Post-Transcriptional Control of the Interferon System

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Abstract

The interferon (IFN) system is a well-controlled network of signaling, transcriptional, and post-transcriptional processes that orchestrate host defense against microbes. The IFN response comprises a multi-array of IFN-stimulated gene products that mediate a variety of biological processes designed to control infection and regulate specific immune responses. In this review, we focus on post-transcriptional mechanisms of gene regulation that occur during the course of IFN induction and during the response of cells to IFN. Post-transcriptional mechanisms involve different levels of regulation such as mRNA stability, alternative splicing, and translation. Such controls offer a fine tuning mechanism for efficient and rapid response and as a negative feedback control in IFN biosynthesis and response.

Introduction

Since the discovery of IFN fifty years ago, research on the regulation of the IFN system began several years later and the description of the "super-induction" phenomenon [1,2] triggered an appreciation of the role of post-transcriptional regulation not only in the IFN system but in general cellular gene expression. In this review, the focus is on the post-transcriptional control of the IFN system during biosynthesis of IFN or during response of cells to IFN. The IFN system consists of latent intracellular enzymes such as 2′-5′ oligoadenylate synthetase (OAS)/ribonuclease L (RNase L), double-stranded RNA dependent protein kinase (PKR), and others acting as the first line of defense against viruses, as evident from studies with specific gene knockout mice [3-6]. Both RNAse L and PKR have post-transcriptional roles that will be discussed here. IFN comes into play as a second line of defense for viruses and amplifies the IFN response with the induction of additional gene products that participate in the antiviral action. Post-transcriptional regulation is one of the several types of regulatory mechanisms that also include receptor-mediated signaling and transcriptional activation. The post-transcriptional control mechanisms confer a stringent mechanism in host defense to viruses and other microbes and contribute to the characteristics of the transient nature of IFN biosynthesis and response. This review is also complementary to other reviews in this special "Biochimie" issue of on the occasion of 50th anniversary of the discovery of IFN.

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Post-transcriptional regulation of IFN-beta: the "Super-Induction" phenomenon and involvement of AU-rich elements

Virus infection of fibroblast and epithelial cells leads to the transient production of type-I IFN, e.g., IFN-α/β, to induce an antiviral state in neighboring non-infected cells. The transient nature of IFN-β expression is due to repression of transcriptional activity and rapid turnover of IFN-β mRNA. Earlier work revealed the important role of post-transcriptional control of IFN production through the observation of a "super-induction" phenomenon. This phenomenon occurs when an inhibitor of RNA or protein synthesis is used in cultured cells during early treatment with an IFN inducer, resulting in increased mRNA levels of IFN [1,2,7,8]. For example, when confluent fibroblast cultures are treated with the double stranded RNA polyI-polyC in the presence of the protein synthesis inhibitor cycloheximide, prolongation of IFN mRNA accumulation occurs [2,7,9]. The super-induction is thought to be largely due to a pos-transcriptional mechanism that leads to stabilization of IFN mRNA [2,7]. It was postulated that a labile repressor, probably encoded by an unstable mRNA, when blocked by the inhibitors of RNA or protein synthesis, may account for the increased stability of IFN mRNA [7,10].

A role of the 3′untranslated region (3′UTR) has been demonstrated by showing that the IFN-β 3′UTR has an inhibitory effect on the translation of IFN-β or *Xenopus* beta globin [11]; likewise, the murine IFN-α 3′UTR also has an inhibitory effect on translation of beta globin mRNA [12]. The translation inhibitory action of the IFN-β 3′UTR is thought to be mediated by interactions of adenylate uridylate (AU)-rich elements (AREs) and the polyA tail, since the removal of either region improves translation efficiency [13]. The role of IFN-β mRNA stability has been investigated by analyzing mRNA accumulation of a reporter gene fused with various portions of the IFN-β 3′UTR [14,15]. The destabilization region was minimally mapped to a 28 nucleotide AU-rich region in the IFN-β 3′UTR; however an additional destabilization region in the 5′UTR has been also identified [15]. Deadenylation has been shown to be a pre-requisite for IFN-β mRNA degradation and mediated by the 3′UTR ARE and an additional sequence in the coding region [16]. Figure 1A shows the mRNA sequence of IFN-β and the putative destabilization regions.

