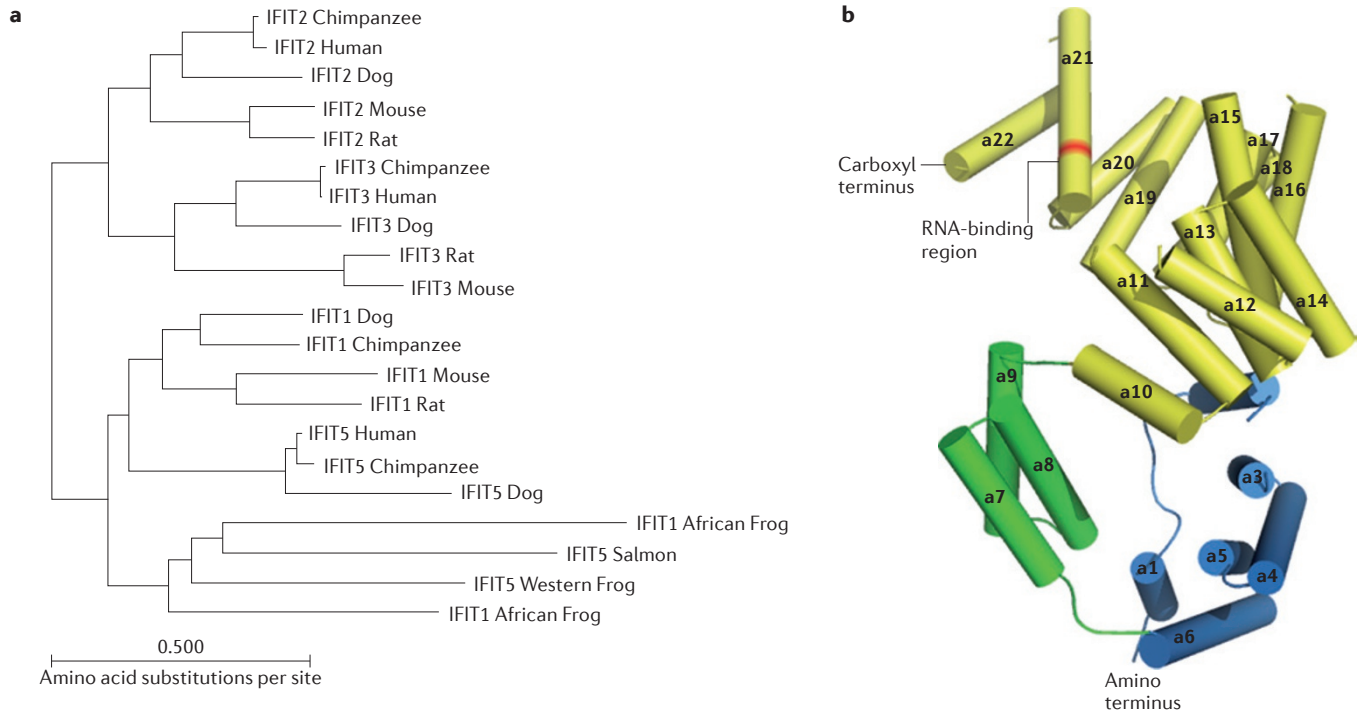


Figure 2 | **Genomic relationship and structure of IFIT proteins.** **a** | The phylogram shows the relationships between proteins of the IFN-induced protein with tetratricopeptide repeats (IFIT) family in different species. All full-length IFIT protein sequences for eight species (human, mouse, rat, chimpanzee, dog, frog, toad and salmon) were obtained from the National Center for Biotechnology Information (NCBI) database. IFIT-like and duplicate amino acid sequences were removed manually or using ElimDupes. Amino acid alignments were generated using CLC Main Workbench. A tree was created from the alignment using the neighbour-joining method and 1,000 bootstrap replicates. The scale of branch length is shown below the tree. **b** | The cartoon diagram shows the structure of the human IFIT2 monomer (PDB ID: 4G1T), with α-helical structural elements shown as cylinders. The amino-terminal region (blue), domain-swapped region (green) and carboxy-terminal region (yellow) are shown. The RNA-binding region is located near the C-terminus and is labelled in red (residue K410). The figure was prepared using PyMOL and is adapted, with permission, from REF. 21

Antiviral activity of IFIT proteins

Given their rapid induction pattern after type I IFN treatment or PRR activation, IFIT proteins are poised to confer inhibitory effects after infection. Recently, progress has been made in identifying how IFIT proteins inhibit the replication of multiple families of viruses through distinct mechanisms of action.

Translation inhibition. Eukaryotic initiation factor 3 (eIF3) is a multisubunit protein complex that functions in translation initiation at several steps, including assembly of the eIF2–GTP–Met-tRNA ternary complex, formation of the 43S pre-initiation complex, mRNA recruitment to the 43S pre-initiation complex, and scanning of the mRNA for the start codon (AUG) (reviewed in REF. 36). Biochemical studies suggest that some IFIT family members reduce the efficiency of cellular cap-dependent protein translation by binding to subunits of the eIF3 translation initiation complex[37]. Human IFIT1 and IFIT2 can block the binding of eIF3 to the eIF2–GTP–Met-tRNA ternary complex by interacting with eIF3E, whereas human IFIT2, and mouse IFIT1 and IFIT2, can block the formation of the 43S–mRNA complex (also known as the 48S complex) by binding to eIF3C[27,37,38] (FIG. 3).

Cap-dependent protein translation
The initiation of translation in eukaryotic cells usually involves the interaction of certain translation initiation factors with an N7-methylguanosine cap at the 5′ end of the mRNA molecule.

Hepatitis C virus (HCV), a positive-stranded RNA virus, contains an internal ribosome entry site (IRES), which regulates the assembly of cap-independent translation initiation complexes on viral mRNA by a sequential pathway requiring eIF3 (REF. 39). Type I IFNs inhibit HCV infection by blocking translation of the HCV RNA[40,41]. Examination of the cellular proteins associated with HCV translation complexes in IFN-treated human cells showed that human IFIT1 is an eIF3-associated factor that fractionates with the initiator ribosome–HCV RNA complex[41]. IFIT1 suppressed the function of the HCV IRES, whereas a mutant IFIT1 protein lacking eIF3-binding activity failed to inhibit HCV replication. Moreover, ectopic expression of IFIT1 decreased HCV infection in hepatocytes[42]. Thus, IFIT1 seems to block HCV replication by targeting eIF3-dependent steps in the viral RNA translation initiation process; these steps include the recognition of the 43S pre-initiation complex by the HCV IRES and the assembly of the 43S–mRNA complex (FIG. 3).

