Virology **261,** 331–339 (1999)
Article ID viro.1999.9880, available online at http://www.idealibrary.com on IDEAL®

Sequences of European Wheat Mosaic Virus and Oat Golden Stripe Virus and Genome Analysis of the Genus *Furovirus*

Aipo Diao,*,† Jianping Chen,† Francine Gitton,*,‡ John F. Antoniw,* Jonathan Mullins,§ Avice M. Hall,¶ and Michael J. Adams*,[1]

*Plant Pathology Department, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, United Kingdom; †Virology Laboratory, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, People's Republic of China; ‡Université Catholique de Louvain, Faculté des Sciences Agronomiques, Unité de Phytopathologie, Place Croix du Sud, 2 Bte 3, B-1348 Louvain-la-Neuve, Belgium; §Department of Biology and Health Science, University of Luton, Park Square, Luton, Beds LU1 3JU, United Kingdom; and ¶Department of Environmental Science, University of Hertfordshire, College Lane, Hatfield, Herts AL10 9AB, United Kingdom

Received April 26, 1999; returned to author for revision May 26, 1999; accepted July 1, 1999

The complete nucleotide sequences of both RNAs of oat golden stripe virus (OGSV) and a wheat-infecting furovirus isolate from France, previously thought to be soil-borne wheat mosaic virus (SBWMV), have been determined. Both viruses had a similar genomic organisation to SBWMV and Chinese wheat mosaic virus, the two other furoviruses previously sequenced but had <70% nucleotides identical to them. The French isolate has been named European wheat mosaic virus (EWMV). Phylogenetic analyses supported the recognition of these isolates as distinct viruses in the genus *Furovirus.* Analysis of the coat protein readthrough domain on RNA2 of all furoviruses strongly predicts two mutually compatible conserved transmembrane domains that may be significant for fungus transmission. The second of these regions is eliminated by a deletion in the isolate of OGSV studied. Leaky opal (UGA) stop codons occur on both RNAs of all four furoviruses characterised and, in common with most other leaky opal codons identified in plant viruses, they are followed by a CGG codon.

INTRODUCTION

Until recently, the genus *Furovirus* included all the rod-shaped plant viruses transmitted by plasmodiophorid fungi, but these have proved to be very diverse in genome organisation. Recent proposals (Torrance and Mayo, 1997), which have been ratified by the ICTV (Pringle, 1999), have revised the genus so that it now includes only those viruses that have two genome segments and that do not have a triple gene block. The best known, and the type member, is soil-borne wheat mosaic virus (SBWMV) for which the complete nucleotide sequences of the two RNA components of an isolate from Nebraska have been described (Shirako and Wilson, 1993). The other viruses included are sorghum chlorotic spot virus and oat golden stripe virus (OGSV), both of which have a serological relationship to SBWMV but differ in their host range and other biological properties (Plumb *et al.,* 1977; Adams *et al.,* 1988; Kendall and Lommel, 1988). Hypochoeris mosaic virus and rice stripe necrosis virus are tentative members of the genus, but their position will have to be reevaluated when sequence data are available. SBWMV has been reported from most winter wheat growing regions of the world including the United States, Brazil, France, Italy, Egypt, Japan, and China. However, recent results have shown that an isolate from China is only 75% (RNA1) and 63% (RNA2) identical to SBWMV and that it should be considered a new member of the genus *Furovirus*, which has been named Chinese wheat mosaic virus (CWMV) (Diao *et al.,* 1999).

We now report the complete sequences of both RNAs of a wheat-infecting furovirus isolate from France and also those of OGSV, showing that both of these should be regarded as distinct viruses in the genus *Furovirus*. Some distinctive features of the genome of furoviruses are analysed further. The French virus has been named European wheat mosaic virus (EWMV).

RESULTS AND DISCUSSION

Genome organisation and sequence comparisons

Both EWMV and OGSV have two RNA species that are organised in a similar manner to SBWMV and CWMV (Fig. 1). Thus RNA1 has three predicted major ORFs; ORF1 encodes a protein with methyltransferase and helicase motifs and concludes with a UGA (opal) termination codon, which can probably be suppressed to generate a larger product incorporating ORF2, encoding the

Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under Accession Nos. AJ132576–AJ132579.

[1] To whom reprint requests should be addressed. Fax: 44-1582-760981. E-mail: mike.adams@bbsrc.ac.uk.

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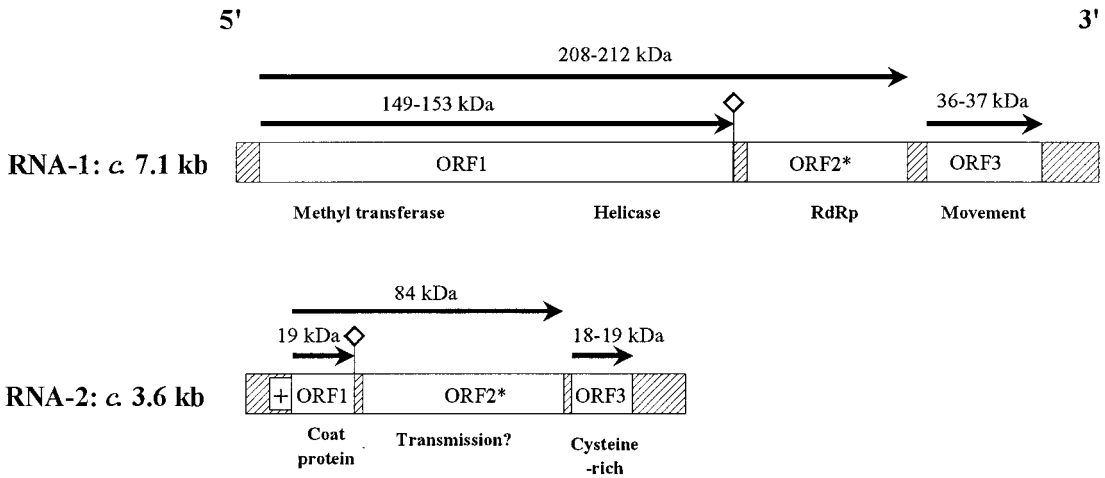

FIG. 1. Genome organisation in the genus *Furovirus*. Predicted open reading frames (ORFs) are shown as boxes (* theoretical only). Putative proteins are shown as lines with arrows; ◇ indicates leaky UGA stop codon; +, possible N-terminal extension of coat protein.

