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Interferon-Stimulated Genes: What Do They All Do?

John W. Schoggins

Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA; email: john.schoggins@utsouthwestern.edu

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Abstract

In the absence of an intact interferon (IFN) response, mammals may be susceptible to lethal viral infection. IFNs are secreted cytokines that activate a signal transduction cascade leading to the induction of hundreds of interferon-stimulated genes (ISGs). Remarkably, approximately 10% of the genes in the human genome have the potential to be regulated by IFNs. What do all of these genes do? It is a complex question without a simple answer. From decades of research, we know that many of the protein products encoded by these ISGs work alone or in concert to achieve one or more cellular outcomes, including antiviral defense, antiproliferative activities, and stimulation of adaptive immunity. The focus of this review is the antiviral activities of the IFN/ISG system. This includes general paradigms of ISG function, supported by specific examples in the literature, as well as methodologies to identify and characterize ISG function.

INTERFERON SIGNALING: WHAT IS AN INTERFERON-STIMULATED GENE?

The simplest definition of an ISG, or interferon-stimulated gene, is any gene induced during an interferon (IFN) response. This includes all type I IFNs (IFNα, β, ε, κ, ω, and others), type II IFN (IFNγ), and type III IFNs (IFNλ1, INFλ2, IFNλ3, IFNλ4). Type I and III IFNs are considered the classical antiviral IFNs, though IFNγ also has well-described viral inhibitory properties (1–6). IFN signaling has been discussed in multiple excellent reviews and is only briefly summarized here (7, 8) (**Figure 1**). IFNs bind cognate cell surface receptors, the heterodimeric IFNAR1/IFNAR2 complex for type I IFNs, the heterodimeric IFNLR1/IL10R2 complex for type III IFNs, and dimers of the heterodimeric IFNGR1/IFNGR2 complex for type II IFN. Type I and III IFNs signal through the JAK/STAT pathway to activate the heterotrimeric transcription factor complex ISGF3, which consists of phosphorylated STAT1/STAT2 and IRF9. Variations on this theme, including unphosphorylated STAT molecules and IRF9-STAT2 homodimers, have also been described (reviewed in 8). Type II IFN also signals through the JAK/STAT pathway, leading to the formation of phosphorylated STAT1 homodimers, also known as IFNγ-activated factor (GAF). Activated ISGF3 and GAF translocate to the nucleus and bind IFN-stimulated response elements and gamma-activated sequences, respectively, in the upstream promoter regions of ISGs. Thus, one could strictly define ISGs as only those genes that are direct targets of ISGF3 and GAF.

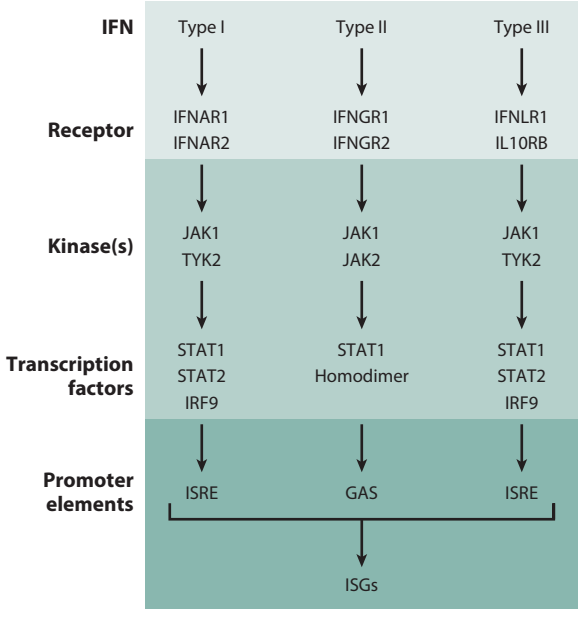


Figure 1

The IFN signaling pathway. The various interferons function as extracellular cytokines that signal through specific dimeric cell surface receptors (IFNAR1/INFAR2 for type I, IFNGR1/IFNGR2 for type II, IFNLR1/IL10RB for type III). Receptor engagement leads to activation of intracellular kinases (JAK1, JAK2, TYK2) that phosphorylate transcription factors STAT1 and STAT2. The activated transcription factors (ISGF3 or STAT1 homodimers) translocate to the nucleus to induce transcription of ISGs by binding ISRE or GAS promoter elements. Abbreviations: GAS, gamma-activated sequence; IFN, interferon; ISG, interferon-stimulated gene; ISRE, interferon-stimulated response element.

However, the system is more complex than this. Many so-called ISGs are also direct targets of interferon regulatory factors (IRF1, IRF3, IRF7), NFκB, or IL-1 signaling (8–13). These ISGs can be induced even in the absence of IFN signaling. To complicate the situation, some of these ISG-inducing factors, such as IRF1 and IRF7, are themselves also IFN-inducible, potentially leading to multiple pathways by which a single ISG can be induced (14–16). Another aspect of ISG expression is that some ISGs are expressed basally in addition to being IFN-inducible, while others appear to be expressed only during an IFN response (17). Should we have nomenclature to distinguish those genes with special expression patterns or kinetics? Possibly. However, recategorizing subsets of ISGs that are either targets of other transcription pathways or expressed in the absence of IFN signaling may just exacerbate an already complicated classification system.

