Cell Research (2008) 18:433-435.

www.nature.com/cr

RESEARCH HIGHLIGHT

Regulation of interferon production and innate antiviral immunity through translational control of IRF-7

Andrea K Erickson[1], Michael Gale Jr[2]

[1]*Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX, 75235-9840, USA;* [2]*Department of Immunology, University of Washington School of Medicine, Seattle, WA, 98195-7650, USA. mgale@u.washington.edu*
Cell Research (2008) **18**:433-435. doi: 10.1038/cr.2008.46; published online 3 April 2008

Innate intracellular immune programs mediate our first line of defense against virus infection and are dependent on type I interferon (IFNs) [1]. IFNs are cytokines that are produced and secreted from virus-infected cells. Autocrine and paracrine engagement by IFNs of the type I IFN receptor initiates tissue-wide and systemic signaling through the cellular Jak-STAT pathway to induce the transcription of hundreds of interferon-stimulated genes (ISGs) (Figure 1A). ISG products confer a variety of functions, including direct antiviral activity, immunomodulatory function, and metabolic control to limit virus infection and promote the adaptive immune response.

The rapid production of IFN is a cornerstone of the innate antiviral response. IFN expression is dependent upon the cellular transcription factors, interferon regulatory factor (IRF)-3 and IRF-7 which become activated as downstream effectors of pathogen recognition receptor (PRR) signaling cascades. PRRs are cellular proteins that include the RIG-I RNA helicase family (RIG-I and MDA5), and the family of Toll-like receptors (TLRs) that engage specific viral products and signal to the nucleus to turn on IFN mRNA expression [2]. IRF-3, which is constitutively expressed in most cells, serves to induce an initial wave of IFN expression from infected cells that is amplified upon subsequent expression and activation of IRF-7, which itself is an ISG [3]. Importantly, IRF-7 is considered the master transcription factor that drives IFN expression by plasma cytoid dendritic cells (pDCs), which are the body's major IFN-producing cells and responsible for systemic IFN-induced antiviral defenses [4]. The nature of IRF-7 as an ISG presents a fundamental problem in immunity wherein high level IFN production and antiviral defenses rely on the rate-limiting processes of nuclear signaling of IRF-7 mRNA transcription and ensuing protein production. How does the cell mediate the rapid IFN production and response against viruses given that most cells lack a preexisting pool of IRF-7 protein? The answer to this question is presented in a recent study by Colina *et al*., who used biochemical, genetic, and cell-based approaches to demonstrate that virus-induced expression of IRF-7 occurs through a translational control program that confers rapid protein production from preexisting but translationally silent mRNA pools [5]. Their results show that the cellular repressor proteins of translation initiation, 4E-BP1 and 4E-BP2, play a central role in regulating the production of type-I IFN thru translational control of IRF-7 expression.

Eukaryotic mRNA translation is controlled primarily at the initiation step when the 40S ribosomal subunit is recruited to the mRNA, which is facilitated by recognition of the 5′-cap structure by the eukaryotic initiation factor (eIF)4F complex. Formation of the eIF4F complex is inhibited by the 4E-BP translational repressors, which when in a hypophosphorylated state bind with high affinity to the cap-binding eIF4E subunit of eIF4F [6]. Hyperphosphorylation of the 4E-BPs is induced in part through mTOR, resulting in release of eIF4E and stimulation of mRNA translation [7]. Virus infection has also been shown to alter 4E-BP phosphorylation, providing a possible link of 4E-BP signaling with virus-induced translational control processes [8]. Colina *et al.* [5] examined the involvement of the 4E-BP translational repressors in the innate antiviral immune response triggered by RNA virus infection. The authors assessed virus and host parameters of infection *in vitro* and *in vivo* using mouse embryonic fibroblasts and mice deficient in 4E-BP1 and 4E-BP2. The authors found that fibroblasts derived from 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ double knock out mice were resistan to infection with vesicular stomatitis virus (VSV) and exhibited decreased levels of viral protein synthesis with approximately 700-fold lower production of infectious virus compared to VSV infection of wild-type cells. The authors proceeded to demonstrate that enhanced IFN production in the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells was responsible for the increased resistance of the cells to virus infection. Similarly, when infected with VSV,

