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The lectures summarized in this publication were presented by their respective authors at a Workshop held on the 7th thru the 9th of May 1990 at the Fundación Juan March.

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Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses

Organized by

F. García-Arenal and P. Palukaitis

D. Baulcome
R. N. Beachy
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253 Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses

Fundación Juan March

Serie Universitaria

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I N D E X

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PROGRAMME

Monday 7th of May

9,15 - Opening address - F. García-Arenal

REPLICATION I - Chairpersons: A.L. Haenni and J.A. García

9,30 - J. BOL - Replication of alfalfa mosaic virus RNAs in vitro and in transgenic plants.

10,00 - M. ROOSSINCK - Molecular genetic analysis of cucumber mosaic virus RNA 1.

10,20 - W.L. GERLACH - The molecular genetics of luteoviruses.

10,40 - I. JUPIN - Mapping sequences required for replication and pathogenesis of Beet Necrotic Yellow Vein Virus RNA-3.

11,00 - POSTERS

13,00 - Lunch

REPLICATION II - Chairpersons: M. Zaitlin and M.A. Mayo

15,00 - A.L. HAENNI - Tymoviruses: from their genome organization to their potential use in anti-viral resistance.

15,30 - S. DING - The tymobox, a molecular tool in studies of Tymoviruses.

15,50 - S. LAIN - RNA helicase activity associated to plum pox potyvirus cylindrical inclusion protein.

16,10 - W.G.DOUGHERTY - Cis and trans substrate specificity of the 49kDa proteinase of tobacco etch virus.

16,40 - Coffee

17,10 - J.A. GARCIA - Expression and function of plum pox potyvirus gene products.

17,40 - R.N. BEACHY - The role of the TMV movement protein in modification of plasmodesmata in Xanthi and Xanthi nc tobacco plants.

18,00 - Y. WATANABE - Functional domains in TMV.

18,30 - E.M.J.JASPARS - The RNA polymerase of alfalfa mosaic virus from completely and incompletely infected cowpea mesophyll protoplasts.

19,00 - End of session

.../...

Tuesday 8th of May

RESISTANCE AND PATHOGENESIS - Chairpersons: J. Bol and D. Baulcombe

9,30 - R.N. BEACHY - Coat protein mediated resistance against TMV: a status report.

10,00 - M. ZAITLIN - Tobacco plants transformed with the TMV 54K sequence are resistant to TMV replication.

10,30 - W.L. GERLACH- Ribozyme activity against plant pathogen RNAs.

11,00 - J.R. DIAZ-RUIZ - Pathogenicity of the tobamovirus pepper mild mottle virus in pepper plants and protoplasts with different genotypes.

11,30 - Coffee

12,00 - C. HEMENWAY - Analysis of the mechanism of coat protein-mediated protection in transgenic plants expressing the potato virus X (PVX) coat Protein (CP) gene.

12,20 - S.BEN THAHAR- Sugarbeet and melon transformation for virus resistance.

12,40 - F. PONZ - Is pepper-potato virus Y actually potato virus Y?.

13,00 - Lunch

SATELLITES - Chairpersons: W.L.Gerlach and J.R. Díaz-Ruiz

15,00 - D. BAULCOMBE- The molecular biology of satellite RNA of cucumber mosaic virus.

15,30 - P.PALUKAITIS- Cucumber mosaic virus and its satellite RNAs: localization of sequences involved in pathogenicity.

16,00 - F. GARCIA-ARENAL - Evolution and variability of the satellite RNA of cucumber mosaic virus.

16,30 - Coffee

17,00 - E. MORIONES - Interaction among strains of tomato aspermy virus and of cucumber mosaic virus satellite RNAs.

17,20 - M.JACQUEMOND- Cucumber mosaic virus satellite RNA replication.

17,40 - J. BURGYAN - Biologically active in vitro transcripts from full-length cloned cDNA to genomic, defective-interfering and satellite RNAs of cymbidium ringspot tombusvirus.

18,00 - G. BRUENING - Self-cleavage and circularization reactions and the replication of the satellite RNA of tobacco ringspot virus.

18,30 - End of session

Wednesday 9th May

GENOME STRUCTURE AND ORGANIZATION - Chairpersons: W.G. Dougherty and E.M.J. Jaspars

9,30 - M.A. MAYO - Identification and expression of the particle proteins of potato leafroll virus.

10,00 - D.L. NUSS - Molecular biology of plant reoviruses.

10,30 - G. BOCCARDO - Molecular organization of cryptic virus dsRNA genomes.

11,00 - H.L. SÄNGER - 7S SRP RNA: The possible cellular target for potato spindle tuber viroid-(PSTVd)related viroids.

11,30 - Coffee

12,00 - T. CANDRESSE - Analysis of the organization and expression of the genome of apple chlorotic leaf spot closterovirus.

12,20 - H.J.M.LINTHORST - Homologies between the genomes of a carlavirus (lily symptomless virus) and a potexvirus (lily virus X) from lily.

12,40 - M. FUCHS - Genome organization and expression of Grapevine Fanleaf Virus.

13,00 - Lunch

15,00 - POSTERS

16,00 - General discussion

17,00 - Closing address - P. Palukaitis

INTRODUCTION

F. GARCÍA ARENAL
Departamento de Patología Vegetal
E.T.S.I. Agrónomos
Madrid (Spain)

P. PALUKAITIS
Department of Plant Pathology
Cornell University
Ithaca, N.Y. (USA)

INTRODUCTION

The multiplication of viruses in their plant hosts is frequently accompanied by an unbalance of plant metabolic and regulatory functions, leading to disease. How viruses replicate in plants, and how they induce disease, is still not well understood in spite of considerable recent progress of plant virology.

Indeed, until comparatively recent times, the extant knowledge on plant virus-coded functions was limited to their structural proteins. During the last eight years, sequence information on the structure of plant virus genomes has accumulated exponentially, and today a wealth of data exists concerning most RNA plant virus groups. This has permitted the comparison of genome organization and expression strategies, as well as the deduction of functions for the putatively encoded protein products. However, in most cases these functions have still not been determined.

Nevertheless, in the last few years the application of two new experimental approaches has permitted a detailed analysis of the mechanisms of expression and pathogenesis of plant viruses, clearifying their biology. First, full length cDNA clones from which infections transcripts can be synthesized are being obtained for an increasing number of plant viruses and their satellites. The manipulation of these clones is rapidly producing new data on genome-encoded functions, their expression during the virus life cycle, and their effect on the plant host.

Second, the use of transgenic plants that constitutively express fragments of viral (or satellite) genomes, or their encoded protein products, has been a breakthrough in plant virus research. The transgenic plants permit the analysis in planta of viral functions and provide an approach to study the mechanisms of viral pathogenesis. Further, the analysis of the resistance associated with plants transformed with parts of viral or satellite genomes can be useful in understanding natural mechanisms of plant resistance, virus interference etc.

Thus, we thought it would be timely to have a meeting on the genome expression and pathogenesis of plant RNA viruses. In contrast to other recently held workshops on plant viruses, we wanted to limit its scope to plant RNA viruses: not only the vast majority of plant viruses have RNA genomes, but progress on understanding of their genome expression and pathogenesis has increased greatly in recent years. Furthermore, we did not want to limit the workshop to single-stranded, plus sense RNA viruses, as the biology of double-stranded and minus-stranded RNA plant viruses, with animal hosts, may provide important clues to plant specific phenomena.

This workshop, sponsored by Fundación Juan March, was held in Madrid, 7th-9th of May 1990.

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ORAL PRESENTATIONS

REPLICATION OF ALFALFA MOSAIC VIRUS RNAs IN VITRO AND IN TRANSGENIC PLANTS

John Bol, Tonja van der Kuyl, Lyda Neeleman, Jean-Michel Dore and Gied Jaspars. Gorlaeus Laboratories, Leiden University, Einsteinweg 5, 2333 CC Leiden, The Netherlands.

The genome of alfalfa mosaic virus (AlMV) is tripartite. RNAs 1 and 2 encode proteins P1 and P2, respectively, which are involved in viral RNA replication. RNA 3 encodes protein P3 with a putative role in cell-to-cell movement, and the viral coat protein (CP). CP is translated from a subgenomic messenger, RNA 4. A mixture of the three genomic RNAs is not infectious unless a few molecules of CP or RNA 4 are added per genomic RNA ("genomic activation").

A cDNA clone of AlMV RNA 3 was transcribed *in vitro* with T7-polymerase into plus-stranded or minus-stranded RNA molecules. An RNA-dependent RNA-polymerase (RdRp) purified from AlMV-infected bean leaves was able to use plus-stranded T7-transcripts as templates for the sunthesis of full-length minus-stranded RNA 3 in an *in vitro* assay. Minus-stranded T7-transcripts were copied by the RdRp into plus-stranded RNA 4 molecules. The RdRp did not recognize non-AlMV RNAs as template. Specific deletions were made in cDNA 3 in order to localize the subgenomic promoter in minus-stranded T7-transcripts and the recognition site for RdRp near the 3'-termini of plus-stranded T7-transcripts. The subgenomic promoter was localized between 10 and 55 nucleotides upstream of the transcription start site for RNA 4 synthesis, while a sequence between 133 and 162 nucleotides from the 3'-end of RNA 3 was sufficient for recognition of plus-stranded templates by the RdRp (ref. 1).

The genes encoding AlMV proteins P1, P2, P3 and CP were constitutively expressed in transgenic tobacco (refs. 2, 3 and 4). Plants expressing P1, P2 or P3 were susceptible to infection with AlMV particles or RNAs. The P1-plants were able to support the replication of a mixture of RNAs 2 and 3 whereas the P2-plants supported the replication of RNAs 1 plus 3. In these incomplete infections the RNAs were normally encapsidated and the plants developed symptoms indistinguishable from those of completely infected plants.

The plants expressing CP were resistant to infection with AlMV particles but susceptible to infection with AlMV RNA. Also, when these plants were inoculated with RNAs 1-3, the CP produced in the plants was able to activate the AlMV genome. Capped T7-transcripts of cDNA with one non-viral G-residue at the 5'-end were infectious to plants and protoplasts when combined with native RNAs 1 and 2 and CP. A transcript with a deletion or inversion in the P3-gene replicated well in protoplasts but not in non-transformed plants or transgenic P3-plants. Thus, there is no proof that P3 produced in these plants is functionally active. Mutant transcripts with a deletion in the CP-gene replicated in protoplasts albeit at a low level, but no replication was observed in non-transformed plants or CP-plants. Apparently, the CP in these transgenic plants was able to activate the AlMV genome but was unable to complement for one or more other essential functions of CP in the AlMV life cycle, e.g. a function in cell-to cell transport. At present a further deletion analysis is made to study *cis*-acting elements in AlMV RNAs which are involved in replication and synthesis of subgenomic RNAs *in vitro*.

References

1. Van der Kuyl, A.C., Langereis, K., Houwing, C.J., Jaspars, E.M.J. and Bol, J.F. *Cis*-acting elements involved in replication of alfalfa mosaic virus RNAs *in vitro*. Virology (1990), in press.
2. Van Dun, C.M.P., Bol, J.F. and Van Vloten-Doting, L. Expression of alfalfa mosaic virus and tobacco rattle virus coat protein genes in transgenic tobacco plants. Virology 159, 299-305 (1987).
3. Van Dun, C.M.P., Van Vloten-Doting, L. and Bol, J.F. Expression of alfalfa mosaic virus cDNA 1 and 2 in transgenic tobacco plants. Virology 163, 572-578 (1988).
4. Van Dun, C.M.P. Expression of viral cDNA in transgenic tobacco. Thesis, Leiden University (1988).

MOLECULAR GENETIC ANALYSIS OF CUCUMBER MOSAIC VIRUS RNA 1

Marilyn Roossinck, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853 USA

Cucumber mosaic virus (CMV) is a broad host range, single-stranded RNA virus with a functionally divided genome. RNA 1 encodes a 111 kD protein which contains the conserved motifs found in all known viral helicases (1). RNA 2 encodes a 97 kD protein which contains the conserved GDD motif found in most viral replicases (2). RNAs 1 and 2 are required and sufficient for replication (3). RNA 3 encodes both the putative movement protein, and the viral coat protein.

Two strains of CMV, Fny-CMV and Sny-CMV, have several replication-related phenotypic differences: time of appearance and severity of symptoms when inoculated onto squash (4); temperature-sensitive replication in muskmelon; and efficiency of replication of satellite RNAs. In addition, several other strains of CMV show some of these phenotypic differences. Pseudorecombinants between Fny- and Sny-CMV were constructed using gel-purified RNAs. These strains were biologically purified and tested for purity with an RNase protection assay, using antisense RNA probes. Using the pseudorecombinants, these phenotypic differences have been mapped to RNA 1. In previous studies with CMV only one phenotypic characteristic, that encoding the lack of systemic movement of N-CMV in squash and tobacco, has been localized to RNA 1 (5).

The RNase protection assays indicated that there were only minor differences in the RNAs 1 from these two strains. Using both DNA sequencing of cDNA clones of Fny- and Sny-CMV, and direct sequencing of viral RNAs, I have examined selected regions of the RNA 1 molecule for sequence variation, both between Fny- and Sny-CMV, and among other phenotypically similar strains. Simultaneously, I am constructing a series of recombinant viruses using a cDNA clone of Sny-CMV RNA 1 and biologically-active cDNA clones of the Fny-CMV RNAs. These recombinant viruses are being used to map precise domains of RNA 1 which are responsible for these phenotypic differences. Results of these analyses will be presented.

1. Habili and Symons (1989) N.A.R. 17:9543-9555.
2. Argos (1988) N.A.R. 16:9909-9915.
3. Nitta *et al.* (1988) J. Gen. Virol. 69:2695-2700.
4. Roossinck and Palukaitis (1990) Mol. Plant-Microbe Interact. 3:(in press).
5. Lakshman and Gonsalves (1985) Phytopath. 75:758-762.

THE MOLECULAR GENETICS OF LUTEOVIRUSES

P Waterhouse, P Keese, M Young, C Zhuomin, J Fillatti, W Gerlach.

CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia.

Luteoviruses comprise a group of plant viruses whose members infect dicot and moncot hosts. They are isometric particles of approximately 25 nm diameter, enclosing a single RNA of approximately 6,000 bases and the viruses are aphid transmitted in a persistent manner.

The genome organisation of a number of luteoviruses has now been described. From this, it can be seen that the luteoviruses fall into two groups with regard to genome organisation. The groups share common features and relatedness at the 3' halves of their genomes, involving the coat protein gene and other open reading frames. However, the two groups have 5' halves containing replicase genes which sometimes show closer relationships to plant viruses outside the luteoviruses than to other luteoviruses.

Luteoviruses use a range of strategies for the genetic expression. Examples are frame shift supression, translational readthrough, gene overlaps and the use of subgenomic promoters. Protein products of unknown reading frames of luteoviruses are being translated from fusion gene constructions in bacteria. The hybrid proteins are being used for antibody production as a means to assay gene expression during luteovirus infection.

The complete sequences for a number of isolates of potato leafroll virus are known. This provides the basis for strategies for construction of synthetic resistance genes for this economically important virus. For barley yellow dwarf virus, the testing of synthetic resistance genes is being approached by the routine use of RNA or virus infections of cereal protoplasts.

Luteovirus molecular biology would be greatly aided by the production of cDNA clones capable of transcribing infectious RNA. This has been achieved for BYDV and is currently underway for PLRV.

Mapping sequences required for replication and pathogenesis of Beet Necrotic Yellow Vein Virus RNA-3

Isabelle Jupin, D. Gilmer, S. Bouzoubaa, K. Richards, H. Guilley and G. Jonard

Institut de Biologie Moléculaire des Plantes
12 rue du Général Zimmer, 67084 Strasbourg, France

Rhizomania of sugar beet is caused by beet necrotic yellow vein virus (BNYVV), a fungus-transmitted plus-strand RNA virus with a quadripartite genome. Each RNA is 5' capped and 3' polyadenylated.

In order to identify the virus sequences involved in the pathogenicity of BNYVV, we obtained infectious transcripts corresponding to RNA-3 and -4. Using site-directed mutagenesis, we have modified RNA-3 and inoculated the altered transcripts to leaves along with RNA-1 and -2. We have now mapped *cis*- regulatory elements on RNA-3 to the 5' 300 nucleotides and 3' 70 nucleotides. The 3'terminal essential domain can be folded into a secondary structure conserved among the 4 RNAs of BNYVV and which probably contains the minus-strand promoter. On the other hand, the 5'noncoding region of the 4 RNAs contains conserved boxes which may play an important role in recognition by the replicase. Moreover, these 5' and 3' terminal sequences are sufficient to permit replication as RNA-3 transcripts with 75% of the central core of the sequence deleted were still viable.We have also demonstrated, using the GUS gene, that these 5' and 3'terminal sequences allow the replication and expression of foreign genes in plants. The first 300 nucleotides also contain the site for initiation of encapsidation.

