Genome organization of a partitivirus from the filamentous ascomycete *Atkinsonella hypoxylon*

Chan-Seok Oh and Bradley I. Hillman*

Department of Plant Pathology, Rutgers, University, New Brunswick, NJ 08903, USA

We have identified viruses in several isolates of the filamentous ascomycete *Atkinsonella hypoxylon*. The virus from one isolate of the fungus, 2H, was selected for genomic characterization. Purified virus particles contained three dsRNAs with sizes estimated by gel electrophoresis to be 2·2, 2·1 and 1·8 kb. A library of cDNA clones representing the three dsRNA segments of isolate 2H was synthesized, mapped and sequenced. The three segments had no significant similarity to each other, as determined by Northern blot analysis, and had sizes of 2180, 2135 and 1790 nt as determined by nucleotide sequence analysis. Long open reading frames were deduced from the sequences of dsRNAs 1 (molecular mass 78 kDa) and 2 (74 kDa), but not from dsRNA 3. Both terminal regions of dsRNA 1 and dsRNA 2 had similar nucleotide sequences, as determined from 5′ RACE clones. Comparisons of the amino acid sequence deduced from dsRNA 1 revealed similarities with viral RNA-dependent RNA polymerases. Translation *in vitro* of full-length cDNA clones representing dsRNAs 1 and 2 each yielded single major products of > 70 kDa by analysis on polyacrylamide gels. Based on properties of its dsRNA segments, the virus of *A. hypoxylon* strain 2H fits into the *Partitiviridae* family, and represents the first member of this family from a fungal host completely characterized at the level of primary nucleotide sequence.

Introduction

Atkinsonella hypoxylon (Peck) Diehl is a filamentous ascomycete that belongs to the tribe Balansieae (family Clavicipitaceae) and causes choke disease on several grasses, including *Danthonia spicata* (L.) Beauv. (poverty oatgrass), *D. compressa* Austin and *D. sericea* Nutt (Diehl, 1950; Leuchtmann & Clay, 1988, 1989*a*, *b*). The fungus grows epiphytically and systemically around the meristems, young leaf bases and inflorescences of its host grasses (Leuchtmann & Clay, 1988). Although *A. hypoxylon* causes choke disease, it often enhances vegetative growth of infected grasses, probably due to diversion of energy from flowering (Clay, 1984) or because of alkaloids produced by the fungus.

dsRNA genetic elements are commonly found in filamentous fungi, either contained in true virus particles or within pleomorphic lipid vesicles (Lemke, 1979). dsRNA segments of mycoviruses range in size from 0·4 to > 10 kb and their capsid polypeptide molecular masses range from 18 to 125 kDa (Buck, 1986). Most isometric dsRNA-containing mycoviruses have capsids composed of one major polypeptide species (Buck, 1986). It is generally assumed that the viral RNA-dependent RNA polymerases (RDRPs), which are present in the virions of dsRNA-containing mycoviruses, are also virus-encoded (Buck, 1979). Isometric dsRNA-containing mycoviruses have been grouped into two virus families, the *Totiviridae* and *Partitiviridae*, based primarily on genome structure and organization (Buck & Ghabrial, 1991 *a*, *b*).

Members of the *Totiviridae* have a single dsRNA segment in the size range 4·6 to 6·7 kb and a single capsid polypeptide of 73 to 88 kDa (Buck & Ghabrial, 1991*b*). *Saccharomyces cerevisiae* viruses L1 and L-A are the most thoroughly characterized viruses in this group. Several complete totivirus sequences have been published, and their genome expression strategies have been examined in considerable detail [reviewed in Wickner (1992) and Ghabrial (1994)].

Members of the *Partitiviridae* have virions containing two monocistronic dsRNA segments, usually of similar size (1·4 to 3·0 kb) and a single capsid polypeptide in the molecular mass range 42 to 73 kDa (Buck & Ghabrial, 1991*a*). Little information is available on the genetic organization of partitiviruses, as no complete partitivirus sequences have been reported. Based on *in vitro*

* Author for correspondence. Fax +1 908 932 9377. e-mail hillman@mbcl.rutgers.edu

Sequences appearing in this paper have been submitted to the GenBank database and assigned the accession numbers L39125 (Segment 1), L39126 (Segment 2) and L39127 (Segment 3).

0001-2966 © 1995 SGM

translation studies, it was determined that one dsRNA segment of *Gaeumannomyces graminis* virus encodes the capsid polypeptide and the other segment encodes an unrelated polypeptide (Romanos *et al.*, 1981). Similar results were reported for the *Rhizoctonia solani* strain 717 virus (Tavantzis & Bandy, 1988).

In a study to screen and characterize extrachromosomal genetic elements of fungal endophytes and closely related epiphytes of turfgrass species, we identified several viruses of *A. hypoxylon*. This paper describes the genomic characterization of one of the viruses.

Methods

Fungal strains. Isolate 2H of *A. hypoxylon* was collected by J. F. White (Auburn University) from stromata on the flowering culms of infected *D. spicata* in Piscataway, New Jersey during the summer of 1990. This and other fungal strains were maintained by continuous subculture on potato dextrose agar (PDA) at room temperature.

dsRNA isolation, cloning, mapping and sequencing of clones. dsRNA was isolated essentially as described by Morris & Dodds (1979) directly from cultures grown on PDA with cellophane between the mycelium and agar. Confirmation of dsRNA composition was by nuclease treatment essentially as described previously (Hillman *et al.*, 1990). Nuclease treated samples were examined on 1% agarose gels. Complementary DNA (cDNA) cloning, examination of clones and sequencing were performed as described previously (Hillman *et al.*, 1992). Briefly, template dsRNA was purified through CF11 chromatography columns, concentrated, and denatured in 90% DMSO. Randomly primed first- and second-strand DNAs were synthesized by MMLV reverse transcriptase and DNA polymerase I. cDNAs were tailed with dCTP, annealed to the dG-tailed vector pUC9 and transformed into *E. coli* strain DH5α cells. Ampicillin-resistant transformants were screened by blue/white colour reactions on X-Gal plates. Clones were mapped using Northern blot, Southern blot and restriction enzyme analysis. Terminal sequences of segments were identified by analysis of 5′ RACE clones (Frohman *et al.*, 1988) with reagents purchased from Life Technologies Inc. Internal primers that were segment- and strand-specific were used to initiate cDNA synthesis from individual dsRNA segments. After tailing with dATP or dCTP, cDNA was amplified by PCR from a second internal primer and the appropriate homopolymeric (dT or dG) anchor primer. Amplification products were cloned into the vector pGEM-3Zf+ and clones were sequenced. DNA sequencing was done by dideoxynucleotide chain termination (Sanger *et al.*, 1977) with [α-^{35}S]dATP and Sequenase (US Biochemical Corp.) (Tabor & Richardson, 1987). Sequence analysis was performed with the aid of the programs Microgenie (Queen & Korn, 1984), CLUSTAL V (Higgins *et al.*, 1992) and PHYLIP (Felsenstein, 1989). Sequence comparisons were done with the GenBank and PIR databases.

