Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus

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cDNA clones representing the ssRNA genome of the NY-RPV isolate of barley yellow dwarf luteovirus (BYDV) were sequenced and 5600 nucleotides of the genome were determined. The deduced genome organization has limited similarity to that of another BYDV isolate, Vic-PAV, but is identical to that of beet western yellows (BWYV) and potato leafroll (PLRV) luteoviruses. NY-RPV has six major positive-sense open reading frames (ORFs) and, by comparison with RNA-dependent RNA polymerase and nucleic acid helicase consensus sequence motifs, it is postulated that NY-RPV ORF2 and ORF3 encode the viral replicase, which is expressed by a translational frame-shift mechanism. The region of the NY-RPV genome

containing the 22K coat protein ORF, the apparently associated internal apparent VPg ORF and the ORF immediately 3'-proximal (ORF6) to the coat protein ORF are organized as reported for other luteoviruses. Evidence is presented showing that ORF6 is expressed by readthrough of the coat protein gene termination codon, and that this protein is associated with the intact virus as a 65K protein. Although NY-RPV infects graminaceous rather than dicotyledonous plants, the taxonomic relationships between BYDV isolates and other luteoviruses deduced from the genome organization and sequence data strongly suggest that NY-RPV is distinct from the PAV-like isolates of BYDV and is more closely related to BWYV and PLRV.

Introduction

Luteoviruses, such as barley yellow dwarf virus (BYDV), beet western yellows virus (BWYV) and potato leafroll virus (PLRV), cause yellowing diseases in a wide range of host plants (Matthews, 1982), are not mechanically transmitted and are limited to the phloem tissue of the host plant. The physical properties of luteoviruses are similar in that they are 24 to 30 nm diameter isometric particles containing a positive-sense ssRNA genome of 5.6 to 6.0 kb (Mayo *et al.*, 1989; Miller *et al.*, 1988a; Veidt *et al.*, 1988; Waterhouse *et al.*, 1988). The genomic RNA has a small protein (VPg) covalently attached to its 5' end (Murphy *et al.*, 1989) but does not have a poly(A) tail (Mayo *et al.*, 1982, 1989; Miller *et al.*, 1988a; Veidt *et al.*, 1988).

Unlike BWYV and PLRV, which typically infect dicotyledonous hosts, BYDV consists of a group of serologically related viruses that infect barley, oats, wheat, rice, maize and other graminaceous hosts (Rochow, 1970). Like other luteoviruses, they are

transmitted only by aphid vectors in a persistent, circulative manner. Isolates of BYDV, originally distinguished and named for their predominant aphid vectors, have been separated into two groups based on their serological relationships (Aapola & Rochow, 1971; Rochow, 1970; Rochow & Carmichael, 1979; Rochow & Duffus, 1981), the cytopathological ultrastructure of infected cells (Gill & Chung, 1970) and the dsRNA profiles identified in infected tissues (Gildow *et al.*, 1983). Based on these criteria, BYDV group 1 includes serotypes MAV, transmitted by *Macrosiphon* (= *Sitobion*) *avenae* Fabr., PAV, transmitted by *M. avenae* and *Rhopalosiphum padi* L., and SGV, transmitted by *Schizaphis graminum* Rond. (Rochow, 1970). Group 2 includes serotypes RMV, transmitted by *R. maidis* Fitch., and RPV, transmitted by *R. padi* (Rochow, 1970).

To help understand the molecular biology of luteoviruses, the nucleotide sequence and genome organization of a PAV serotype of BYDV (Miller *et al.*, 1988a), and BWYV (Veidt *et al.*, 1988) and PLRV (Mayo *et al.*, 1989; Keese *et al.*, 1990) have been determined. The coat protein genes of these luteoviruses (Miller *et al.*, 1988b; Kawchuk *et al.*, 1989; Prill *et al.*, 1989; Veidt *et al.*, 1988), and of isolates representing the two BYDV groups described have also been identified and characterized

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(Vincent *et al.*, 1990). Here, we present the nucleotide sequence and genome organization of the RPV serotype of BYDV. This information is compared to that for a PAV serotype of BYDV, and also to that published for BWYV and PLRV. Analyses of sequence similarity and genome relationships are presented which indicate that the RPV serotype, although it infects and causes yellowing disease in graminaceous hosts, is more closely related to BWYV and PLRV, which typically infect dicotyledonous hosts, than to PAV serotypes of BYDV.

