Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
IFITMs Restrict the Replication of Multiple Pathogenic Viruses

Jill M. Perreira, Christopher R. Chin, Eric M. Feeley and Abraham L. Brass

Microbiology and Physiological Systems (MaPS) Department, University of Massachusetts Medical School, Albert Sherman Center 8 1001, 368 Plantation Street, Worcester, MA 01655, USA

Correspondence to Abraham L. Brass: abraham.brass@umassmed.edu
http://dx.doi.org/10.1016/j.jmb.2013.09.024
Edited by E. Freed and M. Gale

Abstract

The interferon-inducible transmembrane protein (IFITM) family inhibits a growing number of pathogenic viruses, among them influenza A virus, dengue virus, hepatitis C virus, and Ebola virus. This review covers recent developments in our understanding of the IFITM's molecular determinants, potential mechanisms of action, and impact on pathogenesis.

Introduction

To replicate, viruses must gain access to the resource-rich cytosol that lies beyond the cell's plasma membrane. Enveloped viruses breach this barrier by using specialized fusion proteins. Three major classes of viral fusion proteins exist, all similarly containing a fusion peptide that inserts into the cytolemma, thereby anchoring the two membranes side by side [1]. Once transfixed, the juxtaposed membranes are forcefully distorted as the viral envelope protein undergoes a profound conformational change; the two outer leaflets are welded together to form a hemifusion intermediate that rapidly converts into a fusion pore through which the viral contents enter the cytosol. Any means by which the host can block fusion would confer an advantage by preventing both the emergence of an escalating number of progeny viruses and the deployment of viral countermeasures.

Restriction factors are a diverse group of host proteins that are united in the common goal of antagonizing viral replication. Multiple mechanisms of restriction have evolved, with some factors having activity against one virus and others acting broadly across several viral families. The expression of many restriction factors is transcriptionally controlled by the antiviral cytokine, interferon (IFN). Among such IFN-stimulated genes, the related restriction factors, IFN-inducible transmembrane protein (IFITM)1, 2, and 3, inhibit the replication of multiple pathogenic viruses, including influenza A virus (IAV) and influenza B virus, West Nile virus, dengue virus (DENV), severe acute respiratory syndrome coronavirus (SARS CoV), hepatitis C virus (HCV) and the filoviruses, Ebola virus (EBOV) and Marburg virus (MARV; Table 1) [2,3,15]. The antiviral properties of the IFITMs were discovered using orthologous functional genomic strategies [2,8,13,32,41]. Early work showed that the IFITMs resided on the cytolemmal and endosomal membranes and specifically blocked viral pseudoparticles bearing the receptors of restricted viruses, demonstrating that they acted during an early envelope-dependent portion of the viral life cycle [2]. Further studies revealed that the IFITMs block viral replication by preventing viral-host membrane fusion subsequent to viral binding and endocytosis [3,4]. Imaging studies of these events revealed that the invading viruses were trapped by the IFITMs, leading to their ultimate destruction in the host cell's lysosomes and autolysosomes, both of which are expanded with IFITM expression (Fig. 1a and b) [4]. A range of viruses are restricted in this manner, including ones that exploit each of the host cell's endocytic pathways [42]. The kinetics of this entrapment are rapid, with viral entry usually occurring over a 5- to 30-min time span, requiring the IFITMs to already be in place or to rapidly mobilize to meet such threats.
Table 1. Viruses inhibited by IFITM proteins

| Virus               | Family         | Host receptor                                      | Endocytic pathway                     | Where the virus enters the host cell | pH Requirement | Notes                                                                 |
|---------------------|----------------|---------------------------------------------------|----------------------------------------|--------------------------------------|----------------|----------------------------------------------------------------------|
| IAV                 | Orthomyxovirus | α2,6-linked sialic acid (human)                   | Clathrin-mediated endocytosis,        | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| DENV                | Flavivirus     | CD14                                              | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| West Nile virus     | Flavivirus     | Unknown                                           | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| Yellow fever virus  | Flavivirus     | Unknown                                           | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| Omsk hemorrhagic    | Flavivirus     | Unknown                                           | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| fever virus         |                |                                                   |                                        |                                      |                |                                                                    |
| HCV                 | Flavivirus     | CD81, Occludin, SB-R1, Claudin-1                  | Clathrin-mediated endocytosis         | RAB5+ early endosomes                 | pH 6.5         |                                                                    |
| SARS CoV            | Coronavirus    | Angiotensin-converting enzyme 2 (ACE2)            | Clathrin-mediated endocytosis         |                                      | pH 5.5         | Lysosomes (pH-dependent cleavage: cathepsins B and L)               |
| MARV                | Filovirus      | Neimann-Pick C1 (NPC1), T-cell immunoglobulin     | Macropinocytosis                      | NPC1+ lysosomes (pH-dependent        | pH 4.5         |                                                                    |
| EBOV                | Filovirus      | Neimann-Pick C1 (NPC-1), T-cell immunoglobulin    | Macropinocytosis                      | NPC1+ lysosomes (pH-dependent        | pH 4.5         |                                                                    |
| Rift Valley fever   | Bunyavirus     | Unknown                                           | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| virus               |                |                                                   |                                        |                                      |                |                                                                    |
| La Crosse virus      | Bunyavirus     | Unknown                                           | Clathrin-mediated endocytosis         | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| Andes virus          | Bunyavirus     | Unknown                                           | Unknown                               | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| Hantaan virus        | Bunyavirus     | Unknown                                           | Clathrin-dependent endocytosis        | RAB7+ late endosomes                  | pH 5.5         |                                                                    |
| Vesicular stomatitis Indiana virus | Rhabdovirus | LDL receptor                                      | Clathrin-mediated endocytosis         | RAB5+ early endosomes                 | pH 6.5         |                                                                    |
| Rana grylio virus I  | Iridoviridae   | Unknown                                           | Caveolin-mediated endocytosis         | Unknown                               | pH-dependent   | Stable infectivity between pH 4 and pH 9                           |

* a pH values provided have been standardized based on reports of the values found for the relevant endosomal compartments in the primary literature: early endosome, pH 6.5; late endosome, pH 5.5; lysosome, pH 4.5.
Table 1. Viruses inhibited by IFITM proteins.

