Review

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To translate, or not to translate: viral and host mRNA regulation by interferon-stimulated genes

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Type I interferon (IFN) is one of the first lines of cellular defense against viral pathogens. As a result of IFN signaling, a wide array of IFN-stimulated gene (ISG) products is upregulated to target different stages of the viral life cycle. We review recent findings implicating a subset of ISGs in translational regulation of viral and host mRNAs. Translation inhibition is mediated either by binding to viral RNA or by disrupting physiological interactions or levels of the translation complex components. In addition, many of these ISGs localize to translationally silent cytoplasmic granules, such as stress granules and processing bodies, and intersect with the microRNA (miRNA)-mediated silencing pathway to regulate translation of cellular mRNAs.

ISGs block virus replication

In response to an infection, the host recognizes pathogen-associated molecular patterns (PAMPs) of invading microbes in the cell. Viral PAMPs are often nucleic acid-based, derived from their DNA, or from their RNA genomes. Several pattern recognition receptor families located in various cellular compartments work together to sense PAMPs leading to activation of the transcription factors IFN-regulatory factors 3 or 7 (IRF3/7) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (for a recent review, see [1]). The signaling events following PAMP recognition result in dimerization and translocation of IRF3/7 into the nucleus along with NFκB, leading to the transcription and expression of type I IFN and proinflammatory cytokines, which in turn get secreted by the cell. Autocrine or paracrine signaling in response to IFN induces downstream expression of an array of IFN-stimulated genes (ISGs), which function to establish an antiviral state [1].

ISGs act on different stages of the viral life cycle, from entry and replication to assembly and release. In order to productively infect the host and multiply, viruses usurp the host translation machinery to make viral proteins. Translational inhibition is a common mechanism utilized by ISGs to mediate antiviral effects [2]. Indeed, some of the best studied ISGs, protein kinase RNA-activated (PKR) and 2′-5′-oligoadenylate synthetase (OAS)/RNAseL function to block translation to limit virus replication (Box 1). This review focuses on the more recently described ISGs that regulate host or viral translation, localize to translationally silent granules, and interfere with miRNA-mediated silencing of host transcripts.

Regulation of viral and host mRNA translation

Viruses are completely reliant on host cell translational machinery to produce the proteins encoded by their genes. In eukaryotic cells, translation is initiated (summarized in Figure 1 and recently reviewed in [3]) by binding of eukaryotic initiation factor (eIF) 4E to the m7G cap structure at the 5′ end of mRNAs. Meanwhile, poly(A)-binding protein (PABP) binds to the poly(A) tail at the 3′ end of mRNAs. Both eIF4E and PABP interact with the scaffold protein eIF4G, leading to mRNA circularization and recruitment of the 43S preinitiation complex, the minimal constituents of which include the eIF3 complex (13 subunits; a–m), the ternary complex eIF2-GTP-Met-tRNA^{i}, and the 40S ribosomal subunit. The 43S complex then scans the 5′ untranslated region (UTR) of the mRNA until it reaches the translational start codon. Ribosome scanning is aided by the RNA helicase, eIF4A, which disrupts secondary and tertiary structures in the 5′ UTR. The 60S ribosomal subunit then joins the 40S ribosomal subunit to form the 80S ribosome, resulting in translation initiation and elongation; formation of polyribosomes (polysomes) where multiple ribosomes simultaneously translate the same mRNA can then take place. Because translation initiation is a complex and highly ordered process, most of the translational regulation in eukaryotic cells occurs at this step [3]. Several ISG products, such as zinc finger antiviral protein (ZAP), interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), and schlafen 11 (SLFN11), have been shown to affect viral or global protein synthesis, and their modes of action are described in this section. The common strategies shared by these ISGs include direct binding to viral RNA, and interaction with or perturbation of the translation machinery components, preventing translation.

PARPs

Several members of the poly(ADP-ribose) polymerase (PARP) family are ISGs with antiviral activity (Box 2). Among them, the best-characterized antiviral protein is

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Box 1. Early discoveries on the mechanism of action of IFN

Before the discovery of ISGs, it was known that treatment of animal cells with IFN confers upon them resistance to new virus infections. IFN is not directly antiviral; cellular transcription and protein synthesis were found to be required for IFN to work, suggesting that IFN signaling leads to the translation of an inhibitory protein(s). The inhibitory activity targets an early stage of the viral life cycle, specifically the translation of the viral mRNA [99,100]. Protein synthesis in lysates prepared from mouse L cells pretreated with IFN was blocked upon exposure to dsRNA [101,102]. It appeared that a dsRNA-dependent protein kinase(s) and an oligonucleotide inhibitor (pppA2′-5′A2′-5′A) were involved [103–111]. It is now well appreciated that protein kinase RNA-activated (PKR) is a serine–threonine kinase and when activated by dsRNA becomes autophosphorylated and phosphorylates the α subunit of eIF2, leading to the inhibition of host and viral mRNA translation [112–118]. In addition, activation of the 2′-5′-oligoadenylate synthetase (OAS) by dsRNA triggers the synthesis of 2′-5′A from ATP, which causes the dimerization and activation of a latent endoribonuclease (later referred to as RNAse L) [113,119]. RNAseL causes the degradation of viral or cellular RNA leading to translation inhibition [120–122].

