

Subviral Pathogens of Plants and Animals: Viroids and Prions

Edited by

Karl Maramorosch

John J. McKelvey, Jr.

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*The Rockefeller Foundation
New York, New York*

1985



ACADEMIC PRESS, INC.

(Harcourt Brace Jovanovich, Publishers)

*Orlando San Diego New York London
Toronto Montreal Sydney Tokyo*

000013380

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ACADEMIC PRESS, INC.

Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.

24-28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

Subviral pathogens of plants and animals.

"Proceedings of a symposium ... sponsored by the Rockefeller Foundation, held in Bellagio, Italy, June 27-July 2, 1983."--

Includes index.

I. Viroids--Congresses. I. Maramorosch, Karl.
II. McKelvey, John J. III. Rockefeller Foundation.

IV. Title: Prions.

QR500.S83 1984 616'.0194 84-45725

ISBN 0-12-470230-9 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88

9 8 7 6 5 4 3 2 1

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Preface

Prior to 1971 all infectious agents of animal and plant diseases were believed to be limited to bacteria, viruses, fungi, and protozoa, but in 1971 the existence of a new group of pathogens, smaller and less complex than viruses, was discovered. These are autonomously replicating RNA molecules named viroids. In the years intervening between 1971 and the present a dozen plant diseases, earlier believed to be caused by viruses, were found to be associated with viroids. So far, viroid diseases have been linked with higher plants only, not with higher animals, arthropods, or bacteria.

The smallest genomes of independently replicating viruses have a molecular weight of one million. So-called satellite viruses and defective viruses have smaller genomes but they are unable to replicate without helper viruses. Viroid genomes are in the range of 50,000 to 100,000 daltons, and the molecular structure of several has been recently established. The replication of viroids by a rolling circle mechanism, using enzymes from RNA templates, has been unraveled. The viroids are not translated into viroid-specified polypeptides.

Viroids have provided a convenient model for the study of subviral pathogens of animals. Several neurological disorders of man and higher animals, so-called spongiform encephalopathies, were found to be infectious diseases, and their causative agents were described as "slow viruses." The enigmatic scrapie disease of sheep and goats, kuru disease in New Guinea, and Creutzfeldt-Jakob senility of man belong to this group. For several years it was suspected that the "slow viruses" might actually be viroids. Recently it has been demonstrated that the scrapie agent is smaller than viroids and that an essential hydrophobic protein is required for its infectivity. The term prion has been coined for these pathogens. So far, no prion diseases have been found in plants or arthropods. The true nature of prions is still a riddle.

Using viroid research as a model, investigators could proceed most proficiently in determining the physical and chemical nature of the scrapie agent. Experimentation has not borne out the speculation that this agent might be akin to plant viroids. On the contrary, it has been demonstrated that the scrapie pathogen is radically different from either viruses or viroids. Even in highly concentrated preparations the involvement of nucleic acid could not be demonstrated, and the hydrophobic protein alone

seemed to be essential for an expression of infectivity. It remains to be determined whether a small nucleic acid yet to be detected or destroyed by present-day techniques is present in prions. Should the prion's protein constitute the complete pathogen, prions would contradict all established beliefs of molecular biologists. No wonder that recent findings in prion research are not accepted universally. The revolutionary concept that prions are complete pathogenic entities could have far-reaching implications for virology, for human and veterinary medicine, and indeed for basic molecular biology as well.

We have invited several contributors who support the concept of the nature of prions as well as some who oppose it so that a balanced presentation of views, evidence, and diverse conclusions could be made available to the scientific community to stimulate further work on so far unrecognized plant, human, and animal types of subviral pathogens. We hope this research will advance our knowledge of subviral pathogens and contribute to the eventual control of several important diseases in animals and plants.

Twenty chapters have been devoted to the nature of subviral pathogens of plants and animals. The authors, all recognized authorities in their scientific disciplines, have compared the newly emerging concepts and current research results. Important work on the control of plant viroid diseases is now in progress in several countries which may also be relevant for similar research efforts to control the spongiform encephalopathies. Thus the subject of this treatise should be of considerable scientific interest and importance and one that will appeal to an audience representing human and veterinary medicine, virology, zoology, microbiology, plant pathology, entomology, as well as other branches of biology.

The decision to publish this volume was made following an international workshop at the Rockefeller Foundation's Study and Conference Center in Bellagio, Italy, under the sponsorship of the Rockefeller Foundation, the U.S. Public Health Service, as well as universities and research institutes of Australia, Germany, Hungary, Italy, Japan, Netherlands, People's Republic of China, and Peru. We express our sincere gratitude to these sponsors and particularly to the Rockefeller Foundation, not only for providing ideal conditions for the stimulating discussions which took place but also for the preparation of the final typescript of the book. Thanks are due to the contributors for the effort and care with which they have prepared their chapters and to the staff of Academic Press for their part in the production of this volume.

KARL MARAMOROSCH
JOHN J. MCKELVEY, JR.