The 3'-polyadenylate sequence is indispensable for the infectivity of transcripts: suppressed by mutagenesis, it reappears spontaneously during multiplication of the transcripts in the plant. An unexpected feature of these progeny was the presence of a novel short heterogenous U- rich tract separating the poly (A) tail from the 3' end of the heteropolymeric portion of the RNA-3 sequence.

We have also demonstrated using deletions or frameshift mutants that the RNA-3 encoded 25 K polypeptide is responsible for the yellow character of local lesions elicited on leaves by full-length RNA-3 while a second protein may be involved in the necrotic local lesion phenotype of some mutants.

References

Ziegler-Graff *et al.*, (1988) Biologically active transcripts of beet necrotic yellow vein virus RNA-3 and -4. *J. gen. Virol.* **69**, 2347-2357.

Quillet *et al.*, (1989) *In vitro* synthesis of biologically active beet necrotic yellow vein virus RNA. *Virology* **172**, 293-301.

Jupin *et al.*, (1990) Mapping sequences required for productive replication of beet necrotic yellow vein virus RNA 3. *Virology*, in press.

Jupin *et al.*, (1990) Multiplication of beet necrotic yellow vein virus RNA 3 lacking a 3'poly(A) tail is accompanied by reappearance of the poly(A) tail and a novel short U-rich tract preceding it. *Virology*, in press.

TYMOVIRUSES: FROM THEIR GENOME ORGANIZATION TO THEIR POTENTIAL USE IN ANTI-VIRAL RESISTANCE

A.L. HAENNI, G. DRUGEON, J.C. BOYER, G. KADARE, R. GARGOURI-BOUZID, C. DAVID, M. LAMBERT, R. JOSHI, F. AGNES and M.D. DEVIGNES-MORCH
Institut Jacques Monod*, 2 Place Jussieu - Tour 43, 75251 Paris Cedex 05, France

Tymoviruses contain a monopartite RNA genome of '+' polarity. The genome organization of Turnip yellow mosaic virus (TYMV), the type member, has been established by determining the total sequence of the RNA and by *in vitro* translation studies. The RNA contains 3 cistrons. Two overlapping cistrons are translated from the genomic RNA, and correspond to two nonstructural proteins of 206K (K = kilodalton) and 69K. The former protein contains the functional domains (nucleotide-binding domain and polymerase domain) that are highly conserved among most plant and animal RNA viruses; it undergoes a temperature-sensitive proteolytic maturation step, providing the 150K and 78K cleavage products. The function of the highly basic 69K protein is unknown. The third cistron corresponds to the coat protein (20K); it is 3' proximal on the genomic RNA and is only expressed *via* a subgenomic RNA; the subgenomic RNA is synthesized by internal initiation of replication on the '-' RNA strand of genomic size. The genomic organization of TYMV is comparable to that of other tymoviruses whose genome has been sequenced. In addition, the overall strategies used by Belladonna mottle virus, Ononis yellow mosaic virus and Eggplant mosaic virus are similar to those used by TYMV, as established by *in vitro* translation studies. These considerations allow us to classify tymoviruses in the Sindbis-like supergroup.

The 3' proximal 100 nucleotides of the viral RNA inhibit replication *in vitro* of the viral genome and are themselves replicated. Further studies have now demonstrated that even shorter RNA sequences from the 3' end of the viral RNA can inhibit viral RNA replication. This 'sense' RNA approach is now being tested *in vivo*: transgenic rapeseed plants expressing the 3' proximal 100 nucleotides of TYMV have been obtained (work performed in collaboration with M. Tepfer, INRA, Versailles) and will be tested for their capacity to delay virus multiplication. In parallel, pseudo-genomic RNAs have been constructed in view of establishing the size of the regions (promoters) in the genomic RNA that are able to interfere with RNA replication. Finally, a cDNA insert corresponding to the full length TYMV genomic RNA has been obtained and transcribed *in vitro*: the resulting RNA provides the same protein pattern as virion RNA when translated *in vitro*, and is infectious when inoculated onto rapeseed plants.

* The Institut Jacques Monod is an 'Institut Mixte - CNRS, Université Paris VII'

THE TYMOBOX, A MOLECULAR TOOL IN STUDIES OF TYMOVIRUSES

Shouwei Ding, Jennifer Howe[a], Paul Keese[a], Anne Mackenzie, Drew Meek[b], Marlene Osorio-Keese[c], Mary Skotnicki, Pattana Srifah, Marjo Torronen, and Adrian Gibbs.

Research School of Biological Sciences, The Australian National University, Canberra, A.C.T. 2601, Australia.

Tymoviruses are a group of plant viruses which have singlestranded messenger sense genomic RNAs about 6 kb in length. Complete sequences of the genomic RNAs of TYMV (1, 2 and D. Meek, unpublished data), EMV-Trin (3), OYMV-Tin (4), KYMV-JB (5) and ELV (Srifah et al., in preparation) have been recently determined. Partial genomic sequences of several other tymoviruses, including DMV and BdMV-Eur, APLV-Hu, TYMV-Roth, CYVV, WCuMV, KYMV-PD, KYMV-BP have also been determined in our laboratory. It was shown that all of these genomes contain three open-reading frames (ORFs), two of which have their initiation codons close to the 5'-end of the genome and overlap, whereas the virion protein gene is located at the 3' end portion of the genome and is expressed via a subgenomic mRNA (6).

The 5' -terminal sequences of the virion protein mRNAs of ononis yellow mosaic and kennedya yellow mosaic tymoviruses were determined, and also the positions in the genomes of the transcription initiation sites of those mRNAs. Comparisons of the available genomic sequences of tymoviruses revealed two conserved regions, one at the initiation site and another longer sequence of sixteen nucleotides to the 5' side of it. The longer sequence, which we call the tymobox, was tested as a target for a designed ribozyme, which cleaved appropriate genomic fragments of three tymoviruses. A synthetic oligonucleotide with sequence complementary to the tymobox was shown to be a tymovirus-specific probe for diagnosing and identifying tymoviruses, except for wild cucumber mosaic tymovirus. The tymobox sequence was also used as a primer for the second strand DNA synthesis of dsDNA representing the virion protein gene of cacao yellow mosaic tymovirus, a tymovirus with unknown sequence. Thus, the tymobox is a useful tool in molecular studies of tymoviruses (7).

References:

1. Morch,M.D., Boyer,J.C. and Haenni, A.L. 1988 NAR.16,6157.
2. Keese,P., Mackenzie,A. and Gibbs, A. 1989 Virol. 172,536.
3. Osorio-Keese,M.E., Keese,P. and Gibbs,A. 1989 Virol.172,547.
4. Ding,S., Keese,P. and Gibbs, A. 1989 Virol. 172,555.
5. Ding,S., Keese,P. and Gibbs,A. 1990 J. gen. Virol. In Press.
6. Pleij,C.W.A., Neeleman,A. van Vloten-Doting,L. and Bosch,L. 1976, PNAS,USA 73,4437.
7. Ding,S. et al., 1990 NAR. 18,1181.

RNA helicase activity associated to plum pox potyvirus cylindrical inclusion protein.

S. Laín, M.T. Martín, J.L. Riechmann and J.A. García.

Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain.

Most positive strand RNA viruses infecting plants and animals encode proteins with a nucleotide binding motif (NTBM) in their sequence (1). Although there was no reported biochemical evidence on the enzymatic activity of any of these proteins, further sequence analysis led to include them in three superfamilies of helicase-like proteins from very diverse origins (1,2).

The plum pox virus cylindrical inclusion (CI) protein, the NTBM-containing protein encoded by potyviruses, has been purified and shown to be associated with nucleic acid stimulated ATPase and nucleotide dependent RNA unwinding activities. This RNA unwinding activity required the hydrolisis of NTPs to NDP and P_i and thus it can be considered as an RNA helicase activity (3). In the *in vitro* system used, helicase activity was detected only on partial double strand RNA substrates with 3' single strand overhangs, while it failed to unwind an RNA substrate with only 5' overhangs as well as a DNA duplex.

1. Gorbalenya, A.E. and Koonin, E.V. (1989) Nucl. Acids Res. 17, 8413-8440; 2. Laín et al. (1989) Gene 82, 357-362; 3. Geider, L. and Hoffman-Berling H. (1981) Annu. Rev. Biochem. 50, 233-260.

Cis and *trans* Substrate Specificity of the 49kDa Proteinase of Tobacco etch Virus.

William G. Dougherty
Department of Microbiology
Oregon State University
Corvallis, OR., USA 97331-3804

Members of the potato virus Y group express their genetic information from a single-stranded RNA molecule that is approximately 9600 nucleotides in length. The initial translation product would be approximately 350,000 daltons (350kDa) in size; however, rapid co-translational processing and subsequent post-translational processing results in the formation of individual gene products. Two viral-encoded proteolytic activities are involved in tobacco etch virus (TEV) polyprotein processing. They have been referred to as HC-PRO and the 49kDa proteinase. The 49kDa proteinase is responsible for five cleavage events and displays a unique degree of substrate specificity.

Hybrid proteinases have been constructed between the TEV 49kDa proteinase and the tobacco vein mottling virus (TVMV) NIa proteinase. Each recognizes a unique conserved sequence at naturally occurring cleavage sites [TEV = E - Xaa - Xaa - Y - Xaa - Q * S/G; TVMV = V - R/K - F/T - Q * S/G]. We wished to determine the minimum TEV sequence that could be replaced with TVMV sequence such that proteolytic activity was maintained but substrate specificity was altered. All total, 24 proteinases have been constructed and tested for autocatalytic (cis) processing and bi-molecular (trans) cleavage of TEV and TVMV substrates. The results of these studies will be reported.

Expression and function of plum pox potyvirus gene products

J.A. García, S. Laín, M.T. Martín, M.T. Cervera, C. López-Otín and J.L. Riechmann

Plum pox potyvirus is a member of the potyvirus group. Its genome consists of a single-component single-stranded RNA polyadenylated at the 3' end and with a protein linked to the 5' terminus (1,2). The RNA sequence of three different isolates have been elucidated to date (3-5). It contains a unique ORF which spans nearly the entire length of the genome and encodes a single polyprotein which undergoes extensive proteolytic processing to yield the mature products. The small nuclear inclusion protein (NIa) has been shown to be responsible for some of the proteolytic cleavages (6). Amino terminal amino acid sequencing of purified proteins permitted the identification of four cleavage sites in the PPV polyprotein. Two other putative processing places have been postulated on the basis of sequence homology. A binary E. coli expression system has been used to study the pathway for proteolytic processing of the PPV polyprotein. Trans cleavage at the carboxyl end of the cylindrical inclusion protein (CI) occurred, although with less efficiency than at the large nuclear inclusion protein (NIb)-capsid protein (CP) junction. Only cis cleavage was detected at the carboxyl end of the NIa protein. The proteolytic activities at different cleavage sites of several deletion and point mutants of NIa protein have been analysed. The large ΔSX deletion and two different point mutations at His239 abolished the activity at all sites. The effect of other mutations, specially a Glu substitution for Asp274 depended on the particular cleavage site analysed. No evident competitive inhibition of the proteolytic activity of PPV NIa protease by the presence of excess of the different protease mutants could be demonstrated.

The construction of a full-length cDNA clone, from which infectious PPV RNA could be obtained by in vitro transcription, will be also discussed.

1. Laín et al. (1988) Virus Res. 10, 325-342; 2. Riechmann et al. (1989) J. Gen. Virol. 70, 2785-2789; 3. Laín et al. (1989) Virus Res. 13, 157-172; 4. Maiss et al. (1989) J. Gen. Virol. 70, 513-524; 5. Teycheney et al. (1989) Nucl. Acids Res. 17, 10115-10116; 6. García et al. (1989) Virology 170, 362-369.

The role of the TMV movement protein in modification of plasmodesmata in Xanthi and Xanthi nc tobacco plants

Roger N. Beachy, C. Michael Deom, Patricia Moore, Francoise Cellier, Curtis Holt, and Ali Nejidat

Department of Biology, Washington University, St. Louis, Missouri USA 63130
Schmuel Wolf and William Lucas
Department of Botany, University of California, Davis, CA 96 16 USA

The 30 kDa protein encoded by TMV is essential for spread of TMV from cell-to-cell and from leaf to leaf. Because of its role it has be designated the TMV movement protein (MP). In transgenic plants, and in the absence of TMV infection, the MP associates with and modifies the function of the plasmodesmata. In Xanthi tobacco this modification increases the size exclusion limit of plasmodesmata from ~750 Da to greater than 10,000 Da. Such modification is apparently involved in potentiating the spread of TMV from cell-to-cell. While expression of the MP gene in Xanthi nc tobacco alters the size exclusion limit of this cultivar, the degree of modification is less than in Xanthi tobacco unless the temperature is raised to 32°. At this temperature the local lesion response to TMV infection is blocked, and the infection spreads throughout the leaf. Likewise, at 32° the size exclusin limit of plasmodesmata in MP(+) Xanthi nc leaves is increased to greater than 10,000 Da.

A TMV mutant that does not produce MP is able to spread in transgenic, MP(+) Xanthi tobacco plants, and MP(+) Xanthi nc plants at 32°. Complementation is expressed in all but the very young leaf tissue. It is proposed that, although the MP gene is expressed in young leaves, the level of protein accumulation is insufficient to complement the MP(-) TMV to enable its spread from the vascular tissue to surrounding mesophyll cells.

A model will be proposed to accommodate the role of the MP in modification of plasmodesmata in Xanthi and Xanthi nc tobacco.

FUNCTIONAL DOMAINS IN TMV

Yuichiro Watanabe

Department of Biophysics and Biochemistry
Faculty of Science - The University of Tokyo
Bunkyo-Ku, Tokyo 113, JAPAN

Tobacco mosaic virus (TMV) can multiply, spread and propagate in plants through several processes: typically, replication in each cell, cell-to-cell movement and long-distance movement. In vitro expression system of infectious viral RNA and reverse genetic approaches have been showing us the details of functions of virus-coded proteins. The 130K/180K proteins are involved in virus replication probably as replicase components; the 30K protein is related to cell-to-cell movement to adjacent cells; the coat protein aids the virus entity to go through the vascular system. We have been performing time-course analysis of the localization of these proteins in synchronously TMV-infected protoplasts, especially concentrating on the location of the non-structural proteins to investigate further functional details.

Among the cis-elements in RNA sequences, the virus assembly origin is involved in and promotes long-distance movement of virus in plants. The 5'- and 3'- noncoding regions are suspected to be involved in plus- and/or minus- strand RNA syntheses as signals. There were found three consecutive pseudoknots between just downstream termination codon of the coat protein and upstream the t-RNA structure. Deletion analysis showed, however, that almost all this region is not necessary or dispensable for the least virus viability. The point mutation analysis showed that only the most 3'-nearest stem-loop mapped in this region is indispensable for virus viability. As to the 5'-noncoding region, mutants lacking the sequence between 2-8 or 26-72 were inviable.

TMV causes severe diseases in various plants including important crops, but its infection is not totally detrimental to plant survival. Protoplasts, which were infected with TMV, can regenerate into whole plants, accompanied by mosaic symptoms. Plants exhibit various resistance reactions against TMV, as the case may be. Tm-1 gene inhibits the replication of wild-type or coat protein- and/or 30K protein-less TMVs by repressing 130K/180K protein to function. The presence of actinomycin-D in the long standing did not hardly weaken the resistance and the mode of inhibition is constitutive. Tobacco plants with N gene exhibit hypersensitive reactions against almost all of TMV strains found and made so far.

THE RNA POLYMERASE OF ALFALFA MOSAIC VIRUS FROM COMPLETELY AND INCOMPLETELY INFECTED COWPEA MESOPHYLL PROTOPLASTS

E.M.J. Jaspars and Corrie J. Houwing, Gorlaeus Laboratories, Leiden University Einsteinweg 5, NL-2333 CC Leiden, The Netherlands.