The ARE of IFN-β is a Cluster-III ARE, a Class 2 ARE found in many of labile cytokine geness and other genes involved in diverse biological functions [17,18]. Clusters are based on the number of overlapping pentamers in a continuous stretch [17]. Cluster I to IV are similar to Class 2 AREs while Cluster-V is Class 1 AREs; Classes 1 and 2 are based on Chen and Shyu classification [19]. Members of IFN-α which has more than 20 subtypes, contain heterogeneous AREs as they also differ in the ARE patterns in their 3′UTR. Most of the IFN-α AREs belong to Cluster-III ARE such as IFNA5 and IFNA14, but several members also have Cluster-IV (two overlapping pentamers). Many of the new members of the type-I family, such IFN-lambda, have Cluster V (Class 2 ARE). Figure 1B shows examples of the ARE clusters in IFN-α 3′UTRs.

The 3′UTR and particularly AREs are a target for regulation by a number of RNA binding proteins. These RNA binding proteins either participate in mRNA stabilization (e.g. HuR which is a mammalian homolog of embryonic lethal abnormal vision (ELAV) protein) or in mRNA destabilization (e.g. the zinc finger binding protein, tristetraprolin (TTP), and K homology splicing-regulatory protein (KSRP)). For reviews on RNA binding proteins, see references [20-22]. There has not been any details about the identities of RNA binding proteins that bind type-I IFN mRNAs, but, a 65 kDa protein was identified to bind to a 28 nucleotide AU-rich element in the IFN-β 3′UTR and it is inducible by polyI- polyC [23].

Post-transcriptional control of IFN-β occurs as a result of different stimuli, such as viruses and cytokines. Viruses may be able to induce type-I IFN via post-transcriptional mechanisms in

addition to transcriptional induction. HSV-1 infection of fibroblast cell lines leads to stabilization of IFN-β mRNA, as demonstrated by experiments utilizing reporter constructs containing the IFN-β 3′UTR [24]. In particular, the HSV-1 protein ICP27, binds to the AU-rich region and stabilizes IFN-β mRNA [25].

Post-transcriptional control of IFN-γ production

IFN-γ, first reported on in 1965 [27], has been cloned from more than 60 species and based on sequence analysis, portions of its genomic nucleotide structure have been conserved through evolution. This genetic conservation has led a number of laboratories to investigate how expression of this gene is controlled at the transcriptional and post-transcriptional levels. One aspect of the regulation of IFN-γ gene expression that has been investigated by a few laboratories is that of changes in mRNA stability. As described earlier in this review, the presence of ARE motifs in the 3′ UTR region of cytokine contributes to mRNA instability. IFN-γ is no exception to this finding as it has a number of these elements in the 3′ UTR region (Figure 2A). In fact, the portion of the 3′ UTR region that contains these motifs has been highly conserved through evolution, as shown in Figure 2B.

Over the last few years, there have been a number of reports demonstrating that treatment of both NK cells and T cells with various stimuli results in the stabilization of the IFN-γ mRNA. In almost all cases, this increased stability was shown to be dependent upon p38 mitogen activated protein kinase (p38 MAPK) activity, as the stability was not observed when inhibitors of this pathway were included in the assay. These various stimulations include: treatment of T and NK cells with IL-2 + IL-12 [28-32]; IL-12 + IL-18 treatment of NK cells [32]; anti-CD3 + anti-CD28 or anti-CD2 treatment of T cells [33] with a similar study comparing cells obtained from old and young mice [34]; CD4+ memory T cells activated by OX40L [35]; treatment of both CD4+ and CD8+ T cells with IL-2 and bryostatin [36]; and stimulation of Jurkat T cells with anti-LFA1 and anti-CD3 [37]. In addition, our laboratory (H.A.Y.) has derived a mouse where a 100 bp region containing the ARE elements of the 3′ UTR region of the murine IFN-γ mRNA has been deleted. In preliminary experiments, significantly more IFN-γ was expressed *in vitro* and *in vivo* in response to IL-12 or IL-18 treatment (unpublished data), supporting a role for this region of the mRNA in promoting mRNA instability.

Thus far, there have not been any reports directly demonstrating that a specific protein binds to the IFN-γ 3′ UTR region and alters IFN-γ expression. However, in two of the papers mentioned above, there is evidence that the 3′ UTR region is involved in this process. Mavropoulos and co-workers [38] linked the 3′ UTR region of the IFN-γ mRNA to the beta globin gene, transiently transfected the appropriate constructs into HeLa cells and showed that mRNA half-life decreased when activation of the p38 MAPK was inhibited. In another report, Wang and colleagues demonstrated that siRNA to HuR, a protein known to interact with the 3′ UTR regions of cytokine mRNAs, resulted in loss of mRNA stabilization seen in response to treatment of Jurkat cells with anti-LFA1 and anti-CD3 [37]. This paper is the first report of a unique RNA binding protein altering IFN-γ mRNA levels but experiments demonstrating a direct binding of HuR to the IFN-γ mRNA were not shown.

