SIGNALLING IN VIROID PATHOGENESIS

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ABSTRACT

Despite the fact that viroids are the infectious agents with the lowest complexity and best known structure, our understanding of their pathogenic interaction with the host plant is still far from complete. Viroid pathogenesis poses many intriguing questions; these have to be answered without any viroid-specified protein to which to attribute a pathogenic rôle. Here we present a view of viroid pathogenesis in which viroid molecules are considered as pure replicating and pathogenic signals. These act as elicitors of host responses which can also be activated by other afflicting agents. A model is outlined to explain how the viroid-induced response at the cellular level becomes a developmental disease and leads to a plant which is more resistant to subsequent infections.

INTRODUCTION

In the early nineteen-eighties, the idea was first proposed that viroids are non-specific elicitors of a host-coded mechanism of response, that can also be triggered by other agents (Conejero 1981; 1982). At that time, the existence of molecular mechanisms for signal perception and transduction in plant cells was barely being considered. Nowadays, signalling has gained recognition and has become a leading area of interest in plant molecular biology.

Unfortunately, the field is at the beginning of its development, and the cumulated knowledge still has numerous gaps, although this disadvantage is also its charm. In this

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chapter, we are going to attempt to build up a view of viroid pathogenesis within the framework of signalling and signal transduction in plants. We will make suggestions of suitable approaches to increase knowledge of how viroids induce diseases and activate defence reactions in plants. Our approach will be speculative.

VIROID PATHOGENESIS

General considerations

Although viroid replication is a reasonably well understood process (Tabler and Tsagris, this volume), and although there is precise knowledge of their structure and conformation (Gross *et al.*, 1978), the way in which viroid molecules induce diseases and defence reactions is largely unknown.

Viroids have the lowest biological complexity of any pathogen yet known. Their naked RNAs, with a molecular mass of about 10^5 Da, have an informational content of about one tenth that of a minimal virus. Nevertheless, viroids are able to accomplish virus-like functions. They enter the host-plant with the aid of a vector; man with his agricultural practices is probably the main means of transmission. They translocate and spread systemically through the vascular system to reach the target cells. Viroids also replicate autonomously (without the aid of a helper virus), and induce metabolic and developmental alterations reflected in a disease syndrome (plant stunting, leaf malformation and inhibition of root growth). Finally, they are able to induce resistance or protection against subsequent infections.

How do viroids induce these biological processes? Are these biological processes attributable to any specific characteristics of the viroid structure, conformation and mechanism of replication, or are they a response to any specific protein or other kind of molecule induced by infection?

Viroids as non-specific elicitors of responses programmed by the host

Since the evidence indicates that viroids do not code for proteins (Davies *et al.*, 1974; Hall *et al.*, 1974; Semancik *et al.*, 1977; Conejero *et al.*, 1979), it was thought that their pathogenicity had to be exerted through direct interference with some critical cellular targets (Diener, 1979; Semancik and Conejero, 1987). From this, and taking into account the replication and location of viroids in the nucleus (Riesner, 1987; Robertson

and Branch, 1987; Sänger, 1987), the obvious strategy was to look for possible interference of viroid RNA molecules (+RNA) or complementary replicative forms, with either host nucleic acids or proteins, as the primary cause of pathogenicity. Thus it has been suggested that viroids express their pathogenicity by altered regulation of gene expression (Rackwitz *et al.*, 1981; Diener, 1981; Dickson, 1981; Solymosy and Kiss, 1985) and altered translocation of proteins, by base pairing with RNA signal recognition particles (Haas *et al.*, 1988). These interpretations are supported by theoretical considerations, but still await full experimental confirmation.

A different type of approach in our laboratory (Conejero and Granell, 1986) led to evidence indicating that viroids do not incite the developmental syndrome and defence reactions by direct and specific interference with the normal flux of genetic information in the host cell. Rather, the evidence suggested that viroids seem to be elicitors of a general response of the host plant, and that this is mediated by ethylene. This response can also be triggered by agents with no genetic information. It could be said that viroids as pathogens are devoid of "personality".