In vitro transcription and translation. Full-length cDNA clones representing dsRNA 1 (nt 13–2081) and 2 (nt 13–2025) were assembled from several overlapping cDNA clones. Near full-length clones were inserted into the expression vector pTL7SN.3, which has an SP6 transcriptional promoter, the tobacco etch virus (TEV) leader for translation enhancement, and poly(A)-containing terminal sequences (Oh & Carrington, 1989). Transcription with bacteriophage SP6 polymerase and translation in rabbit reticulocyte lysates (Promega) were conducted essentially as described by Carrington & Dougherty (1987). Plasmids were linearized with *Sal*I, which cleaves downstream of the TEV terminal sequence, prior to transcription. Transcripts were translated at 30 °C for 1 h in rabbit reticulocyte lysates in the presence of [^{35}S]methionine, and the radiolabelled products were analysed by SDS–PAGE (Laemmli, 1970) and autoradiography In pulse-chase analysis, [^{35}S]methionine was added at the beginning of the translation

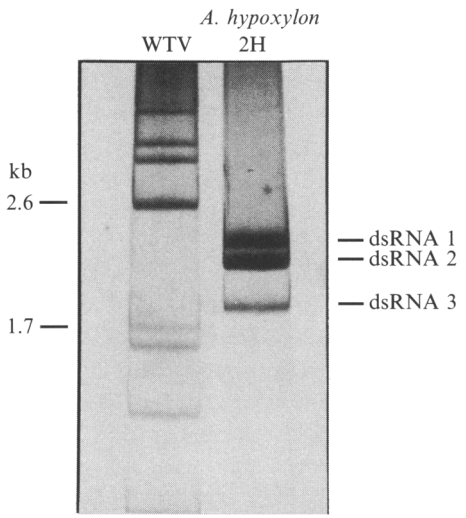

Fig. 1

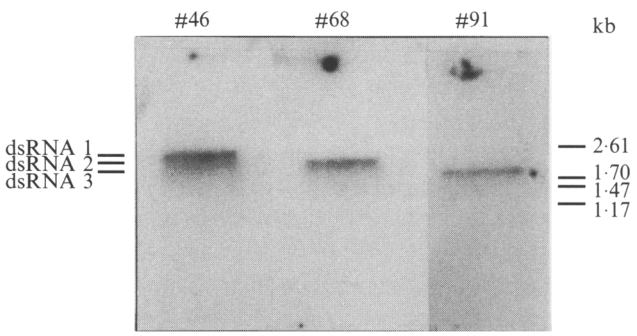

Fig. 2

Fig. 1. dsRNAs extracted from isolate 2H of *A. hypoxylon*. dsRNA was isolated directly from fungal tissue as described in the text, electrophoresed through a 7·5% polyacrylamide gel and silver stained. Wound tumour virus (WTV) dsRNA was used as a size standard (Nuss & Dall, 1990).

Fig. 2. Northern hybridization for assignment of cDNA clones to dsRNA segments. Total dsRNA of *A. hypoxylon* isolate 2H was separated through a 7·5% polyacrylamide gel, blotted to nylon membrane and hybridized with ^{32}P-labelled cDNA clones #46 (1·7 kb insert), #68 (1·7 kb insert) and #91 (0·9 kb insert). Sizes of wound tumour virus (WTV) dsRNAs used as a standard are indicated at the right.

reaction. After 4 min, nonlabelled methionine was added to a final concentration of 15 mM. Samples were withdrawn at 2, 4, 8, 16 and 32 min and analysed as above.

Results

dsRNA isolation and cDNA cloning

For most experiments, dsRNA was isolated directly from fungal tissue rather than from virions. Properties of virions will be described elsewhere (C.-S. Oh & B. I. Hillman, in preparation). Three dsRNAs were evident from *A. hypoxylon* isolate 2H (Fig. 1). Their sizes were approximately 2·2 (dsRNA 1), 2·1 (dsRNA 2) and 1·8 kb (dsRNA 3), as determined by gel electrophoresis. Two sets of cDNA libraries were made from dsRNAs of isolate 2H. The first library was made from total dsRNA, and clones were mapped based on their hybridization to individual dsRNA segments. We found by Northern blot analysis that individual clones hybridized to only one of the three segments, suggesting that each segment was genetically unique (Fig. 2). To complete the library, a second set of cDNA clones was made by gel-purifying individual dsRNA segments 1, 2 and 3, and synthesizing three independent, segment-specific libraries. By this combination of methods, complete sets of overlapping clones representing all three segments, excluding the termini, were obtained. Maps of clones used for the characterization of segments 1–3 are shown in Fig. 3. Randomly primed clones mapping to dsRNAs 1, 2 and 3 were completely sequenced by a combination of subcloning and the use of specific oligonucleotide primers. Terminal sequences of dsRNAs were determined from clones obtained using the 5′ RACE system. Positions of primers used for 5′ RACE reactions are shown with the sequences of the segments in Figs 4, 5 and 6. Only a single 5′ RACE clone terminated beyond the previously available dsRNA 3 sequence, so we do not have confirmation of the termini of that segment. In contrast, multiple co-terminal clones were obtained for segments 1 and 2. Since these segments revealed the presence of large open reading frames (ORFs; see below), results will be presented and discussed relative to the coding strand. Analysis of the clones representing the termini revealed that the first 17 residues at the 5′ termini of segments 1 and 2 were identical, and that 15 of 18 residues at the 3′ termini were identical (Fig. 7). No terminal poly(A) tracks were found, but the 3′ ends had A-rich sequences bounded by CTTC sequences at the termini.

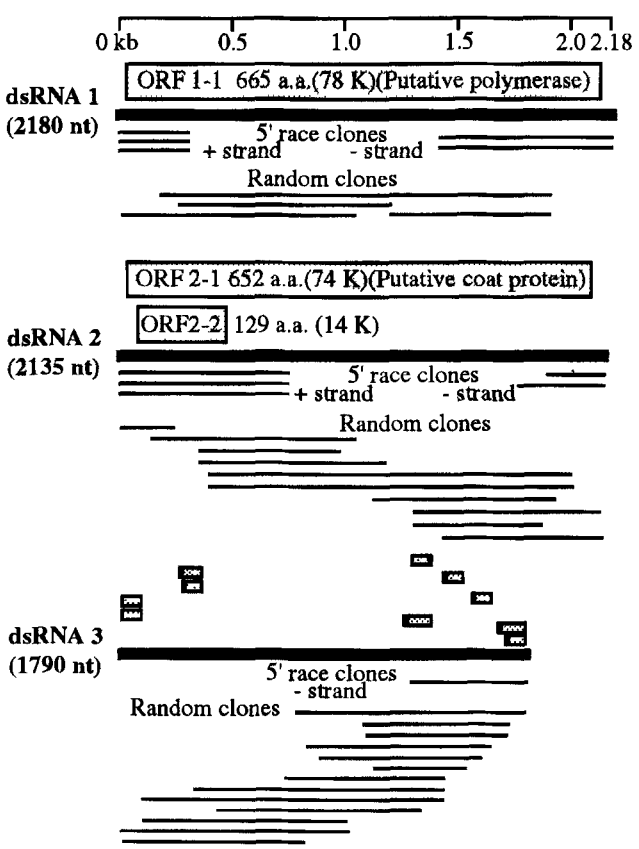

Fig. 3. Overlapping map of cDNA clones representing dsRNAs 1, 2 and 3 of *A. hypoxylon* isolate 2H. For dsRNAs 1 and 2, positions of the large ORFs discussed in the text are indicated. For dsRNA 3, position and direction of all ORFs greater than 25 amino acids in any frame on either strand are shown. Clones were mapped and sequenced as described in the text. Maps are shown using the sequences in Figs 4, 5 and 6 as the positive strands.