4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ double knock out mice had a markedly enhanced survival rate and exhibited an approximately 100-fold lower viral load in their lungs concomitant with increased serum IFN-α levels as compared to wild-type mice. This virus resistant phenotype was also seen when the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ fibroblasts were infected with other RNA viruses that activate the innate antiviral immune response and IFN production through distinct PRR signaling pathways, including RIG-I-dependent or TLR-dependent pathways. These results suggest that the translational repressors must target an mRNA encoding an essential downstream component shared by these pathways whose product is involved in IFN expression.

In order to identify all possible mRNA targets of the 4E-BP1 and 4E-BP2 translational repressors, Colina *et al*. used an innovative adaptation of gene expression microarray analysis to specifically screen for mRNAs that are under translational suppression by 4E-BP1 and 4E-BP2. Total RNA and polyribosome-associated RNA from wild-type and 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ fibroblasts were recovered and analyzed by gene expression microarray. Translationally-active mRNAs within the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells were selected by the criteria that they were enriched within the polyribosome-associated RNA population in these cells compared to the same population in wild-type cells. Among the RNA species present in the polyribosome pools, IRF-7 mRNA exhibited a 12-fold increase in abundance in the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells compared to the wild-type cells. These results were validated using polyribosome distribution analysis where it was found that IRF-7 mRNA associated with polyribosomes and led to increased protein abundance only in 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells, indicating that this mRNA

