VIROIDS AND VIROID DISEASES

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PREFACE

Viroids are the smallest known agents of infectious disease. They are responsible for a number of destructive diseases of cultivated plants, but may also occur and cause disease in animals. Although some of the diseases that viroids cause had been known for decades, these diseases were generally believed to be due to infection by conventional viruses. The unique nature of their causative agents came to light only in 1971, when the agent of the potato spindle tuber disease was shown to be a low molecular weight ribonucleic acid (RNA) with unusual properties that is able, despite its small size, to replicate autonomously in susceptible cells. Recognition of the profound disparity between this pathogen and all other pathogens, including all known viruses, led to the proposal to call such agents *viroids*.

Since then, several other plant diseases have been shown to be caused by similar low molecular weight RNAs, and due to the efforts of a number of investigators much has been learned of the unique physical-chemical and biological properties of viroids. This book endeavors to present a comprehensive and up-to-date account of our knowledge of viroids and of the diseases they cause. Also, and possibly more importantly, the book attempts to point out the many areas of ignorance in our understanding of viroids as biological entities. We do not know, for example, how widespread viroids are in nature, how serious a potential threat they are to agriculture, whether they occur in forms of life other than higher plants, how they replicate, and how they bring about the metabolic aberrations in some of their hosts that lead to disease and, in some cases, death of the infected plant.

Each of these areas of ignorance affords attractive opportunities to advance our knowledge, not only of viroids and of the diseases they cause, but also of profound and general biological mechanisms. Hopefully, this

book will attract additional investigators into the area of viroid research and thereby help to accelerate the acquisition of knowledge regarding this newly recognized group of pathogens.

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T. O. D.

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1. THE DISCOVERY OF VIROIDS

"S' efforcer de se convaincre soi-même de la vérité qu'on a entrevue est le premier pas vers le progrès; persuader les autres est le second."
Louis Pasteur
C.R. hebd. Séanc. Acad. Sci. Paris **82,** 1079 (1876)

In science, unexpected results of routine experiments are the most frequent source of discovery. Many experiments, however, lead to unexpected results, but almost always these have trivial explanations, such as faulty technique or erroneous suppositions. Significant deviations from theory are rare and, as often as not, are overlooked or blamed on faulty experimentation.

The recognition of viroids as a novel type of pathogen followed this pattern. Efforts to purify the agent of the potato spindle tuber disease, which was generally believed to be a conventional plant virus, led to results that were inconsistent with this belief, as well as with widely held concepts of virology and molecular biology. In this chapter, the prerequisites for the recognition of viroids and the chronology of their discovery are discussed.

1.1 PREREQUISITES FOR DISCOVERY OF VIROIDS

Recognition of the basic disparity between viroids and conventional viruses became possible only after certain basic principles of virology and molecular biology had been established. Foremost among these was the realization that the genetic information of viruses resides in their nucleic acid component, a fact that, in the case of plant viruses, has been most dramatically established with the demonstration that RNA isolated from

tobacco mosaic virus is infectious (Gierer and Schramm, 1956; Fraenkel-Conrat, 1956). The isolation of defective tobacco mosaic virus strains from nitrous-acid-treated virus preparations and the demonstration that with these the infectious principle behaves in a manner similar to that of infectious RNA isolated from ordinary virus (Siegel *et al.*, 1962) clearly showed that a virus may be able to persist *in vivo* in the form of free RNA. The discovery of the so-called nonmultiplying or winter forms of tobacco rattle virus and the demonstration that these consist of nonencapsidated viral RNA (Sänger and Brandenburg, 1961; Cadman, 1962) showed that viral pathogens in the form of free RNA occur in nature. Finally, with the demonstration that free, infectious RNA exists also in plants infected with a conventional plant virus (Diener, 1962), the possibility of free RNA viruses occurring naturally became still more plausible. None of these findings, however, suggested the existence of autonomously replicating low molecular weight RNA species or their importance as naturally occurring incitants of damaging diseases of crop plants.

