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The Interferon-Stimulated Genes: Targets of Direct Signaling by Interferons, Double-Stranded RNA, and Viruses

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Abstract The interferon system plays a profound role in determining the outcome of viral infection in mammals. Viruses induce the synthesis of interferon, which, in turn, blocks virus replication by inducing the expression of antiviral proteins encoded by interferon-stimulated genes. It is not widely appreciated that without the participation of interferon, many of the same genes can also be induced by a variety of virus-related agents, such as double-stranded RNA and viral proteins. In this chapter, we discuss different signaling pathways, activated by these agents, that lead to the induction of partially overlapping sets of genes, including the interferon-stimulated genes. We also review the biochemical and cellular properties of the protein products of a selected number of these genes including ISG56, ISG54, and ISG15.

1 Introduction

Interferons (IFN) are defined by their ability to block virus replication. In addition to this property, these potent cytokines have many other effects on cellular physiology, especially in cells of the immune system (Biron and Sen 2001; Samuel 2001). Most, if not all, of these effects are mediated by the products of cellular genes, whose expression is highly induced by interferon treatment of cells. These IFN-stimulated genes (ISGs) number in the hundreds, but the biochemical and cellular functions of only a handful of them have been delineated as yet (Sarkar and Sen 2004). However, much is known about how IFNs induce transcription of these genes, the identities of the proteins that mediate and regulate the signaling pathways and how dysregulation of these pathways in cancer cells or in virus-infected cells contribute to pathogenesis. In this chapter, we review the observations demonstrating that many ISGs can be induced by a number of alternate signaling pathways activated by not only IFNs, but also viral proteins, RNAs and DNAs, bacterial lipopolysaccharides, and most notably double-stranded RNA. In addition, the biochemical and cellular functions of the proteins encoded by a few most highly induced ISGs are discussed.

2 Historical Perspective

Viruses and the interferon system, especially type I IFNs, are connected at many levels. It was clear from the time of its discovery that synthesis and secretion of IFN can be induced by virus infection of cells (Nagano and Kojima 1954; Isaacs and Lindenmann 1957; Watanabe 2004). Since then, much information has been gathered regarding the biochemical pathways, activated by viruses, which lead to IFN gene induction (Garcia-Sastre and Biron 2006). A critical discovery was the finding that synthetic double-stranded RNA could mimic virus infection and induce IFNs. Viral dsRNA is often produced in infected cells as byproducts of viral genome replication (Jacobs and Langland 1996); hence, for a long time it was thought that synthetic dsRNA serves as a surrogate of viral dsRNA. As will be elaborated below, although partially overlapping, the pathways activated by viruses and dsRNA are not identical, nor do they lead to the induction of an identical set of genes. IFNs, of course, inhibit virus replication, creating the powerful loop of antiviral innate immunity: induction of IFNs by virus infection and inhibition of virus replication by IFNs. Since without going through the above IFN loop, dsRNA and virus infection can directly

induce many of the same antiviral genes that IFN can induce, virus replication in the primary infected cell can be directly attenuated by the proteins encoded by these genes (Elco et al. 2005; Sen and Sarkar 2005b). Finally, many viruses, if not all, encode RNA or proteins that can interfere with various components of the IFN system: IFN synthesis, IFN signaling and functions of IFN-induced proteins. Often, the same virus can block more than one such processes, thus ensuring efficacy (Haller et al. 2006). The plethora of interplays outlined above gives rise to the host–virus equilibrium observed in a virus-infected cell. In vivo, additional factors, such as other virus-induced cytokines and activated cells of the immune system, contribute to homeostasis as well.

After the discovery of the ability of dsRNA to induce IFN synthesis, the second major discovery connecting the two was the finding that dsRNA could activate two enzymes, PKR and 2-5 OAS, which are induced by IFNs (Lengyel 1987). Thus, for the action of these enzymes in virus-infected cells, concerted actions of both IFN and dsRNA are needed, one inducing the synthesis of the proteins and the other functioning as their co-factor for imparting enzyme activity (reviewed in Sarkar and Sen 2004). Cloning of many IFN-stimulated genes (ISGs) and subsequent identification of many more ISGs using microarray analyses provided important tools for studying their modes of induction (Der et al. 1998). Such studies surprisingly revealed that dsRNA or virus infection could induce many ISGs directly, without the participation of IFNs (Geiss et al. 2001). In some cell lines, dsRNA and IFNs can synergize their induction as well. The definitive proof of IFN-independent induction of ISGs came from experiments that used cell lines genetically incapable of synthesizing IFNs or responding to IFNs. For example, in human gliomas, the type I IFN locus is often deleted during the development of the disease. Thus, cell lines derived from these tumors cannot produce IFNs, but many ISGs can be induced in them, upon dsRNA treatment or virus infection (Geiss et al. 2001). These genes, although originally classified as ISGs, can be legitimately called dsRNA-stimulated genes (DSGs) or viral-stress-inducible genes (VSIGs) as well.

