became accepted, that the search for host-coded proteins involved in the viroid-incited pathogenic response (PR proteins) commenced in a systematic way.

The first report implicating proteins in viroid pathogenesis was the detection in PSTVd-infected tomato leaves of two proteins with molecular weights 155 and 195 kDa (Zaitlin and Hariharasubramanian, 1972). Five years later, two low molecular weight polypeptides of 15 kDa (CEVd-P1) and 18 kDa (CEVd-P2) were detected in leaves of CEVd-infected *Gynura aurantiaca* (Conejero and Semancik, 1977b).

Enhanced levels of analogous polypeptides were found when the search was extended to additional host plants (Etrog citron, tomato cv. "Rutgers", potato cv. "Marijke") infected with CEVd (Conejero *et al.*, 1979). These polypeptides showed slight variation in size, depending on the host. This fact could be compatible with a viroid-specified nature of these polypeptides and a host-dependent processing reaction. Nevertheless, in one host, *G. aurantiaca*, such polypeptides were also detected in naturally senescing plants, indicating that they were not translation products of the viroid RNA, nor of any mRNA exclusively related to the viroid-host interaction. This finding also excluded the possibility proposed by Matthews (1978) that the polypeptides might be specified by viroid complementary sequences.

Later, the accumulation of several low molecular weight polypeptides was reported for a number of plants infected with several viroids (CEVd, PSTVd, cucumber pale fruit viroid (CPFVd), CSVd) and, in the case of tomato, infected by tobacco mosaic virus (TMV) and cucumber mosaic virus, and the fungus *Cladosporium fulvum* (Camacho-Henriques and Sänger, 1982a,b). More recently, an enhanced level of a high molecular weight protein (140 kDa) was also found in tomato plants infected with TPMVd (Galindo *et al.*, 1984). In addition, decreases in a number of protein species in the range of 14 kDa to 45 kDa were found in different hosts infected by CSVd. Decreased levels of four polypeptides (14.5 kDa to 105 kDa) were associated with pathogenesis in tomato leaves infected either by viroids, viruses or *Cladosporium fulvum* (Camacho-Henriquez and Sänger, 1982b).

All these observations strengthened the idea that the enhanced occurrence of certain proteins which accompanies viroid infection is a host response that may be generalized beyond this particular type of host-pathogen interaction. Thus, these proteins were considered to belong to the class of "pathogenesis-related" or "PR" proteins where the definition includes all proteins which are stimulated in plants as a consequence of pathologically altered metabolism (Van Loon, 1983).

The finding that the polypeptides associated with viroid infection are not specified by the viroid but result from a disease-induced alteration of host metabolism (Conejero *et al.,* 1979; Camacho-Henriquez and Sänger, 1982a,b), prompted the search for other proteins as possible components of the pathological response, as had been described for other systems (Van Loon and Van Kammen, 1970; Gianinazzi *et al.,* 1970). Since the non-denaturing electrophoresis system (Davis, 1964) normally used to detect tobacco PRs excluded basic proteins, a system with the capability of separating cationic proteins (Reisfield *et al.,* 1962) was also tested. This approach led to the detection of ten PR proteins (C1 to C10) in tomato plants infected with CEVd (Granell *et al.,* 1987). Only four cationic PR proteins could be detected in CEVd-infected *G. aurantiaca* (Semancik and Conejero, 1987), again emphasizing the specificity of host control in the biosynthesis of proteins. The basic nature of nine of the tomato proteins contrasts with most PR proteins described so far (Van Loon, 1985). However, the tomato PR proteins do share with tobacco PR proteins the characteristics of being preferentially extracted at low pH and resistant to proteinase digestion (Granell *et al.,* 1987). The tomato PR protein C2, which increases most dramatically, corresponded to the polypeptide previously described as tomato CEVd-P1 (Conejero *et al.,* 1979) or p14 (Camacho-Henriquez and Sänger, 1982b). The basic nature of this protein has been pointed out (Camacho-Henriquez and Sänger 1984) and recently confirmed by sequencing (Lucas *et al.,* 1985). The 69 kDa polypeptide most probably coincides with the 70 kDa polypeptide reported for tomato infected with TPMVd (Diener, 1987) while some of the other SDS-detected proteins matched those reported for tomato infected with PSTVd (Camacho-Henriquez and Sänger, 1982b). These PR proteins were also induced by Ag^+ and ethephon treatments in association with necrotic (localized) lesions or non-necrotic (systemic, viroid-like) reactions (Granell *et al.,* 1987) indicating that their accumulation is not correlated with necrosis. As already discussed, these findings gave support to the idea that PR proteins are components of a non-specific response of the host mediated by ethylene (Granell *et al.,* 1987).