Methods

Virus and RNA isolation. The BYDV isolate used in this work was the New York RPV isolate of Rochow (NY-RPV) (Rochow, 1970), named according to the suggestion of Rochow (1984) that isolates originating in New York State be prefixed by 'NY'. This isolate was maintained at Purdue University in oat plants (*Avena sativa* L. cv. Clintland 64) by mass transfer of viruliferous *R. padi*; the absence of cross-contamination was confirmed by ELISA as described previously (Barbara *et al.*, 1987). Virus was purified and the RNA extracted as described for the MAV-PS1 isolate of BYDV (Barbara *et al.*, 1987).

cDNA. A cDNA library representing the NY-RPV genome was constructed in λ gt11 (Young & Davis, 1983), pUC18 (Vieira & Messing, 1982) and pGEM-3Z (Promega) (Barbara *et al.*, 1987; Vincent *et al.*, 1990). A restriction map representing the viral genome was generated by single and double restriction enzyme digests of the cloned cDNA, and by Southern blot hybridization between different restriction fragments (Maniatis *et al.*, 1982).

c-DNA sequencing. Plasmid DNA isolated by an alkaline lysis technique (Birnboim & Doly, 1979) was sequenced by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) with a modified T7 DNA polymerase (Sequenase; U.S. Biochemical). The cDNA clones representing the viral genome were sequenced by one or more of the following strategies. (i) Restriction fragments representing overlapping regions of the genome were subcloned; (ii) exonuclease III/mung bean nuclease-generated nested deletions were identified in existing cDNA clones (Putney *et al.*, 1981); (iii) specific synthetic sequencing primers were prepared (Laboratory for Macromolecular Structure, Department of Biochemistry, Purdue University). Sequence analyses were performed using Microgenie version 4.0 (Beckman Instruments) and the Genetics Computer Group sequence analysis software, version 6.1 (Devereux *et al.*, 1984).

Western blot analysis. For Western blot analysis of NY-RPV, purified virus particles were dissociated by the addition of an equal volume of 2 × SDS-PAGE sample buffer (Laemmli, 1970), incubated overnight at 4 °C and electrophoresed on a 10% polyacrylamide gel. Viral proteins were detected immunologically with 0.15% Tween 20 used to prevent non-specific binding (Barbara *et al.*, 1987).

Results and Discussion

Sequencing

Overlapping cDNA clones representing NY-RPV genomic RNA were identified by restriction enzyme analysis and hybridization (Fig. 1). From them, 5600 nucleotides of the NY-RPV genome were determined

Fig. 1. Map of inserts from cDNA clones used for sequencing the BYDV NY-RPV isolate. The location of representative restriction sites within the genome is indicated. pRP cDNA clones used to generate the BYDV NY-RPV sequence are shown below the restriction map. The arrows indicate the length and direction of sequencing of these cDNA clones. A, *AccI*; B, *BamHI*; Bg, *BglII*; E, *EcoRI*; H, *HindIII*; Hc, *HincII*; S, *SmaI*; Ss, *SstI*; V, *EcoRV*; X, *XbaI*.

(Fig. 2), of which 100% was determined from sequencing of both strands and 74% was determined from more than one independently derived cDNA clone. The cDNA inserts in clones pRP11A, pRP23, pRP29, pRP95, pRP104, pRP3-2 and pRP1-8 were sequenced in their entirety, whereas only a partial sequence for the remaining clones shown in Fig. 1 was determined. Clones pRP29 and pRP1-8 represent the extreme 5' and 3' ends of the NY-RPV sequence obtained, respectively; no other clones containing sequences coterminal with those obtained from either pRP29 or pRP1-8 were identified. Therefore, only pRP29 was used to determine the 5'-proximal 366 nucleotides and only pRP1-8 was used to determine the 3'-proximal 107 nucleotides.

Sequencing of more than one independently derived cDNA clone representing several regions of the genome identified six varying nucleotides (Fig. 2). Of these, half were C to U transitions and half were transversions, and three result in amino acid changes (Fig. 2); the other three changes, occurring in the 'wobble' position, would not result in any amino acid change. We do not have any indication whether the observed nucleotide differences are cloning artefacts or whether they represent true variability associated with the viral RNA population from which the cDNA library was constructed.

Open reading frames (ORFs)

A computer analysis of the NY-RPV nucleotide sequence identified six ORFs on the positive-sense strand capable of encoding polypeptides greater than

10K, as well as two on the negative-sense strand (Fig. 3). The amino acid sequences encoded by the six major positive-sense ORFs are indicated within the nucleotide sequence in Fig. 2 and are referred to as ORF1 to ORF6 in order of their proximity to the 5' end of the sequence.

ORF1 begins at nucleotide 115, which also begins the first AUG in the genome, and potentially encodes 256 amino acids of M_r 29263. ORF2 begins with the second AUG in the genome at nucleotide 266 and potentially encodes a polypeptide of 654 amino acids (M_r 70575). Thus, ORF2 overlaps the 3' end of ORF1 by 620 nucleotides. The first AUG of ORF3 begins at nucleotide 1813, which is 213 nucleotides downstream of the previous in-frame termination codon, and could encode a polypeptide of 633 amino acids (M_r 71785). The GDD consensus sequence for RNA-dependent RNA polymerase (Kamer & Argos, 1984) has been identified within ORF3 (see below) and thus ORF3 encodes part of the NY-RPV viral replicase.

The coat protein of NY-RPV is encoded by ORF4 (Vincent *et al.*, 1990). Beginning at nucleotide 3687, this gene is 612 bases in length, terminates with an amber termination codon and encodes a protein of 22190. Located completely within ORF4, but in a different reading frame, is 9459 base ORF which is capable of encoding a protein of M_r 17211 (ORF5). Recently the M_r of the VPg of a different RPV isolate has been determined to be 17K (Murphy *et al.*, 1989). As only ORF5 is capable of encoding a protein of M_r 17K, it is probable that it encodes the NY-RPV VPg. ORF6 is a 1197 nucleotide ORF beginning at nucleotide 4302, immediately after the coat protein gene termination codon and in the same reading frame. The first AUG of ORF6 does not occur until nucleotide 4572.