Thus, a common theme from the limited number of reports on IFN-γ mRNA stabilization is that IFN-γ mRNA stability is mediated by a p38 MAPK, a kinase involved in signal transduction in response to many different extracellular signals. Whether or not this kinase targets proteins that may directly interact with the IFN-γ mRNA will likely be elucidated in future studies.

Recently, microRNAs (10-22 nucleotides) have been found to play an important role in regulating gene expression (for reviews see [39,40]. While a direct interaction of a specific miRNA with the IFN-γ mRNA has not been demonstrated, deletion of Dicer, a gene essential

for microRNA processing and maturation, in the T cell compartment resulted in a higher percentage of IFN-γ producing CD4+ T cells that made more IFN-γ in response to stimulation than cells obtained from wild-type mice [41]. Furthermore, computational analysis has revealed that IFN-γ may be a good target for known miRNAs (H.A.Y., unpublished observations). Thus an area of significant future interest will be the possible involvement of miRNAs in the post-transcriptional regulation of IFN expression.

Post-transcriptional control of IFN-γ expression is another possible mechanism by which the host may control levels of IFN-γ protein. While this topic has not been studied in great depth, Kaempfer group [42-44] has reported that the secondary structure of the very 5′UTR portion of the IFN-γ gene has been conserved through evolution, even though the primary nucleotide sequence has not been conserved. The consequence of this evolutionary conservation is that the 5′UTR 14 nucleotides forms a pseudoknot. This pseudoknot strongly activates PKR, an IFN inducible kinase that is activated by double-stranded RNA [42,44]. This active PKR then phosphorylates EIF2α, resulting in the inhibition of further IFN-γ mRNA translation. This translational control has not been observed with type-I IFN since the 5′UTR pseudoknot is not found [42]. The Kaempfer lab also demonstrated that mutations that decrease the stability of this pseudoknot increase IFN-γ protein expression. Thus IFN-γ RNA secondary structure adds an additional level of complexity to our understanding of the mechanisms by which the host regulates expression of this critical immunoregulatory protein.

In summary, mRNA stability, miRNAs and RNA secondary structure all seem to play a role in regulating host IFN-γ gene expression. It is becoming quite evident that numerous mechanisms have evolved to limit prolonged expression of this gene and that RNA structure is an important component in this process.

Role of IFN in post-transcriptional control of cellular gene expression

In addition to suppression of viral mRNA and viral protein synthesis, IFNs possess a multi-array of functions that are reflected in the large number of IFN-stimulated genes [45,46]. IFN possesses both stimulatory and inhibitory functions on the immune system which include post-transcriptional regulation of gene expression, including mRNA stability and translation. Type-I IFNs may increase major histocompatibility antigens (MHC) Class I and II expression, partially by stabilization of the MHC mRNA [47]. The major histocompatibility antigens (MHC class I and II) are increased by IFN treatment of target cells leading to macrophage activation, augmentation of antigen presentation, increased natural killer activity, and cytotoxic T cell activity. Thus, IFNs augment the immune response through MHC I and II mediated processes and this process involves post-transcriptional effects on specific gene expression.

Type-I IFNs have several suppressive effects, including anti-proliferative and down-modulatory effects which include both transcriptional and post-transcriptional regulation of gene expression. For example, IFN-α can reduce c-myc mRNA stability in Daudi cells [48, 49] but increases c-myc mRNA in murine fibroblasts [50]; IFN-β serine 17 can also inhibit c-myc mRNA stability in a colon carcinoma cell line [51]. While it is known that c-myc gene expression is decreased by IFN-γ in a Stat1-dependent manner in embryonic mouse fibroblasts [52], IFN-γ increases c-myc mRNA stability in a human breast cancer cell line [53]. Thus, IFNs have contra-indicatory roles on stability of c-myc mRNA, a transcriptional factor that regulates cell proliferation, and the response seems to be dependent on both of the type of IFN and the target cell.