The signaling pathways used by various agents to induce these genes are distinct, with some overlaps. The single common feature is the cis-element in the promoters of these genes that receives the signaling. It was originally identified as the IFN-stimulated response element (ISRE), to which the IFN-activated trimeric transcription factor, ISGF3, binds and induces transcription. ISGF3 is composed of three proteins, STAT1, STAT2, and IRF9, and it is the latter protein that specifically recognizes ISRE (Stark et al. 1998). IRF9 is a member of the large IFN regulatory factor (IRF) family of proteins, all of which can bind to ISRE or ISRE-like elements. Soon it was discovered that dsRNA or virus infection could activate other IRF members, such as IRF-3, IRF-7, and IRF-5 (Barnes et al. 2002). Upon activation, these proteins translocate

from the cytoplasm to the nucleus, bind to the ISRE sites in the promoters of the target genes, and induce their transcription. Thus, the ISRE–IRF axis is the common link of gene induction by IFNs, viruses, and dsRNA. The latter two agents can efficiently activate other transcription factors, such as NFκB and AP-1, as well (Sen and Sarkar 2005b). Consequently, genes regulated by those transcription factors are induced by dsRNA or virus infection, but not by IFNs. Certain genes, such as the human IFN-β gene, have complex promoters, and they require for induction the co-ordinate actions of IRF-3–IRF-7, NFκB, and AP-1 (Maniatis et al. 1998). Consequently, viruses and dsRNA can induce their transcription, but IFNs cannot. However, induced transcription of genes with simple promoters, such ISG56 and ISG15, is driven by ISRE only, and as a result, all agents that can activate transcription factors containing IRF proteins can efficiently induce these genes. Because of the ease of analysis, we have used the latter genes extensively for delineating the signaling pathways activated by IFNs, dsRNA, and viruses (Peters et al. 2002; Sarkar et al. 2004; Elco et al. 2005). These studies have revealed additional cross-talks among the different inducers of ISGs. Many genes, encoding proteins that are components of the signaling pathways, are ISGs themselves. For example, synthesis of TLR3, a receptor for dsRNA, is strongly induced by IFN (Heinz et al. 2003). Similarly, STAT1, an essential component of the IFN-signaling pathways, is an ISG. Conversely, some ISGs encode proteins, such as SOCS, that block the signaling pathways and limit the duration of the gene induction process (Alexander and Hilton 2004). Thus, ISGs not only affect the antiviral state and other properties of the cell but also regulate, both positively and negatively, their own expression.

3
IFN-Independent Induction of ISGs

Signaling pathways triggered by many viral components, or their mimics, lead to the induction of ISGs. These pathways initiate at different points but converge at various nodes and all cause activation of IRF-3 or IRF-7, the transcription factors that are the common denominators of these pathways (Fig. 1). The Toll-like receptors have emerged as major sensors of viral components (Akira et al. 2006; Stetson and Medzhitov 2006). Viral nucleic acids are recognized by TLRs present in the endosomal membranes (Kawai and Akira 2006; Meylan and Tschopp 2006). TLR3 initiates signaling by binding dsRNA (Alexopoulou et al. 2001), whereas TLR7 and TLR8 recognize viral single-stranded RNA (Diebold et al. 2004); viral DNA is recognized by TLR9 (Tabeta et al. 2004). TLR2 and TLR4, present on the cell surface, are recognized by some viral glycoproteins (Boehme and Compton 2004). In addition to TLR3, two cytoplasmic

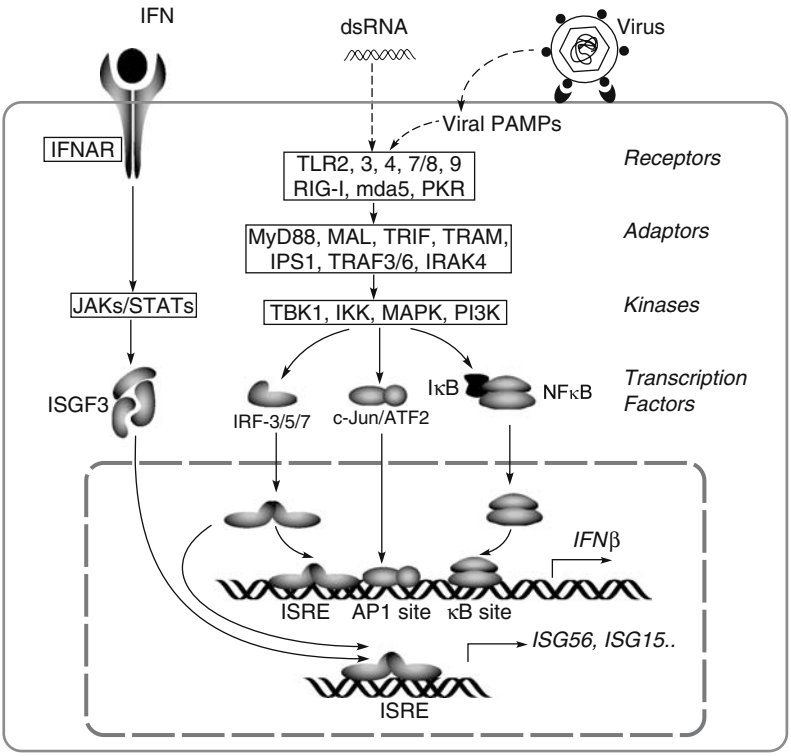

Fig. 1 Signal integration for viral-stress-inducible gene (VSIG) expression. Schematic diagram shows major signaling pathways stimulated by virus, IFN, and dsRNA that induce transcription of a common set of genes. Virus, dsRNA, and viral pathogen-associated molecular patters (PAMP) are sensed by Toll-like receptors or RNA helicases. The signaling pathway finally leads to the induction of interferons as well as several IFN inducible genes (ISGs). IFNs are then secreted and signals through IFN receptor (IFNAR) and JAK/STAT pathway to induce ISGs to generate antiviral state

RNA helicases, RIG-I and Mda-5, can recognize dsRNA and signal (Meylan and Tschopp 2006). It is anticipated that additional cytoplasmic receptors may recognize other viral components and start similar signaling cascades. IRF-3 activation by these pathways requires the action of the protein kinase TBK1, which is recruited to different receptors by different adaptor proteins (Fitzgerald et al. 2003). For TLR3, the critical adaptor protein is TRIF (Yamamoto et al. 2002; Oshiumi et al. 2003), whereas RIG-I and Mda-5 use the mitochondrial

protein IPS-1 (Sen and Sarkar 2005a). TLR7, TLR8, and TLR9 all require the major adaptor of TLR pathways, MyD88. TLR4 uses both MyD88 and TRAM, the latter recruiting TRIF to bring in TBK1 to the signaling complex (Kawai and Akira 2006).