dsRNA 1

dsRNA 1 had a size of 2180 nt (Fig. 4). The deduced amino acid sequence of the dsRNA 1 nucleotide sequence revealed a single large open reading frame (ORF 1-1) from nucleotides 40 to 2034. A protein of molecular mass 78 kDa was calculated from the 665 amino acid residues within ORF 1-1. The amino acid sequence deduced from the nucleotide sequence of dsRNA 1 revealed the presence of conserved motifs known to be landmarks of RDRPs, including the highly conserved GDD motif (Poch *et al.*, 1989). An expanded set of conserved motifs based on dsRNA virus RDRPs (Bruenn, 1993) are underlined and labelled with the deduced amino acid sequence shown in Fig. 4.

dsRNA 2

dsRNA 2 contained 2135 nt (Fig. 5). Two ORFs with the potential to encode proteins greater than 10 kDa were evident (Figs 3 and 5). The larger ORF (2-1) spanned residues 72 to 2027 (652 amino acids) and had the potential to encode a 74 kDa protein. The shorter ORF (2-2) started at nucleotide 82 and ended at nt 468 (129

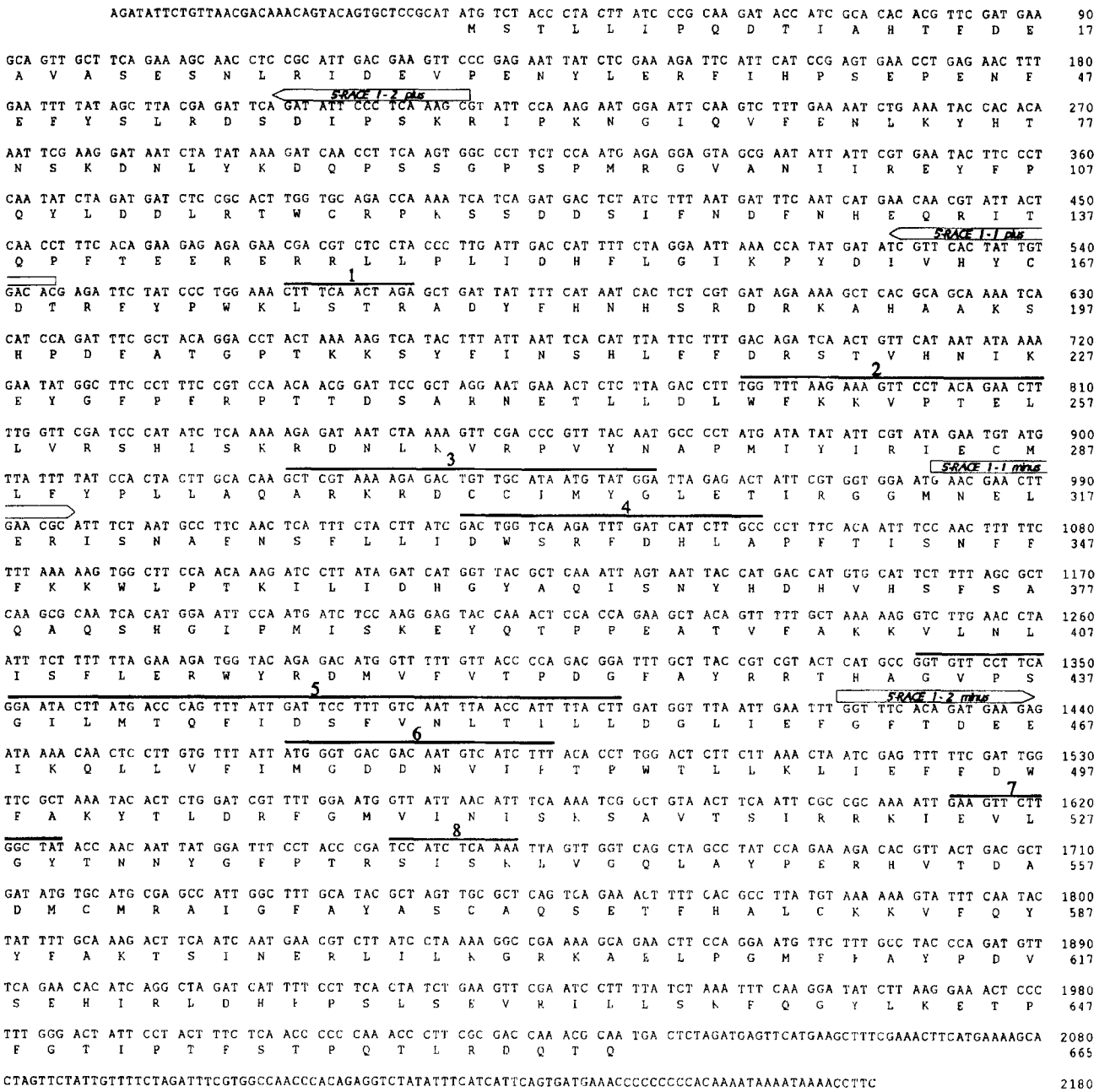

Fig. 4. Nucleotide sequence of dsRNA 1 of *A. hypoxylon* isolate 2H, shown as the (+)-strand cDNA. An open reading frame from nucleotide 40 to 2034 has the potential to encode the 78 kDa protein shown in single-letter code. The positions of conserved RDRP motifs of dsRNA viruses identified by Bruenn (1993) are indicated with lines over the sequence. Positions of first and second internal primers used to generate 5′ RACE clones of both strands are shown, with direction of cDNA synthesis indicated by an arrow.

amino acids), potentially encoding a 14 kDa protein. No clear homologies were identified between either of these deduced proteins and other proteins in the PIR database. The possible relationship between the dsRNA ORF 2-1 product and specific viral proteins was also examined by pairwise comparisons, again without indication of such relationships.

dsRNA 3

Although many overlapping clones representing dsRNA 3 were sequenced, no large ORFs were detected on either strand (Fig. 3). We therefore have not assigned a positive or negative strand to this segment. The sequence of one of its strands is presented in Fig. 6. As suggested earlier,