Whereas we typically think of ISGs as IFN-inducible protein coding messenger RNA (mRNAs), it is important to recognize that IFNs also induce myriad noncoding RNAs including long noncoding RNAs and microRNAs. Recent work is uncovering fascinating biology for some of these RNAs (18–20). Moreover, a large number of genes are also repressed during IFN stimulation, although they are less well characterized than the ISGs. The nomenclature of these genes is also not solidified. They have been called interferon-repressed genes and denoted as IRGs or IRepGs (21–23). However, IRG has also been used as an acronym for interferon-regulated genes, a term that includes the constellation of all RNAs modified by IFN signaling, whether stimulated or repressed (24). If IRGs were used to indicate repressed genes, then we would need a new term to describe the sum collection of ISGs and IRGs, perhaps interferon-modulated genes. However, if IRG more appropriately refers to all genes regulated by IFN, then we would need a unique term to describe the repressed genes, perhaps IDG for interferon-downregulated gene. In fact, in January 2019, I ran a rather nonscientific poll on Twitter to gauge opinions on this nomenclature. There were 86 respondents, and the results were nearly evenly split with respect to how IRG should be used. Thus, without consensus, it is not the intent of this review to prescribe nomenclature but rather to highlight the confusing terminology so that the field can eventually agree upon terms to describe these gene groups.

HOW MANY INTERFERON-STIMULATED GENES ARE THERE?

With the development of microarray technology, the breadth of IFN-induced gene expression was initially assessed in several immortalized human cell lines (25, 26), with similar studies in other species shortly thereafter (27, 28). To date, and with the development of RNA sequencing (RNA-Seq) gene profiling, there are dozens of studies examining the repertoire of ISGs in different species and cell types and with different IFNs (e.g., type I versus II). The Interferome database is an excellent resource that continues to catalog gene expression profiling studies in the context of IFN stimulation (24).

Early microarray studies suggested the existence of >300 ISGs. Our meta-analysis of ISG transcriptomes published before 2007 indicated that approximately 450 genes were commonly induced by type I IFNs across multiple cell backgrounds from diverse mammals (29). Notably, cells of hematopoietic lineage induce many more genes, nearly 1,000 in chimpanzee or mouse cells treated with IFNα (17, 28). We now know that the total number of ISGs may be even higher. Indeed, in a recent study, Shaw et al. (23) used RNA-Seq and cross-comparative analysis to profile the IFN-induced transcriptional response in primary fibroblasts from nine diverse mammals and one bird. In human cells, approximately 10% of the human genome was subject to regulation by IFN. Notably, whereas most other studies implemented a fold change cutoff to designate ISGs, the authors of this study implemented only a statistical cutoff based on false discovery rate. For example, if a particular gene was reproducibly expressed 1.3-fold higher in the presence of IFN,

that gene was considered an ISG. In most other studies, such genes would likely not be considered ISGs because they would fall below a preset 1.5-fold, 2-fold, or even 4-fold induction cutoff. Thus, while the total number of genes induced by IFN is very high, many of these genes have very low levels of induction, which may make it difficult to determine whether they are bona fide antiviral ISGs. Notably, when Shaw et al. (23) compared all ISG transcriptomes from diverse animals, they found only 62 common genes, suggesting that this core ISG set represents an evolutionarily conserved group of ancestral genes critical to host IFN responses.

HOW ARE ANTIVIRAL INTERFERON-STIMULATED GENES IDENTIFIED?

Prior to genome-scale transcriptional profiling, several antiviral ISGs were identified by associating gene expression with suppression of viral infection, followed by functional testing. A classic example is MX1 (also known as MXA), which was originally linked to influenza resistance in genetically tractable mouse models with differing susceptibilities to the virus (30). MX1 was later found to be expressed in IFN-treated cells that resisted influenza A virus infection (31). Functional studies revealed that cells expressing MX1 complementary DNA (cDNA) became highly resistant to influenza A virus infection (32), paving the way for many years of mechanistic work on this classical antiviral ISG. Fast-forward nearly three decades, when similar approaches were recently used to identify the potent human immunodeficiency virus (HIV-1) restriction factor MX2. In these publications, the authors used modern deep-sequencing techniques to show that MX2 expression was associated with IFN-mediated suppression of HIV-1 infection in cultured cells (33, 34). Mechanistically, they found that MX2 inhibited HIV-1 at a post-entry step, prior to the onset of genome amplification.

