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The coat proteins and putative movement proteins of isolates of Prunus necrotic ringspot virus from different host species and geographic origins are extensively conserved

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Abstract

A complete sequence for the RNA 3 of Prunus necrotic ringspot virus (PNRSV) is described (Genbank Accession U57046). Primers from this sequence were used to amplify both the movement protein and coat protein genes of 3 other isolates of PNRSV originating from different host species and geographic locations. Comparisons of these sequences with those of other published sequences for PNRSV and the closely related apple mosaic virus (ApMV) showed that both the movement proteins and coat proteins of isolates of PNRSV are extensively conserved irrespective of either the original host or the geographic origin. The movement protein and coat protein of ApMV and PNRSV are sufficiently conserved to suggest that these two viruses may have evolved from a common ancestor. The amino acid sequence of the two coat proteins shows areas of similarity and difference that would explain the serological continuum reported to occur among isolates of these two viruses. Nevertheless, the movement protein and coat protein of the two viruses are sufficiently different so that ApMV and PNRSV should be considered to be distinct viruses.

Research indicates that Prunus necrotic ringspot virus (PNRSV) exists as many serologically and biologically distinct strains or isolates (Barbara et al., 1978; Fulton, 1981; Crosslin and Mink, 1992). The evidence of variation and diversity is such that it may be thought as virtually axiomatic that each isolate of PNRSV is different and, even possibly, unique.

PNRSV is a member of the genus *Ilarvirus*, which together with the genera *Alfamovirus, Bromovirus,* and *Cucumovirus,* comprise the family *Bromoviridae*. All four genera contain viruses with tripartite genomes. The RNA 1 and RNA 2 code for proteins involved in viral replication and the RNA 3 codes for both a movement protein (3a protein) and the viral coat protein (CP) (Murphy et al., 1995).

Ilarviruses appear to be closely related to alfalfa mosaic virus (AMV), the single member of the genus *Alfamovirus* (Ge and Scott, 1994). In addition to genomic nucleic acids, members of both genera require the presence of CP to successfully initiate infection (Bol et al., 1971; Gonsalves and Garnsey, 1975). Indeed, the CP of AMV has been demonstrated to initiate infections with the nucleic acids of ilarviruses and vice versa (van Vloten-Doting, 1975). The presence of the CP is not required for successful initiation of infection with the genomes of either Cucumoviruses or Bromoviruses (Francki, 1985). The main difference between AMV and the ilarviruses has been that AMV is transmissible by aphids while ilarviruses are not (Hamilton, 1985). More recently, sequence data for 2 members of the subgroup 2 of the ilarviruses indicate the presence of a 2b open reading frame (ORF) which is absent in AMV (Ge et al., 1997).

Work with AMV has shown that a distinct change in symptom expression results from mutation of a single amino acid (aa) in the CP (Neeleman et al., 1991). A similar situation has been demonstrated to exist with

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Figure 1. The complete nucleotide sequence of PNRSV isolate 30/4. Putative translation products of the two ORFs are indicated beneath the sequence. Primers used in PCR are indicated by sequence in underlined, italic type. The 3a gene of other isolates of PNRSV was amplified using a primer complementary to nt 1117-1141 and a primer in the same sense as nt 159-186. The CP gene of other isolates of PNRSV was amplified using a primer complementary to nt 1800-1818 and a primer in the same sense as nt 1034-1051. This sequence has been deposited with GenBank as Accession U57046.

Table 1. Listing of isolates described in this paper. GenBank accession numbers are indicated in parentheses, e.g. (U57046)

Isolate	Original host	Geographic origin
A) Isolates used		
30/4	*Prunus persica* L. Batsch (peach)	South Carolina, USA (U57046)
SW6	*Prunus avium* (L.) L. (cherry)	Michigan, USA (AF013287)
Mission	*Prunus dulcis* (Mill.) Webb (almond)	California, USA (AF013285)
Prune	*Prunus domestica* L. (prune)	California, USA (AF013286)
Can	*Prunus avium* (cherry)	Canada (Mackenzie, unpublished data)
B) Isolates referred to and described in other sources		
PE 5	*Prunus persica* L. Batsch (peach)	South Carolina, USA (L38823)[4]
PV-0096	*Prunus mahaleb* L. (mahaleb)	Germany (S78312)[5]
PV32	*Malus* spp.	USA (U03857)[6] (Y07568)
ApMV-G	-	Germany (S78319)[5]
ApMV	*Malus* spp.	Massachusetts, USA (U15608)[7]

[4]Hammond and Crosslin, 1995
[5]Guo et al., 1995
[6]Sánchez-Navarro and Pállas, 1994
[7]Shiel et al., 1995.

cucumber mosaic virus (CMV), the type member of the genus *Cucumovirus* (Shintaku et al., 1992).

