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Genome properties of beet virus Q, a new furo-like virus from sugarbeet, determined from unpurified virus

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Based solely on the information that beet virus Q (BVQ) contains tubular particles, the entire nucleotide sequence of its tripartite genome was determined from unpurified virus in ca. 40 ml crude sap from locally infected *Chenopodium quinoa*. A starting sequence for RNA 1 was generated using primers corresponding to highly conserved helicase domains in the respective RNAs of furo-, pomo-, peclu-, hordei- and tobraviruses, and was extended by a walking random-primed cDNA approach. The similarity of the 3′ ends of furoviral RNAs allowed starting sequences for BVQ RNAs 2 and 3 to be obtained once the 3′ end of RNA 1 was known. BVQ RNA 1 encodes a protein with a methyltransferase-like, a variable and a helicase-like region, and for a readthrough protein which, in addition, contains an RNA-dependent RNA polymerase region. RNA 2 carries the coat protein gene, a coat protein readthrough protein gene and two additional ORFs which may have arisen by deletions from an originally larger readthrough domain. RNA 3 carries a triple gene block resembling that of several other rod-shaped viruses. The 5′ UTRs of the three RNAs have the potential to form a series of hairpins with C–A and C–C mismatches resembling those found in tymoviral RNAs. The 3′ ends can be folded into tRNA-like structures which are preceded by a long hairpin-like structure and an upstream pseudoknot domain. BVQ belongs to the recently proposed genus *Pomovirus*; it shows evolutionary relationships to furoviruses *in sensu stricto*, peclu-, hordei-, tobra-, tymo-, tobamo-, carla- and potexviruses.

Introduction

During a survey on soil-borne viruses in sugarbeet, two viruses with rod-shaped particles were isolated in addition to beet necrotic yellow vein furovirus (BNYVV) (Lesemann *et al.*, 1989). The Ahlum isolate proved to be serologically closely related to beet soil-borne virus (BSBV), which was first described in England (Ivanovic *et al.*, 1983; Henry *et al.*, 1986) and has since been found in all sugarbeet growing areas worldwide (e.g. Verhoyen *et al.*, 1987; Lindsten, 1989; present authors' unpublished observations). The second virus (Wierthe isolate) was originally thought to be a serologically distantly related strain of BSBV (Lesemann *et al.*, 1989; Barbarossa *et al.*, 1992), but the molecular data presented in this paper suggest that it is a distinct virus for which we propose the name beet virus Q (BVQ). A starter sequence, which was generated with primers specific for highly conserved helicase-encoding domains in RNA 1 of furo-, pomo-, peclu-, hordei- and tobraviruses (Koenig & Loss, 1997), has enabled the entire nucleotide sequence of the tripartite genome of unpurified BVQ to be determined. This paper describes genome properties of BVQ and compares them with those of other, related viruses.

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The EMBL accession numbers of the sequences reported in this paper are AJ223596, AJ223597 and AJ223598 for RNAs 1, 2 and 3, respectively, of BVQ.

Methods

■ **BVQ was maintained on its local lesion host, *Chenopodium quinoa*.** RT–PCR products were obtained using RNA from immunocaptured virus particles or denatured preparations of dsRNA as templates for cDNA synthesis (Koenig *et al.*, 1996, 1997; Koenig & Loss, 1997). They were purified from agarose gels using the QIAquick Gel Extraction kit, cloned into the pT7Blue T-vector (Novagen) and sequenced by means of the Sequenase DNA Sequencing kit version 2

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(United States Biochemical) using [α-^{35}S]dATP in the labelling reaction. 5′- and 3′-terminal sequences were determined using magnetic bead technology, as described by Koenig (1997). Each part of the sequence was read from two to eleven different overlapping clones. The secondary structures of the 5′- and 3′-UTRs were predicted using the genetic algorithm method of RNA folding as described previously, and as implemented in the STAR package for RNA structure predictions (Gultyaev *et al.*, 1995).

Results and Discussion

General properties of BVQ

BVQ was isolated together with BNYVV from a sugarbeet tap root obtained from a rhizomania field near Braunschweig, Germany. Like BNYVV, it causes local lesions on *C. quinoa*, but those of BVQ appear earlier, ca. 5 days after mechanical inoculation. They have a more irregular shape with a tendency to spread along the veins. Systemic infections of *C. quinoa* were not observed. BVQ and BNYVV were separated from originally mixed infections by single lesion transfers. BVQ could not be transmitted to *Nicotiana benthamiana* or *Nicotiana clevelandii*, nor could it be retransmitted by mechanical means to sugarbeet leaves or roots. It is possible that this isolate can be transmitted only by a presumably soil-borne vector, or it may have lost its infectivity for beets after several years of cultivation on *C. quinoa*. The virus proved to be extremely difficult to purify; nevertheless, an antiserum was obtained with a partially purified virus preparation which, despite some additional reactivity with constituents of healthy *C. quinoa*, could be used in immunocapture RT–PCR, immunosorbent electron microscopy (Milne & Lesemann, 1984) and the immunoelectron microscopical decoration test (Milne, 1984). By means of immunoelectron microscopical tests the virus has been detected repeatedly during the past decade in sugarbeet samples from various areas in Germany and abroad, indicating that it is widely spread. Because our knowledge on the biological and pathogenic properties of the virus is limited, we propose the name beet virus Q.