Western blot analysis of purified NY-RPV virions identified three proteins; Fig. 4 shows a typical analysis. Two smaller proteins were identified with estimated M_r s of 19K and 23K; the largest protein had an M_r of approximately 58K to 63K, and often was not detectable (Fig. 4). Both the ability to detect the largest protein and its size were dependent on how often the purified virus preparation had been thawed and refrozen before analysis. When an NY-RPV preparation had been thawed and frozen five times, the 63K protein could not be detected and a faint protein band with an apparent M_r of 58K was identified instead (Fig. 4, lane 1). When this preparation was frozen and thawed once more, the 58K band could no longer be detected.

Of the smaller proteins identified by Western blot analysis, the 23K protein was predominant and migrated in SDS-PAGE as the authentic NY-RPV coat protein (Vincent *et al.*, 1990); the band migrating as a 19K protein was presumably the VPg. No separate ORF is apparent that would account for the largest protein

observed by Western blot analysis of NY-RPV virions. However, readthrough of the coat protein gene termination codon could produce a protein of M_r 65K. The implication is that ORF4 and ORF6 together can encode a 65K protein by readthrough of the ORF4 stop codon, and that this protein is associated with the viral capsid. Similarly, readthrough of the PLRV coat protein gene termination codon recently has been shown (Bahner *et al.*, 1990).

Non-coding regions

The NY-RPV genome contains 401 nucleotides that are not contained within the six major positive-sense coding regions. These non-coding regions are located at the extreme 5' and 3' ends of the genome, and 5' to the coat protein gene (ORF4). We have no evidence that these 5'- and 3'-terminal sequences represent the actual termini of NY-RPV. We identified 114 nucleotides in the 5'-proximal non-coding region and 102 nucleotides in the 3'-proximal non-coding region. Computer analysis of the 3'-terminal non-coding region did not identify any potential tRNA-like structures. For PLRV and BWYV, luteoviruses with genomic organization similar to that of NY-RPV, there are 141, and between 146 and 197 3'-terminal nucleotides, respectively (Keese *et al.*, 1990; Mayo *et al.*, 1989; Veidt *et al.*, 1988). A comparison between the 5' non-coding regions of these luteoviruses showed that although 114 nucleotides were identified at the 5' end of NY-RPV, the length of this region is variable; isolates of PLRV have between 32 and 174 (Keese *et al.*, 1990; Mayo *et al.*, 1989), and BWYV has 31 nucleotides at the 5' terminus (Veidt *et al.*, 1988). However, for these luteoviruses, and also for Vic-PAV (Miller *et al.*, 1988a), a consensus hexanucleotide sequence, AC/UAAAA/C, has been identified in these 5' non-coding regions. This sequence is also found in southern bean mosaic virus (SMBV) (Keese *et al.*, 1990; Wu *et al.*, 1987).

Nearly half of all the non-coding nucleotides in NY-RPV are located between ORF3 and ORF4, i.e. 5' to the coat protein gene. This region is A-U-rich, with 58% of the 185 nucleotides being A or U residues. Uridine forms 42% of the 113 nucleotides immediately upstream of the coat protein gene initiation codon, and A and U residues together make up 62% of the nucleotides in this region. A comparison of similar regions from other luteoviruses indicates that there is a core region consisting of 113 nucleotides upstream of the coat protein gene initiation codon (Fig. 5). This core region contains the identifying features of subgenomic promoters defined for brome mosaic virus (BMV) (Marsh *et al.*, 1988). There is extensive nucleotide identity between the core regions of NY-RPV, BWYV

1 CGGCUCCAAAGCUUAAGCUCACUGUUCGCGCGUGUUCGACGCUUCGACGACAGCCCAUAAACCCGCCUCCGCUUAGCGAUCAACUCGCGUGUUCGUUAUUAACGCAUGUUC * F
ORF1

121 AUCGCCCAACCUUGCGGGCGAGUUCUUGUGUUGACGUCGCCUCCACAGCGCAUCGUUCUACUCGUUAUAGUGUUGAACUCUCGUCUCCGUGUCUAGAUCCACUCUUCACGCGAGCA
ORF1 I A Q P C G R V L V F D V A S H T P S F F T R Y S V E L S L R A L D P L F T R A
ORF2

241 GUAACAGAUUUCGGAUACACCCAAAAGUAAUUGAUUUUUUGUGUGUCUUGGCUUUCUGUCCAAUUCUCCUCACAGGAGAAUUCUACUUCUGGCGCGGUCACCUUAAACCUCCCC
ORF1 V T D F R Y T Q N E I D L F C V S L G F L L P I L L T G E S Y S W R G H L N L P
ORF2 * K S I Y F V C L L A F C C Q F S S Q E N L T L G A V T L T S P