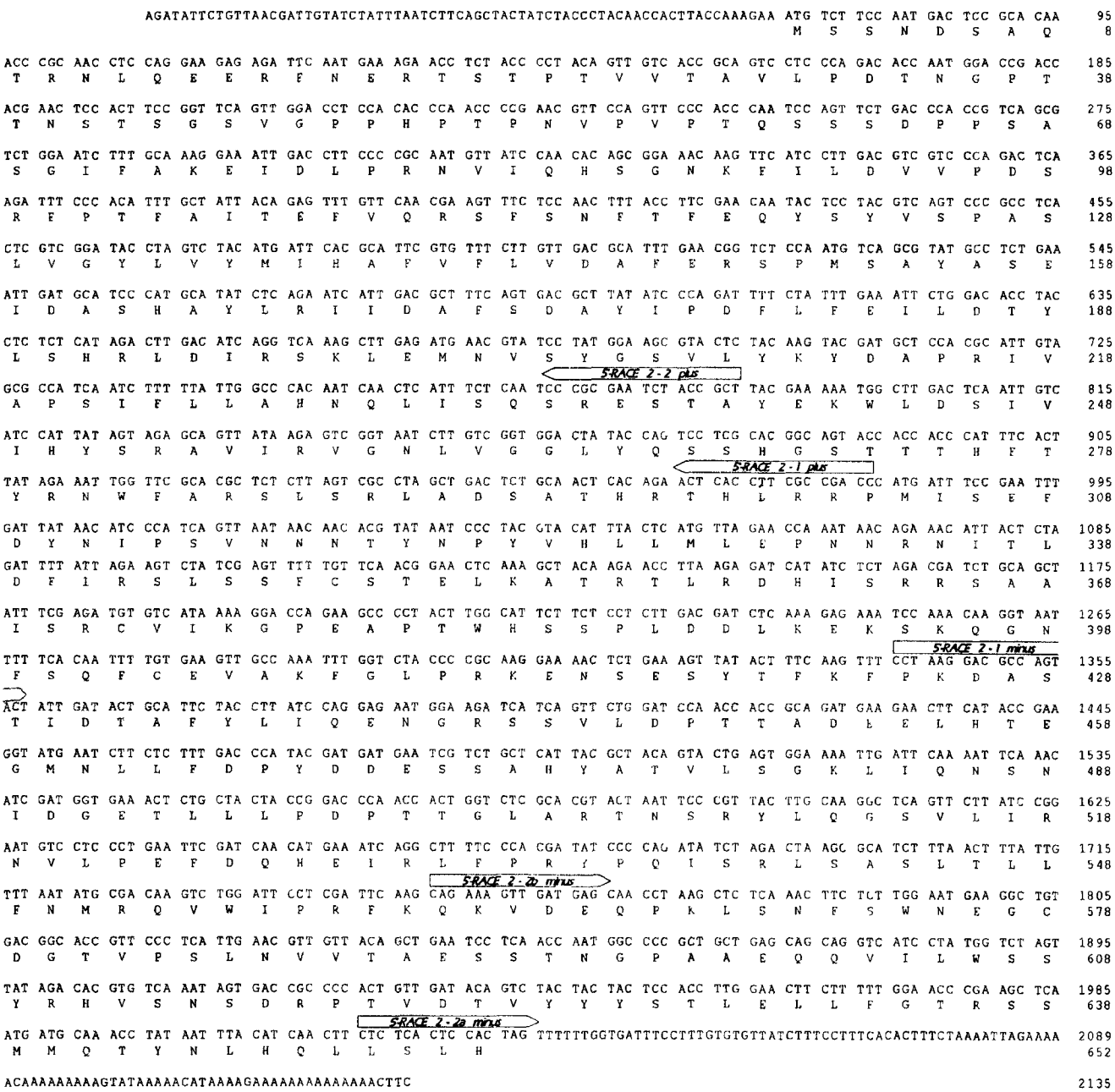

Fig. 5. Nucleotide sequence of dsRNA 2 of *A. hypoxylon* isolate 2H shown as the (+)-strand cDNA. An open reading frame from nucleotide 67 to 2015 has the potential to encode the 74 kDa protein shown in single-letter code. Positions of first and second internal primers used to generate 5′ RACE clones of both strands are shown, with direction of cDNA synthesis indicated by an arrow. Two different oligonucleotides were used as second internal primers to generate 5′ RACE clones from minus-strand template.

we do not know whether the termini of segment 3 are represented in our sequence, so its sequence could be slightly larger than the 1790 nt in Fig. 3. Since this segment lacked apparent coding potential, we did not pursue multiple attempts to clone its termini. There were no obvious similarities between the sequence of segment 3 and the other segments. Additional evidence that the segment 3 map in Fig. 3 in fact represents segment 3 was obtained from PCR experiments, in which products of predicted sizes were amplified from cDNA synthesized from purified segment 3 dsRNA (data not shown).

Cell-free transcription and translation of cDNA clones representing dsRNA 1 and 2

Physical evidence for expression of the translation products deduced from dsRNAs 1 and 2 was obtained from *in vitro* translations in rabbit reticulocyte lysates

```
ATAAGTATAT GACTAATATG TACTTTCGTG AGCGTGACGA AACCAATAGG GGTATAGAAG GGAGGTCTTG AAGTGCAAGT GTAATTTAAA    90
TGTACCTTCT GAATGGTGGG GGTGTGAGAC TTTCAGAGTG ATAGTCGGGT ACCTGGTAAA AAGAAGGGTA TAAGCAGGAG AATTCGCGGT   180
ATTATTGAGA GGAGGTCAAA TAACACGTTT GACGTGAATA ATAGGATTGT CCAAGAAACT ATGAAAATAG CTTCGATAAC TCAGATAATG   270
CAACCGAGGT TTTCAGTTAA CAGGTCTACC ATAATTGGTA ATACGCTGCA GGGCGCGGAA TCACTAAAGT TTTAAGAGGC GATGTTATGG   360
ATCGCTGCTT CTTTAGTAAT AAATACTAAG GCTTCTCGCA TCTAATTTCG TTTTAGCAAA TCTAACCTGG TGACAATTAT TACAAAAATC   450
TTGAATACTA GGTCACAGTA GAGGTGGTTG ATGAGTGTCA AGTAGGTTTT GACAGGCGAA GAGACGGAGG GGGGGGCGGT GGTAATGGAT   540
GGAAGGACTT AAGGGCGAAG GTCTAGAGGA ATCTGTTTGT TCTTCGAGTT CTTTGTAGTT TCCTTCTTCC AGAGCTAGTG CTCGTCTTCG   630
TTCTCGTTTC TAACGGAGGT GTCAACTCAG GGAGCTGTTT TGATACGCTC TTTTATAGCG AGGAAAGAAG TAACGGAGTT TAAGCGCTAG   720
          5'RACE 3-1 minus
GGGCTGACTT CTTCGGAGAA AGGTTTCCGG AAGTTAGTTA TCGCTTACTC TAACTCAGAG CTGGCGGTAA AAGTTGCTAC CCTACGTTGG   810
TCAGTAGAGG TCAACTTGCT CAGGAAGTTT TCCTGTTGGG GGACGCGGCA GTTTTTTGAC TAATGCCATT TTAGGTAGGT CAAGTCAGTT   900
TCTGTTTCGT AGTGAGGTCA TGCCTCTTTT TGAACGAAAG ATGTCTGGTT GGTCTTGACG TATTAGTCAA TTCGTGGAGG GGTAGATTAC   990
ATTTTGATCT ACTATTTAAA CGACGATAAA GTAATGTTAG ATTACTAGTC AAGTCACTGA ATTCGCAGTA AGTCTGAATA GGTGTACACT  1080
GTTCAGGTGT TAGGAGTGCT GCTCCATGTT TAAGTAGTAG TAGGAGTAGT CTACAGGGTA ATCGATCAAA ACTTAGAGTT TCTAAGAGTT  1170
                                                                  5'RACE 3-2 minus
TCGATTTTGA AGTGTTATAG TAAGTTTTAC TATACGTTAA TCAATAGGAG TTACCATTTG ATGGAAAACG GTATGGTATT AAATTCCAAT  1260
GAAAGGAAGT AGTATAAAAA GTCACAAATA TATTTTAAGT GAATTTTCGA GTATTCTTCG TAGTCAGAAA AGGATTTCTA CTATAAGAAA  1350
AGGAATAACT TTGTCTCGAT GACAGCATAG CTGTAAAAAG ACTAAGACGA CAACAATGTC GTAGTCGAGG GTACTGGTTC AAAACAAATG  1440
TTCTTTGGAA AAGAAAATGT TCTGTATCCC AATTTTTCTT GGGAAAAGAT AAAGGGTACT AATTACTCTT ACTATCTAAA CTAAGGTAAA  1530
GTAAAGAAAG TACGTATAGA GTAGAAGTGC TTCTTATGCT TCTCAATACC TTCAGGAGAA AATCTCCCCG TGAGCGTGGA CGGAAAGATA  1620
TCGGGGACGC TAAAGCAATC GCGTAAATGT ACGGAAAGAA AAATGCTTAA AAGGAATGGG CCCAATATCG AACAAGGGAA ATCAACAGTT  1710
TATGAAAATA AGGTTCATTT TTAATATTTA TTTAATTTAT TTTAAACGTG GGTTTTTGGT TTTTTTTAAT GTTCGGGAAG              1790
```