Recognizing a lack of 2′-O methylation. The cellular mRNAs of higher eukaryotes and many viral RNAs are methylated at the *N*-7 and 2′-*O* positions of the 5′ guanosine cap by nuclear and cytoplasmic methyltransferases.

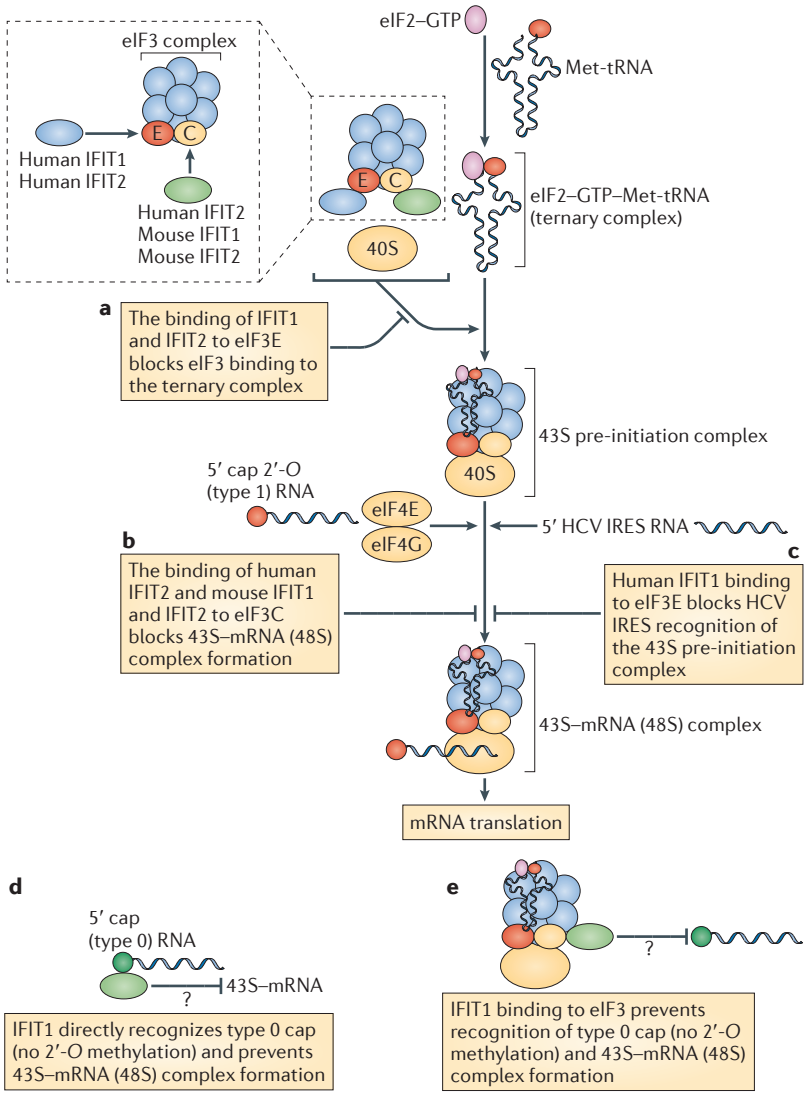


Figure 3 | **IFIT proteins function as antiviral molecules by inhibiting distinct steps in the translation of viral mRNA.** IFN-induced protein with tetratricopeptide repeats (IFIT) proteins bind to subunits of the eukaryotic initiation factor 3 (eIF3) multisubunit complex that regulates translation initiation. Human IFIT1 and IFIT2 bind to eIF3E, and human IFIT2, mouse IFIT1 and mouse IFIT2 bind to eIF3C. The figure shows a schematic diagram of translation initiation and the steps putatively blocked by IFIT family members. To begin translation in mammalian cells, free 40S ribosomal subunits are stabilized by eIF3 and bind to the ternary complex (eIF2–GTP–Met-tRNA) in the presence of eIF1 (not shown). This allows the assembly of the 43S pre-initiation complex, which then binds to mRNA that is capped at the 5′ end and methylated at the *N*-7 and 2′-*O* positions. This interaction is stabilized by eIF4E and eIF4G, and results in the formation of the 43S–mRNA complex, which is competent for AUG (start codon) scanning and mRNA translation. For hepatitis C virus (HCV) genomic RNA with an internal ribosome entry site (IRES), association with eIF4E and eIF4G or other cap-binding factors is not required to stabilize the 43S–mRNA complex. IFIT proteins can inhibit translation through several mechanisms[27,37,38,40,41]. One, the interaction of IFIT1 and IFIT2 with eIF3E blocks the binding of eIF3E to the ternary complex (eIF2–GTP–Met-tRNA) (**a**). Two, the binding of human IFIT2, and mouse IFIT1 and IFIT2, to eIF3C blocks the formation of the 43S–mRNA complex (**b**). Three, the binding of human IFIT1 to eIF3E prevents the recognition of the HCV IRES by the 43S complex. Disruption of eIF3 binding to the HCV IRES also can prevent eIF2 recruitment and suppresses ternary complex formation (**c**). IFIT1 can also inhibit the translation of viral RNA lacking 2′-*O* methylation through two possible mechanisms. One, IFIT1 may directly recognize the type 0 cap structure (no 2′-*O* methylation) on viral RNA and prevent its binding to the 43S pre-initiation complex (**d**). Two, the binding of IFIT1 to eIF3 may preferentially prevent the formation of the 43S–mRNA complex for RNA containing type 0 cap structures (**e**).