Since IFN induces gene expression, other approaches to identify antiviral ISGs have relied on ectopic expression of ISG cDNAs to mimic IFN-mediated induction. Early iterations of this approach used varying numbers of ISGs in targeted screens against individual viruses [e.g., 7 ISGs targeting an alphavirus (35), 23 or 107 ISGs tested for inhibition of hepatitis C virus (36, 37)]. Several groups (29, 38–41) established large-scale ISG screening platforms representing 300–500 common ISGs. Since 2011, these ISG collections have been tested against more than 20 viruses spanning the mammalian viral phylogeny. The power of these large-scale approaches is not only the identification of new ISGs that target specific viruses but also the ability to perform cross-comparative analyses to determine patterns of ISG-mediated suppression. For example, comparative ISG screens across multiple positive-sense and negative-sense RNA viruses suggested that negative-sense RNA viruses were generally less susceptible to ISG-mediated inhibition than positive-sense RNA viruses in the cell lines tested (61). A greater number of ISGs targeted positive-sense RNA viruses, and the magnitude of ISG-mediated suppression was greater than with negative-sense RNA viruses. The implications of these findings are still not clear, but they could be linked to the unique replication strategies of these viruses or their differential abilities to antagonize host antiviral defenses.

In genetic terms, ectopic expression, as just described, is a gain-of-function approach that has been instrumental in helping define ISGs that are individually sufficient for viral suppression. Combinatorial approaches have also been used to demonstrate that co-expressed ISGs can achieve more robust inhibition than a single ISG on its own (29, 42). This is almost certainly how the IFN system works natively. However, cDNA expressional screens do not allow us to ask which genes are necessary for, or contribute to, IFN-mediated inhibition of viral infection. To answer this question, genetic loss-of-function approaches are needed. As with gain-of-function approaches, various iterations of loss-of-function studies have used small interfering RNA (siRNA)- or short

hairpin RNA (shRNA)-mediated gene silencing to test different numbers of ISGs against individual viruses [e.g., 5 ISGs tested for inhibition of an alphavirus (35), 60 ISGs tested for inhibition of hepatitis C virus (HCV) (43), 245 ISGs tested for inhibition of West Nile virus or Sendai virus (44, 45)]. In addition to these targeted approaches, unbiased, genome-scale siRNA screens have also been performed in the context of IFN-mediated suppression of HCV (46, 47). Both of these studies identified not only ISGs but also non-ISG host factors that are required for the IFN response.

Caveats of siRNA-based approaches are the prevalence of off-target effects and the lack of reproducibility across various siRNA screening platforms (48). The development of CRISPR-Cas9 gene editing technology has opened doors to new screening platforms that are more robust at identifying essential genes than their siRNA-based counterparts (49, 50). Two recent studies have used targeted or unbiased CRISPR screening to identify ISGs targeting HIV-1 or flaviviruses. OhAinle et al. (51) generated a CRISPR library containing ~15,000 single guide RNAs that target 1905 ISGs with 8 guides per gene. The library was tested to identify genes required for IFN-mediated inhibition of HIV-1 production. The screen revealed that inhibition of HIV-1 by IFN is largely dictated by a surprisingly small collection of known antiretroviral ISGs, including MX2, TRIM5α, IFITM1, and tetherin/BST2. Richardson et al. (52) recently performed a genome-scale CRISPR knockout screen to identify ISGs that mediate the IFN response to flaviviruses. Somewhat surprisingly, only one true antiviral effector, interferon-inducible protein 6 (IFI6), was identified in this screen. Numerous other genes scored as hits in the screen, including, as expected, almost all genes in the IFN signaling pathway (e.g., IFNAR1, JAK1, TYK2, STAT2, IRF9, etc.). Another cluster of hits in the screen were genes involved in general transcriptional regulation. We suspect that the lack of other known anti-flavivirus ISGs as hits (e.g., IFITM3, IRF1, etc.) in this screen was because genome-wide approach CRISPR screens are designed to reveal those genes with the most potent phenotypes. Since IFN signaling pathway genes are essential for ISG induction, they will likely dominate in any screen involving an IFN response; this appears to be the case in our screen. The fact that IFI6 statistically ranked among these signaling genes is testament to its potent antiviral activity and suggests that it may be among the more important IFN-inducible antiviral effectors controlling flavivirus replication. As CRISPR technology is used with other viruses, it is expected that the repertoire of bona fide antiviral ISGs targeting diverse viruses will become clearer.

WHAT DO INTERFERON-STIMULATED GENES DO?

A major goal in the field of ISG biology is to uncover the molecular mechanisms by which ISG-encoded proteins inhibit viral infection. A key question is, what does mechanism mean? As more ISGs are identified and characterized, we are gaining clarity as to how to define mechanism in different contexts. On one level, we can define mechanism as the mode of inhibition as it relates to a particular step in the infection/replication cycle. For example, we have known for some time now that the antiviral ISG IFITM3 inhibits viral entry (recently reviewed in 1, 53). This was demonstrated with numerous viruses using targeted cell biological and virological tools that can uncouple viral entry from later stages of the replication cycle. Beyond mechanism at the level of the replication cycle, additional work revealed that IFITM3, via a palmitoylated amphipathic alpha helix, blocks membrane fusion of viruses that enter cells through an endocytic route (54–56). Thus, the initial characterization of IFITM3 highlighted inhibition of viral entry as the mechanism, and more detailed study further pinpointed the mechanism to inhibition of viral fusion. As noted by Chemudupati et al. (53), there are still details of IFITM3-mediated inhibition to be uncovered. Indeed, very recently, Spence et al. (57) used site-specific fluorophore tagging and live-cell imaging studies to show that IFITM proteins reside in endocytic vesicles that fuse with incoming viruses. The presence of IFITM proteins increases the trafficking of these virus-containing vesicles to

lysosomes, effectively halting productive entry. Thus, like several other ISGs, the IFITM proteins are great examples of ISG effectors that were first characterized by determining the general replication step affected by ISG expression, followed by more granular studies on the molecular nature of how the effector impacts this step.