361 CUUUCUUAACACCGAACUACUUGGCGGGGCGAGUCGUGGGGUACUUCUCCGCUUCCGUGUAGUGGAGUACUUCGACGAAACCCAGAACUCCGCAUGACCUCCACCAUGUCA
ORF1 L S Y T E L L V R W G G L A V G Y F P A F S A D G D I R Q N P E L R I D L S T M S
ORF2 F L T P N Y L F D G G S Q W G T S L P S P L M V T F D R T Q N S A S T C P P C Q

481 ACCCGCUCUUUCACGAGCAGUUCUACUCAGAUUAUUAACAAGUGGGUUGGCAAAAGCUAUCGUUGGACGACGAAGAGUCUUAUAAAGCGGCAUGAGUCUUUAAAGAUUCCUGCA
ORF1 T R S F Y E Q F L L R Y N T S G L A K A I V G Q K E C F Q S G M E S F K R F L H
ORF2 P A L S T S S S Y S D I I Q V G W Q K L S L D S Q S A F K A A W S L K D S C T

601 UACCGCCUCACGUGCUUUGAAAGCUGCCUUCACGACCUCGUUGGAAAGCCCUUUGGCUCCUGGUCUUAUCUGGACGAGGCUUUGAGGCAACUCUUCUGGCGCGUAGUGGCGGCCAU
ORF1 Y R L T C F E S C L P R P R W E S P L A P G P Y L D R A F E A T L L G R M V G H
ORF2 T A S R A L K A A F H D L V G K A L W L L V L I W T G L L R Q L F S A V W S A I

721 AACCAACUACUUAUACCGGUUUGUCUUCUGAUUACUAGGUUAUUAACAGUUGGUUGUGGAAAGCGUGCGGUGCUUUUUGGAGCGUGCCGGCAUUAUUGCAUCACGCGGU
ORF1 N Q L F L T R L S S D I T R Y L Y N T S G L A V G Y F P A F S A D G D I R Q N P E L R I D L S T M S
ORF2 T N Y S L P V C L L I S L G I I T S W L W K A C R W L F G T L P A L L C I T L V

841 GAAGAAUUAUUCGAAUUCUUAACAUUCAAAGAUUUCUCAAUGAGAAACUGUCUGAGGUUAUGACAGUUAUUCGAUUCUCCAGCACCCUCCGAAAGCGGAGCGUCAUCAUGAUGCGAGC
ORF1 E E Y F P N S Y I Q K I L Q +
ORF2 K N I F R I L T F K R F F N E K T V S G Y D S Y S I P S T P P K R S V I M M R R

(C)
961 CCAAAAUAAAGAGCACAUUGGAUAUGCGAACUGUAUUCGUCUUCUUCGACGAGCGAAAGCUUUGUACAGUUGCUCACAAUUAUAGAGGAGGGUUGCUUUCUUAUUCUACCGACUAG
ORF2 Q N K E H I G Y A N C I R L F D G R N A I V T V A H N I E E G C S F Y S S R T S
(P)

1081 UGUUCCAUCCCAUACAGAAUUCGCGUUAUCUUCGAAAGCAAACCAUGGAUUCGCGAUUCUGGUGGACCAUAAUUGGAAUUGAUUCUGGAGUCAAAGGAGUCCAUUUAC
ORF2 G S I P I T E F R V I F E S K T M D I A I L V G P I N W E S I L G C K G V H F T

1201 CACCGCUGACCGCUUAGCAGAAUGUCCAGCGGCUUUAUCUUCUGAUAGCGAUGGUCAGUGGCGCUCAAACUGGCGAAGAUUUGUGGCUUAUUCGACAACUUCGCCAACUGUCUG
ORF2 T A D R L A E C P A A L Y L D S D G Q W R S N S A K I C G H F D N F A Q V L S

(U)
1321 CAACACUAAAGUCGGUACUCCGUGGCGGUAUCUUAUGGCAAGACUUCUGUUGGCCUCCAAUAAAGGCCAUCCUGGCAAGAAUUAUAAUUAUUGAUGGCUCCUUGCGCAGAAU
ORF2 N T K V G H S G A G Y F Y G K T L V G L H K G H P G K D F N F N L M A P L P G I

1441 ACCCGCUGACUCCCGCGAGUACGUGGAAUCCGACCCCGCCAGGGAUUGGUUUUCCCGAAGAGUCACUGAAUCAAUUGAAGCGCGAUUAGGAGGCAACAAUGUACAAGAA
ORF2 P G L T S P Q Y V V E S D P P Q G L V F P E E V T E S I E A A I K E A T M Y K N

1561 UGUUCUCCGCAUAGAGGAGCUGGCGCCUUAUAAUUAUAAAGCGGCAUACUUGGAAAGACUAGAACGACGAGUCGGGAAACGGGAAGCGGCGGCGCGGCGGUAACAAACGACGAGC
ORF2 V F A N R G R G A F K S K S G I N W E D I E D S G N G K A A A S A V T N A A A
ORF3 K R H Q L G R H R R R V G K R E G G G V R R N K R S S