RNA-dependant RNA polymerase (RdRp). ORF3 encodes a movement protein (Mushegian and Koonin, 1993; Shirako and Wilson, 1993). RNA2 also has three predicted major ORFs; ORF1 encodes the coat protein of ~19 kDa and its sequence concludes with an opal (UGA) termination codon, which can probably be suppressed to generate a larger product incorporating ORF2. In both viruses, there is an in-frame CUG codon upstream of the first AUG at position 208–210 (EWMV) and 204–206 (OGSV) similar to those reported for SBWMV and CWMV, which is believed to initiate a larger (~24 kDa) coat protein (Shirako, 1998). ORF3 encodes a small (18–19 kDa), cysteine-rich protein.

The sizes of the major features and nucleotide and amino acid comparisons among the furovirus sequences are shown in Table 1. Comparisons and alignments with the other furoviruses suggests that the OGSV isolate sequenced had a deletion of ~216 nucleotides in the 3′-terminal third of the coat protein readthrough region (between nucleotides 2007 and 2008). Deletions in this region occur commonly in SBWMV isolates maintained in the laboratory (Chen *et al.,* 1994). Over the entire genome, the sequences of EWMV and OGSV had <70% nucleotides identical to the other furoviruses, with RNA1 being slightly more similar than RNA2. In the 5′-UTRs, EWMV was ~69% (RNA1) and 61% (RNA2) identical to SBWMV and CWMV, whereas OGSV was 48–57% (RNA1) and 56% (RNA2) identical to the other furoviruses. In the 3′-UTRs, EWMV was ~73% (RNA1) and 60% (RNA2) identical to SBWMV and CWMV, whereas OGSV was 59% (RNA1) and 54–62% (RNA2) identical to the other furoviruses. Comparisons of the predicted amino acid sequences showed that the RdRp region was the most similar (~80% identical amino acids), whereas the coat protein readthrough region was the least similar (50–60% identical amino acids). On RNA1, EWMV and OGSV were more distant from SBWMV than was CWMV, but on RNA2, the four viruses seemed to be about equidistant from one another.

Phylogenetic analyses of peptide sequences and taxonomic implications

Phylogenetic analysis of the peptide sequence of the replication protein on RNA1 shows that EWMV and OGSV group with the other members of the genus *Furovirus* and supports their recognition as distinct species. There is a fairly close similarity with members of the genus *Pomovirus* (which have tripartite genomes and also are transmitted by plasmodiophorid fungi). The tree for the RdRp region is shown in Fig. 2a and that for the entire replicase (ORF1 and readthrough portion) is similar but with even larger bootstrap values (data not shown). Analysis of the movement protein (Fig. 2b) indicates a distant relationship to the movement protein of dianthoviruses (CRSV, RCNMV, SCNMV) as suggested by Shirako and Wilson (1993).

The coat proteins of the four furoviruses are quite closely related and have a distant relationship to those in the genus *Pomovirus* (Fig. 2c). A coat protein sequence from a Japanese isolate of SBWMV (Shirako, 1998) grouped more closely with EWMV than with the geographically nearer CWMV, but more sequence of the Japanese isolate would be needed to determine its taxonomic status. In the cysteine-rich protein (Fig. 2d), the analysis demonstrated the distant relationship to hordeivirus γb proteins and the RNA1 3′-terminal gene of pecluviruses that has previously been reported (Diao *et al.,* 1999).

Overall, there seems little difficulty in establishing OGSV and EWMV as distinct members of the genus. OGSV has a different host range to SBWMV (Plumb *et al.,* 1977) and, at

TABLE 1

Comparisons among the Furoviruses Showing the Sizes, Percentage Identical Nucleotides, and Percentage Identical Amino Acids of the Genomes and the Coding Regions

	nt	OGSV	CWMV	SBWMV
Complete genome				
EWMV	10708	62.5	65.8	68.3
OGSV	10343		62.5	64.8
CWMV	10716			70.3
SBWMV	10692			—
RNA1				
EWMV	7025	63.6	68.9	70.3
OGSV	7111		63.3	66.2
CWMV	7147			74.1
SBWMV	7099			—
RNA2				
EWMV	3683	60.3	59.7	64.3
OGSV	3232		60.6	62.0
CWMV	3569			62.6
SBWMV	3593			—