Crosslin and Mink (1992) have demonstrated that the molecular weight of the CPs of some isolates of PNRSV varies by as much as 2 kDa. Thus, it is possible that both the serological and biological variability observed in PNRSV may result from marked variation in the CP of this virus.

In both brome mosaic virus - BMV (genus *Bromovirus*) and CMV the movement protein has a crucial role in host specificity (Mise et al., 1993; Li et al., 1996).

Development of sequence data for the RNA 3 of three isolates of PNRSV (Hammond and Crosslin, 1995; Guo et al., 1995; Sánchez-Navarro and Pállas, 1997) showed that the primary aa sequence of the respective 3a proteins and CPs was very similar. We have sequenced the RNA 3 of a fourth isolate of PNRSV and have used the resulting sequence to design primers for PCR that amplify the 3a protein gene and the CP gene of the virus. These primers were used to amplify both the 3a protein gene and the CP gene of isolates of PNRSV that originate from different host species and geographic locations in an attempt to identify variability in these regions that might be associated with the serological (CP) and biological (CP and 3a protein) variability reported among isolates and strains of PNRSV.

The source and history of the virus isolates used in this work are described in Table 1. Full-length sequence for isolate 30/4 was developed using previously published methods (Ge and Scott, 1994; Scott and Ge, 1995) and the sequence submitted to GenBank. Primers which flank both the 3a protein gene and the CP gene of this sequence were selected (see Figure 1) and used to amplify corresponding fragments from other isolates of PNRSV. The fragments were either cloned into the *p*CR II^{TM} vector using a TA cloning kit (Invitrogen) or 'polished' and ligated into the *Eco*RV site of *p*Bluescript. Sequence fragments were joined using GeneJockey II software(Biosoft). Comparisons of similarity and identity between sequences were made using the program GAP in the GCG software package (Wisconsin Package, 1994). Multiple alignments of the sequences were produced using CLUSTAL W (Thompson et al., 1994) and the sequences were shad-

