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RNA Silencing Suppressor p21 Adopts an Unusual siRNA-Binding Octameric Ring Architecture

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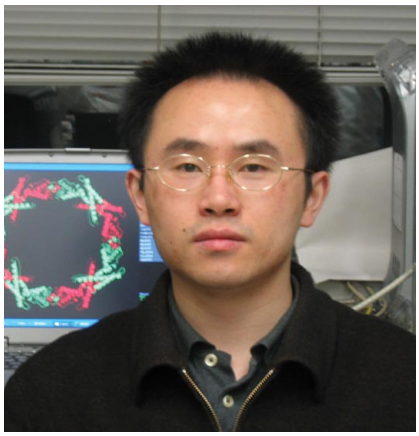
In plants, RNA silencing functions as an innate defense mechanism against virus infection, where viral RNA strands are degraded by an RNA-guided mechanism. Many plant viruses counter-attack by expressing proteins to inhibit this defense pathway. The p21 protein, a RNA silencing suppressor in Beet yellows virus, seems to function by binding siRNAs, a critical mediator in the process. The crystal structure of p21 reveals an octameric ring architecture involving a new mode of protein oligomerization. Putative RNA binding sites inside the ring are suggested by the structure. In addition, biochemical assays show that p21 binds various RNAs besides the siRNA duplex, suggesting the existence of an alternative suppression mechanism.

Unlike animals, plants have no immune system, yet they are still able to recover from virus infection and even acquire resistance. This is because plants have other innate defense mechanisms. RNA silencing, one of the available plant defense mechanisms, targets viral RNA for destruction. Double-stranded RNAs generated during viral replication can be perceived as a signal of virus infection by the plant host and diced into smaller fragments, called small interfering RNAs (siRNAs). These siRNAs, consisting of duplexes of 21 nucleotides in length, are a critical mediator in the silencing process. The siRNAs assemble with effector nucleases and instruct them to specifically cleave viral RNAs, thereby thwarting the proliferation and propagation of the virus. On the other hand, viruses need to evade or suppress this defense in order to survive and propagate. Studies have shown that many plant viruses do possess evolved weapons for counter-defense. The p21 protein is a RNA silencing suppressor recently discovered in Beet yellows virus. Because of its siRNA binding ability, p21is thought to inhibit the silencing process by inactivating siRNA. To understand the function and mechanism of p21, we have determined its crystal structure in its free state, thereby revealing a surprising viral engineered scaffold used in binding siRNA.



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The structure of the p21 monomer is comprised entirely of α-helices and folded into amino-terminal

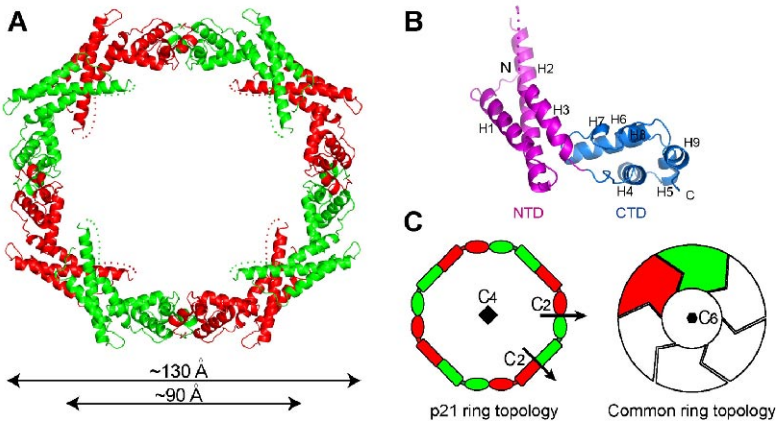

Figure 1. *Crystal structure of p21. (A) The entire octameric ring. Neighboring monomers are alternatively colored with green and red. (B) Structure of the p21 monomer, which consists of two domains. (C) Ring topology of p21 (left panel) compared with other common hexameric ring scaffolds as an example (right panel). Each p21 monomer, colored the same as in (A), is represented by a rectangle (NTD) and an ellipse (CTD).*

