Analysis of RNA role in spreading disease advances study of damaging plant infections

Recent research that links specific pieces of RNA to an infectious organism's duplication and spread could lead the way to the prevention of viroids, pathogens that can kill or damage food crops and other plants.

The findings and the research approach used by Ohio State University scientists also could have applications in the study of how certain viruses spread in humans because the pathogens have some similar characteristics.

The researchers have developed an experimental system to identify specific structural parts of a viroid that are responsible for its multiplication and spread of the disease.

Because no chemical treatments exist that can specifically inhibit viroid infection, an effective way to prevent viroid multiplication and spread is through genetic alterations of susceptible plants. The best approach to such bioengineering is learning exactly how the pathogens function in the first place, said Biao Ding, senior author of the study and professor of plant cellular and molecular biology at Ohio State.

"We're trying to understand how the infection occurs, and how the RNA propagates itself in the cell. But more importantly, even for human diseases, is discovering how a disease spreads. That's where the problem comes in the plant," Ding said.

Viroids resemble viruses, but consist of only small RNA molecules that don't have the protein coat found on viruses and that don't encode any proteins. Viroids so far have been shown to infect only plants.

Ding and colleagues introduced mutations to specific points within the viroid RNA to see how such disruption affected the role of each piece of the structure. They don't have all of the answers yet to explain the entire viroid RNA function, but their approach shows promise for expanding knowledge about how RNA works in the development of an organism and in the spread of multiple diseases, Ding said.

The research is published in a recent issue of the journal *The Plant Cell*.

About 30 species of viroids exist, affecting such plants as tomatoes, potatoes, palm trees and chrysanthemums. One type of viroid has been known to kill millions of palm trees, but more typical effects of the infection are low plant quality and reduced crop yield. The current way to treat viroids is to harvest and destroy infected crops and start over with new plants.

The effects of viroid infection were first noticed in the 1920s but the cause remained unknown until 1971.

"The scientist who discovered the first viroid spent many years trying to find the pathogen," Ding said. "It's not bacteria. It's not a virus. It is really its own kind of pathogen. It doesn't make any proteins or have any protein coat. It's just a piece of RNA."

Unlike the better-known DNA structure – a double helix with base pairs of nucleotides connecting the strands – many RNAs are formed by a single strand that folds back in on itself. As a result, the RNA structure has a series of loops that scientists have long assumed were empty holes with unclear roles in the RNA function.

Research from several labs has recently shown that many of those loops are not holes, but are actually the most important structural parts of the RNA.

“Those loops interact with proteins, other RNAs and small molecules. That helped us decide to look at all of the loops of a viroid RNA and see how each one functions,” Ding said.

The viroid model used for this research is called the Potato spindle tuber viroid, and its infection was studied in a tobacco-like plant called Nicotiana benthamiana for the experiment. Lead study author Xuehua Zhong, at the time a graduate student and now a postdoctoral researcher at Ohio State, led the work to introduce mutations to each of the 27 loops in this viroid to disrupt its structure and see how that disruption affected either viroid replication or the viroid’s ability to spread, or both actions in the case of some loops.

“It looks like all 27 loops are important, but we need to know which ones are important for the RNA’s ability to reproduce itself in the cell, and which ones are important for spreading from one part of the plant to another,” Zhong said. “And we know we can find similar structures in different RNAs, so that means that what we learned can be applied to other types of RNA.”

So far, the researchers have discovered that a change to almost any loop will slow or eliminate replication, except for one loop. In that case, when disrupted, it instead causes an increase in RNA duplication. A few loops also were identified that are required for movement of the viroid from one part of the plant to another, which spreads the infection.

“We still don’t know exactly where a particular mutant fails to spread because there are so many cell layers in the plant. We still need to take a look at each mutant to see where the infection starts in the plant and what kind of cell types are affected,” Ding said.

He also said that although the viroids themselves don’t contain or make proteins, there must be proteins in the plant that are responsible for recognizing the viroid and allowing it to move around.

The viroid mutants that fail to replicate or move around will be valuable materials to help identify these proteins. For example, a protein that recognizes a viroid structure that is required for movement between cell layers would not be expected to bind to the viroid if the structure is mutated to abolish the movement. This also means that mutating the RNA might not be the only bioengineering target. Instead, proteins could be key to designing plants that will not succumb to viroid infection.

“Once you pinpoint a protein, you might be able to change the protein function so it cannot recognize the viroid and allow it to spread,” said Ding, whose lab is developing methods to look for all of the proteins that bind to viroids to promote infection.

“This is really just the beginning,” Ding said. “This is a new focus, not just for us, but for other researchers studying RNAs that can be analyzed in similar ways because their structure is the same or similar. This is really a new way of investigating.”

Source: Ohio State University