Computer Prediction Meets Biological Function: the Role of RNA Structure in Viral RNA Replication

Kyle Johnson, Assistant Professor
Department of Biological Sciences and Director, DNA Analysis Core Facility
Border Biomedical Research Center, UTEP

As part of ongoing studies to determine the mechanism of viral RNA replication, we are investigating the minimal RNA sequences necessary for replication of nodavirus genomic RNAs, and the RNA secondary structures they adopt, using a well-defined reverse-genetic system in yeast cells. Members of the virus family Nodaviridae provide an excellent model system for the study of RNA replication due to their genetic simplicity, their robust yield of replication products, and the ability of their RNAs to replicate in cells from a wide variety of organisms. Nodamura virus (NoV), the prototype of the alphanodavirus genus, contains a bipartite positive strand RNA genome. The larger segment, RNA1, encodes the viral RNA-dependent RNA polymerase (RdRp), while RNA2 encodes the precursor to the viral capsid proteins. Subgenomic RNA3, which encodes a suppressor of RNA interference, is required for replication of RNA2. The role of RNA secondary structure in the genome replication of other RNA viruses has been well established. For the nodaviruses, this role has been studied only for Flock House virus (FHV). A long-range interaction between two regions of RNA1 was required for synthesis of subgenomic RNA3. However, the role of RNA secondary structure in nodavirus genome replication remains unclear. Previous studies with FHV showed that sequences at the 3’ end of RNA2 were critical for RNA replication. The exact role of these sequences is unknown, but they may provide a recognition site for the viral RdRp during RNA replication. If so, we hypothesize that the secondary structure adopted by these RNA sequences is essential for their role in RNA replication. We wondered whether this sequence requirement also held for NoV, which would lend phylogenetic support to the FHV results. We used bioinformatics software to generate predicted secondary structures of a series of 3’-terminal fragments derived from the RNA2 segments of six nodaviruses. All of the programs we used consistently predicted the presence of a conserved stem-loop structure in each of these RNA2 segments. The consistent prediction of this structure encouraged us to target this region for further study to test whether the predicted structure can be verified experimentally and whether it has biological relevance in the viral life cycle. We used nuclease mapping to confirm that this structure does form at the 3’ end of NoV RNA2 in solution. The stem-loop structure was deleted from a cDNA clone of RNA2 using site directed mutagenesis. When the deleted version of NoV RNA2 was tested for its ability to replicate in transformed yeast cells, we observed a dramatic decrease in RNA synthesis. These data suggest that the 3’-terminal region plays a significant role in replication of RNA2. We hypothesize that it may function as a promoter for synthesis of a complementary negative strand replication intermediate.

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For more information, please contact the Colloquium Chair, Dr. Max Shpak, at mshpak@utep.edu or 915.747.8903.