Crystal Structures of Replication-Linked Rnas from Enteroviral Genomes

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The extreme 5′-end of an enteroviral RNA genome contains a highly conserved cloverleaf-like domain that recruits multiple protein factors such as 3CD and PCBP2 to form an essential ribonucleoprotein complex for initiating the genome replication. Using a Fab-assisted RNA crystallography, we have determined the high-resolution crystal structures of such cloverleaf RNA domains from coxsackievirus and rhinovirus. The RNA adopts an H-type four- way junction comprising four stem-loop subdomains (sA, sB, sC, and sD) with co-axially stacked sA-sD and sB-sC helices. In the crystal structures, tertiary interactions between absolutely conserved adenosine in the sC-loop and Py-Py region within the sD stem
organize a near-parallel orientation of the sA-sB and sC-sD helices. Our NMR studies further confirmed that these long-range interactions occur in solution and without the Fab.
Comparison of crystal structures from two enteroviruses (coxsackievirus and rhinovirus) and phylogenetic analyses suggest that the RNA architecture, including the adenosine-Py-Py tertiary contacts, is highly conserved, meaning that our crystal structures likely represent a common structural fold of enteroviral cloverleaf-like domains. The presence of common architectural motifs also allowed us to predict models of seven other enteroviral species using homology modeling. Furthermore, the 3C and PCBP2 binding studies using isothermal titration calorimetry and native gel electrophoresis suggest that these conserved features play important roles in stabilizing the overall RNA structure, influencing protein binding. The H-shaped architecture of the RNA thus facilitates the positioning of the sD and sB loops on opposite ends of the folded RNA for recruiting 3C and PCBP proteins, which perhaps helps avoid a steric clash between these proteins when they assemble into the pre-organized RNA structure during viral genome replication.