

The Crystal Structure of Coxsackievirus 5' RNA Replication Platform

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The extreme 5' untranslated region of the CVB3 (+)-sense RNA genome contains a conserved cloverleaf-like (CL) RNA domain that interacts with the host PCBP and viral 3CD proteins through its stem-loops for initiating genome replication. However, atomic-level detail and interactions of the 5' CL are largely unknown due to the lack of their high-resolution structures. Here, we have reported the crystal structure of intact CVB3 5'CL at 1.9 Å resolution in complex with Fab BL3-6 antibody chaperone. The crystal structure assumes a compact, H-type antiparallel 4-way junction fold composed of one stem and three stem-loop regions. The subdomains assemble into two sets of co-axially stacked helices, with each coaxial stacking forming almost a continuous A-form helix. The sA helix stacks on the sD helix and the sB helix on the sC helix. Remarkably, the crystal structure also revealed unprecedented long-range interactions between the sC-loop and the sD helix's Py-Py region, which agrees well with our NMR results, confirming that these structural features observed in the crystal also exist in the solution. Our crystal structure likely represents a conserved architecture of the enteroviral 5'CLs, including the sC-loop and Py-Py interactions, based on phylogenetic and 3C protein binding analyses. With this being the first high-resolution structure of the enteroviral 5'CLs, our research will pave the way for future studies into the mechanism of enteroviral genome replication and the development of antiviral drugs that target this RNA-centric platform.