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RESEARCH HIGHLIGHT

Crystal structure of IFIT2 (ISG54) predicts functional properties of IFITs

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Interferon carries out its cellular effects, including its antiviral effects, by inducing the synthesis of many new proteins, amongst which is the IFIT (ISG56) family of proteins. The first crystal structure of an IFIT, reported by Yang *et al.*, revealed several functional properties of the protein that may help us to better understand the biological functions of these proteins.

The innate immune system of vertebrates is used to protect them from not only infectious agents but also detrimental environmental stresses. Different receptors can recognize the chemical nature of the offensive agent, microbial or not, and trigger signaling cascades that lead to transcriptional induction of protective proteins. The principle of cell-intrinsic self-defense, coupled with mechanisms to help neighbors, is best exemplified by the interferon (IFN) system, the first line of defense against virus infection. Among many viral stress-inducible proteins are the type I IFNs, which are secreted and induce the synthesis of hundreds of antiviral proteins in uninfected cells, many of which are also directly induced in the infected cells, without the need of IFN. Viral RNAs, both double-stranded and single-stranded, are the chemicals recognized by cellular receptors to trigger the antiviral response; such receptors include specific members of the Toll-like receptor (TLR) family and cytoplasmic RIG-I-like helicase family (RLH). The *IFIT* genes, encoding the P56 (ISG56) proteins, are very prominent among the genes that are induced strongly by IFN, TLR, RLH and other signaling pathways. However, only recently the structural, biochemical and biological properties of these proteins have begun to be investigated. Thus, the recent paper by Yang *et al.* [1], reporting the crystal structure of human IFIT2, is a landmark contribution to the field.

There are multiple members of the IFIT family, four in human: *IFIT1* (*ISG56*), *IFIT2* (*ISG54*), *IFIT3* (*ISG60*) and *IFIT5* (*ISG58*) and three in mouse: *Ifit1*, *Ifit2* and *Ifit3*. The promoters of their genes, which are clustered, contain the IFN-stimulated response elements that are recognized by members of the IRF family of transcription factors. As a consequence, these genes are induced by not only IFN but also many inducers that activate IRFs using different signaling pathways. Surprisingly, the induction of different *IFIT* members is not always regulated coordinately; there is cell type-specific and inducer-specific differential induction of these genes. The primary structures of the IFIT proteins are related, but distinct from each other. Similarly, the cognate members of two species have distinct sequences; for example, human P54 (IFIT2) and mouse P54 (Ifit2) are only 62% homologous. Thus, equating their properties, because of their shared names, is misleading; but this mistake is often made by many authors. All IFIT proteins contain several full and partial tetratricopeptide repeat (TPR) motifs [2].

The first crystal structure of an IFIT protein, the human ISG54 (IFIT2), reveals that the protein exists as domain-swapped dimer and each subunit has 9 helix-turn-helix TPR-like structures [1]. The structure also revealed the existence of a positively-charged nucleotide-binding channel. Although this channel is on the inner surface, the protein can bind RNA with some specificity. Sequence comparison predicts that the domain-swapped dimeric structure may be shared by other IFIT family members. It also opens the possibility of heterodimer formation through this domain, a possibility suggested before. An important conclusion made by Yang *et al.* [1] is that, unlike TPR motifs in other proteins, IFIT protein TPRs may have similarity with a pentatricopeptide repeat (PPR) motif found in plant proteins and known to mediate RNA binding and dimerization. It remains an exciting possibility that the sequence within and surrounding the PRR motifs of different IFIT members dictates their RNA-binding specificities. Future determination of the structure of an IFIT/RNA complex will reveal the structural