Fig. 6. Nucleotide sequence of dsRNA 3 of *A. hypoxylon* isolate 2H. Since there is no evidence for a coding strand, the strand shown as cDNA was chosen arbitrarily. Positions of first and second internal primers used to generate 5′ RACE clones of the minus strand are shown, with direction of cDNA synthesis indicated by an arrow.

(*a*)

```
1  5′ AGATATTCTGTTAACGA  caAaCagTa-
      :::::::::::::::::     : :  :
2  5′ AGATATTCTGTTAACGAttgtAtCtaTt-
```

(*b*)

```
1  -cCccccccAcAAAAtAAAAtAAAAcCTTC 3′
     :      : :::: :::: :::: ::::
2  -aCataaaAgAAAAaAAAAaAAAAaCTTC 3′
```

Fig. 7. Comparison of 5′ (*a*) and 3′ (*b*) termini of dsRNA 1 and dsRNA 2 nucleotide sequences of *A. hypoxylon* 2H virus. Identical residues are indicated by two dots (:) and shown in **bold** type.

using near-full-length clones of these segments (Fig. 8). cDNA clones containing full-length copies of the predicted large ORFs of isolate 2H dsRNAs 1 and 2 were assembled with the SP6 promotor for transcription and the TEV leader sequence to enhance translation *in vitro*. Both constructs resulted in one major product in a standard 1 h translation (Fig. 8*b*) and in time course translations (Fig. 8*c*). Translation of transcripts from the dsRNA 1 construct resulted in a single major protein of > 70 kDa and a few minor bands. The electrophoretic mobility of the major translation product of the dsRNA 1 construct was slightly below the predicted 78 kDa, but within the variation range expected for PAGE. The time course study revealed that the minor bands in the dsRNA 1 translations accumulated early and that the predicted full-length product accumulated later, suggesting that these were immature proteins rather than products of post-translational proteolytic processing.

Translation of the dsRNA 2 construct resulted in a single large protein of nearly the same mobility as the dsRNA 1 product. This protein was approximately the 74 kDa size predicted from the dsRNA 2 nucleotide sequence. No shorter products were detected in these translations.

Sequence comparisons of RDRP among dsRNA viruses

In alignments using larger numbers of RDRP sequences from positive sense, negative sense and dsRNA viruses, we found that the *A. hypoxylon* 2H virus RDRP was more closely related to dsRNA viruses such as totiviruses and partitiviruses than to ssRNA viruses (data not shown). We therefore narrowed our alignments to investigate relationships among these viruses (Fig. 9). Employing the same sequence parameters used by Bruenn (1993) in his investigation of dsRNA RDRP relationships, we found that the *A. hypoxylin* 2H virus RDRP was more closely related to the RDRP of beet cryptic virus [BCV3(2); Xie *et al.*, 1993] than to the RDRP sequences of totiviruses from *Saccharomyces cerevisiae* (L1 and L-A), and *Ustilago maydis* (UmVH1). NB631, a small mitochondrial dsRNA of *Cryphonectria parasitica* (Polashock & Hillman, 1994), was included as an outgroup for developing a phenogram based on these alignments.

Discussion

Of the six isolates of the turfgrass choke pathogen, *A. hypoxylon*, that we have examined, five had resident dsRNA viruses (C.-S. Oh & B. I. Hillman, unpublished). In this paper, we have described the genome organization of the virus from one of these isolates, 2H. We have evidence that the virus population from one of the other isolates, A3, is similar or identical to the 2H virus, but that the other three are different (C.-S. Oh & B. I. Hillman, unpublished). We have made many attempts to pass these viruses by hyphal anastomosis or to eliminate