	nt	kDa	EWMV	OGSV	CWMV	SBWMV
RNA1 ORF1 (Replicase)						
EWMV	3927	148.9	—	62.5	67.7	69.6
OGSV	4038	152.8	*63.3*	—	62.7	67.0
CWMV	4053	153.0	*73.7*	*62.7*	—	72.7
SBWMV	3963	150.0	*75.5*	63.6	*81.1*	—
Readthrough (RdRp)						
EWMV	1524	+58.9	—	73.0	73.8	74.5
OGSV	1524	+58.9	*79.3*	—	70.5	72.3
CWMV	1524	+59.3	*82.5*	*77.6*	—	78.9
SBWMV	1524	+59.2	*82.3*	79.1	*90.4*	—
Movement protein						
EWMV	981	37.2	—	55.9	64.6	67.3
OGSV	975	36.0	*57.3*	—	58.2	58.4
CWMV	990	37.4	*67.2*	*60.3*	—	71.2
SBWMV	984	37.1	*70.2*	*60.0*	*76.8*	—
RNA2 Coat protein						
EWMV	531	19.5	—	68.7	66.5	72.9
OGSV	531	19.4	*72.2*	—	72.3	69.9
CWMV	531	19.2	*71.0*	*77.3*	—	71.0
SBWMV	531	19.3	*79.0*	*77.8*	*76.1*	—
CP readthrough domain						
EWMV	1734	+65.2	—	59.2	57.6	63.1
OGSV	1521	+57.1	*53.2*	—	55.9	59.6
CWMV	1749	+64.7	*50.3*	*53.8*	—	59.6
SBWMV	1734	+64.5	*59.9*	*56.0*	*58.4*	—
Cysteine-rich protein						
EWMV	498	18.0	—	62.8	64.8	70.3
OGSV	522	18.7	*64.0*	—	66.3	62.5
CWMV	522	18.8	*63.4*	*65.9*	—	63.4
SBWMV	525	18.8	*63.0*	*62.4*	*61.5*	—

Note. Amino acids are in italic; identical nucleotides are in the top part of each section.

least on RNA1, was the most distant from it in the molecular comparisons. Earlier results of sequencing the coat protein gene had suggested that OGSV was only a strain of SBWMV (Chen *et al.,* 1996), but this could not be repeated, and those data are now assumed to have resulted from contamination in the RT–PCR reaction. EWMV was also substantially different in these comparisons. Moreover, serological differences between OGSV, EWMV, CWMV, and SBWMV can be shown using polyclonal antisera (Ye *et al.,* 1999; unpublished data). Partial sequence data from a furovirus infecting wheat in Italy shows it to be very similar to the French EWMV reported here (Jianping Yang, and Jianping Chen, unpublished data), which supports the choice of name for the virus.

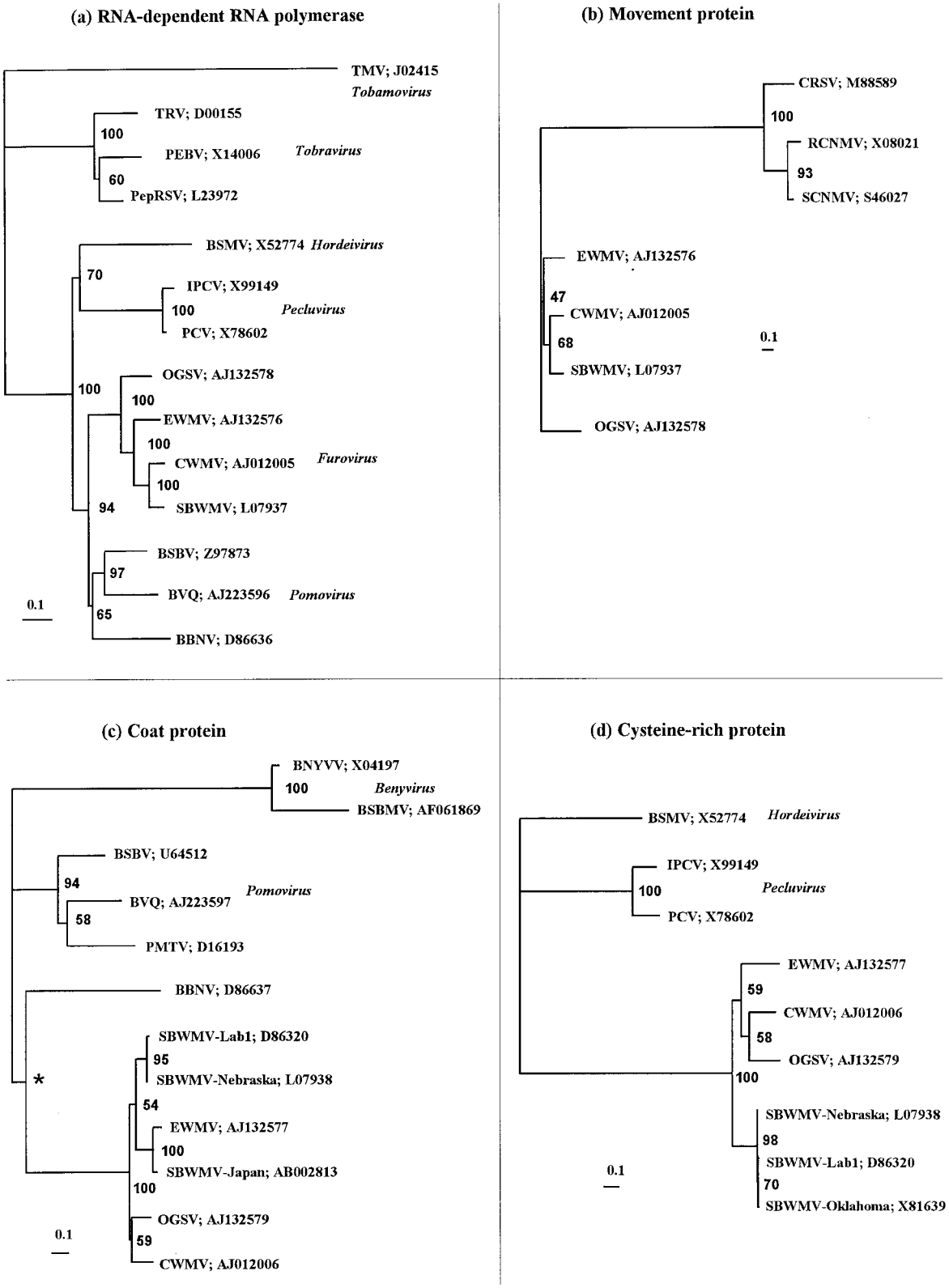

FIG. 2. Phylogenetic trees of the peptide sequences of selected parts of the furovirus genomes and those of related plant viruses obtained using NEIGHBOR analysis and displayed as a Phylogram from VIEWTREE: (a) the RNA-dependent RNA polymerase (tree rooted with TMV); (b) the movement protein (tree rooted with dianthoviruses); (c) the coat protein (tree rooted with benyviruses) and (d) the cysteine-rich protein (tree rooted with BSMV). The values at the forks indicate the number of times out of 100 trees that this grouping occurred after bootstrapping the data. The trees obtained from the original data sets and the consensus trees obtained after bootstrapping were usually identical. Where marked with an asterisk (*) BBNV grouped in the consensus tree with the other species of the genus *Pomovirus* with a bootstrap value of 48. The bars show differences as substitutions per amino acid. BBNV, broad bean necrosis virus; BNYVV, beet necrotic yellow vein virus; BSBMV, beet soil-borne mosaic virus; BSBV,