The idea that at least certain physiological alterations induced by viroid infection were not specific arose when it was found that natural senescence of *Gynura aurantiaca* DC plants was accompanied by the accumulation of the same pathogenesis-related (PR) proteins (P1, 15 kDa and P2, 18 kDa) originally described as associated with citrus exocortis viroid (CEVd) infection (Conejero *et al.*, 1979). Later, (Conejero and Granell, 1986), it was shown that high doses of Ag^+ ions elicited a reaction almost indistinguishable from that induced by CEVd in *Gynura*. It was concluded that the pathological syndrome, defence reactions and the physiological condition of enhanced resistance were components of a general mechanism of response triggered by different types of elicitors.

SIGNALLING IN VIROID PATHOGENESIS

Although a reductionist view would consider that all types of information in biological systems are related to the genetic language, there are informational fluxes that have their own structure and dynamics. This is irrespective of the fact that the genetic machinery can be implicated as a recurrent step, at any time when synthesis of new protein is needed.

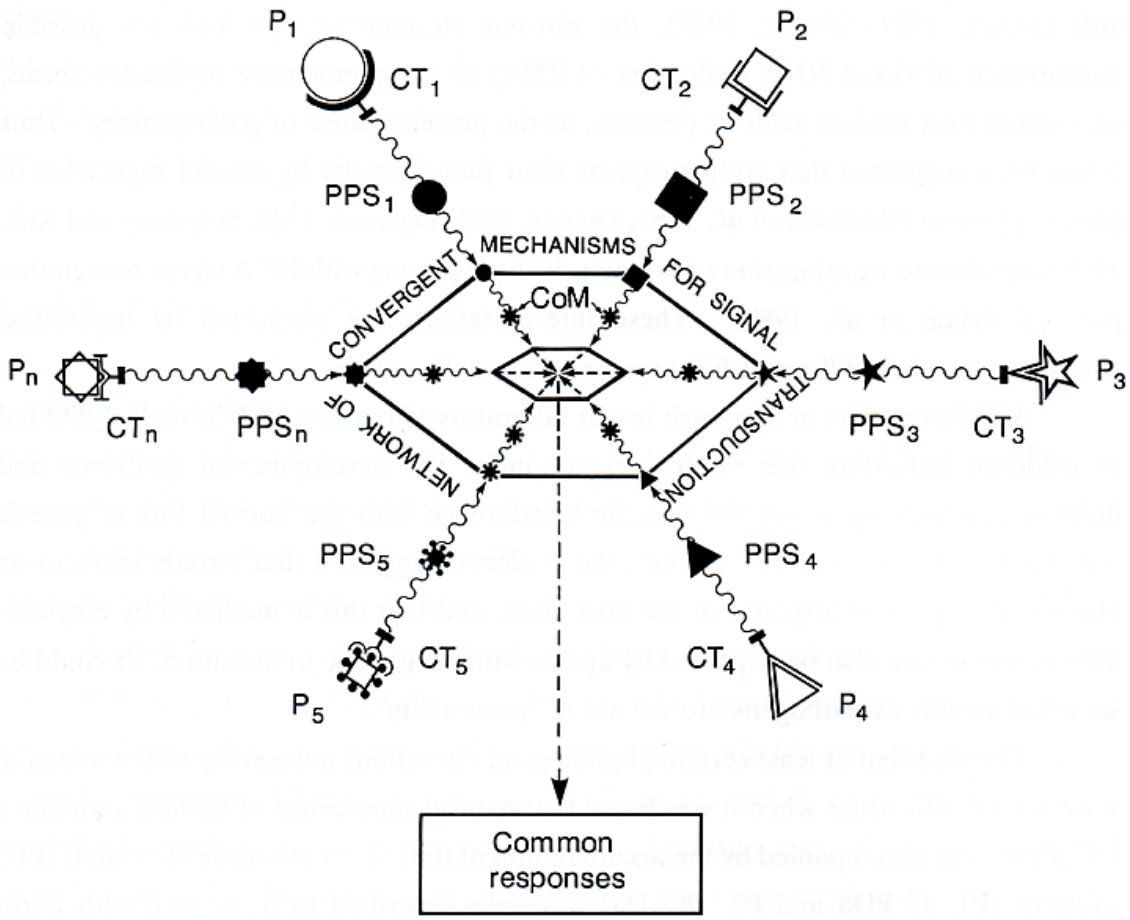

Figure 1. Convergence in transduction and response to pathogenic signals. Originally different pathogenic signals are conveyed, through integrating mechanisms, into a common message leading to elicitation of common responses. P_1-P_n - different pathogens; CT_1-CT_n - cellular targets or sensors; $P_1.CT_1$ etc - primary pathogenic events; PPS_1 etc - primary pathogen signalling; CoM - common messenger.