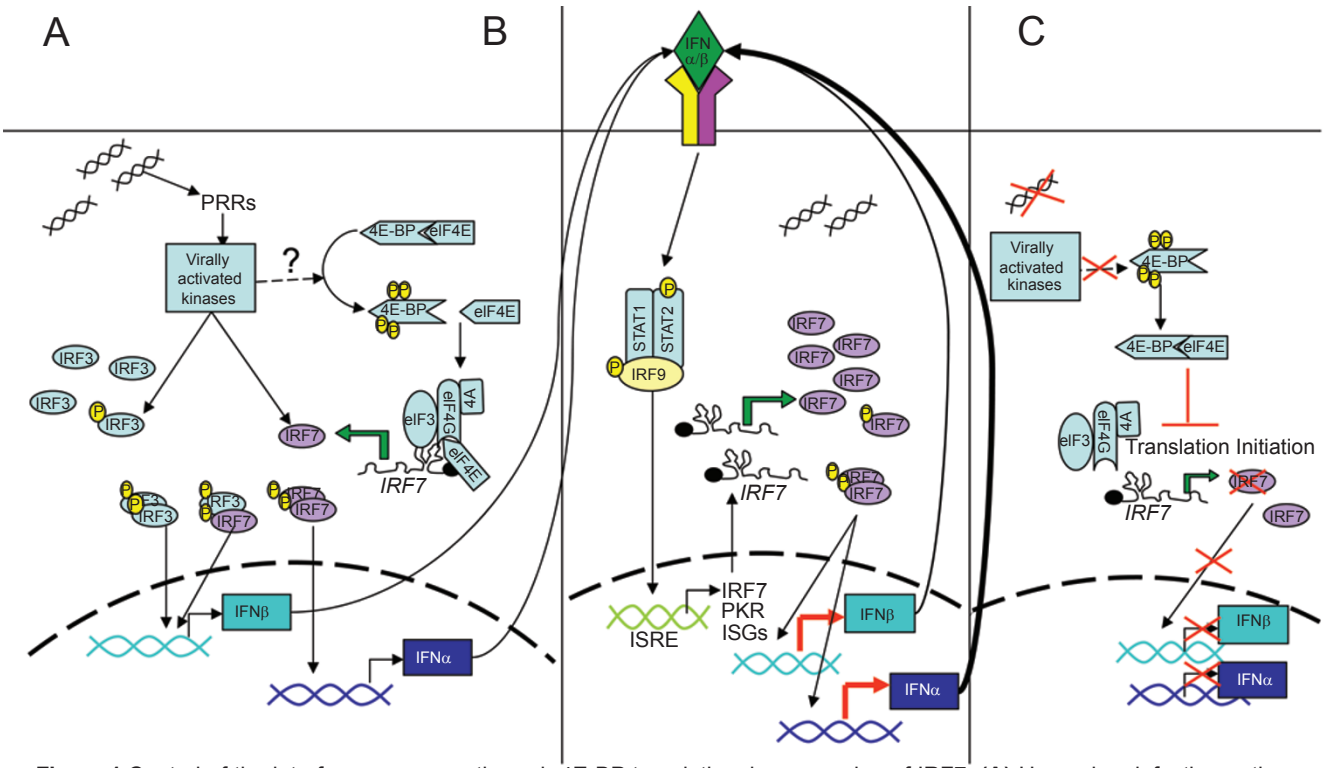

Figure 1 Control of the interferon response through 4E-BP translational suppression of IRF7. **(A)** Upon virus infection pathogen recognition receptors (PRRs) bind to their respective viral ligand resulting in activation of distinct signaling cascades. The mammalian target of rapamycin (mTOR) or an undefined protein kinase may lead to hyperphosphorylation of 4E-BP, allowing release of the eukaryotic translation initiation factor (eIF)4E, which is now free to bind to eIF4G, forming the eIF4F translation initiation complex. This increase in the pool of functional eIF4F allows the translation of mRNAs, such as IRF-7 mRNA, that were only inefficiently translated or translationally silent under conditions of 4E-BP hypophosphorylation. PRR signaling triggers the phosphorylation of IRF-7 and IRF-3 by virus-activated kinases to induce their activation and translocation to the nucleus, resulting in transcriptional activation of type I IFN genes. **(B)** The secreted IFNs bind to their cognate receptor (IFNAR1/2), activating the Jak-STAT pathway wherein the transcription factor, ISGF-3, induces the expression of ISGs including IRF-7, RIG-I and PKR, and many others. ISG products limit virus infection and enhance host immunity. **(C)** After the virus is cleared from the cells, it is imperative that the IFN response be dampened before it is harmful to the host. This may occur in part through translational-suppressive actions of the 4E-BPs on IRF-7 mRNA translation, hence decreasing IFN production and turning off the innate immune response.

was efficiently translated in the absence of the 4E-BPs but rendered translationally silent in their presence in otherwise resting cells. In order to conclusively show that increased IRF-7 protein expression in the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells was responsible for the virus-resistant phenotype and enhanced IFN production the authors used short hairpin RNA to suppress IRF-7 mRNA translation and thereby reduce IRF-7 protein levels in the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells. This approach rendered the cells susceptible to VSV infection due to decreased IFN-α production, thus defining translation control of IRF-7 expression by the 4E-BPs as a critical feature regulating innate antiviral immunity.

The study of Colina *et al*. demonstrates that 4E-BPs translationally regulate a number of cellular mRNAs in addition to IRF-7, raising the question as to how mechanistically translation is governed by the 4E-BPs. It has been shown that mRNAs with extensive secondary structure in the 5′ nontranslated region (NTR) are only inefficiently translated, due in part to reduced efficiency of ribosome binding and/or scanning to the initiator ATG codon of the mRNA open reading frame [9]. In order to determine if this process might mechanistically account for IRF-7 mRNA translational control, the authors used a luciferase reporter gene assay in which the firefly luciferase gene was fused to the 5′ NTR from IRF-7 mRNA. Expression of the 5′ NTR-IRF-7 luciferase gene was enhanced in the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ cells compared to wild-type cells, suggesting that the 5′ UTR of IRF-7 plays a role in the observed 4E-BP translational suppression of IRF-7. While this is compelling data, it does not conclusively demonstrate that RNA secondary structure is the main determinant for translational control of IRF-7 mRNA, due to the lack of RNA structure data and 5′ NTR deletion/mutation analyses that could effectively demonstrate the presence of 5′ NTR secondary structures that impart translation control.