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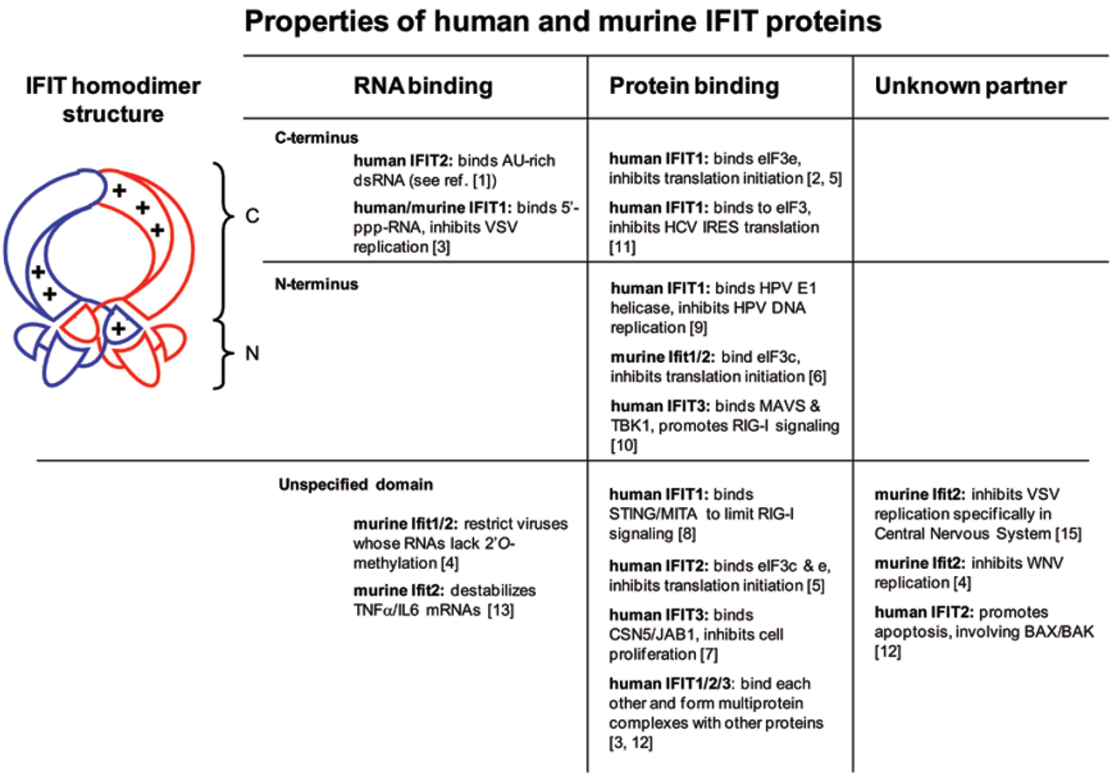

Figure 1 IFIT proteins: structure, binding partners and functions.

requirements of the nucleic acid that fits well in the channel: RNA or DNA, single-stranded or double-stranded. Can a long strand of RNA be decorated with multiple dimers of different IFITs and would such a putative complex have properties different from those of a simple dimer?

It is safe to conclude from the literature that the IFIT proteins can directly bind to specific proteins and RNAs [2]. However, there is conflicting evidence presented in different publications with regards to the nature of the binding partners. Such confusion is not unusual in a rapidly developing field and it, coupled with careful examination of the experimental protocols, when available, can reveal interesting properties of the IFIT proteins. Yang *et al.* [1] reported that IFIT2 can bind dsRNA irrespective of the presence of triphosphates at its 5′ end. Moreover, there is a strong preference for binding to AU-rich dsRNA and AU-rich ARE sequence present in the 3′ UTRs of many unstable mRNAs. These findings are in contrast with those of Pichlmair *et al.* [3] which reported that IFIT2 can bind to RNA only indirectly by complexing with IFIT1 and the complex binds to 5′-triphosphorylated RNA only. The above studies were done *in vitro*, whereas *in vivo* studies by Daffis *et al.* [4] demonstrated that mouse Ifit1 can functionally distinguish mRNAs, which have 2′-*O* methylation, from those that do not. Many protein partners of IFITs have been reported. Human IFIT1 binds to eIF3e, a protein containing a PCI motif, which is known to mediate interactions with TPR motif. Another PCI motif-containing eIF3 subunit, eIF3c, binds to human IFIT2 (which also binds to eIF3e) and murine Ifit1 and Ifit2 [5, 6]. However, Yang *et al.* [1] failed to find an interaction between IFIT2 and eIF3c, which could be due to interference from the epitope tag that they put at the C-terminus of eIF3c, where the IFIT2-interacting PCI domain resides. In addition to eIF3 subunits, different IFIT members interact with subunits of signalosomes and proteasomes that contain PCI motifs ([7] and our unpublished observation). Moreover, human IFIT1 binds to the signaling protein STING/MITA [8] and human HPV E1 protein [9], whereas IFIT3 binds to MAVS and TBK1 [10]. Systematic proteomics studies will be needed in the future to reveal the full repertoire of IFIT-interacting proteins. However, because the IFITs may form heteromers, the binding specificities are expected to be complex.