Biological significance of the PR proteins. The discovery of these proteins posed a number of questions, among which their biochemical function and biological role are the most important, not only for understanding of viroid pathogenesis but also as components of a system of response of plants to pathogenic or stress signals.

Even before the concept of PR proteins had been crystallized, attempts to unravel the biochemical activity displayed by viroid-induced proteins had been made with the

P1 protein from CEVd-infected *G. aurantiaca*. In these studies the potential activities of this protein as ribonuclease or hormone-binding protein were tested with no conclusive results (Flores *et al.*, 1978).

Recently, P-69 has been characterized as an alkaline cysteine proteinase (Vera and Conejero, 1988). It has also been found that P-32 and P-34 (C7 and C6 respectively of Granell *et al.*, 1987) are chitinases (García-Breijo *et al.*, 1989). Also, a ß-1,3-glucanase activity has been assigned to a CEVd-induced PR protein in tomato (F García-Breijo, unpublished results).

Critical to the unravelling of the biological role of these proteins is their *in vivo* localization. The electrophoretic detection of PR proteins in extracts obtained by vacuum infiltration of tomato leaves infected with TMV (Parent and Asselin, 1984), treated with chemicals (Hooft van Huijsduijnen *et al.*, 1986), or after infection with *Cladosporium fulvum* (De Wit and Van der Meer, 1986) prompted the idea of the intercellular localization of PR proteins. Recently Carr *et al.* (1987) have reported the localization of PR1 in tobacco leaves by immunofluorescence. A more direct approach, the immuno-gold-EM technique applied to P1 (p14) (Vera *et al.*, 1988) and P69 (Vera *et al.*, 1989b) led to the discovery of two main locations: the vacuole, in association with inclusion bodies, (a newly described location) and the intercellular spaces of CEVd-infected tomato leaves.

We have already commented on the reputed resistance of PR proteins to degradation by endogenous proteases (Van Loon, 1985). Consistently, they have long half-lives (40-70 h) (Matsuoka and Ohashi, 1986), but there must be some turnover. The fact that they accumulate in the intercellular space (Van Loon, 1985) suggests that their degradation might also occur there. Following this idea, evidence has been obtained (Rodrigo *et al.*, 1989) that some tomato PR proteins are degraded upon incubation of intercellular washing fluids (IWF). This degradation occurs at acidic pH and it has been found that the enzyme responsible is a 37-kDa constitutive aspartyl endoproteinase with a pH optimum of 2.5-3.5. The selective hydrolysis of some of the pathogenesis-related proteins by the 37 kDa aspartyl proteinase lends credence to a possible rôle for this proteinase as part of the regulatory mechanism for the biological action of the PR proteins. The biological relevance of this enzyme in the PR metabolism is reinforced by the fact that an analogous enzyme has been isolated from IWF of tobacco plants (I Rodrigo, P Vera and V Conejero, unpublished results).

Although the biological role of most of these tomato PR proteins is as yet

unknown the following must be stressed:

1. Chitinases and ß-1,3-glucanases can clearly be implicated in defence against pathogens such as bacteria and fungi, containing chitin or ß-1,3-glucans as a component of their structure (Boller *et al.,* 1983).
2. The rôle of P69 proteinase (Vera and Conejero, 1988) either in symptom production or as a defence tool remains to be elucidated.
3. There is no evidence for any biological activity of P1 (p14), the most intensely accumulated PR protein in leaf tissue as a consequence of the viroid induced response. Nevertheless, P1 has been found in leaves from healthy (non-infected) plants and always in association with cell material undergoing disorganization (Vera *et al.,* 1988). This led these authors to propose the idea that P1 is involved in cell degeneration, either naturally activated as a normal event of the biological cycle of the plant (e.g. in lysogenous development of intercellular spaces (Esau, 1972; Fahn, 1972) or by cell ageing) or provoked by afflicting agents. The possible involvement of P1 in the resistance induced in the systemic reaction of the host or with some other biological role cannot be discarded. The biological significance of P1 is also sustained by the fact that this protein is synthesized as a pre-protein, then targetted to the vacuole and to the apoplast (Vera *et al.,* 1988; 1989a) in association with P69 (Vera *et al.,* 1989b). The vacuolar and apoplastic localization of chitinases have also been reported (Mauch and Staehelin, 1989).
4. The reported evidence to date indicates that ethylene is an intermediary step (second messenger) in the coordinate activation of PR synthesis as part of the response to viroid infection (Conejero and Granell, 1986; Granell *et al.,* 1987; Bellés and Conejero, 1989).