1681 GGCAACAAAGGUUAUCGCGACCCCGCGGCGUCCGCAAGUCCCAAAAGAAAGACUGUGUCCUUCUUCGCGGAAGGACCGGACCGCCCGCGUGCAUCCCAACCCACGACCAACUGGGAG
ORF2 A N K V I A T P G V A K S Q K K T A V P S S P K A P Q P P A A S Q P T S T T G S
ORF3 G K Q G Y R G R R R Q V P K E D C C S F F A E G T A A P R C I P T H Q H N W E

1801 CAGAACUCCUUAUUGCAUUGUACUGCAACAUCCCGGACACGGCUGGCCUACUGGACUACGCGAGCAAGAAUUAUAGAACAAUUAUUGAUGGCUCCAGAGGAGCAUUAU
ORF2 R T P I C P I A T S P D T A V P T G P S Q Q E I M N N I M N L L V Q R I D M
ORF3 Q N S Y M S N C Y C I P G H T G C A Y W T I A A R N H E Q H N E S A G P E D R Y

1921 GUCGAAGACUGGAAUUGCAUUGGAGCAGGAGUCCGCGAAUUAAGCUCUGAAGAAACACGCGGCAAGCGGUGGCUCAAAAGAAACGGCCCGCAGCUGGCAAAAGUUCUUCGCGCAUCUAC
ORF2 S K I E K S I V D Q V A N Q A L K K P R G K R G S K K R P A A G K S S S P T S T
ORF3 V E D R E I D S C P S R E S S S E E T T R Q C A W L K E T A R S W Q K F F A D I Y

2041 ACCUGGACGUAUACAACAAACAAAGUCCAGGCUUUGGAAUUCUCCCGGACGUAUCCCGGCGCCCGCAGGCAAGUUGGGGAGAAACUUGUGCC
ORF2 P G T Y Q H P N K G S A S N R L S T E D Q V S K F M D V L A G N V D A G I T L H A G E N S V P
ORF3 T W D V S T S K Q E V P G F E Q V G K F S P Q Y Y P R P R S E S E W G R K L C A

2161 GAACACCCUUCUUGGGUGAGAAAACUGCGGUUUCGGUGGCCCAAGUCGGGCAUCCGCUAAGCUGACAUUCUUCGCGCAGAGCAGCUGGCGUCGACGCUUCCGAGUCAGCU
ORF2 N T L S W V R K L P V S G G P K S G H P L N +
ORF3 E H P L L G E K T A G Y F G W P Q V G A S A E L T S L R L Q A A R W L E R S E S A

(U)
2281 AAAUUCACUACAGUUGCUGUAGAGAGAACGUGAUCAAUUCGACCGGUACAGGCGUACUGAAUUGCAAACCAUACGCGGAGGUGCACACAGGUGAGUUAAGUUGGAAACGUUCAA
ORF3 K I P S D A A R E N V I N R T V Q A Y S N C K T N T P R C T R G G E L S W E T F K

2401 AUUGACUUCUUGAAGCCCAUAAACGCUCCAAUCUGACGCGUGGCUUGGAAUACCGGAGUACUCCGCGGCUCCACCCACUUGGUGGUGGAGACCCAGAUUGGUUUGCCAGUG
ORF3 I D F L E A I K S L Q L D A G V G L P M I T A G I T P T H R G W V E D P D R L P V

2521 UUAGCUCAGCUUACCUUUGACCGCCUACUUAUGCAUGUCAAAGGCAAGCUGGAGCUCUCCCGGAGCAACUGUGAAGGAGAAUUCUGUGAUCCUUAUUCGUCUUCUUGAAGAAACAA
ORF3 L A Q L T F D R L L T M S K A S L E A R S P E Q L V K E N L C D P I R L F V K Q

(U)
2641 GAGCCACAUAAACAGAGUAAAGCUUGAGAGGAGCUUACCGCCUACUACUGUACGUCUUCUUGAUUGAUAUUGGAGCCCGGUGUUUGUUUCAAAGACAAAACAAUCCGAAAUCCGG
ORF3 E P H K Q S K L D E G R Y R L I M S V S L I D Q L V A R V L F Q R Q N K S E I A

2761 UUGUGGACGCGGAUCCGCAAAAACCGGUUUGGAAUUAUCCACCGAGGACCAAGUUAUCAAUUAUUGAUGUCCUCCGCGGGAACGUGGUGGUGUCCGCGGAGGUGGCGGAGCAU
ORF3 L W S A I P S K P G F G L S T E D Q V S K F M D V L A G N V D A G I T L H A G E N S V P
ORF3 L W S A I P S K P G F G L S T E D Q V S K F M D V L A G N V D A G I T L H A G E N S V P

Fig. 2. Nucleotide sequence (RNA form) of the BYDV NY-RPV isolate. The initiation codons for the six major positive-sense ORFs are indicated by an asterisk and the termination codons by +. The deduced amino acid sequence is indicated below the nucleotide sequence. Nucleotides which varied when identified from the sequencing of different clones are indicated in parentheses above the nucleotide sequence; associated changes in the deduced amino acid sequence are indicated in parentheses below the amino acid sequence.

