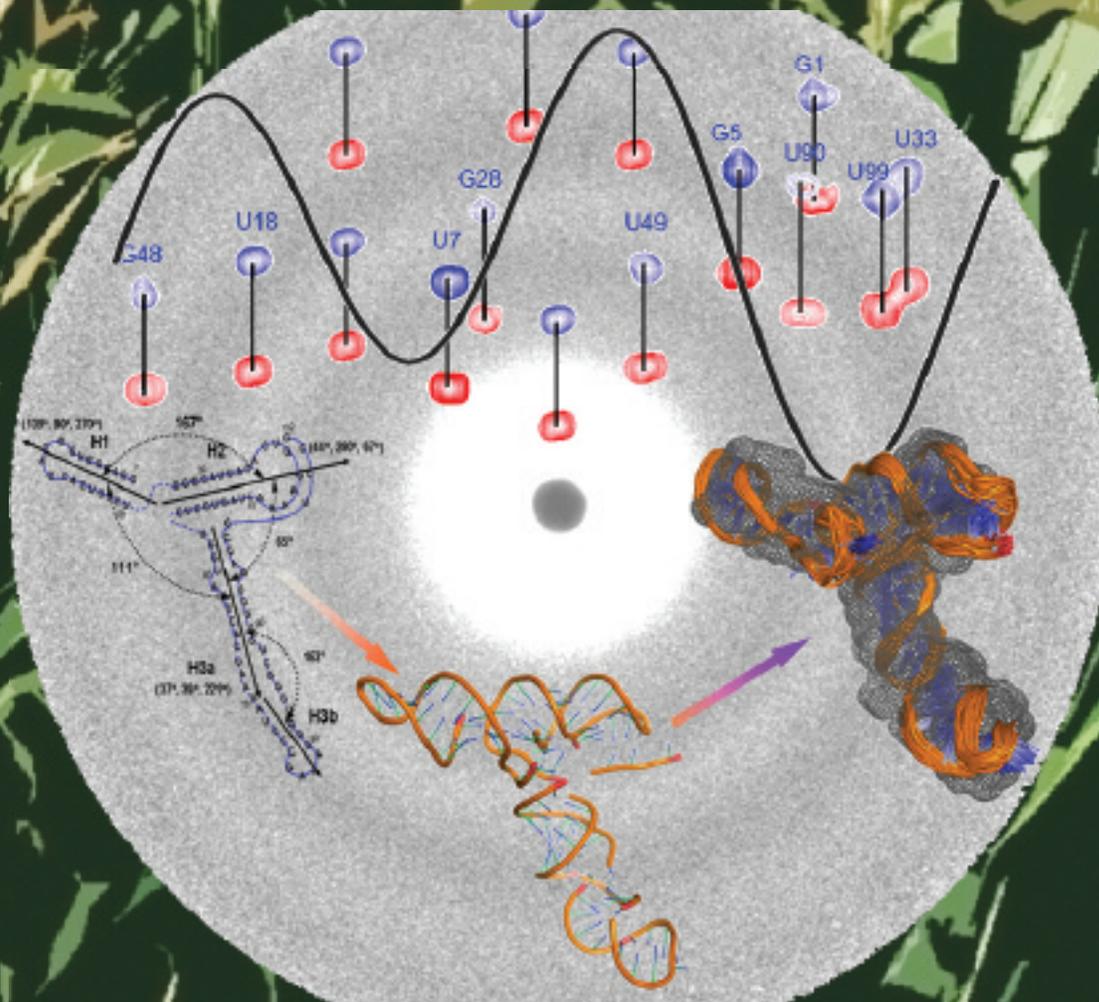


THE PUSH AND PULL OF PLANT VIRUSES

New insights into the way a simple-seeming plant virus, the turnip crinkle virus (TCV), goes about replicating in infected cells have been obtained using solution nuclear magnetic resonance spectroscopy (NMR) and small/wide angle x-ray scattering (SAXS/WAXS) studies with a novel methodology at two APS x-ray beamlines. The findings build on earlier work but provide a clearer understanding of RNA genetics and can even explain the paradox of how RNA translation and protein synthesis operate in parallel even though they pull the machinery, the enzymes and ribosome, of the host cell in different directions. The work may have implications for coping with crop plant viral diseases.



The turnip crinkle virus has just 4054 bases in its genetic code, making it among the smallest and simplest of the single-component RNA viruses that infect plants. Moreover, sub-viral strands of TCV's RNA can use the enzyme polymerase to replicate without the rest of the virus being involved. So molecular biologists find this virus useful as templates for genetic studies of replication and recombination.