All this suggests that the viroid induced PR proteins, despite their different biochemical activities, might have the common biological role of being part of an adaptive response against potential agressors from the environment.

Cell wall components. Three types of biochemical alterations of the cell wall can be considered for possible involvement in the response of the plant to viroid infection: those involved in limiting cell expansion; those implicated in altering the normal cell-to-cell communication and signalling in developmentally active cells and tissues; and breakdown products with the capability of becoming secondary pathogenic signals for neighbouring cells.

The effects of viroid infection on cell walls were realized in cytological studies.

These cytopathic effects received biochemical confirmation when a differential release of protoplasts from healthy and viroid infected tissues was attributed to abnormal ß-1,3-glucan in viroid infected cells (Marton *et al.*, 1982).

More direct information was obtained with purified cell walls from healthy and CEVd-infected tomato cell suspension cultures. Enhanced levels of the key constituents of extensin, hydroxyproline and specific arabinosyl residues, were detected (Wang *et al.*, 1986), and taken to indicate an increased extensin content. An ethylene-induced accumulation of hydroxyproline rich proteins has also been found in diseased plants, and this accumulation has been correlated with the developmental alterations produced (Wang *et al.*, 1986).

A possible mechanistic relationship between the viroid-enhanced ethylene production and its effects in limiting cell expansion growth involving peroxidases and extensin has been outlined (Semancik and Conejero, 1987).

Finally, prominent protrusions have been detected on the surfaces of CEVd-infected cells (Wang *et al.*, 1986). Although the chemical nature of these structures has not yet been determined, it is possible that they are related to the alterations in cell wall composition. A possible relationship between the specific restriction of cell wall growth and the appearance of endocytic invaginations (plasmalemmasomes) reported in viroid-infected cells has also been suggested.

Individual specific reactions integrated in a general non-specific network system of response. The idea that the whole metabolic structure of a plant consists of pathways of sequential steps which are interconnected in a highly reticulate network seems well accepted. This type of organization, together with cascade systems to amplify signals, permits synchronous control of many biochemical functions (Blowers and Trewavas, 1989). It also explains how very different pathogenic signals can elicit common responses (Fig. 1). Otherwise, it is difficult to explain how a viroid is able to elicit a response including coordinate expression of genes encoding chitinases, glucanases, etc. whose biological role is defence against infections agents or stresses other than viroids.

One could hypothesize that these coordinately interconnected defence tools may have been built up in two steps: specific and individual development of each tool by coevolution with a given pathogen or with aggressive environmental conditions, and then the interlinking of the individual specific responses. Thus, what appears to be a non-specific response may result from evolutionary integration of specific individual

components. With this strategy, the battery of defensive weapons would be progressively enriched by new experiences. A prediction of this model would be the possible existence or future development of proteinases and nucleases against viruses and viroids.

The lack of specificity of this response could have biological advantages for the plant. This type of mechanism would broaden the scope of potential pathogens against which the plant would have acquired a certain degree of immunity after having been non-specifically triggered (Fig. 1).

AN INTEGRATIVE MODEL

How a response at the cellular level becomes a developmental disease: intracellular and intercellular stimulus-response processes

The general inhibition of growth in viroid infections is readily understandable. It is proposed that primary infection might occur in the region of differentiation of the procambial initials in the growing apex (Semancik and Conejero, 1987). Then, synthesis and release of ethylene is persistently enhanced during viroid infection resulting in inhibition of growth (Bellés *et al.*, 1989a).

