Structural Studies of Domain IV RNA from Type I Picornaviral Internal Ribosome Entry Sites

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Many single-stranded RNA viruses synthesize viral proteins in host cells through non- canonical cap-independent translation initiation mechanisms. A prominent mechanism adopted by several human and animal viruses utilizes well-structured cis-acting RNA elements called internal ribosome entry sites (IRESs). Though many biochemical studies have appreciated the essential role of IRESs, the lack of high-resolution structures has hampered the elucidation of mechanisms and RNA-protein interactions. The IRES domains are known to interact with the eukaryotic translation initiation factors (eIFs) and 40S ribosomes via a multistep, dynamic assembly process, which makes their structural studies very challenging. Using enterovirus 71 as a model picornavirus, here, we aim to determine the high-resolution structure of domain IV of type I picornaviral IRES, which has been shown to recruit an essential host protein Poly-C Binding Protein-2 (PCBP2) during the translation initiation. Using Fab-assisted RNA crystallography, we have obtained crystals of the Fab-RNA complex that diffracted to 3.2 Å resolution. We phased the data by molecular replacement with the already available Fab structure and started modeling the RNA. Building the intact RNA model was challenging as this moderate-resolution diffraction data generated a poor electron density map. Therefore, our next steps are to screen more conditions and optimize crystallization to improve crystal diffraction. Additionally, we have already purified a recombinant human PCBP2 protein. We will perform binding studies of the dIV and PCBP2 using native gel-electrophoresis and isothermal titration calorimetry (ITC) and advance the dIV-PCBP2 complex for the crystallization trials. We anticipate our crystallographic studies will provide atomic-level information on how picornaviral IRES dIV RNA interacts with its protein counterparts and help develop targeted therapeutics to combat diseases caused by picornaviruses.