

Better together: the role of IFIT protein–protein interactions in the antiviral response

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Abstract

The interferon-induced proteins with tetratricopeptide repeats (IFITs) are a family of antiviral proteins conserved throughout all vertebrates. IFIT1 binds tightly to non-self RNA, particularly capped transcripts lacking methylation on the first cap-proximal nucleotide, and inhibits their translation by out-competing the cellular translation initiation apparatus. This exerts immense selection pressure on cytoplasmic RNA viruses to maintain mechanisms that protect their messenger RNA from IFIT1 recognition. However, it is becoming increasingly clear that protein–protein interactions are necessary for optimal IFIT function. Recently, IFIT1, IFIT2 and IFIT3 have been shown to form a functional complex in which IFIT3 serves as a central scaffold to regulate and/or enhance the antiviral functions of the other two components. Moreover, IFITs interact with other cellular proteins to expand their contribution to regulation of the host antiviral response by modulating innate immune signalling and apoptosis. Here, we summarize recent advances in our understanding of the IFIT complex and review how this impacts on the greater role of IFIT proteins in the innate antiviral response.

INTRODUCTION

The interferon-induced proteins with tetratricopeptide repeats (IFITs) are a family of antiviral RNA-binding proteins, which are among the highest expressed genes during antiviral immune responses. The IFIT gene family is conserved in all vertebrates, having coevolved with the interferon (IFN) system during the genesis of the adaptive branch of the immune system [1]. This implicates IFIT proteins as fundamental components of the immune system.

IFITs have a conserved gene architecture typically comprising two exons, the first of which is very short, under the control of multiple interferon-responsive elements (ISREs) in the promoter region. These include strong binding sites for interferon-stimulated gene factor 3, to promote type I IFN-dependent expression, and weaker signal transducer and activator of transcription (STAT) 1 binding sites allowing type II IFN-driven induction [2, 3]. In addition, IFIT genes can be induced directly by interferon regulatory factor 3 (IRF-3), resulting in induction during the early stages of the immune response, immediately downstream of pattern

recognition receptor (PRR) activation [4]. Therefore, not only are IFITs some of the most highly expressed IFN-stimulated genes (ISGs) [5], but they are also among the earliest to be expressed during an antiviral response. Since the promoter for each IFIT gene is slightly different, there are subtle differences in the induction kinetics for different family members, dependant on stimulus and cell type [6, 7] (reviewed in [8]).

Most mammals encode IFIT1, IFIT1B, IFIT2, IFIT3 and IFIT5, but the exact composition of the IFIT gene locus varies between species, presumably owing to different evolutionary pressures from coevolving viruses [1]. For example, in rodents IFIT5 has been lost and recent phylogenetic analysis has suggested that IFIT1 has also been lost [9]. In mice, to potentially compensate for this loss, *Ifitb* has been duplicated twice, resulting in three paralogues: *Ifit1*, *Ifit1b* and *Ifit1c* [9]. While *Ifit1* largely phenocopies human IFIT1, the functions of the other two murine *Ifit1b* paralogues are unknown. By contrast, in humans, IFIT1B lacks ISREs in its promoter and has been hypothesized to be non-functional [9]. IFIT-like genes have also been identified in non-

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Abbreviations: Bcl, B-cell lymphoma apoptosis regulator; CDK, cyclin-dependent kinase; eIF, eukaryotic translation initiation factor; IFIT, interferon-induced proteins with tetratricopeptide repeats; IFN, interferon; IFNAR, type I interferon receptor; IKK, inhibitor of kappa B kinase; IL, interleukin; IRES, internal ribosome entry site; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; ISRE, interferon-responsive element; JAB, Jun activation domain-binding protein; JAK, Janus kinase; MAC, mitochondrial apoptosis-induced channel; MAVS, mitochondrial antiviral signalling protein; NF- κ B, nuclear factor κ B; RIG, retinoic acid-inducible gene; ROS, reactive oxygen species; SEC, size-exclusion chromatography; SEC-MALS, SEC coupled with multi-angled light scattering; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; TAK, transforming growth factor beta-activated kinase; TBK, TANK-binding kinase; TLR, Toll-like receptor; TNF, tumour necrosis factor; TPR, tetratricopeptide repeat; TRAF, TNF receptor associated factor; TYK, non-receptor tyrosine-protein kinase.

mammalian vertebrates, including reptiles, amphibians and fish; for example, zebrafish have been shown to express at least seven IFITs [1]. By contrast, in birds, almost all IFIT genes have been lost [10], but IFIT5 is conserved and appears to maintain the functions of its human counterpart [11, 12]. Since the nomenclature of IFIT proteins has changed multiple times since their discovery, a summary of IFIT aliases is provided in Table 1.

IFIT proteins are direct antiviral effectors during innate immune responses. This antiviral activity was first attributed to the ability of IFIT1 and IFIT2 to interact with eukaryotic initiation factor (eIF) 3 and inhibit translation at the initiation stage [13, 14]. eIF3 is a large multi-protein complex that acts as a scaffold to coordinate the recruitment of other initiation factors and mRNA to the small ribosomal subunit (40S) [15]. eIF3 is essential for the translation of capped RNAs, including cellular mRNA and some viral RNAs (e.g. coronaviruses and some flaviviruses), as well as those with certain internal ribosome entry sites (IRESs) such as encephalomyocarditis virus (EMCV). However, while different effects of IFIT1 on hepatitis C virus (HCV) IRES-dependent translation have been reported [16, 17], it has no effect on EMCV translation [18], indicating that IFIT proteins may act via different mechanisms.

Later it was discovered that IFITs can inhibit viral infection by binding directly to non-self RNAs to inhibit their translation or replication. This was first described by Pichlmair *et al.*, who identified 5'-ppp RNA-binding proteins in the lysates of IFN-stimulated cells [5]. Later, IFIT1 was shown to bind to capped RNA lacking methylation on the first cap-proximal nucleotide (cap0) with much higher affinity [19–21]. The structures of human IFIT1 and IFIT5 reveal that a positively charged channel, formed in the groove between the globular N- and C-terminal domains,

coordinates the RNA phosphate backbone in a sequence non-specific manner [17, 22–24]. IFIT1 has an additional hydrophobic cavity at the rear of the RNA-binding channel, which accommodates the 5' cap structure [17]. Residues within the channel obstruct efficient binding to 2'-O-methylated nucleotides, accounting for IFIT1's considerably lower affinity for cap1 RNA [17, 25].

By occluding the 5' end of non-self RNA, IFIT1 can compete with the cap-binding complex eIF4F and prevent translation initiation on bound substrates [19]. Since IFIT1 is one of the most abundant IFN-stimulated gene products during the antiviral response, this exerts a significant selection pressure on viruses to maintain mechanisms to protect their messenger RNA from IFIT1 detection. Alphaviruses naturally produce cap0 RNA, but possess a stable stem loop at the 5' end of their genomes that prevents entry into the IFIT1 RNA-binding pocket, even at the expense of optimal viral RNA replication [26]. Destabilization of this stem promotes susceptibility to IFIT1 in a number of alphaviruses, including the emerging human pathogens Chikungunya virus and Venezuelan equine encephalitis virus (VEEV) [26, 27]. By contrast, the members of the families *Flaviviridae* and *Coronaviridae*, which utilize cap-dependent translation, encode their own 2'-O-methyltransferase to generate a 'self' cap1 RNA. Disruption of this activity in the flaviviruses West Nile virus (WNV), Zika virus (ZIKV) and dengue virus (DENV) significantly increases susceptibility to IFIT1 restriction [28–31]. Likewise, the coronaviruses severe acute respiratory syndrome virus and murine hepatitis virus are dependent on functional 2'-O-methyltransferase activity to escape IFIT1 [32, 33]. Similar to alphaviruses, coronaviruses and flaviviruses possess highly structured 5' UTRs to reduce the efficiency of IFIT1 binding [17, 34].

Paramyxoviruses of the genus *Rubulavirus* are strongly restricted by type I IFN in an IFIT1-dependent manner, since it inhibits the translation of particular viral mRNA segments [35, 36]. This was partially attributed to inefficient 2'-O-methylation of these mRNAs by the viral polymerase [36]. However, IFIT1 is incapable of restricting influenza A virus infection, since orthomyxoviruses snatch self caps from host mRNAs, which are resistant to IFIT1 [37]. IFIT1 has also been shown to restrict human papillomavirus replication by sequestering the viral DNA helicase E1 in the cytoplasm [38, 39] and it has been suggested that it inhibits human cytomegalovirus replication in a similar fashion [40]. The antiviral functions of human and murine IFIT proteins are summarized in Table 2.