Here, in an attempt to simplify categorization of ISGs by mechanism of action, the viral replication cycle is divided into three main stages: (*a*) attachment, entry, and trafficking; (*b*) viral protein production and genome amplification; and (*c*) viral particle assembly and egress. Rather than give an encyclopedic list of all ISGs that fall under each grouping, I introduce general mechanistic paradigms, with specific ISGs used as notable or recent examples (**Figure 2**). Readers are referred to numerous other ISG reviews for more in-depth discussion of many of these effectors (53, 58–60).

ATTACHMENT, ENTRY, AND TRAFFICKING

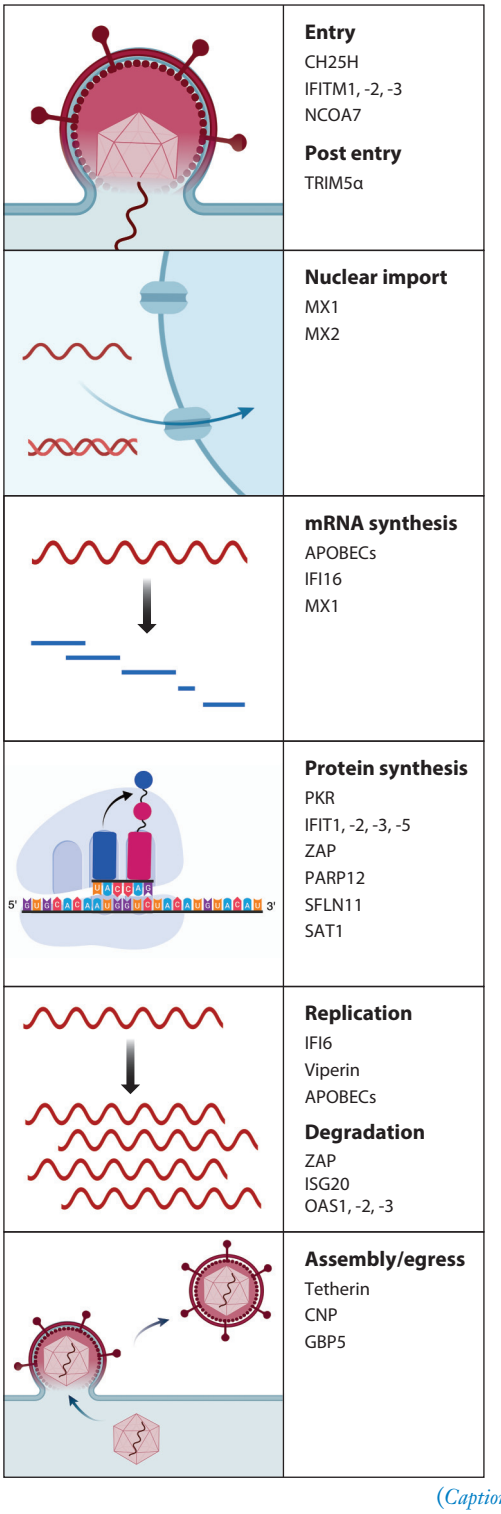
Viruses typically have to make contact with the cell surface to initiate productive infection. One might imagine that targeting viral attachment might be a promising antiviral strategy for the host. However, there are few, if any, bona fide examples of ISGs targeting viral attachment directly. One candidate for this class of effector may be HPSE, an ISG that encodes heparanase. Several studies have shown that ectopic expression of HPSE broadly inhibits multiple alphaviruses, flaviviruses, paramyxoviruses, and noroviruses (29, 42, 61). Heparanase is an enzyme that cleaves the side chains from cell surface heparan sulfate proteoglycans, which are known to have roles in viral attachment. However, heparanase cleavage also releases other heparan sulfate–linked soluble factors (62). It can also modulate signaling pathways independent of its enzymatic activities. Thus, while inhibition of viral attachment is an obvious and attractive mechanism of action for heparanase, additional studies are needed to determine if this speculation holds and whether this gene has a relevant antiviral effect during the IFN response.

In contrast to viral attachment, viral entry is a step commonly targeted by multiple ISG effectors. As noted above, the IFITM proteins are classic examples of ISGs that impair entry of multiple enveloped viruses, with recent studies highlighting a role for these proteins in trafficking viral particles to degradative lysosomes (1, 53, 57, 63). CH25H, the IFN-inducible enzyme that converts cholesterol to 25-hydroxycholesterol (25-HC), is also implicated in suppressing viral entry via the membrane-perturbing functions of 25-HC (64, 65).