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   1  GUUUUUAACAAAUUGAAAUCGUAACAUCCAUUAAUUGGUUGAUUUCUCUUUUACUUGACU   60
  61  AGGUACCCAUUGAAACGGAAUUAAAAAGUCCUAUUUCUCUAAGUAACAUCCCCUUUGUGG  120
 121  GUUUAGAGAUUGUUGGUUGUCUAUUCGUAAUUCCACUAGUUGGUUGAAUAGUGUUUCAGU  180
 181  AUGGCCGAUGUCAGUAAAAAACCCCUCCACUAGUGAUUUCUCAGUAGUGGAGUGUUCUAU  240
                M  S  V  K  N  P  S  T  S  D  F  S  V  V  E  C  S  M
 241  GGACGAAAUGAGCCAGAUAUCAGAGGAUUUGCAUAAACUUAUGCUGUCCGAUGAGAUGAG  300
       D  E  M  S  Q  I  S  E  D  L  H  K  L  M  L  S  D  E  M  R
 301  GGCGUUACCAACGAAAGGUUGUCAUAUCCUGCAUUUAGUUAACCUCCCGAAAUCCAAUAU  360
       A  L  P  T  K  G  C  H  I  L  H  L  V  N  L  P  K  S  N  I
 361  AUUGAGAUUAGCGAGUAAGGAGCAGAAAGGUUUCCUCUCCCGACAGGCCGAUAAAGUAAA  420
       L  R  L  A  S  K  E  Q  K  G  F  L  S  R  Q  A  D  K  V  K
 421  GAAGAAAAUUUACAGAUGUGUAGGCCGAGUAUUCCUCGUUUAUGUACCGAUCAUUCAAGC  480
       K  K  I  Y  R  C  V  G  R  V  F  L  V  Y  V  P  I  I  Q  A
 481  AACGACCUCGGGUCUGAUAAUCCUUAAGUUGCAAAACUCCGAUACAGGAGAGAUUUCAGA  540
       T  T  S  G  L  I  I  L  K  L  Q  N  S  D  T  G  E  I  S  D
 541  CGUCGUGACAGACGCCGAAGUCAACCGCGCCUUUGUUAUUAUGGACAGAUGGGGAAGAUC  600
       V  V  T  D  A  E  V  N  R  A  F  V  I  M  D  R  W  G  R  S
 601  CCUGGUGGAAUCAGCAGAUUUAAAUCUGUUGUAUUCCAUUUCAUGUCCGGACGUCCGACC  660
       L  V  E  S  A  D  L  N  L  L  Y  S  I  S  C  P  D  V  R  P
 661  UGGAGCCAGAGUGGGAGAGAUGAUGGCUUUUUGGGACGAGAGGAUGUCAAGGCAGCAGAC  720
       G  A  R  V  G  E  M  M  A  F  W  D  E  R  M  S  R  Q  Q  T
 721  UUACUUGGAGAAAGGGAAUCCUAUUUUAUUCCCGAUUGCCGAGACAAAACCUUCGAAAUA  780
       Y  L  E  K  G  N  P  I  L  F  P  I  A  E  T  K  P  S  K  Y
 781  UCUUAAUGAUAAGAAGGUGUUGAUGUCUAUGGUCCGAAGUAGGAUAUUGGCAGGUACAGA  840
       L  N  D  K  K  V  L  M  S  M  V  R  S  R  I  L  A  G  T  E
 841  AGGGUGCGAUAUAGCUCCCGAGAAUAUAGAAGUCAAACGAUUAGGGGAUAAUAGGAAGGU  900
       G  C  D  I  A  P  E  N  I  E  V  K  R  L  G  D  N  R  K  V
 901  UUUGACUAUUCAACCGAAAGCCCCGAUCGUAGAGGAAAUUAAGGACGAUGUUGAACCGUU  960
       L  T  I  Q  P  K  A  P  I  V  E  E  I  K  D  D  V  E  P  L
 961  AGGUUCGAAUGGUGAAAAUCAUAUGGAAGAGAAGACCGUGACCGUUAAGGUCGGUAGUUC 1020
       G  S  N  G  E  N  H  M  E  E  K  T  V  T  V  K  V  G  S  S
1021  UGGAAGUGCUUGAGUGACUAUGUCACGAGCGUGCGCUUUGCGCGAGCGUUUUUCUUUCCU 1080
       G  S  A  *
1081  UUCUUCCGAACAUCUCUUUCAUUUGAUAAUGGUUUGCCGAAUUUGCAAUCAUACCCACGC 1140
                                        M  V  C  R  I  C  N  H  T  H  A
1141  UGGUGGAUGCCGUUCUUGCAAGAGGUGCCAUCCGAAUGAUGCUCUGGUCCCACUCAGGGC 1200
       G  G  C  R  S  C  K  R  C  H  P  N  D  A  L  V  P  L  R  A
1201  UCAACAAAGGGCCGCGAAUAACCCGAAUAGGAAUAGGAACCCGAAUAGGGUUUCGAGCGG 1260
       Q  Q  R  A  A  N  N  P  N  R  N  R  N  P  N  R  V  S  S  G
1261  UAUAGGACCUGCGGUCCGACCGCAACCGGUCGUGAAGACCACUUGGACCGUGAGGGGUCC 1320
       I  G  P  A  V  R  P  Q  P  V  V  K  T  T  W  T  V  R  G  P
1321  GAAUGUGCCUCCCCGAAUUCCUAAGGGUUAUGUAGCACAUAAUCACCGAGAGGUGACGAC 1380
       N  V  P  P  R  I  P  K  G  Y  V  A  H  N  H  R  E  V  T  T
1381  GACAGAGGCAGUGAAGUACUUGAGUAUUGACUUCACGACCACUCUCCCCUCAGUUGAUGGG 1440
       T  E  A  V  K  Y  L  S  I  D  F  T  T  T  L  P  Q  L  M  G
1441  UCAGAAUUUGACCUUAUUAACUGUCAUAGUCCGAAUGAACUCUAUGAGUUCGAAUGGUUG 1500
       Q  N  L  T  L  L  T  V  I  V  R  M  N  S  M  S  S  N  G  W
1501  GAUUGGGAUGGUGGAGGACUAUAAGGUGGAUCAACCUGAUGGUCCGAAUGCCCUGUCUAG 1560
       I  G  M  V  E  D  Y  K  V  D  Q  P  D  G  P  N  A  L  S  R
1561  GAAGGGGUUCUUGAAGGACCAACCGAGAGGUUGGCAGUUCGAACCUCCCUCCGAUUUAGA 1620
       K  G  F  L  K  D  Q  P  R  G  W  Q  F  E  P  P  S  D  L  D
1621  UUUCGACACUUUUGCGCGUACGCAUCGUGUCGUCAUCGAAUUCAAGACCGAAGUGCCCGC 1680
       F  D  T  F  A  R  T  H  R  V  V  I  E  F  K  T  E  V  P  A
1681  UGGGGCCAAGGUCUUGGUUAGGGAUUUGUACGUAGUGGUAAGUGACUUACCACGAGUGCA 1740
       G  A  K  V  L  V  R  D  L  Y  V  V  V  S  D  L  P  R  V  Q
1741  AAUUCCGACUGAUGUCUUGCUGGUCGAUGAGGACCUGCUUGAGAUCUAGAGUGAGAUAAG 1800
       I  P  T  D  V  L  L  V  D  E  D  L  L  E  I  *
1801  CACACUCGAAUUUCUCCGAAUGGAAAGUUCGCACCACCGAUAGUGGAUAUUGCGAAAUAG 1860
1861  AUUUCUGAAAGUCGCUUCCCGGCUUUCAUGCUUGGAAAUCUUACCUGCGUUAGCAGAUGC 1920
1921  CCACAACGUGAAGUUGUGGAUGCCCCGUUAGGGAAGC  1957
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ed to show identical and similar amino acids using the program BOXSHADE [BOXSHADE server, Swiss Institute for Experimental Cancer Research, Epalinges, Lausanne).