Contents

Contributors
Preface

Part I Subviral Pathogens

1. The Recognition of Subviral Pathogens
T. O. Diener and Stanley B. Prusiner

Part II Viroid Diseases

2. The Host Range of Viroids
D. Peters and W. T. Runia 21
3. Coconut Cadang-Cadang Viroid
J. W. Randles 39
4. Viroid Etiology of Tinangaja and Its Relationship
with Cadang-Cadang Disease of Coconut 75
Guido Boccardo
5. Hop Stunt Viroid and Hop Viroid Disease 101
Eishiro Shikata

- | | |
|--|-----|
| 6. Viroids and Viroid Diseases in China
<i>Tien Po</i> | 123 |
| 7. Elimination of Potato Spindle Tuber Viroid from Potato
by Cold Treatment and Meristem Culture
<i>L. F. Salazar, L. Schilde-Rentschler, and R. Lizárraga</i> | 137 |
| 8. Control of Viroid Diseases
<i>Karl Maramorosch</i> | 151 |

Part III

Viroid Structure and Replication

- | | |
|---|-----|
| 9. Viroids: Their Structure and Possible Origin
<i>Hans J. Gross</i> | 165 |
| 10. Viroids and snRNAs
<i>F. Solymosy and T. Kiss</i> | 183 |
| 11. <i>In Vivo</i> Intermediates and the Rolling Circle Mechanism
in Viroid Replication
<i>Andrea D. Branch, Kerry K. Willis, George Davatellis,
and Hugh Robertson</i> | 201 |
| 12. On the Mechanism of Replication of Viroids, Virusoids,
and Satellite RNAs
<i>Robert H. Symons, James Haseloff, Jane E. Visvader,
Paul Keese, Peter J. Murphy, Dalip S. Gill,
Karl H. J. Gordon, and George Bruening</i> | 235 |
| 13. Viroid-like RNAs Incorporated in Conventional Virus Capsids
<i>R. I. B. Francki, J. W. Randles, P. W. G. Chu,
J. Rohozinski, and T. Hatta</i> | 265 |
| 14. PM-Antigen: A Disease-Associated Host Protein
in Viroid-Infected Tomato
<i>T. O. Diener, D. R. Smith, and J. Galindo A.</i> | 299 |

15. Construction of Infectious Potato Spindle Tuber Viroid cDNA Clones: Implication for Investigations of Viroid Structure-Function Relationships 315

Robert A. Owens, Michael C. Kiefer, and Dean E. Cress

Part IV

Subviral Pathogens of Animals

16. Prions—Structure, Biology, and Diseases 337

Stanley B. Prusiner, Darlene F. Groth, David C. Bolton, Karen A. Bowman, S. Patricia Cochran, Paul E. Bendheim, and Michael P. McKinley

17. The Enigma of DNA in the Etiology of Scrapie 393

D. H. Adams

18. Biological Properties of Scrapie: An Unconventional Slow Virus 425

Richard I. Carp, Patricia A. Merz, Roger C. Moretz, Robert A. Somerville, Sharon M. Callahan, and Henry M. Wisniewski

19. Viral Etiology of Multiple Sclerosis: A Critique of the Evidence 465

F. Gonzalez-Scarano and N. Nathanson

20. Subacute Spongiform Encephalopathies Caused by Unconventional Viruses 483

D. Carleton Gajdusek

- Index* 545

PART I

Subviral Pathogens

Chapter 1

THE RECOGNITION OF SUBVIRAL PATHOGENS

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I. INTRODUCTION

Until about 12 years ago, it was generally believed that all infectious diseases of plants and animals are caused either by microorganisms (bacteria, fungi, etc.) or by viruses. Since then, disease-causing agents that are smaller and less complex than viruses have come to light. First, in 1971, the potato spindle tuber disease was shown to be caused by small, unencapsidated molecules of autonomously replicating RNA (Diener, 1971). Today, about a dozen diseases of higher plants are known to be caused by similar subviral agents for which the term *viroid* has been adopted. Second, the agent of a neurological disease of sheep and goats, scrapie, which has long been known to possess a number of properties unlike those of viruses, has recently been shown to contain an essential protein and is therefore fundamen-

tally distinct from viroids. The scrapie agent appears to be even smaller than viroids. For this and similar pathogens, the term *prion* has been proposed (Prusiner, 1982).

The discovery of subviral pathogens has opened new vistas in plant pathology, veterinary medicine, and human medicine, as well as in cell and molecular biology. In the following chapters, various aspects of these pathogens will be described in detail; the book as a whole represents an up-to-date account of our present knowledge in this new scientific area.

Purified viroid preparations have been available to biochemists and molecular biologists for about 10 years, permitting application of conventional biochemical procedures, whereas properties of the scrapie agent must still be deduced indirectly by virtue of its biological activity. It is not surprising, therefore, that our knowledge of viroids (particularly of their structural properties) is far greater than that of the scrapie agent. This imbalance is reflected in the unequal number of chapters dedicated to each of the two known types of subviral pathogen. In this introductory chapter we present an overview of the field as it has developed during the last 12 years.

II. VIROIDS AND VIROID DISEASES

Twelve viroids causing eleven naturally occurring diseases have been discovered over the last decade (Table I). Originally the term viroid was introduced on the basis of newly established properties of the infectious agent responsible for the potato spindle tuber disease. These properties were found to differ fundamentally from those of viruses in 4 important respects as listed in Table II.