IFN, particularly Type-I, may exert suppressive action on certain immune response components similar to that reported for IL-4 and IL-10,. For example, IFN-α/β can suppress the pro-inflammatory cytokines TNF-α and IL-8 by both transcriptional and post-transcriptional mechanisms [54,55]. IFN-β induces destabilization of TNF-α and IL-1β mRNA

but increase the stability of IL-1ra mRNA leading to a negative feedback control of the monokine network [55]. A recent report demonstrates a role for the RNA binding protein, TTP, in the cellular response to IFN-β or -γ action on LPS-induced macrophages; TTP downregulates several pro-inflammatory cytokines and chemokines including TNF-α, IL-6, CCCl2 (MCP-1), and CCL3 or MIP-1α [56]. This IFN effect is due to induction of the TTP promoter and it is dependent upon both Stat-1 phosphorylation induced by IFN signaling and by p38 MAPK kinase activation that is triggered by LPS [56]. TTP is a zinc finger protein that promotes the decay of a number of mRNAs that contain Class 2 AREs, such as TNF-α, GM-CSF, IL-3, and COX-2 [57,58]. The p38 MAPK pathway is important in TTP-mediated activity by the fact that its downstream target, MK2, phosphorylates TTP, resulting in the loss of mRNA destabilization activity [59,60].

The suppressive effects of type-I IFN can occur post-transcriptionally via mechanisms different from the regulation of mRNA stability. IFN-α inhibits IgM mu-chain synthesis in IFN-treated sensitive Daudi lymphoma cells as a result of decreased steady-state levels of mu-chain mRNA [49]. This effect has been attributed to a post-transcriptional mechanism, that is not thought to involve increased mRNA decay [49]. The α-chemokine, interleukin-8 (CXCL8) inhibits the antiviral action of IFN-α, presumably by a further downstream post-transcriptional mechanism that affects the OAS/RNase L pathway, though the exact mechanism is not known [61,62].

The role of IFN-regulated RNAse L and PKR in the post-transcriptional control of gene expression

IFN induces gene expression of OAS which, upon binding to viral double-stranded RNA intermediates, becomes activated and synthesizes short 2′-5′oligoadenylates (2-5A). The latter, in turn, activates RNAse L, an enzyme that potently degrades viral mRNAs. This pathway is reviewed by Silverman and Bisbal in this same issue. In recent years, it has been accepted that RNase L participates in the degradation of selected cellular mRNAs [63-67]. Specifically, RNase L has been shown to downregulate PKR mRNA [64]. During the IFN antiviral response in normal cells, PKR mRNA expression is transient, but in RNase L-null cells, extended kinetics of PKR mRNA expression is observed, due to increased mRNA stability [64]. The effect results in prolongation of the PKR-dependent phosphorylation of the subunit of eukaryotic translation initiation factor 2, eIF2α; a process that leads to inhibition of viral protein synthesis [64]. Thus, RNAse L contributes to the transient nature of the IFN response in order to ensure a brief translational arrest imparted by PKR. A similar role of RNAse L negative regulation of the IFN response has also been suggested by the report that a novel IFN-stimulated gene encoding a 43-kDa ubiquitin-specific protease, designated ISG43, is downregulated by RNase L [67]. This regulation occurs at the mRNA stability level since the ISG43 mRNA half life increases in RNase L-null cells [67].

RNAse L can downregulate another functionally important cellular mRNA, myoD, an important transcription factor essential for muscle differentiation. RNase L and its inhibitor RLI are sequentially induced during C2 cell line myoblast differentiation to myotubes [63]. Inhibition or over-expression of RNAse L prolongs or decreases MyoD mRNA half life, respectively[63]. Activated by the localization of a fraction of RNase L in mitochondria that increases with IFN-α treatment, a role of RNAse L in down-regulating miotchondria mRNAs such as those of CYTB), ATPase 6 (ATP6), and cytochrome oxidase II (CO) has been established and proposed as a mechanism of the anti-proliferative action of IFN [66]. This was demonstrated by reducing RNAse L activity through the introduction of an antisense construct or by directly activating RNase L activity by 2-5A [66]. The effects of RNase L on cellular mRNAs appear to be highly restricted to specific mRNAs since no global effects on cellular mRNAs are observed in the studies that dealt with this topic. None of the previous studies

however, demonstrate a direct binding of RNase L or association with the target mRNA, and the mechanism of RNAse L activity remains to be explored.

RNase L has a post-transcriptional regulatory role that is different from mRNA turnover; it can modulate translation termination by interacting with human translation termination factor eRF3/GSPT1 [68]. It is proposed that this interaction brings RNase L into close contact with its target mRNA. Moreover, activated RNase L, may compete with eRF3/GSPT1-PABP, a complex that is essential for termination of mRNA translation and release for subsequent rounds of translation [68].