The coat protein readthrough domain and fungus transmission

Evidence from other fungus-transmitted rod-shaped viruses indicates that the coat protein readthrough domain is incorporated into virions and located at one end of the particle (Haeberlé *et al.,* 1994; Cowan *et al.,* 1997) and that the C-terminal half of the domain is important for fungus transmission. Spontaneous deletions in this region have been shown to prevent transmission of BNYVV by *Polymyxa betae* (Tamada and Kusume, 1991) and of PMTV by *Spongospora subterranea* (Reavy *et al.,* 1998). In experiments with BNYVV, substitution of KTER (at amino acid numbers 553–556) by ATAR completely prevented transmission by *P. betae* (Tamada *et al.,* 1996). This motif does not occur in the readthrough domains of the other fungally transmitted viruses that have been studied (except in the related BSBMV), although SBWMV has KTEIR at amino acids 466–469. Therefore various attempts have been made to identify other conserved regions within this domain that may be associated with fungus transmission (e.g., Koenig *et al.,* 1997; Reavy *et al.,* 1998). It is clear, however, that no simple amino acid motif can be identified that is common to all these viruses. Now that four different furoviruses have been sequenced, this region has been reexamined and the alignment (Fig. 3) shows that there is only limited sequence identity even between these related viruses transmitted by (presumably) the same vector. In particular, the KTEIR sequence in SBWMV does not occur in CWMV, EWMV, or OGSV. Attempts therefore have been made to identify those regions of the protein likely to be exposed on the surface (and thus could be available for interaction with a receptor site on the vector) and also those that might have transmembrane properties. These regions are indicated on Fig. 3, and they show that amino acids likely to be on the surface are not strongly conserved in the C-terminal part of the protein, although two regions identified in an earlier study (Peerenboom *et al.,* 1996) do occur consistently. These are the E[S/T]QR and the T[P/F]EER motifs identified as S1 and S2 on Fig. 3. Two highly conserved regions (T1 and T2) that are strongly predicted transmembrane domains were identified by use of four different software packages and by consideration of likely α helices. Directional alignment of the two helices (Fig. 4) also shows evidence of compatibility between their amino acids, with groupings of amino acids that are either identical or in the same hydrophobicity group and evidence of possible fits between the small glycine residues on one helix and the larger aromatic ones on the other. The deletion in OGSV occurs from the middle of the T2 region and this virus isolate therefore has only one predicted transmembrane region. It is predicted that the region between T1 and T2 would be on the inside of the membrane and therefore that the virus particle would be outside. It therefore seems possible that these regions are involved in attachment to the zoosporangial plasmalemma and assist virus particles to move between the cytoplasm of the plant host and that of the fungus vector. If this is so, the deletion in OGSV would probably eliminate fungus transmission, and this would be consistent with observations on other rod-shaped viruses transmitted by fungi (Tamada and Kusume, 1991; Reavy *et al.,* 1998).

Leaky stop codons

Most of the leaky stop codons so far identified in plant viruses are UAG (amber), but leaky opal (UGA) codons consistently terminate the first ORF of both RNAs of all four furoviruses that have been studied. Leaky opal codons also terminate the replicase genes of members of the genera *Pecluvirus* and *Tobravirus*, the coat protein of pea enation mosaic virus 1 and probably the P9 protein of maize chlorotic mottle virus. Their contexts are shown in Table 2 and demonstrate that most (including all the rod-shaped viruses) have CGG immediately downstream of the opal codon. The experimental work of Skuzeski *et al.,* (1991) with TMV showed that the two codons downstream of the amber codon were significant in determining leakiness and identified CAR-YYA as the optimal 3′ context for leakiness. They also demonstrated that the sequence CAA-UUA downstream of an opal codon could also confer leakiness. However, their experiments used only single base mutations from the wild-type TMV sequence and thus did not examine some of the nucleotide combinations now identified around leaky stop codons. Without further experimental work it is not possible to define a consistent set of rules determining leakiness or to know whether such rules would be identical for both opal and amber codons.

MATERIALS AND METHODS

Virus isolates and sequencing

For EWMV, wheat (cv. Avalon) was grown in soil from a field near Poitiers, Poitou-Charente, France. For OGSV, oats (cv. Peniarth) were grown in soil from Cranbrook, Kent, UK. Both isolates were then maintained in the glasshouse by mechanical inoculation. Infected leaves were stored at −70°C and purified using the methods of

beet soil-borne virus; BSMV, barley stripe mosaic virus; BVQ, beet virus Q; CRSV, carnation ringspot virus; CWMV, Chinese wheat mosaic virus; EWMV, European wheat mosaic virus; IPCV, Indian peanut clump virus; OGSV, oat golden stripe virus; PCV, peanut clump virus; PEBV, pea early-browning virus; PepRSV, pepper ringspot virus; PMTV, potato mop-top virus; RCNMV, red clover necrotic mosaic virus; SBWMV, soil-borne wheat mosaic virus; SCNMV, sweet clover necrotic mosaic virus; TMV, tobacco mosaic virus; TRV, tobacco rattle virus.