Fig. 3. ORFs determined from the positive- and negative-sense orientation of the BYDV NY-RPV nucleotide sequence. Vertical ticks above a line indicate the location of initiation codons; those below a line indicate the location of termination codons. The open boxes represent ORFs and the six major positive-sense ORFs are numbered according to their proximity to the 5' end of the genome. All ORFs are represented as beginning with an AUG initiation codon.

Fig. 4. Immunological detection of the virion-associated proteins from the BYDV NY-RPV isolate. Purified NY-RPV virions were dissociated and electrophoresed on a 10% polyacrylamide gel. After electroblotting onto nitrocellulose, virus-associated proteins were detected immunologically using a polyclonal anti-NY-RPV primary antiserum and an alkaline phosphatase-conjugated secondary antiserum. Sizes of proteins identified from purified NY-RPV preparations are shown to the left, those of protein size standards to the right. Lane 1, purified NY-RPV thawed and frozen five times; lane 2, purified NY-RPV thawed only once.

Fig. 5. Comparison of luteovirus non-coding regions located 5' to the coat protein coding region. Asterisks represent nucleotides identical in the sequences of BYDV NY-RPV isolate, BWYV and PLRV. A vertical bar represents nucleotides identical in all the luteoviruses examined. Numbering is in a direction 5' to the putative coat protein gene initiation codon. Boxed regions represent regions conserved in luteoviruses. RPV, BYDV NY-RPV isolate; BWYV, BWYV FL1 (Veidt *et al.*, 1988); PLRV (Mayo *et al.* 1989); BYDV MAV (P. P. Ueng & R. M. Lister, unpublished results); BYDV PAV (Miller *et al.*, 1988a; P. P. Ueng & R. M. Lister, unpublished results).

and PLRV, and two highly conserved regions found in all luteoviruses sequenced. The high degree of nucleotide conservation, and the presence of A-U-rich tracts and AUCA and AAGA sequence blocks similar to those of BMV subgenomic promoter sequences, suggest that this region may be involved in the formation of subgenomic dsRNAs and/or the regulation of specific protein messages. In fact, the transcriptional start site of a 2.6 kb subgenomic RNA from PLRV has recently been mapped to 34 bp 5' to the coat protein gene translation initiation codon (Tacke *et al.*, 1990), which corresponds to the larger of the two conserved regions. The size of the dsRNAs identified in oat plants infected with NY-RPV (Gildow *et al.*, 1983) is consistent with this region having a role in subgenomic RNA formation.

Comparison with other luteoviruses

The NY-RPV sequence is the second BYDV genome sequence to be reported. The genome of a BYDV PAV serotype from Victoria, Australia, Vic-PAV, has been determined to consist of 5677 nucleotides (Miller *et al.*, 1988a) and encodes six positive-sense ORFs. The genome organizations of NY-RPV and Vic-PAV show significant differences (Fig. 6). Although the RNAs of both contain approximately 5600 nucleotides, there is no similarity in genome organization and little sequence similarity in regions of the genomes 5' to the coat protein genes. The coat protein gene itself is located 3'-proximal in NY-RPV, whereas it is near the centre of the genome of Vic-PAV.

Fig. 6. Genome organization of BYDV NY-RPV and Vic-PAV isolates. The location of major ORFs of NY-RPV and Vic-PAV (Miller *et al.*, 1988a) and the reading frames in which they occur are represented by boxes. The sizes of the polypeptides potentially encoded by each ORF are indicated.

However, similarities between Vic-PAV and NY-RPV are found in the 22K ORFs, in the internal 17K ORFs and in the immediately 3'-proximal ORFs (i.e. NY-RPV ORF6 and Vic-PAV ORF5). The 22K ORFs are the coat protein genes and contain the presumptive 17K VPg gene as an internal ORF. This organization has been found in all luteoviruses examined to date (Vincent *et al.*, 1990). Nucleotide and deduced amino acid sequence identities between NY-RPV and Vic-PAV ORFs determined using Microgenie sequence alignment software were 55.2% and 48.8% for the coat protein coding region, 51.7% and 34.7% for the VPg coding region, and 44.6% and 32.2% for the full ORFs located immediately 3' to the coat protein coding region. The ORF 3'-proximal to the NY-RPV coat protein ORF is proposed to be expressed by readthrough of the coat protein gene termination codon, as with a similar ORF of Vic-PAV and, indeed, with luteoviruses in general. A conserved 14 nucleotide sequence surrounding the luteovirus 22K coat protein gene termination codon has been identified previously, and a role for this sequence in the expression of the readthrough ORF postulated (Smith & Harris, 1990; Veidt *et al.*, 1988). An examination of this region from NY-RPV indicates that this conserved region is actually the 12 nucleotide sequence AAAUAGGUAGAC, with the coat protein gene termination codon indicated by the underlined nucleotides.

The genome organization of NY-RPV, although it differs from that of Vic-PAV, is very similar to that reported for both BWYV (Veidt *et al.*, 1988) and PLRV (Keese *et al.*, 1990; Mayo *et al.*, 1989). Furthermore, the sizes of the putative protein products encoded by each ORF of the latter viruses are similar to those encoded by the ORFs of NY-RPV.

