I. VIROIDS

Various important virus-like diseases in

plants have been shown to be caused by pathogenic

RNAs known as viroids, which have the

following basic properties:

• Viroids are small circular molecules, a few

hundred nucleotides long, with a high

degree of secondary structure.

• Viroids do not code for any polypeptides

and replicate independently of any

associated plant virus.

• Viroids are the smallest known selfreplicating

genetic unit.

Viroids are of practical importance as they

cause several economically significant diseases

and are of general biological interest as being

among the smallest known agents of infectious

disease. The most studied viroid is Potato spindle

tuber viroid (PSTVd). Viroid names are abbreviated

to initials with a “d” added to distinguish

them from abbreviations for virus names.

A. Classification of Viroids

Based on the sequence and predicted structures

of their RNAs, the present 29 known viroids

are classified into two families: the

Pospiviroidae and the Asunviroidae; each family

has several genera (Table 3.1).

B. Pathology of Viroids

1. Macroscopic Disease Symptoms

Viroids infect both dicotyledonous and monocotyledonous

plants. As a group, there is nothing

that distinguishes the disease symptoms produced

by them from those caused by viruses.

Their symptoms include stunting, mottling, leaf

distortion, and necrosis and range from the

slowly developing lethal disease in coconut

palms caused by Coconut cadang-cadang viroid

(CCCVd) to the worldwide symptomless infection

of Hop latent viroid (HLVd).

2. Cytopathic Effects

Various effects of viroid infection on cellular

structures have been reported. For example, in

some infections changes have been observed

in membranous structures called plasmalemmasomes.

Several workers have described pronounced

corrugations and irregular thickness

in cell walls of viroid-infected tissue. Various

degenerative abnormalities have been found in

the chloroplasts of viroid-infected cells.

3. Location of Viroids in Plants

Using confocal laser scanning microscopy

and transmission electron microscopy in conjunction

with in situ hybridisation, both Citrus

exocortis viroid (CEVd) and CCCVd were found

in vascular tissues as well as mesophyll cells.

From experiments involving fractionating

components of viroid-infected cells, it has become

generally accepted that most viroids are located

in the nucleus. The main exception is Avocado

sun blotch viroid (ASBVd),which is found in chloroplasts.

Within nuclei, PSTVd and CCCVd are

located in nucleoli, whereas CEVd accumulates

to higher concentrations in the nucleoplasm.

4. Movement in the Plant

Viruses with defective coat proteins and

naked RNAs move slowly through the plant by

cell-to-cell movement (see Chapter 9). By contrast,

viroids move rapidly from cell to cell of a

host plant in the manner of competent viruses.

The cell-to-cell movement is via plasmodesmata

and is mediated by specific sequences or structural

motifs. Long-distance movement of viroids

is almost certainly through the phloem. The relative

resistance of viroid RNA to nuclease attack

probably facilitates their long-distance movement.

It is also possible that viroid particles

undergoes RNA translocation while bound to

some host protein.

5. Transmission

Viroids are readily transmitted by mechanical

means in most of their hosts. Transmission

in the field is probably mainly by contaminated

tools or similar means. This ease of transmission

of an RNA molecule in the presence of

nucleases is probably due to viroid secondary

structure and to the complexing of viroids to

host components during the transmission process.

Several viroids have been shown to be

pollen and seed transmitted in tomato, potato,

and grapes.

6. Epidemiology

The main methods by which viroids are

spread through crops are by vegetative propagation,

mechanical contamination, and pollen

and seed. The relative importance of these

methods varies with different viroids and

hosts. For example, vegetative propagation is

dominant for PSTVd in potatoes and Chrysanthemum

stunt viroid in chrysanthemums.

Mechanical transmission is a significant factor

for others, such as CEVd in citrus and HSVd in

hops. Seed and pollen transmission are factors

in the spread of ASBVd in avocados.