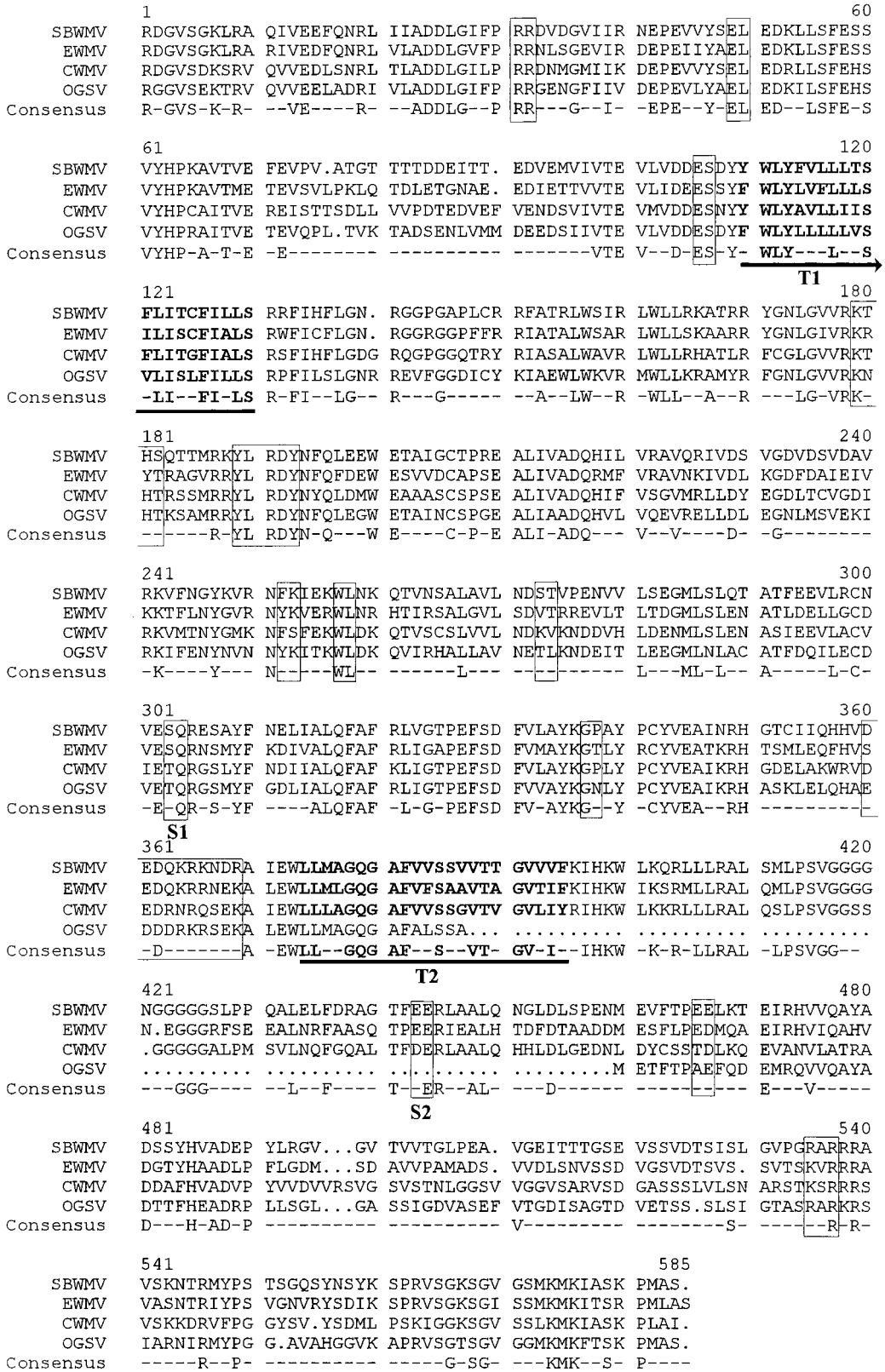

FIG. 3. Alignment of the amino acid sequence of the coat protein readthrough region of the furoviruses. Regions where two or more consecutive amino acids consistently have a surface probability ≥1.25 in GCG PEPTIDESTRUCTURE are boxed. Two substantially conserved regions identified as possible transmembrane domains (T1 and T2) are shown in bold.