PARP13 or zinc finger antiviral protein (ZAP), which is encoded by the zinc finger CCCH-type, antiviral 1 (*ZC3HAV1*) gene. In the remainder of the review, we will refer to the protein as ZAP. ZAP is transcriptionally upregulated by type I IFN signaling and directly induced by phosphorylated IRF3 in virus-infected cells [4,5]. There are at least two splice variants of ZAP – ZAPL (PARP13.1) and ZAPS (PARP13.2) – where the long isoform encodes a PARP domain on the C terminus that is missing in the short isoform [6]. Although both isoforms are induced, ZAPS is upregulated more than ZAPL by virus and type I IFN [7–9]. ZAP was first discovered as a potent antiviral factor against the retrovirus Moloney murine leukemia virus (MLV) in a cDNA library screen [10]. Since then, it has been shown to inhibit a broad range of RNA and DNA viruses, including other retroviruses, alphaviruses, filoviruses, and hepatitis B virus [8,11–14]. It is not understood what determines the broad yet specific antiviral activity of ZAP. It binds viral RNA via its N-terminal zinc fingers, and ZAP-responsive sequence elements in MLV and Sindbis virus have been mapped [15]. ZAP recruits the exosome to target retroviral and specific host mRNAs for degradation [14,16–18] but also acts to block viral genome translation [11]. ZAP dramatically reduces Sindbis virus production, and experiments utilizing temperature-sensitive Sindbis virus mutants that are unable to replicate the RNA genome at nonpermissive temperatures support a mechanism in which ZAP represses translation of the incoming viral genome [11]. ZAP also inhibits translation of luciferase reporters that carry the ZAP-responsive elements from HIV-1 and Sindbis virus [19]. ZAP binds to eIF4A and interferes with the interaction between eIF4A and eIF4G, and as a result blocks translation independently of mRNA degradation [19] (Figure 1).

In addition to ZAP, other members of the PARP family are upregulated by IFN and have also been shown to inhibit alphaviruses. Murine PARP7, PARP10, and the long isoform of PARP12 (mPARP12L) block cellular translation and inhibit replication of Venezuelan equine encephalitis virus (VEEV), another member of the alphavirus genus [20,21]. mPARP12L also blocks infection of a variety of RNA viruses from other families, such as vesicular stomatitis virus (VSV), encephalomyocarditis virus (EMCV), and Rift Valley fever virus (RVFV) [20]. Similar to ZAP, mPARP12L affects protein translation, and the tethering of mPARP12L to a renilla luciferase reporter mRNA inhibits its translation [22]. Furthermore, mass spectrometry identified ribosomal proteins and proteins involved in translation as interacting partners of mPARP12L. mPARP12L interacts with ribosomes in the polysome-containing fractions at 4 hours post-infection (pi) with VEEV but facilitates disassembly of polysomes at later times of infection (12 h pi), which is dependent on its RNA-binding and PARP catalytic activities [21,22] (Figure 1). mPARP12L mutants that are unable to transfer ADP-ribose to substrate proteins, including mPARP12L itself, fail to block translation; however, they are still able to inhibit replication of GFP-expressing VEEV. One possible explanation is that mPARP12L utilizes an unknown mechanism to block VEEV replication that is unrelated to poly-ADP-ribosylation and translational inhibition. The role of the PARP domain in mediating translational inhibition and the catalytic activity-independent antiviral function of mPARP12L warrant further studies.

IFIT1

IFIT proteins are localized in the cytoplasm and lack any obvious enzymatic domain or activity. They contain multiple tetratricopeptide repeats, which are important for protein–protein interactions. IFIT1 (also called p56 and ISG56) is among the better characterized members, and its expression is induced by dsRNA, IRF3, type I IFN, and a variety of viruses [23,24]. Similar to PARP proteins, the IFIT family targets viruses by translational repression [25]. Many cellular and viral mRNAs are methylated at the N-7 and 2′-O positions of the 5′ guanosine cap by nuclear and cytoplasmic methyltransferases, but the function of 2′-O methylation was unclear for many years. Recent studies found that 2′-O methylation of the 5′ cap of viral RNA serves as an immune evasion strategy for viruses that are otherwise recognized by IFIT1.

Human IFIT1 blocks West Nile virus (WNV), Japanese encephalitis virus (JEV), and coronavirus mutants that lack 2′-O-methyltransferase activity, and inhibition occurs by IFIT1 preferentially sequestering capped RNA lacking a 2′-O-methyl group and preventing eukaryotic translation initiation factors from binding to the RNA template [26–30] (Figure 1). Structural studies and binding assays also support a role for IFIT1 in recognizing single-stranded viral RNA bearing a 5′-triphosphate group, which results in translation inhibition, although binding appears to be of lower affinity than IFIT1 binding to 5′ capped RNA lacking 2′-O-methylation [28,31,32]. Intriguingly, alphaviruses do not carry a 2′-O-methylated cap but instead have evolved a stable secondary structure in their 5′ UTRs to evade IFIT1 recognition and translational repression [33]. Taken together, IFIT1 is a critical innate immune effector that inhibits viruses whose RNAs lack a 2′-O-methylated cap.

In addition to directly binding to the viral RNA, IFIT1 binds the eukaryotic translation initiation factor 3e (eIF3e) subunit in a yeast two-hybrid screen and inhibits translation both *in vitro* and *in vivo* by blocking eIF3 stabilization