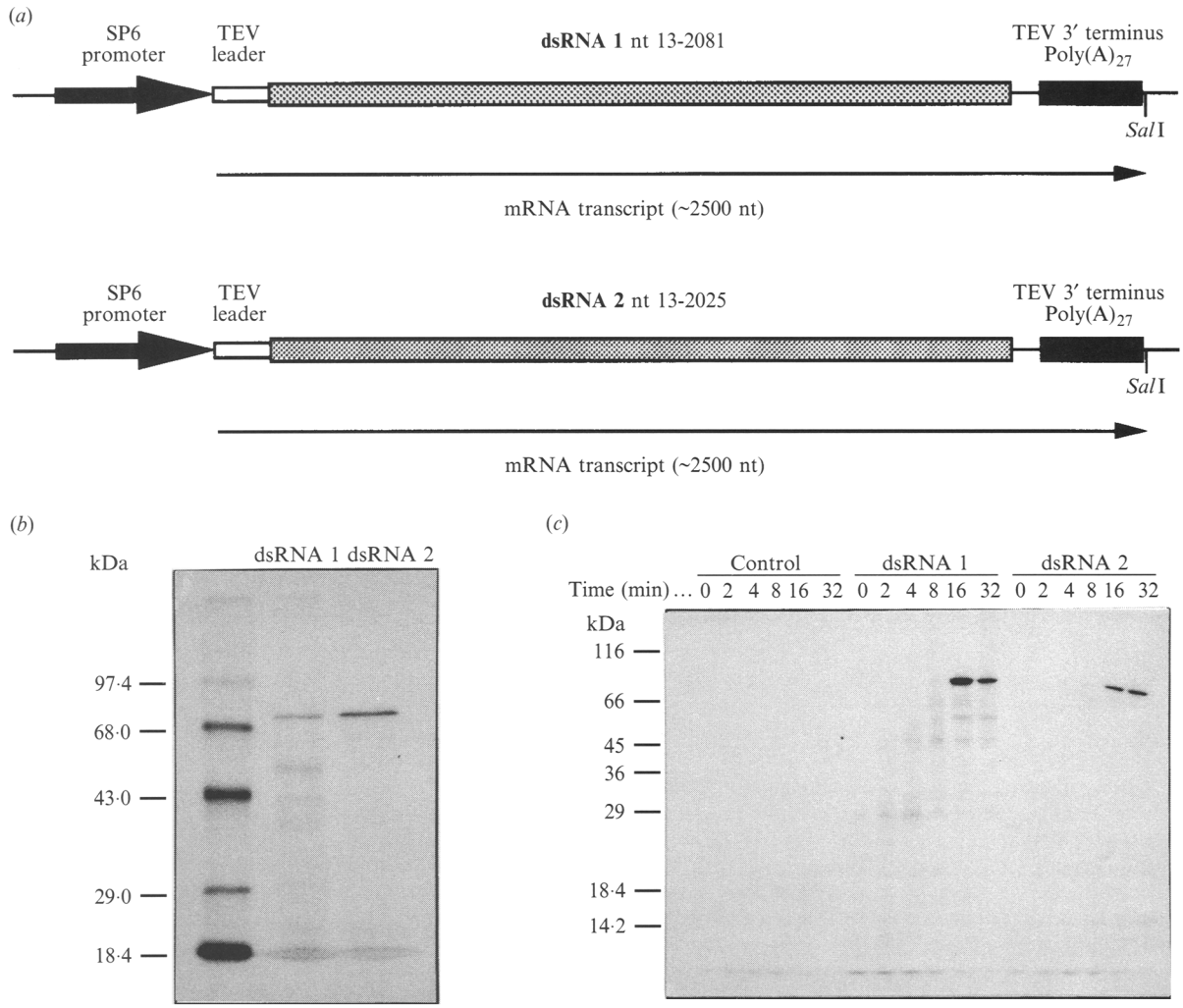

Fig. 8. Translation expression cassette of full-length ORFs 1-1 and 2-1 (Fig. 3) and their *in vitro* translation products. (*a*) Full-length cDNA clones representing dsRNA segments 1 and 2 were constructed as described in the text. (*b*) Cell-free translation of transcripts shown in (*a*). Translation reactions were incubated at 30 °C for 1 h. (*c*) Pulsed-chase analysis. [^{35}S]Methionine was added at the beginning of the translation reactions. After 4 min, nonlabelled methionine was added to a final concentration of 15 mM. Samples were withdrawn at 2, 4, 8, 16 and 32 min. Proteins were analysed by SDS–PAGE and autoradiography. For (*b*) and (*c*), transcription with bacteriophage SP6 polymerase and translation in rabbit reticulocyte lysates were conducted as described in the text. Plasmids were linearized with *Sal*I which cleaves downstream of the TEV terminal sequence, prior to transcription. Transcripts were translated in the rabbit reticulocyte system in the presence of [^{35}S]methionine, and the radiolabelled products were detected by SDS–PAGE and autoradiography.

viruses from the cultures, but have thus far been unsuccessful. Therefore, we do not know the effects of these viruses on the biology of their fungal hosts. Comparative examination of physico-chemical properties of the 2H virus will be presented elsewhere.

Members of the *Partitiviridae* are common in fungal hosts (Ghabrial, 1994). Although the plant cryptic viruses have been included in this family as a separate genus (Ghabrial *et al.*, 1994), nucleotide sequence data have not been available to support this phylogeny. Nucleotide sequence and deduced translation data presented here strongly suggest that the *A. hypoxylon* 2H dsRNA 1 encodes the viral polymerase. dsRNA segment 2 likely encodes the viral capsid protein, since a single major protein of ~ 74 kDa was detected from disrupted virion preparations (data not shown). These results support the conclusions of Romanos *et al.* (1981) and Tavantzis & Bandy (1988) for similar viruses. We have not yet unequivocally confirmed these assignments serologically. Based on *in vitro* translations of segments 2, we have no evidence for expression of the smaller ORF 2-2. As segment 3 appears to have no coding potential for large ORFs, its function in the viral replication cycle is not clear. dsRNA 3 may simply represent a satellite dsRNA segment that is not necessary to viral replication.