IFIT functions have been explored *in vitro*, in cell culture and in mice (Figure 1). *In vitro*, distinct steps of translation initiation, which are mediated by eIF3, have been shown to be inhibited by IFIT1 and IFIT2 [5, 6]. Overall translation inhibition has been demonstrated *in vitro* using reticulocyte lysates and in cell cultures, especially with HCV mRNA translation [11]. The reticulo-

cyte lysate systems are problematic and unreliable because they are often fortified by manufacturers with factors that promote translation, such as eIF3 and mRNA cap methyltransferases, which neutralize the purported effects of IFITs. Experiments with purified eIF3 and other components of translation initiation are much more reliable in this respect. The other strong inhibitory effect of IFIT1 observed *in vitro* is on HPV E1's function in viral DNA synthesis [9]. Cell culture experiments have demonstrated additional functions of IFIT proteins. Human IFIT1 inhibits RIG-I signaling by binding to MITA whereas IFIT3 promotes it [8, 10]. Human IFIT2 also promotes apoptosis [12]. Yang *et al.* [1] claimed antiviral effects of IFIT2 overexpression on replication of NDV and SeV; the effects were not quantified and visually appeared to be marginal [1]. Moreover, without appropriate controls, it is difficult to ascertain whether the observed weak antiviral effects are specific or they reflect general deleterious effects of IFIT2 overexpression on the health of the cells. In contrast, a more convincing effect of mouse Ifit2 overexpression is on TNFα mRNA stability [13]. Pichlmair *et al.* [3] did not observe any antiviral effects of overexpression of individual IFITs, but observed virus-specific stimulatory effects in response to their knockdowns. Using *Ifit1* knockout mice and MEFs derived from them, they demonstrated effects of Ifit1 on VSV replication and pathogenesis. However, the underlying mechanism remains unclear. Daffis *et al.* [4] and Szretter *et al.* [14] demonstrated that 2′-*O*-methylation of viral RNAs, from both RNA and DNA viruses, promotes evasion of the antiviral effects of Ifit1 in a cell type-specific way. Although the specific requirements for RNA recognition by Ifit1 seem to be different in the two reports, both point to the 5′ end of viral RNAs as the potential target of recognition. A major antiviral effect of mouse Ifit2 was demonstrated by Fensterl *et al.* [15] using *Ifit2* knockout mice. These mice were strikingly more susceptible to neuropathogenesis caused by intranasal VSV infection when compared to WT or *Ifit1*$^{-/-}$ mice. Surprisingly, the need of Ifit2 for inhibiting VSV replication was restricted to neurons; VSV replication was inhibited by other mechanisms in other organs of *Ifit2*$^{-/-}$ mice. Now Yang *et al.* [1] have reported the structural basis of RNA binding by human IFIT2, and the physiological function of the RNA-binding property of the corresponding mouse Ifit2 can be tested in the VSV pathogenesis model. Such experiments will connect the crystal structure of IFIT2 to its role in preventing viral pathogenesis.

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