The complete sequence of PNRSV isolate 30/4 (GenBank accession No. U57046) is 1957 nucleotides (nt) in length and contains two ORFs (Figure 1). The putative translation product of the 3a ORF (movement protein) is comprised of 284 aa with an M_r of 31,331 Da. The putative translation product of the 3b ORF (CP) is comprised of 226 aa with an M_r of 25,284 Da.

PCR fragments of the anticipated size ($\sim$ 983 bp for the 3a protein gene and $\sim$ 785 bp for the CP gene) were obtained from purified preparations of all the isolates of PNRSV examined. As the two fragments overlapped, they were joined and the contiguous sequences for the isolates Mission, Prune, and SW6 were submitted to GenBank as accessions AF013285, AF013286 and AF013287, respectively. Multiple alignments of the putative translation products of the fragments, together with the published sequences for the 3a proteins and CPs of other isolates of PNRSV and ApMV, are shown in Figures 2 and 3, respectively.

Comparison of the putative 3a proteins of PNRSV (Figure 2) shows extensive conservation ($>$ 90% identity) among isolates of PNRSV irrespective of the host species (U57046 and L38823 - peach; S78312 and SW6 - cherry) from which the isolate originated. The 3a proteins of the PNRSV isolates shared approximately 55% identity with the 3a protein of ApMV.

The CPs (Figure 3) clearly fall into 2 distinct groups (PNRSV and ApMV) with the major difference between the CP of the two viruses occurring between aa 48 and 70. Regions containing up to 7 consecutive, identical amino acids, e.g. SSNGWIG, are present in both PNRSV and ApMV. The CPs of PNRSV showed $>$ 90% identity among isolates but only 40 - 50% identity with the CP of ApMV. Calculations of the M_r of the putative translation products of the PNRSV fragments show them to vary between 24,913 and 25,315 Da.

Detailed inspection of the sequences for both CP and 3a proteins indicate changes in amino acids at a number of positions, however it was not possible to associate any of these changes with either the host or the geographic location from which the isolates of PNRSV originated.

Here we report an additional complete sequence for the RNA 3 of PNRSV and almost full-length sequences for three other isolates of PNRSV. Both the nucleotide sequence and the putative translation products of the 4 isolates of PNRSV differ only marginally from those previously reported for PNRSV (Hammond and Crosslin, 1995; Guo et al., 1995; Sánchez-Navarro and Pállas, 1997).