4
Induction of ISGs by TLR3 Signaling

TLR3 is present mostly on the endosomal membrane, although in some cell types its presence on the plasma membrane has been noted (Matsumoto et al. 2003). Its ectodomain specifically recognizes endosomal dsRNA through ionic interactions between the negatively charged ligand and positively charged amino acid residues present on both sides of a canyon in which the dsRNA perfectly fits (Bell et al. 2005; Choe et al. 2005; Bell et al. 2006). Extracellular dsRNA has to be endocytosed to reach TLR3, as revealed by the chloroquine sensitivity of the process (de Bouteiller et al. 2005). Because many viruses enter the cell through endocytosis, their genomic RNAs may encounter TLR3 in the endosome. The first step in TLR3 signaling is its dimerization, which presumably leads to a conformational change of its cytoplasmic domain to initiate the signaling process (Fig. 2).

The most novel feature of TLR3 signaling is the need for receptor Tyr-phosphorylation (Sarkar et al. 2003, 2004). Although phosphorylation of specific Tyr residues located in the cytoplasmic domains of receptors for growth factors and cytokines is quite common, this feature is unique for TLR3 among the Toll-like receptors. There are five Tyr residues in the cytoplasmic domain of human TLR3 and several of these residues, if not all, are phosphorylated at the beginning of the signaling process. The functional roles of these residues have been assessed by mutating them, individually or in combinations. At least two of the five are essential for signaling; one of them has to be Tyr759, the other one can be Tyr858 or Tyr733. Tyr-phosphorylation of TLR3 is a ligand-dependent process, but the responsible protein kinase has not yet been identified. Tyr759 or 858, after phosphorylation, can recruit the signaling complex. The main adaptor protein is TRIF, but TRAF3 is needed as well (Hacker et al. 2006; Oganesyan et al. 2006). The different branches of signaling bifurcate from TRIF (Jiang et al. 2004). A complex containing TRAF6, TAB1, TAB2, and TAK1 activates the protein kinases JNK, P38, and IKK (Jiang et al. 2003). Another adaptor, RIP-1, is also recruited by TRIF and it is required for NFκB activation (Meylan et al. 2004). JNK, P38, and IKK activate the transcription factors c-Jun, ATF2, and NFκB, respectively. A separate branch of signaling originating from TRIF is triggered by the recruitment of the protein kinases TBK1 or IKKε,

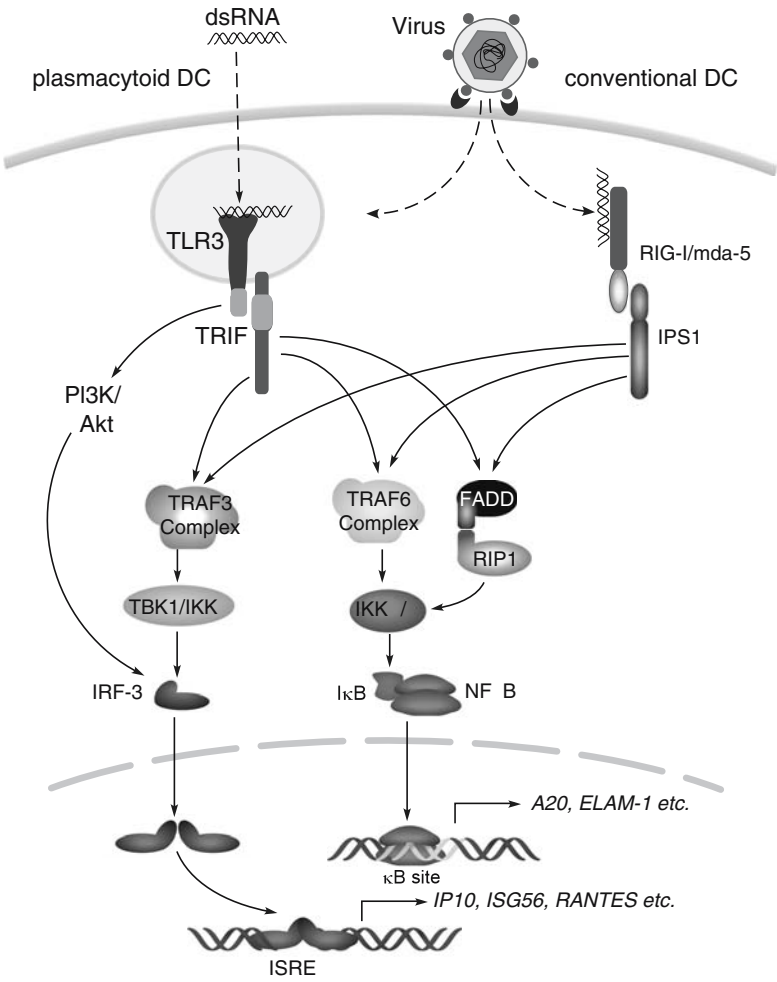

Fig. 2 Signaling pathways activated by viruses and dsRNA. Depending on cell type, viral dsRNA can signal either through Toll-like receptor 3 or RNA helicases: RIG-I/mda-5. Through different sets of adaptors, the signal causes activation of two major transcription factors, IRF-3 and NF-κB, followed by induction of specific sets of genes

which directly phosphorylate IRF-3 (Fitzgerald et al. 2003; Sharma et al. 2003). IRF-3 phosphorylation leads to its dimerization and translocation to the nucleus where it binds to the ISRE sites in the promoters of the target genes

and induces their transcription. The histone deacetylase, HDAC6, is required for IRF-3 to function as a transcription factor (Nusinzon and Horvath 2006).