However, despite considerable investigation into the behaviour of each IFIT protein in isolation, it is becoming increasingly clear that IFIT proteins can associate with each other to form functional hetero-complexes that are greater than the sum of their parts. Here we review recent advances in our understanding of interactions between different human IFIT family members, as well as between IFITs and other cellular proteins. We also discuss how these interactions can modulate and expand the functions of different IFITs,

Table 1. IFIT aliases

A summary of all known aliases for human and murine IFIT genes and proteins. GARG, glucocorticoid-attenuated response gene; IFI, interferon-induced; ISG, interferon-stimulated gene; RIG, retinoic acid inducible gene.

IFIT	Accession no.	Alternative names
IFIT1	NP_001539.3	C56, G10P1, IFI-56, IFI-56K, IFI56, IFIT-1, IFNA11, ISG56, P56
IFIT1B	NP_001010987.1	IFIT1L
IFIT2	NP_001538.4	G10P2, GARG-39, IFI-54, IFI-54K, IFI54, IFIT-2, ISG-54 K, ISG-54K, ISG54, P54
IFIT3	NP_001540.2	GARG-49, IFI60, IFIT4, ISG60, P60, RIG-G
IFIT5	NP_036552.1	ISG58, P58
Ifit1	NP_032357.2	Garg16, Isg56, Ifi56, Ifit1b1, p56
Ifit1b	NP_444447.1	Ifit1b2, Ifit1b2
Ifit1c	NP_001103987.1	Ifit1b3, Ifit1b1
Ifit2	NP_032358.1	Garg-39, Ifi54, p54
Ifit3	NP_034631.1	Garg-49, Ifi49, p49
Ifit3b	NP_001005858.2	Ifit3-like

Table 2. Viral restriction by IFIT proteins

A summary of studies investigating the effect of IFIT proteins during viral infection. Human IFITs are given in upper case, while murine IFITs are in lower case. CDV, canine distemper virus; CHIKV, chikungunya virus; DENV, dengue virus; EBOV, Ebola virus; EMCV, encephalomyocarditis virus; HCMV, human cytomegalovirus; HCoV, human coronavirus; HCV, hepatitis C virus; HPV, human papilloma virus; HSV, herpes simplex virus; IAV, influenza A virus; JEV, Japanese encephalitis virus; LACV, La Crosse encephalitis virus; MERS, Middle East respiratory syndrome; MHV, murine hepatitis virus; MuV, mumps virus; NDV, Newcastle disease virus; OROV, Oropouche virus; PIV, parainfluenza virus; SARS, severe acute respiratory syndrome; SeV, Sendai virus; SINV, Sindbis virus; VACV, vaccinia virus; VEEV, Venezuelan equine encephalitis virus; VSV, vesicular stomatitis virus; WNV, West Nile virus; ZIKV, Zika virus.

Virus	IFIT	Species	Effect
<i>Coronaviridae</i>			
MERS, SARS, HCoV	IFIT1, IFIT2	Human	Antiviral – IFIT1 decreased viral replication and titre by inhibiting translation [17, 20, 32]. IFIT2 significantly decreased viral titres [32]. These effects were moderate for wild-type viruses but severe for cap0 mutant viruses
MERS, SARS, MHV	Ifit1, Ifit2	Mouse	Antiviral – Ifit1 and Ifit2 inhibited replication of cap0 mutant virus [20, 29, 77]. Virus is attenuated <i>in vivo</i> [32, 33]
<i>Filoviridae</i>			
EBOV	Ifit1	Mouse	No effect on viral replication <i>in vitro</i> [37]
<i>Flaviviridae</i>			
HCV	IFIT1	Human	Antiviral – IFIT1 impaired viral replication by inhibiting translation [16]
WNV, ZIKV, DENV	IFIT1, IFIT3	Human	Antiviral – IFIT1 reduced translation of cap0 mutant virus, but not wild-type [29, 37], and was enhanced by coexpression with IFIT3 [25]. IFIT3 restricted wild-type DENV replication [54]
WNV, JEV, DENV	Ifit1, Ifit2	Mouse	Antiviral – Ifit1 inhibits translation and replication of cap0 mutant virus [21, 29, 30], resulting in attenuation <i>in vivo</i> [28, 31]. Ifit2 decreased WNV replication <i>in vitro</i> [29] and neuropathology <i>in vivo</i> [78]
<i>Herpesviridae</i>			
HCMV	IFIT1	Human	Antiviral – decreased viral titre [40]
HSV-1	IFIT3	Human	Antiviral – viral UL41 protein downregulates IFIT3. IFIT3 impaired replication of Δ UL41 virus [58]
<i>Orthomyxoviridae</i>			
IAV	IFIT1, Ifit1	Human, mouse	No effect – IFIT1 decreased polymerase activity <i>in vitro</i> [5] but had no effect on viral titres [37]. Viral replication and pathology was unaffected <i>in vivo</i> [37]
<i>Papillomaviridae</i>			
HPV	IFIT1	Human	Antiviral – IFIT1 bound the viral helicase E1 and impaired DNA replication [38, 39]
<i>Paramyxoviridae</i>			
MuV, PIV2, PIV5	IFIT1	Human	Antiviral – IFIT1 inhibited translation and replication of wild-type rubulaviruses [36]
CDV, SeV, PIV3	IFIT1, IFIT3	Human	No effect on translation or replication of non-rubulavirus family members [36]. IFIT3 had no effect on SeV replication [58]
PIV3	IFIT1	Human	Antiviral – IFIT1 and IFIT3 inhibited viral protein expression and infectivity [79]
NDV	IFIT2, IFIT3, IFIT5	Human	Antiviral – IFIT2 [42], IFIT3 [65, 66] and IFIT5 [66] inhibited viral replication
<i>Peribunyaviridae</i>			
LACV, OROV	Ifit1	Mouse	No effect on viral replication <i>in vitro</i> and <i>in vivo</i> [37]
<i>Picornaviridae</i>			
EMCV	IFIT1, Ifit1, Ifit2	Human, mouse	No effect on viral translation [18] or replication <i>in vitro</i> and <i>in vivo</i> [5, 29, 80, 81]
EMCV	IFIT3	Human	Antiviral – slightly decreased viral titres [82]
<i>Poxviridae</i>			
VACV	IFIT1, Ifit1	Human, mouse	No effect on viral translation or replication <i>in vitro</i> and <i>in vivo</i> [9, 29]
VACV	Ifit1, Ifit2	Mouse	Antiviral – Ifit1 and Ifit2 restricted replication of cap0 mutant virus [9, 29]
<i>Rabdoviridae</i>			
VSV	IFIT1, IFIT2, IFIT3, IFIT5	Human	Antiviral – decreased viral replication [5, 9, 22, 81]. IFIT1 restriction was enhanced by coexpression with IFIT3 [25]
VSV	Ifit1, Ifit2	Mouse	Antiviral – decreased viral titres <i>in vitro</i> and reduced disease pathology <i>in vivo</i> during fatal infection [5]. Ifit2 protected mice from viral neuroinvasion and disease pathology [80]
VSV	Ifit1	Mouse	No effect on viral replication <i>in vitro</i> [9] and no effect on pathology or replication during sublethal infection <i>in vivo</i> [80]
VSV	IFIT1, IFIT2	Human	Proviral – slight increase in viral titres [68]
<i>Togaviridae</i>			
VEEV	IFIT1	Human	Antiviral – TC83 strain was susceptible to translation inhibition by IFIT1 [27]. Effect was enhanced by coexpression with IFIT3 [25]

Table 2. cont.

Virus	IFIT	Species	Effect
VEEV, CHIKV, SINV	Ifit1	Mouse	Antiviral – inhibited translation and replication of viruses with unstable 5' UTR RNA structure [26, 27]
VEEV	Ifit2	Mouse	No effect on viral replication [27].

resulting in multifaceted contributions to the host antiviral defence programme.

INDIVIDUAL IFITS SELF-ASSOCIATE

IFITs are composed of sequential tetratricopeptide repeat (TPR) motifs, which have been well characterized to mediate protein–protein interactions and are frequently found in scaffold proteins [41]. IFIT proteins have been demonstrated to homooligomerize, with the exception of IFIT5, which is exclusively monomeric. Native gel electrophoresis analysis has shown that human IFIT1 and related proteins from rabbit have a propensity to self-associate [19]. The crystal structure of IFIT1 has revealed that it self-associates via a conserved motif in the C terminus, forming an extended tail-to-tail dimer, which dissociates at low concentrations (BioRxiv: <https://doi.org/10.1101/152850>) [34]. While IFIT5 maintains much of the motif that is important for IFIT1 dimerization, it contains an additional C-terminal helix that buries these residues, occluding dimerization (Fig. 1).