Because the smallest known viruses capable of independent replication contain genomes of a size corresponding to a molecular weight (M_r) of about one million, it appeared reasonable to assume twelve years ago that this size represents the minimal amount of genetic information required for a virus to code for virus-specified products and to subjugate the metabolism of the host cell. Indeed, viruses with smaller genomes, although known, are not capable of independent replication but require certain functions provided by a helper virus present in the same cell. In the absence of helper virus, no replication of these "defective" or "satellite" viruses takes place.

Viroids, on the other hand, introduce into their host cells a far smaller amount of genetic information than do viruses, yet their replication does not require the assistance of detectable helper viruses. Because of this, the discovery of viroids came as a surprise and was greeted,

TABLE I
Viroid Diseases

Disease	Viroid	References
1. Potato spindle tuber	PSTV	Diener, 1971
2. Citrus exocortis	CEV	Semancik and Weathers, 1972
3. Chrysanthemum stunt	CSV	Diener and Lawson, 1973
4. Chrysanthemum chlorotic mottle	CCMV	Romaine and Horst, 1975
5. Cucumber pale fruit	CPFV	Van Dorst and Peters, 1974; Sanger. <i>et al.</i> , 1976
6. Coconut cadang-cadang	CCCV	Randles, 1975
7. Hop stunt	HSV	Sasaki and Shikata, 1977
8. Columnea latent	CV	Owens <i>et al.</i> , 1978
9. Avocado sunblotch	ASBV	Dale and Allen, 1979; Thomas and Mohamed, 1979
10. Tomato apical stunt	TASV	Walter, 1981
11. Tomato planta macho	TPMV	Galindo <i>et al.</i> , 1982
12. Burdock stunt	BSV	Chen <i>et al.</i> , 1983

TABLE II
Properties of Viroids Differentiating them from Viruses*

1. The pathogen exists *in vivo* as an unencapsidated RNA; that is, no virion-like particles are detectable in infected tissue.
2. The infectious RNA is of low molecular weight.
3. Despite its small size, the infectious RNA is replicated autonomously in susceptible cells; that is, no helper virus is required.
4. The infectious RNA consists of one molecule only.

*Diener, 1971, 1972a.

initially, with considerable skepticism. Acceptance of the viroid concept was not facilitated by the fact that, at the time, the viroid could not be recognized as a physical entity, but only by virtue of its biological activity, that is, by its capacity to induce disease in susceptible plants. Molecular biologists, particularly, were not too comfortable with such an indirect approach of determining physical-chem-

ical properties of a biological agent. Fortunately, evidence for the correctness of the viroid concept soon became indisputable and work from a number of laboratories resulted in a vast increase in our knowledge of viroids.

Once purified viroid preparations had become available, elucidation of the structure of viroids was rapid. As highlights, the following events might be mentioned: determination of the thermal denaturation properties of a viroid (Diener, 1972b); electron microscopic visualization of a native viroid (Sogo *et al.*, 1973); and of fully denatured viroids (McClements, 1975; Sanger *et al.*, 1976; McClements and Kaesberg, 1977), leading to the important discovery that viroids are single-stranded, covalently closed circular RNA molecules with extensive regions of intramolecular complementarity. Detailed quantitative thermodynamic and kinetic studies of their thermal denaturation (Langowski *et al.*, 1978; Domdey *et al.*, 1978; Henco *et al.*, 1979; Gross and Riesner, 1980) revealed that viroids exist in their native conformations as extended rodlike structures characterized by a series of double-helical sections and internal loops. These structural studies culminated in the determination of the complete nucleotide sequence and most probable secondary structure of the potato spindle tuber viroid (PSTV) (Gross *et al.*, 1978). Thus, in less than 10 years, viroids advanced from entities whose very existence was doubted by some to RNA pathogens whose molecular structure is completely known.

In contrast to our extensive knowledge of viroid structure, functional aspects of viroid-host relationships are still inadequately understood. Consensus exists, however, that viroids are (1) not translated into viroid-specified polypeptides (for example, Davies *et al.*, 1974; Semancik *et al.*, 1977; Conejero and Semancik, 1977; Symons, 1981; Zelcer *et al.*, 1981); (2) replicated by host enzymes from RNA templates (Grill and Semancik, 1978; Rackwitz *et al.*, 1981; Boege *et al.*, 1982); and (3) probably replicated by a rolling circletype mechanism with the circular viroid (or its complement) serving as a template, resulting in the synthesis of oligomeric strands of the viroid and its complement (Owens and Cress, 1980; Branch *et al.*, 1981; Rohde and Sanger, 1981; Owens and Diener, 1982).

Today, with the growing library of viroid nucleotide sequences, we appear to be at the threshold of being able to correlate particular viroid regions with biological properties and to investigate the effects of specific nucleotide exchanges, insertions, or deletions on host specificity, viroid replication efficiency, and pathogenicity. Undoubtedly, availability of infectious, viroid-complementary, recombinant DNA clones, as reported in this volume, will be