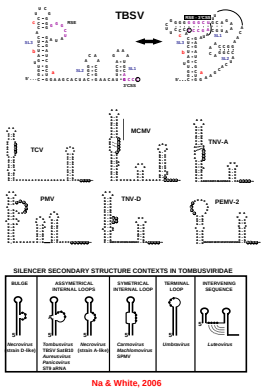
Can *cis*-acting Elements in TBSV be used to Predict Similar Structures in Other Members of the Family *Tombusviridae*?

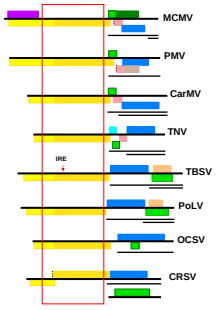
Kay Scheets and Stephanie Shryock, Botany Dept., Oklahoma State University, Stillwater, OK 74078

RNA secondary structures have been shown to be important *cis*-acting factors in the life cycle of RNA viruses. Recent work has analyzed the structure/function of the Replication Silencer Element (RSE) and 3' Complementary Silencer Sequence (3'CSS) controlling the switch between translation and replication of *Tomato bushy stunt virus* (TBSV) [1]. Similar structures were proposed for other members of the family *Tombusviridae* and related genera [1].



Na & White, 2006

Another important structure required for efficient replication of TBSV is the Internal Replication Enhancer (IRE) which is always found in DI-RNAs of tombusviruses (region II) [2-4]. The IRE is located within the RdRp readthrough ORF, and there is some sequence/structure relatedness for all members of this genus. Members of the family *Tombusviridae* show their greatest protein homology within the RdRp. We were interested to see if secondary RNA structures could be identified in similar locations of other viruses in this family.

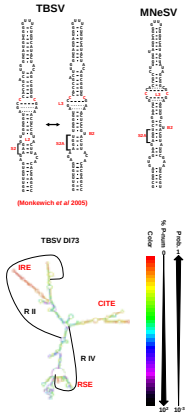


Genome organizations of the type members of each genus are shown. The regions with the highest protein sequence similarity for RdRps are boxed in red.

Methods

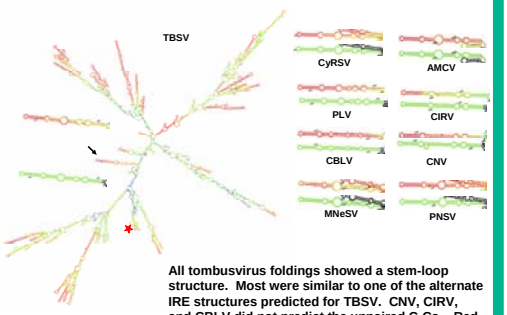
The 95 nt region comprising the long stem-loop identified in TBSV was used to select the related regions from all family members with complete genomes listed in GenBank using ClustalW. This identified a region approximately 300 nt downstream of the readthrough stop codon for all viruses except the necroviruses due to their low protein homology in this region. For necroviruses, the 95 nt segments were selected based on the protein alignments. The complete genomes were submitted to the mfold server [5] with pairing number (p-num) analysis and then with the 95 nt region highlighted for easy identification. Additionally, the 95 nt regions were folded.

Internal Replication Enhancer (IRE)



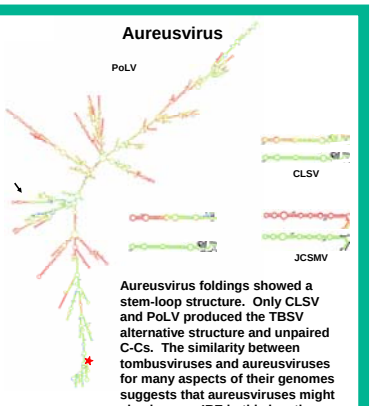
Structural analysis supports two alternative IRE structures for TBSV [2]. The red C's in L3 are important for specific binding of TBSV's p33 [2, 3]. MNeSV is an example of folding with the alternate structure [6]. TBSV DI-RNAs have sequence from four regions of TBSV genome (RI to R IV). Red abbreviations indicate structures with identified functions [4].

Tombusvirus



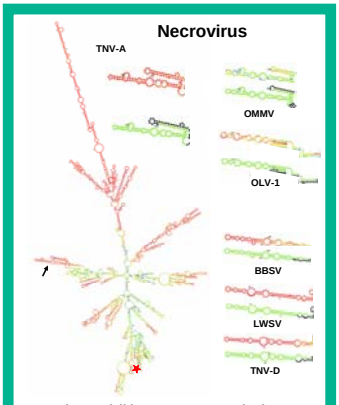
All tombusvirus foldings showed a stem-loop structure. Most were similar to one of the alternate IRE structures predicted for TBSV. CNV, CIRV, and CBLV did not predict the unpaired C-Cs. Red star marks the 5'/3' ends.