The putative aa sequences of the CPs of isolates of PNRSV that we have examined are highly conserved despite the fact that the isolates originated from diverse host species and geographic locations. This conservation is in complete contrast to the hypothesis which initiated this work, namely: variation in the primary aa sequence of the CP might be responsible for the serological and biological variability associated with PNRSV. The conservation present in these data indicates that the known serological variability of PNRSV is probably a function of protein secondary structure and the poor immunogenic characteristics of ilarviruses rather than of major differences in the aa that comprise the primary sequence of the CP. Moreover, it should be noted that a computerized analysis (GCG programs PEPTIDESTRUCTURE and PLOTSTRUCTURE) of the potential antigenic structures of these CPs (data not shown) showed only minor differences between the proteins.

However, the possibility of changes in the CP affecting symptom expression cannot be discounted. Neeleman et al. (1991) detected only 5 differences between the 221 aa of the CPs of strains 425 and YSMV of AMV. Of these it was the replacement at position 29 of a glutamine residue with an arginine residue which caused a marked change in symptoms from mild chlorosis to necrotic local lesions. There are typically more than 4 but less than 10 differences in the amino acids among the CPs of the isolates of PNRSV that we have examined. It was not possible to associate any of these changes with either the original host from which these isolates came or the geographic origin of the isolates, e.g., U57046 and L38823 both originated from peach trees grown in South Carolina but the latter did not have the asparagine and arginine (NR) residues at positions 43 and 44. Neither were we able to associate any of the changes with information that we had gathered concerning either the expression of symptoms by these isolates on *Cucumis sativus* or the range of host species that they would infect. However, Hammond et al. (1996) have recently reported that both serotype and symptom type of 6 isolates of PNRSV can be distinguished based on specific nucleotides and amino acids.

Mise et al. (1993) showed that the 3a protein controls the ability of two bromoviruses to infect either monocotyledonous or dicotyledonous hosts. Li et al.