| Virus                              | Cathepsin processing | IFITM specificity                | References |
|------------------------------------|----------------------|----------------------------------|------------|
| IAV                                | No                   | IFITM3 > IFITM2 > IFITM1         | [2–7]      |
| DENV                               | No                   | IFITM3/IFITM1 > IFITM2           | [2,8–12]   |
| West Nile virus                    | No                   | IFITM3 > IFITM1 > IFITM2         | [2,8,11,13,14] |
| Yellow fever virus                 | No                   | IFITM3 > IFITM1 > IFITM2         | [2,11,13,14] |
| Omsk hemorrhagic fever virus       | No                   | IFITM3 > IFITM1 > IFITM2         | [2,11,14]  |
| HCV                                | No                   | IFITM1, not IFITM3               | [2,13–16]  |
| SARS CoV                           | Yes                  | A549: IFITM3/IFITM2 > IFITM1 Vero E6: | [3,17] |
| MARV                               | Yes                  | A549: IFITM3 > IFITM2 > IFITM1   | [3,18]     |
| EBOV                               | Yes                  | A549: IFITM1/IFITM3 > IFITM2 Vero E6: | [3,18–20] |
| Rift Valley fever virus            | No                   | IFITM1/IFITM2/IFITM3            | [21,22]    |
| La Crosse virus                    | No                   | IFITM1/IFITM2/IFITM3            | [21,23,24] |
| Andes virus                        | No                   | IFITM1/IFITM2/IFITM3            | [21,25]    |
| Hantaan virus                      | No                   | IFITM1/IFITM2/IFITM3            | [21,26,27] |
| Vesicular stomatitis               | No                   | IFITM1 > IFITM2 > IFITM3        | [2,5,21,22,28,29] |
| Scaphthalmus maximus rhabdovirus   | Unknown              | IFITM1 (Paralichthys olivaceus)  | [30,31]    |
| HIV-1                              | No                   | SUPT1: IFITM3 > IFITM2/IFITM3    | [13,32–35] |
| JSRV                               | No                   | HTX: IFITM1 > IFITM2 > IFITM1    | [5,36,37]  |
| Reovirus                           | No                   | IFITM3                          | [38,39]    |
| Rana grylio virus                  | Unknown              | IFITM1 (Paralichthys olivaceus)  | [30,40]    |
Consistent with an early role in intrinsic immunity, the IFITM1, 2, and 3 proteins are ubiquitously expressed, with IFITM2 and IFITM3 present at baseline in most primary and transformed cells (Tables 2 and 3). In contrast, IFITM1’s basal expression is considerably less. The levels of these three IFITMs are increased by IFN-α or IFN-γ. Remarkably, the depletion of IFITM3 alone results in the loss of 50–80% of the in vitro anti-IAV actions of IFN [2,61]. Furthermore, mice null for only Ifitm3 are more susceptible to IAV infection, testifying to the importance of IFITM-mediated restriction in vivo [65,101]. In sum, these data suggest that the IFITMs may prevent or ameliorate multiple viral illnesses. Indeed, such is the case for influenza infection, where a human allele of IFITM3, rs12252-C, is associated with worse influenza
infections, suggesting that the IFITM3 status of a population may influence the course of seasonal influenza epidemics and pandemics [65,66]. Recent studies regarding the IFITMs have focused on their structure–function as well as their potential mechanism of action. In addition, several efforts have reported new families of IFITM-sensitive viruses. Herein, we discuss these results together with previous work and compare and contrast the various models proposed to explain how the IFITMs protect our cells.

The IFITM and CD225 Families

In addition to IFITM3, four more members of the IFITM family are present in both man (IFITM1, 2, 5, and 10; Fig. 2a and Table 3) and mouse (Ifitm1, 2, 5, and 6). The IFITMs each contain two hydrophobic membrane-associated domains separated by a conserved intracellular loop (CIL, Fig. 2a). The IFITM family belongs to a larger family of membrane-associated proteins, the CD225/pfam04505 protein superfamily, with greater than 300 members sharing homology across their first membrane-associated domains and CILs. Interestingly, while IFITM5 has been shown to prevent infection by multiple viruses, it is also restricted in its expression to osteoblasts, where it plays a role in bone formation in vitro and in vivo [69,102]. Consistent with these data, a variant allele of IFITM5 is associated with a human brittle bone disease, osteogenesis imperfecta type V [61,87]. Remarkably, the protein encoded by this rare IFITM5 allele possesses an additional five amino acids at its N-terminus; this is in keeping with our unpublished data showing the IFITMs do not function in a wild-type manner when epitope tagged at their N-termini.

Additional human CD225 family members include PRRT1, PRRT2, TMEM91, TMEM233, TUSC5, SYNDIG1, and SYNDIG1L, with little if anything known about the functions of these proteins (Fig. 3a and Table 3). However, PRRT2, which is expressed in the nervous system, has received considerable attention for its genetic association with multiple movement disorder syndromes, many of which arise in the setting of its haploinsufficiency [87–89]. Therefore, among the 12 CD225 family members present in humans, there are currently three proteins with human disease associations: IFITM3, PRRT2, and IFITM5 (Table 3). Many additional CD225 proteins are found among prokaryotes, including several plant pathogens, suggesting that the CD225 domain confers a selective advantage across kingdoms. Interestingly, while both fish and amphibians express CD225 family members, none have been found in plants, fungi, insects, or worms, leading to the fascinating hypothesis that a gene encoding a prokaryotic CD225 family member may have been transferred to an ancestral metazoan [103].

Membrane Topology and Cellular Localization

Two hydrophobic domains are separated by a CIL in each of the IFITMs. Our appreciation of whether these hydrophobic domains are transmembrane or intramembrane continues to evolve over time [42,104]. Initial studies suggested that IFITM3 possessed two transmembrane domains predicated on the extracellular accessibility of epitopes located on either the N- or C-terminus [2]. However, mass spectrometry (MS) analyses by several groups (Table 4; Ref. [104]; PhosphoSite) have detected prevalent ubiquitylation of IFITM3’s lysine 24, which could occur only if the N-terminal domain (NTD) was cytosolic (Table 4; Ref. [104]). Similarly, a tyrosine in the NTD of IFITM3, Y20, was also shown to undergo phosphorylation a clathrin-mediated endocytosis motif (see below) [33]. In support of these latter results, large-scale MS studies have also reported detecting Y20-P, in addition to several other posttranslational modifications occurring in the respective NTDs of the other human CD225 proteins. For example, PRRT2’s S239 has been reported to undergo phosphorylation by nine groups at PhosphoSite Plus. Furthermore, an engineered IFITM3 possessing both an N-terminal myristoylation site and a C-terminal prenylation site was modified by these cytosolic-resident enzymes and restricted IAV [104]. Collectively, these data argue for both N- and C-termini residing intracellularly, similar to the reticulon and caveolin proteins [104,110], with both hydrophobic regions being intramembranous (IM1 and IM2, Fig. 2b). However, the strongest data exists for the N-terminus’ orientation. We note that IFITMs residing in the cytosolic leaflet of the membrane would shield it both from neutralization by invading viruses and from degradation by lysosomal enzymes. An intramembranous topology also has potential implications for altering the membrane’s biophysical properties, a point discussed below. Of importance, this topology remains controversial, and to reflect that, we refer the reader to a recent review that also presents the alternatively proposed transmembrane topology in graphic form [42].

Endogenous IFITM1 is predominantly located in the plasma membrane and in early endosomes [3,4], where it resides in lipid rafts and interacts with the cell surface proteins CD19 and CD81, the latter being a co-receptor for HCV [111–113]. In contrast, the majority of endogenous IFITM2 and 3 are present in late endosomes, lysosomes, and autolysosomes, colocalizing with Rab7, CD63, and LAMP1 [3,4,109]. Overexpression of IFITM1, 2, or 3 results in the exogenous proteins localizing to a highly acidified and expanded late endosomal and lysosomal compartment [3,4]. However, even when overexpressed, a substantial amount of IFITM1 can still be detected near the cell surface [3,15,61]. We...
| Virus                        | Family         | Host receptor                      | Endocytic pathway                           | Where the virus enters the host cell | pH Requirement | Cathepsin processing | IFITM specificity | References                  |
|-----------------------------|----------------|-----------------------------------|---------------------------------------------|--------------------------------------|----------------|----------------------|-------------------|--------------------------|
| Lymphocytic choriomeningitis virus | Arenavirus     | Dystroglycan (DG)                 | Clathrin independent, caveolin independent, dynamin independent, Potentially Rab5/Rab7 independent | Late Endosome Lysosome               | pH 4.5         | Unknown              | Immune            | [2,43–46,50,124]       |
| Lassa virus                 | Arenavirus     | Preferred: dystroglycan(DG); potential: DC-SIGN, LSECtin, Tyro3/Axl/Mer (TAM) | Clathrin independent, caveolin independent, dynamin independent, Potentially Rab5/Rab7 independent | Late Endosome Lysosome               | pH 4.5         | Unknown              | Immune            | [2,44–46,50,124]       |
| Machupo virus               | Arenavirus     | Transferrin receptor 1 (TfR1)     | Clathrin-mediated endocytosis               | RAB7+ late endosomes after acidic pH induced dissociation of GP1 and GP2 | pH 5.5         | No                   | Immune            | [2,43,45,47,124]       |
| Junin virus                 | Arenavirus     | Unknown                           | Clathrin-mediated endocytosis               | RAB7+ late endosomes after acidic pH induced dissociation of GP1 and GP2 | pH 5.5         | No                   | Immune            | [48–50,124]            |
| MLV                         | Gammaretrovirus | Murine cationic amino acid transporter-1 (mCAT-1) | Clathrin independent                        | Cell surface                         | None           | No                   | Immune            | [2,51–53]               |
| SeV                         | Paramyxovirus  | Sialic acid-containing ganglioside receptors (SA-R); alternate: asialoglycoprotein receptor (ASGP-R) | Clathrin independent                      | Cell surface                         | None           | No                   | Immune            | [54–57]                 |
| CCHFV                       | Bunyavirus     | Nucleolin                         | Clathrin-mediated endocytosis               | RAB5+ early endosomes                 | pH 6.5         | No                   | Immune            | [21,58–60]             |
have observed that the overexpression of epitope-tagged IFITMs can produce partial mislocalization and diminution of their antiviral function. For example, epitope-tagged IFITM1 appears more in the late endosomes and lysosomes than the endogenous protein. As noted, in our experience, N-terminal epitope tags alter the intracellular localization of the IFITMs more than C-terminal tags. Moreover, the addition of green fluorescent protein or discosoma species red protein to either terminus of IFITM3 results in chimeric proteins that do not restrict viral replication and are retained in the cellular interior (our unpublished data). The molecular determinants underlying IFITM cellular localization are discussed in Structure and Function.