These fluxes begin with the "perception" of a change in the environment ("primary signal" or "stimulus"). The signal is "transduced" through intermediary steps ("secondary" or higher order "signals" or "messengers") into a message which is understandable by cellular targets with the capability of giving a "short-term" response, or by the genetic machinery when a more permanent response is required.

The signals can be very distinct; examples include radiation, temperature, pressure, non-biotic chemicals, certain regulatory molecules, cell-to-cell structural contacts, structural components of pathogens and breakdown products of cell structures. The capacity of response to certain of these signals is on the basis of the morphogenic

processes associated with ontogeny. This capacity would also explain the homeostasis and adaptability that characterize living systems. In this respect plants are no exception. Because of their lack of mobility, they have to accommodate to the environment and their capacity to respond to external signals needs to be highly developed.

Convergence in the mechanisms of signal transduction and response to signals

Have all different signals specific perception and transduction systems? Although there is not enough experimental evidence to answer this question, it is probably not the case. In general, the evolution of these systems appears to have been guided by a principle of mechanistic economy when diversification has not been essential. This tendency has led to development of common mechanisms of information processing, and the idea that plants are endowed with a common mechanism of response to different afflicting agents is in accordance to this principle. But economy is not the only advantage of having a number of interlinked response reactions to form a general system of response (Fig. 1). Such a system would have at least two additional advantages compared with independent transduction chains: control would be easier, and attack by certain pathogens would elicit defence against subsequent infection by other pathogens.

Viroids as signals

Viroids are signals because they induce host responses. Since the molecular message of viroids is not translated into specific proteins that could help in their replication or pathogenicity, we can say that viroids are "pure signals". This is an essential difference from viruses. The "signalling language" of viroids, however, is like that of viruses in that it is recognizable by the host enzymatic machinery as a replicative order or request: "please replicate me" (Fraser, 1987). In susceptible hosts the viroid is also recognized as a "pathogenic message".

Do these signals have any special structural traits enabling them to exert biological functions without the aid of any viroid-specified protein? It is an increasingly accepted idea that viroids probably associate *in vivo* with host proteins in order to accomplish some aspects of their infectivity and pathogenicity, but full experimental confirmation is still awaited.

From the point of view of their nucleotide composition viroids have no special characteristics, such as strange or modified purine or pyrimidine bases. Viroid RNAs, however, have very singular structural features: they are covalently closed circular RNA

molecules which under non-denaturing conditions adopt a very compact conformation that confers a rod-like appearance. This conformation is maintained by means of a high proportion of intra-molecular base-pairing, interrupted only in certain regions where the lack of association gives rise to the so called "loops". A number of theoretical models have been developed in attempts to correlate these structural features with the replicability and pathogenicity of viroids (Sänger, 1982; Flores, 1984; Schnölzer *et al.*, 1985; Visvader and Symons, 1985).

The approach has not been restricted to studies of the differential behaviour of naturally existing viroids and viroid strains in combination with different hosts. New technologies for the construction of viroid cDNA clones and infectious RNA transcripts, site-specific mutagenesis and construction of chimeric viroid cDNAs combining fragments from different viroids or strains of a viroid have permitted new approaches (for a review see Owens and Hammond, 1987).

However, a number of difficulties have to be overcome. There is uncertainty about the native conformation of viroids. Viroids might adopt very different conformations when interacting with host components during replication or pathogenesis. Secondly, it is very difficult to relate precisely localized point mutations with loosely defined and evaluated biological properties such as replicability and pathogenicity. A more accurate knowledge of the basic component steps of viroid-host interaction will be necessary to establish meaningful structure-function relationships.

Are the signals between viroid and plant extracellular or intracellular?