PKR, an important effecter arm of the antiviral action against many viruses including EMCV, VSV, and HSV-1 [69-71], is a serine/threonine kinase and its mechanism of action is mediated through inhibition of translation initiation due to phosphorylation of EIF 2α. A detailed description of the PKR pathway is given in this issue by Meurs and Esteban. It is expected that PKR can mediate several pathways as a result of translational control. Specific expression of selected proteins (Fas, p53, Bax and others) that trigger apoptosis by engagement of the caspase pathway, can be mediated by PKR activation [72]. PKR also has novel protein substrates and cellular functions in calcium signaling, nuclear factor-k B (NF-kB) signaling, IgE class switching, and cellular growth [5,73]. Though translation arrest is transient and global, particularly for housekeeping proteins, translation of stress-induced transcripts encoding heat shock proteins and selected transcription factors (e.g, GCN4 and ATF4) are not inhibited and may actually increase as a result of stress [74]. PKR-mediated stabilization of mRNA, which is dependent on the presence of 3′UTR AU-rich sequences, has been demonstrated during heat shock, with heat shock 70 (HSP70) mRNA [75].

Other types of post-transcriptional control in the IFN system

An interesting observation regarding PKR and an example of alternative splicing in the IFN system, is the finding of a spliced variant with deletion of exon 7 of the PKR gene [76] - in transformed Jurkat T cells, but not normal T cells. This PKR variant has a dominant negative function that inhibits PKR autophosphorylation and eIF2α subunit phosphorylation [77]. Whether this is a general phenomenon in transformed cells is not known. Another example of alternative splicing operating in the IFN system is IFN regulatory factor (IRF) 3 (IRF-3) which, along with IRF-7, is indispensable for IFN-β promoter activation. There is an alternatively spliced form, called IRF-3a, that lacks the DNA binding domain and is unable to bind its target sites in the IFN promoter. Thus this variant acts as a negative modulator that inhibits virus-induced activation of the IFN-β promoter [78]. Alternative splicing can also be induced by IFN-β resulting in the production of soluble isoforms of CD28 and CTLA-4 and these altered forms of the proteins can in turn affect T cell activation [79].

RNA editing by the IFN-inducible form of adenosine deaminase, ADAR1, may also be considered a post-transcriptional mechanism of gene regulation. This enzyme recognizes RNA with a double-stranded structure and causes Adenosine (A) deamination to Inosine (I). Inosine is recognized as guanosine by the cell machinery [80,81]. Reviews citing work from the Samuel laboratory focused on this topic is found elsewhere [80-82].

The role of post-translational effects of IFN-α has been demonstrated by examples such as the reduction of eIF5A activity in a tumor cell line [72]. The eIF5A activity is modulated by a series of post-translational modifications that culminates in the formation of the unusual amino acid hypusine. that the synthesis of this amino acid has been correlated with cell proliferation and apoptosis [83,84]. Another post-translational effect of IFN is ubiquitin conjugation, a process that targets proteins for degradation in the 26 S proteasome. ISG15 is an IFN-inducible ubiquitin-like protein that has been experimentally linked to the proteasome [85]. A possible ramification of this pathway has been recently demonstrated in which virus- and IFN-induced

ISG15 conjugation leads to inhibition of IRF-3 degradation and therefore increased IRF-3 activity and an enhanced antiviral innate response [86].

Conclusions

IFNs are key gene products that play diverse roles in host defense and regulation of the immune system. Regulation of the IFN system, whether during IFN biosynthesis or during the cellular response to IFN, occurs at many different levels. This complex regulation requires immediate signaling events, rapid transcriptional activation, and post-transcriptional control. The post-transcriptional mechanism itself occurs at multiple levels, including mRNA stability, alternative splicing, translation, and post-translational effects. IFNs themselves stimulate a large number of IFN-stimulated genes, notably RNAse L, PKR, and ADAR1 and these genes participate in the post-transcriptional regulation of the host immune response. Stringent control of the IFN system allows swift elimination of microbes and beneficial immunomodulatory functions prior to the onset of deleterious consequence for the host (Figure 3). Understanding these pathways can lead to new approaches to harnessing the IFN system for further therapeutic applications.