A few hours after inoculation of cowpea protoplasts with a mixture of the genome RNAs (RNAs 1, 2 and 3) and the subgenomic coat protein messenger (RNA 4) of alfalfa mosaic virus the presence of a viral RNA polymerase could be demonstrated. During the next 12 hours a strong increase of the activity of this enzyme took place. If we omitted the RNAs 3 and 4 from the inoculum and added a small amount of coat protein a similar increase of the activity of the viral RNA polymerase could be observed. If no coat protein was added to an inoculum of RNAs 1 and 2 no trace of viral RNA polymerase could be detected. Evidently, formation of the enzyme is dependent on parental coat protein, but not on the synthesis in the infected cell of the gene products of RNA 3 (the 32K non-structural protein and the coat protein). In completely (with RNA 3) and in incompletely infected protoplasts (without RNA 3) the viral RNA polymerase was associated with chloroplasts but also with small non-pigmented particles which could be vesicles of the chloroplast envelopes. In both cases the enzyme-membrane complexes produced in vitro mainly RNAs of plus polarity which were found annealed to their endogenous templates. When the viral RNA polymerases from both kinds of infected protoplasts were solubilized (with the aid of the non-ionic detergent dodecyl-maltoside) and were made template-dependent (by treatment with micrococcal nuclease) they were able to synthesize full-length minus strands on added plus-strand RNAs of alfalfa mosaic virus. In both cases this synthesis was equally inhibited by small amounts of coat protein.

So, in vitro no significant difference was detectable between the viral RNA polymerases from complete and incomplete infections except that in the latter case the endogenous template for the synthesis of RNAs 3 and 4 was lacking. However, when we looked at the level of viral RNAs in vivo, we found, as Nassuth and Bol did earlier (Virology 124, 75-85, 1983), that the incompletely infected protoplasts were accumulating much less plus-strand RNA than the completely infected protoplasts. In one experiment where the protoplasts were inoculated with RNAs 1 and 2 plus 4 coat protein subunits per RNA molecule the accumulation of plus-strand RNA was so low that virtually all RNA of plus polarity was found in double strands.

Therefore, we have considered the possibility that the coat protein is necessary for releasing plus-strand RNAs from replication complexes. If there is no coat protein in an inoculum parental replication complexes could be formed but would not be able to produce single-strand RNAs, and thus RNA synthesis would stop at a very early stage. Experiments to test this and other hypotheses concerning the role of the coat protein in viral RNA synthesis have been done and the results will be discussed.

Coat Protein Mediated Resistance Against TMV: A Status Report

Roger N. Beachy, Ali Nejidat, Curtis Holt, Lisa Wisniewski and Gregg W. Clark, Department of Biology, Washington University, St. Louis, MO, USA, 63130

We have continued studies to elucidate the nature of the mechanism(s) that are involved in coat protein (CP) mediated resistance to limit TMV infection in transgenic tobacco and tomato plants. To date all of our data are derived from studies with the CP gene of the U_1 (common) strain of TMV as expressed from the P35S from cauliflower mosaic virus and the PSSU derived from a gene encoding ribulose bisphosphate carboxylase. The gene was expressed in Xanthi and Xanthi nc tobacco, and in a susceptible cultivar of tomato.

It is generally true that greater levels of CP accumulation lead to greater levels of resistance. Resistance is reflected in a reduced number of sites of infection and reduced rate of virus spread throughout the plant. This was documented by several approaches, including the use of a TMV mutant in which the cistron encoding the 30 kDa (movement) protein was replaced by a β-glucuronidase. CP(+) plant lines in which the CP gene was driven by the PSSU were also produced. While these plants and protoplasts derived therefrom were resistant to infection by TMV, there was little resistance to systemic infection.

Plants that express the U_1 CP gene were inoculated with several different tobamoviruses. There was a high level of resistance against TMV, tomato mosaic virus, pepper mild mottle virus, and tobacco mild green mosaic virus, less resistance against ondontoglassum ringspot virus, and much less, but significant resistance against ribgrass mosaic virus.

An attempt will be made to integrate our level of knowledge about CP-mediated resistance an experimentally testable model.

TOBACCO PLANTS TRANSFORMED WITH THE TMV 54K SEQUENCE ARE RESISTANT TO TMV REPLICATION

Milton Zaitlin, Daniel B. Golemboski, John P. Carr and George P. Lomonossoff*

Department of Plant Pathology
Cornell University, Ithaca, New York 14853 USA

TMV RNA encodes 4 polypeptides of 183k, 126k, 30k and 17.5k. The 183k protein is a readthrough of the 126k protein; both are thought to be a part of the viral replicase complex. In addition, we have contended in the past that the open reading frame for a 54k protein in the readthrough region of the 183k protein represents a fifth viral-encoded protein, although it has not been detected in tissues by us (unpublished) nor by Saito et al. (Mol. Gen. Genet. 205: 82, 1986). However, there is a polyribosome-associated subgenomic RNA with an open reading frame for this protein (Sulzinski et al. (Virology 145: 132, 1985), which implies a 54k protein should be translated in vivo.

In experiments designed to seek a function for the 54k protein, we transformed plants with a cDNA clone containing TMV nucleotides 3472 to 4916, which encompass all of the 54k protein codon except for 3 nucleotides at the 3' end. Surprisingly, we found that the transformed plants were completely resistant to TMV (Strain U1) when inoculated with either virions or RNA at concentrations of up to 500µg/ml or 300µg/ml respectively, the highest concentrations tested. The copy number of the 54k sequence in the plants ranged from 1 to 5 in individual transformants, but the level of resistance was not influenced by the copy number. A sequence-specific 54k RNA transcript of the expected size was observed, but no 54k protein was detected by Western blotting, immunoprecipitation or radiolabeling; these studies are continuing, however.

The transformed plants were resistant to a yellow mutant of Strain U1 (YSI/1, Garcia-Arenal et al. Virology 132: 131, 1984) but were not resistant to TMV strains U2 or L or to a strain of cucumber mosaic virus (Fny).

Resistance to TMV U1 was also expressed at the protoplast level, where no virus replication was obtained when protoplasts were electroporated with TMV RNA. Protoplasts from the transformed plants did support the replication of strain U2.

The 54k region of the TMV genome contains amino acid sequence motifs conserved among many RNA viruses of both plants and animals. Without knowledge of whether the resistance is brought about by protein or RNA it is difficult to formulate an hypothesis for the mode of action of the resistance, but it is very probable that inhibition of replicase assembly or function is involved.

*Permanent address: John Innes Institute and AFRC Institute of Plant Science Research, Norwich, UK

RIBOZYME ACTIVITY AGAINST PLANT PATHOGEN RNAs

M Young, W L Gerlach

CSIRO Division of Plant Industry , GPO Box 1600, Canberra ACT 2601, Australia.

Ribozymes are catalytic RNA molecules. One form involves adaptation of the autolytic cleavage activity of the plus strand of satellite RNA of tobacco ringspot virus, to produce new RNA ribozymes capable of cleavage of RNA substrates.

Such sequences can be placed in gene cassettes for transcription *in vitro* and *in vivo*. Amongst forms which we have prepared are sequences which target the RNA of the plant pathogens tobacco mosaic virus (TMV) and citrus exocortis viroid (CEV).

The CEV ribozyme is capable of cleaving CEV RNA *in vitro*, albeit at a slow rate. This appears to be related to the secondary structure of the molecule. TMV target RNA is cleaved at a faster rate, presumbably due to a lesser degree of secondary structure. The efficacy of a particular ribozyme is not simply related to the length of the hybridizing arms.

A local-lesion host of *Nicotiana tabacum* has been used for the production of transgenic plants expressing TMV ribozymes and corresponding antisense controls. In two experiments the individual transformants have varied in their degree of resistance to tobacco mosaic virus. However, in both experiments the mean lesion number in the population of plants producing ribozymes is lower than the mean for the antisense controls and the most resistant plants are in the ribozyme populations. Further experiments involve the testing of these gene constructions in a systemic host, cell culture system and the construction of new ribozyme genes with potentially greater activity.

Pathogenicity of the Tobamovirus pepper mild mottle virus in pepper plants and protoplasts with different genotypes*.

J.R. Díaz-Ruíz
UEI Fitopatología, Centro de Investigaciones Biológicas, CSIC.
Velázquez, 144. 28006-Madrid.

Pepper mild mottle virus (PMMV), a proposed member of the Tobamovirus group, was found to be the main disease-causing agent of protected pepper crops in the Southeastern region of Spain, because it is able to infect pepper cultivars with incorporated resistance genes to other tobamoviruses. Although the virus produces slight symptoms on the leaves, it causes malformation and reduction in size on the fruits. Similar diseases of pepper have been reported in several countries, mainly mediterranean, and distinct PMMV pathotypes (P_0, P_1, $P_{1,2}$ and $P_{1,2,3}$) have been described, based upon their ability to overcome the resistance genes L^-, L^1, L^2 and L^3 in Capsicum spp., respectively.

We have determined that most PMMVs isolated in Spain during 1982-1985 belong to the $P_{1,2}$ pathotype, based on the hypersensitive responses of Capsicum spp. which carry the different resistance genes. Since one method of controlling plant viral diseases is through the incorporation of hypersensitive resistance genes into cultivated crop varieties, we have characterized one of these spanish isolates (PMMV-S) and compared it to an italian isolate (PMMV-I) belonging to the $P_{1,2,3}$ pathotype. To understand the pathogenicity of PMMV and its interaction with the host resistance genes, we have analyzed the synthesis of viral coat protein (CP) in susceptible and resistant tobacco and pepper plants inoculated with purified PMMV-S or tobacco mosaic virus (TMV) and in protoplasts from these plants electroporated with the viral RNAs. We have also analyzed the messenger activity of PMMV-S genomic RNA, in parellel with TMV RNA, in a cell-free rabbit reticulocyte system. For further analysis, we have determined and compared the nucleotide sequence of PMMV-S and PMMV-I CP genes.

The results indicate that PMMV pathotypes establish infection in tobacco plants less efficiently than TMV, suggesting that their movement and/or replication is impaired in these hosts.

Resistance, recorded as localized hypersensitive reaction and absence of viral CP in the non-inoculated upper leaves, was expressed at 25°C against PMMV-S and TMV. However, in tobacco plants with the NN genotype mantained at 32°C, TMV become systemic while systemic spread of PMMV-S remained inhibited. Conversely, in pepper plants with the L^3L^3 genotype, PMMV-S spreads systemically while TMV remain localized, suggesting that at this temperature the hypersensitive response depends on the virus-host resistance gene combination.

Resistance was not expressed in isolated protoplasts from these plants, suggesting that the regulation of the hypersensitive resistance genes is different in cells of intact leaves that in protoplasts and that it may be related to viral cell-to-cell movement. However, in NN tobacco protoplasts, the detection of PMMV-S CP was delayed 14-18 h. compared to TMV CP, while in L^3L^3 pepper protoplasts, no significant differences in the kinetic of PMMV-S and TMV CPs synthesis, up to 48 h, were observed.

In vitro translation studies of PMMV-S and TMV genomic RNAs showed that the efficiency of translation and the polypeptide products made were equivalents, suggesting that the two RNAs are equally stable and that the milder symptoms and lesser replication of PMMV-S in tobacco plants, probably is not the result of differences in the primary expression of the 5' proximal genes of its genome.

Nucleotide sequence analysis of PMMV-S and PMMV-I CP genes predicts a polypeptide chain of 156 amino acids and reveals a high degree of similarity. Although 27 nucleotide changes were found, only 4 resulted in amino acid changes. If the viral CP play a role in the induction of the hypersensitive response, these changes may be involved in the differential ability of these viruses to overcome the hypersensitive resistance in pepper plants.

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Analysis of the Mechanism of Coat Protein-Mediated Protection in Transgenic Plants Expressing the Potato Virus X (PVX) Coat Protein (CP) Gene

Cynthia Hemenway, Jim Weiss, Jenny Lodge, Carl Braun, Cliff Lawson, Wojciech Kaniewski, and Nilgun Tumer

Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

Protection against PVX infection has been demonstrated in transgenic tobacco (1) and potato plants that express the PVX CP gene. In addition, potato plants expressing both the PVX and the potato virus Y (PVY) coat protein genes have shown good protection in both greenhouse (2) and field test situations. Experiments are also underway to analyze protection against both PVX and PVX RNA in protoplasts derived from transgenic and control plants, specifically in terms of virus replication.

To analyze further the mechanism of coat protein-mediated protection and to understand the molecular events during PVX infection, a full-length cDNA for PVX has been isolated. This cDNA clone has been used to generate transcripts in vitro that are infectious on both local lesion and systemic hosts (3). We have recently improved the infectibility of these transcripts by reducing the number of extraneous nucleotides at the 3' end (downstream of the polyA tail) from 9 to 4 nucleotides. Current experiments at the whole plant and protoplast level are focused on analyzing the functions of the various open reading frames encoded by the PVX genome. Specifically, we are investigating which open reading frames encode proteins involved in replication and movement.

1. Hemenway, C., Fang, R.X., Kaniewski, W.K., Chua, N.-H., and Tumer, N.E. (1988) Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. The EMBO J., **7**, 1273-1280.
2. Lawson, C., Kaniewski, W., Haley, L., Rozman, R., Newell, C., Sanders, P., and Tumer, N.E. (1990) Engineering resistance to mixed virus infection in a commercial potato cultivar: resistance to potato virus X and potato virus Y in transgenic Russet Burbank. Bio/Technology, **8**, 127-134.
3. Hemenway, C., Weiss, J., O'Connell, K., and Tumer, N.E. (1990) Characterization of infectious transcripts from a potato virus X cDNA clone. Virology, **175**, 365-371.

Sugarbeet and melon transformation for virus resistance

M. DE BOTH, P. PEREZ, D. GERENTES, J. KALLERHOFF, J. PERRET, S. BEN TAHAR

Genetic transformation of crop plants allows the introduction of novel characters such as virus resistance, provided that an efficient method for the regeneration of whole plant is available.

A regeneration system for melon and sugarbeet through organogenesis was developed, on different tissus (1), (2).

By using *Agrobacterium* mediated transformation, transgenic plants expressing ß-glucuronidase (3) have been obtained, this expression was also found in the R1 progeny of transgenic melon. The method is currently being applied to the introduction of Cucumber Mosaic Virus coat protein genes (4) in order to obtain cross-protection against this virus in melon.

To obtain sugarbeet variety resistant to rhizomania. Potential resistance genes were constructed from BNYVV sequences such as the capsid protein and antisens RNA. BNYVV coat protein mediates protection in sugarbeet protoplasts (5).

This communication will focus on the present state of the art.

(1) - JEFFERSON R.A. (1987) - Plant Molecular Biology Reporter 5 : 387-405

(2) - PEREZ P., GERENTES D., KALLERHOFF J., HOSEMANS D., BEN TAHAR S. and PERRET J. (1990) - 53rd Winter Congress IIRB, Brussels 14-15 February. In press.

(3) BEN TAHAR S., DE BOTH M. and THION L. (1989) - Proceedings cucurbitaceae 89. Evaluation and enhancement of cucurbit germplasm. Charleston pp 21-17.

(4) - NOEL M.J.T. and BEN TAHAR S. (1989) - Nucl. Acid. Res. 17 : 10492.

(5) - KALLERHOFF J., PEREZ P., BOUZOUBAA S., BEN TAHAR S. and PERRET J. (1990) - submitted in Plant Cell Reports.

Is pepper-Potato Virus Y actually Potato Virus Y?

M.J. Soto[1], R. Sánchez-Monge[2], G. Salcedo[2], A. Fereres[1], F. Ponz[1].
1: Dpto. Protección Vegetal, CIT-INIA, Apartado 8111, 28080 Madrid. Spain
2: Dpto. Bioquímica, ETSI Agrónomos, Universidad Politécnica, Ciudad Universitaria s/n. 28040 Madrid. Spain

Potato virus Y (PVY) is the type member of the potyvirus group of plant viruses. It is a widespread virus infecting important Solanaceous crops, such as potato, pepper, tomato, or tobacco (1). Strains that have received more attention by researchers are the ones naturally infecting potato, divided in three groups (O, C, and N) based on symptomatology induced in several potato cultivars, whereas strains from other plants have been much less studied at the biological and molecular level. Pepper-infecting strains have been classified in pathotypes (0, 1, and 1-2), according to their ability to overcome certain resistance genes (2). We have undertaken the characterization of pepper-PVY, and its comparison with the better-studied potato strains.

The classification of potyviruses into strains and distinct viruses has recently been revised, taking into consideration information coming from different aspects of their biology and incorporating molecular analysis (3). The results obtained so far by us cast some doubts on the identity of pepper-PVY as real PVY. Host range, serological behaviour using several PVY monoclonal antibodies, and biochemical analysis of the coat protein of this virus do not fit well with conserved traits of well-characterized PVY strains. If further characterization supports our present view, pepper-PVY should be reclassified as a different virus.

REFERENCES

1.- de Bokx, J.A., and Huttinga, H. 1981. CMI/AAB Descriptions of Plant Viruses, No. 242.