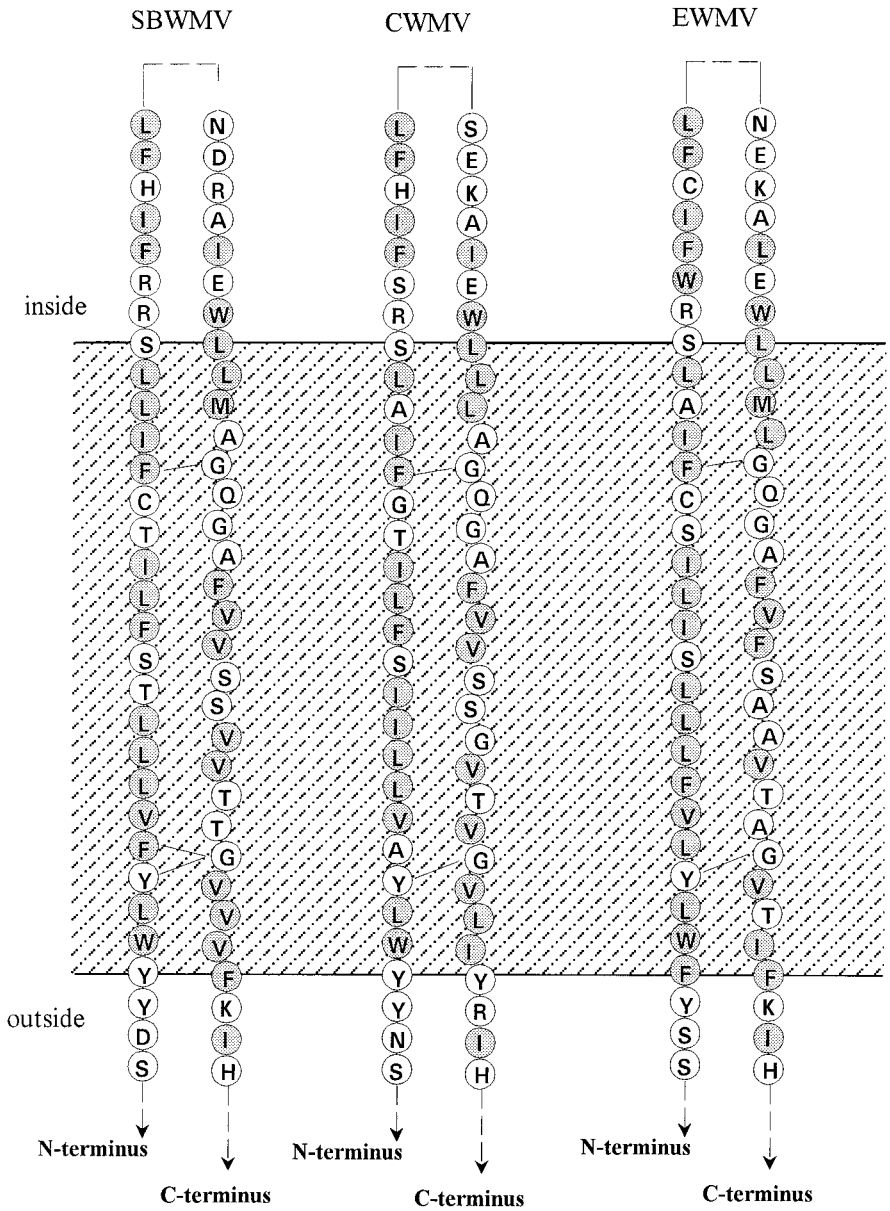

FIG. 4. Directional alignments of the two putative transmembrane regions of SBWMV, CWMV and EWMV, showing their predicted alignment within the membrane. Highly hydrophobic residues are shaded and glycine-aromatic residue fits are marked by lines.

Diao *et al.* (1999; for EWMV) or Adams *et al.* (1988; for OGSV).

Viral RNAs were extracted from the purified virus preparation using the method described by Chen *et al.* (1994). A similar sequencing strategy was adopted for all four RNA species presented here. First, a genome fragment was amplified by RT–PCR, using primers designed to conserved regions of the genome and this was cloned and sequenced. Primers designed from this sequence then were used in RT–PCR reactions to produce fragments extending toward the 5′ and 3′ termini. To ensure that the terminal nucleotides were included, a DNA primer SP6R (5′-CCCCCCTATAGTGTCACCTAAAT-3′) was ligated to the 3′terminus of viral RNA and also to the 3′ terminus of first strand cDNA. Primer SP6 (5′-GATTTAGGTGACACTATAG-3′), complementary to SP6R, then was used in combination with primers designed to internal sequences to amplify, clone, and sequence the entire genome. Sequences were obtained in both direction from overlapping clones and additional clones were obtained to confirm the terminal sequences and regions of ambiguity. For the 3′ terminus, the ligation reaction used 10 U T4 RNA ligase (Biolabs) in a 20-μl reaction, which contained *ca.* 1 μg viral RNAs, 200 pmol SP6R, 50mM Tris–HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 10 μg/ml BSA, 25% PEG (MW 8000), and 10 U RNase inhibitor (GIBCO), incubated at 37°C for 15 min. The RNA/SP6R ligation product was purified using a

TABLE 2

Context of the Leaky Opal (UGA) Stop Codons in Furovirus Genomes and in All Other Plant Viruses Where They Have Been Identified

Virus and segment	Gene	Accession no.					
Furovirus							
EWMV RNA1	replicase	AJ132576	gcg	aaa	UGA	cgg	uuu
EWMV RNA2	coat protein	AJ132577	acc	agu	UGA	cgg	gac
OGSV RNA1	replicase	AJ132578	cag	aaa	UGA	cgg	uuu
OGSV RNA2	coat protein	AJ132579	agu	gcc	UGA	cgg	ggc
CWMV RNA1	replicase	AJ012005	gac	aaa	UGA	cgg	uuu
CWMV RNA2	coat protein	AJ012006	ucg	agu	UGA	cgg	gau
SBWMV RNA1	replicase	L07937	acu	aaa	UGA	cgg	uuu
SBWMV RNA2	coat protein	L07938	ucg	agu	UGA	cgg	gac
Others							
BBNV RNA1	replicase	D86636	ccu	aaa	UGA	cgg	ugu
IPCV RNA1	replicase	X99149	acc	aaa	UGA	cgg	uuu
PCV RNA1	replicase	X78602	acc	aaa	UGA	cgg	uuu
PEBV RNA1	replicase	X14006	aug	aaa	UGA	cgg	ugu
PePRSV RNA1	replicase	L23972	gcc	uua	UGA	cgg	ugu
TRV RNA1	replicase	D00155	guc	uua	UGA	cgg	uuu
MCMV RNA	P9	X14736	uuc	aac	UGA	gcu	gga
PEMV-1 RNA	coat protein	L04573	ucc	cuc	UGA	ggg	gac

QIAGEN RNeasy Plant Minikit following the manufacturer's protocol. For the 5′ terminus, first-strand cDNA synthesis used a reverse primer with purified viral RNA as the template and the resulting RNA/cDNA hybrid was purified using the QIAquick Gel Extraction Kit (QIAGEN) and denatured by heating at 100°C for 2 min. Single-stranded cDNA was then ligated with primer SP6R as described above, and the ligation product used as a template for PCR with primer SP6 and a reverse primer.