In addition to tentatively assigning gene functions to

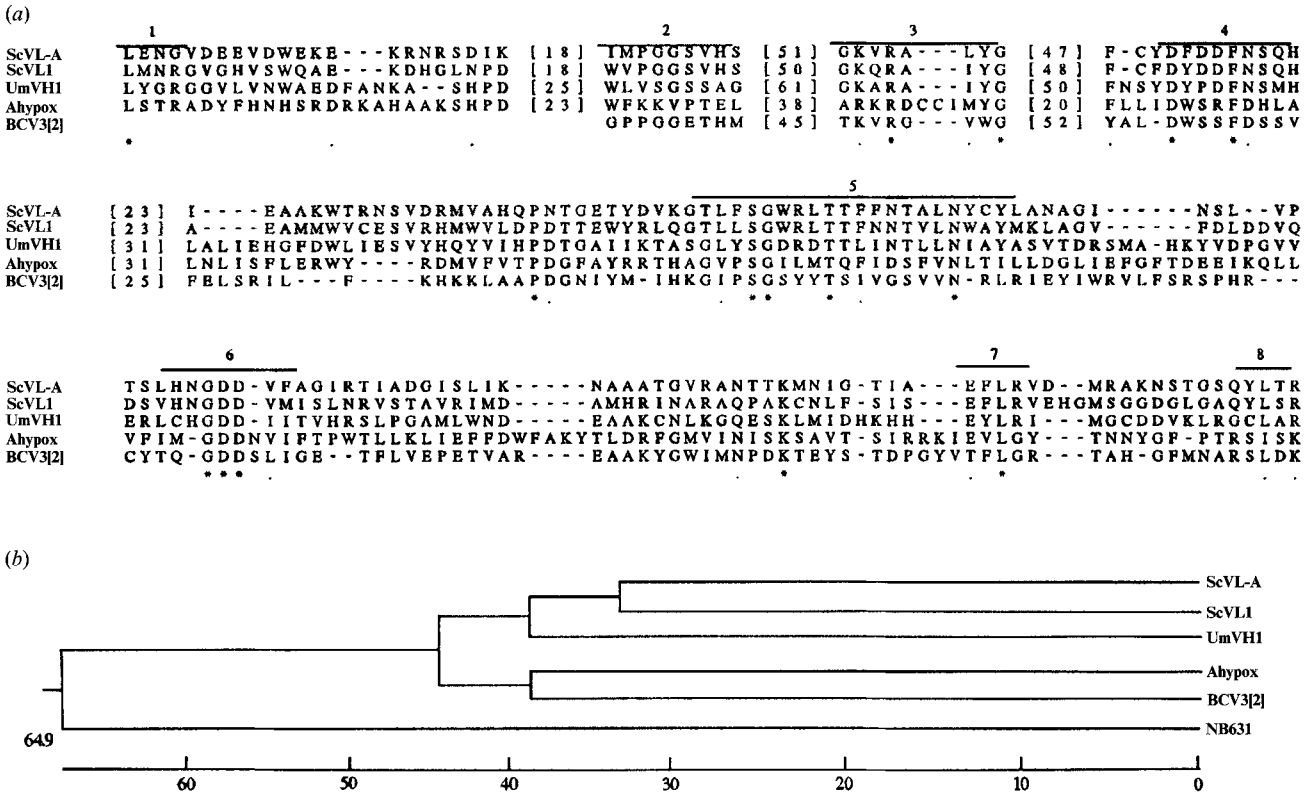

Fig. 9. Comparison of *A. hypoxylon* 2H virus putative RDRP with putative RDRPs of other dsRNA viruses. (*a*) Amino acid sequences within the conserved RDRP motifs suggested by Bruenn (1993) from the *S. cerevisiae* viruses ScVL1 and ScVL-A, *U. maydis* virus UmVH1 and beet cryptic virus 3 dsRNA 2 [BCV3(2)] were aligned with similar sequences of the *A. hypoxylon* 2H virus (Ahypox). Designated motifs 1–8 are those suggested by Bruenn (1993), as in Fig. 4. Conserved residues are indicated by an asterisk (*) and similar residues with dots (.). The number of residues between the conserved motifs is indicated in parentheses. (*b*) Dendrogram based on alignments in (*a*). The homologous sequence of a small dsRNA of *Cryphonectria parasitica* (NB631) (Polashock & Hillman, 1994) was included as an outgroup.

segments 1 and 2 of the *A. hypoxylon* isolate 2H virus, we have determined that their terminal sequences are conserved. We found no close similarity between 3′ termini of the *A. hypoxylon* dsRNAs and BCV3[2] (Xie *et al.*, 1993). Both viruses contain the 5′-terminal sequence AGA, however, and both have A-rich sequences near the 3′ termini. The A-rich sequences of BCV3[2] were suggested as possibly involved in polyadenylation (Xie *et al.*, 1993). We have no information regarding possible presence of poly(A) tails on the mRNAs presumably associated with the *A. hypoxylon* 2H virus.

Many other multisegmented viruses have the property of conserved terminal sequences, including dsRNA-containing viruses (e.g. Anzola *et al.*, 1987), negative-strand RNA-containing viruses (e.g. Stoeckle *et al.*, 1987) and positive-strand RNA-containing viruses (e.g. Ahlquist *et al.*, 1984). In the case of reoviruses, differences in conserved terminal sequences have been conjectured to represent sorting signals that ensure packaging of exactly one copy of each segment per virion, in addition to providing proper *cis*-acting sequences for replication of that segment (Anzola *et al.*, 1987). Unlike reovirus segments, no inverted repeats sequences were associated with termini of *A. hypoxylon* segments 1 and 2 and they were not predicted to form panhandle structures. Furthermore, viruses similar to the *A. hypoxylon* 2H virus are thought to encapsidate a single dsRNA per particle (Wood & Bozarth, 1972; Buck *et al.*, 1981). We have no data regarding specific encapsidation of dsRNAs in this system, and the role of conserved terminal sequences of segments 1 and 2 of *A. hypoxylon* isolate 2H is yet to be determined.

Bruenn (1993) found that RDRP sequences of dsRNA viruses such as totiviruses and BCV were more closely related to each other than to RDRP sequences of ssRNA viruses. This is in contrast to RDRP sequences of some other dsRNA-containing viruses, including many of the *C. parasitica* viruses, which are most closely related to the ssRNA-containing plant virus family *Potyviridae* (Koonin *et al.*, 1991; Hillman *et al.*, 1994), and T and W dsRNA viruses of *S. cerevisiae* (Matsumoto & Wickner, 1991; Rodriguez-Cousino *et al.*, 1991; Esteban *et al.*, 1992) as well as NB631 of *C. parasitica* (Polashock & Hillman, 1994), which are most closely related ssRNA-

containing coliphages. Our alignments support these conclusions, extend the dsRNA RDRP alignments to the fungal partitiviruses, and support the inclusion of plant cryptic viruses within the *Partitiviridae*.

The sequence similarity between the *A. hypoxylon* 2H virus and BCV3 is intriguing. The epiphyte *A. hypoxylon* is closely related to fungal endophytes such as *Acremonium* spp., which inhabit plants intercellularly without causing disease (Schardl *et al.*, 1991). As more information is generated, it will be interesting to examine similarities and differences between other plant and fungus-infecting partitiviruses.

We thank Dr T. Michael A. Wilson for his participation in the initial stages of the research and Dr James White for isolate 2H of *A. hypoxylon*. We thank the Rutgers Turfgrass Research Fund for supporting this work.

References

AHLQUIST, P., DASGUPTA, R. & KAESBERG, P. (1984). Nucleotide sequence of brome mosaic virus genome and its implications for virus replication. *Journal of Molecular and Cellular Biology* **172**, 369–383.

ANZOLA, J. V., XU, Z., ASAMIZU, T. & NUSS, D. L. (1987). Segment-specific inverted repeats found adjacent to conserved terminal sequences in wound tumor virus genome and defective interfering RNAs. *Proceedings of the National Academy of Sciences, USA* **84**, 8301–8305.

BRUENN, J. A. (1993). A closely related group of RNA-dependent RNA polymerases from double-stranded RNA viruses. *Nucleic Acids Research* **21**, 5667–5669.

BUCK, K. W. (1979). Virion-associated RNA polymerases of double-stranded RNA mycoviruses. In *Fungal Viruses*, pp. 62–77. Edited by H. P. Molitoris, M. Hollings & H. A. Wood. New York: Springer Verlag.

BUCK, K. W. (1986). Fungal virology – an overview. In *Fungal Virology*, pp. 1–84. Edited by K. W. Buck. Boca Raton: CRC Press.

BUCK, K. W. & GHABRIAL, S. A. (1991*a*). Partitiviridae. In *Classification and Nomenclature of Viruses. Fifth Report of the International Committee for the Taxonomy of Viruses. Archives of Virology*, Supplementum 2, pp. 208–211. Edited by R. I. B. Francki, C. M. Fauquet, D. L. Knudsen & F. Brown. New York: Springer Verlag.

BUCK, K. W. & GHABRIAL, S. A. (1991*b*). Totiviridae. In *Classification and Nomenclature of Viruses. Fifth Report of the International Committee for the Taxonomy of Viruses. Archives of Virology*, Supplementum 2, pp. 203–205. Edited by R. I. B. Francki, C. M. Fauquet, D. L. Knudsen & F. Brown. New York: Springer Verlag.

BUCK, K. W., ALMOND, M. R., MCFADDEN, J. J. P., ROMANOS, M. A. & RAWLINSON, C. J. (1981). Properties of thirteen viruses and virus variants obtained from eight isolates of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*. *Journal of General Virology* **57**, 157–168.

CARRINGTON, J. C. & DOUGHERTY, W. G. (1987). Small nuclear inclusion protein encoded by a plant potyvirus genome is a protease. *Journal of Virology* **61**, 2540–2548.