Of additional interest is that pDCs were found to express only a low level of 4E-BPs. As pDCs express high constitutive IRF-7 [4], Colina *et al.* suggest that this may be a result of inherently low levels of 4E-BPs in the pDCs. VSV infection to trigger TLR7 signaling or treatment to trigger TLR9 signaling in pDCs from the 4E-BP1$^{-/-}$ 4E-BP2$^{-/-}$ mice resulted in their increased production of IFN-α as compared to pDCs from wild-type mice, suggesting that even at low levels the 4E-BPs can control IRF-7 protein production. Taken together, the results of Colina *et al*. support a model in which 4E-BPs impart translational control of IRF-7 expression. In the case of resting cells, including pDCs, IRF-7 mRNA will be expressed at a low level but will be translationally silent through 4E-BPs binding to eIF4E (Figure 1A). In response to virus infection of fibroblasts and epithelial cells, PRR signaling confers IRF-3 activation and a level of IFN production that drives increased expression of IRF-7 mRNA (Figure 1B). Additional virus-induced signals, perhaps mediated by mTOR or other pathways, converge on the 4E-BPs to mediate their hyperphophorylation. This would lead to release of eIF4E to stimulate mRNA translation wherein increased levels of eIF4E will support translation of mRNAs with structured 5′ NTRs, possibly including IRF-7 mRNA, that otherwise impede ribosome binding or scanning [9]. This model invokes the ensuing IFN production as a positive feedback mechanism of IRF-7 expression to sustain IFN gene transcription and antiviral defenses, while providing for discrete control of these processes through translational regulation (Figure 1C).

The study by Colina *et al*. elegantly demonstrates the importance of translational control mechanisms in regulation of the host response to viral infection. It is important to elucidate the pathways that govern 4E-PB phosphorylation and binding to eIF4E during virus infection, as their nature could reveal the virus-responsive protein kinases or phosphatases that regulate 4E-BP function. Such factors would present therapeutic targets for enhancement of antiviral immunity or suppression of autoimmune diseases associated with IFN production [10].

References

1 Loo YM, Gale M Jr. Viral regulation and evasion of the host response. *Curr Top Microbiol Immunol* 2007; **316**:295-313.
2 Saito T, Gale M Jr. Principles of intracellular viral recognition. *Curr Opin Immunol* 2006; **19**:17-23.
3 Tamura T, Yanai H, Savitsky D, Taniguchi T. The IRF Family Transcription Factors in Immunity and Oncogenesis. *Annu Rev Immunol* 2007 Dec 20; doi:10.1146/annurev.immunol.26.021607.090400.
4 Honda K, Yanai H, Negishi H, *et al.* IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 2005; **434**:772-777.
5 Colina R, Costa-Mattioli M, Dowling RJ, *et al.* Translational control of the innate immune response through IRF-7. *Nature* 2008 Feb 13; doi:10.1038/nature06730.
6 Sonenberg N, Hinnebusch AG. New modes of translational control in development, behavior, and disease. *Mol Cell* 2007; **28**:721-729.
7 Sonenberg N, Dever TE. Eukaryotic translation initiation factors and regulators. *Curr Opin Struct Biol* 2003; **13:**56-63.
8 Clemens MJ. Translational control in virus-infected cells: models for cellular stress responses. *Semin Cell Dev Biol* 2005; **16**:13-20.
9 Koromilas AE, Lazaris-Karatzas A, Sonenberg N. mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO J* 1992; **11**:4153-4158.
10 Gottenberg JE, Chiocchia G. Dendritic cells and interferon-mediated autoimmunity. *Biochimie* 2007; **89**:856-871.