MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 1, No. 8, pp. 317-325, 1988

*Current Review*

# Sequence Determinants of Symptoms in the Genomes of Plant Viruses, Viroids, and Satellites

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Received 13 June 1988. Accepted 24 October 1988.

Progress in understanding the molecular basis of pathogenic host-microbe interactions has come from two lines of research, the study of the host and the study of the pathogen. Studies of host factors for resistance or tolerance have resulted in descriptions of genetic alleles, but individual gene sequences have not been characterized. Molecular descriptions of pathogen disease-inducing genes, on the other hand, recently have been accumulating rapidly. This is especially evident with the viruses, which are particularly well suited to the application of current techniques in molecular biology. Most of this advance in our knowledge of their genomes and gene products derives from sequence studies and from *in vitro* manipulations predicated on the accumulating sequence data.

The altered physiology of the diseased state is linked ultimately to gene-product-level events, but the many steps linking genes to symptoms may mislead us, as Zaitlin cautioned in an earlier review (1979), into "describing the consequences of disease and not its causes." The induction of disease symptoms is presumed to be a host-interactive viral function. Other viral functions have been associated with genes involved with the production and dissemination of virus particles. If a host-interactive, symptom-inducing function was the primary role of one particular viral gene, then we might expect to find mutations in that gene that alter the symptoms of viral infection without affecting virus titer. Differential effects on symptoms and titer do result from allelic variations in host components of the interaction. However, for viral determinants that are well characterized, symptomatic impact and titer are found to vary in parallel. This suggests that the molecular basis of symptomatology will in most cases not be identified with a single gene. Rather, symptoms will generally arise from the dynamic interplay of the entire array of pathogen products with as yet undefined host components. If variation in pathogen titer were not such a strongly determining factor, effects reflected in symptoms could be ascribed far more directly to primary genetic determinants.

During the course of experiments in the 1960s, primarily investigations of the genetic code, Fraenkel-Conrat (e.g., Funatsu and Fraenkel-Conrat 1964) noted a correlation between disease symptomatology and the coat protein of TMV. (See Table 1 for virus name abbreviations.) A series of amino acid substitutions in the coat protein were associated with a switch from mosaic symptoms to necrotic local lesions in *Nicotiana sylvestris*. Although this work suggested that the mutations could be responsible for the altered host response, secondary mutations in genes other than the coat protein gene could have been responsible as well. The new phenotypes also could have arisen from co-infections by several interdependent viral mutants, only one of which carried the altered coat gene. The likelihood of these more involved explanations could not be assessed by using the technology of the 1960s, so a pursuit of the primary determinant was not possible then.

Today, virologists are in command of much more powerful techniques. The advent of cloning has provided genetically homogeneous inocula for many viruses, viroids, and satellites. Sequencing data are now routinely available to guide *in vitro* mutagenesis studies of specific genetic loci. The clonal derivation of inocula from a single copy of an altered viral genome ensures that an experimental change introduced *in vitro* will be the only variable under investigation, with the remainder of the genome held invariant. Of course, genetic heterogeneity may appear soon after inoculation, depending on the endogenous error rate in replication. By using these techniques, the more involved explanations for the Fraenkel-Conrat findings can be ruled out, and, consequently, the coat protein gene of TMV indeed has been conclusively demonstrated to be one of the primary determinants of host response.

Complete genome sequences are known for members of 17 virus groups (Table 1). For seven of these, the fidelity of the sequence data is assured by virtue of the infectivity of genomic copies derived as transcripts *in vitro*. Genomic sequences of viral strains derived from *in vitro* mutation or from clones of related isolates can be cut at opportune restriction endonuclease sites, and the pieces then may be reciprocally exchanged. Multiple exchanges reconstitute pairs of viable chimeras completely identical in sequence, save for the subsection under study. The impact of such precise alterations on symptoms can then be unambiguously assigned to particular genomic domains of function. The application of this analysis will expand in step with the continuous addition of viral groups and strains to the list in Table 1.

