"Viroids": A New Kind of Pathogen?

Although the pioneering research on tobacco mosaic virus occupies a secure niche in the history of virology, viral diseases of plants now receive relatively little attention. For example, potato spindle tuber disease and citrus exocortis disease are hardly household words—except perhaps in the households of potato farmers and citrus growers. Plant virologists have long assumed that these diseases were of viral origin, but attempts to isolate the causative viruses by conventional techniques were not successful. Now three groups of investigators have identified the elusive infectious material—low-molecular-weight RNA—and they think that this RNA belongs to a new class of pathogenic agents.

Theodor O. Diener, of the Plant Virology Laboratory of the United States Department of Agriculture's Agricultural Research Service, Beltsville, Maryland, has been investigating the etiology of potato spindle tuber disease. Extracts of diseased potato plants can be used to infect healthy plants; the extracts also produce distinctive symptoms in the tomato plant, the species that Diener usually employs for his experiments. Because the infectious material is extremely potent and is present in very low concentrations, it could be detected only by its biological activity—a fact that severely hindered the initial investigations of its properties. By means of density gradient centrifugation, however, Diener determined that the infectious material sedimented much too slowly to be a typical nucleoprotein virus consisting of a large RNA moiety and a protein coat. Other evidence, including the observation that the infectivity was eliminated by ribonuclease but not by deoxyribonuclease, indicated that the material was RNA.

At first, Diener believed that he was working with a double-stranded RNA, with a conventional viral genome (a molecular weight of at least 1 million), which was released from virions by the mild extraction procedure. Further experiments failed to verify this hypothesis. Diener could find no evidence of nucleoprotein particles in his infected plants. Using a combination of density gradient centrifugation and gel electrophoresis to determine the molecular weight of the RNA, Diener found a value of 50,000 for the major infectious species. In addition, although the material behaved like double-stranded RNA in some systems, in others it behaved more like single-stranded RNA. Diener has now isolated enough of the RNA to determine its susceptibility to thermal denaturation. The results are consistent with a structure like that of transfer RNA, where a single strand is folded in such a way that it has partial double-stranded character. Diener believes that this infectious entity—a single-stranded, free RNA with an unusually low molecular weight—is the first known representative of a new class of pathogens that he has named "viroids."

R. P. Singh and his colleagues at the Canada Department of Agriculture Research Station in Fredericton, New Brunswick, have also reported that potato spindle tuber disease is caused by a small agent, which appears to be very similar to the one described by Diener. Although Singh agrees with Diener about the other properties of the RNA, he has proposed a lower molecular weight—25,000 to 35,000.

According to J. S. Semancik and L. G. Weathers of the Department of Plant Pathology, University of California, Riverside, a low-molecular-weight, heat-stable RNA is the cause of citrus exocortis disease. Their data also indicate that this RNA has a "transfer RNA–like" conformation. In fact, the citrus exocortis agent may be identical to the "viroid" of potato spindle tuber disease. Extracts of infected plants of the test species *Gynura aurantiaca*, used by Semancik and Weathers, produced symptoms in tomato plants that were indistinguishable from those produced by extracts of potato plants with potato spindle tuber disease. The infected *Gynura* and tomato plants contained a small RNA that was not found in healthy plants. Both RNA's, which were detectable by ultraviolet absorption as well as by infectivity, migrated at the same rate in gel electrophoresis experiments. Furthermore, when Semancik and Weathers compared the citrus exocortis RNA with a sample of material supplied to them by Diener, the two appeared to have identical properties. Semancik and Weathers, however, report a molecular weight of 120,000 for both; they attribute these discrepancies in molecular weight to differences in the techniques used in the two laboratories.

The existence of a low-molecular-weight RNA capable of replicating in several genetically different hosts has potentially significant implications for molecular biology and virology. Even an RNA with a molecular weight of 120,000 is only about one-tenth the size of the RNA genome of the smallest known self-replicating plant virus. Such an RNA can code for a protein containing about 100 amino acids—a protein thought to be too small to be an enzyme with the ability to synthesize RNA. Diener originally hypothesized that the "viroid" could be a satellite RNA that required a helper virus for its replication. However, he has not been able to find any evidence for the existence of such a helper.

The three groups of investigators are now considering the question of whether this unusual species of RNA functions and replicates in a typical viral manner, possibly with the aid of plant enzymes, or whether it may act as a modifier of host gene expression. Because the mechanism of action of the RNA has not yet been elucidated, and because the name "viroid" implies "like a virus" to Semancik and Weathers, they now prefer to call the material "infectious" or "pathogenic" RNA.

Diener has identified a second "viroid," with properties somewhat different from those of the potato spindle tuber agent, as the pathogen causing chrysanthemum stunt disease, but he thinks that these agents are not unique to plants. He has proposed that they are implicated in the etiology of scrapie, a disease of the central nervous system of sheep, because of similarities in the properties of the scrapie agent and "viroids." At present, there is substantial disagreement concerning the chemical nature of the scrapie pathogen, which has never been isolated. Investigators in at least one laboratory, that of Richard Marsh at the University of Wisconsin, Madison, have begun experiments to determine whether there is any relation between "viroids" and scrapie. Marsh feels that this line of research is promising enough to pursue, but admits that he is pessimistic about the outcome. Nevertheless, there is a possibility that plant virology, in addition to its significant contributions to the understanding of fundamental cell processes, may also help to identify the still unknown causes of certain animal diseases.—JEAN L. MARX