Strategy for determining the complete nucleotide sequence of the tripartite genome of BVQ

Because BVQ is extremely difficult to purify, a strategy was adopted for analysing its genome properties which is based partially on previous experiences with BSBV (Koenig, 1997; Koenig & Loss, 1997; Koenig *et al.*, 1997) and is outlined in Fig. 1. A starting sequence for BVQ RNA 1 was obtained with primers UF8 (5′ dGATGGTGTACCCGGATGTGGAAAGTC 3′) and UF9 (5′ dATGCGCCATCAACGCTTCATCAAAATG 3′) (Koenig & Loss, 1997), which were derived from the coding sequences for helicase motifs I (sense) and II (antisense), respectively, of RNA 1 of potato mop-top furovirus (PMTV) (B. Reavy, personal communication). These sequences are highly conserved in RNA 1 of furoviruses (soil-borne wheat mosaic furovirus, SBWMV) (Shirako & Wilson, 1993); pomoviruses (BSBV) (Koenig & Loss, 1997); pecluviruses (peanut clump virus, PCV) (Herzog *et al.*, 1994); hordeiviruses (barley stripe mosaic hordeivirus, BSMV) (Gustafson *et al.*, 1989); and tobraviruses (Uhde *et al.*, 1998). By means of random-primed cDNAs prepared against ssRNA from immunocaptured virus particles or denatured preparations of dsRNA, and PCR using the respective random primer used for cDNA synthesis in combination with a specific primer derived from the known part of the sequence, we were able to obtain step by step almost the entire sequence of RNA 1 of BVQ. The 5′ and 3′ ends of this RNA were obtained after dG-homopolymer tailing of minute amounts of cDNAs which, for washing purposes, had been trapped on avidin-coated magnetic beads via 3′-biotinylated oligonucleotides (Koenig, 1997; Fig. 1: ACEMBBO). Because the 3′ ends of all furo-, pomo- and pecluvirus RNAs investigated so far are known to be similar for a given virus (e.g. Shirako & Wilson, 1993; Herzog *et al.*, 1994; Koenig & Loss, 1997), primer T was designed to be complementary to the 3′ end of BVQ RNA 1, which had been determined by means of ACEMBBO after dG-homopolymer tailing. Random-primed cDNAs to denatured preparations of dsRNA were amplified by means of PCR with the respective random primer used in the cDNA synthesis together with primer T. Of the various PCR products obtained, some had a sequence identical to that of the 3′ end of RNA 1 of BVQ, but in others the sequence was different. Their extension by the random-primed cDNA approach outlined above revealed that starting sequences had been obtained for RNAs 2 and 3 of BVQ, which allowed their entire sequences to be obtained, including the true 3′ ends (Koenig, 1997) as described in Fig. 1. The determination of the entire sequence of the tripartite genome of BVQ, for which almost the only information available (except for its host range) was that it had tubular particles, was achieved with ca. 40 ml of crude sap from locally infected *C. quinoa*. A similar strategy should also be applicable to other viruses for which purification presents difficulties.

BVQ RNA 1

RNA 1 of BVQ consists of 6003 nucleotides (nt) (Fig. 2) and contains coding sequences for enzymes presumably involved in virus replication. A large ORF starts at AUG 121 and is interrupted at position 4051 by a UAA stop codon which terminates the first coding region (ORF 1). It then continues in-frame for an additional 1521 nt until it reaches another UAA stop codon at nt 5575, which terminates the putative read-through ORF 2 (Fig. 2, upper part). The 5′ and 3′ context of the first UAA stop codon (CAAUAACGGUUUGGGUC, stop codon underlined) is almost identical to that of BSBV RNA 1 (CAAUAACGGUGUGGGUC) and is very similar to that of the UGA stop codons (AAAUGACGGUUUGGGUC) at the corresponding positions in RNA 1 of SBWMV (Shirako & Wilson, 1993) and PCV (Herzog *et al.*, 1994). Presumably, this context confers leakiness on UGA and possibly also UAA stop codons. Since no further ORFs for peptides of more than 3 kDa