(NTD) and carboxy-terminal (CTD) domains (**Figure 1B**). But at the higher structural level, p21 forms an octameric ring, which adopts a previously unknown topology (**Figure 1A**). Normally, protein ring structures are formed by a single type of asymmetric (involving a different part of the protein, head-to-tail) association process between adjacent subunits. In stark contrast, the eight p21 monomer subunits associate through two types of symmetric alignments, namely via a head-to-head (NTD-NTD) and tail-to-tail (CTD-CTD) association. This structure represents a new theme in the cyclic oligomerization of proteins. **Figure 1C** compares the topology of p21 and other common ring structures.

To search for the RNA binding site, we noticed that several basic residues, exposed within the inner surface of the ring, are highly conserved among p21 homologs, suggesting that they might be involved in RNA binding through electrostatic interactions with negatively charged phosphates in RNA (**Figure 2**). In addition, the large inner cavity of the ring (~90 Å in diameter) provides enough space for RNA binding.

The structure of p21 differs significantly from a previously characterized suppressor, p19 from tombusvirus, which is dimeric and strictly recognizes an siRNA duplex of specific length. These structural differences suggest that their RNA binding mechanisms might also be different. Indeed, biochemical analysis showed that p21 is a general nucleic acid binding protein, interacting with 21-nt or longer single- and double-stranded RNAs (**Figure 3**). Because RNA silencing involves various types of RNA, it is possible that p21 might suppress RNA silencing by interacting with RNAs besides siRNA.

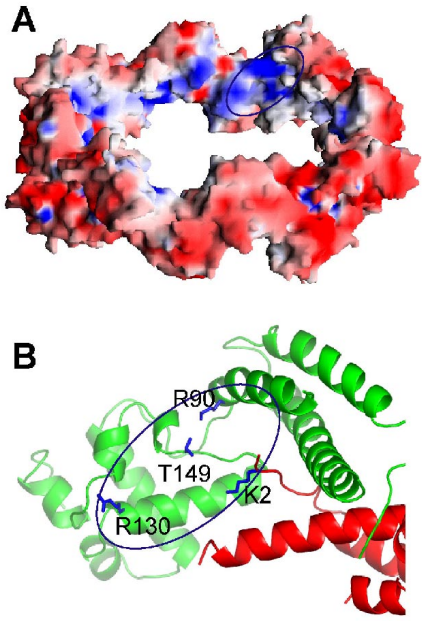

Figure 2. *A putative RNA binding surface inside the ring, which is positively charged (A) and clustered with several conserved residues (B).*

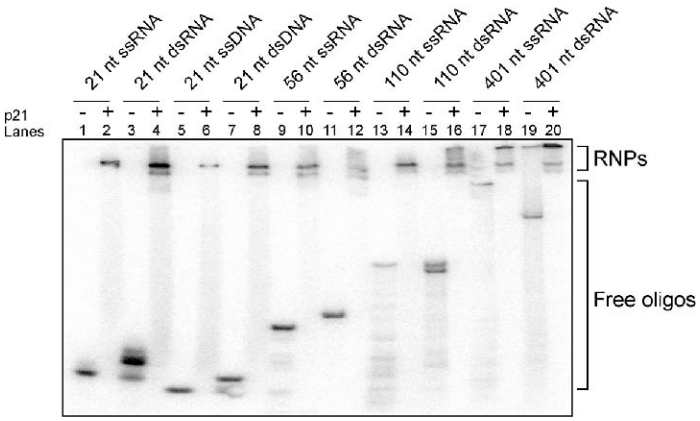

Figure 3. *RNA binding assay of p21 showing that p21 forms complexes with various RNAs. Individual RNAs migrate slower in the native gel after forming protein complex (RNP).*