It is more difficult to explain how the pathogenic response at the cellular level has effects at higher levels of organization, thus producing the distorted pattern of leaf development, for example. A precise system of cell-to-cell communication is required for the control of the normal pattern of growth and differentiation of the leaf. Failure or changes of some critical cell-to-cell connections, produced by alterations of the cell wall structure or other signalling events, could distort developmental patterns. A differential susceptibility of cells to becoming infected and to producing a pathogenic response would contribute to the macroscopical anisotropy of development. This hypothesis would also explain how different pathogenic agents such as a viroid (CEVd) and Ag^+ induce the same macroscopic syndrome, if both agents elicited the same general response in the cells. But we still need to explain how two agents with different pathways of access to cells ($AgNO_3$ is applied by spraying, viroids travel through the vascular system) incite indistinguishable developmental aberrations. This difficulty can be overcome if it is assumed that the same juvenile cells with a special reactivity and with the same developmental role are preferentially affected by both agents. We are dealing with an adaptive morphogenetic response to viroids which, following our

reductionist ideas, conveys information through common "informational channels". This is true not only at the cellular level (integrating membranes, common second messengers and coordinate activation of certain genes) but also at the supracellular level (commonality of morphogenetic information fluxes which can be distorted in the same way by alteration of certain especially susceptible key cells).

The model involves juvenile compatible cells which have the capacity to become infected and to display a pathogenic response (i.e. to undergo affliction and produce defence reactions) (Semancik and Conejero, 1987). Of these cells, a few would be the primary target cells: those at which the viroid molecules arrive from outside. Also, a small proportion of these cells would be infected by viroid molecules coming from other cells. But the bulk of infection is probably through cell division. The infected cells undergo a series of biochemical changes as a consequence of which they become physiologically and structurally altered. In the language of the signalling model, viroids as "primary signals" are amplified through replication, then "sensed", most probably at the membrane level, thus triggering a network of transductional events leading to cell response. The cell becomes a viroid-diseased cell, then transmits mismessages of aberrant developmental information horizontally, to other cells. Disease mismessages are also transmitted vertically by cell division.

All this conforms the developmental syndrome, which may also involve non-infected cells brought in by secondary signalling. This reflects the existence of a cascade system of signal transduction in the supracellular network, analogous to that at the cellular level (Fig. 2). In this case, one would predict, for example, that ethylene synthesis and PR protein production would occur in "secondarily stressed" but non-infected cells of infected leaves.

How does cellular response lead to a more resistant plant?

Apart from the known phenomena of specific cross-protection among viroids (interference with the establishment of a severe strain of a viroid by prior infection and specific interaction with an homologous milder strain) there are other possible mechanisms of resistance and protection in which signalling and response may be implicated. Chitinases, ß-1,3-glucanases and other PR proteins potentially implicated in defence reactions have already been discussed and may have specific defensive significance (Vera *et al.*, 1989a,b; Mauch and Staehelin, 1989), although none is known against viroids. Non-specific physiological resistance may also occur as a consequence

of the stress imposed by infection in either infected or non-infected neighbouring cells which have been signalled. These cells lose the juvenile condition that enables them to be compatible and responsive to viroid infection. This may be the kind of protection produced by Ag^+ and ethephon treatments at low doses. The lack of specificity in the so-called cross-protection between non-homologous viroids (Niblett *et al.*, 1978) could also be an example of non-specific physiological resistance.

In systems reacting hypersensitively (which is not in general the case for viroid infections) there is another type of defensive phenomenon, the so-called induced systemic resistance (as in TMV infection of *N*-gene tobacco). The necrotic localized reaction in fully expanded inoculated leaves induces resistance to virus establishment in younger, upper leaves. Gianinazzi *et al.* (1970) and Van Loon and Van Kammen (1970) suggested that PR proteins could be involved in this phenomenon.

A similar reaction was observed in experiments following the mimicking procedure previously described for tobacco plants (Van Loon, 1977). Fully expanded leaves of *Gynura aurantiaca* plants were pricked with 0.2 M ethephon prior to infection with CEVd. This treatment impaired the establishment of viroid infection. This is taken as additional proof of the general scope of this mechanism of defence. In this case, however, no PR protein was associated with the induced resistance (JM Bellés, unpublished results). This is in accordance with the reported lack of correlation between acquired systemic resistance to viruses and PR proteins (for a review see Fraser, 1987).