Specificity of Action

IFITMs inhibit the entry of a number of viruses, with enveloped RNA viruses reported most frequently (Table 1). Remarkably, the IFITMs block the entry of viruses from each of the three classes of viral fusion proteins. Furthermore, susceptible viruses are prevented from entering via the cell surface, the early and late endosomes, as well from the lysosome. While overlap exists among which IFITMs inhibit which viruses, some specificity is apparent, with IFITM1 better preventing infection by early endosomal-entering viruses and IFITM3 exerting more resistance to viruses that enter in the late endosomes or lysosomes, including IAV, DENV, and the Bunyaviridae, including Rift Valley fever virus (RVFV). These late-entering viruses share common features including their dependence on greater endosomal acidification (pH<6) and the actions of Rab7, a host protein required for late endosomal trafficking and acidification [114]. IFITM2 behaves similarly to IFITM3 in terms of viral specificity, albeit with weaker effect, and so it will not be the focus of additional comment [2].

IFITM3 is less proficient than IFITM1 at inhibiting viruses that enter at the cell surface or in the early Rab5+ endosomes (pH>6). For example, IFITM1 prevents human immunodeficiency virus type 1 (HIV-1) entry in both T cell lines and HeLa cells [13,32]. In contrast, we saw no effect on HIV-1 when IFITM3 was overexpressed in HeLa cells (TZM-bl cell line, National Institutes of Health AIDS Reagent Resource) and only a twofold effect with its overexpression in Jurkat T cells (our unpublished data; Ref. [2]), suggesting that it plays a minor role in regulating HIV-1 entry. An additional example is IFITM3’s modest restriction of vesicular stomatitis virus-g protein-mediated entry, which is Rab5 dependent and requires a pH of 6.5 or less to fuse. IFITM1 also blocks HCV, a hepacivirus that enters in Rab5+ endosomes with a pH requirement of 6.5 [15]. Our previous work overexpressing IFITM3 in the HCV JFH1-permissive cell line, HuH7.5.1, showed no effect on HCV infection [2]. Similarly, IFITM1, but not IFITM3, halted infection by pseudoparticles bearing the Jaagsiekte sheep retrovirus (JSRV) envelope, which also fuses in the Rab5+ early endosomes at pH 6.3 [5]. Furthermore, although bunyaviruses express a similar glycoprotein (GP) that mediates fusion, they differed in their respective susceptibilities to IFITM3 [Lacrosse virus > RVFV > Andes virus > Hantaan virus > Crimean Congo hemorrhagic fever virus (CCHFV)] [21]. RVFV was only restricted by IFITM3, and CCHFV showed no inhibition by IFITM1, 2, or 3. Interestingly, IFITM3 was equally as effective in blocking the same virus from infecting different cell types, while IFITM1’s efficacy varied more across cell lines; this suggests that IFITM1’s actions were cell-type specific (Table 1). The difference in localization may provide one explanation as to why the viral specificities of IFITM1 and 3 differ, as they may preferentially inhibit viruses that enter where they are located. However, an exception to this rule is IFITM1s outperforming IFITM3 in inhibiting the very late-entering viruses, SARS CoV, EBOV, and MARV [3]. In addition, chimeric IFITM1 proteins containing the NTD of IFITM3 are located similarly to wild-type IFITM3 but do not curtail IAV as potently, suggesting that location alone may not fully explain these observations.

The list of IFITM-resistant viruses is short by comparison, although a bias against reporting negative data may be contributory (Table 2). Both the Moloney leukemia virus (MLV) and Sendai virus (SeV) envelopes fuse at the cell surface in a pH-independent manner and both are immune to the IFITMs [2,54]. Among the reported pH-independent viruses that fuse at the plasmalemma, only HIV-1 is blocked by IFITM1, making this perhaps the best site to avoid IFITM-mediated restriction. The largest enrichment of IFITM-resistant viral envelopes occurs among the arenaviruses, including both the old world (Lassa virus, lymphocytic choriomeningitis virus) and new world (Machupo, Junin) classes. Due to the pathogenicity of these viruses, this work has been done almost exclusively using pseudoparticles bearing the viral GP receptors [2,3,21]. To date, the arenaviruses and the bunyavirus CCHFV are the only late endosomal-entering viruses immune to IFITM3. Intriguingly, the only attribute shared between the two classes of arenavirus pseudoparticles is the GP spike because the old world arena viruses enter in a clathrin-independent manner after binding to the α-dystroglycan receptor, while the new world viruses bind to the transferrin receptor and enter the host cell via clathrin-mediated endocytosis [115].

Structure and Function

Efforts to elucidate the structure and function of the IFITMs have focused on IFITM3. For brevity, we now highlight a few of these insights (Fig. 2).
IFITM3’s NTD and Y20: The N-termini of IFITM2 and 3 share strong homology across their first 21 amino acids, a region absent in IFITM1 (Fig. 2). Mutation of a single tyrosine, Y20, within this region produces a 50% or greater loss in restriction, coincident with the mutant isoform’s mislocalization to the cell periphery [33,61]. This work demonstrates that Y20 is required for the proper trafficking of IFITM3 to the late endosomes and lysosomes and thus is a major functional determinant within this IFITM2/3-specific portion of the NTD. Moreover, Y20 appears to be a key component of the amino acid cluster, YXXφ, which is similar to many bona fide clathrin-mediated endocytosis motifs (CMEMs) [6,33]. It follows that the lack of this CMEM within IFITM1 contributes to its differential localization, given that both IFITM1 and 3 are comparably palmitoylated on either C72 (IFITM3) or C50 (IFITM1, see section below) [109]. The localization of IFITM1/3 chimeras is also strongly influenced by their respective NTDs [61].

IFITM3’s IM1 and IFITM complexes: While searching for proteins that interact with IFITM3 using affinity purification coupled with MS, it was noted that the IFITMs formed homo- and heteromeric complexes [61]. These associations persisted after high-speed ultracentrifugation, suggesting that they were direct in nature. Alanine scanning (AS) mutagenesis identified two redundant phenylalanine residues in IFITM3’s IM1, F75 and F78, which were needed for both complex formation and restriction. The existence of IFITM multimers whose disruption coincides with decreased restriction and whose formation is dependent on residues within IM1 suggests that such interactions may alter the properties of the host cell membrane.