Whereas *N*-7 methylation is essential for RNA translation and stability, the function of 2′-*O* methylation had remained uncertain[43,44]. Recent studies showed that a West Nile virus (WNV) mutant lacking 2′-*O* methyltransferase activity was attenuated in wild-type cells and mice but was pathogenic in the absence of *Ifit1* expression[45,46]. The mutant virus lacking 2′-*O* methyltransferase activity had higher levels of replication in the peripheral tissues of *Ifit1*$^{-/-}$ mice than in wild-type mice after subcutaneous infection, and the lethal dose (LD_{50}) of this virus was 16,000-fold lower in *Ifit1*$^{-/-}$ mice than in wild-type mice. 2′-*O* methylation of viral RNA did not affect IFN induction in WNV-infected cells but instead modulated the antiviral effects of IFIT proteins. Moreover, poxvirus and coronavirus mutants that lacked 2′-*O* methyltransferase activity were more sensitive to the antiviral actions of IFIT proteins than their wild-type counterparts[45,47]. It remains unclear whether IFIT proteins inhibit viruses that lack 2′-*O* methylation at the stage of protein translation by directly recognizing non-2′-*O*-methylated viral RNA, thereby preventing the recognition of viral RNA by the 43S pre-initiation complex, or by serving as a scaffold for other proteins that regulate translation (FIG. 3). Wild-type alphaviruses of the *Togaviridae* family, which are positive-stranded cytoplasmic RNA viruses, lack 2′-*O* methylation on their viral RNA[48] and, thus, should be sensitive to IFIT-mediated restriction. Although further mechanistic studies are warranted, in support of this hypothesis ectopic expression of IFIT1 inhibited infection by Sindbis virus, and, reciprocally, silencing of *Ifit1* expression resulted in enhanced infection[49].

5′-ppp RNA recognition. A recent study indicates that human IFIT1 can also function as a sensor for viral RNA by recognizing an uncapped 5′-ppp and sequestering the RNA from the actively replicating pool[50] (FIG. 4). Using a proteomics approach with 5′-ppp RNA as bait, a mass spectrometry analysis identified IFIT1 as a primary binding partner. Subsequent experiments showed that only IFIT1 interacts directly with 5′-ppp on RNA, whereas IFIT2 and IFIT3 form a complex with IFIT1 that is required for antiviral function. These IFIT-dependent interactions were relevant in protecting against RNA viruses displaying a 5′-ppp, as silencing of IFIT1, IFIT2 and IFIT3 expression in HeLa cells enhanced the replication of the negative-stranded RNA viruses Rift Valley fever virus (RVFV), vesicular stomatitis virus (VSV) and influenza A virus to varying degrees, despite the fact that the production of mRNA encoding IFNβ was unaffected. By contrast, ectopic expression of individual IFIT proteins in cells did not confer an inhibitory effect on these viruses, suggesting that the IFIT protein complex is required for this antiviral activity. Studies with *Ifit1*$^{-/-}$ mouse fibroblasts and myeloid cells also showed enhanced replication of VSV despite wild-type production levels of type I IFNs and other pro-inflammatory cytokines. *In vivo*, *Ifit1*$^{-/-}$ mice were more vulnerable to infection with VSV, with higher virus-induced mortality observed. However, and in apparent conflict, experiments by a second group using the same VSV strain but an independently