2.- Marchoux, G., and Gebre-Selassie, K. 1989. Phytoma, No. 404, pp. 49-52.

3.- Shukla, D.D. and Ward, C.W. 1989. Arch. Virol. **106**, 171-200.

THE MOLECULAR BIOLOGY OF SATELLITE RNA OF CUCUMBER MOSAIC VIRUS

David Baulcombe, The Sainsbury laboratory, Norwich NR2 3HY, U.K.

Satellite RNA of cucumber mosaic virus (CMV) modifies the symptoms of CMV infection either by attenuating the effects of the virus or by inducing the appearance of symptoms that are more severe and distinct from the viral symptoms. The Y satellite RNA of CMV manifests these effects in three ways: there is attenuation of viral effects evidenced by the reduction of viral RNA accumulation and elimination of viral symptoms that cause stunting and affect leaf morphology; superimposed on this viral attenuation there are bright yellow mosaic symptoms on tobacco or systemic necrosis on tomato.

The yellow mosaic symptoms result from a reduction in the amount of chlorophyll in the affected leaves and is paralleled by a reduction in the level of Δ amino laevulinic acid (Δala), which is a precursor of chlorophyll biosynthesis.

The biochemical basis of the tomato necrosis is not known. However there is genetical variation between tomato cultivars and related species. Analysis of this genetical variation will indicate the number and nature of genes affecting the necrotic symptoms and will also permit the isolation of these genes for study at the molecular level.

An extensive mutation analysis of satellite RNA has identified sequences affecting the ability of satellite RNA to modify symptoms. This analysis leads to the following conclusions:
a) yellow mosaic and necrotic symptoms are affected by separate parts of the molecule.
b) satellite RNA-encoded peptides are not involved in symptom induction.
c) the only sequences definitively identified as playing a part in induction of yellow mosaic symptoms are outside a stem-loop structure that has been implicated in that effect.
d) it is likely that structural interactions of the satellite RNA trigger the symptom-induction effects. A "gain of function" mutation strategy will be described that will allow these structural interactions to be studied.

It is clear that although the satellite RNA triggers the novel symptoms in the infected plants, the helper virus also imposes some specificity on the response of the host plant. This helper virus effect is being studied using modified viruses from transcripts of full length cDNA clones.

CUCUMBER MOSAIC VIRUS AND ITS SATELLITE RNAs: LOCALIZATION OF SEQUENCES INVOLVED IN PATHOGENICITY.

Peter Palukaitis and David E. Sleat, Dept. of Plant Pathology, Cornell University, Ithaca, NY USA

The satellite RNAs of cucumber mosaic virus (sat RNAs of CMV) usually alter the pathogenicity induced by their helper virus, CMV. Using RNA transcripts derived from cDNA clones of several satellite RNAs of CMV, we have shown that the domains specifying necrosis and chlorosis in tomato are localized within different halves of the satellite RNA molecule (1). Further mapping of the domain for necrosis induction showed that sequences between nucleotides 292 and 308 are involved in the induction of necrosis. Specifically, site-directed mutagenesis involving three nucleotide substitutions was used to change the phenotype of the WL1-sat RNA from ameliorative to necrogenic on tomato (2). However, while such a mutant satellite RNA could induce necrosis in tomato when supported by helper viruses of CMV subgroup II, necrosis was not induced when the mutant satellite RNA was co-inoculated with CMV strains belonging to subgroup I. Such subgroup specificity is not observed with a naturally necrogenic satellite RNA (3). These results indicate that other sequences in both the satellite RNA and the helper virus are involved in the induction of necrosis in tomato.

Two satellite RNAs were shown to be able to induce chlorosis in tobacco only in the presence of subgroup II CMV strains. Using pseudorecombinants of CMV strains belonging to the two subgroups, the induction of chlorosis was only observed when RNA 2 of subgroup II CMV strains was present, suggesting that some interaction between the satellite RNA and RNA 2 (or its gene product) is involved in the induction of chlorosis in tobacco (4).

An analysis of the nucleotide sequences of two satellite RNAs that induce chlorosis in tobacco and three satellite RNAs that induce chlorosis in tomato suggests that the host specificity for chlorosis is associated with nucleotide 149. Site-directed mutagenesis of this nucleotide in a cDNA clone of a satellite RNA which is chlorogenic on tomato and ameliorative on tobacco, from a U to a C, altered the phenotype of the transcribed satellite RNA to ameliorative on tomato and chlorogenic on tobacco. Further mapping of the the sequences involved in chlorogenicity will be described.

1. Kurath and Palukaitis (1989) Mol. Plant-Microbe Interact. 2: 91-96.
2. Sleat and Palukaitis (1990) Proc. Natl. Acad. Sci. USA 87: (in press).
3. Kurath and Palukaitis (1987) Virology 159: 199-208.
4. Sleat and Palukaitis (1990) Virology 176: (in press).

EVOLUTION AND VARIABILITY OF THE SATELLITE RNA OF CUCUMBER MOSAIC VIRUS

F. García-Arenal, A. Fraile and M.A. Aranda

Depto. de Patología Vegetal, E.T.S.I. Agrónomos, 28040-Madrid, Spain

Cucumber mosaic virus (CMV) satellite RNAs (CMV-sat RNAs) are able to variously modify the symptoms induced by CMV, and it has been shown that some phenotypes may be determined by the nature of 1-3 possitions in the sat RNA molecule. The study of the variation and evolution of CMV-sat RNAs may illustrate traits of their molecule relevant to their replication and pathogenesis, and to their possible use as control agents for CMV.

We have analyzed the potential of CMV-sat RNA to vary by two different approaches. First, the sequence of 24 CMV-sat RNAs were compared, showing a pattern of evolution similar to that described for other RNA genomes: divergence occurs through different evolutionary lines, that co-circulate, and there is no correlation between genetic proximity of strains and their origin. The analysis of variable possitions in the molecule suggest that (functional?) constraints to variation maintain it within narrow limits.

In addition we have compared different sat RNAs collected during an epidemic of CMV on tomato in the spring of 1989. CMV-sat RNAs were characterized by the analysis of mismatches in heteroduplexex formed with transcripts of clones derived from a reference strain. The data show that CMV sat RNA may evolve quickly during a single epidemy: high genetic divergence was shown to occur along a single evolutionary line by mutation accumulation. The effect of the observed structural changes on the biology of the sat RNA has been analyzed.

INTERACTION AMONG STRAINS OF TOMATO ASPERMY VIRUS AND OF CUCUMBER MOSAIC VIRUS SATELLITE RNAS

E. Moriones and A. Fraile

Depto. de Patología Vegetal, E.T.S.I. Agrónomos, 28040-Madrid, Spain

Tomato aspermy virus (TAV) has been reported to support the replication of the satellite RNAs of cucumber mosaic virus (CMV-sat RNAs). Although differences between both interactions have been described, a detailed analysis of the relationship between TAV and CMV-sat RNA has not been reported.

The interaction of six CMV-sat RNAs, differing in their sequence and biology, with two strains of TAV (1-TAV and V-TAV) was studied on tobacco and tomato. CMV-sat RNAs, including a strain necrogenic for tomato, had no effect on the symptoms induced by TAV on tomato; on the other hand they attenuated (including a strain chlorogenic for tobacco) the symptoms induced in tobacco. The analysis of virus-encapsidated RNA shows 1-TAV to be a helper as efficient as CMV, whereas V-TAV always encapsidates a much lesser proportion of sat RNA. This was shown to be due to two different phenomena: while V-TAV supports the replication of some sat RNAs to a lesser degree than 1-TAV, it is as good a helper with other strains of CMV-sat RNA, but encapsidates them less effectively. In none of the helper/sat combinations did the presence of CMV-sat RNA affect the replication of TAV. Thus, the interaction TAV/CMV-sat RNA varies according to the strains of sat RNA and helper, and differs from that described for CMV.

CUCUMBER MOSAIC VIRUS SATELLITE RNA REPLICATION

Didier TOUSCH*, Mireille JACQUEMOND** and Mark TEPFER*

* INRA, Laboratoire de Biologie Cellulaire, 78026 Versailles Cédex, France.
** INRA, Station de Pathologie Végétale, BP 94, 84143 Montfavet Cédex, France.

Cucumber mosaic virus satellite RNA is considered to be a natural parasite of its helper virus : when present in a CMV inoculum, this non-infectious molecule strongly replicates in the plant, leading to an important reduction of viral genomic RNA synthesis. Symptoms developed by almost all host plants are then attenuated.

We have previously shown in transgenic tobacco plants that transcripts based on monomeric forms of CMV satellite RNA are recognized by CMV replicase to produce large quantities of biologically active satellite RNA, and that such genes confer tolerance to CMV (Jacquemond, Amselem and Tepfer, 1988, MPMI 1, 311-316). These results are difficult to reconcile with replication of CMV satellite RNA via a rolling circle mechanism as is the case for the satellite RNA of tobacco ringspot virus (Bruening et al., 1988 in Plant Molecular Biology, NATO ASI series A : Life Sciences, Vol. 140).

We have more recently transferred other genes to tobacco, based on either the necrogenic satellite RNA I17N, or the non-necrogenic satellite RNA R. The corresponding cDNAs were inserted in both orientations, giving rise to genes whose transcripts bear either (+) or (-) sense satellite RNA sequences. On infection with satellite RNA-free I17F CMV strain, plants bearing any of the four genes, I17N(+), I17N(-), R(+) or R(-), produced large quantities of satellite RNA and displayed attenuated symptoms. Whatever the construction transferred, the encapsidated satellite RNA was a (+) sense one and shared the sequence and the biological properties of its corresponding native satellite RNA. These results suggest that the CMV replication complex is equally capable of recognizing (+) or (-) satellite RNA sequences in the precursor transcripts.

If such an hypothesis is correct, intermediate replicative forms, partially double stranded, would be found in a preparation of total RNAs from infected plants. We are currently studying these particular forms using a single stranded probe specific for the additional sequence present at one end of the cDNA transferred which is from the vector used for cDNA cloning (Jacquemond and Lauquin, 1988, BBRC 151, 388-395).

The homopolymeric regions flanking the satellite cDNA ,and thus the satellite RNA precursor transcript, may be essential for recognition by the CMV replicase. Collmer and Kaper (1985, Virology 145, 249-259) have shown that the ds replicative forms of the satellite RNA have an unpaired G residue at the 3' end of the (-) strand. They proposed that (+) strand synthesis is initiated on the adjacent penultimate C residue of the (-) strand. The oligo(dC) tail added at the 5' end of the cDNA would lead, upon replication, to synthesis of an oligo(dG) tract adjacent to the ultimate 3' C of the corresponding (-) strand. In order to study the role of these flanking sequences, we are deleting part or all of the homopolymeric regions.

BIOLOGICALLY ACTIVE IN VITRO TRANSCRIPTS FROM FULL-LENGTH CLONED cDNA TO GENOMIC, DEFECTIVE-INTERFERING AND SATELLITE RNAs OF CYMBIDIUM RINGSPOT TOMBUSVIRUS.

Jòszef Burgyan*, Peter D. Nagy*, Luisa Rubino and Marcello Russo

Dipartimento di Patologia Vegetale, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Italy.

Cymbidium ringspot virus (CyRSV) is a tombusvirus containing singlestranded, positive-sense RNA of 4733 nt. Strategy of expression is based on the formation of two subgenomic RNAs (2118 and 936 nt), which can be translated in vitro to give two proteins of Mr 41,000 (coat protein) and 22,000, respectively. Full-length genomic RNA encodes a protein which is likely to be the viral replicase.

In addition to the subgenomic RNAs, a defective-interfering (DI) and/or satellite RNA are formed. Both molecules are able to attenuate symptoms produced by the helper virus. Sequence analysis of genomic and DI RNAs shows that the 5' and 3' ends are fully conserved. Moreover, most of the stretches entering the composition of DI RNA have a similar start, which is also present at the 5' end of satellite RNA.

Full-length clones of genomic, DI and satellite RNAs were prepared, from which RNA was synthesized. Upon inoculation to test plants, genomic RNA transcript replicated inducing the typical symptoms of CyRSV infections. DI and satellite RNA transcripts replicated only in the presence of the helper virus genome.

DI RNA is not formed in plants inoculated with genomic RNA; however, after three passages, DI RNA is formed de novo. Molecules of different size are formed, the larger of which are precursors of the smaller, stable molecules.

*Permanent address: Research Institute for Plant Protection, P.O. Bow 102, H-1525 Budapest, Hungary.

SELF-CLEAVAGE AND CIRCULARIZATION REACTIONS AND THE REPLICATION OF THE SATELLITE RNA OF TOBACCO RINGSPOT VIRUS

Hans van Tol, Jamal M. Buzayan, Boni K.G. Passmore, and George Bruening, Department of Plant Pathology, University of California, Davis, CA 95616.

Tobacco ringspot virus (TobRV) supports the replication of a satellite RNA (sTobRV RNA). The positive polarity form of this RNA, designated as sTobRV(+)RNA), is polarity that is most abundantly encapsidated in TobRV capsid protein. The predominant encapsided forms are the linear monomer, of 359nt, and, in lesser amounts, linear multimers of this sequence. Both polarities of sTobRV RNA appear as monomeric, multimeric, and circular forms in infected tissue, which is consistent with rolling circle models for sTobRV RNA replication. The expected first step in rolling circle replication is the circularization of linear sTobRV(+)RNA. Although linear sTobRV(+)RNA spontaneously circularizes to only a very limited extent in vitro, the circular and linear forms of this molecule are roughly equally abundant in extracts of infected tissue. In contrast, monomeric sTobRV(-)RNA efficiently circularizes in vitro. Thus this ligation reaction is a candidate step in the sTobRV RNA replication cycle. Research from this laboratory has identified a four base pair stem which is located within the sequences that are required for the sTobRV(-)RNA ligation reaction in vitro. The central two base pairs of the stem were modified by substituting two bases in one strand and, in another construction, in the other strand. When both these pairs of mutations were introduced, the resulting double-mutant is expected to have the two central base pairs restored. Neither of the two-base, singly-mutated forms of sTobRV(+)RNA replicated when co-inoculated with TobRV, and each corresponding sTobRV(-)RNA spontaneously circularized to only a very limited extent. In contrast, co-inoculated, doubly-mutated sTobRV RNA increased, apparently as efficiently as the wildtype satellite RNA. The doubly-mutated sTobRV(-)RNA also circularized as efficiently as the wildtype RNA. These results suggest that spontaneous circularization of sTobRV(-)RNA is necessary for the biological activity of the satellite RNA.

Identification and Expression of the Particle Proteins of Potato Leafroll Virus

M.A. Mayo, Scottish Crop Research Institute
Invergowrie, Dundee DD2 5DA, UK

Like other luteoviruses (1), potato leafroll virus (PLRV) has particles that contain Mr 23000 coat protein and a 6kb RNA, and that are transmitted by aphids in a circulative, probably non-propagative, fashion (2). Recently published sequences (3,4,5) have shown that the PLRV genome contains two regions of coding sequence, each containing 3 open reading frames (ORF), that are separated by a 200 nt non-coding sequence. The products of in vitro translation correspond in size to those encoded by the 5' group of ORFs; the putative RNA polymerase is encoded by the most 3' ORF of this group. A relatively abundant sub-genomic RNA, which is 3' co-terminal with the genome RNA, is present in infected cells; presumably it is the mRNA for proteins encoded by the 3' group of ORFs. Although the 200 nt non-coding region that is 5' of the coat protein gene has some sequence features of sub-genomic RNA promoters, the size estimated for the sub-genomic RNA suggests that its 5' end is within the RNA polymerase gene.

PLRV coat protein is encoded by the 5'-most of the 3' group of ORFs and is followed in-frame by an ORF for a Mr 56000 protein (P5). Indirect evidence suggests that this ORF is expressed by translational read-through of the amber termination codon of the coat protein gene (3,4). Immunoblotting with a monoclonal antibody to coat protein and an antiserum raised against a fusion protein containing part of P5 has shown that a read-through protein, which comprises coat protein joined to P5, is made in infected cells. Virus particles purified by the standard method contain a shorter, Mr 53000 read-through protein, but particles isolated rapidly from infected protoplasts contain some larger and some full-length read-through protein molecules. Possibly the read-through protein is partially degraded during routine virus purification.

Strain V of PLRV is distinct from most isolates in being poorly aphid-transmissible and in failing to react with two of a panel of ten monoclonal antibodies to PLRV particles (6). However, the amino acid sequence of PLRV-V coat protein differs from those of coat proteins of aphid-transmissible isolates by only one conservative change. It may therefore be that when incorporated into virus particles, the P5 part of the read-through protein plays a key role in the transmission of the PLRV particles by aphids. Further work is needed to characterize the P5 of PLRV-V.