Cysteine palmitoylation: To prevent viral fusion, the IFITMs must traffic to vulnerable regions. As

### Table 3. Human CD225 protein superfamily members

| Gene name | Entrez gene ID | Function | Cellular expression pattern |
|-----------|----------------|----------|-----------------------------|
| IFITM1    | 8519           | Viral restriction; cell adhesion; tumor suppression | Cell surface, early endosomes > late endosomes |
| IFITM2    | 10581          | Viral restriction; cell adhesion; tumor suppression | Late endosomes and lysosomes |
| IFITM3    | 10410          | Viral restriction; cell adhesion; tumor suppression | Late endosomes and lysosomes |
| IFITM5    | 387733         | Bone mineralization; regulates association of CD9 with FKS06 binding protein (FKBP11)-CD81–prostaglandin F2 receptor negative regulator (FPRP) complex | Cell surface, most intense membrane localization at cell-to-cell junctions (HEK293 cells overexpressing the mouse homolog) |
| IFITM10   | 402778         | Unknown | Unknown |
| PRRT1     | 80863          | Interacts with AMPA receptors; oncogenesis | Unknown |
| PRRT2     | 112476         | Unknown; truncating mutations cause of paroxysmal kinesigenic dystonia (PKD) and infantile convulsions with choreoathetosis (ICCA) syndrome | Unknown |
| TUSC5     | 286753         | Responsive to insulin, glucose, glucocorticoids and/or PPARγ agonists; potential role in fat cell physiology; down-regulated in breast adenocarcinoma | Unknown |
| TMEM91    | 641649         | Unknown | Unknown |
| TMEM233   | 387890         | Unknown | Unknown |
| SYNDIG1   | 79953          | Regulates synaptic AMPAR content; regulates AMPAR- and NMDAR-mediated transmission; may play a role in regulating synaptogenesis | Unknown |
| SYNDIG1L  | 646658         | Unknown; possible role in the pathogenesis of Huntington disease; suggested role in striatal function | cis-Golgi (mouse protein fused to yellow fluorescence protein in Chinese ovary cells and HeLa cells) |
discussed, IFITM1 is primarily located in the plasma membrane and in early endosomes, and IFITM3 resides in the late endosomes and lysosomes. Palmitoylation of proteins on cysteines directs them to cellular membranes and in some instances into the endosomal pathway [116]. Preventing the addition of a palmitate to C72 diminished IFITM3’s antiviral actions and resulted in the mutant’s more central cellular location [61,109]. Similar results were also reported for murine Ifitm1 [54]. Thus, palmitoylation of a single conserved residue in the CD225 domain plays an important part in preventing viral entry and correctly positioning IFITM1 and 3 [61,109].

The CD225 domain: While this domain defines one of the largest families of membrane-associated proteins, little data exist regarding its function. The structural and functional data for the CD225 domain of IFITM3, presented below, reveal a strong link between proper intracellular location and restriction, thus lending support for a location-restriction rule. This simply predicts that any perturbation or mutation that alters the location of the IFITMs in respect to the cytosolic membrane or endosomal pathway will decrease restriction [2,4,61,104,109]. AS mutagenesis in six residue increments identified the CD225 domain as being the most important region of IFITM3 in terms of its cellular localization and expression level [61]. The importance of F75 and F78 for IFITM complex formation, together with the role of C72’s palmitoylation for localization, provides the earliest structural and functional associations for the CD225 domain and demonstrates this domain’s importance for intrinsic immunity [2,4,61,109]. Additional CD225 domain molecular determinants required for IFITM3’s restriction of IAV and DENV were found within IM1, with IFITM3 AS mutants 67AS (amino acids 67–72)

Table 3 (continued)

| Tissue expression pattern | Human genetic determinants | References |
|---------------------------|---------------------------|------------|
| Ubiquitous                | Unknown                   | [2,61–63]  |
| Ubiquitous                | Unknown                   | [61,63,64] |
| Ubiquitous                | rs12522-C; rs388188        | [2,33,61,63,65–68] |
| Bone; localized to mineralizing nodules (WT protein expressed in rat osteoblast cultures) | c.14C > T, 5-amino-acid N-terminal addition. Associated with a brittle bone disease, osteogenesis imperfecta type 5 | [3,69–84] |
| Adrenal gland, blood, bone, brain, connective tissue, eye, intestine, kidney, larynx, liver, lung, mammary gland, mouth, pancreas, placenta, prostate, salivary gland, skin, testis, trachea | Unknown | [85,86] |
| Brain, cervix, esophagus, intestine, kidney | Unknown | [87–96] |
| Embryonic tissue, Nervous system (globus pallidus, cerebellum, subthalamic nucleus, cerebellar peduncles, caudate nucleus, spinal cord, cerebral cortex, hippocampus). Low levels in heart, lung, kidney, skin | rs199662641; rs200926711; rs76335820; c.649–650insC; c.776dupG; c.649dupC. Associated with paroxysmal kinesigenic dyskinesia (PKD), benign familial infantile seizure/epilepsy (BFIS)/BFIE, infantile convulsions with choreoathetosis syndrome (ICCA) | [87–96] |
| White/Brown Adipose Tissue, Peripheral nervous system somatosensory neurons, mammary gland | Unknown | [97,98] |
| Blood, bone, brain, cervix, connective tissue, eye, heart, intestine, kidney, lung, placenta, prostate, skin, spleen, testis, thyroid, uterus | Unknown | [99] |
| Bone, brain, eye, lung, muscle, pancreas, thyroid | Unknown | [99] |
| Brain, embryonic tissue, esophagus, eye, heart, intestine, kidney, lung, ovary, pancreas, prostate, stomach, testis, thymus, umbilical cord | Unknown | [99] |
| Brain, connective tissue, eye, kidney, lung, nerve, pancreas | Unknown | [100] |

Review: IFITMs Inhibit Pathogenic Viruses
Fig. 2. (a) Alignment of the human IFITM1, IFITM2, IFITM3, IFITM5, and IFITM10 protein sequences (ClustalW2). The amino acids are color coded as follows: red, hydrophobic amino acids; green, polar amino acids; pink, basic amino acids; blue, acidic amino acids. The CD225 domain and the adjacent IM2s of the aligned proteins are outlined in red. Gaps introduced to maximize alignment are indicated by dashes. (b) Cartoon of IFITM3 in the endosomal membrane. The molecular determinants required for the IFITM3-mediated restriction, sites of posttranslational modifications, and nonsynonymous single-nucleotide polymorphisms (NS-SNP) are indicated in the key (bottom right). The NTD, intramembrane domain 1 (IM1), CIL, and intramembrane domain 2 (IM2) are indicated. IM1 and the CIL are in red font to convey that they comprise the canonical CD225 domain. The outer and inner leaflets of the endosomal membrane are noted. (c) Sequence logo for the CD225 domains and IM2s of the human IFITMs in (a). The respective amino acid properties color coded as above. Blue numbers indicate residues in IFITM3 found to be required for restriction, localization, or expression. These and similar figures were generated using Weblogo and the CLUSTALW freeware programs.
and 73AS (amino acids 73–78) being defective in intracellular localization and antiviral activity; subsequent work has revealed that N69 is likely the most critical residue in this region for both functions (our unpublished data; Ref. [61]; Fig. 3). Again, several mutations revealed a strong link between proper intracellular location and restriction. Within IFITM3’s CIL, R85, R87, Y99, and K104 were all needed for viral restriction. Y99 has been reported to be phosphorylated on PhosphoSitePlus; however, similar to Y20, both the functional significance and the possible regulation of this PTM remain to be determined. Y99’s mutation decreased restriction of IAV more so than DENV, demonstrating that differing structural requirements may be necessary for controlling various infections [61]. Interestingly, Y99A, along with R87A, were mutations that lowered restriction but had intracellular distributions indistinguishable from wild-type IFITM3, thus separating these phenotypes and demonstrating that the CD225 domain functions in both proper positioning and restriction.