Another recently identified ISG effector that targets viral entry is human nuclear receptor coactivator 7 (NCOA7). In a study correlating ISG expression to HIV-1 permissivity, the same study that led to the identification of MX2 (34), NCOA7 was identified as a potential antiviral candidate. Both ectopic expression and gene silencing experiments revealed that NCOA7 inhibits infection of multiple viruses that enter cells via endocytosis. Mechanistically, NCOA7 binds the vacuolar H^+-ATPase, which promotes vesicle acidification and degradation of endocytosed cargo (66).

Post-entry viral processes, prior to the onset of viral gene expression, can also be targeted by ISG effectors. For example, after retroviral entry, the viral capsid can be directly targeted by TRIM5α. This potent and multifunctional protein forms a hexagonal lattice that assembles on the capsid and mediates proteasome-dependent destruction of the viral core (67–70). Through sensing the capsid lattice, TRIM5a can also initiate an antiviral signaling cascade via the TAK1 kinase complex (71).

Another post-entry process targeted by the IFN response is trafficking of viruses that replicate in the nucleus. Certain ISG effectors specifically block delivery of viral machinery through the nuclear pore complex. For influenza A virus and HIV-1, respectively, this process has been shown



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

ISG targeting distinct steps in the viral replication cycle. Viral replication steps are shown in boxes. Examples of ISG effectors targeting viral entry, viral genome nuclear import, viral gene or protein synthesis, viral genome replication, or virion assembly/egress are indicated next to each box. Abbreviations: APOBEC, apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like; ISG, interferon-stimulated gene; mRNA, messenger RNA; PKR, protein kinase R; ZAP, zinc antiviral protein. Figure generated from Biorender.

to be targeted by the classic ISG MX1 and the more recently characterized MX2 (also MxA and MxB, respectively). MX1 also targets early viral gene expression of some cytoplasmic negative-sense RNA viruses as noted below. The MX proteins were discussed in the preceding section and have been recently reviewed (72).

VIRAL PROTEIN PRODUCTION AND GENOME AMPLIFICATION

Regardless of the composition of the viral genome (DNA or RNA, single-stranded or double-stranded), all viruses must make mRNA and protein to replicate. The IFN/ISG system has evolved a number of distinct, and often sophisticated, mechanisms to target viral gene expression, protein production, and genome amplification. Several classic and more recent examples of each of these ISG-mediated antiviral paradigms are highlighted here.

Viral Gene Expression

The majority of DNA viruses and retroviruses use host DNA-dependent RNA polymerases (usually RNA polymerase II and rarely RNA polymerase III) to generate viral mRNA. Direct inhibition of transcription of these viral DNAs is not a common ISG effector mechanism, likely because of the deleterious effects on host gene expression that would occur if the activity of cellular RNA polymerases were inhibited. Nonetheless, an early study on the anti-herpesvirus effects of IFN concluded that early viral gene expression was suppressed by IFN treatment (73). More recently IFI16, an IFN-inducible DNA sensor, was shown to interfere with transcription of a human cytomegalovirus gene, although this effect occurred independent of IFN (74). Other host factors, some of which are modestly induced by IFN, are reported to suppress transcription of viral genes (reviewed in 53). However, relative to other stages of the replication cycle, transcription of DNA virus genomes or retroviral DNA intermediates does not appear to be a dominant target of IFN action.

Negative-sense, single-stranded RNA viruses use their RNA-dependent RNA polymerase to transcribe viral genes from the genomic template. Viral gene expression in this context has been shown to be targeted by ISG effectors. As noted above, a classic example is MX1, which targets transcriptional elongation (reviewed in 72). A recent study uncovered an antiviral effector that targets Ebola virus transcription. A proteomics approach was used to define the set of human host proteins that interact with Ebola virus proteins NP, VP35, VP40, GP, VP30, VP24, and L (75). VP30, which regulates viral RNA synthesis, was found to interact with RBBP6, a ubiquitin E3 ligase with multiple functions including roles in cell cycle progression and transcription. Structural studies demonstrated that RBBP6 interacted directly with VP30, thereby preventing interactions of VP30 with viral NP and impairing viral transcription. This unique example of molecular mimicry expands the landscape of host antiviral mechanisms targeting viral gene expression. Notably, RBBP6 also appears to be an ISG. It is present in the Interferome database and is modestly induced in IFN-treated primary fibroblasts from humans, cows, sheep, and pigs (23). Although Batra et al. (75) did not examine RBBP6-VP30 interactions in the context of IFN

treatment, it is tempting to speculate whether RBBP6 contributes to cell intrinsic defense in both unstimulated and IFN-stimulated cells.