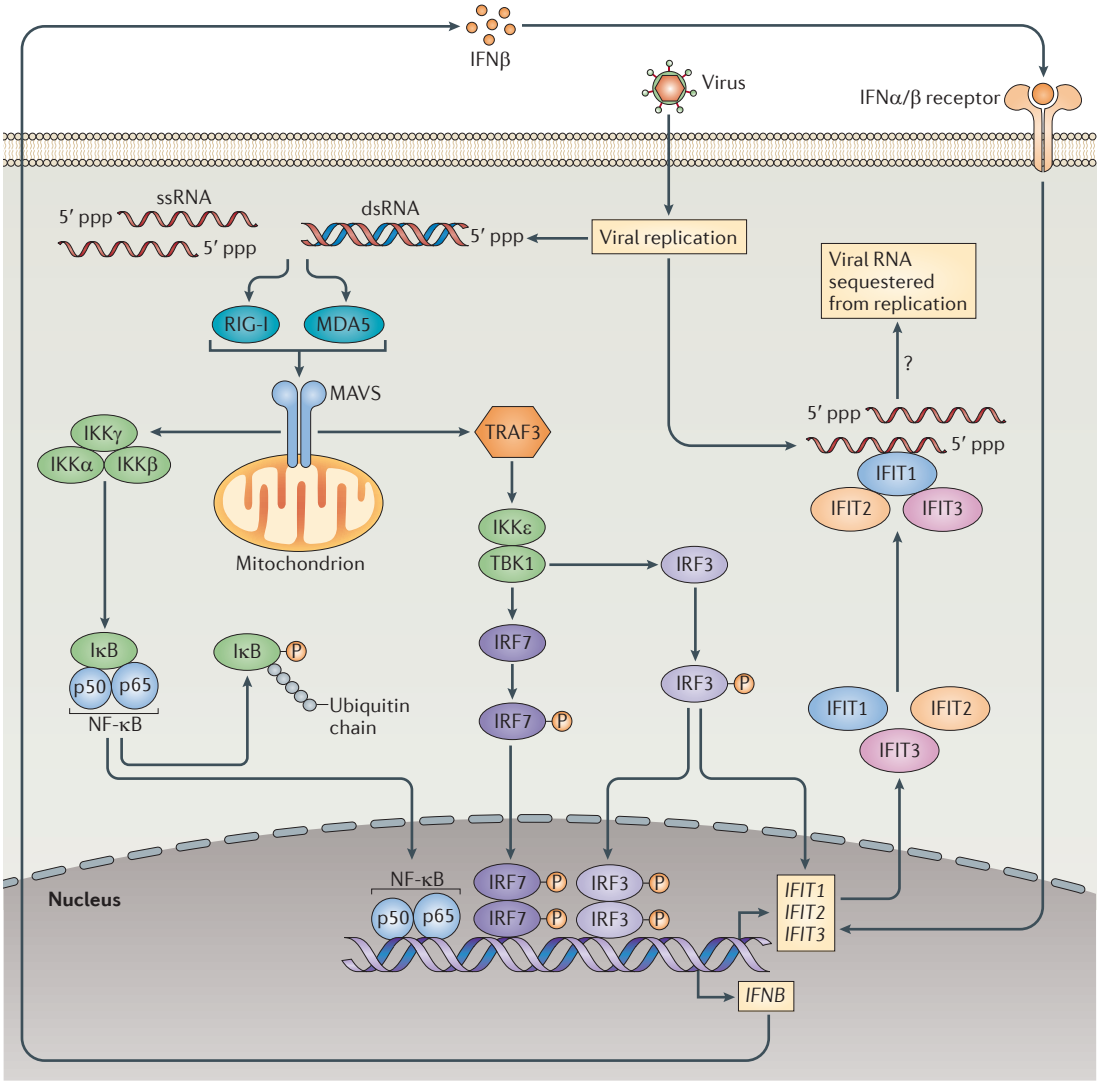


Figure 4 | **IFIT proteins recognize the 5′-ppp of viral RNA and inhibit infection.** Viral infection by negative-stranded RNA viruses (such as Rift Valley fever virus, vesicular stomatitis virus and influenza A virus) generates single- or double-stranded RNA with uncapped 5′-ppp motifs. These RNA molecules are recognized by the cytoplasmic sensors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). RIG-I and MDA5 induce the expression of IFN-stimulated genes (ISGs) — including IFN-induced protein with tetratricopeptide repeats 1 (IFIT1), IFIT2 and IFIT3 — through both interferon-β (IFNβ)-dependent pathways and IFNβ-independent (for example, IFN-regulatory factor 3 (IRF3)-dependent) pathways. IFIT1 functions as a sensor of viral RNA containing the 5′-ppp motif, resulting in the assembly of an IFIT1–IFIT2–IFIT3 complex. This presumably inhibits viral infection by sequestering RNA from the actively replicating pool or by promoting RNA degradation. Data are conflicting regarding whether IFIT proteins also promote or inhibit the host inflammatory response, possibly by changing the relative amount of viral RNA in the cell with free 5′-ppp ends. dsRNA, double-stranded RNA; IκB, NF-κB inhibitor; IKK, IκB kinase; MAVS, mitochondrial antiviral signalling protein; NF-κB, nuclear factor-κB; ssRNA, single-stranded RNA; TBK1, TANK-binding kinase 1; TRAF3, TNFR-associated factor 3, Part of this figure is adapted, with permission, from REF. 81

Internal ribosome entry site
(IRES). An RNA sequence that allows for the recruitment of the translation machinery in a manner that is independent of the 5′ end of the mRNA (cap-independent translation).

2′-*O* methylation
A modification of cellular and/or viral RNA. In mammalian cells, this modification seems to prevent translation inhibition by IFIT proteins.

Lethal dose
(LD_{50}). The LD_{50} test was introduced for the biological standardization of dangerous drugs or agents. It refers to the concentration or dose of a given agent that is lethal to 50% of the tested population.

Uncapped 5′-ppp
Refers to the 5′ end of an RNA molecule that is not modified by a nucleotide cap. Uncapped 5′-ppp motifs are present on the negative and/or positive RNA strand intermediates of some RNA viruses and are recognized specifically by host pattern-recognition receptors (such as RIG-I) to trigger immune responses.

generated *Ifit1*$^{-/-}$ mouse showed no differences in mortality compared with wild-type mice over a wide range of VSV doses[51]. In this study, VSV infection was uniformly lethal in *Ifit2*$^{-/-}$ mice, a phenotype that was associated with enhanced viral replication in neurons of the brain but not in cells from other organs, such as the lungs and liver. Finally, a third study showed that silencing of *IFIT3* expression in human A549 lung adenocarcinoma cells resulted in decreased IFNα-dependent antiviral activity against VSV. Moreover, ectopic expression of IFIT3 inhibited infection not only by VSV but also by encephalomyocarditis virus, a picornavirus that encodes the genome-linked protein Vpg, which binds to the 5′ end of the viral RNA and probably blocks the uncapped 5′-ppp[52]. Clearly, studies with additional RNA and DNA viruses and IFIT-deficient cells and mice are warranted to establish the mechanisms by which IFIT proteins control different families of viruses.

Binding to viral proteins. IFIT1 can inhibit infection by human papillomavirus (HPV) — a large DNA virus — through a distinct mechanism: by binding to the viral helicase E1, which is required for replication[53,54]. E1 is a multifunctional viral protein with ATPase and DNA helicase activities. IFIT1 sequesters HPV E1 in the cytoplasm, partitioning it from the replication complex, which is localized to the nucleus. HPV replication is sensitive to the antiviral effects of type I IFNs, but silencing of IFIT1 expression using short hairpin RNA (shRNA) resulted in a loss of this inhibitory activity. In contrast to the wild-type E1 gene, transfection of a mutated E1 gene — encoding a mutant E1 protein that lacks residue 399 and cannot bind to IFIT1 — supported the replication of HPV DNA even in the presence of inhibitory levels of type I IFNs[54].

IFIT-mediated effects on inflammatory responses

In addition to their antiviral effector functions, IFIT proteins might have immunomodulatory activity, although the data as to the net effect of individual IFIT proteins on cellular immune responses are not consistent. Two reports have suggested that IFIT proteins negatively regulate the host inflammatory and antiviral responses. One showed that ectopic expression of IFIT2 in mouse macrophages inhibited LPS-induced expression of tumour necrosis factor (TNF), interleukin-6 (IL-6) and CXC-chemokine ligand 2 (CXCL2; also known as MIP2) and that this effect was mediated post-transcriptionally, possibly through effects on mRNA stability[55]. More recently, human IFIT1 and IFIT2 were reported to bind to and inhibit stimulator of IFN genes (STING; also known as MITA), which functions as a mitochondrial adaptor protein that recruits TANK-binding kinase 1 (TBK1) and IRF3 to a complex with mitochondrial antiviral signalling protein (MAVS; also known as IPS1, CARDIF and VISA), resulting in the downstream induction of IFNβ expression in response to viral RNA or DNA[56]. Ectopic expression of IFIT1 in human embryonic kidney 293T cells and macrophages inhibited the activation of IRF3 and nuclear factor-κB (NF-κB) and the transcription of *IFNB* in response to polyinosinic–polycytidylic acid (polyI:C) and prevented polyI:C-induced inhibition of VSV infection. Moreover, silencing of IFIT1 expression inhibited VSV infection, presumably by modulating the IRF3- and IFN-dependent responses. A biochemical analysis indicated that IFIT1 disrupted the physical interaction between STING and MAVS or TBK1.