1. Waterhouse, P.M., Gildow, F.E. & Johnstone, G.R. (1988). AAB Descriptions of Plant Viruses No. 339.

2. Harrison, B.D. (1984). CMI/AAB Descriptions of Plant Viruses No. 291.

3. Mayo, M.A., Robinson, D.J., Jolly, C.A. & Hyman, L. (1989). J. Gen. Virol. 70, 1037-1051.

4. Van der Wilk, F., Huisman, M.J., Cornelissen, B.J.C., Huttinga, H. & Goldbach, R. (1989). FEBS Letters 245, 51-56.

5. Keese, P., Martin, R.R., Kawchuk, L.M. & Gerlach, W.L. (1990). J. Gen. Virol. 71, 719-724.

6. Massalski, P.R. & Harrison, B.D. (1987). J. Gen. Virol. 68, 1813-1821.

MOLECULAR BIOLOGY OF PLANT REOVIRUSES

Donald L. Nuss, Dept. of Mol. Oncol. & Virology, Roche Inst. of Mol. Biology, Roche Research Center, Nutley, NJ 07110 USA

Plant-infecting members of the family *Reoviridae* are divided into two genera, *Phytoreovirus* and *Fijivirus*. Members of the genus *Phytoreovirus* have genomes consisting of 12 segments of double-stranded (ds)RNA with a total molecular weight of approximately 16 x 10^6, and are transmitted by leafhoppers. In comparison, members of the genus *Fijivirus* have genomes consisting of 10 segments and are transmitted by planthoppers. As a group, these viruses have considerable potential as experimental systems with which to study a range of biological and molecular processes. The fact that they replicate both in plant hosts and in their insect vectors, coupled with the availability of cultured cell lines derived from the insect vector, makes these viruses particularly well suited for examining the intimate nature of virus-insect vector-plant host interactions. Infection of the insect vector is noncytopathic and persistent, while infection of the plant host is tissue specific and results in numerous symptoms, including neoplasia. Consequently, these viruses also provide opportunities for examining the molecular basis of viral persistence, cytopathology, and disease symptom expression in both the plant and animal kingdoms. Furthermore, characterization of defective interfering RNAs associated with one plant reovirus has revealed a number of basic principles involved in the sorting and packaging of segmented RNA genomes.

Plant reoviruses also have several limitations as experimental systems. They do not cause local lesions on their plant hosts or plaques on vector cell monolayers. As a result, they are not amenable to the genetic reassortment analysis so successfully applied to the study of vertebrate reoviruses. In addition, the complexity of plant reovirus genomes makes initiation of infection with synthetic transcripts a formidable task. Therefore, efforts to understand the functional and structural properties of plant reovirus genomic segments and their encoded gene products have, so far, relied on the *in vitro* expression and sequence analysis of cDNA copies of genomic segments. This approach has provided considerable information regarding the genomic organization and coding strategy for two members of the genus *Phytoreovirus*, wound tumor virus (WTV) and rice dwarf virus (RDV), and is beginning to yield similar information for a member of the genus *Fijivirus*; maize rough dwarf virus (MRDV). Comparative sequence analysis has already revealed that several members of the genus *Phytoreovirus* share sequence similarity with each other, but not with MRDV, thus validating the existing plant reovirus classification scheme. Sequence comparisons between WTV and RDV are expected to be particularly informative since these viruses, although related at the nucleotide and amino acid level, differ in plant host range, tissue specificity, vector range and disease symptom expression.

MOLECULAR ORGANIZATION OF CRYPTIC VIRUS dsRNA GENOMES

G. BOCCARDO[1], T. CANDRESSE[2], P. PALUKAITIS[3], D.D. DUNIGAN[3], M. d'AQUILIO[1] and J. DUNEZ[2].

Istituto di Fitovirologia Applicata del C.N.R., strada delle Cacce 73, 10135 Torino, Italy (1); Station de Pathologie Végétale, INRA BP 81, 33883 Villenave d'Ornon, France (2) and Department of Plant Pathology, Cornell University, Ithaca, New York 14854, USA (3).

Cryptic viruses (1) are a group of plant viruses sharing some characteristics with mycoviruses belonging to the Partitiviridae family (2), noticeably in having an RNA-dependent RNA polymerase activity (replicase) within the particles (3) and apparently coding for one product for each genome segment (4).

RNA fingerprinting analysis of isolated dsRNA genome segments has revealed that little or no nucleotide sequence homology exists between the segments constituting the genomes of white clover cryptic virus 1 and 2 (WCCV 1, WCCV 2), representing the two subgroups within the cryptic viruses. Wandering spot analysis on isolated dsRNA segments from WCCV 1 has shown that these are polyadenylated and share at both terminal regions a limited stretch of nucleotides in common. Analysis of the nearly full-length cloned cDNAs for each of the dsRNA segments of WCCV 1 genome confirmed the absence of major nucleotide sequence homology between them and revealed that the poly(A) homopolymer is located at the 3' terminus of each coding strand. *In vitro* transcription and translation of the cloned cDNAs showed that each RNA contains a single open reading frame accounting for almost the complete coding potentiality, the smaller RNA encoding the coat protein (M_r 52 KDa), and the larger a protein (M_r 65 kDa), likely to represent the viral replicase. *In vitro* translation of native dsRNA cryptic virus genomes showed that these dsRNA possess messenger activity and that each segment encodes for one product, as reported for other cryptic viruses (4).

(1) Boccardo *et al.*(1987). Adv. Virus Res. 22, 171-214.
(2) Brown, F. (1989), Intervirology 80, 181-186.
(3) Boccardo, G., & Accotto, G.P. (1988). Virology 163, 413-419.
(4) Accotto *et al.*(1990) J. Gen. Virol. 71, 433-437.

7S SRP RNA: THE POSSIBLE CELLULAR TARGET FOR POTATO SPINDLE TUBER VIROID-(PSTVd)RELATED VIROIDS.

Heinz L. Sänger and Bernd Haas, Max-Planck-Institut für Biochemie, Abteilung Viroidforschung, D-8033 Martinsried bei München, F.R.G.

In search for possible viroid-specific cellular targets we found (1) that the 299 nucleotides long 7S RNA from leaf tissue of tomato is structurally very similar to the 7SL SRP RNA from mammals (2) which suggests similar functions for both RNAs. Under this premiss the plant 7S RNA is an integral part of the signal recognition particles (SRP) which are responsible for the intracellular translocation of distinct proteins including membrane proteins (2). Sequence comparison has revealed five contiguous regions in the tomato leaf 7S SRP RNA which could theoretically form a stable hybrid with corresponding sequences of PSTVd (1,3). In the rod-like viroid molecule these regions include the lower strand of the domain which has previously been considered to be responsible for modulating viroid pathogenicity (4). In case such viroid-7S SRP RNA interaction occurs in vivo, it could interfere with the biological function of the SRP-mediated protein translocation and thus cause disease. This would not only explain the cytopathic abnormalities of cell wall structures but also the macroscopic symptoms observed in viroid-infected plants.

Since we could, so far, not achieve direct evidence that the proposed viroid-7S SRP RNA interaction actually accurs in vivo, we attempted to obtain further indirect support for our concept. Therefore, we sequenced the 7S (SRP) RNAs from additional viroid host plants. In all the viroid-host plant combinations compared so far, we found a similar sequence complementarity between the corresponding viroids and 7S SRP RNAs similar to that between PSTVd and tomato 7S SRP RNA. Thus the basic structural precondition for a possible interaction between viroids and the 7S SRP RNA as the relevant host target are fullfilled. In addition, the differences in virulence of certain PSTVd isolates can also be explained by this molecular interaction.

Despite of their different sequence, all PSTVd isolates can theoretically form with the 7S SRP RNA a nearly identical complex whose thermodynamic stability is very similar. But a correlation can be made between the virulence of the PSTVd isolates and the melting behaviour of the region considered to be involved in their pathogenic interaction with the host target.

Computer simulation has revealed that with increasing virulence of the PSTVd isolates, the intramolecular thermal stability of a distinct part within the complex-forming region becomes increasingly destabilized and hence increasingly accessable for a possible interaction with the cellular target. Thus it can now be assumed that the severity of the disease as caused by the various PSTVd isolates can be determined thermodynamically and kinetically i.e. by the rate of complex formation, by the stability of the complex, and by the number of the resulting complexes.

1. Haas et al. (1988) EMBO J. 7, 4063-4074.
2. Siegel, V. & Walter, P. (1988) Cell 52, 39-49.
3. Symons, B. (1989) Nature 338, 542-543.
4. Schnölzer, M. et al. (1985) EMBO J. 4, 2181-2190.

ANALYSIS OF THE ORGANIZATION AND EXPRESSION OF THE GENOME OF APPLE CHLOROTIC LEAF SPOT CLOSTEROVIRUS.

Sylvie German, Thierry Candresse, Moshe Bar Joseph(*), Maryvonne Lanneau and Jean Dunez

Station de Pathologie Végétale, INRA, BP 131, 33140 Pont de la Maye, France and (*) Institute of Plant Protection, ARO, Volcani Center, PO Box 6, 50250 Bet-Dagan, Israel.

Apple chlorotic leaf spot virus (ACLSV), a member of the closterovirus group induces a variety of symptoms on fruit trees (1), including severe graft incompatibility in some *Prunus* combinations, causing important problems in nurseries. ACLSV belongs to the subgroup A of closteroviruses, characterized by a small (ca. 730 nm) particle and genome size.

The viral particles are composed of a single type of protein subunits of 23 kDa encapsidating a single genomic RNA molecule (2). The complete, 7555-nucleotide long sequence of ACLSV genomic RNA has been determined from cloned cDNA. ACLSV RNA is polyadenylated and harbors, from 5' to 3', three overlapping open reading frames (ORFs) coding for proteins of 216, 50 and 28 kDa. It has untranslated regions of 151 (5') and 190 (3') nucleotides.

In vitro translation of the genomic RNA yields a major product of approximately 200 kDa. Analysis of the protein encoded by this ORF reveals homologies with the replication-associated proteins of "alpha-like" plant RNA viruses. The closest homologies are found with the proteins of potex- and carlaviruses. These results show that ACLSV should be regarded as a member of the "alpha-like" superfamily of plant viruses.

Direct sequencing of the coat protein and *in vitro* translation of RNAs obtained by *in vitro* transcription of cloned cDNAs have allowed the mapping of the coat protein gene to the 28 kDa ORF and demonstrate that the 22 kDa coat protein is produced by internal initiation on the second ATG codon of this ORF.

ACLSV infected plants contain five species of dsRNAs molecules. As demonstrated by *in vitro* translation of these denatured dsRNAs, three of these species, of approximately 7.5, 2.5 and 1.2 kbp respectively, probably represent the double stranded forms of the genomic RNA and of subgenomic messenger RNAs for the 50 kDa ORF and for the coat protein gene. Preliminary evidence suggests that, surprisingly, the two other species could be 5' coterminal with the genomic RNA. The precise structure and function of these unusual molecules is currently under investigation.

(1) Lister, R.M. (1970). CMI/AAB Description of Plant Viruses N° 30.
(2) Yoshikawa, N. & Takahashi, T. (1988). J. Gen. Virol. 69, 241-245.

HOMOLOGIES BETWEEN THE GENOMES OF A CARLAVIRUS (LILY SYMPTOMLESS VIRUS) AND A POTEXVIRUS (LILY VIRUS X) FROM LILY

Johan Memelink, Cornelia I.M. van der Vlugt[1], Huub J.M. Linthorst*, Antonius F.L.M. Derks[1], Cees J. Asjes[1] and John F. Bol

Department of Biochemistry, Leiden University, Gorlaeus Laboratories, Einsteinweg 5, 2333 CC Leiden and [1]Bulb Research Centre, Vennestraat 22, P.O. Box 85, 2160 AB Lisse, The Netherlands

cDNA clones complementary to the 3'-terminal regions of the genomic RNAs of the carlavirus lily symptomless virus (LSV) and the potexvirus lily virus X (LVX) have been sequenced. The carlavirus RNA sequence contains five open reading frames (ORFs) coding for proteins of Mr 25374, 11631, 6960, 32041 (coat protein) and 16121, which are very similar in size, amino acid sequence and relative position in the genome to proteins encoded by two different carlaviruses from potato. The first four of these proteins also show considerable amino acid sequence similarity to proteins encoded by RNA of potexviruses, and the relative position of the ORFs on the carlavirus genome strongly resembles that in the potexvirus genomes. The LVX cDNA clone contains three ORFs encoding proteins of Mr 23574, 11767 and 21569 (coat protein). A small ORF immediately 5' of the coat protein ORF that has been found in other potexviruses is not present in the LVX genome. Thus, the data confirm the close taxonomic relationship between carlaviruses and potexviruses and reveal some differences in genome organization among the potexviruses.

Genome organization and expression of Grapevine Fanleaf Virus

M. Fuchs *, M.A. Serghini °, M. Pinck °, C. Ritzenthaler °, F. Hans °, B. Walter *, L. Pinck °

* INRA, Station de Recherches Vigne et Vin, Laboratoire de Pathologie Végétale, 28 rue de Herrlisheim 68021 Colmar, France and ° Institut de Biologie Moléculaire des Plantes du CNRS, Laboratoire de Virologie, 12 rue du général Zimmer, 67084 Strasbourg, France

Grapevine fanleaf virus (GFLV), a member of the nepovirus group, induces one of the most widespread and damaging viral diseases of grapevine. The viral genome consists of two positive single-stranded RNA molecules which are encapsidated in isometric particles. GFLV RNAs are polyadenylated and have a protein covalently linked to their 5' ends. The F13 strain (GFLV-F13) contains an additionnal RNA species (RNA-3) with satellite RNA properties (**1**).

In order to further characterize the genetic organization of the nepoviruses, we determined the complete nucleotide sequence of the GFLV-F13 genome. Sequence alignements allowed tentative assignements of viral functions to be made: RNA-1 (7344 nt) codes for a 253 K polypeptide which has extensive regions of identity with putative replicases, proteases and NTP binding proteins. RNA-2 (3774 nt) encodes a 122 K polypeptide (P2) which shows weak homologies with movement proteins and contains the coat protein (56 K) which was precisely located within the C-terminus of P2. The proteolytic cleavage of the polypetide P2, probably catalysed by a specific protease induced by RNA1, occurs at an Arg/Gly site which is the first reported for plant viruses expressing their genomic RNA by polyprotein synthesis (**2**). These results favor similar genetic organization and expression strategies for GFLV and picorna-like viruses.

RNA-3 (1114 nt) codes a 37 K protein (P3) which has strong homologies with the large satellite RNA associated with arabis mosaic virus (ArMV), but very limited resemblance with tomato black ring virus satellite RNAs (**3**). Full-length cDNA clones of the GFLV satellite RNA were transcribed *in vitro* by T3 polymerase. The biological activity of these transcripts was demonstrated by co-inoculation on *C. quinoa* with the genomic RNAs of either GFLV or ArMV two strains naturally devoid of a satellite RNA. Either of these genomic RNAs could act as helper for satellite RNA replication. The influence of the satellite RNA on the severity of symptoms is now under investigation.

Considering all these data, genetically engineered resistance based on the expression either of the coat protein or the satellite RNA are possible strategies to control the fanleaf degeneration of grapevines.

References

1. Pinck, L., Fuchs, M., Pinck, M., Ravelonandro, M. and Walter, B. 1988. A satellite RNA in grapevine fanleaf virus strain F13. J. gen. Virol. 69, 233-239.
2. Serghini, M.A., Fuchs, M., Pinck, M., Reinbolt, J., Walter, B. and Pinck, L. 1990. RNA2 of grapevine fanleaf virus: sequence analysis and coat protein location. J. gen. Virol. (in press).
3. Fuchs, M., Pinck, M., Serghini, M.A., Ravelonandro, M., Walter, B. and Pinck, L. 1989. The nucleotide sequence of satellite RNA in grapevine fanleaf virus, strain F13. J. gen. Virol. 70, 955-962.