There is considerable interest in evolutionary relationships between viruses based on amino acid sequence motifs associated with the RNA-dependent RNA polymerases (viral replicases) (Habibi & Symons, 1989; Kamer & Argos, 1984; Poch *et al.*, 1989) and nucleic acid helicases (Gorbalenya *et al.*, 1988; Gorbalenya & Koonin, 1989; Habibi & Symons, 1989; Hodgman, 1988a, b). These relationships are based both on sequence similarity within motifs and on the distance separating motifs. Using such a classification scheme, positive-sense RNA plant viruses have been placed into three supergroups, with the luteoviruses in supergroup B (Habibi & Symons, 1989).

The Vic-PAV replicase shows substantial similarity to the replicase of carnation mottle virus (Miller *et al.*, 1988a), and the replicases of BWYV and PLRV show homology to that of SBMV (Mayo *et al.*, 1989; Veidt *et al.*, 1988), all members of supergroup B. A BESTFIT comparative analysis (Devereux *et al.*, 1984) of the amino acid sequence deduced from NY-RPV ORF3 with that of the putative replicase-encoding region of SBMV (Wu *et al.*, 1987) has identified a region containing the GDD amino acid motif for RNA-dependent RNA polymerases (Kamer & Argos, 1984). This region is also similar to regions of both PLRV and BWYV (Fig. 7). The four amino acid sequence motifs of supergroup B positive-sense RNA viral replicases (Habibi & Symons, 1989) (VPTDCSGFDWS-53-GVQKSGSYNTSSTNSRV-11-WAIAMGDDALEA-22-FCSRIF) were also identified within this region (Fig. 7), although the last motif was not represented in SBMV. The NY-RPV replicase motifs showed greater similarity in both sequence and gap distances to comparable regions in BWYV and PLRV, than to those in Vic-PAV.

Seven amino acid sequence motifs have been identified for proteins that have helicase functions (Habibi & Symons, 1989; Hodgman, 1988a, b), and have been used to evaluate the taxonomic relationships between luteoviruses and other positive-sense RNA plant viruses (Habibi & Symons, 1989). The NY-RPV sequence contains three of the seven helicase motifs. These regions resemble the 'A' and 'B' sites of the catalytic site of nucleoside triphosphate-utilizing enzymes (Gorbalenya *et al.*, 1988; Gorbalenya & Koonin, 1989) and supergroup B motif VI (Habibi & Symons, 1989). The potential 'A' and 'B' sites are located in ORF2 (356-AGYFY-GKT-41-LVPFEE-240), and motif VI has been identified in ORF3 (351-QSKLDEGRYRLIMSVS-267). The NY-RPV helicase motifs were compared with those from other luteoviruses (Habibi & Symons, 1989). Motif VI has been found in all luteoviruses, but although all other luteoviruses contain motif IV, no such region was identified in NY-RPV. Regions comparable to the 'A' and 'B' motifs were not identified in PLRV or Vic-PAV,

RPV	326	ARSPEQLVKENLCDPIRLFKVQEPHK	QSKLDEGRYRLIMSVS	LIDQLV
PLRV	308	DMSAEELVQEGLCDPIRLFKVQEPHK	QSKLDEGRYRLIMSVS	LVDQLV
BWYV	276	DMGPEELVRNGLCDPIRLFKVQEPHK	QAKLDEGRYRLIMSVS	LVDQLV
RPV	326	ARSPEQLVKENLCDPIRLFKVQEPHK	QSKLDEGRYRLIMSVS	LIDQLV
SBMV	600	ALSPTMVEMLGCDPVLFVKQEPHK	SRKLKEFRYRLISSVS	IVDQLV
Vic-PAV	121	SAGKRAMYHKAISLKTVPYHQDAN	VQAFLLKKEKHWMTKDI	APRLIC
RPV	374	ARVLFORQNKSEIALWSAIPSKPGFGLSTEVQVSKFMDVLAVNGVGSPEE		
PLRV	356	ARVLFORQNKREISLWRSVPSPKPGFGLSTDTQTAEFLCQLQVSGA. PEE		
BWYV	324	ARVLFORQNKREIALWRAIPSKPGFGLSTEQVLDVESLARQVGTITTE		
RPV	374	ARVLFORQNKSEIALWSAIPSKPGFGLSTEVQVSKFMDVLAVNGVGSPEE		
SBMV	648	ERMLFGAQNLEIAEWQSIPIPKPGMGLSVIHQADAFDRLRVXHTVCPAA		
Vic-PAV	169	PRSKRYNIILGTRLKFNEKKIMHAIDSVEGSPTVLSGYDNFKGRIIAKK		
RPV	424	VCDNWRDLL	VPTDCSGFDWS	VSDWMLADDMEVRNRLTIDCNELTRHLR
PLRV	406	LCANHKEYT	RPTDCSGFDWS	VRYWMLDEDDMEVRNRLTFNNQLTKRLR
BWYV	374	VVANWKNYL	TPTDCSGFDWS	VADWMLHDDMIVRNRLTIDLNPAERLR
RPV	424	VCDNWRDLL	VPTDCSGFDWS	VSDWMLADDMEVRNRLTIDCNELTRHLR
SBMV	698		EADISGFDWS	VQDWELWADVEMRIVLGSFPPMARAAR
Vic-PAV	219	WQKFACFVA	IGVDASRFQDQ	VSEQAQKWEHGIYNGIFGDSEMALEH
RPV	472	AVWLQGISNSVLCLSDGTMLAQRPV	GVOKSGSYNTSSTNSRV	RVMAAY
PLRV	454	AAWLKICISNSVLCLSDGTMLLAQTPV	GVOKSGSYNTSSNSRI	RVMAAY
BWYV	422	SCWLRICISNSVLCLSDGTMLLAQH	GVOKSGSYNTSSNSRI	RVMAAF
RPV	472	AVWLQGISNSVLCLSDGTMLAQRPV	GVOKSGSYNTSSTNSRV	RVMAAY
SBMV	736	N.RPSCFMNSVLQSLNGQLQQLP	GIMKSGSYCTSSSTNSRI	RCIMAE
Vic-PAV	267	QITNNIKMFVEDKMLRFVRGRHMS	GDINTSMGNKLIMCGMM	HAYLKK
RPV	520	HCGAS	WATAMGDDALEA	.PDTLSKYKDLGFKVEVSGELE
PLRV	502	HCGAD	WAMAMGDDALEA	.PNSDLEEKTLGFKVEVGRELE
BWYV	470	HTGAI	WAMAMGDDALES	.NPADLAAYKKGFKVEVSGELE
RPV	520	HCGAS	WATAMGDDALEA	.PDTLSKYKDLGFKVEVSGELE
SBMV	783	LIGSP	WCIAMGDDVEG	FVEGAREKYAGLGHLDYKPCPA
Vic-PAV	315	LGVEA	ELCNGGDDCTII	TDRANEKLFDMYHFLQYGFNM