1.2 CHRONOLOGY OF DISCOVERY

In retrospect, it is evident that the recognition of viroids as representatives of a novel class of pathogens that are distinct from viruses required convincing evidence in five crucial areas. It was necessary to demonstrate that:

1. The pathogen exists *in vivo* as a nonencapsidated nucleic acid.
2. Viruslike particles are not detectable in infected tissue.
3. The pathogen is a low molecular weight nucleic acid.
4. The infectious nucleic acid replicates autonomously, that is, without assistance from a helper virus.
5. The infectious nucleic acid consists of one molecular species only.

So far the following viroids have been identified and named after the diseases they cause:

1. Potato spindle tuber viroid (PSTV).
2. Citrus exocortis viroid (CEV).
3. Chrysanthemum stunt viroid (CSV).

4. Chrysanthemum chlorotic mottle viroid (ChCMV).
5. Coconut cadang-cadang viroid (CCCV).
6. Cucumber pale fruit viroid (CPFV).
7. Hop stunt viroid (HSV).

It is interesting to examine the chronology by which evidence in the five crucial areas listed above was reported with each viroid. So far, evidence supporting all five criteria has been reported only for PSTV, whereas evidence with the more recently discovered viroids is still lacking in one or more of the crucial areas.

Here, only the earliest record giving convincing evidence is listed for each viroid; confirming reports and a more detailed discussion of the experimental evidence will be presented in Chapter 4.

1967. On the basis of its low sedimentation rate and sensitivity to ribonuclease, Diener and Raymer (1967) conclude that PSTV is a free RNA. Existence of viruslike particles is considered unlikely because phenol treatment of crude extracts does not change the sedimentation properties of the infectious material, since the latter has the expected buoyant density of RNA, and because the low sedimentation rate cannot be explained on the basis of low density (lipid content) of a putative virion.

1968. The existence of virions in PSTV-infected tissue becomes even less likely when Diener (1968) shows that, *in situ*, PSTV is sensitive to ribonuclease.

With citrus exocortis disease, Semancik and Weathers (1968b) present evidence that the causative agent sediments at a low rate and is sensitive to ribonuclease. Also, no viruslike particles can be identified by electron microscopy in CEV-infected tissue (Semancik and Weathers, 1968a).

1970. Zaitlin and Hariharasubramanian (1970) report that no proteins that can be construed as coat proteins are identifiable in PSTV-infected tissue.

1971. From a combined analysis of PSTV in density gradients and polyacrylamide gels, Diener (1971b) concludes that PSTV is a low molecular weight RNA. In the same report, extensive evidence to indicate that no helper virus is involved in the replication of PSTV is presented. The name *viroid* is proposed as a generic term for PSTV and pathogenic nucleic acids with similar properties (Diener, 1971b).

Diener and Smith (1971) report further evidence for the low molecular weight of PSTV by showing that the RNA is able to penetrate into gels of high polyacrylamide concentration, from which high molecular weight RNAs are excluded.

1972. Because the infectivity dilution curve of PSTV suggests a single-hit event, Diener (1972b) concludes that PSTV consists of a single molecular species.

Semancik and Weathers (1972b), on the basis of its mobility in polyacrylamide gels, conclude that CEV is a low molecular weight RNA. They show that its buoyant density is that expected of an RNA (Semancik and Weathers, 1972a).

Diener and Lawson (1972) present evidence that the chrysanthemum stunt disease is viroid caused by demonstrating that the infectious agent sediments at a low rate, is sensitive to ribonuclease, and moves with low molecular weight RNAs in polyacrylamide gels.

1973. Semancik *et al.* (1973b) investigate the resistance of CEV to ionizing radiation and report a target volume of about 10^5 daltons, suggesting that CEV consists of a single molecular species.

Hollings and Stone (1973) determine that CSV is highly resistant to ultraviolet light irradiation.

Sogo *et al.* (1973) achieve the first visualization of a viroid (PSTV) by electron microscopy and establish its low molecular weight by direct length measurements.

1974. On the basis of its low rate of sedimentation and sensitivity to ribonuclease, Romaine and Horst (1974) conclude that the agent of the chrysanthemum chlorotic mottle disease is a viroid.

Diener *et al.* (1974) report that PSTV has a very high resistance to inactivation by ultraviolet light irradiation, suggesting that PSTV consists of one molecular species only.