As mentioned above, Tyr759 of TLR3 is absolutely needed for complete signaling by this receptor. When this residue is mutated to Phe, NFκB- and IRF-3-driven genes are not induced by dsRNA. Surprisingly, in dsRNA-treated cells expressing the mutant receptor, NFκB is released from IκB and translocated to the nucleus but it does not drive gene transcription (our unpublished observation); similarly, IRF-3 is dimerized and translocated to the nucleus but it is transcriptionally inactive (Sarkar et al. 2004). Investigation of the underlying molecular mechanisms has revealed that the activation of both transcription factors, IRF-3 and NFκB, is a two-step process and the second step is defective in cells expressing the Y759F mutant of TLR3. Phosphorylated Tyr759 recruits PI3 kinase, probably indirectly, to the TLR3 complex, PI3 kinase is activated, it phosphorylates Akt, which leads to additional phosphorylation of the TBK1-activated IRF-3. The tyrosine kinase, Src, which is known to be activated by Akt, may be a participant in this pathway, because Src is activated by TLR3 signaling and its presence is needed for gene induction by TLR3 (our unpublished observation). As expected from the above description, inhibitors of PI3 kinase, Akt and Src, both block IRF-3-mediated gene induction by TLR3. They have the same effect as the Tyr759 mutation of TLR3, namely incomplete phosphorylation of IRF-3, as revealed by two-dimensional gel analysis of nuclear IRF-3 isolated from dsRNA-treated cells expressing Y759F TLR3. Chromatin immunoprecipitation assays demonstrate that unlike fully phosphorylated IRF-3, underphosphorylated IRF-3 cannot bind tightly to the promoter and interact with co-activators, such as CBP (Sarkar et al. 2004).

In the NFκB pathways, the first step of activation is mediated by the phosphorylation of IκB by the IKK complex and the consequent release of NFκB and its translocation to the nucleus. TLR3 Tyr759 is not required for the above process or for the phosphorylation of NFκB P65 protein in Ser276 and Ser536 resides. However, it is required for additional phosphorylation of P65 as revealed by two-dimensional gel analysis of nuclear P65. Underphosphorylated P65 cannot bind to the promoters of the target genes tightly and drive their transcription. Surprisingly, the PI3 kinase pathway is not required for the second step of P65 phosphorylation (our unpublished observation). The above studies highlight the two-step nature of the activation of both IRF-3 and NFκB, although the details are different. The first step is initiated by the phosphorylation of Tyr858 of TLR3, leading to the release of NFκB from IκB and the dimerization of IRF-3 as a result of its partial phosphorylation. The second step is initiated by the phosphotyrosine 759 of TLR3. It leads to further phosphorylation of IRF3 and its full activation and complete phosphorylation of NFκB P65 and its full activation.

5
Partially Overlapping Repertoire of Genes Induced by Viruses, IFN, and dsRNA

In order to identify the primary target genes induced by viruses, type I IFNs and dsRNA, microarray analyses have been performed by many investigators (reviewed in Sarkar and Sen 2004). However, it is important to realize that because the different inducing agents can induce one another, the results of the above analyses, unless carefully designed, are often difficult to interpret. For example, virus replication can produce both dsRNA and IFNs, and dsRNA treatment can produce IFN, thus complicating the pictures. Mutant cell lines have helped to circumvent this problem considerably. Human cell lines that cannot synthesize IFNs, because of the deletion of the type I IFN locus in their genomes, have been particularly useful to identify dsRNA-regulated genes. Such an analysis using cDNA microarray identified 175 dsRNA-stimulated genes in a human glioma cell line. On the other hand, expression of 95 other genes was repressed by dsRNA treatment of these cells (Geiss et al. 2001). A subset of dsRNA-induced genes was also induced by inflammatory cytokine, IFNs, and viruses, indicating that these genes have broad functions (Elco et al. 2005). Induction of some of these genes required ongoing protein synthesis, while others did not, and the dsRNA-induced genes functionally covered all aspects of cellular metabolism. Mutant cell lines that are incapable of responding to IFNs or dsRNA have also been effectively used to untangle the overlapping signaling pathways. When cells from mice carrying targeted gene disruptions are used for this purpose, the results are unequivocal. In contrast, the results from mutagenized human cell lines, selected for IFN-unresponsiveness or dsRNA-unresponsiveness, need cautious interpretations. For example, the HT1080-derived U series of cells have been widely used for examining the roles of specific components of the IFN-signaling pathways in the cellular responses to other inducers. Using these lines, we initially concluded that none of the components of the type I IFN signaling pathways, other than STAT1, is needed for gene induction by dsRNA (Bandyopadhyay et al. 1995). The need of STAT1 was based upon the observed failure of U3A cells to respond to dsRNA. But recent in-depth analysis revealed that the original conclusion was erroneous; STAT1 is not needed for dsRNA signaling; U3A cells cannot respond to dsRNA because, unlike the parental line, they do not express TLR3. STAT1 restoration in U3A cells did not restore basal TLR3 expression and dsRNA-responsiveness, indicating that a different mutation is responsible for this phenotype. Ectopic expression of TLR3 or induction of the resident TLR3 gene by IFN treatment imparted dsRNA responsiveness to U3A cells (C.P. Elco and G.C. Sen, unpublished observation). The same was true for HeLaM cells, providing an explanation

for our old observation that IFN pretreatment makes these cells responsive to dsRNA (Tiwari et al. 1987). The above example should remind investigators that the mutagenized cell lines carry many mutations in addition to the ones for which they have been selected and a given phenotype and the known mutation cannot be causally connected, without rigorous testing.