CONCLUSIONS AND PROSPECTS

The model outlined in Figure 2 is an attempt to summarize and integrate the data and ideas that have been discussed in the preceeding pages under the concept of host-pathogen interaction as a case of pathogenic signalling and homeostatic response in plants. Although the model has numerous gaps, these are due to lack of experimental data rather than to inconsistency with what is presently known. Deeper knowledge of many mechanistic details is still required, but the model provides a framework within which a number of questions relating to viroid pathogenesis can be considered.

1. How can some host plants actively replicate viroids without any visible pathological syndrome?

2. Why do viroids not "need" to act as messengers to incite disease?
3. How can viroids with very different primary structures produce indistinguishable disease syndromes on a given host?
4. Why is the intensity but not the type of syndrome expressed by the host modulated by the viroid structure?
5. How can the so called cross-protection between rather non-homologous viroids be explained?

Principally, we need to elucidate which is the initial pathogenic alteration or interference produced by viroid molecules; which are the steps leading to the signal that triggers the non-specific network of reactions forming the host response; which are the components of this informational flux; how it is connected with control of gene expression; and what kind of relationship exists between the pathogenic and defence reactions.

Viroids, as the simplest pathogens, offer unique opportunities to study these questions. These opportunities are enhanced by the ease with which viroid molecules can now be engineered. Studies with viroids may give insights into how plants interact with more complex pathogens such as viruses and fungi. However, a major obstacle to progress in studies of plant-viroid interactions is the lack of host systems which are genetically well-defined for variation in response, in terms of resistance/susceptibility and severity of the disease syndrome.

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REFERENCES

Bellés JM, Granell A, Durán-Vila N, Conejero V (1989a) ACC synthesis as the activated step responsible for the rise of ethylene production accompanying citrus exocortis viroid infection in tomato plants. J Phytopathol 125:198-208

Bellés JM, Vera P, Durán-Vila N, Conejero V (1989b) Ethylene production in tomato cultures infected with citrus exocortis viroid (CEV). Can J Plant Pathol (in press)

Bellés JM, Conejero V (1989) Ethylene mediation of the viroid-like syndrome induced by Ag^+ ions in *Gynura aurantiaca* DC plants. J Phytopathol 124:275-284

Blowers DP, Trewavas AJ (1989) Second messengers: their existence and relationship to protein kinases. In: Boss WF, Morré DJ (eds) Second messengers in plant growth and development. Alan R Liss, New York, in press

Boller T, Gehri A, Mauch F, Vögeli U (1983) Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. Planta 157:22-31

Boss WF, Morré DJ (1989) Second messengers in plant growth and development. Alan R Liss, New York, in press

Brown EF, Newton RP (1981) Cyclic AMP and higher plants. Phytochemistry 20:2453-2463

Camacho-Henriquez A, Sänger HL (1982a) Gelelectrophoretic analysis of phenol-extractable leaf proteins from different viroid/host combinations. Arch Virol 74:167-180

Camacho-Henriquez A, Sänger HL (1982b) Analysis of acid-extractable tomato leaf proteins after infection with a viroid, two viruses and a fungus, and partial purification of the "pathogenesis-related" protein p14. Arch Virol 74:181-196

Camacho-Henriquez A, Sänger HL (1984) Purification and partial characterization of the major "pathogenesis-related" tomato leaf protein p14 from potato spindle tuber viroid (PSTV)-infected tomato leaves. Arch Virol 81:263-284

Carr JP, Dixon DC, Nikolau BJ, Voelkerding KV, Klessig DF (1987) Synthesis and localization of pathogenesis-related proteins in tobacco. Mol Cell Bol 7:1580-1583

Conejero V (1981) Stimulation of a viroid-like syndrome and inhibition of symptom expression by Ag^+ ion. Abstr 5th Int Congr Virol Strasbourg, France, p 228

Conejero V (1982) Do viroids elicit host pre-existing mechanisms of response? Abstr IV Int Conf Comparative Control of Viral Diseases Banff, Alberta, Canada (Abstr W 12-1)