CLAY, K. (1984). The effect of the fungus *Atkinsonella hypoxylon* (Clavicepitacae) on the reproductive system and demography of the grass *Danthonia spicata*. *New Phytologist* **98**, 165–175.

DIEHL, W. W. (1950). *Balansia and the Balansiae in America*. Agriculture Monograph no. 4. United States Department of Agriculture, Washington, DC.

ESTEBAN, L. M., RODRIGUEZ-COUZINO, N. & ESTEBAN, R. (1992). T double-stranded RNA sequence reveals that T and W dsRNAs form a new RNA family in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **267**, 10874–10881.

FELSENSTEIN, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

FROHMAN, M. A., DUSH, M. K. & MARTIN, G. R. (1988). Rapid amplification of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proceedings of the National Academy of Sciences, USA* **85**, 8998–9002.

GHABRIAL, S. A. (1994). New developments in fungal virology. *Advances in Virus Research* **43**, 303–388.

GHABRIAL, S. A., BOZARTH, R. R., BUCK, K. W., YAMASHITA, S., MARTELLI, G. P. & MILNE, R. J. (1994). *Partitiviridae*. In *Sixth Report of the International Committee for the Taxonomy of Viruses*. Edited by F. A. Murphy, C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. P. Mayo & M. D. Summers. New York: Springer Verlag (in press).

HIGGINS, D. G., BLEASBY, A. J. & FUCHS, R. (1992). CLUSTAL V: improved software for multiple sequence alignments. *CABIOS* **8**, 189–191.

HILLMAN, B. I., SHAPIRA, R. & NUSS, D. L. (1990). Hypovirulence-associated suppression of host functions in *Cryphonectria parasitica* can be partially relieved by high light intensity. *Phytopathology* **80**, 950–956.

HILLMAN, B. I., TIAN, Y., BEDKER, P. J. & BROWN, M. P. (1992). A North American hypovirulent isolate of the chestnut blight fungus with European isolate-related dsRNA. *Journal of General Virology* **73**, 681–686.

HILLMAN, B. I., FULBRIGHT, D. W., NUSS, D. L. & VAN ALFEN, N. K. (1994). *Hypoviridae*. In *Sixth Report of the International Committee for the Taxonomy of Viruses*. Edited by F. A. Murphy, C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. P. Mayo & M. D. Summers. New York: Springer Verlag (in press).

KOONIN, E. V., CHOI, G. H., NUSS, D. L., SHAPIRA, R. & CARRINGTON, J. C. (1991). Evidence for common ancestry of a chestnut blight hypovirulence-associated double-stranded RNA and a group of positive-strand RNA plant viruses. *Proceedings of the National Academy of Sciences, USA* **88**, 10647–10651.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

LEMKE, P. A. (1979). Co-evolution of fungi and their viruses. In *Fungal Viruses*, pp. 2–7. Edited by H. P. Molitoris, M. Hollings & H. A. Wood. New York: Springer Verlag.

LEUCHTMANN, A. & CLAY, K. (1988). Experimental infection of host grasses and sedges with *Atkinsonella hypoxylon* and *Balansia cyperi* (Balansiae, Clavicipitaceae). *Mycologia* **80**, 291–297.

LEUCHTMANN, A. & CLAY, K. (1989*a*). Morphological, cultural, and mating studies on *Atkinsonella*, including *A. texensis*. *Mycologia* **81**, 692–701.

LEUCHTMANN, A. & CLAY, K. (1989*b*). Isozyme variation in the fungus *Atkinsonella hypoxylon* within and among populations of its host grasses. *Canadian Journal of Botany* **67**, 2600–2607.

MATSUMOTO, Y. & WICKNER, R. B. (1991). Yeast 20S RNA replicon. Replication intermediates and encoded putative RNA polymerase. *Journal of Biological Chemistry* **266**, 12779–12783.

MORRIS, T. J. & DODDS, J. A. (1979). Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* **69**, 845–858.

NUSS, D. L. & DALL, D. J. (1990). Structural and functional properties of plant reovirus genomes. *Advances in Virus Research* **38**, 249–306.

OH, C.-S. & CARRINGTON, J. C. (1989). Identification of essential residues in potyvirus proteinase HC-Pro by site-directed mutagenesis. *Virology* **173**, 692–699.

POCH, O., SAUVAGEUT, I., DELARUE, M. & TORDO, N. (1989). Identification of four conserved motifs among RNA-dependent RNA polymerase encoding elements. *EMBO Journal* **8**, 3867–3874.

POLASHOCK, J. J. & HILLMAN, B. I. (1994). A small mitochondrial double-stranded (ds) RNA element associated with a hypovirulent strain of the chestnut blight fungus and ancestrally related to yeast cytoplasmic T and W dsRNAs. *Proceedings of the National Academy of Sciences, USA* **91**, 8680–8684.

QUEEN, C. & KORN, L. J. (1984). A comprehensive sequence analysis program for the IBM personal computer. *Nucleic Acids Research* **12**, 581–599.

RODRIGUEZ-COUSINO, N., ESTEBAN, L. M. & ESTEBAN, R. (1991). Molecular cloning and characterization of W double-stranded RNA, a linear molecule present in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **266**, 12772–12778.

ROMANOS, M. A., BUCK, K. W. & RAWLINSON, C. J. (1981). A satellite double-stranded RNA in a virus from *Gaeumannomyces graminis*. *Journal of General Virology* **57**, 375–383.

SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). Sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* **74**, 5463–5467.

SCHARDL, C. L., LIU, J.-S., WHITE, J. F. JR., FINKEL, R. A., AN, Z. & SIEGEL, M. (1991). Molecular phylogenetic relationships of nonpathogenic grass mycosymbionts and clavicipitaceous plant pathogens. *Plant Systematics and Evolution* **178**, 27–41.

STOECKLE, M. Y., SHAW, M. W. & CHOPPIN, P. W. (1987). Segment-specific and common nucleotide sequences in the noncoding regions of influenza B virus genome RNAs. *Proceedings of the National Academy of Sciences, USA* **84**, 2703–2707.

TABOR, S. & RICHARDSON, C. C. (1987). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proceedings of the National Academy of Sciences, USA* **84**, 4767–4771.

TAVANTZIS, S. M. & BANDY, B. P. (1988). Properties of a mycovirus from *Rhizoctonia solani* and its virion-associated RNA polymerase. *Journal of General Virology* **69**, 1465–1477.

WICKNER, R. B. (1992). Double-stranded and single-stranded RNA viruses of *Saccharomyces cerevisiae*. *Annual Review of Microbiology* **46**, 347–375.

WOOD, H. A. & BOZARTH, R. F. (1972). Properties of virus-like particles of *Penicillium chrysogenum*: One double-stranded RNA molecule per particle. *Virology* **47**, 604–609.

XIE, W. S., ANTONIW, J. F. & WHITE, R. F. (1993). Nucleotide sequence of beet cryptic virus 3 dsRNA2 which encodes a putative RNA-dependent RNA polymerase. *Journal of General Virology* **74**, 1467–1470.

(*Received 24 November 1994; Accepted 1 February 1995*)