By contrast, IFIT2 dimerizes by exchanging two-and-a-half TPRs in the N-terminal region between each monomer [42], resulting in a compact side-by-side dimer bound by a

larger, more stable interaction surface (Fig. 2). While there is no structure currently available for human IFIT3, it seems likely that IFIT3 can homodimerize by a similar mechanism. The N-terminal domain of IFIT3 shares 70% sequence identity with that of IFIT2 and residues that are critical for homodimerization of IFIT2 are absolutely conserved in IFIT3 [42]. Analysis by size-exclusion chromatography (SEC) coupled with multi-angled light scattering (SEC-MALS) has shown that IFIT2 is predominantly dimeric with some higher order tetra- and pentameric species resulting from aggregation of dimers, while IFIT3 is mostly dimeric with a small proportion of dissociated monomers [34].

IFIT1 ASSOCIATES WITH IFIT2/3 TO FORM A HETEROTRIMER

A complex containing IFIT1, IFIT2 and IFIT3 was first observed in glycerol gradient sedimentation experiments on IFN-stimulated HeLa cell extracts [43]. The IFIT complex migrated with a molecular weight of 150–200 kDa, which is equivalent to a trimer or tetramer of IFIT proteins. Later, mass spectrometry analysis of pulldown experiments showed that each human IFIT protein could co-precipitate

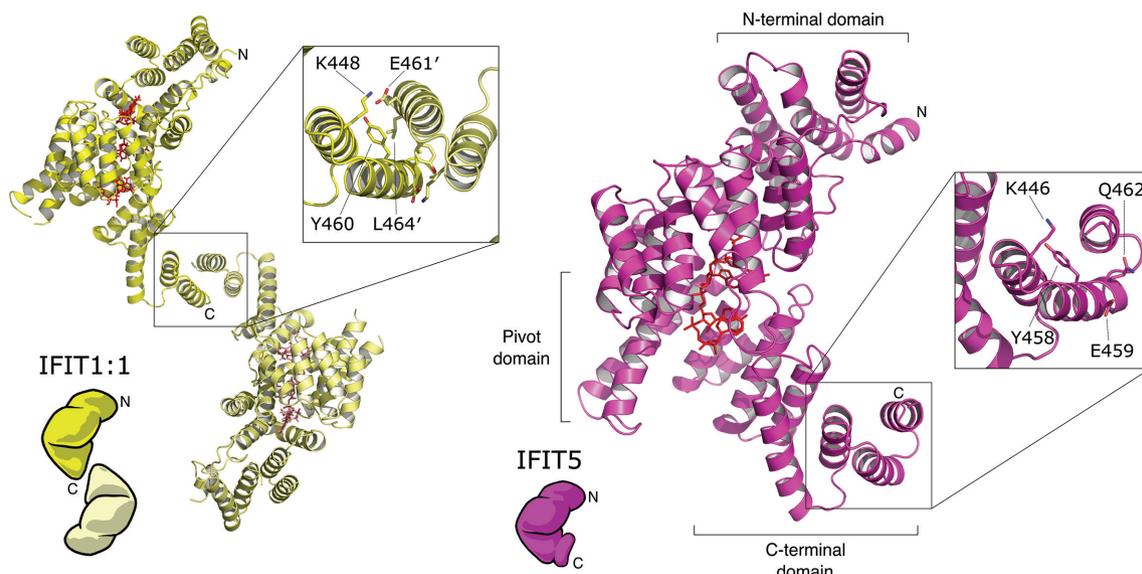


Fig. 1. IFIT1 homodimerizes via a C-terminal YExL motif that is buried in IFIT5. Crystal structures of IFIT1 (PDB: 5W5H) (BioRxiv: <https://doi.org/10.1101/152850>) and IFIT5 (PDB: 4HOT) [24], with schematic cartoons representing IFIT1 (yellow) and IFIT5 (magenta). For IFIT1, the dimerization interface is enlarged and interacting side-chains are labelled. For IFIT5, the C-terminal domain is enlarged and conserved side-chains are labelled. Bound RNA is shown in red.

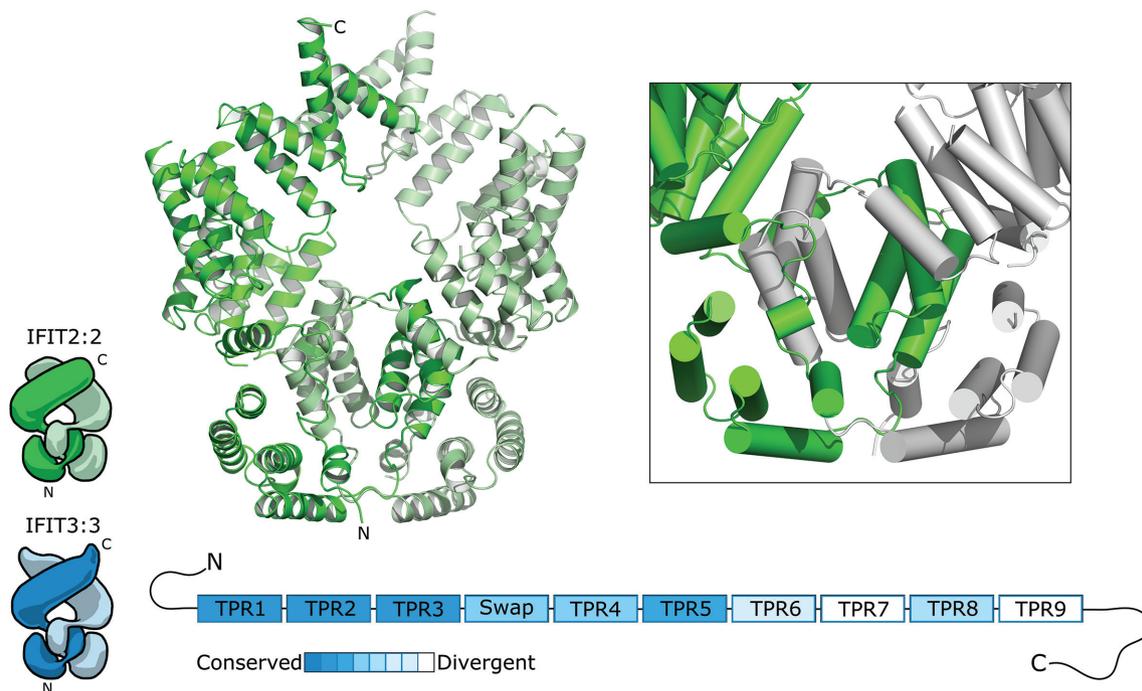


Fig. 2. IFIT2 homodimerizes via an N-terminal domain swap that is conserved in IFIT3. Crystal structure of IFIT2 (PDB: 4G1T) [42] with schematic cartoons of IFIT2 (green) and IFIT3 (blue). The domain-swapped dimerization interface is enlarged in the box. IFIT3 is shown as a domain diagram with the regions conserved between IFIT2 and IFIT3 shown in dark blue and the divergent regions shown in light blue. The TPR motifs are annotated as in [42] and the putative domain swap region is labelled 'Swap'.

the other IFIT family members, with the exception of IFIT5 [5, 20]. In the same study, it was shown by SEC that purified IFIT1 can interact with IFIT2 and IFIT3; it was therefore hypothesized that IFIT1, IFIT2 and IFIT3 can form a stoichiometric complex via these interactions. The composition and assembly pathway of this complex has more recently been probed in greater detail (Fig. 3).

While IFIT1 can interact with both IFIT2 and IFIT3, the nature of these interactions is very different. IFIT1 interacts rapidly with IFIT3 even at low temperatures, while the association with IFIT2 is time- and temperature-dependent [34]. Equally, in pulldown assays IFIT1 interacts strongly with IFIT3 but poorly with IFIT2 [25]. When analysed by SEC-MALS, IFIT1:IFIT2 complexes comprised a mixture of peaks of different molecular weights, indicating that IFIT1 and IFIT2 interact in a number of different ways and there is no single interaction site to produce a defined complex. This is consistent with previous pulldown experiments, which suggested that the interaction between IFIT1 and IFIT2 is relatively non-specific, since both N- and C-terminal truncations of IFIT2 can co-precipitate with IFIT1 [43]. By contrast, the IFIT1:IFIT3 complex was almost completely tetrameric, with a small population of heterodimers, which was consistent with the small population of monomeric IFIT3 detected in purified preparations [34].

