Structural Studies of the Flexible Filamentous Plant Viruses

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The Plant Viruses of Interest

In the *Potyviridae* family:
- Wheat streak mosaic virus (WSMV)

In the *Alphaflexiviridae* family:
- Potato virus x (PVX)
- Papaya mosaic virus (PapMV)
WSMV, PVX, and PapMV

- Significant agricultural pathogens
- Structure determination is important for viral assembly and disassembly studies
- Important for structural comparisons across all potyviruses and potexviruses – particularly WSMV
- Hybrid method of structure determination: combination of cryo-electron microscopy (cryo-EM) and X-ray fiber diffraction
- Currently have low to medium resolution models
Cryo-EM models of A) WSMV at 25 Å resolution and B) PVX at 21 Å resolution. Bars = 25 Å.
Ultimate Goal: Improve the Current Understanding of the Structural Commonalities Between All Flexible Filamentous Plant Viruses

Approached this in two ways:

- Produce a higher resolution model of WSMV by improving solution conditions
- Perform a comparative structural analysis between PVX and PapMV through sequence comparisons and structure docking
Improving WSMV Solution Conditions

- Extremely flexible
- Tendency to tangle with other virions
- Not amenable to helical reconstruction
- Difficult to produce fiber diffraction samples
Producing a 3-D Model by Cryo-EM

Selecting Virus Segments

Cryo-electron micrographs of virus

Select straight well-defined regions

Bar = 100 nm
Producing a 3-D Model by Cryo-EM

Selecting Virus Segments

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Identify Solution Conditions That Will Straighten and Orient the Virus

In previous semesters:

• Alteration of pH and divalent cation concentration
• Preliminary testing of fragmentation by freeze-thawing
  • Aided in straightening particles
  • However, better fragmentation method needed

This semester:

• Refine freeze-thaw fragmentation procedure
• Explore other fragmentation methods
Fragmentation Methods Tested:

- Freeze-thawing
- Passing sample through small gauge needle
- Sonication
All Methods Yielded Similar Results

20 Cycles of Freeze-Thawing. Bar = 100 nm

10 Passes Through Needle. Bar = 100 nm.

10 Seconds of Sonication. Bar = 100 nm
Original Sample

Bar = 100 nm
Produce New Virus Samples For Experimentation

- Grow new wheat crops
  - Takes ~4-6 weeks to sow, inoculate with WSMV, and harvest

- Screen samples by TEM prior to experimentation
New Sample: WSMV 13/10
Next Sample: WSMV 13/11

Bar = 100 nm
Sample Preparations

- From mid-fall to early spring, 6 new virus preparations were made
- Inability of WSMV to propagate and assemble in any
- No intact virus samples available for experimentation

Decided to shift focus of project…
Comparative Structural Analysis

• New direction: evaluating structural elements common to the flexible filamentous viruses

• Focused on the coat protein structures of PVX and PapMV.

• Methods developed to be later applied to WSMV
What is known about PapMV and PVX?

- Know the structure of C-terminally truncated PapMV coat protein by x-ray crystallography (Yang et al., 2012)
  - 7 alpha helices
  - Truncated structure fits in the outer domain of subunit
  - Several functionally important amino acids determined
    - Lys97 for RNA-subunit binding
    - Phe13 and hydrophobic pocket of 8 amino acids for subunit polymerization
- Have a 21 Å resolution structure of PVX
- Know the sequences of both
Comparison of sequences
Creating a Homology Model of the PVX Coat Protein

Imposed the secondary and tertiary structure of the PapMV coat protein onto the PVX sequence
Structure Docking

- *Segger* (Pintilie et al., 2010) can segment a virus model into subunits

- Can then dock structure of interest into the subunit
  - Either automatically
  - Manually

Docked PVX homology model into a segmented PVX cryo-EM subunit model

From Yang et al., 2012
Automatic Docking of PVX Coat Protein Homology Model
Manual Docking of PVX Coat Protein Homology Model
• Strong similarities between PVX and PapMV in sequence and their likely secondary structures
• Functionally important amino acids are conserved
• Manual docking of homology model → new and believable orientation
• Strong similarities between the two coat protein structures
• Likely translates to similar mechanisms in which they perform biological functions
Future directions…

- Exploring various docking programs
  - Rigid-body docking vs. flexible-body docking

- Improving WSMV solution conditions
  - Overcoming difficulty to propagate
  - Obtaining a better resolution cryo-EM model
  - Extending the comparative analysis of PapMV and PVX to new WSMV model
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