Conejero V, Granell A (1986) Stimulation of a viroid-like syndrome and the impairment of viroid infection in *Gynura aurantiaca* DC plants by treatment with silver ions. Physiol Mol Plant Pathol 29:317-323

Conejero V, Semancik JS (1977a) Exocortis viroid: alteration in the proteins of *Gynura aurantiaca* accompanying viroid infection. Virology 77, 221-232

Conejero V, Semancik JS (1977b) Analysis of the proteins in crude plant extracts by polyacrylamide slab gel electrophoresis. Phytopathology 67:1424-1426

Conejero V, Picazo I, Segado P (1979) Citrus exocortis viroid (CEV): protein alterations in different hosts following viroid infection. Virology 97:454-456

Davies JW, Kaesberg P, Diener TO (1974) Potato spindle tuber viroid. XII. An investigation of viroid RNA as messenger for protein synthesis. Virology 61:281-286

Davis BJ (1964) Disc electrophoresis. II. Method and application to human serum proteins. Annu NY Acad Sci 121:404-427

De Wit PJGM, Van Der Meer GM (1986) Accumulation of the pathogenesis-related tomato leaf protein P14 as an early indicator of incompatibility in the interaction between *Cladosporium fulvum* (syn *Fulvia fulva*) and tomato. Physiol Plant Pathol 24:17-23

Diener TO (1979) Viroids and viroid diseases. Wiley, New York

Diener TO (1981) Are viroids escaped introns? Proc Natl Acad Sci USA 78:5014-5015

Diener TO (ed) (1987) The viroids. Plenum, New York

Dickson E (1981) A model for the involvement of viroids in RNA splicing. Virology

115:216-221
Esau K (1972) Plant Anatomy, 2nd edn., Wiley, New York
Fahn A (1972) Plant Anatomy, 2nd edn., Pergamon, Oxford
Flores R (1984) Is the conformation of viroids involved in their pathogenicity? J Theor Biol 108:519-527
Flores R, Rodríguez JL (1981) Altered pattern of root formation on cuttings of *Gynura aurantiaca* infected by citrus exocortis viroid. Phytopathology 71:964-966
Flores R, Chroboczek J, Semancik JS (1978) Some properties of the CEV-P1 protein from citrus exocortis viroid-infected *Gynura aurantiaca* DC. Physiol Plant Pathol 13:193-201
Fraser RSS (1987) Biochemistry of virus-infected plants. Research Studies Press, Letchworth; Wiley, New York
Galindo JA, Smith DR, Diener TO (1984) A disease-associated host protein in viroid-infected tomato. Physiol Plant Pathol 24:257-275
García-Breijo F, Garro R, Conejero V (1989) Two pathogenesis-related (PR) proteins C7 (P32) and C6 (P34) from citrus exocortis viroid (CEV)-infected tomato plants are chitinases. Physiol Mol Plant Pathol (in press)
Gianinazzi S, Martin C, Vallée JC (1970) Hypersensibilité aux virus, température et protéines solubles chez le *Nicotiana* Xanthi nc. Apparition de nouvelles macromolécules lors de la répression de la synthèse virale. CR Acad Sci Paris 270D:2383-2386
Granell A, Bellés JM, Conejero V (1987) Induction of pathogenesis-related proteins in tomato by citrus exocortis viroid, silver ion and ethephon. Physiol Mol Plant Pathol 31:83-90
Gross HJ, Domdey H, Losow C, Jank P, Raba M, Alberty H, Sänger HL (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. Nature 273:203-208
Guy M, Kende H (1984) Conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by isolated vacuoles of *Pisum sativum* L. Planta 160:281-287
Haas B, Klanner A, Ramm K, Sänger HL (1988) The 7S RNA from tomato leaf tissue resembles a signal recognition particle RNA and exhibits a remarkable sequence complementarity to viroids. EMBO J 7:4063-4074
Hall TC, Wepprich RK, Davies JW, Weathers LG, Semancik JS (1974) Functional distinctions between the ribonucleic acids from citrus exocortis viroid and plant viruses: Cell-free translation and amino-acylation reactions. Virology 61:486-492
Hanks RW, Feldman AW (1972) Changes in amounts of auxin-like growth promoter, gibberellin, and inhibition in citrus infected with exocortis virus. In: Price WC (ed) Proceedings of the 5th conference of the international organization of citrus virologists, University of Florida, Gainesville, p 244
Hari V (1980) Ultrastructure of potato spindle tuber viroid-infected tomato leaf tissue. Phytopathology 70:385-387
Hepler PK, Wayne RO (1985) Calcium and plant development. Annu Rev Plant Physiol 36:397-439
Hiddinga HJ, Jenssen Crum C, Hu J, Roth DA (1988) Viroid-induced phosphorylation of a host protein related to a dsRNA-dependent protein kinase. Science 241:451-453
Hooft Van Huijsduijnen RAM, Alblas SW, De Rijk RJ, Bol JF (1986) Induction by salicylic acid of pathogenesis-related proteins and resistance to alfalfa mosaic virus infection in various plant species. J Gen Virol 67: 2135-2143
Klaff P, Gruner R, Hecker R, Sättler A, Theissen G, Riesner R (1989) Reconstituted