A series of mutant cell lines was used to investigate the gene induction pathways activated by Sendai virus infection. Microarray analysis revealed that the same genes were induced by SeV in the presence or the absence of TLR3 (Elco et al. 2005). In TLR3-expressing cells, dsRNA and SeV did not induce completely overlapping sets of genes, although many genes were induced by both indicating that the two inducers have distinct properties. Induction of some of the virally induced genes required IFN signaling, suggesting that they are probably induced secondarily by IFN produced upon virus infection. Induction of some genes required NFκB, others required IRF-3, whereas a third group required both transcription factors. An unexpected observation was that increasing levels of IRF-3 inhibited the induction of some, but not all, NFκB-dependent genes (Elco et al. 2005). The molecular basis of this cross-talk between the two signaling pathways remains unknown.

6
The ISG56 Family

Members of the ISG56 (IFIT1) gene family are very strongly induced in response to IFN, dsRNA, or virus infection. These genes show up at or near the top of all microarray databases inquiring into the nature of cellular genes induced upon infection with a variety of viruses (Sarkar and Sen 2004). Our early investigation demonstrated that type I IFN, dsRNA, or infection with encephalomyocarditis virus or vesicular stomatitis virus could independently induce ISG56 mRNA and the encoded protein, P56, in human glioma cells (Kusari et al. 1987; Tiwari et al. 1987). Because the human ISG56 gene promoter contains two ISREs and no other identifiable transcription factor binding sites, it has been very useful for analyzing, in isolation, the IRF-mediated signaling pathways activated by different inducers. There are four members of the human ISG56 gene family IFIT-1 or ISG56, IFIT-2 or ISG54, IFIT-4 or ISG60, and IFIT-5 or ISG58. In mouse, there are three genes, ISG56 (IFIT1), ISG54 (IFIT2), and ISG49 (IFIT-3). Phylogenetically, human/mouse ISG56, human/mouse ISG54, and human ISG60/mouse ISG49 are the cognate genes in the two species.

Induction patterns of human and mouse ISG56 and ISG54 have been studied in vitro and in vivo (Terenzi et al. 2005, 2006; F. Terenzi et al., unpublished

observations). Although they are usually induced with similar characteristics, interesting and unexpected differences have been noted. In human HT1080 cells, IFN-β treatment strongly induced both mRNAs, but the level of ISG54 mRNA declined quickly, whereas the level of ISG56 mRNA remained constant over a 24-h period. In response to dsRNA, the ISG56 mRNA was induced much more efficiently, but the kinetics of induction of the two mRNAs was very similar. Sendai virus infection induced both mRNAs strongly, but the levels of both mRNAs declined rapidly. In contrast, in another cell line (HEK293), SeV caused strong and sustained induction of both mRNAs for 24 h. These results demonstrated inducer-specific and cell-type-specific differential regulations of ISG56 and ISG54 induction, some of which could be functioning at a post-transcriptional level.

The mouse ISG56 and ISG54 genes are induced by IFN β and dsRNA equally well in bone-marrow-derived macrophages, but in mouse embryo fibroblasts dsRNA cannot induce them, probably because these cells do not express TLR3. Interesting differences were noted when IFNα, IFNβ, dsRNA, or VSV were injected to mouse tail veins. In most tissues, both genes were induced in response to all inducers, but in spleen B cells, only ISG54 was induced. In liver, both genes were induced by dsRNA and IFNβ, whereas only ISG56 was induced by IFNα and VSV. These results indicate that the regulation of induction of the two closely related genes, ISG56 and ISG54, is complex and governed by both tissue-specific and inducer-specific processes (F. Terenzi et al., unpublished observation).

All members of the ISG56 family of genes encode proteins with multiple tetratricopeptide (TPR) motifs. These are degenerate protein–protein interaction motifs and they often function in combinations. Each TPR adopts a helix-turn-helix structure and adjacent TPR motifs pack in parallel, forming a spiral of repeating anti-parallel helices (Lamb et al. 1995; Blatch and Lassle 1999). Most TPR-containing proteins bind to cellular multi-protein complexes and regulate their functions. Although human and mouse ISG56 proteins have only 50% sequence identity, they both have six identifiable TPR motifs that are located along the linear protein sequences at similar positions. In contrast, the ISG54 proteins of both species have four TPR motifs located in the N-terminal halves of the protein (Sarkar and Sen 2004).

One multi-protein complex, to which the ISG56 related proteins bind, is the translation initiation factor eIF-3. Binding of the P56 proteins to eIF3 causes inhibition of the protein synthesis initiation (Guo et al. 2000). A 12-subunit protein complex, eIF3 catalyzes many steps of initiation of protein synthesis. One of these steps is the stabilization of the ternary complex, eIF2.GTP.tRNA-Met_i; this function of eIF3 is inhibited by both HuP56 and HuP54, but not by