To compare the prevalence of these functionally important residues, the CLUSTAL and Weblogo programs were used to construct probability diagrams for amino acids in the IM1, CIL, and IM2 regions of the human IFITMs (IFITM1, 2, 3, 5, and 10; Fig. 2c) with or without an additional seven human CD225 proteins (Fig. 3b) [117]. These analyses revealed that across the IFITMs, N69, C72, F75, R85, and K104 were among the residues with the highest conservation, consistent with their functional importance to IFITM3-mediated restriction. D56, D86, and D92 are conserved across the CD225 proteins and were found to be required for IFITM3 expression, suggesting important roles in stability [61]. D56 is situated immediately prior to IM1 and thus may maintain membrane topology. Though the IM1s of the IFITMs contain a higher percentage of conserved polar residues than found in the IM2s, only N69 was found to be required for restriction by IFITM3, suggesting an alternative functional role and/or redundancy (Fig. 2c). IM2 is not a designated portion of the CD225 domain since its sequence conservation is low; however, in all the human CD225 proteins, the segment following the CIL is hydrophobic, and in many instances, a leucine/isoleucine zipper is demonstrable. Consideration should be given to extending the CD225 domain to include a distal hydrophobic segment predicted to be

![Fig. 3. (a) Alignment of the CD225 domains and IM2s of the human CD225 proteins (ClustalW2). The amino acids are color coded as above. (b) Sequence logo for the human CD225 proteins. Blue numbers indicate residues in IFITM3 found to be required for restriction, localization, or expression. This figure was generated using Weblogo.](image-url)
membrane associated. AS mutagenesis of IFITM3’s IM2 revealed that the amino acid side chains within this region were not required for either restriction or localization [61]. However, because a hydrophobic-based mutagenesis strategy was employed, the resulting alterations may have been too conservative. Therefore, IM2 may indeed serve a role in decreasing membrane fluidity based on its hydrophobic properties and potential leucine zipper motif (discussed below).

### IFITM’s Role In Vivo

IFITM3’s potent restriction of IAV in vitro suggested that it might also play an important role in vivo; such is the case, as mice deficient in Ifitm3 succumb more readily to IAV infection when compared to wild-type littermates [65,101]. While these experiments involved the animals’ first exposure to IAV, an additional in vivo role for Ifitm3 in protecting long-term memory CD8+ T cells during re-infection has also been reported [118].

#### Table 4. IFITM protein family posttranslational modifications

| Protein | Posttranslational modification (PTM) | Functional role | References |
|---------|-------------------------------------|-----------------|------------|
| IFITM1  | Mouse: S-palmitoylation cysteines 49, 50, 83, 103 | Localization to membrane | [54] |
|         | Phosphorylation lysine 67            | Unknown         | [105–107] |
| IFITM2  | Phosphorylation serine 9             | Unknown         | [108] |
|         | Ubiquitination lysine 87             | Unknown         | [105–107] |
| IFITM3  | Phosphorylation tyrosine 20          | Required for proper trafficking from the cell surface and localization to late endosomes and lysosomes; required for restriction of IAV and DENV, not present in IFITM1. | [33,61], PhosphoSitePlus |
|         | Ubiquitination lysine 24             | Most robustly ubiquitinated of the four lysines found in IFITM3. The combined mutation of K24, K83, K88, and K104 to alanine increased protein stability, augmented the formation of autolysosomes, and promoted restriction of IAV | [54,104], PhosphoSitePlus |
|         | S-palmitoylation cysteines 71,72,105 | Mutation of C72 to alanine disrupts proper cellular localization and decreases restriction for IAV and DENV. Mutation of either C71 or C105 to alanine has no effect on these attributes | [61,109] |
|         | Ubiquitination lysine 83             | Mutation to alanine had no effect on restriction | [61,104] |
|         | Ubiquitination lysine 88             | Mutation to alanine had no effect on restriction | [61,104], PhosphoSitePlus |
|         | Phosphorylation tyrosine 99          | Mutation to alanine decreased restriction of DENV more than IAV but did not alter localization | [61], PhosphoSitePlus |
|         | Ubiquitination lysine 104            | Mutation to alanine decreased restriction (IAV > DENV) without altering localization | [61,104], PhosphoSitePlus |

* For PTMs with greater than three citations on PhosphoSitePlus, the reader is referred to the website using the following link where those citations are visible upon clicking the specific PTM (http://www.phosphosite.org/homeAction.do).
Review: IFITMs Inhibit Pathogenic Viruses

Fig. 4 (legend on previous page)
Mechanism of Action

Notably, when Everitt et al. investigated how IFITM3 might influence the clinical course of humans infected with IAV, they found that a minor allele, SNP rs12252-C, was enriched in patients hospitalized due to pandemic H1N1/09 infection in England and Scotland [65]. While the mechanistic role of this allele remains under investigation, the rs12252 C/C SNP alters a predicted splice acceptor site suggesting several scenarios. While comparatively rare in Caucasion populations, the rs12252-C allele is considerably more prevalent in Han Chinese individuals. Therefore, it was noteworthy when in an independent study rs12252-C was subsequently found to be enriched for in patients with severe influenza (69% severe influenza versus 25% mild) [66]. Based on these results, the rs12252 allele is estimated to confer a sixfold higher risk for severe influenza. Therefore, populations expressing higher percentages of the rs12252 allele such as in regions of China and Japan may be more at risk for seasonal influenza epidemics and pandemics [66]. It follows that IFITM3 genotyping may be beneficial to clinicians serving these populations because it could help with risk stratification including decisions for closer observation and administration of therapy. Moreover, these results suggest that IFITM levels and/or actions may influence the clinical course of additional illnesses caused by IFITM-sensitive viruses.

IFITMs block viral fusion and prevent the cytosolic entry of viral genomes. Instead of successfully fusing with the host membrane, IFITM-sensitive viruses are arrested at the cell surface and trapped in the endosomal pathway, resulting in their travel to the lysosomes or autolysosomes, where they are endosomal pathway, resulting in their travel to the lysosomes or autolysosomes, where they are destroyed (Fig. 1a and b). The end result, the sequential inhibition and destruction of the virion, serves to lower the inoculum and is an effective means of preventing invasion by an array of viruses that exploit the endocytic pathway.

How do the IFITMs prevent viral entry? In addressing this, several points must be considered: (i) the IFITM's inhibition of an array of viruses that enter at various cell locations using divergent fusion machinery; (ii) the ability of some viruses to resist one IFITM but not another; (iii) the IFITM immunity of SeV, MLV, and all of the arenaviruses envelopes thus tested; (iv) previous data showing that their overarching effect is the inhibition of viral fusion subsequent to viral binding and endocytosis; and (v) data concerning the molecular determinants required for restriction. Recently, we and others have postulated that the IFITMs alter the physical properties of the host cell's membrane, thus interfering with viral fusion [5,54,61,119]. In this "tough-membrane" model, we postulate that intramembranous interactions between the IMI domains of adjacent IFITMs alter both the fluidity and the bending modulus of the host cell membrane, making it resistant to the viral fusion machinery (Fig. 4) [61]. Additionally, the intramembranous insertions of the IMs may asymmetrically compress the outer leaflet, generating a curvature directed away from the viral fusion machinery's drawing force. Precedent for this last part comes from studies of the reticulons, which shape the endoplasmic reticulum into a tubular structure by inserting their two IM domains into the outer leaflet of the membrane [110]. The tough-membrane model predicts that IFITMs must be at the site of viral entry, which has been shown to be the case in mutagenesis studies. Moreover, it accounts for the specificity of IFITM actions, with IFITM1 primarily blocking early endosomal-entering viruses and IFITM3 acting on late endosomal-entering pathogens. In addition, this scenario would account for the rapid action of the IFITMs and their effective inhibition of diverse viruses.

