A Structural Study of IFIT2/ISG54 Suggests its Functional Mechanism on its Anti-Viral Activity and Cellular Functions

IFIT1/ISG56 family members are well-known as interferon stimulated genes with antiviral activities. However, the mechanisms of the antiviral activities of these proteins were not clear. We determined the structure of IFIT2/ISG54, the first structure reported in the protein family. Our results showed that ISG54 is a domain swapped dimer in solution. It is an RNA binding protein which can specifically bind AU-rich RNAs. The specific RNA binding ability of ISG54 is required for its anti-viral activity. Furthermore, our results showed that ISG54 can bind some cellular RNA elements, suggesting its functional mechanism in cells.

Upon infection by virus, interferon is produced by cells, which then stimulates the expression of many genes to fight the infection. These genes are called Interferon Stimulated Genes (ISGs) [1]. The functions of some ISGs have been studied, such as ISG15 [2]. ISG56 family proteins are well-known anti-viral proteins [3], but the mechanisms of these proteins involved in their anti-viral effect were not clear. It was reported that ISG56 and ISG54 proteins can bind protein translational elongation factors, such as eIF3c and eIF3e, thus blocking the viral protein translation process [4]. However, two other investigations showed that ISG56 or ISG54 inhibit viruses with specific modification at the viral mRNA 5' end [5, 6]. To understand the exact mechanism of ISG56 family proteins, we determined the structure of human ISG54 which is the first structure reported in the ISG56 family using the facilities in BL-5A of the PF [7].

ISG54 is a super-helical structure built up by TPR repeat-like helices (Fig. 1). However, ISG54 is not a monomer but unexpectedly a domain swapped dimer through some helices in the middle of the protein, making the whole structure resemble the letter X. Interestingly, when the structure is shown as a surface electrostatic presentation, the inner surface of the C-terminal part of the super-helical protein is exclusively positively charged. This structural feature strongly suggests that ISG54 is a nucleic acid binding protein. Therefore, we performed an EMSA experiment to test the binding of ISG54 with some model RNAs. Our results showed that ISG54 can bind AU-rich double-strand model RNAs but not GC-rich RNAs. Phosphate modification at the 5' end or methylation state on the Cap structure of viral mRNA is not required for the binding. To test whether the RNA binding ability of ISG54 is required for its antiviral activity, we used VSV and Sendai virus as model systems to perform the anti-viral activity experiment. The results showed that over-expression of wild-type ISG54 can block the replication of these viruses in HEK293 cells; however, disruption of RNA binding ability of ISG54 impaired or decreased the anti-viral activity of this protein, suggesting that the RNA binding ability of ISG54 is required for anti-VSV and Sendai virus activity. Besides the anti-viral activity, we also considered the potential mechanisms of ISG54 in its cellular functions. The crystal structure was determined by the SAD method by selenium derivative by using the KEK radiation facilities and Shanghai synchrotron radiation facilities.

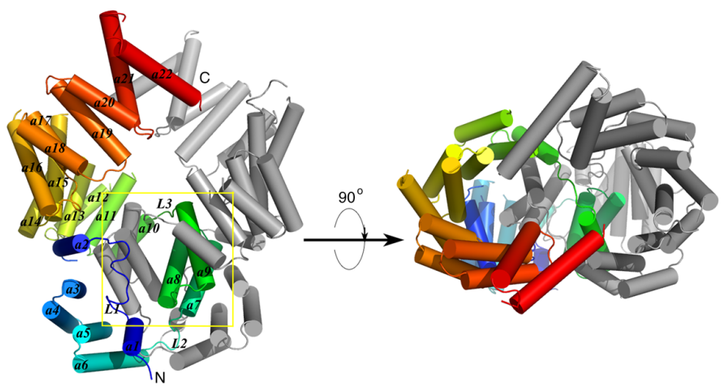

Figure 1: IFIT2/ISG54 is a domain swapped dimer with a super-helical structure at its C-terminal. One monomer is shown in gray and the other one is shown in spectral colors, from blue at the N-terminal to red at the C-terminal. The secondary structure elements are labeled. In the ISG54 dimer, helixes 7-9 from one monomer insert into the other monomer, forming the domain swapped interface, as shown in the yellow box. Left panel: side view of the structure; Right panel: top view.

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