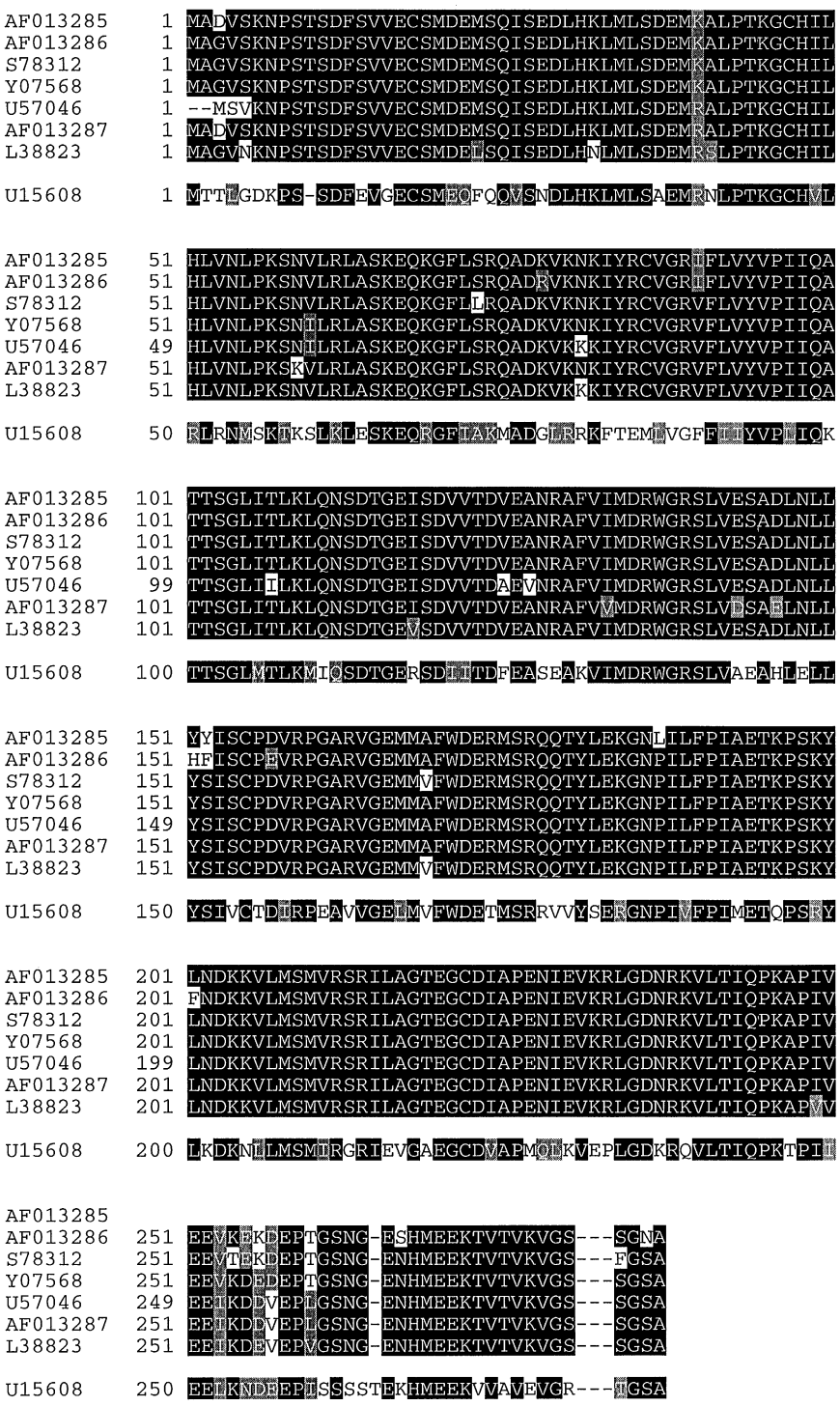

Figure 2. A multiple alignment of the putative translation products (movement proteins) of the 3a ORF of PNRSV (GenBank accessions U57046, S78312, L38823, AF013285, AF013286, AF013287 and ApMV (GenBank accession U15608). Refer to Table 1 for details of the isolates. The alignment was generated using CLUSTAL W and shaded using BOXSHADE. Identical amino acids are surrounded by black boxes and similar amino acids are surrounded by grey boxes. Amino acids that share no similarity are unshaded.