Although provocative, these data conflict with the results of experiments in human HeLa cells in which silencing of IFIT1 and IFIT2 expression resulted in increased levels of VSV infection[50]. This study also showed that the modulation of IFIT protein levels did not alter type I IFN responses in mouse fibroblasts, macrophages or dendritic cells[50]. Moreover, other groups reported recently that silencing of mouse *Ifit1* suppresses the expression of inflammatory genes in response to LPS-mediated TLR4 activation[57] and that ectopic expression of IFIT3 enhances IRF3-mediated gene expression[58]. In the latter study, a TPR motif of IFIT3 interacted with the amino terminus of TBK1, and bridged TBK1 to MAVS on mitochondria, such that the host antiviral responses were boosted in the presence of IFIT3. Given these ostensibly conflicting results, more investigation is required to evaluate the network of immunomodulatory effects of individual IFIT proteins in cell culture and *in vivo*.

Anti-proliferative effects of IFIT proteins

Type I IFNs can have anti-proliferative effects in cell culture[59]. Because of their ability to bind components of the eIF3 complex and inhibit host protein translation, IFIT proteins might contribute to the restriction of cell division imposed by IFN signalling. Independently, IFIT proteins may modulate the expression of negative regulators of the cell cycle, leading to the accumulation of cells at the G1–S phase transition[60]. Indeed, ectopic expression of IFIT3 in U937 human myeloid cells resulted in the sequestration of JUN activation domain-binding protein 1 (JAB1; also known as COPS5), which limited ubiquitin- and proteasome-dependent degradation of cyclin-dependent kinase inhibitor 1B (also known as p27 and KIP1). In other studies, IFIT1 was shown to bind and sequester the ribosomal protein L15 (RPL15). Ectopic expression of IFIT1 or silencing of *RPL15* had an anti-proliferative effect on human gastric cancer cells, and higher IFIT1 levels correlated with enhanced sensitivity to IFN-induced inhibition of proliferation[61]. Finally, expression of human IFIT2, independently of IFN-mediated stimulation, was shown recently to promote cell apoptosis via a mitochondrial pathway. In this study, IFIT2 formed a complex with IFIT1 and IFIT3, and IFIT3 was shown to negatively regulate the pro-apoptotic effects of IFIT2 (REF. 62). Thus, IFIT proteins as a complex seem to regulate cell apoptosis after the induction of type I IFN responses or other cell stress pathways.

Summary of IFIT protein functions

IFIT genes are rapidly induced in many virus-infected cells through IFN-dependent and -independent pathways. Over the past decade, it has become clear that this family of related proteins inhibits viral infections through multiple mechanisms, for example by suppressing translation initiation, binding uncapped or incompletely capped viral RNA, and sequestering viral proteins or RNA in the cytoplasm. Moreover, recent functional studies suggest that IFIT family members might also regulate cell-intrinsic and cell-extrinsic immune responses, through pathways that remain to be defined and/or corroborated. As new structural and functional insights are gained about individual IFIT family members, it is likely that we will begin to appreciate the basis and complexity of the ligand interactions that explain the distinct functions of IFIT proteins in controlling viral pathogenesis and, possibly, in minimizing immune-mediated damage to the host.

The IFITM family

The gene and protein family. Although IFIT and IFITM proteins have quite distinct mechanisms of action, there are some underlying similarities in terms of family structure. Both families comprise multiple closely related

Genome-linked protein Vpg
Vpg is a protein attached to the 5′ end of RNA during RNA synthesis by several families of positive-stranded RNA viruses, including *Picornaviridae* and *Caliciviridae*. These proteins have pivotal roles in the replication cycle of these viruses, including effects on viral protein synthesis.

members that lack obvious enzymatic activities. Most vertebrate animals have two or more IFITM genes. The human IFITM locus is located on chromosome 11 and is composed of four functional genes: *IFITM1*, *IFITM2*, *IFITM3* and *IFITM5*. *IFITM4P* is a pseudogene. Mouse *Ifitm1*, *Ifitm2, Ifitm3* and *Ifitm5* are located on chromosome 7 and are orthologues of their human counterparts. In addition, mice have two other IFITM genes: *Ifitm6*, which is also located on chromosome 7; and *Ifitm7*, a retrogene located on chromosome 16. As in humans, mouse *Ifitm4p* is a pseudogene[63].

IFITM proteins have a common topology that comprises short luminal N- and C-termini, two antiparallel transmembrane domains and a short conserved cytoplasmic domain (FIG. 5). The first transmembrane domain, which is the more conserved, includes two cysteine residues, at least one of which is modified by palmitoylation[64]. Although several groups have confirmed this topology by flow cytometric recognition of N- and C-terminal tags, an alternative topology was proposed recently. According to this second model, the putative transmembrane regions associate with the inner leaflet of the membrane, and both N- and C-terminal domains are located in the cytoplasm[65]. Evidence for this model (FIG. 5a) includes the absence of *N*-linked glycans in the putative ectodomains despite the presence of native or engineered *N*-linked glycosylation sites, and the observation that the N-terminal domain can be ubiquitylated. *N*-linked glycosylation and ubiquitin modifications typically are found in the luminal and cytosolic domains of transmembrane proteins, respectively.

Expression. In contrast to the IFIT proteins, IFITM proteins are expressed basally, in the absence of IFN induction, in both primary tissues and cell lines[66]. IFITM1, IFITM2 and IFITM3 are expressed ubiquitously in humans, whereas IFITM5 is expressed primarily in osteoblasts. The expression of all four human IFITM proteins is induced robustly by both type I and type II IFNs. In mice, however, expression of *Ifitm3* is the most strongly induced by IFNs, whereas other IFITM genes are less responsive to IFN treatment. The expression of human *IFITM3* and mouse *Ifitm3* is also induced by IFNγ and by members of the gp130 family of cytokines (such as oncostatin M and IL-6), which all use similar JAK–STAT signalling mechanisms. This observation suggests that the induction of IFITM3 expression in a more targeted, IFN-independent manner might be possible through the ligation of tissue-specific receptors by gp130 family cytokines. Studies on the induction of IFITM genes after the ligation of PRRs might also identify additional IFN-independent mechanisms of expression.