Decreasing membrane fluidity may impede the lateral movements of membrane-associated proteins and thus inhibit both the sequential interaction of viral envelopes with multiple host receptors, as is required for HIV-1 and HCV, and the coalescence of multiple viral envelopes, as has been demonstrated for IAV entry (Fig. 4a–c) [120]. It is also possible that viral membranes that originate from the host and contain IFITMs will have decreased fluidity. Although speculative, this scenario could also explain why some viruses are immune to the IFITMs; perhaps the IFITM-resistant viral fusion machinery possesses intrinsic properties (strength, cooperativity) that permit them to overcome the increased rigidity of the host membrane. Alternatively, viruses that either depend on a single host receptor (MLV and SeV) or more readily achieve the clustering of the required envelopes to fuse may also overcome restriction. For viruses that are susceptible to the IFITMs but only rely on one host receptor, that is, JSRV, one possibility is that an additional co-receptor requirement may await detection. An obvious concern with this scenario is that while a more viral-resistant membrane would be advantageous, it could potentially interfere with normal cellular physiology, for example, endocytic trafficking, vesicular fusion, and cytokinesis, all of which are impacted by membrane fluidity.

How do a relatively few IFITM molecules alter the cell's membrane surfaces? One explanation may come from IFITM1 locating to lipid rafts, an area of the membrane where many viruses bind to host receptors and are endocytosed [112]. This suggests that IFITMs may have been selected to concentrate at, or rapidly move to, viral attachment and entry zones that are enriched for host receptors; one means of achieving this may be seen with IFITM1's association with the tetraspanins, including CD19 and CD81, which reside in lipid rafts [15]. Binding to proteins that are enriched in lipid rafts could efficiently home the IFITMs to a location...
where they can block multiple viruses without needing to interact with each virus' host receptor; these associations could potentially involve a predicted leucine zipper in IM2. Together, the paired interactions of IM1 and 2 could foreseeably tether each end of the IFITMs to create a protective mesh stretching across viral entry zones (Fig. 4d). Indeed, a meshwork of tetraspanins, the tetraspanin web, has been previously proposed [121]; however, in contrast to earlier pro-viral models of tetraspanin interactions, this scenario would instead be antiviral. For IFITM3, located in late endosomes, this model predicts its association with viral entry zones as the pathogen-bearing endosomes sequentially mature along the endocytic pathway.

While such a direct model of IFITM-induced restriction addresses some issues, we note that the IFITMs cause changes in the endosomal environment, including its expansion and acidification, suggesting that modulation of endosomal conditions may also contribute to viral inhibition [3,4,21,104,122]. In light of this, it has recently been proposed that IFITMs antagonize the host cell's lipid homeostasis, resulting in the mislocalization of high levels of cholesterol to the late endosomes producing a block to viral fusion [119]. While cholesterol itself is required by many viruses for fusion, it also can inhibit infection. IFITM1, 2, and 3 are reported to strongly bind to the endocytic trafficking protein, vesicle-associated membrane protein-A (VAPA). The IFITM–VAPA interaction interferes with the binding of VAPA to oxysterol binding protein (OSBP) [123]. OSBP is a known regulator of cholesterol trafficking and has been shown to bind to numerous host proteins, including VAPA. Therefore, a competition may exist between OSBP and the IFITMs for VAPA, with higher IFITM levels sending more cholesterol to the late endosomes and blocking viral fusion. An attractive aspect of this model is that the general alteration of membrane cholesterol levels readily explains how a relatively small number of IFITMs can prevent different viruses from entering from the late endosomes and lysosomes. However, it is unclear how the mislocalization of cholesterol to the late endosomal compartment would inhibit early endosomal-entering viruses such as HIV-1, HCV, and JSRV. Similarly, this model cannot fully explain IFITM specificity because the interaction of either IFITM1 or 3 with VAPA does not appear to direct excess cholesterol to a particular compartment; that is, IFITM1 is not shown to relocate cholesterol to the early endosomes to block HCV and JSRV. While these early attempts to explain the antiviral effects of the IFITMs may prove useful, much additional experimentation is required, perhaps leading to the integration of several components of these models. For example, increased cholesterol in combination with IFITM complexes could further toughen the host membrane against viral entry (Fig. 4d, right panel). These unanswered mechanistic issues notwithstanding, the IFITMs represent a broadly acting and previously unappreciated class of restriction factor that traps and degrades invading pathogens, thereby protecting the host individually as well as at a population level.

Acknowledgments

We thank University of Massachusetts Medical School (UMMS) colleagues, R. Fish, B. Hobbs, L. Benson, T. Brailey, and J. Barrett, and Ragon Institute colleagues, M. Boyarina, K. Donnelly, and P. Richtmeyer. This work was generously supported by a grant (1R01AI091786) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, to A.L.B. A.L.B. is grateful to the Charles H. Hood Foundation, the Burroughs Wellcome Fund, the Phillip T. and Susan M. Ragon Foundation, and the Harvard and UMMS Centers for AIDS Research for their generous support.

Received 19 August 2013; Received in revised form 18 September 2013; Accepted 19 September 2013
Available online 25 September 2013

Keywords:
interferon effector genes;
http://pfam.sanger.ac.uk/family/PF04505

Abbreviations used:
IFITM, interferon-inducible transmembrane protein; IFN, interferon; IAV, influenza A virus; DENV, dengue virus; SARS CoV, severe acute respiratory syndrome coronavirus; HCV, hepatitis C virus; EBOV, Ebola virus; MARV, Marburg virus; CIL, conserved intracellular loop; MS, mass spectrometry; NTD, N-terminal domain; RVFV, Rift Valley fever virus; HIV-1, human immunodeficiency virus type 1; JSRV, Jaagsieke sheep retrovirus; GP, glycoprotein; CCHFV, Crimean Congo hemorrhagic fever virus; MLV, Moloney leukemia virus; SeV, Sendai virus; CMEM, clathrin-mediated endocytosis motif; AS, alanine scanning; VAPA, vesicle-associated membrane protein-A; OSBP, oxysterol binding protein.

References

[1] Harrison SC. Mechanism of membrane fusion by viral envelope proteins. Adv Virus Res 2005;64:231–61.
[2] Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 2009;139:1243–54.
[3] Huang IC, Bailey CC, Weyer JL, Radoshitzky SR, Becker MM, Chiang JJ, et al. Distinct patterns of IFITM-mediated
restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog 2011;7:e1001258.

[4] Feeley EM, Sims JS, John SP, Chin CR, Pertel T, Chen LM, et al. IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog 2011;7:e1002337.

[5] Li K, Markosyan RM, Zheng YM, Golfetto O, Bungart G, Li M, et al. IFITM proteins restrict viral membrane hemifusion. PLoS Pathog 2013;9:e1003124.

[6] Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 2003;72:395–447.

[7] Leung HS, Li OT, Chan RW, Chan MC, Nicholls JM, Poon LL. Entry of influenza A virus with a alpha2,6-linked sialic acid binding preference requires host fibronectin. J Virol 2012;86:10704–13.

[8] Jiang D, Weidner JM, Qing M, Pan XB, Guo H, Xu C, et al. Identification of five interferon-induced cellular proteins that inhibit West Nile virus and dengue virus infections. J Virol 2010;84:8332–41.

[9] Chen YC, Wang SY, King CC. Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. J Virol 1999;73:2650–7.

[10] Zaitseva E, Yang ST, Melikov K, Pourmal S, Chernomordik LV. Dengue virus ensures its fusion in late endosomes using compartment-specific lipids. PLoS Pathog 2010;6:e1001131.

[11] Smit JM, Moesker B, Rodenhuis-Zybert I, Wilschut J. Identification of five interferon-induced cellular proteins that inhibit West Nile virus and dengue virus infections. J Virol 2011;85:2126–7.