Viral Protein Translation

In the viral replication cycle, translation is a critical Achilles's heel that IFN targets through multiple mechanisms. A classic example of an ISG that inhibits viral protein production is protein kinase R (PKR), a double-stranded RNA-dependent kinase that phosphorylates EIF2a to modulate multiple cellular processes including translation (76). The IFIT family (which includes IFIT1, IFIT2, IFIT3, and IFIT5 in humans) uses multiple mechanisms to target viral protein production, such as binding eiF3C or eiF3E to suppress translation initiation. A molecular signature recognized by some IFITs is a type 0 cap structure lacking 2′-*O*-methylation. This was demonstrated by showing robust IFIT1-mediated suppression of viruses genetically modified to lack 2′-*O*-methylation (reviewed in 77, 78). Another classic inhibitor of viral translation is the zinc antiviral protein (ZAP), an ISG effector with broad antiviral activity that was recently reviewed and is discussed briefly below (53).

While PKR, IFITs, and ZAP are some of the more well-characterized ISGs targeting translation, recent efforts are uncovering additional molecules that fall into this class of antiviral effectors. ZAP is a member of the poly(ADP-ribose) polymerase (PARP) family and is also known as PARP13. Several other PARP members, including the ISGs PARP7, PARP10, and PARP12, were shown to suppress viral replication (79). Further study of the long isoform of PARP12 (PARP12L) demonstrated that this protein forms complexes with the ribosome and suppresses both cellular and viral translation via its catalytic and autoribosylation activities (80). Another mechanism of translation inhibition was discovered in studies on the ISG schlafen 11 (SLFN11). This protein is able to suppress rare transfer RNA codons that are often used by viruses such as HIV-1 (81). More recently, the ISG SAT1, which encodes the polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase, was shown to inhibit primary translation of flavivirus and alphavirus genomes (82). Mounce et al. (82) showed that polyamines stimulate viral RNA synthesis and that their depletion via chemical inhibition or SAT1 expression limits viral replication. Thus, targeting polyamine synthesis is yet another tool in the IFN-induced antiviral armamentarium. A final recent example of an ISG targeting viral protein synthesis is Shiftless, an antiviral effector also known as C19orf66, RyDEN, and IRAV. Wang et al. (83) demonstrated that Shiftless inhibits programmed −1 ribosomal frameshifting, which is required for maintaining a proper ratio of Gag to Gag-Pol levels during HIV-1 infection. Previous studies also showed that Shiftless/C19orf66 suppresses diverse RNA viruses (29, 84, 85), although it is not known whether those effects are mediated through a similar programmed −1 ribosomal frameshifting mechanism. Based on the number and diversity of mechanisms used by ISG effectors to target translation, it is likely that additional factors targeting this critical step will be uncovered.

Viral Genome Amplification

All viruses must replicate their genomes to produce new viral progeny and propagate infection. Indeed, viral genome amplification, also referred to as replication, is frequently used as a generic assay to assess antiviral ISG activity. For example, control or ISG-expressing cells can be infected with a virus, and viral genome copies can be quantitated by PCR. This assay itself is not necessarily a direct indicator that an ISG targets viral genome amplification, since inhibition of any step prior to genome amplification would also result in fewer viral genome copies. However, for many viruses, tools and assays are available to test whether an ISG specifically targets viral genome replication.

A recently characterized, and rather remarkable, example of an ISG effector suppressing viral replication is viperin (encoded by gene *RSAD2*). Viperin is a radical *S*-adenosyl-L-methionine (SAM) domain-containing enzyme that uses a 4Fe-4S cluster to cleave SAM. Discovered almost 20 years ago, this ISG has been shown to inhibit numerous viruses, often with distinct and unrelated mechanisms such as suppression of viral replication or egress (reviewed in 1). Gizzi et al. (86) demonstrated that viperin catalyzes the formation of 3′-deoxy-3′,4′-didehydro-CTP (ddhCTP) from cytidine triphosphate (CTP) in a radical SAM-dependent reaction. Cells ectopically expressing viperin or treated with IFN had elevated ddhCTP levels. Mechanistically, ddhCTP was shown to incorporate into newly synthesized viral RNA and function as a chain terminator for the viral RNA-dependent RNA polymerase of flaviviruses. However, in primer extension assays, the polymerases of two picornaviruses, human rhinovirus C and poliovirus, were poorly inhibited by ddhCTP, highlighting the potential specificity of this mechanism for certain viral polymerases. Of note, a distinct picornavirus, enterovirus A71, is reported to be suppressed by viperin (87). Thus, viperin may target viruses via ddhCTP-dependent and ddhCTP-independent mechanisms (88).

Another antiviral mechanism that targets viral replication, albeit indirectly, is suppression of autophagy. While autophagy contributes to innate immune control of viral infection, many viruses are known to coopt the autophagic machinery to promote their replication (89). It appears this hijacking mechanism can be targeted by a recently characterized antiviral ISG. In an shRNA-based screen to identify IFN-inducible factors targeting a paramyxovirus, the protein tudor domain-containing 7 (TDRD7) suppressed Sendai virus replication (45). Sendai virus was shown to induce autophagy as a requirement for optimal viral replication. TDRD7 suppressed virus-induced autophagy by inhibiting the phosphorylation of AMPK, a kinase that activates the autophagy machinery. Similar results were observed with related viruses, parainfluenza virus type 3 and measles virus. However, an unrelated picornavirus, encephalomyocarditis virus (EMCV), was not inhibited by TDRD7. Rather, EMCV replication was slightly enhanced in cells ectopically expressing TDRD7. This result is somewhat surprising since picornaviruses, including EMCV, are thought to utilize the autophagy machinery to promote replication (90). Further, previous ISG screens suggest poliovirus, also a picornavirus, is inhibited by TDRD7 (61). Thus, additional studies are needed to clarify viral specificity during TDRD7-mediated suppression of autophagy.