Differential scanning fluorimetry analysis, which quantitatively probes the melting profile of proteins in solution, revealed that individual IFITs are thermally unstable [34]. IFIT3 in particular has a melting temperature of less than 35 °C, indicating that in isolation these proteins would be highly unstructured or partially melted at physiological temperatures. However, interaction between IFIT1 and IFIT3 confers stability to the entire complex, increasing melting temperatures by up to 8 °C. By contrast, the IFIT1:IFIT2 complex was just as unstable as its constituents. This indicates that IFIT1 readily and specifically binds to IFIT3, forming a larger complex that is thermodynamically preferred.

IFIT1 and IFIT3 interact via a specific and highly conserved C-terminal YExxL motif [25, 34], originally identified as the site responsible for the concentration-dependent homodimerization of IFIT1 (BioRxiv: <https://doi.org/10.1101/152850>). Mutation of this YExxL motif abrogates interaction between IFIT1 and IFIT3 when analysed by SEC [34]. Isothermal titration calorimetry experiments showed a low nanomolar affinity between IFIT1 and IFIT3, both in terms of the full-length IFIT3 protein and the isolated C-terminal domain (IFIT3_{CTD}) [25]. In agreement with the size-exclusion data, there was no interaction between IFIT1 and C-terminally truncated IFIT3 or a mutant in which several residues within and surrounding the YExxL motif were

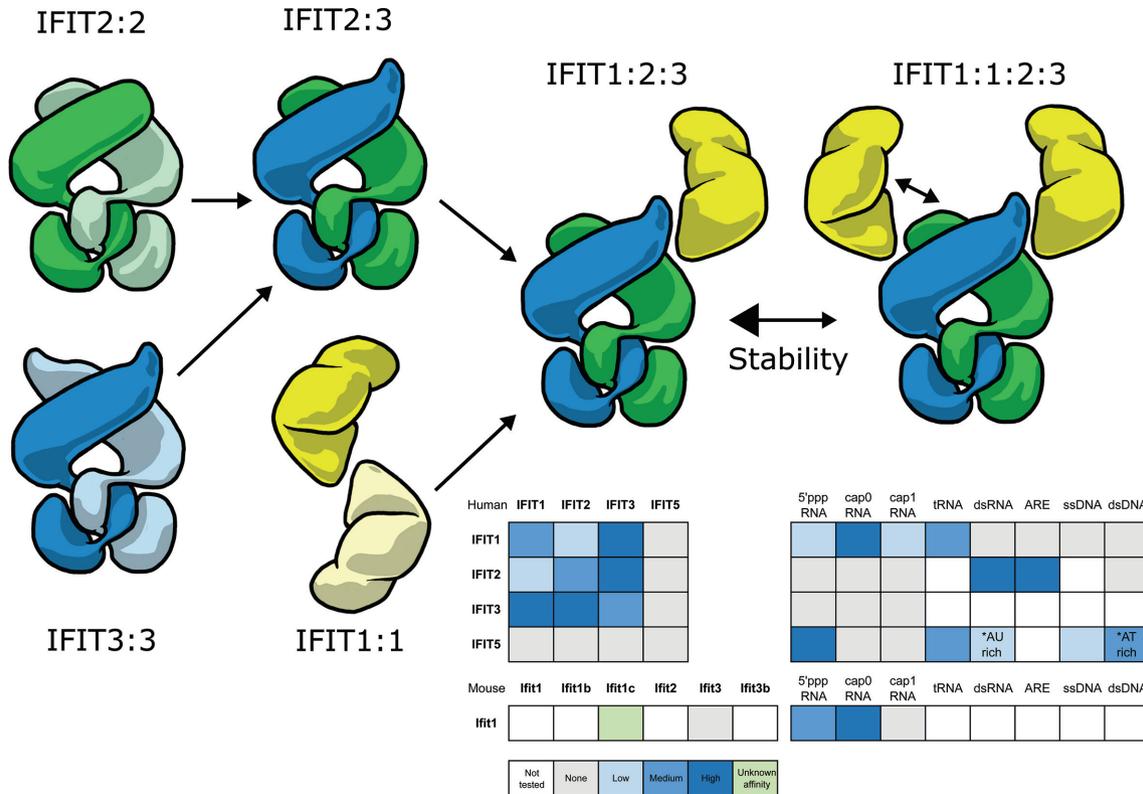


Fig. 3. Assembly pathway for the IFIT1:IFIT2:IFIT3 complex. Schematic cartoons showing the IFIT complex assembly pathway. Weak interactions are indicated with reversible arrows. The heatmap illustrates the relative affinities of human IFIT proteins for each other, as well as for their RNA substrates. This is also shown for murine Ifit1 for comparison. ARE, AU-rich element-containing RNA. White, interaction not tested; grey, no interaction detected when tested; light to dark blue, increasing affinity of interaction; green, interaction reported but no affinity information available.

mutated. Therefore, IFIT3 readily displaces IFIT1 homodimers by utilizing the same binding interface but with higher affinity. Unlike IFIT1 homodimers, the C-terminal helix in IFIT5 cannot be displaced by IFIT3 (Fig. 4) [5].

A recent co-crystal structure of IFIT1 and two-and-a-half C-terminal helices from IFIT3 has begun to shed light on the mechanism of IFIT assembly (Fig. 4) [25]. This structure reveals that an IFIT1:IFIT3 interface, overlapping the IFIT1 homodimerization site, buries over 1000 Å² of the IFIT1 surface. The orientation of the IFIT3 C-terminal domain is very similar to that of the corresponding C-terminal region in the IFIT1 homodimer (Fig. 2b; RMSD=0.522 Å for 372 atoms, C-terminal helices only). Importantly, IFIT3 has a C-terminal extension relative to IFIT1 that has not been fully modelled in this structure and appears to loop over the C-terminal domain of IFIT1. This is supported by hydrogen–deuterium exchange assays, which map additional interaction sites between IFIT1 and IFIT3 across much of the C terminus and regions of the pivot domain of IFIT1. This greatly expands the interaction interface with IFIT1, potentially accounting for the higher affinity binding between IFIT1 and IFIT3 over IFIT1 homodimerization.

By interacting with IFIT3 through its C terminus, IFIT1 is able to associate with a heterodimer of IFIT2 and IFIT3 (likely stabilized through a domain swap in the N terminus), to form the trimeric complex that was observed in cells, as described above. This trimer was the most thermally stable of all the complexes when analysed by differential scanning fluorimetry, suggesting that this is a favourable state for these proteins to adopt [34]. *In vitro*, addition of a molar excess of IFIT1 to the trimer results in the formation of a tetramer, possibly formed by the non-specific interaction interface(s) between IFIT1 and IFIT2. As such, this tetramer is unstable and readily dissociates back into the favoured trimer. It is possible that if IFIT1 is expressed at very high levels, the tetrameric complex may reversibly form in the cell, although the functional significance of this larger complex is unclear.

IFIT3 ACTS AS A COFACTOR TO STIMULATE IFIT1 STABILITY AND ACTIVITY IN CELLS

In viral infection, IFIT3 promotes IFIT1 restriction of susceptible viruses. Flaviviruses WNV and ZIKV with defective 2'-O-methyltransferases were more susceptible to IFIT1-