[12] Chan YK, Huang IC, Farzan M. IFITM proteins restrict antibody-dependent enhancement of dengue virus infection. PLoS One 2012;7:e34508.

[13] Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, et al. A diverse range of gene products are essential for Ebola virus infection. Nature 2011;472:481–9.

[14] Wilkins C, Woodward J, Lau DT, Barnes A, Joyce M, McFarlane N, et al. IFITM1 is a tight junction protein that inhibits hepatitis C virus entry. Hepatology 2013;57:461–9.

[15] Harris HJ, Clerc F, Farquhar MJ, Goodall M, Hu K, Rassam P, et al. Hepatoma polarization limits CD81 and hepatitis C virus dynamics. Cell Microbiol 2013;15:430–45.

[16] Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplastic tail deleted. J Virol 2007;81:8722–9.

[17] Hunt CL, Lennemann NJ, Maury W. Filovirus entry: a novelty in the viral fusion world. Viruses 2012;4:258–75.

[18] Carette JE, Raaben M, Wong AC, Herbert AS, Obermoser G, Mulherkar N, et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 2011;477:340–3.

[19] Cote M, Misasi J, Ren T, Bruchez A, Lee K, Filone CM, et al. Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. Nature 2011;477:344–8.

[20] Muthasani R, Tran JP, Retterer C, Radoshitzky SR, Kota KP, Altamura LA, et al. IFITM-2 and IFITM-3 but not IFITM-1 restrict Rift Valley fever virus. J Virol 2013;87:8451–64.

[21] Harmon B, Schudel BR, Maar D, Kozina C, Ikegami T, Tseng CT, et al. Rift Valley fever virus strain MP-12 enters mammalian host cells via caveola-mediated endocytosis. J Virol 2012;86:12954–70.

[22] Hollidge BS, Nedelsky NB, Salzano MV, Fraser JW, Gonzalez-Scarano F, Soldan SS. Orthobunyavirus entry into neurons and other mammalian cells occurs via clathrin-mediated endocytosis and requires trafficking into early endosomes. J Virol 2012;86:7988–9001.

[23] McMillan M, Hoffmann JA, Arzilli M, Rambaut A, Draviam V, Wang D, et al. IFITM proteins restrict viral membrane fusion. J Virol 2012;86:10704–13.

[24] Zakaawa J, Takashima I, Hashimoto N. Cell fusion by hantahara HFRS virus is inhibited by IFITM1. J Virol Methods 2009;157:57–63.

[25] Holliday EM, de Jong JCM, de Ix RO, Mankertz J, Hoornstra R. IFITM proteins restrict vesicular stomatitis virus infection. J Virol 2010;84:12817–22.

[26] Harris HJ, Clerte C, Farquhar MJ, Goodall M, Hu K, Rassam P, et al. IFITM proteins restrict antibody-dependent enhancement of dengue virus infection. J Virol 2012;86:10704–13.

[27] McLaughlin J, O’Neill HJ, Coyle PV. Low pH-induced cytopathic effect—a survey of seven hantavirus strains. J Virol Methods 1999;81:193–7.

[28] Weidner JM, Jiang D, Pan XB, Chang J, Block TM, Guo JT. Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. J Virol 2010;84:12646–57.

[29] Finkelstein D, Werman A, Novick D, Barak S, Rubinstein M. LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. Proc Natl Acad Sci U S A 2013;110:7306–11.

[30] Zhu R, Wang J, Lei XY, Gui JF, Zhang QY. Evidence for Parachuteovirus oviparous IFITM1 antiviral effect by impeding viral entry into target cells. Fish Shellfish Immunol 2013;35:918–26.

[31] Zhang QY, Tao JJ, Gui L, Zhou GZ, Ruan HM, Li ZQ, et al. Isolation and characterization of Scophthalmus maximus rhabdovirus. Dis Aquat Organ 2007;74:95–105.

[32] Lu J, Pan Q, Rong L, He W, Liu SL, Liang C. The IFITM proteins inhibit HIV-1 infection. J Virol 2011;85:2126–37.

[33] Jin X, Pan Q, Ding S, Rong L, Liu SL, Geng Y, et al. The terminal region of IFITM3 modulates its antiviral activity by regulating IFITM3 cellular localization. J Virol 2012;86:13697–707.

[34] Yoshi H, Kamiyama H, Goto K, Oishi K, Katunuma N, Tanaka Y, et al. CD4-independent human immunodeficiency virus type 1 infection involves participation of endocytosis and cathepsin B. PLoS One 2011;6:e19352.

[35] Sloan RD, Kuhl BD, Mesplede T, Munch J, Donahue DA, Wainberg MA. Productive entry of HIV-1 during cell-to-cell transmission via dynamin-dependent endocytosis. J Virol 2013;87:8110–23.

[36] Bertrand P, Cote M, Zheng YM, Albritton LM, Liu SL. Jaagsiekte sheep retrovirus utilizes a pH-dependent endocytosis pathway for entry. J Virol 2008;82:2555–9.

[37] Cote M, Zheng YM, Liu SL. Receptor binding and low pH coactivate oncogenic retrovirus envelope-mediated fusion. J Virol 2009;83:11447–55.

[38] Kaneko AA, Bowman CH, Chin CR, Brass AL, Holm GH. Interferon inducible transmembrane protein 3 (IFITM3) restricts reovirus cell entry. J Biol Chem 2013;288:17261–71.

[39] Danthi P, Holm GH, Stehle T, Dermody TS. Reovirus receptors, cell entry, and proapoptotic signaling. Adv Exp Med Biol 2013;790:42–71.

[40] Zhan QY, Xiao F, Li ZQ, Gui JF, Mao J, Chinchar VG. Characterization of an iridovirus from the cultured pig frog Rana grylio with lethal syndrome. Dis Aquat Organ 2001;48:27–36.

[41] Shapira SD, Gatt-Viks I, Shum BO, Dricot A, de Grace MM, Wu L, et al. A physical and regulatory map of host-influenza...
Review: IFITMs Inhibit Pathogenic Viruses

interactions reveals pathways in H1N1 infection. Cell 2009;139:1255–67.

[42] Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat Rev Immunol 2013;13:46–57.

[43] Rojek JM, Kunz S. Cell entry by human pathogenic arenaviruses. Cell Microbiol 2008;10:828–35.

[44] Kunz S. Receptor binding and cell entry of Old World arenaviruses reveal novel aspects of virus–host interaction. Virology 2009;387:245–9.

[45] Burri DJ, Pasqual G, Rochat C, Seidah NG, Pasquato A, Campbell KP, et al. Cell entry of Lassa virus induces tyrosine phosphorylation of dystroglycan. Cell Microbiol 2013;15:689–700.

[46] Coffin JM. Virions at the gates: receptors and the host-virus interaction reveals pathways in H1N1 infection. Cell 2009;139:1255–67.

[47] Zhang Y, Li L, Liu X, Dong S, Wang W, Huo T, et al. Crystal structure of Junin virus nucleoprotein. J Gen Virol 2013;94:2175–83.

[48] Helguera G, Jemielity S, Abraham J, Cordo SM, Martinez MG, Rodriguez JA, et al. An antibody recognizing the apical domain of human transferrin receptor 1 efficiently inhibits the entry of all new world hemorrhagic fever arenaviruses. J Virol 2012;86:4024–8.

[49] Martinez MG, Forlenza MB, Candurra NA. Involvement of cellular proteins in Junin arenavirus entry. Biotechnol J 2009;4:866–70.

[50] Kumar P, Nachagari D, Fields C, Franks J, Albritten LM. Host cell cathepsins potentiate Moloney murine leukemia virus infection. J Virol 2007;81:10506–14.

[51] Voelkel C, Galla M, Dannhauser PN, Maetzig T, Sodeik B, Kumar P, Nachagari D, Fields C, Franks J, Albritton LM. IFITM3 restricts the morbidity and mortality associated with influenza. Nature 2012;484:519–23.