As a prerequisite for replication, retroviruses use a virally encoded reverse-transcriptase to generate DNA from their RNA genome. This process has been shown to be targeted by ISGs, with a key example being the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) proteins, a family of C-to-U editing enzymes. One potent antiviral member of this family, APOBEC3G, hypermutates single-stranded retroviral genomes, leading to impairment of viral gene expression and replication (70, 91). It can also function in a deaminase-independent manner by binding viral genomes and preventing reverse transcription (92, 93).

In another recent example of viral replication suppression, IFI6 was shown to potently inhibit flavivirus replication (52). IFI6 is a small, highly hydrophobic protein that was found to localize to the endoplasmic reticulum (ER), the site of flavivirus replication. Mechanistically, IFI6 suppressed the ability of flaviviruses to form replication organelles, which are characterized by single-membrane invaginations at the ER that are thought to house the viral replication machinery. Indirect evidence suggested that IFI6 did not inhibit viral polymerase activity but rather interfered with the membrane-perturbing function of these viruses. Notably, IFI6 did not inhibit the related hepatitis C virus, which also replicates at the ER but does so in double-membrane vesicular structures that bud outward from the ER. Thus, IFI6 may derive its specificity from the physical characteristics and/or composition of the flavivirus replication organelles it targets.

The IFN response uses several ISG effectors to directly degrade viral RNAs or viral genomes, thereby potently suppressing viral replication. The most well-studied examples are the

oligoadenylate synthetases (OAS proteins), ZAP, and interferon-stimulated gene 20 (ISG20). The OAS proteins are enzymes that catalyze the formation of 2′–5′-linked oligoadenylates that activate latent cellular RNase L, which efficiently degrades viral genomes (94, 95). ZAP, which was described above as an inhibitor of viral translation, has also been shown to target viral mRNAs for exosome-mediated degradation (96). ISG20 is a 3′ to 5′ exonuclease that has been shown to suppress replication of multiple viruses by degrading viral genes or genomes (97). It also appears to suppress alphavirus replication by inducing a type I IFN response, independent of its exonuclease activity (98).

Viral Particle Assembly and Egress

The late stages of the viral replication cycle include virion assembly, morphogenesis, trafficking, and egress from the cell. There appear to be fewer ISGs targeting these steps relative to the earlier phases of the viral replication cycle. Many of these effectors appear to be specific for HIV-1 and other retroviruses. It is unclear why. Perhaps we have not yet identified a broader spectrum of similar ISGs targeting other viruses because of technical difficulties in uncoupling late viral stages from earlier ones. Alternatively, these ISGs may simply not exist.

Among the known ISG effectors targeting late stages of the viral replication cycle, the most well characterized is tetherin (encoded by BST2). Tetherin is a transmembrane protein that has a rather remarkable ability to anchor budding virions to the cell surface and prevent them from being released. This antiviral mechanism was first discovered with HIV-1 and has since been found to extend to numerous other enveloped viruses (53, 99). Other examples in this class of effectors include CNP (2′,3′-cyclic-nucleotide 3′-phosphodiesterase), which binds Gag and inhibits HIV-1 particle assembly (39), and GBP5 (guanylate binding protein 5), which impairs incorporation of the viral envelope glycoprotein Env into virions (100).

INTERFERON-STIMULATED GENES THAT PROMOTE VIRAL INFECTION

There is a small but growing class of ISGs that have the unexpected property of promoting viral infection. Two examples are LY6E and MCOLN2. A logical hypothesis is that these effectors negatively regulate IFN induction, signaling, or response, similar to other known negative regulators such as SOCS1, USP18, AXL, or ADAR1 (101–107). However, rather than negatively regulating IFN, they appear to have direct enhancing effects on viral infection. LY6E and MCOLN2 were originally identified in ectopic expression-based ISG screening platforms (29, 61). MCOLN2 (mucolipin-2) is an endosomal cation channel that enhances entry of several enveloped RNA viruses that fuse with late endosomes (108). MCOLN2 specifically enhances the trafficking efficiency of these viruses. Whether this effect is a direct or an indirect effect of diverting the virus from an otherwise less productive route of entry is still not known. Viral entry is also the target of LY6E (lymphocyte antigen 6 family member E), an IFN-inducible glycosylphosphatidylinositol-anchored protein and member of the LY6/uPAR family. Similar to MCOLN2, LY6E also promotes infection of viruses that use endocytic entry pathways, although the mechanism is slightly different (109–111). For influenza A virus, LY6E enhances viral uncoating, which occurs after endosomal escape (110). This leads to increased nuclear translocation of the viral genome and ultimately more efficient infection across a population of cells.