Aureusvirus



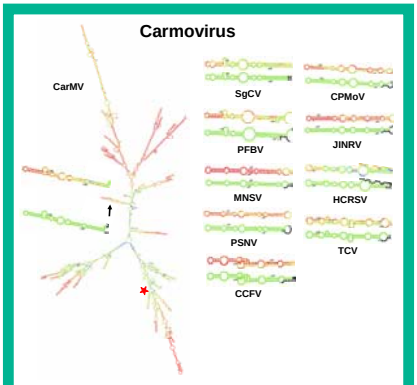
Aureusvirus foldings showed a stem-loop structure. Only CLSV and PoLV produced the TBSV alternative structure and unpaired C-Cs. The similarity between tombusviruses and aureusviruses for many aspects of their genomes suggests that aureusviruses might also have an IRE in this location.

Necrovirus



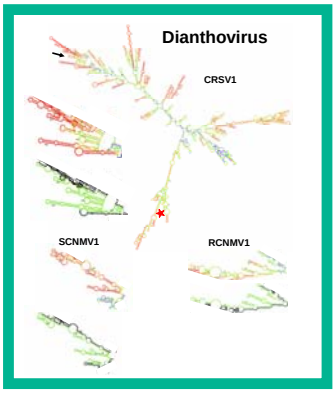
Necroviruses fell into two groups; single stem loops or shorter stem loops with an extention pairing to other regions. None showed similarity to the TBSV IRE.

Carmovirus



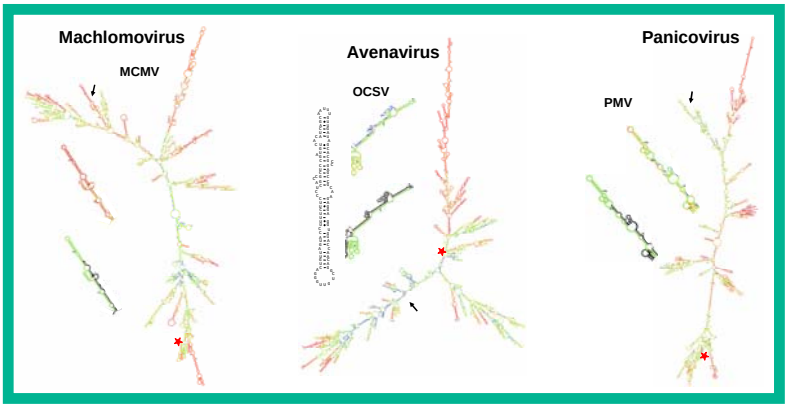
All carmoviruses but CCFV produced stem-loop structures. Although there was some structural similarity among some members, there was no overall structure apparent, and none showed similarity to the TBSV IRE.

Dianthovirus



The 95 nt regions of the three monotypic genera and dianthoviruses did not produce discrete stem loop structures in the whole genome foldings. The foldings of the individual 95 nt regions of dianthoviruses, MCMV and PMV produced complex structures. OCSV 95 nt folding produced a stem-loop structure, but it did not resemble the TBSV IRE.

Machlomovirus / Avenavirus / Panicovirus



Conclusion

Except for the closely related aureusviruses, there is no indication that other members of the family *Tombusviridae* contain a structure that might function as an IRE located in a similar part of the genome. It is interesting to note that the structure predicted for JCSMV has the lowest p-num values for aureusviruses but not the best structural match to TBSV IRE.

References

1 Na, H., White, K.A. 2006. Structure and prevalence of replication silencer—3' terminus RNA interactions in *Tombusviridae*. Virology 345, 305-316.

2 Monkewich, S., Lin, H.-X., Fabian, M.R., Xu, W., Na, H., Ray, D., Chernysheva, O.A., Nagy, P.D., White, K.A., 2005. The p92 polymerase coding region contains an internal RNA element required at an early step in tombusvirus genome replication. J. Virol. 79, 4848-4858.

3 Pogany, J., White, K.A., Nagy, P.D., 2005. Specific binding of tombusvirus replication protein p33 to an internal replication element in the viral RNA is essential for replication. J. Virol. 79, 4859-4869.

4 White, K.A., Nagy, P.D., 2004. Advances in the molecular biology of tombusviruses: Gene expression, genome replication, and recombination. Prog. Nucleic Acid Res. Mol. Biol. 78, 187-226.

5 Zuker, M., 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Research 31, 3406-3415. http://www.bioinfo.rpi.edu/applications/mfold/

6 Scheets, K. and Redinbaugh, M. G. (in press) Infectious cDNA transcripts of *Maize necrotic streak virus*: infectivity and translational characteristics. Virology