To answer this question we need to distinguish between initiation of the infection in the "primary target cells" and the subsequent progress of the infection by cell division. Viroids are extracellular signals whether they arrive in a cell from outside the host plant, or from previously infected cells by systemic spread. However, when spread in association with cell division (progeny viroids are split between daughter cells), viroids must be considered as intracellular signals. In this regard, it is important to note the following: for viroids to be perceived as pathogenic signals they may have to be present at a certain level; this level seems not to be reached before replication; the bulk of the viroid population in the developing leaf tissue (in which the symptoms arise) is probably spread through cell division, so viroids are probably maintained inside the cells most of the time; and there are data indicating that viroids are pathogenic intracellularly. It has been shown that CEVd induces enhanced biosynthesis of ethylene and PR proteins in

permanently infected tomato cell cultures (Bellés *et al.*, 1989b).

Are viroids primary signals or secondary messengers?

Again, it is necessary to dissect the question and consider the replicative and pathogenic messages separately. As far as the replicative message is concerned viroids (+RNA) are at the same time the primary signals, recognized by host RNA polymerases, and the response (product) of the replicating machinery. Minus-RNA copies can be regarded as the amplifying intermediary step of the transduction chain. This intermediary form could be considered as a "second messenger". This transducing circuit is different from the more common form where a signal is perceived at the plasma membrane. In this case the second messengers convey information from plasma membrane to cytosol and nuclei (for a review see Boss and Morré, 1989).

As pointed out above, viroids need to be amplified through replication not only to be infectious but also to switch on a pathogenic response. This might imply that viroids as pathogens also have the character of secondary messengers; amplification is a distinctive feature of conventional second messengers (Boss and Morré, 1989). Nevertheless, this role cannot be attributed to viroids since the product of the amplification of the pathogenic message (progeny viroid molecules) and the primary infecting signals are identical. No pathogenic signalling appears to derive from replication as a process (Conejero and Granell, 1986; Diener, 1987; Semancik and Conejero, 1987).

Recognition as infectious signals and perception as pathogenic signals: the question of specificity

Viroids are not infectious to all plants; only certain hosts are susceptible. One may conclude that viroids are specific in both their establishment as infectious entities (recognition, replication and spread) and in their capacity to incite a disease in a given host.

Specificity in viroid-host interactions, as in other systems, cannot be discussed without considering the biological meaning. We need to distinguish a differential affinity for cellular targets leading to damage (fortuitous specificity), from a selective recognition generated by coevolution of viroids (or any pathogen) with the host plant as a consequence of a history of interactions. The fact that a plant is a host or non-host for a given viroid does not imply *a priori* any adaptive or integrating process responsible

for the compatibility or non-compatibility. In the case of an ancient viroid-host relationship, the possibility of specific resistance, tolerance or susceptibility can be entertained. Finally, it is interesting to point out that the mechanisms of replication and pathogenesis seem to be non-specific.

It is possible, however, that viroids may have been native components in certain plants. They may have become infectious through evolution, by loss of control of their synthesis, thus leading to a kind of "auto" or "self-infection". In these plants newborn viroids ("protoviroids") would still be native components. These replicating RNAs might be transferred in some unknown way to related plants in the wild. More recently, with the intensification of agricultural practices, viroids would probably have been transmitted to phylogenetically-related cultivated plants with the conserved capability to "recognize" viroids as "self". Viroids would not be normal constituents of these plants but they would have a certain degree of structural homology with host components enabling them to reach and penetrate competent cells and to be replicated by host enzymes. One can speculate that there could also be a specific viroid-host interaction during the establishment of the infection.

How the viroid-cell compatibility is determined is a challenging question. The specificity determinants could be at the level of viroid entrance to the cell, the accessibility of or recognition by the replicative machinery, or specificity could be determined by a combination of both types. In any case, the determinants or compatibility factors would only be present in a combination suitable for initiation of infection in certain meristematic cells at a given phase of the cell cycle (Semancik and Conejero, 1987).

Since the pathogenic encounter of plants and viroids is thought to be relatively recent (Diener, 1987), it is conceivable that the pathogenic components of viroid-host interaction may lack the specificity traits that one would expect from coevolution of very old partners, as seems to be the case for viruses, bacteria and fungi and their host plants.

In conclusion, it is possible to explain a specific recognition of viroids as "self" by the host during the establishment of the infection, leading to compatibility. Disease, however, would be a reflection of the sensing or perception of viroid molecules as "non-self" by the host, and the subsequent pathogenic reaction. If a given host has not the capacity, either to perceive viroids as "non-self" or to react after the perception, then it would be a non-susceptible (tolerant) host.