[52] Lee S, Zhao Y, Anderson WF. Receptor-mediated Moloney murine leukemia virus entry can occur independently of the clathrin-coated-pit-mediated endocytic pathway. J Virol 2012;86:6024–8.

[53] Lee S, Zhao Y, Anderson WF. Receptor-mediated Moloney murine leukemia virus entry can occur independently of the clathrin-coated-pit-mediated endocytic pathway. J Virol 2012;86:6024–8.

[54] Hach JC, McMichael T, Chesarino NM, Yount JS. Palmitoylation on conserved and non-conserved cysteines of murine IFITM1 regulates its stability and anti-influenza A virus activity. J Virol 2013;87:9923–7.

[55] Bitzer M, Lauer U, Baumann C, Spiegel M, Gregor M, Yang G, Xu Y, Chen X, Hu G. IFITM1 plays an essential role in the antiproliferative action of interferon-gamma. Oncogene 2007;26:594–603.

[56] Zhang Z, Liu J, Li M, Yang H, Zhang C. Evolutionary dynamics of the interferon-induced transmembrane gene family in vertebrates. PLoS One 2012;7:e49265.

[57] Coffin JM. Virions at the gates: receptors and the host-virus interactions reveals pathways in H1N1 infection. Cell 2009;139:1255–67.

[58] Seo GS, Lee JK, Yu JI, Yun KJ, Chae SC, Choi SC. Identification of the polymorphisms in IFITM3 gene and their association in a Korean population with ulcerative colitis. Exp Mol Med 2010;42:99–104.

[59] Shen C, Wu XR, Jiao WW, Sun L, Feng WX, Xiao J, et al. A functional promoter polymorphism of IFITM3 is associated with susceptibility to pediatric tuberculosis in Han Chinese population. PLoS One 2013;8:e67816.

[60] Hanagata N, Li X. Osteoblast-enriched membrane protein IFITM5 regulates the association of CD9 with an FKRBP11–CD81–FPRP complex and stimulates expression of interferon-induced genes. Biochem Biophys Res Commun 2011;409:378–84.

[61] Semler O, Garbes L, Keupp K, Swan D, Zimmermann K, Becker J, et al. A mutation in the 5′-UTR of IFITM5 creates an in-frame start codon and causes autosomal-dominant osteogenesis imperfecta type V. Am J Hum Genet 2012;91:349–57.

[62] Ruach F, Moffatt P, Cheung M, Roughley P, Lalic L, Lund AM, et al. Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C > T mutation in all patients. J Med Genet 2013;50:21–4.

[63] Cho TJ, Lee KE, Lee SK, Song SJ, Kim KJ, Jeon D, et al. A single recurrent mutation in the 5′-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Hum Genet 2012;91:343–8.

[64] Takagi M, Sato S, Hara K, Tani C, Miyazaki O, Nishimura G, et al. A recurrent mutation in the 5′-UTR of IFITM5 creates osteogenesis imperfecta type V. Am J Hum Genet 2013;91:343–8.

[65] Kasabai B, Gaumond MH, Moffatt P. Regulation of the bone-restricted IFITM-like (Bril) gene transcription by Sp and Gli family members and CpG methylation. J Biol Chem 2013;288:13278–94.

[66] Grover M, Campeau PM, Lietman CD, Lu JT, Gibbs RA, Schlesinger AE, et al. Osteogenesis imperfecta without features of type V caused by a mutation in the IFITM5 gene. J Bone Miner Res 2013 [Epub ahead of print].
Review: IFITMs Inhibit Pathogenic Viruses

Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SC, Dawson BC, et al. Phenotypic variability of osteogenesis imperfecta type V caused by an IFITM5 mutation. J Bone Miner Res 2013;28:1523–30.

Hanagata N, Li X, Morita H, Takemura T, Li J, Minowa T. Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. J Bone Miner Metab 2011;29:279–90.

Liu Y, Liu H, Titus L, Boden SD. Natural antisense transcripts enhance bone formation by increasing sense IFITM5 transcription. Bone 2012;51:933–8.

Balasubramanian M, Parker MJ, Dalton A, Giunta C, Hanagata N, Li X, Morita H, Takemura T, Li J, Minowa T. PRRT2 gene mutations in familial and sporadic paroxysmal kinesigenic dyskinesia cases. Mov Disord 2013;28:1313–4.

Jing XY, Li XH, Yuan P, Deng J, Hu B, Wang Y. A novel mutation and functional implications of 5 variants in the PRRT2 gene in 20 paroxysmal kinesigenic dyskinesia pedigrees. Parkinsonism Relat Disord 2013;19:639–42.

Fabbri M, Marini C, Bisulli F, Di Vito L, Elia A, Guerrini R, et al. Clinical and polygraphic study of familial paroxysmal kinesigenic dyskinesia with PRRT2 mutation. Epileptic Disord 2013;15:123–7.

Wang JL, Mao X, Hu ZM, Li JD, Li N, Guo JF, et al. Mutation analysis of PRRT2 in two Chinese BFIS families and nomenclature of PRRT2 related paroxysmal diseases. Neurosci Lett 2013;552C:40–5.

Oort PJ, Warden CH, Baumann TK, Knotts TA, Adams SH. Characterization of Tusc5, an adipocyte gene co-expressed in peripheral neurons. Mol Cell Endocrinol 2007;276:24–35.

Bubnov V, Moskalev E, Petrovskiy Y, Bauer A, Hoheisel J, Zaporozhan V. Hypermethylation of TUSC5 genes in breast cancer tissue. Exp Oncol 2012;34:370–2.
lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. J Immunol 1992;149:2841–50.

[113] Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, et al. Binding of hepatitis C virus to CD81. Science 1998;282:938–41.

[114] Lozach PY, Huotari J, Helenius A. Late-penetrating viruses. Curr Opin Virol 2011;1:35–43.

[115] Nunberg JH, York J. The curious case of arenavirus entry, and its inhibition. Viruses 2012;4:83–101.

[116] Chamberlain LH, Lemonidis K, Sanchez-Perez M, Werno MW, Gorleku OA, Greaves J. Palmitoylation and the trafficking of peripheral membrane proteins. Biochem Soc Trans 2013;41:62–6.

[117] Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator. Genome Res 2004;14:1188–90.

[118] Wakim LM, Gupta N, Mintern JD, Villadangos JA. Enhanced survival of lung tissue-resident memory CD8(+) T cells during infection with influenza virus due to selective expression of IFITM3. Nat Immunol 2013;14:238–45.

[119] Amini-Bavil-Olyaee S, Choi YJ, Lee JH, Shi M, Huang IC, Farzan M, et al. The antiviral effector IFITM3 disrupts intracellular cholesterol homeostasis to block viral entry. Cell Host Microbe 2013;13:452–64.

[120] Ivanovic T, Choi JI, Whelan SP, van Oijen AM, Harrison SC. Influenza-virus membrane fusion by cooperative foldback of stochastically induced hemagglutin intermediates. Elife 2013;2:e00333.

[121] Boucheix C, Rubinstein E. Tetraspanins. Cell Mol Life Sci 2001;58:1189–205.

[122] Wee YS, Roundy KM, Weis JJ, Weis JH. Interferon-inducible transmembrane proteins of the innate immune response act as membrane organizers by influencing clathrin and v-ATPase localization and function. Innate Immun 2012;18:834–45.

[123] Wyles JP, McMaster CR, Ridgway ND. Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol-binding protein to modify export from the endoplasmic reticulum. J Biol Chem 2002;277:29908–18.

[124] Rojek JM, Sanchez AB, Nguyen NT, de la Torre JC, Kunz S. Different mechanisms of cell entry by human-pathogenic Old World and New World arenaviruses. J Virol 2008;82:7677–87.