1975. Randles (1975) provides evidence for the association of two RNA species with coconut cadang-cadang disease and suggests that the disease may be viroid incited.

Dickson *et al.* (1975) publish the first RNA fingerprints of viroids (PSTV and CEV) and conclude from their complexity that each viroid is a single, distinct molecular species.

1976. Sänger *et al.* (1976) present evidence that the agent of the cucumber pale fruit disease is a viroid as had been claimed earlier on the basis of unpublished evidence (Van Dorst and Peters, 1974). Evidence

presented includes rate of sedimentation, sensitivity to ribonuclease, gel mobility, and length measurements by electron microscopy.

Dickson (1976) presents two-dimensional fingerprint patterns of CSV and the cadang-cadang-associated RNA, and conclude that each is a single distinct, low molecular weight RNA species.

1977. Sasaki and Shikata (1977b) report that the agent of the hop stunt disease sediments at a low rate and is sensitive to ribonuclease. They conclude that the agent is a viroid.

Conejero and Semancik (1977) provide evidence that no protein that has the expected properties of a viral coat protein can be identified in CEV-infected tissue, making it unlikely that viruslike particles exist in such tissue.

Thus, for all viroids so far identified, convincing evidence indicates that they are nonencapsidated RNAs. Some evidence has been presented with all but two viroids (ChCMV and CPFV) that viruslike particles are absent in infected tissue. The low molecular weight of all viroids except ChCMV and HSV has been reasonably well established, but the question of autonomous replication has so far been investigated only with PSTV. Evidence that the viroid is a single molecular species is available for PSTV, CEV, CVS, and CCCV, but not for CPFV, ChCMV, or HSV.

With the elucidation of the unique molecular structure of viroids (see Chapter 7), peculiar structural features, such as circularity and a very high degree of intramolecular complementarity, promise to be useful criteria for the identification of an RNA as a viroid. So far, however, it is not yet certain whether all viroids will prove to possess these structural features.

1.3 DEFINITION OF VIROID

By necessity, the following definition of the term *viroid* is operational, that is, it is based on presently recognized properties of the pathogens:

Viroids are low molecular weight nucleic acids that are present in certain organisms afflicted with specific maladies. Viroids are not detectable in healthy individuals of the same species but, when introduced into such individuals, they replicate autonomously and cause the appearance of the characteristic disease syndrome. Unlike viral nucleic acids, viroids are not encapsidated. Viroids are highly resistant to heat, as well as to ultraviolet and ionizing radiation. They

contain extensive regions of intramolecular complementarity and exist as covalently closed circular structures.

Undoubtedly, with increased knowledge of the properties of viroids, this definition will require modification, but in light of present knowledge, it adequately distinguishes viroids from all other pathogens known, including conventional viruses.

1.4 THE TERM VIROID

Although usage of the term *viroid* to denote the pathogens that are the subject of this book is now universal, certain objections to the term were raised at an earlier time. These centered around the following two arguments:

1. Properties of the newly discovered pathogens are not sufficiently different from those of conventional viruses. These pathogens simply constitute very small viruses. No new term, therefore, is needed.

This argument was advanced by McKinney (1973), who regarded viroids as the most primitive or the most degenerate plant viruses known. As has been pointed out (Diener, 1973a), no evidence exists as to whether viroids are or are not related to viruses, and the more recent elucidation of the unique structural properties of viroids (see Chapter 7) makes it unlikely that they evolved from conventional viruses or that they constitute highly degenerate viruses (see Section 11.2). In light of these and other properties of viroids, a new term definitely appears to be needed.

2. A new term is called for, but *Viroid* is not suitable because:
   a. It implies similarity with viruses.
   b. The term has been preempted by earlier usage in different connotations.

Objection (a), which evidently is the opposite of the objection discussed under argument (1), was raised by Semancik *et al.* (1973b), who introduced the term *pathogene* "to provide a position of divergence from viral processes implicit in the terms viroid . . . and metavirus (Hanneman and Singh, 1972)." *Viroid*, however, may be interpreted to indicate similarity of