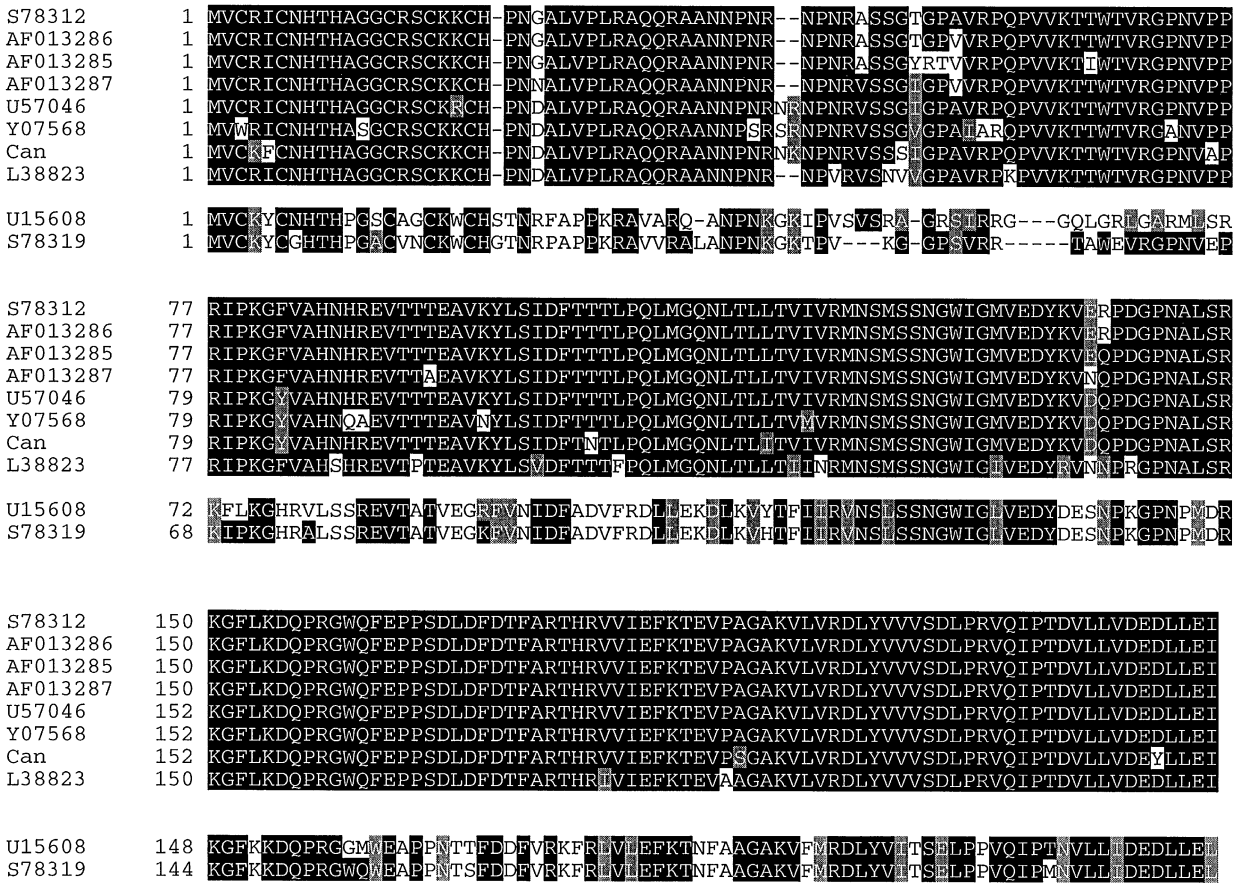

Figure 3. A multiple alignment of the putative translation products of the CP genes of isolates of PNRSV (GenBank accessions U57046, S78312, L38823, AF013285, AF013286, AF013287) and ApMV (GenBank accessions U15608, S78319) from different sources. Refer to Table 1 for details of the isolates. The alignment was generated using CLUSTAL W and shaded using BOXSHADE. Identical amino acids are surrounded by black boxes and similar amino acids are surrounded by grey boxes. Amino acids that share no similarity are unshaded.

(1996) showed that mutations in the 3a gene of CMV altered the host range of the virus. We were unable to identify any specific changes in the 3a proteins that could be associated with the host from which the isolate of PNRSV originated.

Clearly it is impossible to arrive at a specific conclusion as to the role of minor variations in amino acid sequence of the 3a proteins and the CP of PNRSV on host specificity. Here we have examined sequences from diverse hosts and geographic locations and the variation may be such that only when a substantial number of isolates from a single host have been sequenced will we be able to identify the contribution of specific amino acid changes to host specificity.

However, there are clear differences between both the 3a proteins and CPs of PNRSV and ApMV, viruses that have long been considered distinct but serologically related. Both viruses infect hops (*Humulus* spp.) and roses (*Rosa* spp.) and in these hosts there has been great confusion over distinguishing the causal agents of some diseases e.g., rose mosaic disease. Indeed, had PNRSV and ApMV been initially isolated from either hops or roses rather than *Prunus* and *Malus*, respectively, then they would undoubtedly have been considered strains of a single virus (Mink, 1992).

In earlier work we reported that the two ilarviruses which infect citrus shared 71% similarity between their movement proteins while their coat proteins shared 66.5% similarity (Scott and Ge, 1995) and on this basis we suggested that they continue to be considered distinct viruses. PNRSV and ApMV share approximately 71% similarity between their 3a proteins and - 68% similarity between their CPs and as such should be considered to be distinct viruses. However, inspec-

tion of the CP sequences (Figure 3) and the movement proteins (Figure 2) shows large regions of sequence where amino acids occur in common. The occurrence of these regions would support the idea that PNRSV and ApMV, even though distinct viruses, have evolved from a common ancestor.

Moreover, the common aa sequences in the CPs and the area of dissimilarity between the CPs of PNRSV and ApMV at aa 59 – 64 (Figure 2), would offer an explanation for the serological relationships reported between these two viruses. Clearly antibodies binding to epitopes in the disimilar region would show the viruses to be serologically unrelated while antibodies binding to epitopes in the regions with conserved aa sequences would show some degree of serological relationship.

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The generous gift of PNRSV isolates in prune and almond by J.K. Uyemoto is gratefully acknowledged. Sequence data for the RNA 3 of isolate 30/4 have been deposited with GenBank Data Library as Accession No. U57046 and sequences for the isolates Mission, Prune, and SW6 were submitted to GenBank as accessions AF013285, AF013286 and AF013287. This is technical contribution No. 4221 of the South Carolina Agricultural Experiment Station.