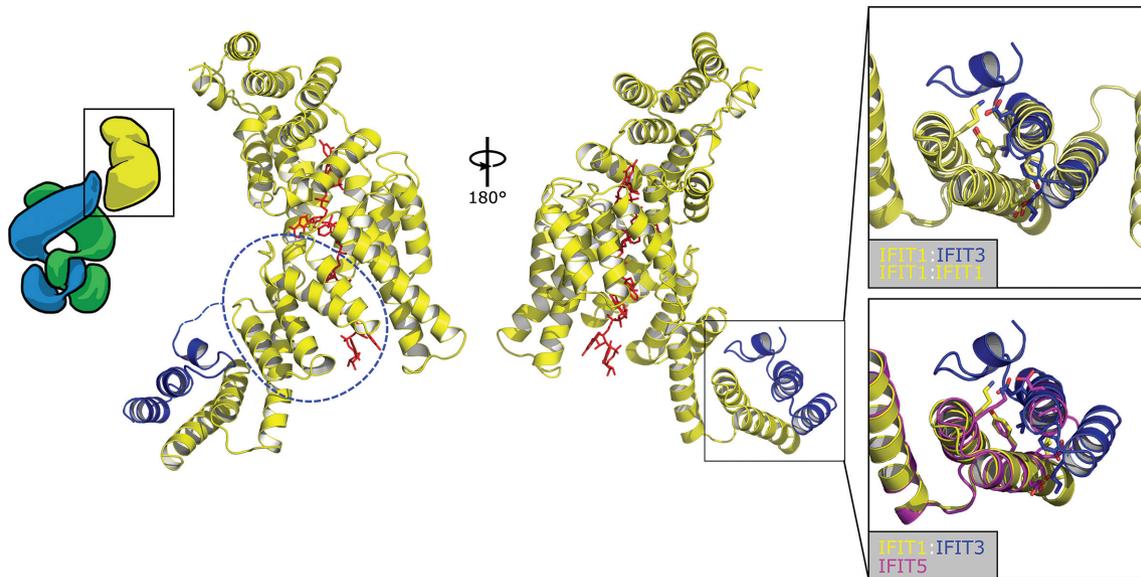


Fig. 4. IFIT1 and IFIT3 interact via a conserved C-terminal motif. Crystal structure of IFIT1 (yellow) complexed with one-and-a-half C-terminal TPRs from IFIT3 (blue) (PDB: 6C6K) [25]. Dotted blue lines indicate the regions with which the unmodelled C-terminal helices of IFIT3 may interact with IFIT1, as discussed in the text. The interface between IFIT1 and IFIT3 is enlarged and is superimposed with the IFIT1 (upper panel) homodimer or the C terminus of IFIT5 (lower panel) for comparison. Key side-chains involved in the interaction are shown.

mediated restriction when IFIT3 was overexpressed [25]. This was also shown for a cell culture-adapted strain of the alphavirus VEEV, which has previously been shown to be bound by IFIT1 [26]. However, wild-type cap1 flaviviruses were not inhibited by IFIT1, with or without IFIT3. This is consistent with *in vitro* translation data showing that IFIT1 can only inhibit cap1 mRNAs at micromolar concentrations and this does not appear to be enhanced by IFIT3 binding [34].

Transgenic IFIT3 expression increased the steady-state level of endogenous IFIT1 in a dose-dependent manner [25]. This was corroborated by an independent overexpression study and was dependent on the integrity of the YExxL motif in the C terminus of IFIT1 and IFIT3; the mutation or deletion of this domain results in poor IFIT1 expression [34]. This is a result of enhanced IFIT1 stability at the protein level, prolonging its half-life from less than 2 h to over 24 h [25]. This may be due to the intrinsic physical stability of the IFIT1 : IFIT3 complex, as described above, or to additional protection by IFIT3, for example shielding IFIT1 from active degradation. Therefore, one of the ways that IFIT3 enhances IFIT1 activity is by increasing the concentration of IFIT1 in cells and preventing its turnover by locking it in a stable complex.

However, IFIT1 translation inhibition activity is also enhanced *in vitro*, where protein levels can be controlled. In lysate-based and cell-based translation assays, IFIT1 complexed with IFIT3 inhibited the translation of reporter genes at twofold lower concentrations compared to IFIT1 alone

[34]. This was observed for both the IFIT1 : IFIT3 complex and the IFIT1 : IFIT2 : IFIT3 trimer, and correlated with a twofold enhancement of cap0 RNA binding determined by both primer extension inhibition [34] and filter binding assays ([25] IFIT1 : IFIT3 only). Binding to IFIT3 also appears to increase the specificity of IFIT1 for cap0 RNA, since IFIT1 : IFIT3 complexes have significantly lower affinity for both uncapped and cap1 RNA [25]. Importantly, the interaction between IFIT1 and IFIT2 had no effect on IFIT1 activity and only marginally stabilized IFIT1 expression in cells [34], indicating that this is unlikely to be a functional interaction in the cell.

In primer extension inhibition assays, the binding of IFIT1 to a model cap0 RNA produces cDNA products that are 6–7 nucleotides shorter than the full-length signal [19]. This IFIT1 ‘toeprint’ is consistent with the number of nucleotides bound in the positively charged IFIT RNA-binding cleft. It was previously shown that rabbit IFIT1B, which can stably homooligomerize, makes a secondary toeprint that is 12–13 nucleotides shorter than the full-length signal, suggested to be caused by additional IFIT1B molecules making stable contacts with the RNA [19]. However, using a similar method, IFIT1 : IFIT3 complexes only cause a single toeprint on model RNAs, suggesting that the complex does not make additional contacts with the RNA to increase overall avidity [34].

Instead, IFIT3 appears to induce a tighter conformation of IFIT1 on the RNA. When examining a structured ZIKV 5’ UTR in the toeprint assays, a proportion of IFIT1 appeared

to be displaced by the reverse transcriptase, resulting in a full-length cDNA product being produced even at high concentrations of IFIT1 [34]. When complexed with IFIT3, IFIT1 was resistant to this displacement, allowing a saturation of binding that was not possible with IFIT1 alone. This indicates that IFIT1:IFIT3 complexes are more stably bound on the 5' end of the RNA, leading to the hypothesis that IFIT3 may slow the off-rate of IFIT1 from the RNA. IFIT5 was shown to cycle between 'open' and 'closed' conformations, providing a mechanism for 5'-ppp RNA binding by this protein [24]. If a similar mechanism is required for IFIT1-cap0 RNA binding, then it is possible that the interactions between IFIT3 and the pivot region of IFIT1 described above may alter the flexibility of IFIT1, locking it in a closed conformation following RNA binding.

In the structure of the partial IFIT1:IFIT3 complex, the C terminus of IFIT1 is rotated towards the RNA-binding pocket by ~4 Å when compared to homodimeric IFIT1 [25]. While this movement was hypothesized to account for the increased affinity of the complex for cap0 mRNA, it is much smaller than the conformational change observed between 5'-ppp RNA bound and unbound forms of IFIT5. It is important to note, however, that while the IFIT1:IFIT3_{CTD} complex was co-crystallized with a 10nt RNA, shorter (4–6nt) RNA oligonucleotides were bound for the crystal structures for IFIT1 and IFIT5. It is possible that these extra nucleotides offer additional electrostatic interactions that 'pull' IFIT1 closed, independently of the action of IFIT3. This 10 nt bound structure likely represents a more accurate view of the closed conformation of IFIT1, since it naturally binds to long messenger RNA substrates. Further study of the IFIT1:IFIT2:IFIT3 complex, and in particular greater knowledge of its structural arrangement and the dynamics of RNA binding, is crucial to understanding how IFIT1 RNA recognition is enhanced and refined by IFIT3.

There is evidence to suggest that an uncharacterized human IFIT protein, IFIT1B, may also interact with the complex. IFIT1B has not been well studied, due to a perception in the field that human IFIT1B may not be expressed during infection, owing to a lack of ISREs in its promoter [2]; this, coupled with difficulties in expressing the protein recombinantly, means that the activity and interactions of human IFIT1B are not well understood. Despite this, mass spectrometry-based pulldown experiments have identified peptides corresponding to IFIT1B co-precipitating with both IFIT1 and IFIT3, but not with IFIT2 [5]. The YExxL motif and many of the surrounding residues are absolutely conserved between IFIT1 and IFIT1B. Therefore, it is possible that IFIT1B could use the same interface to interact with the IFIT2:IFIT3 complex to form an alternative trimer with different activity to the IFIT1 complex. The orthologous rabbit IFIT1B has previously been shown to interact with and inhibit translation on RNAs bearing cap0 and, surprisingly, cap1 structures *in vitro* [19], indicating that it is capable of inhibiting the translation of self mRNAs. It will be of interest to examine the impact of rabbit IFIT3 on the

specificity and affinity of rabbit IFIT1B for different RNA substrates.

It is important to note that the nature of the IFIT complex is very different in rodents, which have historically been used to model IFIT-dependent antiviral responses *in vivo* [5, 37]. In mice, while there is no true homologue of human IFIT1, there are three *Ifit1b*-like proteins [9]. These all contain the conserved C-terminal YExxL motif, indicating that they should form homo- and heterodimers. Mass spectrometry-based pulldown experiments have shown that murine *Ifit1* can precipitate peptides corresponding to *Ifit1c* [20], an uncharacterized murine *Ifit1b*-like protein with unknown function and expression profile. By contrast, murine *Ifit1* did not precipitate with *Ifit2* or *Ifit3* [20], which has been confirmed in pulldowns of endogenous protein from primary cells [44]. This is because in rodents *Ifit3* has undergone a large C-terminal deletion, which removes the critical YExxL motif responsible for interaction with IFIT1. When *Ifit3* is artificially extended with the human IFIT3_{CTD}, interaction is restored [25]. While work still needs to be done to identify and characterize the functional *Ifit* complexes in mice, it is clear that the IFIT complexes formed in humans and rodents are quite different; this should be taken into consideration when comparing small animal models to human disease.