At this point the question arises: which is the structural or dynamic cellular target that interacts with viroid molecules to trigger the pathogenic response? At present there is no answer to this question; little experimental effort has been devoted to it. Nevertheless, it is worthwhile to comment on work which has looked for complexes of viroids with cell components.

From these studies the clearest conclusion is that the bulk of the viroid population seems to be present in the nucleolus (Schumacher *et al.*, 1983). Thus the possibility that the primary pathogenic encounter takes place in the nucleolus has been proposed (Riesner 1987). Experiments *in vitro* have demonstrated an affinity of potato spindle tuber viroid (PSTVd) for histones and for a non-histone protein (Wolf *et al.*, 1985; Klaff *et al.*, 1989). However, the physiological significance of this finding is unknown.

Recently, Hass *et al.* (1988) have found a sequence complementarity between the 5' terminus of tomato signal recognition particle (SRP) RNA and the "lower strand" of the rod-like RNA secondary structure model of PSTVd and four other viroids (CEVd, chrysanthemum stunt viroid (CSVd), tomato apical stunt viroid (TASVd) and tomato planta macho viroid (TPMVd)), which also replicate and incite disease in tomato plants. Interestingly, this region includes the virulence-modulating (VM) region (Schnölzer *et al.*, 1985) or "P domain" (Visvader and Symons, 1985; 1986). On this basis Haas *et al.* (1988) suggest that SRP RNA would be a possible primary cellular target with which viroid molecules could interact, thus inciting disease. Two of the potential mechanisms proposed involve direct interaction of viroid RNA either with SRP RNA or with SRP protein. In both cases the pathogenesis is explained as an alteration of translocation and integration of membrane proteins into endoplasmic reticulum in differentiating tissue at the growing apex. This would result in abnormal formation of cellular membranes in meristematic cells and initiate the development of symptoms like leaf malformations and retardation of growth.

It is interesting to note that the authors integrate in their model the fact that the mature PSTVd molecules accumulate in the nucleolus with a specific stage of cell division in which the interaction would be possible. In this stage the nucleolus and the nuclear membrane disintegrate and mature viroid molecules could be transiently released into the cytoplasm (Haas *et al.*, 1988). The problem which remains is how to reconcile the exquisite and precise nature of the viroid-SRP RNA interaction that would imply 30 bp complementarity, with the reputed lack of specificity of viroids as

pathogenic signals or the commonality of the response elicited by viroids.

If the hypothetical viroid-SRP interaction were the real initiator of pathogenesis, one would have to admit a specific primary signalling and a non-specific response in viroid pathogenesis. If that were the case, it is not difficult to imagine that a distortion at the level of the plasma membrane or other cell membrane could trigger a general mechanism of response, which could be activated at the same membrane level by signals from other pathogenic stress factors. In this concept the primary pathogenic event could be less specific than that proposed by Haas *et al.* (1988). Any interaction giving rise directly or indirectly to signals (biochemical changes) capable of being sensed or perceived by the appropriate cell membrane would trigger the same response, through release or production of common second messengers.

Intermediary messengers of the viroid pathogenic signal

There is increasing information in support of the idea that in the signal transduction systems of plants, the plasma membrane has an important role in conveying information from outside the cell to the cytoplasm (Blowers and Trewavas, 1989). There, a series of transduction fluxes lead up to the responding elements. These compounds are called second messengers. The identification of second messengers in plants and their role in signal transduction is only commencing.

Much effort has been devoted to confirming the postulated rôle of cAMP as a second messenger in plants. Neither adenylate cyclase (Yunghaus and Morré, 1977) nor cAMP-dependent protein kinase, the physiological receptor for cAMP, have been found in plants (Brown and Newton, 1981). Furthermore, cAMP has never been found to be required for any physiological response (Hepler and Wayne, 1985). Later, a number of components of plant metabolism have been suggested to play a rôle as second messengers in plant systems: Ca^{2+}, K^{+}, H^{+}, NAD^{+}/NADH, polyamines, diacylglycerol and phosphoinositides (Boss and Morré, 1989). Among these the most clearly accepted as a second messenger is the Ca^{2+}-calmodulin system (Marmé, 1989).