the mouse homologs. Another function of eIF3 is to facilitate the formation of the 48S complex composed of the 40S ribosomal subunit and the 20S complex containing eIF3, ternary complex, eIF4F, and mRNA. The latter function of eIF3 is blocked by both mouse P56 and mouse P54; it is also blocked by HuP54. None of these proteins blocks the formation of the 20S complex or many other functions of eIF3. The high selectivity of the affected eIF3 functions probably reflects the fact that different regions of this large protein complex mediate different functions and the P56 proteins bind only to specific regions of eIF3 and affect the functions carried out by those regions. Support for the above concept comes from the observation that different members of the P56 family bind to specific subunits of eIF3 (Hui et al. 2003, 2005). Human P56 binds to the eIF3e protein (also known as Int-6 or P48). This protein contains both a nuclear localization signal and a nuclear export signal and its presence in both the cytoplasm and the nucleus has been noted (Guo and Sen 2000). Although its cytoplasmic function is easily attributable to its property as a subunit of eIF3, its nuclear function remains an enigma. In this context, it is worth noting that this protein was discovered as the product of the mouse Int-6 locus, whose disruption by the integration of a mouse mammary tumor virus genome causes mammary carcinoma in mice; however, the biochemical basis of that pathogenesis is unknown (Marchetti et al. 1995). HuP56 interacts with the shared C-terminal domain of both the cytoplasmic and the nuclear isoforms of eIF3e. This domain contains a PCI motif that is responsible for the P56 interaction. The PCI motif, a long α-helix, is present in different subunits of three large protein complexes: the regulatory subunit of proteasome (P), the COP9/signalosome (C) complex, and the translation initiation (I) factor 3 (Hofmann and Bucher 1998). Similar to eIF3e, eIF3c contains a PCI motif, and both mouse P56 and mouse P54 interact with eIF3c, but not with eIF3e. Human P56 does not interact with eIF3c, human P54 interacts with both subunits, and human P58 and P60 interact with neither (Terenzi et al. 2006). The interaction between eIF3c and HuP54 is mediated by at least two domains of eIF3c: the PCI domain at the C-terminal and another domain present at its N-terminal. In contrast, using its own N-terminal region, mouse P54 interacts only with the PCI domain of eIF3c and mouse P56 interacts only with the N-terminal region of eIF3c. Thus, different P56-family proteins can interact with more than one region of eIF3c (Hui et al. 2005; Terenzi et al. 2005, 2006). P56 can inhibit translation of not only capped cellular mRNAs but also viral mRNAs, such as hepatitis C mRNAs, translation of which is initiated at internal ribosomal entry sites (IRESs). There is strong evidence for the regulation of Hepatitis C protein synthesis by P56 in IFN-treated or untreated virus-infected cells (Wang et al. 2003).

7
Functions of ISG15

ISG15 is another human gene that is strongly induced by IFNs, dsRNA, and viruses (Martensen and Justesen 2004). It encodes a 15-kDa ubiquitin-like protein, P15 or ISG15. Like ubiquitin, ISG15 protein is linked to target proteins by isopeptide linkages between lysine side chains of the targets and its own carboxyl terminus. Many ISG15-conjugated proteins are present in IFN-treated cells; some are constitutively expressed and some are IFN-induced, such as ISG56. They functionally cover many aspects of cell metabolism (Zhao et al. 2005). Like ubiquitination, ISG15ylation of proteins requires the participation of three families of enzymes, E1, E2, and E3. These have been identified as UbeIL, UbcH8, and HERC5, respectively (Yuan and Krug 2001; Zhao et al. 2005; Dastur et al. 2006); However, additional enzymes with the same properties may exist. The genes encoding these enzymes are IFN-inducible as well, and the proteins themselves are targets of ISG15ylation. It is not yet known how the target proteins of ISG15ylation are selected because they apparently do not contain any common signature motifs. An enzyme, UBP43, which can remove ISG15 from these target proteins has also been identified, although its specificity is not as stringent as originally claimed (Malakhova et al. 2006).

The primary protein product of ISG15 is a 17-kDa precursor, which is cleaved to produce P15, a 15-kDa protein, upon the removal of eight carboxyl-terminal residues (Potter et al. 1999). The functional consequences of ISG15ylation of proteins remain elusive. Some information is available in this regard in the context of cells infected with different viruses. For example, the NS1B protein of influenza B virus specifically blocks ISGylation of proteins, suggesting that this process allows the virus to evade an antiviral effect of ISG15 (Yuan and Krug 2001). In another study, ISG15 was implicated to be the protein that mediates the action of IFN against HIV-1 morphogenesis (Su et al. 1995; Okumura et al. 2006). IFN inhibits the release of HIV-1 virions without affecting viral protein synthesis and this effect can be mimicked by ectopic expression of ISG15 and its activating enzymes. HIV-1 morphogenesis requires ubiquitination of its Gag protein and its interaction with the cellular protein TsgI01. ISG15 inhibits the interaction between the two proteins as well as their ubiquitination. A potential role of ISG15 in blocking the replication of Sindbis virus in mice was suggested in a study utilizing a chimeric Sindbis virus to express ISG15. ISG15 expression protected mice against Sindbis virus-induced lethality and virus replication (Lenschow et al. 2005). In contrast, $ISG15^{-/-}$ mice were perfectly capable of mounting an IFN-induced antiviral response against vesicular stomatitis virus and lymphocytic choriomeningitis virus (Osiak et al. 2005). In the future, further

evaluation of these genetically modified mice, for their ability to mount antiviral effects against other viruses, may be illuminating.

8 Future Perspectives

With the realization of the diversity of viral agents that can induce the same genes by triggering different converging pathways, the time is right to evaluate the relative contributions of these pathways in virus-infected organisms. Already there are indications that, even for a single virus, different pathways may be dominant in different cell types. It is conceivable that such differences play major roles in determining pathogenesis, or the lack of it, when the same organism is infected with the same virus, but by different routes. It is likely that many of these viral-stress-inducible genes are induced by other infectious agents as well. Functionally, the products of these genes are probably designed to protect cells from many types of extracellular stresses. For limiting the spread of viral infection, they may directly block the synthesis of viral components or may cause premature apoptosis of the infected cell to abort virus replication. Future investigations will reveal the individual properties of these proteins and the evasive mechanisms that some viruses employ to counteract them.