Antiviral activity of IFITM proteins

IFITM proteins were identified more than 25 years ago, and their responsiveness to type I and type II IFNs is well described[67]. IFITM proteins have been ascribed roles in diverse biological processes, such as immune cell signalling, germ cell homing and maturation, and bone mineralization[68]. In B cells, human IFITM1 was shown to associate directly with the tetraspanin CD81 and indirectly with the B cell receptor components CD19 and CD21, although the significance of these interactions remains unclear[69,70]. Despite abundant evidence for their strong induction by IFNs, for years most studies of IFITM family proteins focused on their role in development[66]. However, these investigations were called into question by the observation that mice homozygous for a deletion of the entire IFITM locus (*IfitmDel* mice) had no apparent developmental defects, or indeed any overt phenotype[71].

An antiviral role for IFITM3 was discovered in an RNA interference screen for factors that modulate influenza A virus infection[72]. Depletion of IFITM3 using small interfering RNA or shRNA enhanced influenza A virus infection, and ectopic expression of IFITM1, IFITM2 or IFITM3 markedly inhibited influenza A virus replication. Surprisingly, retroviruses pseudotyped with influenza A virus haemagglutinin were affected similarly to influenza A virus by IFITM depletion and ectopic expression, whereas retroviruses pseudotyped with the entry proteins of murine leukaemia virus, Lassa virus or Machupo virus were not affected by the presence or absence of IFITM proteins. This observation localized the restriction of influenza A virus by IFITM proteins to a haemagglutinin-mediated step in the virus replication cycle. Subsequent studies established that, uniquely among antiviral proteins, IFITM proteins interfere with a step in viral replication preceding fusion of the viral and cellular membranes[73,74].

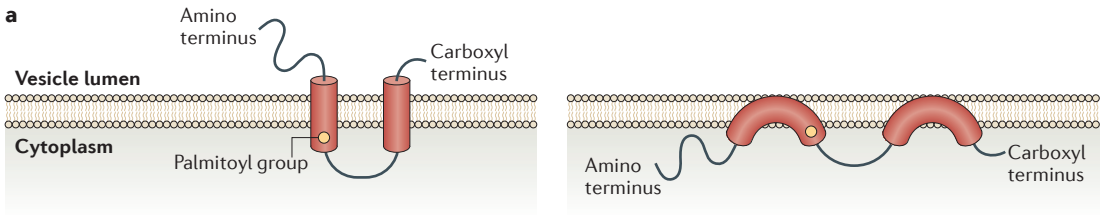
There are several implications of this early restriction step. First, IFITM-mediated restriction precedes the induction of type I IFNs in infected cells, which might explain the high basal level of expression of IFITM proteins in many tissues. IFN induction, however, can amplify IFITM expression and protect uninfected cells in a paracrine manner, and acute-phase cytokines such as IL-6 might induce IFITM expression systemically. Second, viral escape from restriction by IFITM proteins could be more challenging than escape from inhibitory factors that function at later stages of the viral replication cycle. For example, viral proteins such as HIV-1 Vif and Vpu, which are generated after viral entry, allow the virus to evade host responses mediated by APOBEC3G or BST2 (which affect viral replication and assembly) by degrading these restriction factors. In comparison, because IFITM-mediated restriction precedes infection, there is no opportunity for the *de novo* synthesis of viral inhibitors. Thus, the virion must carry a protein that counteracts IFITM-mediated restriction (which is unlikely given the relatively small amount of viral protein that is delivered to a cell) or alter its site of fusion with host cell membranes (FIG. 6).

In addition to influenza A virus, IFITM proteins restrict infection by several other enveloped viruses[14,72,74–76]. These include flaviviruses (dengue virus and WNV), filoviruses (Marburg virus and Ebola virus) and coronaviruses (such as severe acute respiratory syndrome (SARS) coronavirus). By contrast, infection by alphaviruses, arenaviruses and murine leukaemia virus (a retrovirus) seems to be unaffected by IFITM protein expression. VSV is weakly restricted by IFITM proteins, and HIV-1 might be restricted in a cell-type specific

Pseudotyped
A pseudotyped virus expresses envelope proteins from a foreign or heterologous virus.

manner[14,77]. These varying degrees of restriction are also observed for retroviruses pseudotyped with the entry proteins of different viruses. Viruses that are restricted by IFITM proteins tend to fuse with host cell membranes in a late endosome or lysosome. Indeed, when retroviruses bearing the entry protein of the SARS coronavirus were induced by trypsin to fuse at the plasma membrane, IFITM-mediated restriction was bypassed, establishing that the site of viral fusion is crucial for the antiviral activity of IFITM proteins[74].

There seems to be specialization among the antiviral functions of IFITM proteins[74]. In particular, IFITM3 is especially effective in controlling influenza A virus, as *Ifitm3*$^{-/-}$ mice challenged with an H1N1 influenza virus strain sustained higher viral loads and succumbed more rapidly to disease[78]. *Ifitm3*$^{-/-}$ mice had a viral infection phenotype indistinguishable from that of *IfitmDel* mice (which lack *Ifitm1*, *Ifitm2*, *Ifitm3*, *Ifitm5* and *Ifitm6*), which suggests that the other mouse IFITM proteins do not have a