POSTER PRESENTATIONS

LEAKY AMBER CODONS OF PLANT VIRAL mRNAs: ANALYSIS OF THE CODON CONTEXT REQUIREMENTS FOR READTHROUGH

Geert Angenon, Johan Botterman*, Marc Van Montagu, and Ann Depicker

*Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium); * Plant Genetic Systems N.V., Plateaustraat 22, B-9000 Gent (Belgium)*

One of the expression strategies used by RNA viruses is the partial suppression of a stop codon (usually the UAG amber codon). This allows viruses to make two proteins out of a limited amount of genetic information: the "normal" gene product which ends at the stop codon and a readthrough protein. For tobacco mosaic virus (TMV), it has been shown that both products (replicase subunits) are made *in vivo* (Beier *et al.*, 1984; Saito *et al.*, 1986) and are necessary for an effective replication of the virus (Ishikawa *et al.*, 1986). Based on the genomic nucleotide sequence, it is highly likely that the same strategy is used by a range of other RNA viruses (BNYVV, CarMV, TNV, TCV, MCMV, BYDV, BWYV, PLV, CNV, CRV, TYMV, TRV, and PEBV). The nucleotides surrounding the suppressed amber codons (the codon context) shows a certain similarity. For some viruses a stretch of homology can be detected (e.g. in TMV, turnip yellow mosaic virus, and beet necrotic yellow vein virus the context is CAAUAGCAA). The only feature which all these viruses have in common is two adenosines preceding the amber codon.

We examined the possibility that the amber codons present in these viral RNAs can only be suppressed if they are embedded in a specific codon context. We constructed plant expression vectors containing the *bar* gene (encoding phosphinothricin acetyltransferase (PAT)) without stop codon followed by an neomycin phosphotransferase II (*nptII*) gene without a start codon. The two marker genes are linked by oligonucleotides containing a TAG stop codon in different codon contexts. Suppression of the amber codon is monitored via the detection of a PAT-NPTII fusion protein. The constructions were introduced in a monocotyledonous and two dicotyledonous plants (*Oryza sativa*, *Nicotiana tabacum*, and *Arabidopsis thaliana*) via electroporation of protoplasts or *Agrobacterium*-mediated transformation.

Results will be presented which show that suppression of an amber codon is indeed codon context dependent. An amber codon in the TMV context (CAAUAGCAA) is suppressed with an efficiency of 10%, whereas no suppression could be detected with several constructions in which some nucleotides of the TMV context were changed.

Also, a statistical analysis of the nucleotides surrounding the amber stop codon in published plant gene sequences was made. This shows that the nucleotide distribution is not random and that the nucleotides of the TMV context are generally avoided.

References

Beier, H., Barciszewska, M., and Sickinger, H.-D. (1984). EMBO J. 3, 1091-1096.

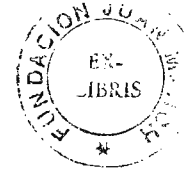
Ishikawa, M., Meshi, T., Motoyoshi, F., Takamatsu, N., and Okada, Y. (1986). Nucl. Acids Res. 14, 8291-8305.

Saito, T., Watanabe, Y., Meshi, T., and Okada, Y. (1986). Mol. Gen. Genet. 205, 82-89.

CLONING OF POTATO VIRUS Y AND POTATO LEAFROLL VIRUS COAT PROTEIN GENES

Vera Thole, Tamás Dalmay, József Burgyán and Ervin Balázs

Agricultural Biotechnology Center Gödöllő H-2101 P.O.Box. 170. HUNGARY



Recent advances in the genetic engineering enable the researcher to produce transgenic plants. Coat protein mediated cross protection was shown to be very effective in different host virus combinations. To produce virus resistant potato we have cloned an altered virulence strain of potato virus Y (PVY) and an isolate of potato leafroll virus (PLRV). Cloning strategy for the coat protein genes of the two virus was different. In the case of PLRV we have used two synthetised oligonucleotides (21nt each) as primers. Sequences for the oligonucleotides were taken from the known sequences (Prill et al. 1989) Cloning of PVY coat protein gene was performed with oligo dT priming. Viral specific cDNAs were cloned into pUC 18. Sequence analysis showed 4-5 % differences compared to the published sequences. Further cloning into Agrobacterium vector are in progress to produce transgenic plants containing both coat protein genes.

SOME MOLECULAR FEATURES OF THE WALNUT STRAIN OF CHERRY LEAFROLL VIRUS.

M. J. Borja[1], A. Rowhani[2], D. Alexander[3], F. Sanchez[1], A. Frias[1], G. Bruening[2] and F.Ponz[1]
1: Dpto. Protección Vegetal, CIT-INIA, Apartado 8111, 28080 Madrid. SPAIN.
2: Dpt. Plant Pathology, U.C. Davis, Davis, CA 95616. USA.
3: Calgene Inc., 1920 Fifth St., Davis, CA 95616. USA.

Cherry Leaf Roll Virus (CLRV) is a nepovirus (1). The walnut strain of CLRV is the causal agent of a lethal disease of English walnuts known as blackline (2). Viral cDNA was cloned and two plasmids were selected that had different restriction maps: pCLRV5.2 and pCLRV6.0 . Although the differences between them suggested they correspond to different RNAs, Northern analysis showed cross-hybridization of both cDNAs with both viral RNAs, even under very restringent hybridization conditions. Southern analysis of different restriction fragments from both clones revealed the existence of an extensive homology region, approximately 2 Kb long, between both RNAs, at the 3' ends . The partial nucleotide sequence of this region reveals it is a non-coding one preceding the poly (A) tail. The assignation of the cDNA clones to the viral components was performed by Northern analysis using plasmids carrying inserts in which the homology region had been deleted.

We have previously described the restriction of CLRV by the satellite RNA of tobacco ringspot virus (sTobRV RNA), another nepovirus, both in herbaceous hosts and in walnuts (3&4). We have also described that sTobRV RNA inhibits the in vitro translation of CLRV genomic RNAs (3). The possible direct interaction between CLRV RNAs and sTobRV RNA has been investigated. We have found cross-hybridization between the viral RNAs and the heterologous satellite.

REFERENCES

(1) A.T. Jones. 1985. CMI/AAB Descriptions of Plant Viruses CLRV nº 306.

(2) A.Rowhani & S.M.Mircetich.1988. Phytopathology78, 817.

(3) F. Ponz, A. Rowhani, S.M. Mircetich & G. Bruening. 1987. Virology 160, 183.

(4) A. Rowhani, F. Ponz, S.M. Mircetich & G. Bruening. 1988. Annual Meeting of the American Phytopathological Society, San Diego, California, USA.

CHARACTERIZATION OF THE BEAN YELLOW MOSAIC VIRUS GENOME

Kit Boye, Poul Erik Jensen and Knud Henningsen, Department of Genetics, Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Copenhagen F, Denmark.

Bean Yellow Mosaic Virus (BYMV) is a member of the potyvirus group. Potyviruses have filamentous virions containing a single RNA species approximately 9500 nucleotides in length with a VPg covalently attached to the 5' end (1). The RNA encodes one open reading frame translated into a large precursor polyprotein. The polyprotein is cleaved after translation to release the mature viral proteins (2). The BYMV genome expresses at least five proteins; a helper component, a cytoplasmic inclusion protein, a coat protein (CP), a proteinase and a putative RNA-dependent RNA polymerase polymerase (3).

Our work is concentrated on studies of the BYMV RNA replication. Genomic BYMV RNA has been purified and used in cDNA synthesis. The cDNA has been cloned in pUC vectors. The genes encoding a possible VPg, the CP and the RNA polymerase has been sequenced. The BYMV polymerase gene has been inserted into vectors for expression in *E. coli* together with the BYMV proteinase gene. Using the BYMV proteinase enzyme to cleave the polymerase from the precursor protein a native polymerase enzyme should be obtained (4).

The BYMV polymerase produced in bacteria can be used in the development of a polymerase *in vitro* assay system (4). Such a system would be very usefull in characterizing the polymerase enzyme and in studying the effects of *in vitro* mutagenesis of the gene. A polymerase able to bind to the BYMV RNA but ineffective in transcribing it could possibly suggest a new approach for obtaining virus resistant plants.

(1): V. Hari (1981): Virology *112*, 391-9.
(2): J.C. Carrington and W.G. Dougherty (1987): J. Virol. *61*, 2540-8.
(3): C.-A. Chang *et al*. (1988): J. Gen. Virol. *69*, 1117-22.
(4): M.A. Rothstein *et al*. (1988): Virology *164*, 301-8.

INTERFERENCE WITH TURNIP YELLOW MOSAIC VIRUS REPLICATION BY GENOME-LIKE FRAGMENTS AND ENGINEERED DEFECTIVE INTERFERING RNAs.

Jean-Christophe Boyer[1], Bruno Zaccomer[2], Marie-Dominique Morch[1], Mark Tepfer[2] and Anne-Lise Haenni[1].

1- Laboratoire des Systèmes Végétaux, Institut Jacques Monod*, 2 place Jussieu 75251 Paris, France.
2- Laboratoire de Biologie Cellulaire, CNRA, route de Saint-Cyr, 78000 Versailles, France.

Turnip Yellow Mosaic Virus (TYMV) is the type member of the Tymoviruses. Its monopartite genome is a 6318 nucleotide long positive-stranded RNA. It was previously shown in our laboratory that TYMV RNA replication can be inhibited by 90% when transcripts from cDNA clones corresponding to the 3' end of the genomic RNA are introduced in an *in vitro* replication assay (this is referred to as the "sense RNA approach"[1]). Along the same line, we have constructed Defective Interfering (DI) RNAs encompassing different combinations of binding sites for the TYMV replicase. We have analysed the behaviour of both genome-like fragments and engineered DI genomes when used as simple templates for the replicase on one hand, and as inhibitory competitors for the binding of this enzyme in the presence of the genomic RNA on the other hand.

1-Genome-like fragments

All the transcripts encompassing at least one binding site for the viral replicase give rise to replication products *in vitro*.

Both "sense" and "antisense" RNAs directed against the 3' end of the genomic RNA appear to be efficient inhibitors of viral replication *in vitro*: however, while a 90% inhibition can be achieved for a 100/1 molar ratio between an antisense transcript and TYMV genomic RNA, the "sense" approach seems more efficient than the previous one for lower molar ratios.

2-DI RNAs

Due to the orientation of their binding sites for the replicase, amplification of the DI transcripts was expected through *in vitro* replication. However, while DI RNAs clearly inhibit TYMV RNA replication *in vitro*, they do not seem to be more powerful inhibitors than sense or antisense RNAs. Moreover, the migration pattern of the DI RNA replication products together with their Northern blot analysis strongly suggests that there is very little or no amplification of DI RNAs *in vitro*. This observation seems to reveal the limitations of this *in vitro* system and makes its *in vivo* transposition necessary.

Therefore, we are currently developing a transient expression system in rapeseed protoplasts that we will co-transfect with TYMV RNA and the various transcripts.

Reference:
1. M.D. Morch, R.L. Joshi, T.M. Denial, A.L. Haenni (1987) Nucl Acids Res.15: 4123-4130.

*The "Institut Jacques Monod" is an "Institut Mixte, CNRS-Université Paris VII".

A FIRST PHYLOGENY OF VIROIDS AND VIROID-LIKE SATELLITE RNAs.

Joaquin Dopazo, Santiago F. Elena, Ricardo Flores[1] and Andrés Moya.
Departament de Genètica y Servei de Bioinformàtica, Universitat de Valencia.
[1] Instituto de Agroquímica y Tecnología de los Alimentos. CSIC. Valencia. SPAIN.

Since its discovery, the molecular biology and pathology of viroids have been amply studied. Several hypothesis suggested that viroids could stem from pre-existent cellular RNAs or from viruses that had suffered a degenerative process. More recently, with the discovery that certain RNAs possess catalytic properties, the idea that RNA preceded DNA as a carrier of genetic information have gained supporters. This has lead to the proposal of an alternative hypothesis for the origin of the viroids by Diener (1989), who has suggested that viroids as well as certain small plant pathogenic RNAs (viroid-like satellite RNAs) may represent derived molecular fossils from the primitive RNA world.

We report in the present communication a phylogenetic reconstruction of twenty viroids and viroid-like satellite RNAs. To evaluate the monophyletic character as well as the true tree structure of the derived phylogeny we have follow two different approaches: bootstrap and statistical geometry. The results clearly differentiate five groups of viroids that we named *Avoviroids* (ASBVd), *Potaviroids* (PSTVd, TPMVd, CLVd, CEVd, TASVd and CSVd), *Cocoviroids* (CCCVd, HLVd and CTIVd), *Appleviroids* (ASSVd, GYSVd and GV1Bd) and *Hopviroids* (HSVd) in addition to satellite RNAs. Branches connecting these groups are almost what is expected for a monophyletic group in a bootstrap resampling, and the monophyletic relationships among these groups are clearly confirmed by the statistical geometry.

Diener, T.O. (1989). *Proc.Natl.Acad.Sci.USA*. 86:9370-9374.

PROPERTIES OF A NUCLEAR SYSTEM FROM GYNURA AURANTIACA INFECTED BY CITRUS EXOCORTIS VIROID SUITABLE FOR STUDYING VIROID REPLICATION IN VITRO.

R. Flores and J.F. Marcos. Unidad de Biología Molecular y Celular de Plantas. IATA (CSIC). Calle Jaime Roig 11, 46010 Valencia, Spain.

An RNA polymerase activity was detected in nuclei-rich fractions from Gynura aurantiaca infected by citrus exocortis viroid (CEV). This polymerase catalyzed the in vitro synthesis of several RNAs, shown to be viroid-specific since they were absent in control experiments with healthy plants and molecular hybridization analysis demonstrated that they contained CEV-specific sequences most of which were of the same polarity as the viroid RNA. The nature of the viroid-specific RNA species was studied by chromatography on non-ionic cellulose, digestion with RNase under low and high ionic strength conditions, and analysis by polyacrylamide gel electrophoresis in non-denaturing and denaturing systems. The results indicated that these in vitro synthesized RNAs contain unit and longer-than-unit linear viroid strands forming multistranded complexes with single- and double- stranded regions; they have therefore, the same structural properties deduced for the RNAs synthesized in vivo isolated from viroid-infected tissues, which are the presumed replicative intermediates of the rolling circle mechanism proposed for viroid replication. The synthesis of the CEV-specific RNA species was very much reduced in the presence of 1μM-α-amanitin, suggesting the involvement in this process of an RNA polymerase II-like enzyme acting on an RNA template. A soluble fraction containing the polymerase-template complex responsible for the synthesis of the CEV-specific RNAs, was isolated by treatment of the nuclei-rich preparations with heparin and DNase. Preliminar experiments to characterize this complex by gel exclusion chromatography and density gradient centrifugation showed that it has a size clearly bigger than the CEV RNA.

DOMAIN OF THE CUCUMBER MOSAIC VIRUS SATELLITE-RNA SEQUENCE INVOLVED IN THE NECROTIC RESPONSE OF INFECTED TOMATO PLANTS

Mireille Jacquemond*, Didier TOUSCH** and Mark TEPFER**

* INRA, Station de Pathologie Végétale, BP 94, 84143 Montfavet Cédex, France.
** INRA, Laboratoire de Biologie Cellulaire, 78026 Versailles Cédex, France.

Cucumber mosaic virus (CMV) strains bearing a satellite RNA are attenuated on almost all host plants. The major exception to this rule is specific to tomato, where certain satellite RNAs also attenuate symptom gravity whereas others modify symptoms of viral infection, leading in some cases to a spectacular lethal necrosis. Nonetheless, these necrogenic satellite RNAs have typical attenuation properties on essentially all other host plants.

The two satellite RNAs studied, I17N and R, are the most closely related members of the necrogenic and non-necrogenic groups, differing by only four substitutions and a single-base deletion (Jacquemond and Lauquin, 1988, BBRC, 151, 388-395). In order to determine which differences are responsible for the necrotic response, we have first created recombinants between I17N and R satellite-cDNAs using restriction sites located at positions 152 (Sca I) or 256 (Hinf I). The biological properties of the *in vitro* RNA transcripts have been tested by co-inoculation with the satellite RNA-free strain I17F on young tomato plants. The results show that only the two variations located towards the 3' end of the molecule (a A/C substitution at position 295 and the deletion of a C at position 326 when comparing R to I17N) are responsible for its necrogenic capacity.

In a second step we have modified specific bases by site-directed mutagenesis in the sequence of a non-necrogenic cDNA differing from the original necrogenic I17N only at positions 295 and 326. Insertion of a C at position 326 does not seem to affect its attenuation properties (confirmation of this requires sequencing of progeny RNA). Site-directed mutagenesis at position 295 - still unsuccessfull under conditions where mutagenesis at position 326 was readily achieved - will allow us to determine if a single change is sufficient to determine the necrogenic response.