and cellular viroid-protein complexes. J Gen Virol 70:2257-2270
Kojima M, Murai M, Shikata E (1983) Cytopathic changes in viroid-infected leaf tissues. J Fac Agric Hokkaido Univ 61:219-223
Lieberman M (1979) Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30:533-591
Lucas J, Camacho-Henriquez A, Lottspeich F, Henschen A, Sänger HL (1985) Amino acid sequence of the "pathogenesis-related" leaf protein p14 from viroid-infected tomato reveals a new type of structurally unfamiliar proteins. EMBO J 4:2745-2749
Mauch F, Staehelin LA (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and ß-1,3-glucanase in bean leaves. Plant Cell 1:447-457
Marmé D (1989) The role of calcium and calmodulin in signal transduction. In: Boss WF, Morré DJ (eds) Second messengers in plant growth and development. Alan R Liss Inc, New York, in press
Marton L, Durán-Vila N, Lin J, Semancik JS (1982) Properties of cell cultures containing the citrus exocortis viroid. Virology 122:229-238
Matsuoka M, Ohashi Y (1986) Induction of pathogenesis-related proteins in tobacco leaves. Plant Physiol 80:505-510
Matthews REF (1978) Are viroids negative-strand viruses? Nature 276:850
Niblett CL, Dickson E, Fernow KH, Horst RK, Zaitlin M (1978) Cross protection among four viroids. Virology 61:486-492
Owens RA, Hammond RW (1987) Viroid function: Molecular biology of viroid-host interactions. In: Diener TO (ed) The viroids. Plenum, New York, p 167
Paliwal YC, Singh RP (1981) Cytopathological changes induced by potato spindle tuber viroid in *Scopolia sinensis*. Can J Bot 59:677-682
Parent JG, Asselin A (1984) Detection of pathogenesis-related proteins (PR or b) and other proteins in the intercellular fluid of hypersensitive plants infected with tobacco mosaic virus. Can J Bot 62:564-569
Rackwitz HR, Rohde W, Sänger HL (1981) DNA-dependent RNA polymerase II of plant origin transcribes viroid RNA into full-length copies. Nature 291:297-301
Ranjeva R, Boudet AM (1987) Phosphorylation of proteins in plants: regulatory effect and potential involvement in stimulus/response coupling. Annu Rev Plant Physiol 38:73-93
Reisfeld RA, Lewis VJ, Williams DJ (1962) Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature 195:156-160
Riesner D (1987) Physical-chemical properties. Structure formation. In: Diener TO (ed) The viroids. Plenum, New York, p 63
Robertson HD, Branch AD (1987) The viroid replication process. In: Semanck JS (ed), Viroids and viroid-like pathogens. CRC Press, Boca Raton, p 50
Rodrigo I, Vera P, Conejero V (1989) Degradation of tomato pathogenesis-related proteins by an endogenous 37-kDa aspartyl endoproteinase. Eur J Biochem (in press)
Rodríguez JL, García-Martínez JL, Flores R (1978) The relationship between plant growth substance content and infection of *Gynura aurantiaca* DC by citrus exocortis viroid. Physiol Mol Plant Pathol 13:355-363
Sänger HL (1982) Biology, structure, functions and possible origin of viroids. In: Parthier B, Boulter D (eds) Encyclopedia of plant physiology. New Series Vol. 14B Springer-Verlag, Berlin, p 368
Sänger HL (1987) Viroid function. Viroid replication. In: Diener TO (ed) The viroids. Plenum, New York, p 117