For most viroid diseases, the reservoir of

inoculum appears to be within the crop itself,

which raises the question as to where the

viroid diseases came from. The evidence suggests

that many viroid diseases are of relatively

recent origin. None of the recognised

viroid diseases was known to exist before

1900, and many were first described since

1940. The sudden appearance and rapid

spread of a new viroid disease can probably

be accounted for by viroids being readily

transmitted by mechanical means and many

modern crops being grown as large-scale

monocultures. Thus, from time to time a

viroid present in a natural host and probably

causing no disease might escape into a nearby

susceptible commercial crop and spread rapidly

within it. If the viroid and crop plant

had not evolved together, disease would be

a likely outcome. There is direct evidence for

such a sequence of events with the tomato

planta macho disease in Mexico.

C. Properties of Viroid RNAs

The properties of the RNAs of the two

families of viroids are summarised in Table 3.2.

1. Sequence and Structure

The nucleotide sequences of most members

of the viroid group and those of numerous

viroid variants are now known. They range in

size from 246 to 375 nucleotides (see Table 3.1).

Members of both families have circular RNA

molecules, but those of the Pospiviroidae are

rodlike, whereas those of the Avsunviroidae are

branched (Figure 3.1).

It should be remembered that these structures

have been derived either from computer

predictions or from in vitro experiments and

that, in vivo, viroids may be associated with

host proteins and have other structures. However,

other evidence points to at least a partial

rod-shaped structure in vivo in that viable

duplications or deletions preserve this type of

structure. Viroids have tertiary structure that

is thought to be important in interactions with

host proteins.

The predicted rodlike structures of the Pospiviroidae

have five structural-functional domains

that are common to all members (Figure 3.1B;

see Box 3.1). These were thought to have specific

functions, but the situation is now considered

to be more complex. For instance,

symptom expression is thought to be controlled

by determinants located within the TL, P, V,

and TR domains.

2. Replication

Even if it was assumed that the three outof-

phase potential reading frames were fully utilized,

viroids do not contain enough information

to code for an RNA replicase. It is now generally

accepted that viroids are not translated to give

any polypeptides. Table 3.2 shows that members

of the Pospiviroidae replicate in the nucleus and

members of the Avsunviroidae in chloroplasts.

It is likely that viroids of both families have

sequences and/or structure motif(s) for import

into their replication organelle.

Viroids replicate via an RNA template,

most probably by a rolling circle mechanism.

Figure 3.2 illustrates two rolling circle models.

In the asymmetric pathway, the infecting circular

(þ)-strand monomer is transcribed into linear

multimeric (–) strands, which then are the

template for the synthesis of linear multimeric

(þ) strands. In the symmetric pathway, the linear

multimeric (–) strands are processed and

ligated to give (–) monomer circles that are

the template for linear multimeric (þ)-strand

synthesis. In both cases, the multimeric (þ)

strand is processed to give monomeric circles.

As the symmetric pathway involves both (þ)-

and (–)-strand circular forms and the asymmetric

pathway only (þ)-strand circular forms, the

two mechanisms can be distinguished by the

presence or absence of the (–)-strand circular

form. This RNA species has not been found in

plants infected with PSTVd, and thus the replication

of this viroid is considered to follow the

asymmetric pathway. In contrast, (–)-strand circular

monomer RNA forms have been found

in ASBVd infections, which suggests that replication

of this viroid follows the symmetric

pathway.

The rolling circle replication of viroids

involves the following features:

• Nuclear replication of members of the

Pospiviroidae is by DNA-dependent RNA

polymerase II. Chloroplastic replication of

members of the Avsunviroidae is by a

chloroplastic DNA-dependent RNA

polymerase.

• Initiation of replication of PSTVd maps to

the left terminal loop (see Figure 3.1B). The

replication initiation site for members of the

Avsunviroidae has still to be determined.

Processing of the long products of rolling circle

replication of members of the Pospiviroidae to

give monomeric (þ) RNAs is effected by a host

RNase activity in the nucleolus.

• Cleavage of the long replication products

of members of the Asunviroidae is by a

hammerhead ribozyme (see Box 3.2)

• Circularisation of the monomeric

molecules of nuclear viroids is thought

to be calalysed by host RNA ligase.

It is unclear if a chloroplastic RNA

ligase exists for members of the

Avsunviroidae or whether the reaction is

autocatalytic.