b

	Amino terminus / Transmembrane 1
Human IFTIM1	MHKEEHEVAVLGAPPS--TILPRSTVINIHSETSVP-DHVVWSLFNTLFLNWCCLGFIAFAY
Mouse IFTIM1	MPKEQQEVVVLGSPHI--STSATATTINMP-EISTP-DHVVWSLFNTLFMNFCCLGFVAYAY
Chicken IFTIM1	MQSYPQHTSINMP--SYGQDVTTTIPISPQPPPKDFVLWSLFNFVLCNAFCLGLCALSY
Human IFTIM2	MNHIVQT-FSPVNSGQPPNYEMLKEEQEVAMLGAPHN--PAPPTSTVIHIRSETSVP-DHVVWSLFNTLFMNTCCLGFIAFAY
Mouse IFTIM2	MSHNSQAFLSTNAG-LPPSYETIKEEYGVTELGEPSN--SAVVRTTVINMPREVSVP-DHVVWSLFNTLFFNACCLGFVAYAY
Human IFTIM3	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHN--PAPPTSTVIHIRSETSVP-DHVVWSLFNTLFMNPCCLGFIAFAY
Mouse IFTIM3	MNHTSQAFITAASGGQPPNYERIKEEYEVAEMGAPHG--SASVRTTVINMPREVSVP-DHVVWSLFNTLFMNFCCLGFIAYAY
Human IFTIM5	MDTAYPREDTRAPTP--SKAGAHTALTLGAPHPPPRDHLIWSVFSTLYLNLCCLGFLALAY
Mouse IFTIM5	MDTSYPREDPRAPSSRKADAAAHTALSMGTPGPTPRDHMLWSVFSTMYLNLCCLGFLALVH
Chicken IFTIM5	MDTSYPREDYLPMT--SHKRDSSPTTAT--SAPPRDHLIWSIFNTIYMNFCCLGFVALAF
Mouse IFTIM6	MVKRDPD---SAPVPSTVVCINSDVIQP-DHITWSTFNTVFMNGCCLGFIAYIY
Mouse IFTIM7	MPKDQHEVVVMGTPHT--STSSTTTIITMP-EISKP-DYVVWSLFNTLFMNFCCLGFIAYAY

	Cytoplasmic / Transmembrane 2 / Carboxyl terminus
Human IFTIM1	SVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTIGFILLLVFGSVTVYHIMLQIIQEKRGY
Mouse IFTIM1	SVKSRDRKMVGDTTGAQAFASTAKCLNISSLFFTILTAIVVIVVCAIR
Chicken IFTIM1	SIKSRDRIIAKDFVGASSYGRTAKIFNIFAFCVGLLVTILSIVLVFLYLPLYTVRP
Human IFTIM2	SVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGIFMTILLVIIPVLVVQAQR
Mouse IFTIM2	SVKSRDRKMVGDVVGAQAYASTAKCLNISSLIFSILMVIICIIIFSTTSVVVFQSFAQRTPHSGF
Human IFTIM3	SVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTILLIVIPVLIFQAYG
Mouse IFTIM3	SVKSRDRKMVGDVTGAQAYASTAKCLNISTLVLSILMVVITIVSVIIIVLNAQNLHT
Human IFTIM5	SIKARDQKVVGDLEAARRFGSKAKCYNILAAMWTLVPPLLLLGLVVTGALHLARLAKDSAAFFSTKFDDADYD
Mouse IFTIM5	SVKARDQKMAGNLEAARQYGSKAKCYNILAAMWTLVPPLLLLGLVVTGALHLSKLAKDSAAFFSTKFDEEDYN
Chicken IFTIM5	SVKARDRKVAGDVEAARRFSSKARCYNALATAGSVLLPILLAALVVTGVLHLSKLAQDSVGFFSSQFSASDDEDK
Mouse IFTIM6	SVKSRDRKMVGDMTGAQSHASTAKILNILALVISLIFYIMLIVLYSFNLLGNQR
Mouse IFTIM7	SVKSRDRKMVGDMTGAQAFASTARCLNISCLILSVVMVILFITFFATRR

Figure 5 | **Proposed topologies and sequence alignment of IFITM orthologues and paralogues.** **a** | Two topologies have been proposed for proteins of the IFN-induced transmembrane protein (IFITM) family. In the first model, the amino and carboxyl termini are located in the lumen of IFITM-containing vesicles, and the hydrophobic regions fully traverse the membrane (left). Yount *et al.*[65] have proposed an alternative model in which both termini are oriented towards the cytoplasm, and the hydrophobic domains are embedded in the membrane without traversing it (right). A yellow dot in both models indicates the site of a palmitoyl group that is important for protein stability and restriction activity[64]. **b** | An alignment of human, mouse and chicken IFITM proteins is shown. Red indicates conservation of a residue in at least nine of the twelve IFITM proteins shown. Note that the conservation of the first transmembrane domain and the cytoplasmic domain is based on the first topology model. The site of palmitoyl addition is highlighted in orange. Green and blue highlighting indicates species-specific signature residues of humans and mice, respectively, possibly suggesting interaction with a cofactor that similarly diverged in each species.

Viral fusion
A process required by enveloped viruses for entry and replication in host cells. Fusion often occurs in endosomal or early lysosomal compartments after pH- and/or protease-dependent changes.

significant role in controlling influenza A virus infection[79]. Consistent with these data, patients who were hospitalized owing to severe infection with the 2009 pandemic H1N1 strain of influenza A virus were enriched for a single-nucleotide polymorphism that decreased expression of full-length IFITM3 (REF. 78). Although analogous *in vivo* studies of other viruses that are restricted by IFITM proteins remain to be carried out, cell-culture experiments indicate that IFITM1 restricts filoviruses and SARS coronavirus more effectively than IFITM3 does[74]. More impressively, mouse IFITM6 did not prevent influenza A virus infection, but efficiently limited infection mediated by filovirus entry proteins.

