Structural Basis for the Coevolution of Tomato Mosaic Virus and the Resistance Protein Tm-1

The tomato mosaic virus (ToMV) resistance protein Tm-1 binds the helicase domain of ToMV replication proteins (ToMV-Hel) and thereby inhibits RNA replication. Herein, we report crystal structures for the complex between the N-terminal inhibitory domains of Tm-1 and ToMV-Hel. The complex contains a Tm-1 dimer and two ToMV-Hel monomers, with the interfaces between them bridged by an ATP. Residues in ToMV-Hel and Tm-1 involved in antagonistic coevolution are also found at the interface. The crystal structures gave us an atomic view of the step-by-step coevolutionary arms race between a plant resistance protein and a viral protein.

Viruses evolve so rapidly that they can escape host defense systems. To counter rapidly evolving viruses, the sequences of many host restriction factor genes are subject to positive selection and, consequently, rapidly mutate [1, 2]. Molecular evolutionary approaches have revealed residues important for resistance in host defense protein sequences. The use of such information in conjunction with the tertiary structures of related proteins greatly facilitates our understanding of virus–host evolutionary arms races [3].

We previously found that tomato mosaic virus (ToMV) and the resistance gene *Tm-1* have coevolved [4]. Here, we report atomic details of the coevolutionary arms race between them as deduced from several crystal structures and molecular dynamics simulations [5]. The ToMV resistance protein Tm-1 is a 754-amino acid protein that binds ToMV replication protein and inhibits ToMV RNA replication [6]. Tm-1 protein contains at least two domains: an uncharacterized N-terminal region (M1-K431) and a TIM barrel-like C-terminal domain. An *Escherichia coli*-expressed N-terminal fragment of Tm-1 (residues 1–431; Tm-1(431)) inhibits ToMV RNA replication *in vitro* [7]. We first determined the crystal structure of Tm-1(431) [Fig. 1(a)]. Tm-1(431) is a dimer and the residues T79–D112, which were under positive selection meaning that the sequences have rapidly changed [4], are exposed to the surface of the molecule and form a flexible loop and an α-helix [5]. A *Tm-1*-resistance-breaking ToMV mutant LT1 harbors mutations that cause Q979 to E and H984 to Y substitutions in the helicase domain of replication proteins (ToMV-Hel) [4]. We also determined the crystal structure of ToMV-Hel [Fig. 1(b)]. Q979 and E984 of ToMV-Hel are also exposed to the surface [8], where Tm-1 presumably targets.

We then crystallized a complex of Tm-1(431) and ToMV-Hel [Fig. 1(c)]. Since ATP is required for the ToMV-Hel–Tm-1(431) complex formation [5] but is also hydrolyzed by ToMV-Hel [9], ATPγS was supplied for crystallization. The asymmetric unit of the crystal contains a tetrameric complex with a 2:2 stoichiometry consisting of a Tm-1(431) homodimer and two monomeric ToMV-Hel molecules [5]. Each interface of ToMV-Hel and Tm-1(431) consists of the N-terminal region of the Tm-1 NN domain and two regions of ToMV-Hel, i.e., I1094–Y1109 in the C-terminal region and H975–M986, which forms a loop and an α-helix connecting the 1A and 2A domains. At least 21 residues in Tm-1(431) and 23 residues in ToMV-Hel directly contact each other. Residues involved in the antagonistic coevolution such as E979 and H984 of ToMV-Hel and T79–D112 of Tm-1(431) are found in the interface. Notably, an ATPγS molecule is found in each ToMV-Hel–Tm-1(431) interface, in addition to those found in the two ToMV-Hel NTPase active sites. Additional biochemical experiments suggested that the ATP molecule in the interface is necessary for the interaction between ToMV-Hel and Tm-1(431).

A naturally occurring amino acid change (I91 to T) in Tm-1 makes it a stronger inhibitor of ToMV RNA replication, which enables it to inhibit the replication of LT1 [4]. We also solved the structure of the ToMV-Hel–Tm-1(431/I91T) complex crystallized in the presence of ATPγS [5]. The overall structure of this complex is very similar to that of the ToMV-Hel–Tm-1(431) complex. In the ToMV-Hel–Tm-1(431/I91T) structure, T91 is positioned at the center of the interface with ToMV-Hel and is involved in a hydrogen bond network containing water molecules. The structural information reasonably explains how the I91 to T substitution strengthens the inhibitory activity of Tm-1.

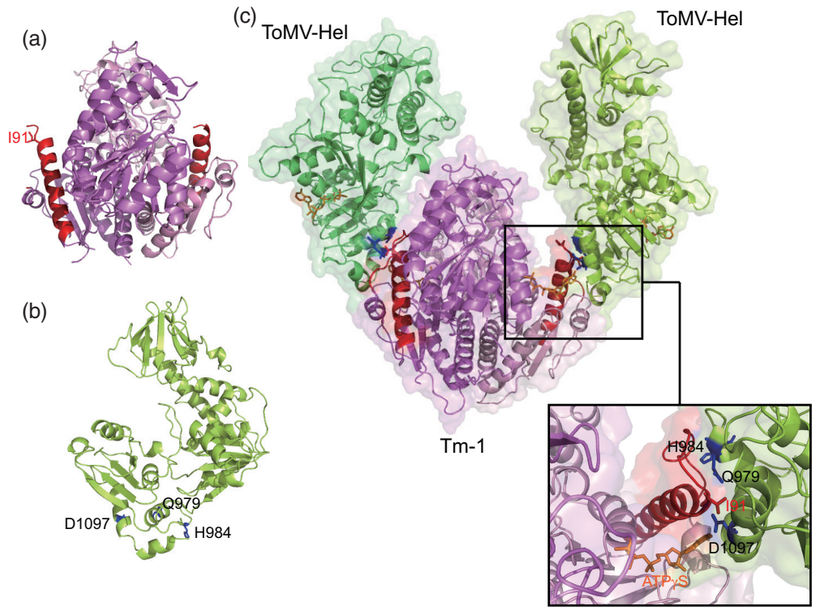

Figure 1: Crystal structures of ToMV-Hel and Tm-1(431). (a) Crystal structure of Tm-1(431). Residues T79-D112, which are under positive selection, are colored red. (b) Crystal structure of ToMV-Hel. The residues that are mutated in resistance-breaking mutants are shown as blue sticks. (c) Crystal structure of ToMV-Hel and Tm-1(431) complex. A close-up view of the interaction interface between ToMV-Hel and Tm-1 is shown on the lower right.

ToMV-LT1-derived mutants which additionally have E979 to K (LT1(E979K)) or D1097 to Y (LT1(D1097Y)) substitution in the replication proteins escape from the inhibition by Tm-1(I91T) [4]. Together with molecular dynamics simulations, we showed how viral mutations in LT1 (Q979 to E and H984 to Y) affect the interaction with Tm-1, and those in LT1(E979K) and LT1(D1097Y) affect the interaction with Tm-1(I91T). All of these substitutions compromised the interaction with Tm-1 or Tm-1(I91T). Taken together, we could visualize the molecular arms race between ToMV and Tm-1, comprising (i) inhibition by host, (ii) viral escape from the inhibition, (iii) acquisition of stronger inhibitory ability to inhibit the escaped virus mutants, and (iv) further viral escape from the inhibition.

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BEAMLINES

BL-5A, BL-17A, and AR-NW12A

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