References

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124:783–801

Alexander WS, Hilton DJ (2004) The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol 22:503–529

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413:732–738

Bandyopadhyay SK, Leonard GT Jr, Bandyopadhyay T, Stark GR, Sen GC (1995) Transcriptional induction by double-stranded RNA is mediated by interferon-stimulated response elements without activation of interferon-stimulated gene factor 3. J Biol Chem 270:19624–19629

Barnes B, Lubyova B, Pitha PM (2002) On the role of IRF in host defense. J Interferon Cytokine Res 22:59–71

Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Segal DM, Davies DR (2005) The molecular structure of the Toll-like receptor 3 ligand-binding domain. Proc Natl Acad Sci USA 102:10976–10980

Bell JK, Askins J, Hall PR, Davies DR, Segal DM (2006) The dsRNA binding site of human Toll-like receptor 3. Proc Natl Acad Sci U S A 103:8792–8797

Biron C, Sen GC (2001) Interferons and other cytokines. Fields virology. Knipe D, Howley P, Griffin D, Lamb R, Martin M, Straus S (eds) Lippincott Williams & Wilkins, Philadelphia pp 321–351

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21:932–939

Boehme KW, Compton T (2004) Innate sensing of viruses by toll-like receptors. J Virol 78:7867–7873

Choe J, Kelker MS, Wilson IA (2005) Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science 309:581–585

Dastur A, Beaudenon S, Kelley M, Krug RM, Huibregtse JM (2006) Herc5, an interferon-induced HECTE3 enzyme, is required for conjugation of ISG15 in human cells. J Biol Chem 281:4334–4338

De Bouteiller O, Merck E, Hasan UA, Hubac S, Benguigui B, Trinchieri G, Bates EE, Caux C (2005) Recognition of double-stranded RNA by human toll-like receptor 3 and downstream receptor signaling requires multimerization and an acidic pH. J Biol Chem 280:38133–38145

Der SD, Zhou A, Williams BR, Silverman RH (1998) Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc Natl Acad Sci U S A 95:15623–15628

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303:1529–1531

Elco CP, Guenther JM, Williams BR, Sen GC (2005) Analysis of genes induced by Sendai virus infection of mutant cell lines reveals essential roles of interferon regulatory factor 3, NF-kappaB, and interferon but not toll-like receptor J Virol 79:3920–3929

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T (2003) IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 4:491–496

Garcia-Sastre A, Biron CA (2006) Type 1 Interferons and the virus-host relationship: a lesson in detente. Science 312:879–882

Geiss G, Jin G, Guo J, Bumgarner R, Katze MG, Sen GC (2001) A comprehensive view of regulation of gene expression by double-stranded RNA-mediated cell signaling. J Biol Chem 276:30178–30182

Guo J, Sen GC (2000) Characterization of the interaction between the interferon-induced protein P56 and the Int6 protein encoded by a locus of insertion of the mouse mammary tumor virus. J Virol 74:1892–1899

Guo J, Hui DJ, Merrick WC, Sen GC (2000) A new pathway of translational regulation mediated by eukaryotic initiation factor 3. EMBO J 19:6891–6899

Hacker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, Kamps MP, Raz E, Wagner H, Hacker G, Mann M, Karin M (2006) Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature 439:204–207

Haller O, Kochs G, Weber F (2006) The interferon response circuit: induction and suppression by pathogenic viruses. Virology 344:119–130

Heinz S, Haehnel V, Karaghiosoff M, Schwarzfischer L, Muller M, Krause SW, Rehli M (2003) Species-specific regulation of Toll-like receptor 3 genes in men and mice. J Biol Chem 278:21502–21509

Hofmann K, Bucher P (1998) The PCI domain: a common theme in three multiprotein complexes. Trends Biochem Sci 23:204–205

Hui DJ, Bhasker CR, Merrick WC, Sen GC (2003) Viral stress inducible protein P56 inhibits translation by blocking the interaction of eIF3 with the ternary complex (eIF22GTP2Met-tRNAi). J Biol Chem 278:39477–39482

Hui DJ, Terenzi F, Merrick WC, Sen GC (2005) Mouse p56 blocks a distinct function of eukaryotic initiation factor 3 in translation initiation. J Biol Chem 280:3433–3440

Isaacs A, Lindenmann J (1957) Proc R Soc Lond B Biol Sci 147:258–267

Jacobs BL, Langland JO (1996) When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 219:339–349

Jiang Z, Zamanian-Daryoush M, Nie H, Silva AM, Williams BR, Li X (2003) Poly(I-C)-induced Toll-like receptor 3 (TLR3)-mediated activation of NFkappa B and MAP kinase is through an interleukin-1 receptor-associated kinase (IRAK)-independent pathway employing the signaling components TLR3-TRAF6-TAK1-TAB2-PKR. J Biol Chem 278:16713–16719

Jiang Z, Mak TW, Sen G, Li X (2004) Toll-like receptor 3-mediated activation of NF-kappaB and IRF3 diverges at Toll-IL-1 receptor domain-containing adapter inducing IFN-beta. Proc Natl Acad Sci U S A 101:3533–3538

Kawai T, Akira S (2006) Innate immune recognition of viral infection. Nat Immunol 7:131–137

Kusari J, Szabo P, Grzeschik KH, Sen GC (1987) Chromosomal localization of the interferon-inducible human gene encoding mRNA 561. J Interferon Res 7:53–59

Lamb JR, Tugendreich S, Hieter P (1995) Tetratrico peptide repeat interactions: to TPR or not to TPR? Trends Biochem Sci 20:257–259

Lengyel P (1987) Double-stranded RNA and interferon action. J Interferon Res 7:511–519