Although we have mechanistic insight into the step of the viral entry that both of these factors target, we still do not have a clear picture of the biological relevance of this effect. The enhancing effect of LY6E is evolutionarily conserved across diverse mammals, suggesting conservation

Table 1 Examples of interferon-stimulated gene effector enzymes that generate bioactive molecules

Gene	Protein	Molecule generated	Function
OAS1, *-2*, *-3*	oligoadenylate synthetase-1, -2, -3	2′–5′-linked oligoadenylates	activate RNase L to degrade viral RNA (transcription/replication)
CH25H	cholesterol 25-hydroxylase	25-hydroxycholesterol	inhibit viral membrane fusion (entry)
RSAD2	viperin	3′-deoxy-3′,4′-didehydro-CTP	chain terminator during viral RNA synthesis (replication)
CGAS	cyclic GMP-AMP synthase	cyclic GMP-AMP	activate STING, induce IFN

Abbreviations: IFN, interferon; STING, stimulator of interferon genes.

of function. One explanation is that the viruses have evolved to hijack these effectors, similar to the way some coronaviruses have been reported to use IFITM3 to gain entry into the cells (112, 113). If so, then we might predict that, like IFITM3, LY6E and/or MCOLN2 may also possess antiviral activity in certain contexts. For LY6E-mediated effects on HIV-1, recent data support this prediction (114). It is also possible that the cell uses these molecules to promote viral uptake. For example, increased viral uptake into antigen-presenting cells may stimulate a more robust adaptive immune response, leading to better host control of the infecting virus. Testing these distinct hypotheses will likely require targeted knockout approaches in genetically tractable (e.g., mouse) models of viral pathogenesis.

PUTTING IT ALL TOGETHER

As new antiviral ISGs are discovered and known ISGs are further characterized, several themes are emerging from the collective knowledge of their antiviral mechanisms. As discussed at length in this review, almost any stage of the viral replication cycle can be inhibited by antiviral effectors. Some stages are more commonly targeted than others, at least based on our current knowledge. For example, for most viruses except maybe the retroviruses, early stages of the replication cycle (entry, gene expression, genome replication) are more commonly targeted than late stages (virion assembly, morphogenesis, egress). As better viral tools are developed to study late stages of infection, it is certainly probable that additional ISGs targeting these steps will be uncovered.

Beyond the categorization of ISG effectors relative to the viral replication cycle, other functional groupings are emerging from recent studies. For example, it is becoming clear that IFN induces a growing class of antiviral enzymes, each of which catalyzes the formation of a molecular product that confers antiviral activity through distinct mechanisms. Examples in this group include OAS proteins, CH25H, viperin, and the viral sensor cGAS, which is also an ISG (115) (**Table 1**). Another class of effectors are membrane-localized proteins that inhibit some viral process involving the use and/or manipulation of cellular lipids and membranes (**Table 2**). Examples

Table 2 Interferon-stimulated gene effectors targeting viral membrane processes

Gene	Protein	Function
BST2	tetherin	tether virion to cell membrane (egress)
CH25H	cholesterol 25-hydroxylase	inhibit viral membrane fusion (entry)
IFI6	IFN-induced protein 6	inhibit flavivirus-induced ER membrane rearrangements (replication)
IFITMs (*-1*, *-2*, *-3*, *-5*)	IFN-induced transmembrane proteins	promote viral trafficking to endosomes (entry)
NCOA7	nuclear receptor coactivator 7	impair virus fusion to endosome (entry)

Abbreviations: ER, endoplasmic reticulum; IFN, interferon.

of this group include IFITMs, NCOA7, CH25H, IFI6, and tetherin, each of which is discussed above.

In summary, the question of what all these ISGs do is complex and lacks a simple answer. IFN has one function that we know of—to bind to a cell surface receptor. This single molecular interaction then triggers a signal transduction cascade that leads to the production of the true workhorses of the system, the ISGs. The protein products encoded by these ISGs can work alone, but likely work in concert, to alter the cellular state for viral defense. Since the first ISGs were cloned over 30 years ago, researchers from diverse fields have been cataloguing the functions of ISG-encoded proteins. For the effectors that have well-defined mechanisms of action, it often took years and occasionally decades of work by numerous labs to get there. Given the diversity of viruses that are of interest to not only the biomedical community but also the agricultural and fishing industries, it is possible that we have only scratched the surface with respect to understanding the true mechanistic breadth of the antiviral IFN response. This possibility is further underscored by the growing body of literature showing that many ISG effectors evolve rapidly, giving rise to species-specific restriction factors with potentially unique viral specificities (116). As new technologies reveal more antiviral ISGs in both model and nonmodel systems, an important next step will be to determine their mechanisms of action, which has been the primary focus of this review. Beyond mechanism, another important goal is evaluating the relevance of these effectors in modulating viral pathogenesis, preferably with robust in vivo models or human studies. It may take several more decades of research to get there, but the hope is that we ultimately arrive at a clear understanding of the panoply of molecular mechanisms that govern one of nature's most sophisticated antiviral defense systems.

DISCLOSURE STATEMENT

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