References

Barbara DJ, Clark MF, Thresh JM and Casper R (1978) Rapid detection and serotyping of Prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. Annals of Applied Biology 90: 395–399

Bol JF, van Vloten-Doting L and Jaspars EMJ (1971) A functional equivalence of top component RNA and coat protein in the initiation of infection by alfalfa mosaic virus. Virology 46: 73–85

Crosslin JM and Mink GI (1992) Biophysical differences among Prunus necrotic ringspot ilarviruses. Phytopathology 82: 200–206

Francki RIB (1985) The viruses and their taxonomy. In: Francki RIB (ed) The Plant Viruses Vol 1:Polyhedral virions with tripartite genomes (pp 1–19) Plenum Press, New York and London

Fulton RW (1981) Ilarviruses. In: Kurstak E (ed) Handbook of Plant Virus Infections and Comparative Diagnosis (pp 378–413) Elsevier/North Holland Biomedical Press, Amsterdam

Ge X and Scott SW (1994) The nucleotide sequence of citrus leaf rugose ilarvirus RNA-2. Journal of General Virology 75: 2841–2846

Ge X, Scott SW and Zimmerman MT (1997) The complete sequence of the genomic RNAs of spinach latent virus. Archives of Virology 142: 1213–1226

Gonsalves D and Garnsey SM (1975) Functional equivalence of an RNA component and coat protein for infectivity of citrus leaf rugose virus. Virology 64: 23–31

Guo D, Maiss E, Adam G and Casper R (1995) Prunus necrotic ringspot ilarvirus: Nucleotide sequence of RNA 3 and the relationship to other ilarviruses based on coat protein comparison. Journal of General Virology 76: 1073–1079

Hamilton, RIB (1985) Virus transmission. In: Francki RIB (ed) The Plant Viruses Vol 1: Polyhedral virions with tripartite genomes (pp 1–19) Plenum Press, New York and London

Hammond RW and Crosslin JM (1995) The complete nucleotide sequence of RNA 3 of a peach isolate of prunus necrotic ringspot virus. Virology 208: 349–353

Hammond RW, Crosslin JM and Mink GI (1996) Primer design for strain-specific detection of Prunus necrotic ringspot virus by PCR. Phytopathology 86: (Abstract)

Li Q, Ryu KH and Palukaitis P (1996) Cucumber mosaic virus 3a gene: Analysis of site-directed mutants that affect movement, RNA binding and host range. X^{th} International Congress of Virology, Jerusalem, Israel, 11–16 August, 1996, p 34

Mink GI (1992) Ilarvirus Vectors. In: Harris K (ed) Advances in Disease Vector Research 9: 261–281

Mise K, Allison RF, Janda M and Ahlquist P (1993) Bromovirus movement protein genes play a crucial role in host specificity. Journal of Virology 67: 2815–2823

Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP, Mayo MA and Summers MD (1995) Virus Taxonomy: Classification and Nomenclature of Viruses. Sixth report of the International Committee on Taxonomy of Viruses. Archives of Virology, Supplementum 10: 450–457

Neeleman L, van der Kuyl AC and Bol JF (1991) Role of Alfalfa mosaic virus coat protein gene in symptom formation. Virology 181: 687–693

Sánchez-Navarro JA and Pállas V (1997) Evolutionary relationships in the ilarviruses: nucleotide sequence of prunus necrotic ringspot virus RNA 3. Archives of Virology 142: 749–763

Scott SW and Ge X (1995) The complete nucleotide sequence of the RNA 3 of citrus leaf rugose and citrus variegation ilarviruses. Journal of General Virology 76: 957–963

Shiel PJ, Alrefai RH, Domier LL, Korban SS and Berger PH (1995) The complete nucleotide sequence of apple mosaic virus RNA. Archives of Virology 144: 1247–1256

Shintaku MH, Zhang L and Palukaitis P (1992) A single amino acid substitution in the coat protein of cucumber mosaic virus induces chlorosis in tobacco. The Plant Cell 4: 751–757

Thompson JD, Higgins DG and Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680

van Vloten-Doting L (1975) Coat protein is required for infectivity of tobacco streak virus: Biological equivalence of the coat proteins of tobacco streak virus and alfalfa mosaic virus. Virology 65: 215–225

Wisconsin Package Version 8, Program manual (1994) Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA