The Crystal Structure of A PTE RNA Element from A Cactus Virus That Binds Human Eif4e.

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The 3' cap-independent translation enhancers (3'-CITEs) at the 3' untranslated region (UTR) of tombusvirus genomes promote translation of viral RNA genome that bypass the requirement of 5'-cap to recruit various host translation initiation factors through the cap- independent mechanism. In the absence of a 5'-cap, they have been proposed to participate in long-range interaction with 5' UTR to circularize the mRNA and hence prepare the genome for translation. These 3'-CITEs are known to bind the ribosomal subunits or translation initiation factors. However, the lack of a high-resolution, three-dimensional structure has limited our understanding of how these structured RNA elements mimic the 5'-cap to recruit the eukaryotic initiation factors. In this study, we have determined the crystal structure of a PTE (Panicum Mosaic Virus-like 3'-CITE) from the Saguaro Cactus Virus (SCV) genome which was made possible by a Fab chaperone-assisted crystallography. Our structure reveals a T-shaped three-way junction that constitutes an unusual pseudoknot architecture between the G-rich bulge and the pyrimidine-rich domain. Consistent with the previous biochemical studies, the hypermodified G18 nucleotide flips from the rest of the PTE structure to create the binding site for eIF4E. The similar crystal structures of G18C, G18A, and G18U mutants compared to the wild-type structure demonstrate that the PTE structure is preorganized. Furthermore, despite being a plant virus, the binding of PTE with human eIF4E shown by our isothermal titration calorimetry (ITC) and gel electrophoresis mobility shift assays supports the hypothesis that this PTE domain represents a structured RNA mimic of the mRNA 5′cap.