ISOLATION OF RECOMBINANT DNA CLONES ENCODING HYDROLASES OF TOBACCO

H.J.M. Linthorst, L.C. van Loon[1], J. van Roekel[2], C. van Rossum, B.J.C. Cornelissen[2] and J.F. Bol, Biochemistry Department, Leiden University, Leiden; [1]Department of Plant Physiology, Agricultural University, Wageningen and [2]Mogen International NV, Leiden, The Netherlands.

Infection of Samsun NN tobacco with tobacco mosaic virus results in the induced synthesis of so-called pathogenesis-related (PR-) proteins. cDNA clones from mRNA for PR-proteins P/Q (extracellular chitinase) and intracellular chitinase were isolated using a differential hybridization technique, and sequenced. The basic chitinase cDNA clone shows an open reading frame coding for a protein which is very homologous to a intracellular chitinase from tobacco (Shinshi et al., 1987). In the deduced amino acid sequence of the extracellular chitinase a 23 residues long signal peptide is present followed by a 231 amino acid residues long mature protein. This protein is homologous to intracellular chitinase but lacks the approximately 50 residues long N-terminal region which has been shown to be a chitin-binding region in e.g. weat germ agglutinin, intracellular chitinases, certain lectins and wound-induced proteins. After transformation of tobacco with chimaeric genes containing the 35S CaMV promoter and either extra- or intracellular chitinase coding regions, transgenic plants were shown to accumulate the chitinases without prior induction. PR-P/Q appeared to be localized in the intercellular fluid of the leaves.
With a probe homologous to an intracellular β-1,3-glucanase cDNA from tobacco (Shinshi et al., 1988) clones were isolated from a genomic tobacco library and sequenced. The sequence of the open reading frame of one of the clones, was shown to be identical to one of the cDNA clones of Shinshi et al. (1988). The coding region of the gene is devided over two exons containing the putative signal peptide and the mature pro-protein reading frames, respectively. The other clone contains a pseudogene. Furthermore, cDNA clones from an expression library were isolated with an antiserum against PR-2/N and used as probe in Northern and Southern hybridization.

Shinshi et al., (1987). PNAS 84, 89-93.
Shinshi et al., (1988). PNAS 85, 5541-5545.

BIOLOGY OF MAIZE RAYADO FINO VIRUS (MRFV).

Espinoza, A.M., Gámez, R., León, P., Ramírez, P., Rivera, C., and Maçaya, G.

Centro de Investigaciones en Biología Celular y Molecular, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, 2060 San José, Costa Rica.

Maize Rayado Fino Virus (MRFV) is the type member of the rafiviruses, a new group that also includes oat blue dwarf virus (OBDV) and Bermuda grass etched-line virus (BELV). It is the only known indigenous virus of maize in Mesoamerica.

MRFV has the striking characteristic of being able to multiply both in maize and in its insect vector, the leafhopper Dalbulus maidis. In the regions where rayado fino disease is prevalent, there is an alternation between endemic and epidemic phases. MRFV is transmitted in a persistent manner by its vector and often occurs in field infections associated with mollicutes (the maize stunt complex) transmitted by the same vector. MRFV was first described in Costa Rica and El Salvador and has since been detected from the southern part of the United States to Brazil. Its distribution overlaps that of its vector, and both virus and vector have very narrow and overlapping host ranges. Several serologically diferent strains of MRFV have been isolated from different regions.

Maize cultivars differ in their susceptibility and sensitivity to MRFV. Losses of 40 - 50% of the weight of mature ears have been recorded on individual plants of locally adapted Central American cultivars, but losses may reach 100% in some newly developed cultivars. There are no satisfactory control measures for MRFV and no immune genotype have been found. Some cultivars and landraces express tolerance, and locally adapted material appears to be more tolerant and to show lowered degree of incidence in a population. The perennial tetrapliod teosinte Zea perennis is immune, and the perennial diploid Z. diploperennis is tolerant to MRFV.

MRFV is a small isometric particle of ca. 30 nm in diameter. It has a single-stranded RNA genome of 2.0 - 2.1 x 10^6 molecular weight, and does no appear to posses a 5' linked VPG or poly A tail. The viral capsid is composed of two proteins of 22K (K = Kilodaltons) and 28K, in a molar ratio ranging from 3:1 to 7:1. These two proteins contain common peptide sequences suggesting that either the 22K protein derives from the 28K by proteolytic conversion or that the 28K originates from an undefined read-through mechanism. In reticulocyte lysates, the MRFV genomic RNA directs the synthesis of a large number of peptides, ranging from 15,000 to 165,000 molecular weight, with two large peptides of 110,000 and 165,000 predominating. No polypeptides are detected that comigrate with the capsid protein and no capsid protein is detected by immuno-precipitation of the *in vitro* translation products. No evidence exists for the presence of an encapsidated subgenomic RNA.

Present work includes cDNA cloning and sequencing of the MRFV genome, search for subgenomic RNAs produced *in vivo*, and the study of viral replication and expression both in the plant host and insect vector (in collaboration with R. M. Hammond, USDA-ARS Beltsville Agricultural Research Center).

References:

1. Gámez, R., and León, P. (1988) Maize rayado fino virus and related viruses. In: The Plant Viruses, Vol. 3. (R. Koenig, ed.) Plenum Press, New York. pp. 213-233.

2. Espinoza, A.M., Ramírez, P., and León, P. (1988) Cell-free translation of maize rayado fino virus genomic RNA. J. Gen. Virol. 69; 757-762.

ESTABLISMENT OF RESISTANCE TO PEA EARLY BROWNING VIRUS IN PLANTS.

Steen Guldager Petersen[1] and Bernhardt Borkhardt[2]
[1]Department of Genetics, The Royal Danish Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen F, Denmark, [2]Institute of Plant Pathology, Danish Research Service for Plant and Soil Science, Lottenborgvej 2, DK-2800 Lyngby, Denmark.

Pea Early Browning Virus (PEBV) is a member of the tobra Virus group. The geographical distribution of PEBV is Western Europe, especially The Netherlands and England. The genome consists of two RNA species, RNA1 and RNA2. The RNA2 contains the gene for the coat protein (1). For Tobacco Mosaic Virus it has been shown, that plant transformation with the gene encoding the coat protein can confer virus resistance to the recipient plant (2).

RNA from Pea Early Browning Virus strain SP5 (PEBV) has been extracted and cDNA has been synthesized both using random priming and oligo-dT priming. In the last case the RNA first were poly-A-tailed by the enzyme poly-A-Polymerase. The cDNA has been cloned in the vectors pGEM-3 and PUC-18. The cDNA clones has been characterized by gel electroforesis and northern and southern hybridization.

A 2.4 kb clone derived from the RNA2 has been sequenced. The sequence contains two open reading frames (ORFs) which could encode proteins consisting of 212 amino acids (ORF212) and 255 amino acids (3). Comparison of the amino acid sequence of ORF212 with the amino acid sequence of the coat protein of three different Tobacco Rattle Viruses (4,5,6) strongly indicate that ORF212 encodes the coat protein of PEBV.

The coat protein gene has been cloned in different vectors for expression in protoplasts and tobacco and eventually pea plants. In one vector construct (designated 121-A) the beta-glucuronidase gene (GUS) was deleted from the Agrobacterium vector pBI-121 prior to insertion of the PEBV coat protein gene. Transformed into plants this vector express kanamycin resistance and the coat protein of PEBV. In another construct (designated 121-B) a fragment containing the PEBV coat protein gene flanked by the CaMV 35S promotor and the NOS-terminator was inserted into the Hind III site of pBI-121. This vector express kanamycin resistance, beta-glucuronidase activity and the coat protein in transformed plants. The coat protein gene has been cloned in both directions in the two constructs for production of sense and antisense mRNA to the coat protein.

Two species of tobacco, Nicotiana tabacum and Nicotiana benthamiana have been transformed with 121-A and plants resistant to kanamycin have been obtained. The transformations have been confirmed by southern hybridization.

The transgenic plants will be tested for expression of the coat protein by northern hybridization and ELIZA and will as well be tested for resistance to PEBV. Transformation by 121-B is also prepared.

References:

1. Huges G., Davies J.W. and Wood K.R: (1986): J. Gen. Virol. 67, 2125-2133.
2. Powell-Abel P., Nelsom R.S., De B., Hoffman N., Rogers S.G., Fraley R.T. and Beachy R.N. (1986): Science 232, 738-743.
3. Petersen S.G., Lehmbeck J. and Borkhardt B. (1989): Plant Molecular Biology 13, 735-737.
4. Bergh S.T., Koziel M.G., Huang S., Thomas R.A., Gilley D.P. and Siegel A. (1985): Nucleic Acids Res. 13, 8507-8518.
5. Cornelissen B.J.C., Linthorst H.J.M., Brederode F.T. and Bol J.F. (1986): Nucleic Acids Res. 14, 2157-2169.
6. Angenent G.C., Linthorst H.J.M., Belkum A.F., Cornelissen B.J.C. and Bol J.F. (1986): Nucleic Acids Res. 14, 4673-4682.

CHARACTERIZATION OF BROME MOSAIC VIRUS RNA-DEPENDENT RNA POLYMERASE

R. Quadt and E.M.J. Jaspars

Department of Biochemistry, Gorlaeus Laboratories, Leiden University
P.O.B. 9502, 2300 RA Leiden, The Netherlands

Positive-strand RNA viruses represent the vast majority of viral plant pathogens. The tricornavirus group forms a useful model system to study viral RNA replication since the genome organization and replication strategy of viruses from this group are representative for many plant - viruses.

The molecular basis of RNA replication of plus-strand RNA plant viruses is poorly understood. One approach to gain more insight in this process is to characterize the key enzyme involved i.e. RNA-dependent RNA polymerase (RdRp). The purification of RdRps from virus-infected plants has proven to be most troublesome because of a complexity of factors. Nevertheless, we succeeded in obtaining an extensively purified RdRp preparation from BMV-infected barley. Data on polypeptide composition, the role of viral proteins and specific interactions of the RdRp with viral RNAs will be presented.

An alternative system for the analysis of plant-viroid interactions: Transformation of *Arabidopsis thaliana* with Viroid cDNA.

D. Martínez, J.M. Martínez-Zapater, F. Ponz and J. Romero.
Dpto. Protección Vegetal, CIT-INIA, Apartado 8111, 28080 Madrid. Spain.

Arabidopsis thaliana is a crucifer plant whose special biological and genetical characteristics make it an excellent model system for the molecular analysis of plant physiological functions difficult to study in other systems (1). Viroids are plant infectious entitites depending completely on plant-encoded functions for their replication (2). None of these functions has been positively identified to date. Initial trials to infect *Arabidopsis* with viroids by mechanical inoculation were not succesful. Therefore, we took a different approach to force the interaction of a viroid with the *Arabidopsis* cell.

Dimeric Potato Spindle Tuber Viroid (PSTV)-cDNA (a generous gift of Dr. Owens, Bestville, USA) was subcloned in both orientations in the Bam HI site of the plant transformation binary plasmid pBI121. So, the viroid insert was placed under the control of the constituvely expressed Cauliflower Mosaic Virus 35S promoter as part of a hybrid construct, in which the untranslated leader sequence of the reporter gene GUS, was interrupted by the viroid sequence. The recombinant plasmids were used to transform *Arabidopsis* roots by a standard procedure (3). Kanamicin resistant calli were analyzed for GUS activity. Different results were obtained for calli and regenerated plants, expressing different polarities of the viroid dimer. This result suggests that the fate of the hybrid transcripts is different in the *Arabidopsis* cell for the different constructs. Several species of the genus *Nicotiana* have been reported as symptomless hosts of PSTV (4). Consequently, we have also transformed tobacco plants with the same constructs in order to be able to compare *Arabidopsis* results with those obtained in a positive control (host plant). In tobacco, another viroid (Hop Stunt Viroid) has already been succesfully expressed from an artificial gene in transgenic plants (5). The analysis of the forms taken by PSTV RNA in all combinations of the transformed and regenerated plants will be presented and disccussed.

REFERENCES

1.- Meyerowitz, E.M. 1987. Ann. Rev. Genet. **21**, 93-111.
2.- Diener, T. 1987. The Viroids. Plenum Press (New York).
3.- Valkenes, D., van Montagu, M., and van Lusebellens, M. 1988. Proc. Nat. Acad. Sci. **85**, 5536-5540.
4.- Singh, R.P. 1973. Amer. Potato J. **50**, 111-123.
5.- Yamada, J., Yoshioka, M., Sano, T., Shikato, E., and Okada Y. 1989. Molec. Plant Microbe Inter. **2**, 169-174.

PROCESSING OF VIROIDS IN VITRO AND IN VIVO

H.L. Sänger, M. Tsagris, M. Tabler, W. Schiebel, C. Anderl and B. Haas, Max-Planck-Institut für Biochemie, Abteilung Viroidforschung, D-8033 Martinsried bei München, F.R.G.

The processing of viroids has attracted considerable attention after it had been found that precursor-like RNA transcripts of avocado sunblotch viroid (ASBVd) are able to undergo autocatalytic self-cleavage (1,2). This property which ASBVd has in common with certain viral satellites RNAs (3,4) is based on the presence of specific sequence motifs in these RNAs that can form the active "hammerhead" structure, a configuration which renders these molecules self-cleavable (1). It must be stressed, however, that in this and many other respects, ASBVd is an exception amongst the 17 sequenced viroid "species" and their many sequence variants known at present. All these other viroids are characterized by a largely conserved supposedly processing-relevant domain in the center of their rod-shaped secondary structure and potato spindle tuber viroid (PSTVd) is the prototype of these "normal" viroids.

Our studies on the processing of different multimeric linear precursor-like PSTVd-specific RNA transcripts revealed that they do not process autocatalytically under conditions where various other RNAs do (5). But upon addition of extracts from plant nuclei these synthetic PSTVd RNA multimers are enzymatically cleaved and ligated to monomeric circles indistinguishable from the mature viroid naturally accumulating in infected plant tissue (6). In our recent investigations we utilized as processing substrate linear monomeric PSTVd-specific RNA constructs of unit length which were 3'-terminally extended by 5'-terminal sequences of different length. We also used transcripts carrying specific mutations in these duplicated regions. We tested the infectivity of all these transcripts and we analyzed their processability in vitro in the presence of nuclear extracts and of various isolated enzymes. Our results demonstrate that intracellular viroid processing is evidently dependent on specific sequences embedded in the precursors in particular structural configurations which are recognized by the relevant host enzyme(s). Although circular monomers capable of initiating viroid infections are generated also from synthetic precursor-like transcripts in vivo, their processing seems to require less specific structural preconditions. This concept is corroborated by the results of corresponding in vitro studies.

1: Hutchins, C.J. et al. (1986), Nucleic Acids Res. 14, 3627-3640. 2: Forster, A.C. et al. (1988), Nature 334, 265-267. 3: Prody, G. et al. (1986), Science 231, 1577-1588. 4: Forster, A.C. & Symons, R.H. (1987), Cell 49, 211-220. 5: Tsagris, E. et al. (1987), Virology 157, 227-231. 6: Tsagris E. et al. (1987), EMBO J. 6, 2173-2183.

ANALYSIS OF RESISTANCE BREAKING STRAINS OF POTATO VIRUS X

S. Santa Cruz, D.C. Baulcombe, T.A. Kavanagh*,.
Sainsbury Laboratory, Norwich. *Trinity College, Dublin.

Potato Virus X (PVX) is a common and agronomically important pathogen of potato. Isolates of the virus are commonly classified into four groups on the basis of their interaction with two potato resistance genes Nx and Nb (Cockerham, 1970).

Full length cDNAs have been obtained for three isolates representing two strain groups. The cDNA for a group III isolate (3UK) has been used to generate transcripts which cause symptoms identical to those produced by the parent isolate on both indicator species and a range of potato cultivars.

Two group IV isolates, CP_4 (Jones, 1985) and HB (Moriera and Jones, 1980), have also been cloned. Hybrid cDNAs produced by *in vitro* recombination have been transcribed and used to infect indicator species. Analysis of the effects of chimaeric transcripts on potato cultivars has allowed the determinant for Nx induction to be mapped to the 3' region of the 3UK isolate genome.

REFERENCES:

Cockerham G (1970) Genetical Studies on Resistance to Potato Virus X and Y. Heredity, **25**, 309-348.

Jones R A C (1985) Further Studies on Resistance breaking Strains of Potato Virus X. Plant Pathology. **34**, 182-189.

Moriera A, Jones R A C (1985) Properties of a Resistance Breaking Strain of Potato Virus X. Annals of Applied Biology. **95**, 93-103.