IFIT2 AND IFIT3 FORM A HETERODIMER

IFIT2 forms a domain-swapped dimer, in which helices 7–9 are exchanged between each monomeric unit, forming a strong association in the N terminus [42]. IFIT2 can also associate with itself weakly via the C terminus by non-specific interactions that may mediate higher-order aggregation [43]. IFIT3 can associate with the first four TPRs of IFIT2, including helices 7–9 in the domain-swapping region [43]. However, *in vitro*-purified IFIT2 and IFIT3 only interact when heated to physiological temperatures [34]. As described above, this brings IFIT2 and IFIT3 above their melting points, indicating that partial unfolding of the proteins is required to promote the formation of a heterocomplex, possibly by the exchange of N-terminal TPRs. The resultant IFIT2:IFIT3 heterodimer is significantly more stable than either homodimer, and therefore thermodynamically preferable.

In mouse, endogenous *Ifit2* and *Ifit3* have also been shown to interact in pulldown experiments from primary cell lysates [44]. Since the N terminus is highly conserved between mouse and human orthologues, the interaction interfaces have been preserved even while there have been major modifications to other parts of the gene. Therefore, it is likely that murine *Ifit2* and *Ifit3* may interact by the same mechanism as in humans. Intriguingly, in mouse there is an additional *Ifit3* paralogue (*Ifit3b*), which is nearly identical to its parent gene [2]. Two of the five amino acid substitutions that distinguish *Ifit3* and *Ifit3b* are clustered at the putative binding interface. This raises the possibility that there may be two functionally distinct *Ifit2*:*Ifit3*

heterodimers in mouse, which could broaden the functional capacity of this complex.

IFIT2 : IFIT3 MODULATES APOPTOSIS

IFIT2 has been identified as a pro-apoptotic factor in various human cell lines [43, 45]. Consequently, a number of studies have investigated the ability of IFIT2 to kill cancer cells, including leukaemia [46], colorectal carcinoma [47, 48], hepatocellular carcinoma [49], osteosarcoma [50] and oral squamous cell carcinoma [51, 52]. Overexpression of IFIT2 leads to the activation of caspase-3 and the disruption of plasma membrane asymmetry, which is characteristic of apoptotic cell death [43, 51, 52], while inhibition of the proteasome-dependent turnover of IFIT2 promotes the formation of pro-apoptotic IFIT2 aggregates in the perinuclear region [45]. Likewise, derepression of micro-RNA-mediated downregulation of IFIT2 sensitizes cells to a number of extrinsic pro-apoptotic stimuli, including serum starvation and chemotherapeutic drugs [50, 51].

IFIT2-mediated apoptosis is independent of DNA damage responses [45, 48]. Instead, IFIT2 acts via the mitochondrial pathway, in which the balance between pro- and anti-apoptotic Bcl-2 family proteins regulates the permeability of the outer mitochondrial membrane (Fig. 5) [53]. Upon apoptotic stress, BH3-only proteins induce the aggregation of Bax and Bak at the outer mitochondrial membrane, forming

a pore called the mitochondrial apoptosis-induced channel, which releases cytochrome c and other mitochondrial proteins into the cytoplasm. This results in the formation of the apoptosome complex, which activates caspase-9, which in turn activates the executioner caspase-3, a broadly specific protease whose activity leads to DNA fragmentation and cell death. IFIT2 was shown to localize to the outer mitochondrial membrane and activate Bax and Bak [43]. Expression of anti-apoptotic Bcl-2 homologues such as Bcl-xl and the adenovirus protein E1B-19K inhibited IFIT2-dependent apoptosis. Treatment of cells with curcumin, which also promotes mitochondrial apoptosis, induces the expression of IFIT2, which in turn exacerbates curcumin-dependent apoptosis [46].

By contrast, during DENV infection, the knockdown of IFIT3 enhanced virus-dependent apoptosis, while the overexpression of IFIT3 rescued cell survival [54]. Similarly, in overexpression studies, co-expression of IFIT3 was shown to ameliorate IFIT2-dependent apoptosis [43]. It would be interesting to investigate whether this is due to independent pro-survival signals from IFIT3 or whether the IFIT2 : IFIT3 interaction is necessary to modulate IFIT2-dependent apoptosis. For example, IFIT3 may be able to rescue IFIT2 from unstable aggregation by sequestering it in the favourable heterodimeric complex. Alternatively, interaction with IFIT3 may disrupt the interfaces that allow IFIT2 to interact with pro-apoptotic effectors. Further interaction

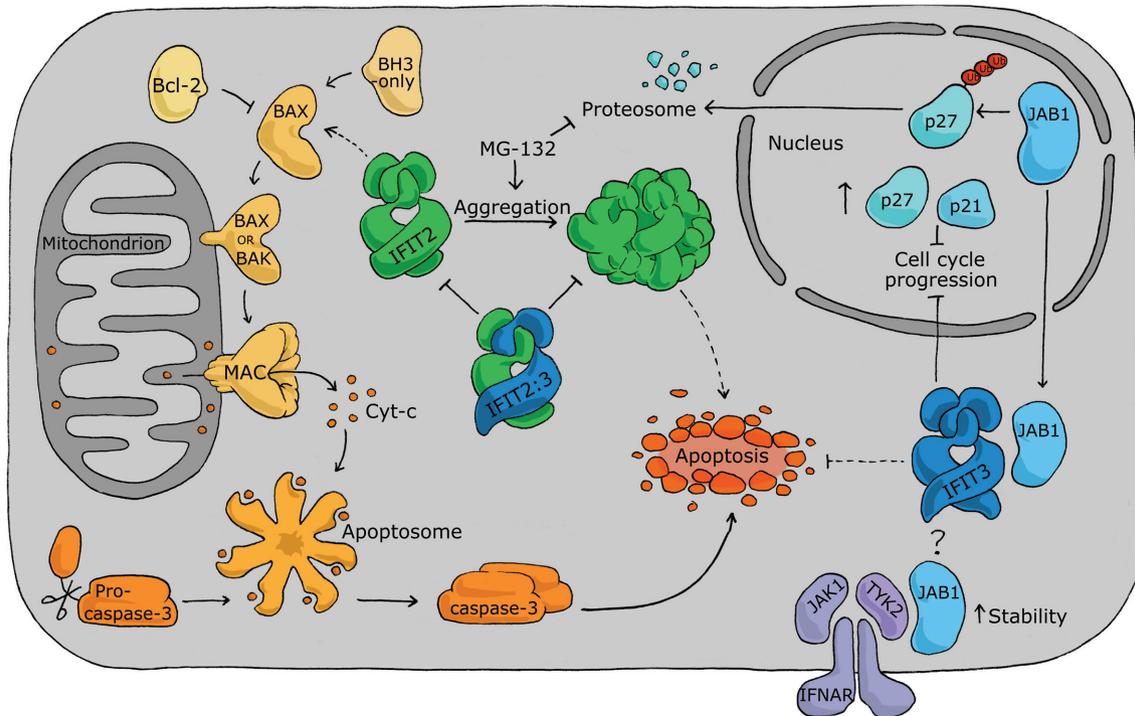


Fig. 5. IFIT2 and IFIT3 modulate apoptosis and cell proliferation. Schematic showing the pathways with which IFIT2 and IFIT3 interact to modulate apoptosis and cell cycle progression. The dashed arrows represent pathways with unclear mechanisms and the question marks show possible roles that have not yet been explored in detail.

studies will be necessary to determine which proteins IFIT2 interacts with directly to induce apoptosis and the nature of these interactions in the context of the IFIT2:IFIT3 heterodimer.

IFIT proteins are not equally expressed during the immune response, and the expression patterns are tissue- and stimulus-specific [6, 7]. While IFIT2 and IFIT3 can be induced by type I and type II IFNs, there appear to be differences in the extent to which they respond to these stimuli. IFIT3 expression has been shown to be dependent on STAT-2, resulting in strong upregulation by IFN α [54], while IFIT2 expression is reliant on the activation of STAT-1 downstream of type II IFN receptor signalling [47]. Similarly, IFIT2 expression has been shown to be dependent on inhibitor of kappa B kinase (IKK) ϵ , which phosphorylates STAT-1 at serine 708, promoting the expression of a subset of ISGs, while IFIT1 and IFIT3 are not IKK ϵ -sensitive [55]. Therefore, by targeting IKK ϵ , WNV was able to selectively downregulate IFIT2 expression, possibly to escape cell death and promote viral replication. Hence, over the course of an infection, the balance between pro- and anti-apoptotic signals may be fine-tuned by modulating the balance of IFIT2 versus IFIT3 in the cell.