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A

IFN-β

5′acauucuaacugcaaccuuucgaagccuuugcucuggcacaacaggua gua ggc gacacuguucguguugucaacaugaccaacaagugu
cuccuccaaauugcucuccuguugugcuuccaccuacagcucuuuccauga gc uacaacuugc uuggauuccuacaaagaagcagcaauu
uuca guguca gaagcucc uguggcaa uuga auuggga ggc uuga auuacugcccucaaggaca ggaugaacuuuugacauccccuga gga gauuaa
gcagcugcagcagua guucca gaa ggaggac gcc gca uugaccauc ua ugaga uagc uccagaacac ac uuuugc uauuuuuuca gacaagauucaucu
agcac ugg cuggaauga gac ua uuguuga gaaccucc uggc uaauguuua uca uca gauaaccauc ugaagacagucc uggaa gaaaaacu
gga gaaa gaaga uuucaccagg ggaaaaacucauga gcaguc ugc acc ugaaaaga uauuaugggga gga uucugcauuacc ugaa ggccaag
gagucagucacugugccuggaccauagucagaguggaaa*uccuaaggaacuuuuacuucauuaacagacuuuac*agguuaccuc**cgaacu**
gaagaucuccuagccugugccucugggacuggacaauugcuucaagcauucuucaaccagcagaugcuguuuaaggugacugaugc
uaauguacugcauaugaaaggacacuagaagauuuugaaauuuuuauuaaauuaugaguuauuuuuauuuaauuuaaauuuua
uuuuggaaaauaaauuauuuuuggugcaaaaguca 3′

B

IFNA1:
CUG_AUUU_AUCUA_UUUA_AAU_AUUUAUUUA_ACUAUUCAUAAG_AUUUA_AAUUAUUUUUGUU

IFNA2:
CU_AUUUA_AAU_AUUUUU_AAAAU_A**UUAUUUAUUUA**ACUA_UUUA_UAAAACAAC

IFNA4:
CAUCU_AUUUAUUUA_AAUA_UUUAUUUA_UUUUUAAA_AUUUA_AAUUA_UUUUUU_AUGUGAUA

IFNA5:
AUCU_AUUUA_AAU_AUUUAUUUAUUUA_UAAG_AUUUA_AAUUA_UUUUA_AACUUA

IFN14:
UUUCAUCU_AUUUAUUUA_AAU**AUUUAUUUAUUUA**ACUA_UUUUU_AUUA_UUUA_AAUUA_UUUUU_
UAUG

IFN8:
UCU_AUUUAUUUA_AAUCUUU_AUUUA_GUUAACUAUCUAUAG GGCUUAAAUUAGUUUUGUU

IFNA10:
UCUUUUGUUU AAA**UAUUUAUUUA**AUUAUUUUUAAAAUUUAUGUAAUAUCA

IFN16:
UCU **AUUUAUUUA** AA**UAUUUAUUUAUUUA**ACUA_UUUUUA_AGGUUUAAAUCA

IFN17:
UCAUCU_AUUUAUUUA_AAA**UAUUUAUUUAAUUAUUU**UUAAG_AUUUA_AAUUA_UUUUUUUAU_

IFN21:
UCUAUUGAAA**UAUUUAUUUAUUUA**UUAG_AUUUA_AAUUAUUUUUGUCCAUG

IFNW1 (IFN omega):
AUGUU**AUUUA**UUUUUACUC**AUUUAUUUA**UUCUUAC_AUUUUA_UCAUAUUUAUACU**AUUUA**U
AUUCU

IL28A inUerleukin 28A (IFN, lambda 2):
UGCAACCUGA GA_UUUUAUUUA_UAAAUUAGC CACUUGUCUU A_AUUUA_UUGC