TCV also uses several structural elements of messenger RNA to make translation of its genetic code more efficient. This allows it to replicate rapidly in the infected host plant without being subjected to the host cell self-defense system. Some of these structural elements also work together to trigger translation and boost efficiency. Now, researchers from the National Cancer Institute at Frederick; Johns Hopkins University; Argonne; the University of Maryland College Park; and the National Institutes of Health have obtained the three-dimensional solution structure of one such structural element: a ribosome-binding element in TCV. The researchers developed a novel method that allowed them to combine the benefits of SAXS/WAXS with nuclear magnetic resonance imaging (Fig. 1). The SAXS/WAXS experiments were performed at XSD beamlines 12-ID-C,D and and Bio-CAT beamline 18-ID at the APS.

TCV lacks the known translational enhancers, the so-called 5' cap and the 3' poly(A) tail, which are normally used for starting protein synthesis in animals, plants, and fungi. As such, understanding how translation is initiated and enhanced to make TCV infectious to host plants seemingly relies on the structural element located in the region after the protein synthesis stop signal (stop codon). This region is called the 3' untranslated region (3' UTR). This

structural element helps the virus to hijack the host plant's protein factory, the ribosome, and use it to replicate the viral proteins without hindrance from the plant's self-defense mechanisms. One of the problems facing researchers trying to understand the underlying molecular biology is that the two key processes, translation and replication, seem to be mutually exclusive as they operate in the opposite direction, one synthesizing RNA, the other proteins.

The 3' UTR in TCV has a cap-independent translation element (CITE), within which is a ribosome-binding structural element (RBSE). It is this section that hijacks the large subunit of the host ribosome to make viral proteins. There is a large symmetric loop within the RBSE, which plays a key role in coordinating translation and replication. The structure determination achieved by the researchers in this study offers the global structure of this loop, the 102-nucleotide RBSE RNA. The structure reveals that it shares almost every structural feature with transfer RNA (tRNA) in solution, as if the 102-nt RBSE were a large cousin of tRNA. There are two hairpins, H1 and H2, linked by a 7-nucleotide unit, which resembles to the variation loop in tRNA even in its sequence, H3, and is accessible to interactions with the ribosome.

This new global structure provides an insight into how the unit can bind to the ribosome and might explain how the system can switch between replication and protein production by revealing that H1 and H2 work together in translation but independently of H3, which is involved in protein synthesis through ribosome binding.

This is the first experimental determination of a functional element in the 3' UTR of RNA from any organism and could open up research into viral infectivity that has implications beyond this

simple plant virus. The researchers feel that the key to the success of the study were the SAXS/WAXS experiments performed at APS. — *David Bradley*

See: Xiaobing Zuo¹, Jinbu Wang¹, Ping Yu¹, Dan Eyler², Huan Xu^{1†}, Mary R. Starich¹, David M. Tiede³, Anne E. Simon⁴, Wojciech Kasprzak¹, Charles D. Schwieters⁵, Bruce A. Shapiro¹, and Yun-Xing Wang^{1*}, "Solution structure of the cap-independent translational enhancer and ribosome-binding element in the 30 UTR of turnip crinkle virus," *Proc. Nat. Acad. Sci. USA* **107**(4), 1385 (January 26, 2010). DOI: 10.1073/pnas.0908140107

Author affiliations: ¹National Cancer Institute at Frederick, ²Johns Hopkins University, ³Argonne National Laboratory, ⁴University of Maryland College Park, ⁵National Institutes of Health. [†]Present address: Fudan University

Correspondence:
*wangyunx@mail.nih.gov.

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12-ID-C,D • XSD • Chemistry, materials science, physics • Grazing incidence small-angle scattering, small-angle x-ray scattering, surface diffraction, wide-angle x-ray scattering • 4.5-36 keV • On-site • Accepting general users

18-ID • Bio-CAT • Life Sciences • Fiber diffraction, microdiffraction, microfluorescence (hard x-ray), small-angle x-ray scattering, time-resolved x-ray scattering, micro x-ray absorption fine structure • 3.5-35 keV • On-site • Accepting general users

< Fig. 1. A new method (G2G, "Global measurements to Global structure") has been developed for structure determination of large RNAs in solution using residual dipolar coupling (RDC) from NMR measurements, represented by the blue and red contour linked by black lines with residue labels; and SAXS/WAXS measurements, represented by gray co-centered circular rings in the background. The black "wave" is the RDC-structural periodicity correlation curve that was used to extract the orientation of the RNA duplexes. At the lower left is a two-dimensional (2-D) drawing of the topology of the 102-nt RNA. The low-center model is a rendering of the 2-D drawing of the topology in three-dimensional (3-D) space; at the lower right is the refined 3-D ensemble of the 102-nt RNA structures that were restrained with RDC and SAXS data. (For a detailed description of the G2G method, please see *J. Mol. Biol.* 393, 717 (2009). Cornfield photo courtesy of Sam Mugraby, Photos8.com, www.photos8.com.)