Characterization of the Capsid Protein of Arabis mosaic virus

Herta Steinkellner, Gottfried Himmler, Diethard Mattanovich and Hermann W.D. Katinger

Institute of Applied Microbiology, University of Agriculture, Peter Jordanstrasse 82, A-1190 Wien, Austria

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Arabis mosaic virus (AMV) is a member of the Nepovirusgroup. The genome consists of two RNA species (RNA1 and RNA2) which are encapsidated separately in icosahedral particels. In protoplasts RNA1 of Nepoviruses replicates independently indicating that information necessary for viral replication is encoded by RNA1. RNA2 contains the gene for the viral cell-to-cell spread as well as the capsid protein gene at the 3'-end of the viral RNA.
cDNA of RNA2 of AMV was made (1). The sequence of the 3'end was determined and comparison with Tomato black ring virus and Grapevine crome mosaic virus, the two other Nepoviruses sequenced so far (2,3), show a homology of 30%.
The NH_2-terminal amino acid sequence of the AMV capsid protein has been determined by sequential Edmann degradation of the purified viral capsid protein. The first amino acids are ATXMVYVLKG. A search on the translated sequence of RNA2 gave a similar amino acid sequence with a hypothetical translation product of 585 amino acids long (mol. weight 64kDa). The size of the capsid protein, however, estimated by SDS-PAGE electrophoresis, is 54kDa. A search on the predicted start position of the coat protein gene on RNA2, shows an already known potential cleavage site (Q/A) for cystein proteases, which are thought to be active in nepoviruses (2).
Expression vectors are constructed to characterize the exact position of the AMV capsid protein and to define the cleavage site of the nepoviral proteinase.

(1)Steinkellner,H. Himmler,G. Laimer,M. Mattanovich,D. BisztraiG., und Katinger H.(1989).Kostruktion von cDNA von Arabis mosaic virus und deren Anwendung für Diagnose. Mitt. Klosterneuburg.6, 242-246
(2)Brault,V. Hibrand,L. Candresse,T. Le Gall,O. and Dunez,J. (1989).Nucleotide Sequence and Genetic Organisation of Hungarian grapevine chrome mosaic nepevirus RNA2. Nuc.Ac.Res.17 19 7809-7819.
(3)Meyer,M. Hemmer,O. Mayo,M.A. and Fritsch C.(1986).The Nucleotide Sequence of Tomato black ring virus RNA2. J. Gen. Virol. 67, 1257-1271.
(4)Takemoto,Y. Nagahara,Y. Fukuyama,K. Tskihara,T. and Iwaki,M. (1985).Crystallization and Preliminary Characterization of Arabis mosaic virus. Virology 145, 191-194.

cDNA Cloning and sequencing of AMCV (Artichoke Mottled Crinkle Virus)

M. TAVAZZA, A. LUCIOLI, A. CALOGERO, E.BENVENUTO, R. TAVAZZA, R.ORDAS

Artichoke Mottled Crinkle Virus (AMCV) is a member of the Tombusvirus group (1). In nature AMCV infects the globe artichoke (*Cynara scolymus*) causing plant size reduction, leaf distorsion and decrease in the number of flower heads (2).
AMCV has a monopartite positive sense ssRNA genome, which is not polyadenylated at the 3' end(3). The translation strategy of AMCV involves the production of two 3' coterminal subgenomic RNAs of ~2,2 and 0,9 Kb, as in other members of the Tombusvirus group (4).
We report here the cDNA cloning and sequencing of most of the viral genome. The size of the AMCV genome is about 4750 nt and it contains five long open reading frames (ORF) which code for proteins with Mr 33K, 92K, 41K, 21K and 20K. The 33K protein is the product of ORF1 and the 92K protein is derived by readthrough of the amber termination codon of the 33K protein. ORF3 encodes for the coat protein and it starts at ~2.7 kb downstream from the 5' end and stops at ~1 kb upstream from the 3' end, thus it is not located near the 3' end. ORF4 (21K) and ORF5 (20K) are two nested ORFs, in different frames, following the coat protein gene.
Genomic organization of AMCV is similar to the genomic organization of other members of the Tombusvirus group which have been studied: cymbidium ringspot virus (5), tomato bushy stunt virus cherry strain (6) and cucumber necrosis virus (7).
Nicotiana clevelandii was transformed with a chimeric gene encoding for AMCV coat protein in both orientations using Ti plasmid-derived plant transformation vector (pBI 121.1); regenerant plants were analyzed with npt II assay and Northern blot. The progeny of the self-fertilized transgenic plants will be tested to determine if they could suppress symptoms caused by AMCV infection.
ORF4 and ORF5 have been cloned, in both orientations, in pBI 121.1 and transformation experiments on *Nicotiana clevelandii* are in progress.

1. Matthews REF. Intervirology 17: 4-199 (1982).
2. Martelli GP. Phytopath. Medit. 4: 58-60 (1965).
3. Tavazza M, Lucioli A, Ancora G, Benvenuto E. Plant Mol. Biol. 13: 685-692 (1989).
4. Russo M, Burgyan J, Carrington J C, Hillman BI, Morris TJ. J.Gen.Virology 69: 401-406 (1988).
5. Grieco F, Burgyan J, Russo M. Nucl. Acids Res. 17: 6383 (1989)
6. Hillman BI, Hearne P, Rochon D, Morris TJ. Virology 169: 42-50 (1989).
7. Rochon D, Tremaine JH : Complete Nucleotide Sequence of the Cucumber Necrosis Virus Genome . Virology 169: 251-259 (1989).

STRATEGIES TO INTERFERE WITH VIRUS REPLICATION IN TRANSGENIC PLANTS

B Zaccomer, F Cellier and M Tepfer, Laboratoire de Biologie Cellulaire, INRA-Centre de Versailles, 78026 Versailles Cedex, France.

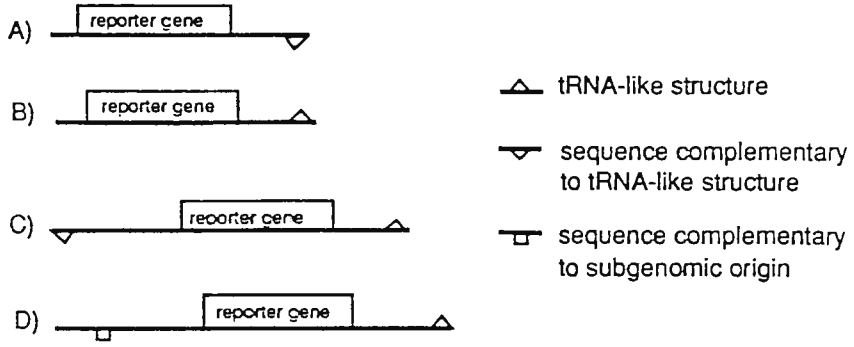
MD Morch and AL Haenni, Laboratoire de Biochimie du Développement, Institut Jacques Monod, CNRS-Université Paris VII, 2 place Jussieu, 75251 Paris Cedex 05, France.

Turnip yellow mosaic virus (TYMV) is the type member of the tymovirus group; its genome is composed of a single molecule of (+) sense RNA, with a tRNA-like structure at the 3' end. The tRNA-like region is recognized by the TYMV replicase in initiating the synthesis of (-) strand RNA from the (+) sense template. Two others regions are recognized by the replicase on the minus strand in initiating the synthesis of new (+) genomic strand and subgenomic RNA.

We have introduced into *Brassica napus* plants three kinds of genes that might interfere with replication of TYMV. Transcripts of certain genes bear regions which are complementary to the the 3' region of the virus (fig.A), such antisense ricRNA (replication interfering complementary RNA) could mask the site recognized by the replicase in initiating (-) strand synthesis.

A second strategy concerns the use of (+) sense RNA as a competitor for the replicase. Morch et al. (Nucl. Acids Res. (1987)15: 4123–4129) have shown that RNAs bearing the tRNA-like region are competitive inhibitors of the replication of TYMV genomic RNA *in vitro*. We have introduced genes into *B. napus* allowing us to test this "sense" strategy *in vivo* as well (fig. B).

The third strategy consists of introducing in plants genes coding for artificial defective interfering RNAs (DI RNA). These DI RNAs were constructed using either the tRNA-like structure or its complementary sequence and the subgenomic origin (fig. C and D). The transcripts containing these DI RNAs should be amplified in infected plant cells and could interfere with virus replication.



CONCLUSIONS AND PERSPECTIVES

F. GARCÍA ARENAL
Departamento de Patología Vegetal
E.T.S.I. Agrónomos
Madrid (Spain)

P. PALUKAITIS
Department of Plant Pathology
Cornell University
Ithaca, N.Y. (USA)

CONCLUSIONS AND PERSPECTIVES

This meeting, organized under the auspices of the Fundación Juan March, brought together 50 scientists from 15 countries to present data and discuss recent advances in "Genome Expression and Pathogenesis of Plant RNA Viruses".

The meeting served several purposes: (1) disseminating the most recent unpublished data to a receptive audience of both well-established and young scientists; (2) providing a forum for the presentation of quality research being done by Spanish scientists, only a few of whom have had the opportunity to present their data in such small meetings under the auspices of other European organizations; and (3) providing a venue for interaction between the Spanish scientists and the foreign visitors, as well as between the younger and the well-established scientists.

The topics that were presented during the three-day meeting cover four broad areas of research: (1) replication and functions of viral gene products; (2) the molecular biology, interactions and evolution of satellite RNAs; (3) viral genome structure and organization; and (4) resistance to virus infection and the molecular basis of pathogenesis. Interesting new data were presented in all four areas, even though the last European meeting covering many of the same topics was held only nine months earlier, indicating a field of research undergoing rapid development.

Those viruses in which most of the previous advancements had been made, continue to provide experimental systems for research on the "cutting edge". However, some of the more interesting developments also occur with viruses only recently fully characterized. A combination of these two avenues of research has led to a better understanding of some viral gene functions and more details of the specific mechanisms involved in other viral gene functions. This is true for genes involved in various aspects of both replication and processing. Such work has been aided greatly by the use of biologically active cDNA clones.

Various speakers presented data relating the interactions of specific sequences in viral pathogens with components fo their hosts leading to replications, pathogenicity and/or resistance to virus infection. The use of viral genome segments in transgenic plants to study viral-host interactions continues, with many unexpected results. The expression of viral coat protein in such plants produces different levels of resistance with different viruses, suggesting that more than one mechanism may be involved.

Differences in the expression of pathogenicity occur as the result of as few as one-to-three nucleotide changes. Some viral RNAs show rapid evolution while others remanin quite conserved, probably as the result of selection. Thus, considerable progress has been made in understanding the molecular basis of virus mutation and the parameters involved in selection of sequence variants.

Many previously uncharacterized viruses (some only known to exist as undefined infectious agents a short time ago) have been purified and substantially characterized at the molecular level. The analysis of such data in comparison with those already published show both the conservation of genome organization and gene function, as well as the diversity of genetic information capable of encoding similar functions. These subtle differences will be very important in elucidating active sites of such genes and domains involved in viral-protein: host interactions.

A discussion period at the end of the meeting permitted a venue for elaboration on topics not completely covered during the meeting, the presentation of additional data, and discourse on various topics of general interest to the group, some polemical in nature.

Overall, considerable advances have been made in all of the four areas of research covered by the meeting. Progress has been, and will continue to be, very rapid, and major breakthroughs can be expected in the next few years in all of these areas.

The organizers and the participants are grateful to the Fundación Juan March for providing funds, facilities and the oportunity to hold this meeting.

LIST OF PARTICIPANTS

LIST OF PARTICIPANTS

P. Abad Campos	- Cátedra de Patología Vegetal. E.T.S.I. Agrónomos. Universidad Politécnica. Valencia (Spain).
G. Angenon	- Rijksuniversiteit Gent. Laboratorium voor Genetika. Gent (Belgium).
E. Balázs	- Agricultural Biotechnology Center. Gödöllö (Hungary).
D. Baulcombe	- The Sainsbury Laboratory. John Innes Institut Norwich (U.K.).
R.N. Beachy	- Department of Biology. Washington University. St. Louis, MO. (USA).
S. Ben Tahar	- Laboratoire de Biologie Cellulaire et Moléculaire. Aubière (France).
G. Boccardo	- Istituto di Fitovirologia Applicata del C.N.R. Torino (Italy).
J. Bol	- Gorlaeus Laboratories. Leiden University. Leiden (The Netherlands).
M.J. Borja	- Dptº. Protección Vegetal. CIT-INIA. Madrid (Spain).
K. Boye	- Department of Genetics. Royal Veterinary and Agricultural University. Copenhagen (Denmark).
J.C. Boyer	- Laboratoire des Systèmes Végétaux. Institut Jacques Monod. Paris (France).
G. Bruening	- Department of Plant Pathology. University of California. Davis. CA. (USA).
J. Burgyan	- Research Institute for Plant Protection. Budapest. (Hungary).
T. Candresse	- Station de Pathologie Végétale. INRA. Villenave d'Ornon (France).
J.R. Díaz-Ruiz	- UEI Fitopatología. Centro de Investigaciones Biológicas. C.S.I.C. Madrid (Spain).
S. Ding	- Research School of Biological Sciences. Australian National University. Canberra. (Australia).
J. Dopazo	- Departament de Genètica y Servei de Bioinformàtica. Universitat de Valencia (Spain).
W.G. Dougherty	- Department of Microbiology. Oregon State University. Corvallis. OR. (USA).

R. Flores - Unidad de Biología Molecular y Celular de Plantas. IATA (CSIC). Valencia (Spain).

M. Fuchs - INRA. Station de Recherches Vigne et Vin. Laboratoire de Pathologie Végétale. Colmar, (France).

J.A. García - Centro de Biología Molecular. C.S.I.C. Universidad Autónoma. Madrid (Spain).

F. García-Arenal - Deptº. de Patología Vegetal. E.T.S.I. Agrónomos. Madrid (Spain).

W.L. Gerlach - CSIRO. Division of Plant Industry. Canberra (Australia).

A.L. Haenni - Institut Jacques Monod. Paris Cedex (France).

C. Hemenway - Monsato Company. St. Louis, MO. (USA).

M. Jacquemond - INRA. Station de Pathologie Végétale. Montfavet Cédex (France).

E.M.J. Jaspars - Gorlaeus Laboratories. Leiden University. Leiden (The Netherlands).

I. Jupin - Institut de Biologie Moléculaire des Plantes. Strasbourg (France).

S. Lain - Centro de Biología Molecular (CSIC-UAM). Universidad Autónoma. Madrid (Spain).

H.J.M. Linthorst - Biochemistry Department. Leiden University. Leiden (The Netherlands).

G. Macaya - Centro de Investigaciones en Biología Celular y Molecular. Universidad de Costa Rica. San José (Costa Rica).

M.A. Mayo - Scottish Crop Research Institute. Invergowrie, Dundee (U.K.)

E. Moriones - Deptº. de Patología Vegetal. E.T.S.I. Agrónomos. Madrid (Spain).

D.L. Nuss - Dept. Of Molecular Oncology and Virology. Roche Institute of Molecular Biology. Roche Research Center. Nutley, NJ. (USA).

P. Palukaitis - Dept. of Plant Pathology. Cornell University. Ithaca, NY (USA).

C. Patiño - C.N.B. - C.S.I.C. Madrid (Spain).

S.G. Petersen - Department of Genetics. The Royal Danish Veterinary and Agricultural University. Copenhagen (Denmark).

F. Ponz	- Dptº. Protección Vegetal. CIT-INIA. Madrid (Spain).
R. Quadt	- Department of Biochemistry. Gorlaeus Laboratories. Leiden University. Leiden (The Netherlands).
J. Romero	- Dptº. Protección Vegetal. CIT-INIA. Madrid. (Spain).
M. Roossinck	- Dept. of Plant Pathology. Cornell University. Ithaca, NY. (USA).
H.L. Sänger	- Max-Planck-Institut für Biochemie. Martinsried bei München (F.R.G.).
S. Santa Cruz	- Sainsbury Laboratory. Norwich (U.K.).
H. Steinkellner	- Institute of Applied Microbiology. University of Agriculture. Wien (Austria).
M. Tavazza	- ENEA - Agrobiology Dept. Roma (Italy).
M.P. Vallés	- Estación Experimental de Aula Dei. Dptº. de Genética. Zaragoza. (Spain).
Y. Watanabe	- Department of Biophysics and Biochemistry. Faculty of Science. The University of Tokyo. Bunkyo-Ku, Tokyo (Japan).
B. Zaccomer	- Laboratoire de Biologie Cellulaire. INRA-Centre de Versailles. Versailles Cedex (France).
M. Zaitlin	- Department of Plant Pathology. Cornell University. Ithaca, NY.(USA).

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Course on DNA - Protein Interaction.

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251 **Lecture Course on Approaches to Plant Development.**
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