Independent of IFIT2, IFIT3 has also been investigated for its antiproliferative effects. IFIT3 interacts with Jun activation domain-binding protein 1 (JAB1), causing JAB1 to relocalize to the cytoplasm (Fig. 5) [56]. JAB1 is typically localized in the nucleus, where it promotes the degradation of the cyclin-dependent kinase (CDK) inhibitor p27. Retention of JAB1 in the cytoplasm by IFIT3 results in an accumulation of p27 in the nucleus, which halts cell cycle progression, resulting in an accumulation of cells stuck at the G₁/S transition checkpoint [56]. In the same study, it was noted that stable IFIT3-expressing cell lines downregulated c-myc expression, leading to the derepression of p21, another CDK inhibitor involved in blocking cell cycle progression. Downregulation of c-myc and upregulation of p21 and p27 by IFIT3 overexpression was confirmed in a subsequent study [57]. Interestingly, when herpes simplex virus 1 (HSV-1) is deficient in the tegument protein UL41, which downregulates IFIT3 by affecting mRNA stability, the virus becomes susceptible to IFIT3 restriction [58]. Since the efficiency of HSV replication is affected by cell cycle arrest, this could be a possible mechanism for IFIT3-dependent antiviral activity.

Intriguingly, JAB1 can interact with TYK2, a component of the IFN receptor complex, and enhance the efficiency of type I IFN signalling by promoting the stability of the type I IFN receptor [59]. JAB1 acts by preventing the activation and recruitment of ubiquitin ligases, which promote proteasome-dependent turnover of the IFN receptor complex. It seems possible, therefore, that IFIT3-dependent relocalization of JAB1 to the cytoplasm may promote this interaction with TYK2 to potentiate IFN signalling.

A ROLE FOR IFIT2 IN INFLAMMATION

Studies in mice on non-viral pathogens have suggested that Ifit2 may be involved in negatively regulating inflammation in two ways: downregulation of Toll-like receptor (TLR) 4 responses and modulation of reactive oxygen species (ROS) production. While TLR4 is typically considered to be responsible for sensing bacterial and fungal cell wall carbohydrates, it can also recognize viral glycoproteins [60]. Two studies have suggested that murine Ifit2 may limit TLR4-driven expression of proinflammatory cytokines, particularly TNF α and IL-6 [61, 62]. This was ascribed to the ability of Ifit2 to modulate the stability of cytokine mRNA, possibly by binding directly to RNA with AU-rich elements in their 3' UTRs, as has been shown for human IFIT2 [42]. However, a third study linked murine Ifit2 knockout to impaired TLR4 responses [44], and so further work is necessary to clarify the exact role that Ifit2 plays in this pathway.

Similarly, the production of ROS has been associated with cell death and pathology during infection with a number of RNA viruses (reviewed in [63]). In phagocytes, NADPH oxidase (NOX) at the outer mitochondrial membrane catalyzes the production of superoxide anions. NOX activity is regulated by a number of cytoplasmic subunits, including p67^{phox}. Ifit2 was shown to co-precipitate with p67^{phox} along with a mitochondria-associated heat shock chaperone hsc70 [61]. This complex was suggested to promote the activation of p67^{phox} and thus negative regulation of NOX activity, limiting superoxide production. In this way, during *Candida* infection, the knockout of Ifit2 limited the production of ROS from primary phagocytes [61]. While in fungal infection this may result in impaired pathogen clearance, it may be beneficial during viral infection. During influenza virus infection, excessive production of ROS contributes to inflammation and cytopathic effect [64]. Since p67^{phox} and hsc70 are both TPR-containing proteins, it has been suggested that IFIT2 binds via TPR–TPR interactions, however the exact binding sites have not yet been mapped.

A better understanding of the biochemical basis of the protein–protein interactions that mediate IFIT2's many functions is necessary to understand how IFIT2 is able to carry out such varied roles during the immune response. In particular, it is important to clarify whether the anti-inflammatory roles described in mouse translates to the human IFIT2 orthologue and to determine the impact of IFIT2:IFIT3 heterodimerization on these functions.

IFIT1, IFIT2 AND IFIT3 MODULATE THE MAVS SIGNALLING AXIS TO REGULATE IFN INDUCTION

IFIT1, IFIT2 and IFIT3 have been investigated independently for their ability to modulate the signalling pathways that lead to IRF-3 activation, particularly downstream of RIG-I-like receptor activation by double-stranded RNA in the cytoplasm (Fig. 6). IFIT3 was shown to potentiate RIG-I signalling, resulting in increased type I IFN and

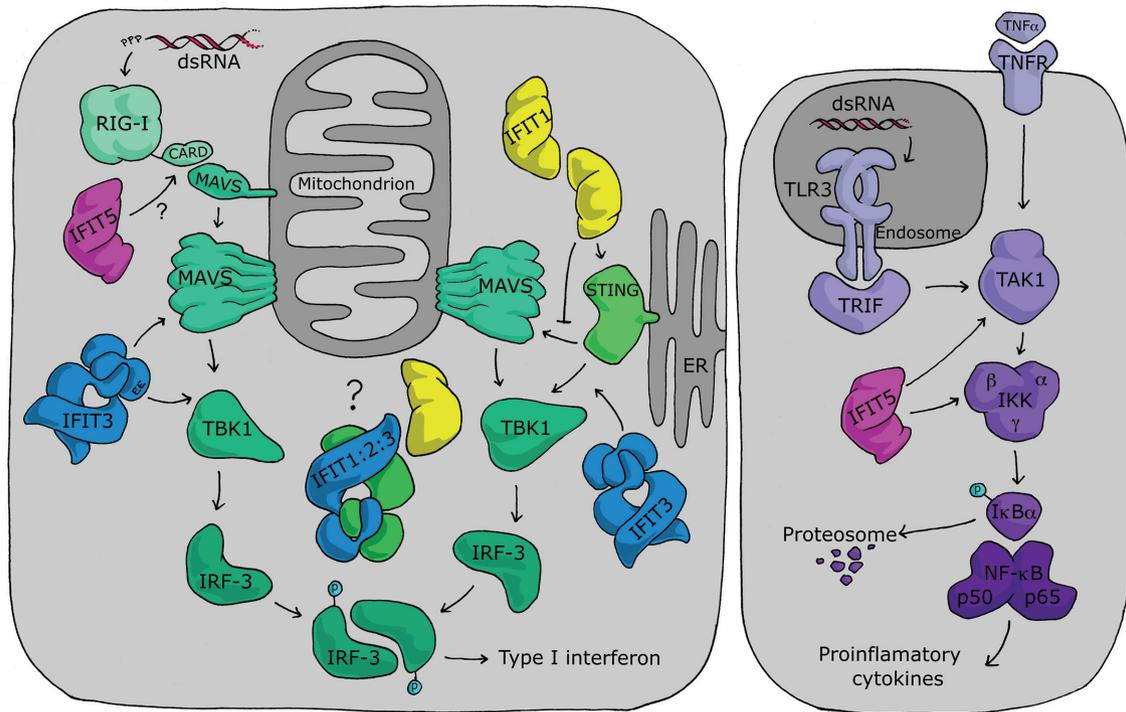


Fig. 6. IFIT1, IFIT2, IFIT3 and IFIT5 regulate different aspects of innate immune signalling. Schematic showing the pathways that IFIT family members modulate during innate immune signalling. The question mark highlights the unknown role of the entire IFIT1:IFIT2:IFIT3 complex in this process, compared to individual components.

proinflammatory cytokine production during Sendai virus infection both in immortalized and primary cell lines [65–67]. Like IFIT2, IFIT3 localizes to the outer mitochondrial membrane and the N-terminal domain of IFIT3 coprecipitates with mitochondrial antiviral signalling protein (MAVS) and the associated TANK-binding kinase 1 (TBK1), as well as numerous other members of the MAVS signalling complex [65]. Interaction with TBK1 was dependent on two glutamate residues in the N terminus of IFIT3 (EE164/165) that engage the positively charged N terminus of TBK1, while MAVS bound at a separate N-terminal site. Thus, IFIT3 is capable of recruiting both MAVS and TBK1, acting as a scaffold to bridge and enhance their interaction, increasing the signalling activity of the complex. Since IFIT2 and IFIT3 likely interact via an N-terminal domain swap, it would be interesting to determine what effect heterodimerization has on the interaction between IFIT3 and the MAVS complex; for example, in IFIT2 the amino acid corresponding to the second glutamate in the IFIT3:TBK1 interaction motif is substituted for lysine, which could electrostatically interfere with TBK1 recruitment.