IL28B (IFN lambda 3):
UGCAACCUGA GAUUUU_AUUUA_UAAAUUAGCCACUUGUCUUA_AUUUA_UUGC

IL29 inUerleukin 29 (IFN, lambda 1)
ACCUU_AUUUA_UGCGCUGAGCCCUACUCCUUCCUUA**AUUUA**UUUCCUCUCACC
CUUU_AUUUA_UG

IFNE1(IFN epsilon 1):
GUAAUACA_AUUUA_UAGUACAAUCACAUUGCUUUGAUUUUGUGUAUAUAUAT_ATTT_ATCTG

Figure 1. ARE variations in Type-I IFN 3′UTRs
A The mRNA sequence of the human IFN-β is shown. Sequence in bold refers to 3′UTR.
Underlined are putative ARE patterns such as pentamers and hexamers. Sequence in italics is
destabilization region in the coding region as defined in Ref. [16]. **B** Sequences of putative
ARE regions in the 3′UTRs of type-I IFN including several sub-types of IFN-α. Underlined
are AU-rich patterns such as pentamers or hexamers while sequences in Bold are ARE that
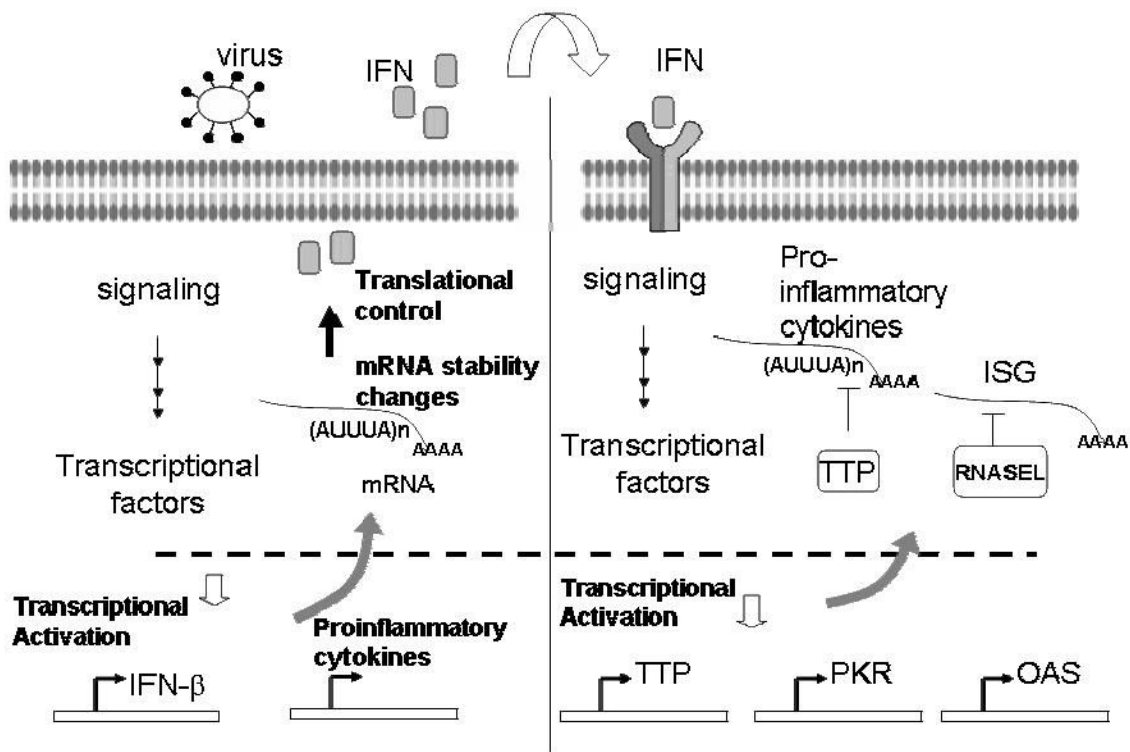matches possibly functional 13 nucleotide consensus [18,46,87].

A

IFN-γ

5′cacauuguuucugaucaucugaagaucagcuauuagaagagaaagaucaguuaaguccuuuggaccugauc
agcuugauacaagaacuacugauuucaacuucuuuggcuuaauucucucggaaacgaugaaauauacaagu
uauaucuuggcuuuucagcucugcaucguuuuggguucuuuggcuguuacugccaggacccauauguaaa
agaagcagaaaaaccuuaagaaauauuuuaaugcaggucauucagaugauguagcggauaauggaacucuuuucu
uaggcauuuugaagaauuggaaagaggagagugacagaaaaauaaugcagagccaaauugucuccuuuuac
uucaaacuuuuuaaaaacuuuaaagaugaccagagcauccaaaagagugugggagaccaucaaggaagacau
gaaugucaaguuuuucaauagcaacaaaaagaaacgagaugacuucgaaaagcugacuaauuauucgguaa
cugacuugaauguccaacgcaaagcaauacaugaacucauccaagugaugggcugaacugucgccagcagcu
aaaacagggaagcgaaaaaggagucagaugcuguuuucgaggucgaagagcaucccaguaa**ugguugucc
ugccugcaauauuuuga**<u>auuuuaaa</u>ucuaaaucu<u>auuuau</u>uaauauuuuaacauuauuuau
auggggaauuau<u>auuuuu</u>agacucaucaaucaaauaagu<u>auuua</u>uaauagcaacuuuugu
guaaugaaaaugaauaucuauuaauauauguauuauuuuauaauuccuauauccugugac
ugucucacuuaauccuuuguuuucugacuaauuaggcaaggcuaugugauuacaaggcu
uuaucucaggggccaacuaggcagccaaccuaagcaagaucccaugggguugugugguuua
uuucacuugaugauacaaugaacacuuuauaagugaaggugauacuauccaguuacugccg
guuugaaaauaugccugcaaucugagccagugcuuuaauggcaugucagacagaacuug
aaugugucaggugacccugaugaaaacauagaucucaggagagauuucaugccuggugcuu
ccaaauauuuguugacaacugugacuguacccaaauggaaaguaacucauuuguuaaaau
uaucaauaucuaauauauaugaauaaaguguaaguucacaac3′**