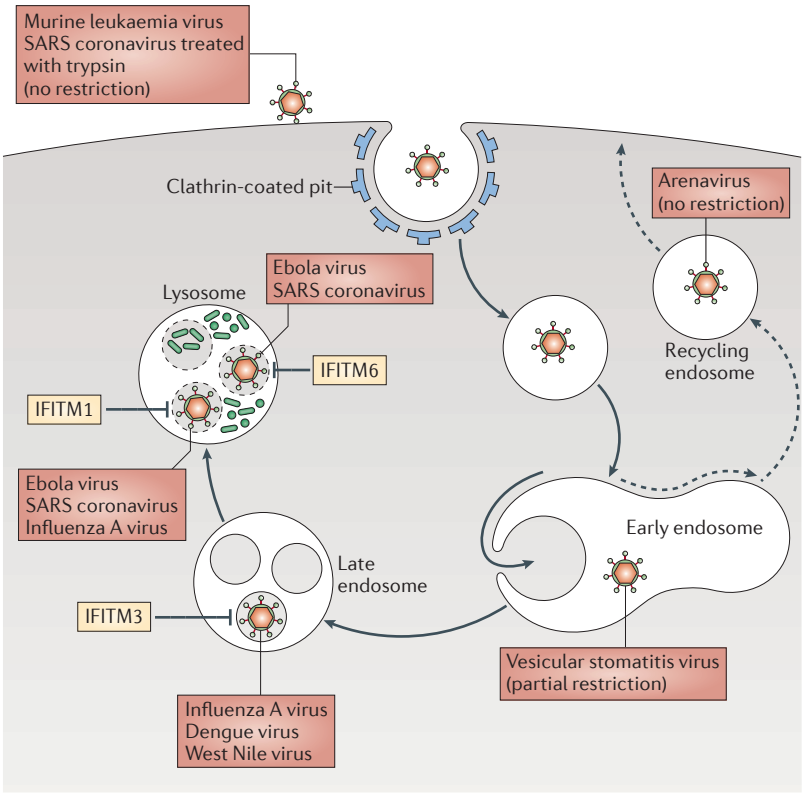


Figure 6 | **Correlation between the site of virus fusion and susceptibility to IFITM-mediated restriction.** Viruses fuse with host-cell membranes in different compartments within the endocytic pathway, and IFN-induced transmembrane protein (IFITM)-mediated restriction activity correlates with the site of fusion. For example, arenaviruses (such as Junin virus and Machupo virus) follow the recycling pathway of their common receptor, transferrin receptor 1 (REF. 82). These viruses are not susceptible to IFITM-mediated restriction. By contrast, viruses such as influenza A virus fuse in late endosomes and are restricted by IFITM proteins, particularly by IFITM3 (REF. 72). Viruses such as severe acute respiratory syndrome (SARS) coronavirus, Ebola virus and influenza A virus depend on lysosomal cathepsins and other lysosome-resident proteins for fusion, and these viruses are restricted mainly by IFITM1 (REF. 74). Mouse IFITM6 is more specialized and restricts the entry of Ebola virus and SARS coronavirus, but not influenza A virus. Trypsin treatment of SARS coronavirus allows it to fuse at the plasma membrane and bypass IFITM-mediated restriction. Retroviruses pseudotyped with entry proteins from these viruses show identical patterns of restriction, implicating the entry process in the antiviral activity of IFITM proteins. Note that the diagram is schematic and ignores much of the diversity of cellular compartments and the complexity of cellular trafficking.

The mechanisms underlying the antiviral activity of IFITM proteins remain uncertain. However, several possibilities have been excluded[73,74]. Ectopic expression of IFITM proteins does not alter the expression of virus receptors, affect the pH of endosomal compartments or interfere with the cathepsin activity that is necessary for the fusion of some restricted viruses. Although IFITM proteins can be detected on the plasma membrane, particularly when overexpressed or induced by IFNs, they are enriched in intracellular compartments, including late endosomes, where restricted viruses fuse. Two models have been proposed to explain the antiviral activity of IFITM proteins[73,74] (FIG. 6). In the first model, IFITM proteins are hypothesized to modify endosomal or lysosomal vesicles such that they become inhospitable to viral fusion. IFITM proteins could achieve this by altering the lipid components of the vesicle membrane, by enriching vesicles with nonspecific proteases that inactivate entry proteins or, as proposed recently[80], by interfering with the activity of the V-type proton ATPase, which is responsible for endosomal acidification. In the second model, IFITM proteins could alter the rate or pattern of vesicle trafficking such that viruses are redirected to a non-fusogenic pathway. The expression of IFITM proteins in many cell lines induces large vacuoles, suggesting that these proteins in some way interfere with vesicle trafficking, fusion or resolution[73]. However, the presence and size of these vacuoles do not correlate with the efficiency of restriction, and morphological changes were not observed when endogenous IFITM proteins were depleted, despite the increased levels of influenza A virus replication in these cells[72,74]. As in the case of the IFIT proteins, the absence of obvious enzymatic domains in the IFITM proteins suggests that cellular cofactors are necessary for antiviral activity. Consistent with this possibility, IFITM proteins have species-specific signature sequences that are localized at the cytoplasmic base of both transmembrane domains (FIG. 5b).

Summary of IFITM protein function

IFITM proteins are a family of small transmembrane proteins that are induced strongly by IFNs, but that are also expressed basally in several cell types and lines. Although other functions have been proposed, the primary role of IFITM proteins seems to be antiviral. IFITM3 in particular significantly contributes to the control of influenza A virus *in vivo*, and tissue-culture studies suggest that several of the other IFITM proteins help to restrict infection by other enveloped viruses. The expression of IFITM proteins makes cells refractory to steps in the viral infection cycle that precede viral fusion, but the mechanisms by which these proteins mediate such functions remain incompletely defined. It also remains poorly understood how IFITM proteins differentially restrict distinct viruses, and whether they can modulate the replication of other pathogens, including non-enveloped viruses, bacteria and parasites. As in the case of the IFIT proteins, additional work to characterize the activity and regulation of IFITM proteins may suggest more tailored approaches for controlling infection by specific pathogens.

Overall summary

It may be unfortunate that IFIT and IFITM family proteins share such similar acronyms, because, although both are IFN induced, they control virus infection through distinct mechanisms. IFIT proteins function in the cytoplasm, whereas IFITM proteins traverse the membrane and are enriched in late endosomes and lysosomes. IFIT proteins suppress the initiation of translation, bind to and sequester uncapped viral RNA, and sequester at least one viral protein (HPV E1) in the cytoplasm. IFITM proteins, by contrast, prevent several enveloped viruses from fusing with endosomal or lysosomal membranes and penetrating the cytoplasm. Moreover, IFIT proteins are expressed poorly, if at all, in the absence of inflammatory or danger signals, whereas IFITM proteins are expressed basally in many tissues. IFITM proteins generally are induced to greater levels than IFIT proteins by IFNγ, and possibly by members of the gp130 family of cytokines (such as IL-6). However, although there are many differences, there are some parallels between IFIT and IFITM proteins. Compared with the APOBEC family of restriction factors, the IFIT and IFITM families target a wider range of viruses. Moreover, and similarly to the APOBEC proteins, the IFIT and IFITM families comprise specialized paralogues, perhaps reflecting an evolutionary arms race with pathogens. A deeper understanding of the antiviral activity and mechanism of action of the members of each family may facilitate the development of broad-spectrum antiviral agents that mimic or amplify their activities.

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Competing interests statement
The authors declare no competing financial interests.

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