To obtain the starting sequence for EWMV RNA1, a set of primers was designed on the basis of the helicase domain using a strategy similar to that used for beet soil-borne virus by Koenig and Loss (1997). The upstream primer FU3177 (5′-GATGGTGTTCCCGGCTGTGGAAAATC-3′) was in equivalent position to primer UF8 of Koenig and Loss (1997) but matched the corresponding sequence of SBWMV Nebraska isolate (L07937, nts 3177–3202). The sequence of the downstream primer, RC131 (5′-GAAACACCCTGTCGTAGAAGTCCTGC-3′), was chosen from the sequence of CWMV (AJ012005, nts 4235–4260).

To obtain the starting sequence for EWMV RNA2, the upstream primer Ye5 (5′-CTTGACATGTGGGAAGC-3′), corresponding to nts 1443–1459 on CWMV RNA2 (AJ012006) was used in combination with SP6 on viral RNA ligated to primer SP6R, to amplify ~2 kb at the 3′ terminus. A second upstream primer FFSB2–1 (5′-TGCTTAATGGCGTGAGTAAATTAGG-3′), corresponding to nts 482–506 on SBWMV, RNA2 (L07938) was also used to help extend the sequence toward the 5′ terminus.

To obtain the starting sequence for OGSV RNA1, the upstream primer FU3177 (as used for EWMV RNA1) was used in combination with SP6 on viral RNA ligated to primer SP6R, to amplify ~1.4 kb at the 3′ terminus. A second upstream primer FCSB 10 (5′-AGGGCTTATGCGATGGCTATCC-3′), corresponding to nts 750–771 on CWMV RNA1 (AJ012005) was also used to help extend the sequence toward the 5′ terminus.

To obtain the starting sequence for OGSV RNA2, the upstream primer Ogf5 (5′-ATTTGGGCATTTTTCCGAGACG-3′), corresponding (with one mismatch) to nts 938–959 on SBWMV RNA2 (L07938), was used in combination with SP6 on viral RNA ligated to primer SP6R, in an attempt to amplify ~2.6 kb at the 3′ terminus. Only a fragment of 0.6 kb was cloned but this was sufficient to provide the starting sequence.

PCR products were cloned using the pGEM T-Easy vector system (Promega Inc., Southampton, UK), and competent cells JM109 were transformed. Plasmids were extracted using the QIAprep spin miniprep kit (Qiagen Ltd., Crawley, UK), and the clones were then sequenced on an ABI automated sequencer, using cycle sequencing with the ABI PRISM Dye terminator cycle ready reaction kit according to the manufacturer's instructions.

Sequence analyses

The Wisconsin (GCG) package (version 8; Genetics Computer Group, 1994) program GAP was used for pairwise comparisons of nucleotide and amino acid sequences, using a gap creation penalty of 5.00 (nucleotides) or 3.00 (amino acids) and a gap extension penalty of 0.30 (nucleotides) or 0.10 (amino acids).

Phylogenetic analyses were done using programs in PHYLIP version 3.572c (Felsenstein, 1993). Sequences from other viruses were obtained from, and translated by, the files and software provided with Descriptions of Plant Viruses on CD-ROM (Adams *et al.,* 1998). Peptide sequences were aligned with the GCG program PILEUP, using a gap weight of 3.000 and a gap length weight of 0.100. Genetic distances between pairs of sequences were calculated using the program PROTDIST using the Dayhoff PAM method. Phylogenetic trees were constructed by a distance method (NEIGHBOR) using the original data set and 100 bootstrap data sets generated by the program SEQBOOT from the original set. In all cases the consensus tree was generated by the program CONSENSE. Trees were displayed using the program TREEVIEW (Page, 1996).

Transmembrane regions were predicted from peptide sequences by the programs HMMTOP (Tusnády and Simon, 1998), TMHMM (Sonnhammer *et al.,* 1998), TMPred (Hofmann and Stoffel, 1993), and TopPred 2 (von Heijne, 1992) and by considering likely α helices using the scales of Chou and Fasman (1974) and of Deleage and Roux (1987). All the information was used to produce a consensus model.

ACKNOWLEDGMENTS

We thank Mrs Margaret Perry for help with the sequencing. This work was partly funded by an EU collaborative project (INCO IC18-CT960049), Chinese High-tech Program (863), Zhejiang Provincial Foundation for Natural Sciences (RC9604), and the China Foundation for Natural Sciences (39600098). Aipo Diao is a student registered for the degree of PhD at the University of Hertfordshire, Hatfield, UK. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. The import and maintenance of EWMV was done under the provision of the MAFF plant health licence 37/2414.

REFERENCES

Adams M. J., Jones, P., and Swaby, A.G. (1988). Purification and some properties of oat golden stripe virus. *Ann. Appl. Biol.* **112**, 285–290.

Adams, M. J., Antoniw, J. F., Barker, H., Jones, A. T., Murant, A. F., and Robinson, D. (1998). "Descriptions of Plant Viruses on CD-ROM." Association of Applied Biologists, Wellesbourne.

Chen, J. P., Macfarlane, S. A., and Wilson, T. M. A. (1994). Detection and sequence analysis of a spontaneous deletion mutant of soil-borne wheat mosaic virus RNA2 associated with increased symptom severity. *Virology* **202,** 921–929.

Chen, J., Shi, N., Wilson, T. M. A., Antoniw, J. F., Macfarlane, S. A., and Adams, M. J. (1996). Sequence analysis of wheat and oat furovirus capsid protein genes suggests that oat golden stripe virus is a strain of soil-borne wheat mosaic virus. *Virus Res.* **41,** 179–183.

Chou, P. Y., and Fasman, G. D. (1974). Prediction of protein conformation. *Biochemistry* **13,** 211–222.

Cowan, G. H., Torrance, L., and Reavy, B. (1997). Detection of potato mop-top virus capsid readthrough protein in virus particles. *J. Gen. Virol.* **78,** 1779–1783.