B

```
Human       ucCUGCCuGCAaUAUUUGAA.UU.UU.AAAUC.U.AAAuCUAUUUAUUAAUAUUUaAcA.uUA.UUUAuA~~~~
Marmoset    ucCUGCCuGCAaUAUUUGAA.UU.UU.AAAUC.U.AAAuCUAUUUAUUAAUAUUUaAcA.uUA.UUUAuA~~~~
Rabbit      ~~~~aCCuGCAaUAUUUGAA.UU.UU..uAUC.U.AAAuCUAUUUAUUAAUAUUUaAcA.uUA.UUUAuA~~~~
Cow         ucCUGCCuGCAaUAUUUGAA.UU.UUuAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcA.u~~
Dog         ucCUGCCuGCAaUAUUUGAA.UU.UUuAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcAuu~~
Elk         ucCUGCCuGCAaUAUUUGAA.UU.UUuAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcAuu~~
Goat        ucCUGCCuGCAaUAUUUGAA.UU.UUaAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcAuu~~
Sheep       ucCUGCCuGCAaUAUUUGAA.UU.UUaAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcAuu~~
Horse       ucCUGCCuaGCAaUAUUUGAA.UU.UUuAAAUC.U.AAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcA.u~~
Pig         ucCUGCCuGCAaUAUUUGAA.UU.UUuAAAUC.cAAuCUAUUUAUUAAUAUUU.A.A..UAuUUUAcA.u~~
Groundhog   ucCUGCCaGCAcUAUUUGAA.UU.UU.AAAU..UgAAAuC....UAUU..UA.UU.A.A..UA.UUUA.A~~~~
Hamster     ~~~UGCCaGCAaUAUUUG.A.aU.UU.AAAaCuU.AAAUCUAUUUAUUAAUAUUUaAcA.uUA.UUUAuA.ugg
Cotton Rat  ~~~~~~~~~~~~~AUUUG.A.UU.UU.AAAUC.U.AAACUAUUUAUUAAUAUUUaAcA.uUA.UUUAu..u~~
House mouse ~~CUGCCaGCAcUAUUUGAAuUU.UU.AAAUC.U.AAAcCUAUUUAUUAAUAUUUaA.AacUA.UUUAuA.u.g
Rat         ~~CUGCCaGCAcUAUUUGAA.UU.UU.AAAaC.U.AAAcCUAUUUAUUAAUAUUUaA.AuuUA.UUUAuA.u.g
Gerbil      ucCUGCCaGCAcUAUUUGAA..UcUU.gAUC.U.AAAcCUAUUUAUUAAUAUUUaA.AauUA.UUUAuA~~~~
Consensus   --CUGCC-GCA-UAUUUGAA-UU-UU-AAAUC-U-AAA-CUAUUUAUUAAUAUUU-A-A--UA-UUUA-A----
```

Figure 2.
A The human IFN-γ mRNA sequence. Underlined are putative ARE regions with the 3′ UTR region shown in bold. **B** Alignment of IFN-γ 3′ UTR regions containing AU-rich motifs.

Figure 3. Post-transcriptional control of gene expression during IFN response
Viruses induce the transcription of type-I IFN in several cell types. The biosynthesis of IFN-β is well-regulated and involves post-transcriptional mechanisms of regulation such as mRNA stability and translation control. The IFN-β mRNA contains $ARE(AUUUA)_n$ in the Figure, which is subject to modulation by RNA binding proteins. IFN produced as a result of virus infection binds to IFN receptors in nearby healthy cells to protect them from virus infection. IFN initiates a cascade of events that leads to transcription of many genes (IFN-stimulated genes, ISG) and also pro-inflammatory cytokines. Some of these gene products act as negative feedback inhibitors that attenuate IFN and cytokine response. The RNA binding protein, tristetraprolin (TTP), may destabilize mRNAs that belong to the pro-inflammatory cytokines, particularly in the presence of inflammatory stimulus. RNAse L which requires the activation of the IFN-inducible oligoadenylate synthetase (OAS) act as a negative feedback inhibitor resulting in downmodulation of a selected class of ISG mRNAs including the PKR and ISG43. Additional details are found in Text. Also, details of the individual pathways are found elsewhere in this Review Issue.