Schnölzer M, Haas B, Ramm K, Hofmann H, Sänger HL (1985) Correlation between structure and pathogenicity of potato spindle tuber viroid (PSTV). EMBO J 4:2181-2190

Schumacher J, Sänger HL, Riesner D (1983) Subcellular localization of viroids in highly purified nuclei from tomato leaf tissue. EMBO J 2:1549-1555

Semancik JS, Conejero V (1987) Viroid pathogenesis and expression of biological activity. In: Semancik JS (ed) Viroids and viroid-like pathogens. CRC Press, Boca Raton, p 71

Semancik JS, Conejero V, Gerhart J (1977) Citrus exocortis viroid: survey of protein synthesis in *Xenopus laevis* oocytes following addition of viroid RNA. Virology 80:218-221

Semancik JS, Vanderwoude WJ (1976) Exocortis viroid: cythopathic effects at the plasma membrane in association with pathogenic RNA. Virology 69:719-726

Solymosy F, Kiss T (1985) Viroids and snRNAs. In: Maramorosch D, McKelvey JJ (eds) Subviral pathogens of plants and animals: viroids and prions. Academic, New York, p 183

Van Loon LC (1977) Induction by 2-chloroethylphosphonic acid of viral-like lesions, associated proteins and systemic resistance in tobacco. Virology 80:417-420

Van Loon LC (ed) (1983) Workshop on pathogenesis-related (b) proteins in plants. Neth J Plant Pathol 89:239-325

Van Loon LC (1985) Pathogenesis-related proteins. Plant Mol Biol 4:111-116

Van Loon LC, Van Kammen (1970) Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN'. II. Changes in protein constitution after infection with tobacco mosaic virus. Virology 40:199-211

Vera P, Conejero V (1988) Pathogenesis-related proteins of tomato. P69 as an alkaline endoproteinase. Plant Physiol 87:58-63

Vera P, Conejero V (1989) Citrus exocortis viroid (CEV) infection alters the *in vitro* pattern of protein phosphorylation of tomato leaf proteins. Mol Plant-Microbe Interactions, in press

Vera P, Hernández-Yago J, Conejero V (1988) Immunocytological localization of the major "pathogenesis-related" (PR) protein of tomato plants. Plant Sci 55:223-230

Vera P, Hernández-Yago J, Conejero V (1989a) "Pathogenesis-related" P1 (p14) protein. Vacuolar and apoplastic localization in leaf tissue from tomato plants infected with citrus exocortis viroid: *in vitro* synthesis and processing. J Gen Virol 70:1933-1942

Vera P, Hernández-Yago J, Conejero V (1989b) Immunogold localization of the citrus exocortis viroid-induced pathogenesis-related proteinase P69 in tomato leaves. Plant Physiol 91:119-123

Visvader JE, Symons RH (1985) Eleven new sequence variants of citrus exocortis viroid and the correlation of sequence with pathogenicity. Nucleic Acids Res 13:2907-2920

Visvader JE, Symons RH (1986) Replication of *in vitro* constructed viroid mutants: location of the pathogenicity-modulating domain of citrus exocortis viroid. EMBO J 5:2051-2055

Wahn K, Rosenberg-de Gomez F, Sänger HL (1980) Cythopathic changes in leaf tissue of *Gynura aurantiaca* infected with the viroid of citrus exocortis disease. J Gen Virol 49:355-365

Wang MC, Lin JJ, Durán-Vila N, Semancik JS (1986) Alteration in cell-wall composition and structure in viroid-infected cells. Physiol Mol Plant Pathol

28:107-127
Wolf P, Gilz R, Schumacher J, Riesner D (1985) Complexes of viroids with histones and other protein. Nucleic Acids Res 13:355-367
Yang SF, Hoffman N (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155-159
Yunghaus WN, Morré DJ (1977) Adenylate cyclase activity is not found in soybean hypocotyl and onion meristem. Plant Physiol 60:144-149
Zaitlin M, Hariharasubramanian V (1972) A gel electrophoretic analysis of proteins from plants infected with tobacco mosaic and potato spindle tuber viruses. Virology 47:296-305