IFIT1 has been shown to promote type I IFN expression during infection with various alphaviruses, which limited virus spread in plaque assays [27]. However, one study has suggested that IFIT1 downregulates IFN induction by disrupting the interaction between MAVS and TBK1, in

marked contrast to IFIT3 [68]. IFIT1 does not interact directly with either of these proteins, but instead binds stimulator of interferon genes (STING), a multifunctional adaptor protein that has previously been shown to enhance the MAVS–TBK1 interaction and promote IFN production [69]. It has been suggested that binding to IFIT1 occludes the MAVS- and TBK1-binding sites on STING, since IFIT1 overexpression prevents their co-precipitation. While IFIT2 could also interact with STING, it had little effect on MAVS signalling, suggesting that it binds in an alternative way that is either unobtrusive or easily displaced. It is important to note that the studies on IFIT3 described above were performed in HEK293T cells that do not express functional STING [70], and therefore any effects of STING on the association between MAVS and TBK1 would have been overlooked in this system. Interestingly, IFIT1 was found to be phosphorylated during Sendai virus infection, but the site and effect of this modification remain unclear [68]. Since a C-terminal tyrosine is essential for interaction between IFIT1 and IFIT3 [34], it would be interesting to determine whether this site is prone to phosphorylation, and what impact this could have on complex formation. Additionally, when overexpressed in HeLa cells a small proportion of IFIT1 was shown to be ISGylated, although the consequences of this modification and its impact on complex formation are not known [71].

A recent study has shown that IFIT3 can also bind to STING, promoting the recruitment of TBK1 to STING downstream of cytoplasmic DNA sensing [72]. Monocytes derived from patients with systemic lupus erythematosus (SLE), an autoinflammatory disease, expressed higher IFIT3 than control donors, which resulted in enhanced expression of IFN β . In knockout cell lines, IFIT3 was shown to function upstream of TBK1 but downstream of STING, and overexpressed IFIT3 could co-precipitate with both of these components. Therefore, IFIT3 was proposed to act as a scaffold to promote the interaction of TBK1 and STING, enhancing the activation of IRF-3 to promote the expression of type I IFN. Elevated IFIT3 expression was linked to disease pathology, consistent with a described role for IFIT3 in promoting pathological inflammation in SLE [73]. Since the binding site for STING has not yet been mapped, it is currently unclear whether IFIT3 can bind to MAVS and STING simultaneously, recruiting TBK1 to the entire complex, or whether MAVS and STING compete for IFIT3 binding, depending on the nature of the viral stimulus, i.e. RNA versus DNA.

Since IFIT1, IFIT2 and IFIT3 can interact with components of the STING–MAVS–TBK1 signalosome, but when examined in isolation have variable effects, the effect of the entire IFIT1:IFIT2:IFIT3 holo-complex at this stage in the pathway certainly warrants further investigation. It is possible that IFIT3 could prevent the inhibitory effect of IFIT1, for example by competing with IFIT1 for STING binding or by maintaining IFIT1 in a conformation that is not conducive to STING interaction. Alternatively, it is possible that the larger trimeric IFIT1:IFIT2:IFIT3 complex could inhibit IFIT3-mediated enhancement of the MAVS signalling pathway by sterically hindering the assembly of the STING–MAVS–TBK1 complex. This could serve to prevent the over-activation of inflammatory responses during the antiviral response. Differential temporal regulation of IFIT1 and IFIT3 could result in sequential activation then repression of the same signalling pathway by modulating the repertoire of IFIT complexes in the cell at a given time during infection.

There is also evidence to suggest that IFIT5 may play a role in this pathway, independent of the IFIT1:IFIT2:IFIT3 complex. Knockdown of IFIT5 reduced Sendai virus-dependent expression of IFN- β and IFIT1 mRNA, while overexpression potentiated expression [66]. Overexpressed IFIT5 partially localized to mitochondria and could interact with MAVS, RIG-I and TRAF6 in pull-down assays, in addition to interacting weakly with TBK1. As such, it was proposed that IFIT5 functions upstream of MAVS by recruiting RIG-I to the MAVS complex, complementing the role of IFIT3 in promoting downstream recruitment of TBK1, to stimulate signalling [74]. However, a previous report suggested that while IFIT5 and RIG-I colocalized in the cell, they were mainly observed at actin filaments and did not interact directly [23]. Considering these opposing studies, further investigation is

necessary to determine the exact role of IFIT5 in the RIG-I pathway.

IFIT5 STIMULATES THE NF- κ B PATHWAY

IFIT5 has been implicated in the modulation of NF- κ B signalling, which controls the inflammatory arm of the innate immune response (Fig. 6). NF- κ B is constitutively present in the cytoplasm associated with the inhibitor of kappa B alpha (I κ B α) complex. Upon activation of the pathway, either by the sensing of viral signatures by TLRs in the endosome or the engagement of cell surface cytokine receptors such as the TNF α receptor, the IKK complex becomes phosphorylated and promotes the degradation of I κ B α , and the subsequent derepression of NF- κ B. Active NF- κ B then translocates into the nucleus to promote the transcription of proinflammatory cytokines and type I IFN (reviewed in [75]). In overexpression and knockdown experiments, IFIT5 enhanced the expression of NF- κ B-responsive cytokines, including IL-8 and CCL5 [66]. This is consistent with the results of a meta-analysis of gene expression profiles in which IFIT5 was posited as a modulator of NF- κ B/RelA function after injury [76].

IFIT5 acts by sustaining the activation of the IKK complex to promote the derepression and nuclear translocation of NF- κ B [74]. IFIT5 does this by binding directly to IKK β , a component of the IKK complex, along with transforming growth factor beta-activated kinase 1 (TAK1), a kinase responsible for phosphorylating and activating IKK. IFIT5 appears to act specifically at this stage in the pathway, since it could not interact with upstream or downstream proteins, including NF- κ B itself. The pivot region of IFIT5 mediated interaction with IKK β , while the pivot and C-terminal domains were able to bind to TAK1. IFIT5 promoted TAK1 co-precipitation with IKK β and vice versa, while IFIT5 knockdown reduced interaction between TAK1 and IKK β , resulting in a concomitant reduction in IKK β phosphorylation and reduced NF- κ B activity. Therefore, IFIT5 acts as a molecular scaffold in the NF- κ B pathway by bridging TAK1 and the IKK complex to facilitate more efficient activation of NF- κ B.

In separate studies, IFIT5 has been shown to bind to RIG-I/MAVS and TAK1/IKK β . However, it is still unclear how IFIT5 balances its role in these two pathways, for example to avoid unproductively recruiting RIG-I to IKK β or TAK1 to MAVS. Detailed mapping of the interaction sites of IFIT5 binding partners and an understanding of the relative affinities of each of these proteins for IFIT5 may help to untangle this question. Since the C terminus of IFIT5 undergoes a large conformational change upon RNA binding [24], it is possible that this may affect binding to TAK1, which binds across the pivot and C-terminal domain [74]. In this case, RNA binding may act as a switch between NF- κ B and IRF-3 signalling, promoting differential responses in infected cells, which contain 5'-ppp RNA ligand, and stimulated bystander cells, which do not.

CONCLUSIONS

While the IFIT family comprises only five proteins, which are highly conserved at both the sequence and the structural level, IFITs have evolved diverse functions to facilitate innate antiviral immunity. By interacting with RNA and proteins, IFITs can restrict viral translation and modulate innate immune signalling, cell death and inflammation. However, this family has also developed lineage-specific IFIT complexes, which are differentially regulated across the duration of the antiviral response. In humans, IFIT1, IFIT2 and IFIT3 associate into a stable trimeric complex in which IFIT3 is the central hub that enhances and regulates IFIT1 RNA binding, while modulating IFIT2-driven apoptosis. This has the potential to offer enormous functional plasticity to an otherwise small complement of similar proteins. Understanding the cooperativity between different IFIT family members and the dynamics of complex formation over the course of infection will give important insights into the nuances of IFIT antiviral function.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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