Lenschow DJ, Giannakopoulos NV, Gunn LJ, Johnston C, O'Guin AK, Schmidt RE, Levine B, Virgin HWT (2005) Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo. J Virol 79:13974–13983

Malakhova OA, Kim KI, Luo JK, Zou W, Kumar KG, Fuchs SY, Shuai K, Zhang DE (2006) UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. EMBO J 25:2358–2367

Maniatis T, Falvo JV, Kim TH, Kim TK, Lin CH, Parekh BS, Wathelet MG (1998) Structure and function of the interferon-beta enhanceosome. Cold Spring Harb Symp Quant Biol 63:609–620

Marchetti A, Buttitta F, Miyazaki S, Gallahan D, Smith GH, Callahan R (1995) Int-6, a highly conserved, widely expressed gene, is mutated by mouse mammary tumor virus in mammary preneoplasia. J Virol 69:1932–1938

Martensen PM, Justesen J (2004) Small ISGs coming forward. J Interferon Cytokine Res 24:1–19

Matsumoto M, Funami K, Tanabe M, Oshiumi H, Shingai M, Seto Y, Yamamoto A, Seya T (2003) Subcellular localization of Toll-like receptor 3 in human dendritic cells. J Immunol 171:3154–3162

Meylan E, Tschopp J (2006) Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. Mol Cell 22:561–569

Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, Tschopp J (2004) RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat Immunol 5:503–507

Nagano Y, Kojima Y (1954) CR Seances Soc Biol Fil 148:1700–1702

Nusinzon I, Horvath CM (2006) Positive and negative regulation of the innate antiviral response and beta interferon gene expression by deacetylation. Mol Cell Biol 26:3106–3113

Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, Perry A, Cheng G (2006) Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. Nature 439:208–211

Okumura A, Lu G, Pitha-Rowe I, Pitha PM (2006) Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG. Proc Natl Acad Sci U S A 103:1440–1445

Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol 4:161–167

Osiak A, Utermohlen O, Niendorf S, Horak I, Knobeloch KP (2005) ISG15, an interferon-stimulated ubiquitin-like protein, is not essential for STAT1 signaling and responses against vesicular stomatitis and lymphocytic choriomeningitis virus. Mol Cell Biol 25:6338–6345

Peters KL, Smith HL, Stark GR, Sen GC (2002) IRF-3-dependent NFkappa B- and JNK-independent activation of the 561 and IFN-beta genes in response to double-stranded RNA. Proc Natl Acad Sci U S A 99:6322–6327

Potter JL, Narasimhan J, Mende-Mueller L, Haas AL (1999) Precursor processing of pro-ISG15/UCRP, an interferon-beta-induced ubiquitin-like protein. J Biol Chem 274:25061–25068

Samuel CE (2001) Antiviral actions of interferons. Clin Microbiol Rev 14:778–809

Sarkar SN, Sen GC (2004) Novel functions of proteins encoded by viral stress-inducible genes. Pharmacol Ther 103:245–259

Sarkar SN, Peters KL, Elco CP, Sakamoto S, Pal S, Sen GC (2004) Novel roles of TLR3 tyrosine phosphorylation and PI3 kinase in double-stranded RNA signaling. Nat Struct Mol Biol 11:1060–1067

Sarkar SN, Smith HL, Rowe TM, Sen GC (2003) Double-stranded RNA signaling by Toll-like receptor 3 requires specific tyrosine residues in its cytoplasmic domain. J Biol Chem 278:4393–4396

Sen GC, Sarkar SN (2005a) Hitching RIG to action. Nat Immunol 6:1074–1076

Sen GC, Sarkar SN (2005b) Transcriptional signaling by double-stranded RNA: role of TLR. Cytokine Growth Factor Rev 16:1–14

Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J (2003) Triggering the interferon antiviral response through an IKK-related pathway. Science 300:1148–1151

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD (1998) How cells respond to interferons. Annu Rev Biochem 67:227–264

Stetson DB, Medzhitov R (2006) Antiviral defense: interferons and beyond. J Exp Med 203:1837–1841

Su Y, Popik W, Pitha PM (1995) Inhibition of human immunodeficiency virus type 1 replication by a Tat-activated, transduced interferon gene: targeted expression to human immunodeficiency virus type 1-infected cells. J Virol 69:110–121

Tabeta K, Georgel P, Janssen E, Du X, Hoebe K, Crozat K, Mudd S, Shamel L, Sovath S, Goode J, Alexopoulou L, Flavell RA, Beutler B (2004) Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc Natl Acad Sci U S A 101:3516–3521

Terenzi F, Pal S, Sen GC (2005) Induction and mode of action of the viral stress-inducible murine proteins P56 and P54. Virology 340:116–124

Terenzi F, Hui DJ, Merrick WC, Sen GC (2006) Distinct induction patterns and functions of two closely related interferon-inducible human genes ISG54 and ISG56. J Biol Chem 281:34064-34071

Tiwari RK, Kusari J, Sen GC (1987) Functional equivalents of interferon-mediated signals needed for induction of an mRNA can be generated by double-stranded RNA and growth factors. EMBO J 6:3373–3378

Wang C, Pflugheber J, Sumpter R Jr, Sodora DL, Hui D, Sen GC, Gale M Jr (2003) Alpha interferon induces distinct translational control programs to suppress hepatitis C virus RNA replication. J Virol 77:3898–3912

Watanabe Y (2004) Fifty years of interference. Nat Immunol 5:1193

Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. J Immunol 169:6668–6672

Yuan W, Krug RM (2001) Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. EMBO J 20:362–371

Zhao C, Denison C, Huibregtse JM, Gygi S, Krug RM (2005) Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. Proc Natl Acad Sci USA 102:10200–10205