Another good candidate as a second messenger for signal transduction in plant systems is ethylene. This is a main component of the hormonal system which controls plant development and is involved in plant response to different kinds of stress (Lieberman, 1979; Yang and Hoffman, 1984). The location of its biosynthetic machinery either in the plasma membrane or the tonoplast (Guy and Kende, 1984) would be consistent with the idea that its biosynthesis and release could amplify and transduce

signals perceived by a sensor located at these membranes.

Until very recently almost all experimental effort on how second messengers might be involved in signal transduction in host-viroid interactions has been carried out in our laboratory. Our concern has been to demonstrate the role of ethylene as a mediator of the viroid-induced response. A number of considerations and data support this idea:

1. Treatment of *Gynura aurantiaca* plants with silver nitrate (500-1000 mg/l) produces a developmental syndrome in the host which is characteristic of CEVd-infected plants, including stunting, leaf malformations, inhibition of rooting and the accumulation of the same PR proteins (P1 and P2) (Conejero and Granell, 1986). In tomato plants, silver nitrate and CEVd both induced the ten cationic PRs (Granell *et al.,* 1987).
2. The viroid-like effects elicited by Ag^+ ions include the induction of a cross-protection-like phenomenon, since Ag^+ at low concentration impairs symptom expression in CEVd-infected plants. The characteristics of this protection suggest that it is produced by interference with the establishment of the viroid infection (Granell *et al.,* 1987).
3. Both CEVd infection and Ag^+ (Conejero and Granell, 1986) stimulate ethylene production.
4. Treatment of plants with 2-chloroethylphosphonic acid, (ethephon, an ethylene releasing compound) at high doses (5000 mg/l) induces the viroid-like syndrome and the same PR proteins in both *Gynura* (Semancik and Conejero, 1987) and tomato plants (Granell *et al.,* 1987).
5. The relative intensity of PR production in three tomato cultivars (Rutgers, Rentita and Hilda 72) as a response to ethephon treatment was the same as that in response to CEVd infection (Granell *et al.,* 1987).
6. Ethephon at a low rate (500 mg/l) is able to impair the establishment of viroid (CEVd) infection (Semancik and Conejero, 1987).
7. Inhibition of either of the last two steps of ethylene biosynthesis: the conversion of S-adenosylmethionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC) and the conversion of ACC to ethylene, inhibits PR production and the expression of symptoms in silver-treated *G. aurantiaca* plants. The same inhibitory effect was obtained with norbornadiene (an antagonist of ethylene action) (Bellés and Conejero, 1989).

Ethylene is not the only phytohormone to have been implicated in viroid pathogenesis. A decrease in gibberellins related to the stunting reaction in CEVd-infected citrus trees (Hanks and Feldman, 1972) and *G. aurantiaca* plants (Rodríguez *et al.*, 1978) is the clearest effect reported. The effects on auxin-like substances and in ABA in both type of hosts were contradictory (Semancik and Conejero, 1987). In infected citrus, auxin-like substances decreased and an inhibitor presumed to be abscisic acid increased. In *Gynura*, however, an IAA-like auxin remained unchanged. Low levels of an additional auxin-like substance appeared and ABA was not affected by the presence of viroid. Also, a reduced root initiation in CEVd-infected *Gynura* was correlated with a reduction in a diffusible auxin-like substances from the apical buds (Flores and Rodrígues, 1981). That these or other hormonal changes could be involved in transducing viroid pathogenic signalling into the cellular response would be in accordance with the notion of plant growth substances as general second messengers (Blowers and Trewavas, 1989).

More recently, attempts to fill the gaps in our knowledge of viroid pathogenesis have been directed to protein phosphorylation. This is reputedly a key element in the signal transduction network of plants (Ranjeva and Boudet, 1987) and was chosen by two research groups working with different viroids infecting tomato plants: PSTVd (Hiddinga *et al.*, 1988) and CEVd (Vera and Conejero, 1989). The work on PSTVd concentrated on the viroid-induced enhancement of phosphorylation of a 68 kDa host protein. According to the authors, this is homologous to human double stranded (ds) RNA-dependent protein kinase (Hiddinga *et al.*, 1988). In addition to this effect, the work on CEVd described increased phosphorylation of other proteins of 40, 30, 27, 24, 23 and 22 kDa. The CEVd-induced effect on protein phosphorylation was Mn^{2+}-dependent (Vera and Conejero, 1989).

Due to their secondary structure, viroids can mimic a ds-RNA. Therefore, the dsRNA-dependence of the enhanced phosphorylation of the 68 kDa homologous to the human kinase was interpreted as a dsRNA-mimicking effect of PSTVd. Thus the activation of the 68 kDa kinase would be the primary event in viroid pathogenesis (Hiddinga *et al.*, 1988). Unfortunately, results obtained with CEVd (Vera and Conejero, 1989) differ from those with PSTV in that the phosphorylation was not dependent on exogenous dsRNA molecules, and the protein did not cross-react with antibodies against human dsRNA-dependent protein kinase 68. The apparent discrepancy between these results needs further study. The identification of the target proteins, the kinases and

phosphatases involved, and their connections with other steps of the transduction network also remain open questions.

THE RESPONSE

Macroscopic components of the response

The response of hosts to pathogens consists of active reactions specifically directed to arrest the establishment of the infection or to avoid or counteract the pathological effects; the disease itself is also part of the response. Furthermore, even if it is necessary or possible to distinguish pathological reactions from defence reactions for analytical purposes, this is not always the best strategy. Sometimes the two main aspects of the response are so intimately related as to be impossible to separate clearly. Host physiological and structural changes involved in disease expression may eventually also become components of resistance mechanisms.

In this chapter, the developmental syndrome, the induced systemic resistance and the physiological condition of resistance that the stress associated with disease produces in certain cells or tissues, will be considered as components of the viroid-elicited response (Fig. 2).

Cytopathic changes

Under this heading we will not attempt a complete review of the subject; for this see Semancik and Conejero (1987). Our aim is to emphasize those data most relevant to our main argument:

1. The absence in infected plants of any effect(s) known to be *exclusively* viroid-induced. Many of the observed host responses to the presence of the viroid resemble responses which result from either virus infection or physiological stress.
2. Even though the nucleus has been generally accepted as the site of viroid replication and, potentially, the site where the primary pathogenic event could take place, there seems to be a general consensus that there is no cytopathic structural alteration at the nuclear level. This fact further supports the idea of segregation of viroid replication and pathogenesis (Conejero and Granell, 1986).
3. Alterations have been described in cell walls (Wahn *et al.*, 1980), membranes such as the plasmalemma (Semancik and Vanderwoude, 1976; Wahn *et al.*, 1980;

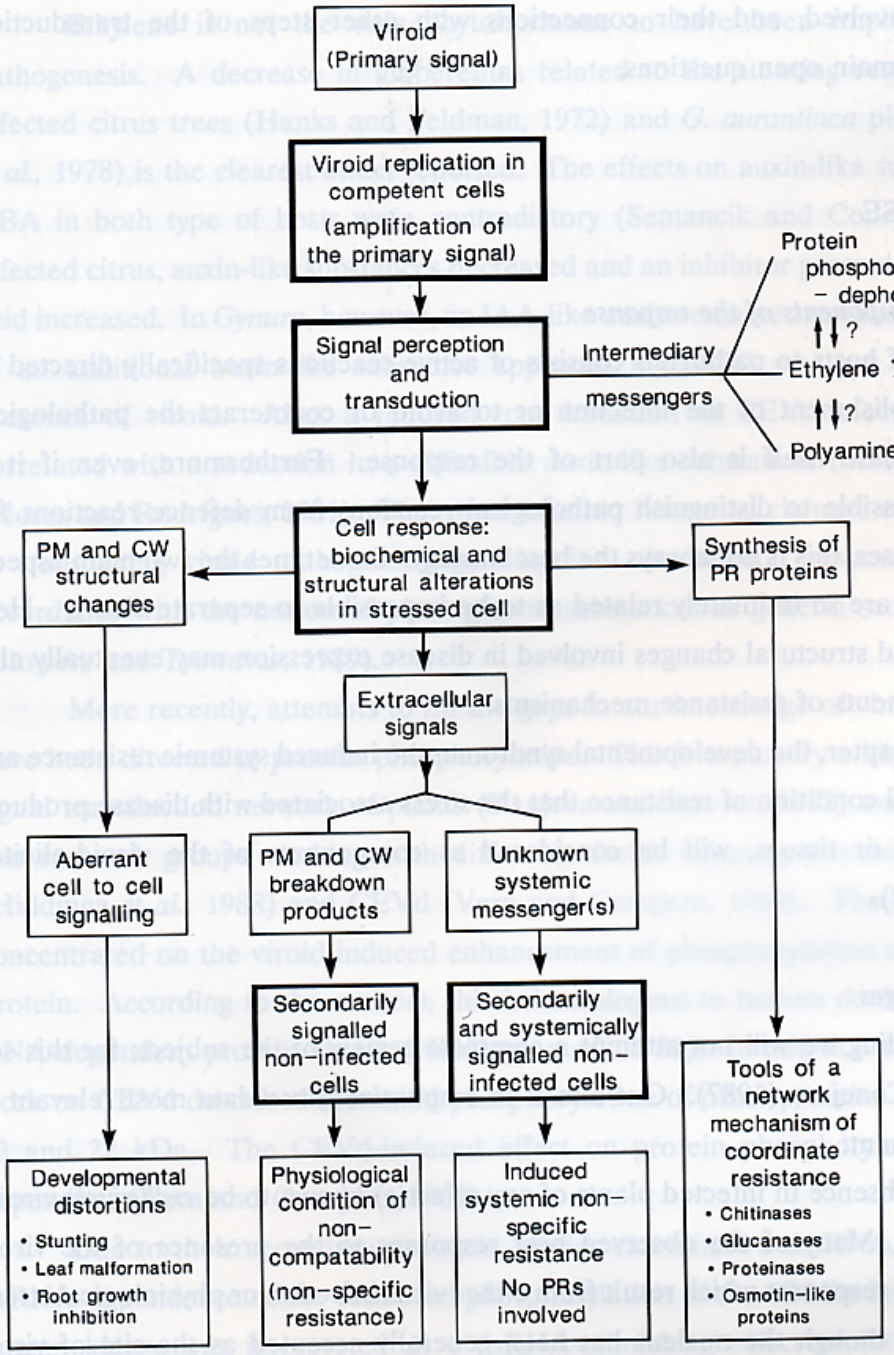

Figure 2. A model to explain how the viroid-induced response at the cellular level becomes a developmental disease and leads to a plant which is more resistant to subsequent infections.

Hari, 1980) the tonoplast (Kojima *et al.,* 1983; Paliwal and Singh, 1981) and the thylakoids (Kojima *et al.,* 1983). Together with the rôle of the cell wall and membranes in recognition reactions for a number of regulatory processes, this has led to speculation that the viroid itself or a viroid-induced signal may be capable of interacting with these reactive interfaces (Semancik and Conejero, 1987).

Molecular components of the response

This is perhaps an over-ambitious heading, considering the lack of knowledge on this subject. For the scanty and scattered data available, terms like "biochemical alterations" or "metabolic changes" might be more appropriate. But the point is that we need to envisage the molecular changes involved in viroid pathogenesis within the framework of signalling, and not only as a more adequate way of organizing the cumulated data. We need this framework as the new paradigm under which to approach future research. We need to know the primary pathogenic event, the intermediate steps, the end products and their mutual regulatory interconnections. Also, a major interesting aspect of these networks is which of their components become regulatory bridges implicating the genetic machinery in signal transduction as well as in the final response.

We will now summarize the data that biochemical studies on viroid pathogenesis have produced. We shall omit the biochemical changes that have already been discussed as intermediary steps of the transduction flux, and restrict the discussion to the end products of the network i.e. the molecular components of the response.

Nucleic acids. Apart from viroid molecules (+RNA) and the intermediary forms involved in their replication (±dsRNA), there is no reported evidence on any qualitative or significant quantitative alteration in the normal nucleic acid constituents of the host cell (Diener, 1987). This restricts the involvement of nucleic acids as components of the pathogenic process to the viroid-related RNA forms, as already discussed.

PR proteins as non-specific components of the response. Attempts to demonstrate the functionality of viroids as mRNAs in *in vitro* (Davies *et al.,* 1974; Hall *et al.,* 1974) as well as in semi *in vivo* (Semancik *et al.,* 1977) protein synthesizing systems gave negative results. *In vivo* studies on viroid infected tissues (Zaitlin and Hariharasubramanian, 1972; Conejero and Semancik, 1977a) did not provide any support for this putative role of viroids. However, the *in vivo* studies revealed that alterations in host protein metabolism are implicated in viroid pathogenesis. Nevertheless, it was not until the idea that viroids do not specify protein synthesis either *in vitro* or *in vivo*