Molecular Basis for Flexibility in a Flexible Filamentous Plant Virus Discovered From Research on a Common Taiwan Bamboo Virus

An international research team led by Dr. Na-Sheng Lin, Distinguished Research Fellow at the Institute of Plant and Microbial Biology, Academia Sinica, Dr. Yau-Heiu Hsu, Distinguished Professor of the Graduate Institute of Biotechnology, National Chung-Hsing University, and Dr. Edward H. Egelman, Professor of the Department of Biochemistry and Molecular Genetics, University of Virginia, recently determined the filamentous structure of the flexible virus, *Bamboo mosaic virus* (BaMV) at the near-atomic level. Previous structural studies of filamentous viruses began more than 75 years ago but never succeeded, owing to the virus' extreme flexibility. The new finding solves the mystery of how the flexible virus particles are maintained even as the structure deforms due to mechanical forces. Flexible filamentous plant viruses cause more than half the viral crop damage in the world, but are also potentially useful for applications in agriculture and biotechnology. The research was published in *Nature Structural and Molecular Biology* on July 13, 2015.

Filamentous plant viruses are broadly classified into rigid (rod-like) and flexible viruses. The first virus to be identified was a rod-like virus – *Tobacco mosaic virus* (TMV). Starting in 1936, the structure of TMV was analyzed by X-ray fiber diffraction because, due to its rigidity, virions (viral particles) align easily into a liquid crystalline state. In contrast, it was not previously possible to obtain an atomic model for the flexible plant viruses, such as BaMV, because these viruses cannot be crystallized and are too flexible to be analyzed using high resolution X-ray fiber diffraction or cryo-electron microscopy (Cryo-EM).

In this study, the structure of BaMV was determined at the near-atomic level using CryoEM, with images captured using a direct electron detector, followed by 3D reconstruction using Iterative Helical Real Space Reconstruction (IHRSR) software developed by Dr. Egelman. The protein model construction began by docking a crystallized fragment, capsid protein (CP) without N or C termini, from *Papaya mosaic virus*, to generate an initial model. Next, the missing N and C termini were built using simulation software, Rosetta, and a novel enumerative backbone sampling protocol on the amino acid sequence of BaMV CP. In addition, a model of single stranded viral RNA encapsidated by CPs was obtained by docking the *Rift valley fever virus* RNA structure into the density map. By combining the protein and RNA structural data, the first near atomic structural model of flexible filamentous virus was determined. The structural model of BaMV shows that the CPs are left-handed and loosely packed, as opposed to the right-handed and tightly packed virion assembly observed for TMV. Its N terminus is well exposed, as expected, and the C terminus is

pointing toward the center of the virion. The model explains how a flexible virion can be maintained as it is deformed due to mechanical forces.

This new finding will pave the way for research into virion assembly, structural stability, and the virus infectious cycle. Moreover, this discovery can be further applied to both biotechnology (for the design of vaccines and protein carriers) and nanotechnology in engineering, contributing to the field of structural virology.

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The full article, entitled “The Molecular Basis for Flexibility in the Flexible Filamentous Plant Viruses”, is available on the *Nature Structural and Molecular Biology* website at: http://www.nature.com/nsmb/journal/v22/n7/index.html