Fig. 7. A comparative analysis of luteovirus putative replicase amino acid sequences. An amino acid sequence from the putative BYDV NY-RPV replicase was identified by BESTFIT analysis (Devereux *et al.*, 1984) with the SBMV putative replicase amino acid sequence. The NY-RPV region identified was then used in BESTFIT analyses with the amino acid sequence of the putative replicases from BWYV and PLRV. The NY-RPV sequence is represented twice to allow direct comparison with other viruses in the analysis. The comparable region from BYDV Vic-PAV (Miller *et al.*, 1988a) has been included for comparison. Vertical dashes represent identical amino acids and vertical dots indicate chemically related amino acids. Asterisks represent the consensus sequence of RNA-dependent RNA polymerases (Kamer & Argos, 1984). Boxed regions represent the helicase and replicase motifs identified in NY-RPV ORF3. The Vic-PAV helicase motif VI is located between amino acids 173 and 188. The numbering represents the amino acid position from the beginning of the ORF in which the GDD sequence is located. RPV, NY-RPV isolate; PLRV (Mayo *et al.*, 1989); BWYV (Veidt *et al.*, 1988); SBMV (Wu *et al.*, 1987); Vic-PAV (Miller *et al.*, 1988a).

but similar sequence and gap lengths were found in BWYV (356-SPYFNGKT-38-VFAQED-194) (Veidt *et al.*, 1988).

It has been proposed that the luteovirus viral replicase is expressed as a result of a translational frameshift allowing two overlapping ORFs to produce one protein (Keese *et al.*, 1990; Mayo *et al.*, 1989; Miller *et al.*, 1988a; Veidt *et al.*, 1988). As both ORF2 and ORF3 within the NY-RPV genome are associated with helicase function, it seems probable that both the helicase and the viral replicase are expressed by a translational frameshift mechanism. For the luteovirus Vic-PAV, a region for site-specific ribosomal slippage has been identified within the 16 nucleotide overlap between these ORFs. For other luteoviruses, including NY-RPV, the region of overlap is considerably greater, including up to several hundred nucleotides, but contains no obvious site for ribosome slippage.

Taxonomic considerations

The genome of the NY-RPV isolate of BYDV shares significant properties with other luteoviruses. These include (i) a replicase-encoding region presumably expressed by translational frameshift, (ii) an ORF, presumably encoding the VPg, completely contained within the 22K coat protein gene and (iii) a highly conserved nucleotide sequence surrounding the 22K coat protein gene termination codon, which potentially allows the formation of a larger fusion protein. There is also considerable similarity between all luteovirus 22K coat proteins, although the NY-RPV coat protein is more like those from BWYV and PLRV than those from the MAV-PSI and P-PAV isolates of BYDV (Vincent *et al.*, 1990). The greater similarity between the NY-RPV coat protein gene sequence and those of BWYV and PLRV, the similarity between the genome organization of NY-RPV, BWYV and PLRV, and the relative dissimilarity between the genome organization of NY-RPV and Vic-PAV are all consistent with the hypothesis that NY-RPV is a strain of BWYV (Casper, 1988). Hence, NY-RPV should be classified as distinct from BYDV strains similar to Vic-PAV. However, differences in aphid vectors (i.e. *R. padi* and *Myzus persicae*) and host range (i.e. monocotyledonous and predominantly dicotyledonous hosts), suggest that NY-RPV should also be classified as distinct from BWYV and PLRV.

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