**TMV.** An extensive body of observations on the symptomatology of TMV has been accumulating since the late 1800s, often in the context of host resistance genes. In tobacco, the presence of the N-prime (N′) gene, originally from *N. sylvestris*, restricts infection of most strains of TMV (although not common strain U1) to necrotic lesions only on the inoculated leaves (e.g., Fraser 1983). Another gene, the N gene, originally from *N. glutinosa*, results the same limited necrotic response from almost every strain of TMV (Samuel 1931). The infection is restricted to the inoculated leaves unless the temperature rises above about 32° C. At higher temperatures, resistance breaks down, and a systemic infection ensues. The mild vein-yellowing mosaic, merging to areas of chlorosis surrounding "green islands" in

**Table 1.** Listing of completely sequenced viruses, viroids, and satellites

| Group | Virus/strain | Cloned sequence infectuous | Reported by: |
|---|---|---|---|
| Geminivirus | | | |
| | Tomato golden mosaic (TGMV) | + | Hamilton *et al.* 1984 |
| | Cassava latent (CLV) | + | Stanley and Davies 1985 |
| | Beet curly top | + | Stanley *et al.* 1986 |
| | Digitaria streak | + | Donson *et al.* 1988 |
| | Bean golden mosaic | + | Morinaga *et al.* 1987 |
| | Maize streak (MSV) | | |
| | Kenyan | + | Grimsley *et al.* 1987 |
| | South African | + | Lazarowitz 1988 |
| | Wheat dwarf | + | Hayes *et al.* 1988b |
| | Chloris striate mosaic | − | Anderson *et al.* 1988 |
| | Mung bean yellow mosaic | − | Ikegami *et al.* 1988 |
| | Miscanthus streak | − | Chatani *et al.* 1988 |
| Caulimovirus | | | |
| | Cauliflower mosaic (CaMV) | | |
| | CM-1841 | + | Gardner *et al.* 1981 |
| | Xinjing | + | Fang *et al.* 1985 |
| | Cabb S | − | Franck *et al.* 1980 |
| | D/H | − | Balazs *et al.* 1982 |
| | Carnation etch ring | + | Hull *et al.* 1986 |
| | Figwort mosaic | + | Richins *et al.* 1987 |
| Bromovirus | | | |
| | Brome mosaic (BMV) | + | Ahlquist *et al.* 1984 |
| | Cowpea chlorotic mottle | + | Allison *et al.* 1988 |
| Tobamovirus | | | |
| | Tobacco mosaic (TMV) | | |
| | L, L-11 | + | Meshi *et al.* 1986 |
| | U1 | + | Dawson *et al.* 1986 |
| Comovirus | | | |
| Hordeivirus | Cowpea mosaic | + | Vas *et al.* 1988 |
| Carmovirus | Barley stripe mosaic | + | A. O. Jackson (pers. commun.) |
| | Carnation mottle | − | Guilley *et al.* 1985 |
| | Maize chlorotic mottle | − | Lommel and Nutter 1988 |
| Potyvirus | Turnip crinkle | + | T. J. Morris (pers. commun.) |
| | Tobacco etch | − | Allison *et al.* 1986 |
| Cucumovirus | Tobacco vein mottle | − | Domier *et al.* 1986 |
| Tobravirus | Cucumber mosaic (CMV) | − | Davies and Symons 1988 |
| Potexvirus | Tobacco rattle | − | Hamilton *et al.* 1987 |
| | White clover mosaic | − | Forster *et al.* 1988 |
| Furovirus | Potato virus x | − | Huisman *et al.* 1988 |
| Luteovirus | Beet necrotic yellow vein | − | Bouzoubaa *et al.* 1987 |
| Tymovirus | Barley yellow dwarf | − | Miller *et al.* 1988 |
| Nepovirus | Turnip yellow mosaic | − | Morch *et al.* 1988 |
| Sobemovirus | Tomato black ring | − | Greif *et al.* 1988 |
| Alfalfa mosaic virus (AlMV) | Southern bean mosaic | − | Wu *et al.* 1987 |
| | | − | Barker *et al.* 1983 |
| Viroids | | | |
| | Potato spindle tuber (PSTV) | + | Sänger 1984 |
| | Citrus exocortis (CEV) | + | Gross *et al.* 1982 |
| | Hop stunt | + | Ohno *et al.* 1983a |
| | Tomato apical stunt | + | Owens *et al.* 1986 |
| | Cucumber pale fruit | + | Puchta *et al.* 1988 |
| | Avocado sun blotch | − | Symons 1981 |
| | Chrysanthemum stunt | − | Haseloff and Symons 1981 |
| | Coconut cadang cadang | − | Haseloff *et al.* 1982 |
| | Coconut tinangaja | − | Keese and Symons 1987 |
| | Tomato planta macho | − | Kiefer *et al.* 1983 |
| | Grapevine yellow speckles | − | Koltunow and Rezaian 1988 |
| | Apple scar skin | − | Hashimoto and Koganezawa 1987 |

continued on next page

**Table 1.** (Continued from previous page)

| Group | Virus/strain | Cloned sequence infectuous | Reported by: |
|---|---|---|---|
| Satellites | | | |
| | Cucumber mosaic | + | Kaper *et al.* 1988a |
| | Tobacco necrosis | + | Van Emmelo *et al.* 1987 |
| | Tobacco ringspot | + | Gerlach *et al.* 1986 |
| | Turnip crinkle | + | Simon and Howell 1986 |
| | Arabis mosaic | | |
| | Lilac | + | Lui *et al.* 1988 |
| | Hop | − | Kaper *et al.* 1988b |
| | Peanut stunt | − | Collmer *et al.* 1985 |
| | Panicum mosaic | − | Masuta *et al.* 1987 |
| | Solanum nodiflorum | − | Haseloff and Symons 1982 |
| | Lucerne transient streak | − | Keese *et al.* 1983 |
| | Velvet tobacco mottle | − | Kiberstis *et al.* 1985 |
| | Subterranean clover mottle | − | Francki 1987 |
| | Tomato black ring | − | Meyer *et al.* 1984 |
| | Tobacco mosaic | − | A. J. Dodds (pers. commun.) |

systemically infected leaves, is characteristic of infection in susceptible hosts (genetically n/n: "little-n" plants), similar to the symptom shown on the right side of Figure 1.

Tm-1, Tm-2, and Tm-2-2 genes (described in Pelham 1972) confer resistance in *Lycopersicon esculentum* against tobamoviruses, including the tomato strain of TMV. Isolates of TMV that induce mosaic symptoms in susceptible cultivars will cause no symptoms in Tm-1-genotype tomatoes. Tm-1 confers tolerance; virus titer may be unaffected or may be reduced, depending on viral strain. In cultivars homozygous for Tm-2, infection will be restricted to necrotic lesions at the inoculation site; heterozygous (Tm-2/+) hosts show a systemic veinal necrosis, and necrosis gives way to systemic mosaic at higher temperatures, just as in N-gene-mediated resistance in tobacco.

With the advent of infectious, clone-derived TMV RNA (Meshi *et al.* 1986; Dawson *et al.* 1986), genomic domains in TMV responsible for induction of the above host-specific responses, as well as for other viral traits, have been assigned to the three primary viral reading frames: the coat protein gene, the (putative) replicase gene, and the movement-protein gene (m-protein, also known as 30K protein).

**Coat protein gene.** By exchanging coat protein genes between two strains of TMV that induce distinct symptoms, Saito *et al.* (1987) have definitively assigned this gene as a determinant of host response. Subsequently, Knorr and Dawson (1988) pursued this analysis to the nucleotide sequence level. They described a strain, D1, that carries a single base change altering coat protein amino acid 148 (Fig. 2b). This mutation alters the interaction of strain U1 with *N. tabacum* 'Xanthi' (genotype n/n), evoking chlorotic lesions at the inoculation site that develop necrotic centers, preceding systemic white ("bleached") lesions (Fig. 1, left) on uninoculated leaves. The mutation evokes an altered response, e.g., necrotic lesions limited to the inoculation site, in *N. sylvestris*. A second unmapped mutation (D7) gives the same symptom but was not found within 300 bases of the D1 site. These infections revert to systemic mosaic above 35° (D. Knorr, personal communication), a few degrees above the temperature at which N'-gene-mediated restriction of infection spread normally gives way to development of systemic symptoms (Fraser 1983).

**Fig. 1.** Symptoms of a TMV mutant (strain D1) and its parent (TMV 204) on *N. tabacum* 'Xanthi.' A single nucleotide change (Fig. 1b) alters symptoms from the mosaic phenotype of the parent (right) to systemic small bleached lesions of strain D1 (left).

a. TMV strain LS-1: systemic spread restricted above 32°

*TyrCysProLeuSerLeu*
UACUGCCCUUUGUCAUUA
UACUGCUCUUUGUCAUUA
*TyrCysSerLeuSerLeu*

b. TMV strain D1: systemic spread restricted below 35°

*SerSerSerGlyLeuVal*
AGCUCUUCUGGUUUGGUU
AGCUCUUUUGGUUUGGUU
*SerSerPheGlyLeuVal*

c. CaMV strain 58: systemic spread restricted above 21°

*GluLeuAsnSerThrVal*
GAGCTTAACAGCACAGTT
GAGCTTAAGAGCACAGTT
*GluLeuLysSerThrVal*

**Fig. 2.** Novel symptomatic types (left) resulting from point mutations (right). Two sequences are shown in each case, an upper (parental) sequence and the mutated sequence below. The parental viral strains systemically infect these hosts under the conditions that restrict movement of the mutant strains to the site of inoculation. **a**, TMV m-protein amino acids 151–156 (in *N. tabacum*); **b**, TMV capsid protein amino acids 146–151 (in *N. sylvestris*); **c**, CaMV gene VI product amino acids 79–84 (in *D. stramonium*).

The work of Knorr and Dawson (1988) suggests that many of the coat-protein point mutations cataloged by Funatsu and Fraenkel-Conrat (1964) also may have been primary determinants of the N′-gene mediated response. The distribution of these substitutions along the length of the coat protein gene resembles the distribution of substitutions in the neuraminidase and hemaglutinin genes of influenza virus variants (Air and Laver 1986). Influenza virus and its host typify a gene-for-gene interaction in which host resistance is mediated by specific molecular recognition, and virulence derives from viral mutations that evade recognition. Genetic data for the induction of strain-specific resistance in specific plant-pathogen pairs had also been fit to a gene-for-gene recognition model (Keen 1982). The analogy implies that if the N′ gene locus encodes specific recognition elements, their recognition potential is quite comprehensive, comparable in complexity to that of the mammalian system for resistance to pathogenic viral variants. Alternatively, these coat protein mutations may not be directly recognized by the host, but result only in impaired viral coat protein function. The mechanism whereby this later scenario might give rise to necrosis in the N′ background has not been investigated.

Resistance mediated by the N gene is induced against TMV determinant(s) other than the coat protein. This was initially inferred from the local lesion restriction in *N. tabacum* 'Xanthi'-nc not only of wild-type TMV, but also of mutant strains of TMV that produced nonfunctional (Siegel *et al.* 1962) or undetected levels (Sarkar and Smitamana 1981) of coat protein. The deduction was confirmed by Takamatsu *et al.* (1987), who observed the same response with a viral RNA derived from a clone (pLCD 29) containing a precise coat protein deletion (from RNA positions 5710-6160). Defective TMV strains that can not make functional coat protein move poorly within the host (Siegel *et al.* 1962; Sarkar and Smitamana 1981) as though cell-to-cell movement continued but vascular movement was blocked. This class of mutation has been investigated in detail by Dawson *et al.* (1988), who analyzed a series of deletions, each of which destroys the site at which the coat protein binds to the viral RNA. The various mutations each have distinct symptomatic phenotypes. Each mutation produces a different truncated coat protein that cannot be sequestered into virions.

**Putative replicase gene.** A change of two amino acids in the (putative) replicase gene of TMV-L (Meshi *et al.* 1988a) was found to be responsible for the "resistance-breaking" phenotype of a virus derived from a parent strain avirulent in tomatoes carrying the Tm-1 gene. Subcloning analysis shows that one of these changes, the glutamine-to-glutamic acid substitution at amino acid position 979, is sufficient to switch the phenotype to systemic mosaic. However, a concise interpretation is not obvious because the other change or related changes reappear in the progeny virus population arising from infection with the single-site mutant.

The replicase gene is claimed to be the site of an attenuating mutation in TMV-L (Nishiguchi *et al.* 1985). These workers found 10 base substitutions in the comparison of genome sequences of TMV-L and its milder derivative, TMV L-11a. Only three of them would result in changes in the inferred amino acid sequence, and all of those map to the replicase gene. This conclusion, however, leaves unaddressed the possibility that mutations may express effects through (noncoding) functions of the RNA itself. TMV L-11a is a strain used to cross-protect greenhouse tomatoes from severe strains of TMV; its titer is about 20% that of virulent strain TMV-L.

**Movement protein.** TMV LS-1 was the first symptom variant to be mapped by sequencing of the viral RNA genome. The mutation was found to be in the gene for the m-protein (Nishiguchi *et al.* 1978). At 22° the LS-1 strain causes milder systemic symptoms than the TMV-L parent strain from which it was derived (Deom *et al.* 1987); at 32° it causes small chlorotic lesions restricted to the inoculation site only. A search for amino acid sequence changes responsible for this temperature-sensitive phenotype was first approached by peptide mapping of coat and m-proteins (Leonard and Zaitlin 1982). The only peptide variant found was in the m-protein. The RNA sequence of the gene encoding this protein was then compared with that of the parent strain (Ohno *et al.* 1983b), and only one change was found (Fig. 2a).

Deom *et al.* (1987) have since shown that the LS-1 phenotype is completely suppressed by complementation, using only the product of the parental-type m-protein gene. In this experiment the m-protein was provided *in trans* by the host, expressed from a copy of the m-protein gene integrated in the plant genome. In these transgenic plants infection by LS-1 is supported equally well at 22° or 32°, and the milder LS-1 infection reverts to that of the parental L strain. The conclusion that the single base change in the m-protein is solely responsible for the LS-1 phenotype was also affirmed by Meshi *et al.* (1987), who generated the attenuated LS-1 phenotype by engineering the single LS-1 mutation into the full-length TMV-L RNA. The LS-1 mutation has been used to show that the function of the m-protein is the facilitation of viral cell-to-cell movement.

A viral mutation that overcomes Tm-2 resistance has also been mapped to the gene for the m-protein (Meshi *et al.* 1988b). As in Tm-1 "resistance-breaking," a pair of amino acid substitutions was initially identified.

**Geminiviruses.** The symptomatic effects of deletions in the coat protein gene have clarified the role of that protein in the replication cycles of geminiviruses CLV and TGMV. The CLV coat protein *per se* was found not to be essential for symptom induction or movement through the host. However, substitutions in the coat gene must maintain the overall size of the circular viral DNA to retain infectivity. For example, a CLV strain carrying a deletion of 727 base pairs within the coat protein gene on DNA 1 (Ward *et al.* 1988) was nonviable. However, the restoration of CLV DNA 1 to full length by the addition of 785 base pairs of noncoding bacterial DNA completely restored the capacity of this coat-proteinless construct to spread through the host, evoking chlorotic lesions on inoculated leaves of *N. benthamiana* and curling of the leaves that expand subsequently. Similarly, the coat protein of TGMV can be replaced by an equivalent length of DNA (Hayes *et al.* 1988c). TGMV becomes progressively less severe and less able to move through its host as progressively longer sections of the coat protein gene are removed (Gardiner *et al.* 1988; Hayes *et al.* 1988a). The patchy symptoms of these movement-impaired deletions resemble those of the analogous TMV mutants (Dawson *et al.* 1988). The deletion of 88 base pairs from a second TGMV reading frame (AL3; Elmer *et al.* 1988) also leads to attenuation.

The geminiviruses contrast with TMV, for which viability is independent of both genome length and of functional coat protein, but where coat protein is necessary for unrestricted movement. As with TMV, the coat-protein-independent viability of the (two-component) geminiviruses is an attribute that recommends them as recombinant vectors for the transport and expression of novel RNA sequences (Ward *et al.* 1988; Hayes *et al.* 1988c). (Monopartite MSV, however, is inactivated by the insertion of a termination codon into its coat protein gene [Boulton *et al.* 1988]). A CAT gene cartridge has been used to replace the coat protein gene of CLV, maintaining the original length of DNA 1 (Ward *et al.* 1988). The resulting unhindered systemic spread of the CLV vector gives rise to high CAT expression levels *in planta*. This contrasts with the analogous constructs in BMV (French *et al.* 1986) and TMV (Takamatsu *et al.* 1987) from which potentially high levels of CAT expression cannot be reached *in planta* because the lack of coat protein leaves them movement-impaired.

**CaMV.** As illustrated above with TMV, multiple loci can independently determine aspects of symptoms in a given plant virus. This is also seen in chimeric recombinants derived from phenotypically distinct strains of CaMV (Daubert *et al.* 1984; Schoelz and Shepherd 1988). Their diverse symptomatologies cannot be attributed simply to a single locus. Analysis of these recombinants has revealed CaMV gene VI to be a host response determinant (Daubert *et al.* 1984; Schoelz *et al.* 1987). The product of CaMV gene VI, the most prominent viral-coded product in infected cells, is found as an insoluble aggregate in cytoplasmic inclusions. Its primary function is unknown. Insertions of four amino acids into the translational reading frame of CaMV strain cabbage-B lead to attenuation of symptom severity in *Brassica campestris* (Daubert *et al.* 1983); these mutants showed decreased viral titer. A single base-pair change in gene VI altering amino acid 81 (Fig. 2c) changes the infection symptom of CaMV from systemic mosaic to localized necrotic lesions on *Datura stramonium* (Routh and Daubert, unpublished). Infection with this altered virus (CaMV strain 58) is affected by environmental conditions: below 21° C strain 58 induces systemic vein necrosis in *D. stramonium* (Fig. 3, left).

**Fig. 3.** Symptoms of CaMV strain 58 and its parent on *D. stramonium* at 18° C: a single nucleotide change (Fig. 1c) alters the chlorotic spots (right) of the parent strain to necrotic spots/vein necrosis of strain 58 (left).

Another mode of symptom induction mediated by CaMV gene VI has been described (Baughman *et al.* 1988; Goldberg *et al.* 1987). In this experiment, the product of gene VI is expressed from a copy of the gene integrated by *Agrobacterium* transformation into the chromosome of *N. tabacum*. In *N. tabacum*, infection by these strains of CaMV (CM-1841; cabbage-B) is restricted to the inoculation site; the response of a transgenic, systemic host to the production of this protein has not been reported. The authors note that every cell in these transformed hosts should be expressing the gene VI product. If the response of the host was a direct consequence of the presence of the foreign protein, a uniform symptom, e.g., a general chlorosis, would be expected. However, the host often responds with a pattern of alternating yellowed or bleached or green sectors, described as flecked or blotched symptoms. The connection between the visible leaf-level response and the gene VI product remains to be established. For example, it is conceivable that the production of these patterns may be a nonspecific response, evoked by the appearance in the cytoplasm of any insoluble, aggregating protein.

**AlMV.** A temperature-sensitive strain of AlMV, giving a systemic necrosis in N-gene tobacco in place of the wild-type systemic chlorosis, has been interpreted as resulting from a defect in the replication apparatus. The putative cause is mutation of asparagine at position 126 to aspartic acid in the coat protein gene (cited in Huisman *et al.* 1987). However, the AlMV strain described here was not constituted from cloned and sequenced components, so the formal possibility still exists that other mutations on RNA 3 may contribute to the phenotype. A system perhaps better suited to the correlation of symptomatic consequences with precisely defined replicase mutations is the infectious, cloned BMV system (e.g., Ahlquist *et al.* 1984).

**Satellites.** RNAs that replicate only in the presence of a supporting virus are termed satellite RNAs, if they are found within the capsid protein of the supporting virus, or satellite viruses if they encode their own capsid protein (Francki 1985). The RNAs of these satellites are small (324–1,375 bases) and are not homologous with the genome sequence of their supporting viruses. Defective-interfering (DI) RNAs also exhibit dependence on supporting virus infection and become encapsidated within the supporting capsid. However, DI RNA has extensive homology with the genomic sequence of the supporting virus.

The sequences of satellites associated with 13 different viruses have been reported (Table 1); the sequence of a DI RNA that dramatically ameliorates the symptoms of the supporting virus (Hillman *et al.* 1985) has also been reported (Hillman *et al.* 1987). Five satellite RNAs have been cloned in functional form. The use of cloned forms of both the satellite and its supporting virus will ensure that the basic supporting virus inoculum is satellite-free. Previously, this concern has complicated assessments of the impact of satellites on virus-induced symptoms.

Fourteen isolates of the CMV satellite RNA have been sequenced (cited in Hidaka *et al.* 1988). There are a large number of single-base differences between them, however, and sequence comparisons do little to define distinct domains of function that correlate with satellite-controlled attenuation or potentiation of viral symptoms (Garcia-Arenal *et al.* 1987; Kaper *et al.* 1988a). Firmer conclusions are derived from the analysis of broader domains of the

satellite sequence. In studies of chimeras between a severe satellite (S-sat) and a milder isolate (D-sat), C. Collmer (personal communication) has localized the domain for necrosis in tomato to the 3′ half of the molecule. G. Kurath and P. Palukaitis (personal communication) have made similar observations with the D-sat; they have also localized yellow chlorosis of tomato induced by the B-sat isolate to the 5′ half of the satellite RNA. For the Y-sat (Hidaka *et al.* 1988) future analysis will focus on a characteristic 57-base insertion that appears to differentiate it from from milder isolates.

Investigators of the symptomatologies induced by satellite variants must continually take into account the potential of these RNA sequences to mutate during infection (cf. Collmer and Kaper 1988). Further, these symptoms can also be altered by the strain of the CMV-supporting virus, as well as by the species of host plant and by environmental conditions (summarized in Palukaitis 1988). An example of this potential complexity is found in Kaper and Waterworth (1977). They demonstrated a satellite interaction that increased symptoms of infection by CMV strain S in tomato, whereas it had the opposite effect on the same viral strain in tobacco.

**Viroids.** Twelve viroids have been sequenced (see Table 1); clones of five of these have been shown to be infectious. Comparative sequence analysis of these viroids provides an example of fine structure domain mapping (Keese and Symons 1985). A correlation of the sequences and characteristic symptoms induced by six distinct PSTV isolates (Sänger 1984; Schnolzer *et al.* 1985) identified a section of the viroid sequence that modulates symptoms. Visvader and Symons (1986) located the pathogenicity domain in CEV by constructing chimeras from clones of severe and mild isolates. The severe isolate generates a lower infectious titer than the mild one. The chimeras were found to be similar in titer to the mild parental type. Severity, coupled with elevated titer, was induced to reappear with one of the chimeras by elevating the inoculum concentration. Although reversion *in planta* of altered sequences is a concern with mutational studies of viroids, in this case the sequences were found to remain unchanged.

Precise identification of the essential symptom-modifying nucleotide(s) in these variable domains will require point mutational analysis. However, viroid sequences apparently are not designed to store information that will later be expressed as protein structure; the sequence serves to specify viroid secondary structure (Diener 1987) through which they appear to express their pathogenicity. Thus, the only acceptable sequence alterations will be those that the structure can accommodate, leaving other mutations (including most of those that have been attempted [cf. Owens *et al.* 1986; Ishikawa *et al.* 1985; Hammond and Owens 1987]) nonviable.

By combining two lethal mutations (C to U, at position 285, with G to A at position 76) in the type strain of PSTV, Hammond and Owens (1987) have produced an alteration in viroid pathogenicity. In the resulting strain, a base-paired A:U replaces a G:C pair in the proposed native structure. This strain (PSTV H + H′) exibits milder symptoms than its parent and accumulates to less than 10% of the titer of the parent strain. Comparison of this strain with the series of PSTV strains that have similar titers but variable severities (Sänger 1984) suggests that PSTV H + H′ may show lower severity as a consequence of its lower titer, not because of an alteration in intrinsic pathogenicity.

The "intermediate" strain of PSTV differs from the "lethal" strain, described by Schnolzer *et al.* (1985), by substitutions at only four base positions. Hammond (unpublished) has produced a series of mutants derived from the intermediate strain by sequential replacements at these positions with the nucleotides of the severe strain. Several of the resulting constructs are viable. They carry sequence alterations that would be expected to destablize the secondary structure of the viroid. Thus, they serve to test hypotheses that would associate viroid sequence/structure with symptomatology. The viable constructs should fold into structures with less base-paired helical content (higher "free-energy" structures) than that of the intermediate strain. These constructs show symptomatic severities equal to or less than that of the milder parent type.

**Outlook.** By correlating genomic sequence features with symptomatic phenotypes, researchers are now identifying viral genes and viroid sequences that influence symptom expression. Viruses carrying single-site changes that alter symptoms are found to be altered in titer also, compared with the titers of their parental strains. Thus, there are no known examples of viral genes that act solely to alter symptoms, e.g., without also affecting viral production or spread. However, "constant-titer" analysis of symptom induction can be approached in systems of subviral agents. For example, a host component may be inferred to be the primary interaction site of the viroid sequence characterized by Sänger (1984) in a series of similar-titer, variable-severity viroid mutants. Similarly, a host-specific interaction in the infection of tomato aspermy cucumovirus may be the primary target of the satellite RNA of CMV. In this system (Harrison *et al.* 1987), viral symptoms were greatly decreased by the presence of the satellite, whereas the virus titer was unaltered.

Multiple, independent loci in the viral genome can determine aspects of symptomatology (e.g., in TMV, CaMV). In viruses that contain multipartite genomes, multiple symptomatic determinants are revealed in the phenotypes of strains made by the reciprocal exchange of component RNAs (pseudo-recombinants; reviewed by Van Loon 1987). As described in this review, genes encoding the capsid protein, the replicase, and the inclusion component have all been operationally identified as viral determinants of pathogenic symptoms. The m-protein gene has been identified with the attenuation of symptom expression. To date, the m-protein is the only viral gene product that has been seen to interact directly with the host (in its association with plasmodesmata [Tomenius *et al.* 1987]). There is no evidence bearing on the specific interaction with host molecules of the products of the other viral genes that determine pathogenic symptoms. Until such evidence appears, we must consider the possibility that pathogenic responses may not be the direct consequence of specific protein-protein interactions between particular pathogen and host components, but that they could arise through less direct, higher order reactions, on levels of complexity similar to the wound response, stress responses, or senescence.

Characterization of the interactions among all the components of the viral replication cycle *in vivo* is a significant challenge. More difficult still will be the

description of the factors that mediate the compatible coexistance of this cycle with the host on which it depends. A third order of complexity appears if we consider pathogenicity to be a special case, a deviation from the coadapted accommodation of a host and parasite. The infliction of disease symptoms by obligate parasites, like viruses or viroids, on their own supporting hosts does seem *a priori* to be an aberration, a departure from the rule that the success of the parasite is balanced by the success of the host.

Strongly symptom-inducing viruses, viroids, and satellites may be departures from the norm, deviations that will afford insight into the workings of more normal, more compatible plant-parasite relationships. By using these pathogens as tools, the complexities of the pathogen replication cycle interaction with the host can be modified, manipulated, and, eventually, better understood.


## ACKNOWLEDGMENTS

I would like to thank those scientists who improved this review by making available information that was at the time unpublished.


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