New and Notable

A Single-Molecule View on the Disassembly of Tobacco Mosaic Virus

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Viruses are common microbes that significantly affect human health. A virus particle typically comprises a long nucleic-acid chain coated by a densely packed protein capsid. Once getting inside living cells or other organisms after infection, the virus particles quickly uncoat the protein capsid and replicate themselves.

Although great efforts have been devoted to exploring the disassembly/assembly mechanism of viruses in the past few decades (1–3), a molecular picture of virus disassembly is still lacking. In this issue of *Biophysical Journal*, the article "Single-Molecule Force Spectroscopy Study on the Mechanism of RNA Disassembly in Tobacco Mosaic Virus" by Liu et al. (4) describes an AFM-based study on the disassembly of tobacco mosaic virus (TMV) at the single-virus-particle level. Although AFM has recently evolved into a general tool to study the conformational dynamics of biomacromolecules and the unbinding of ligand-receptor pairs or chemical bonds, manipulating an intact virus made of a gigantic protein-nucleic acid complex is challenging (5–9). This is because the nucleic acid is normally coated/protected by the coat proteins, making it inaccessible from the outside. In addition, it is difficult to control the pulling geometry of a virus particle in single-molecule AFM experiments.

In 2010, Liu et al. (10) successfully developed a method for the study of single tubular virus particles using single-molecule AFM. They were able to perpendicularly immobilize cysteine-labeled TMV particles on gold-coated surfaces via the 3′-end of the virus. Subsequently, the amino-coated cantilever tip can preferentially grasp the negatively charged 5′-end of RNA and stretch it. Thanks to this smart experimental design, Liu et al. (10) were able to unambiguously observe the RNA disassembly from single TMV nanoparticles in the 5′-end to 3′-end direction.

In Liu et al. (4), they found that the TMV particles disassembled in a stepwise fashion, showing sawtooth-like force plateaus (see their Fig. 1, (4)). Liu et al. (4) provided detailed studies on the disassembly of TMV particles at neutral pH and low calcium concentrations, mimicking the in vivo conditions of the host cells (4), and found that both neutral pH and low calcium concentrations can facilitate the disassembly of TMV particles, which is a necessary step for their replication.

Liu et al. (4) found that increasing pH from 4.7 to 7.0 destabilizes the TMV structure and leads to lower plateau forces. By performing dynamic force spectroscopy measurements and inspecting the structure of TMV particles (11), Liu et al. (4) found that such a decrease in unbinding forces may be due to the conformational change of coat proteins. At pH 4.7, the inner loops of TMV coat proteins adopt an extended conformation, making the RNA binding sites well protected inside the protein capsid (see their Fig. 5). However, at pH 7, these loops became disordered, making the RNA binding sites more accessible by solvent. Therefore, a pH switch from 4.7 to 7.0 destabilizes RNA-coat protein interactions. Interestingly, the disordered loops of TMV at pH 7.0 could lead to long-range structural hindrance for RNA moving outward from the protein capsid. They also decreased the reassembly probability significantly.

Liu et al. (4) also found that the 5′-end of the RNA of TMV can be exposed upon lowering the calcium concentrations. Some of the force-extension curves of TMV disassembly clearly show features of stretching uncoated RNA preceding the force plateau. These curves are of shorter persistence lengths. It was previously shown that the persistence length is very sensitive to different structural properties (12). The lower the calcium concentration, the higher the number of force-extension traces. Moreover, neutral pH could further help the dissociation of the coat proteins from 5′-end of the RNA. Intriguingly, although removing calcium could lead to the dissociation of the protein capsid at the 5′-end, the stability of the rest of the TMV remains unchanged in the absence of calcium.

Then, how does the rest of the TMV particle disassemble in vivo? By comparing the maximum work a ribosome can perform during translocation and the free energy that is required to mechanically dissociate the RNA from the coat proteins, Liu et al. (4) proposed that replication or translation motor (replisome) should be able to pull out the remaining genetic RNA from the protein capsid. However, at a low pH of 4.7, such cotranslational disassembly is inhibited.

Taken together, the single-molecule AFM measurements revealed the molecular mechanism for the disassembly of RNA from protein capsid of TMV particles inside the plant cell (Fig. 1): Once a TMV particle enters a plant cell during infection, increase in pH and decrease in calcium concentrations leads to exposing the 5′-end of TMV and weakening the RNA-coat protein interactions of the remaining part. Then the replisome is loaded onto the exposed RNA. The subsequent movement of replisome along the RNA mechanically drives the disassembly of the rest of the TMV

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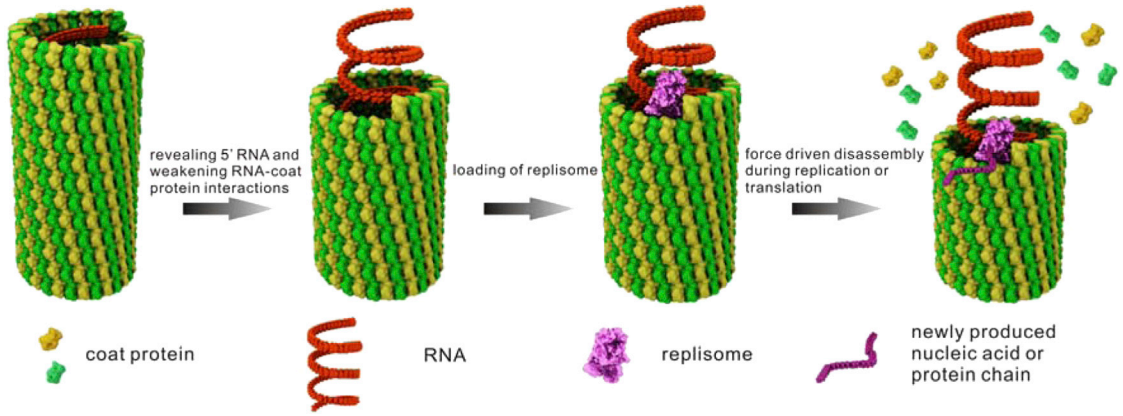

FIGURE 1 Schematic of the disassembly of TMV particle in vivo at neutral pH and low calcium concentrations.

particle. Because the guest RNA molecules are prone to be digested inside host cells, such a cotranslational disassembly mechanism could effectively protect the genetic RNA of TMV.

The work by Liu et al. (4) paves a way to study the disassembly of tubular virus particles using single-molecule AFM, which is not only of great fundamental interest but also of valuable practical application. For example, the acting mechanisms of antiviral drugs can be directly revealed. Tailoring the disassembly/assembly of virus particles could also extend their applications as novel biomaterials. However, even on the fundamental level, such a study has just begun.

There are many unanswered questions remaining:

1. Why do the 5′-end fragment and the remaining part of the RNA exhibit different calcium-dependent interactions with the same coat proteins?
2. Does the protein capsid retain its structure after the RNA is disassembled?
3. Can we directly correlate the peaks and valleys of the force plateaus with the RNA sequences with high precision?
4. Is the pH-dependent or calcium-dependent disassembly mechanism general among various viruses (13)?

Addressing these questions will certainly improve our understanding of the structure and evolution of viruses.

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