Deleage, G., and Roux, B. (1987). An algorithm for protein secondary structure prediction based on class prediction. *Protein Eng.* **1,** 289–294.

Diao, A., Chen, J., Ye, R., Zheng, T., Yu, S., Antoniw, J. F., and Adams, M. J. (1999). Complete sequence and genome properties of Chinese wheat mosaic virus, a new furovirus from China. *J. Gen. Virol.* **80,** 1141–1145.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.572c. Distributed by the author, Department of Genetics, University of Washington, Seattle.

Genetics Computer Group (1994). "Program Manual for the Wisconsin Package Version 8." 575 Science Dr., Madison, WS, 53711.

Haeberlé, A. M., Stussigaraud, C., Schmitt, C., Garaud, J. C., Richards, K. E., Guilley, H., and Jonard, G. (1994). Detection by immunogold labelling of P75 readthrough protein near an extremity of beet necrotic yellow vein virus-particles. *Arch. Virol.* **134,** 195–203.

Hofmann, K., and Stoffel, W. (1993). TMbase—A database of membrane spanning proteins segments. *Biol. Chem. Hoppe-Seyler* **374,** 166.

Kendall, T. L., and Lommel, S. A. (1988). Fungus-vectored viruses of wheat in Kansas. *In* "Viruses with Fungal Vectors" (J. I. Cooper and M. J. C. Asher, Eds.), pp. 37–60. Association of Applied Biologists, Wellesbourne.

Koenig, R., Commandeur, U., Loss, S., Beier, C., Kaufmann, A., and Lesemann, D.-E. (1997). Beet soil-borne virus RNA 2: Similarities and dissimilarities to the coat protein gene-carrying RNAs of other furoviruses. *J. Gen. Virol.* **78,** 469–477.

Koenig, R., and Loss, S. (1997). Beet soil-borne virus RNA 1: Genetic analysis enabled by a starting sequence generated with primers to highly conserved helicase-encoding domains. *J. Gen. Virol.* **78,** 3161–3165.

Mushegian, A. R., and Koonin, E. V. (1993). Cell-to-cell movement of plant viruses. Insights from amino acid sequence comparisons of movement proteins and from analogies with cellular transport systems. *Arch. Virol.* **133,** 239–257.

Page, R. D. M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Comp. Appl. Biosci.* **12,** 357–358.

Peerenboom, E., Jacobi, V., Antoniw, J. F., Schlichter, U. H. A., Cartwright, E. J., Steinbiss, H.-H., and Adams, M. J. (1996). The complete nucleotide sequence of RNA-2 of a fungally-transmitted UK isolate of barley mild mosaic bymovirus (BaMMV) and identification of amino acid combinations possibly involved in fungus transmission. *Virus Res.* **40,** 149–159.

Plumb, R. T., Catherall, P. L., Chamberlain, J. A., and Macfarlane, I. (1977). A new virus of oats in England and Wales. *Ann. Phytopathol.* **9,** 365–370.

Pringle, C. R. (1999). Virus Taxonomy—1999. *Arch. Virol.* **144,** 421–429.

Reavy, B., Arif, M., Cowan, G. H., and Torrance, L. (1998). Association of sequences in the coat protein/readthrough domain of potato mop-top virus with transmission by *Spongospora subterranea*. *J. Gen. Virol.* **79,** 2343–2347.

Shirako, Y. (1998). Non-AUG translation initiation in a plant RNA virus: A forty amino acid extension is added to the N terminus of the soil-borne wheat mosaic virus capsid protein. *J. Virol.* **72,** 1677–1682.

Shirako, Y., and Wilson, T. M. A. (1993). Complete nucleotide sequence and organization of the bipartite RNA genome of soil-borne wheat mosaic virus. *Virology* **195,** 16–32.

Skuzeski, J. M., Nichols, L. M., Gesteland, R. F., and Atkins, J. F. (1991). The signal for a leaky UAG stop codon in several plant viruses includes two downstream codons. *J. Mol. Biol.* **218,** 365–373.

Sonnhammer, E. L. L., von Heijne, G., and Krogh, A. (1998). A hidden Markov model for predicting transmembrane helices in protein sequences. *In* "Proceedings of the Sixth International Conference on Intelligent Systems for Molecular Biology" (J. Glasgow, T. Littlejohn, F. M. J. Lathrop, D. Sankoff, and C. Sensen, Eds.), pp. 175–182. AAAI Press, Menlo Park, CA.

Tamada, T., and Kusume, T. (1991). Evidence that the 75K readthrough protein of beet necrotic yelow vein virus RNA-2 is essential for transmission by the fungus *Polymyxa betae*. *J. Gen. Virol.* **72,** 1497–1504.

Tamada, T., Schmitt, C., Saito, M., Guilley, H., Richards, K., and Jonard, G. (1996). High resolution analysis of the readthrough domain of beet necrotic yellow vein virus readthrough protein: A KTER motif is important for efficient transmission of the virus by *Polymyxa betae*. *J. Gen. Virol.* **77,** 1359–1367.

Torrance, L., and Mayo, M. A. (1997). Proposed re-classification of furoviruses. *Arch. Virol.* **142,** 435–439.

Tusnády, G. E., and Simon, I. (1998). Principles governing amino acid composition of integral membrane proteins: Applications to topology prediction. *J. Mol. Biol.* **283,** 489–506.

von Heijne, G. J. (1992). Membrane protein structure prediction, hydrophobicity analysis and the positive-inside rule. *J. Mol. Biol.* **225,** 487–494.

Ye, R., Zheng, T. Chen, J., Diao, A., Adams, M. J., Yu, S., and Antoniw, J. F. (1999). Characterization and partial sequence of a new furovirus of wheat in China. *Plant Pathol.,* **48,** in press.