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NMR studies on the solution structures of VBS RNAs of Yeast Saccaromyces cerevisiae virus

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In the plus strand of ScV-M1 (1.9 kb) of the yeast double-stranded RNA virus, Saccharomyces cerevisiae virus, two adjacent viral protein binding sites (VBS), named SL1 and SL2, are necessary and sufficient for viral protein binding and for replication of the viral plus strand. NMR studies on the VBS RNAs, SL1 and ¹³C, ¹⁵N doubly labeled SL2 will be presented. Several 3D HCCH experiments have accomplished full assignment of SL2 RNA. These VBSs consist of stem-loop structure with the same 5'-nt loop sequences. The hairpin-loop at the end of the second stems of both of SL1 and SL2 consists of five bases, GAUUC. Base stacking continues for three nucleotides on the 5'-side of the loop. The final structure contains a single hydrogen bond involving the guanine imino proton and the carbonyl O2 of the cytosine between the nucleotides on the 5'-and 3'-ends of the loop, although they do not form a Watson-Crick base pair. All three pyrimidine bases in the loop point to the direction of the major groove, which implies that viral protein may recognize the major groove of the loop region. The residues at and near the bulges exhibit dynamic sugar conformations. In case of SL1 RNA having a mononucleotide bulge moiety, bulged adenine residue is stacked in the stem, but there are NOEs suggesting that the bulged adenine spends part of the time as a bulged-out conformation. The structures of the two VBSs, SL1 and SL2, are similar but SL2 which contains a larger internal loop appears to be more flexible. The comparison between SL2 RNA structures with and without incorporating residual dipolar couplings into the structure determination will be made.