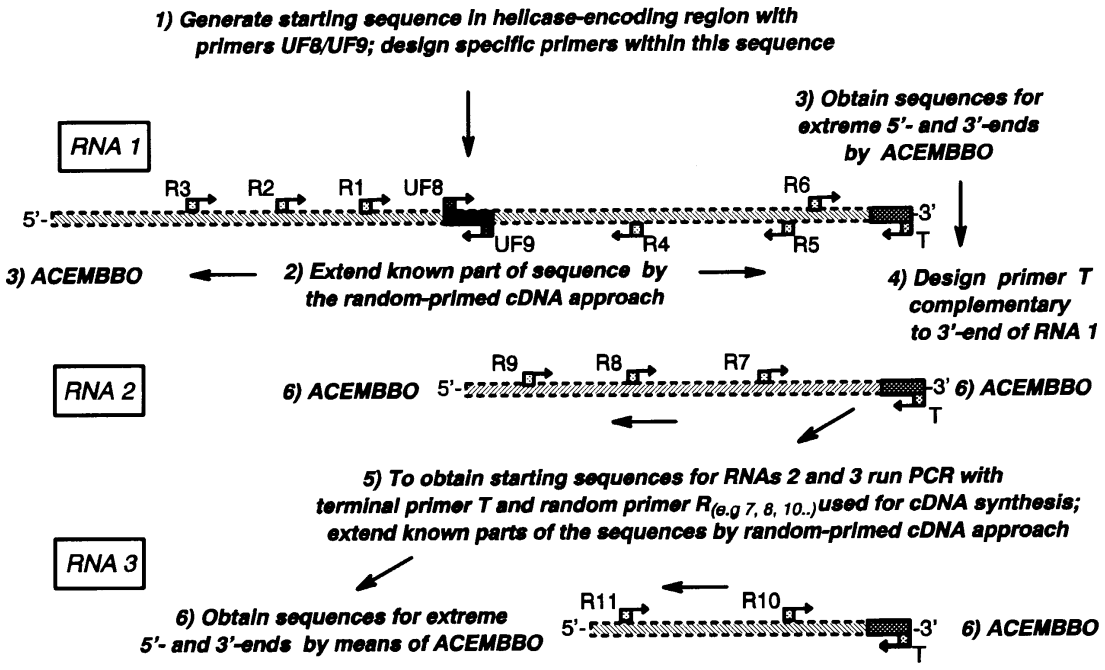

Fig. 1. Diagram illustrating the strategy used for the determination of the complete nucleotide sequence of unpurified BVQ. ACEMBBO denotes the amplification of cDNA ends assisted by magnetic beads and biotinylated oligonucleotides (Koenig, 1997).

are found on the plus strand of BVQ RNA 1, its genetic organization resembles that of BSBV RNA 1 and is different from those of all other rod-shaped viruses analysed so far (Koenig & Loss, 1997).

The putative proteins encoded in ORFs 1 and 2 have molecular masses of 149 kDa (149K protein) and 207 kDa (207K protein), respectively. They show considerable amino acid sequence identity with the corresponding proteins not only of BSBV but also SBWMV (Fig. 2, lower part). In addition, these two proteins have previously been shown to exhibit sequence identities with the corresponding proteins of other rod-shaped viruses such as the pecluvirus PCV, the hordeivirus BSMV and the tobravirus tobacco rattle virus (Koenig & Loss, 1997). The typical methyltransferase, helicase and RNA-dependent RNA polymerase motifs (Koonin & Dolja, 1993) are readily recognized in the N- and C-terminal parts of the putative BVQ 149K protein and in the readthrough portion of its 207K protein, respectively (Fig. 2, lower part). Also, outside these motifs, blocks of highly conserved sequences are found. Even in the previously identified variable region in the central part of the smaller RNA 1-encoded proteins (Koenig & Loss, 1997), some conserved residues are recognized for the BVQ, BSBV and SBWMV proteins (Fig. 2, lower part).

BVQ RNA 2

RNA 2 of BVQ consists of 2913 nt (Fig. 3). The 5′ proximal ORF encodes a protein with a molecular mass of 19 kDa, which is apparently the viral coat protein. It contains in its central and C-terminal parts, respectively, the RF and FE motifs, which are typical for the coat proteins of rod-shaped plant viruses (Koonin & Dolja, 1993), and additional conserved sequences which are shared especially with PMTV and BSBV coat proteins (Fig. 3). A conserved sequence, EDSALNVAHQL, found in the coat proteins of BVQ (amino acids 121–131), BSBV and PMTV, but not in those of SBWMV and other rod-shaped viruses, is especially conspicuous. One of the MAbs which Pereira *et al.* (1994) have prepared for PMTV is specific for an epitope in this region. This MAb (SCR 70) does not bind to intact PMTV particles but only to denatured PMTV coat protein after Western blotting, indicating that the epitope is located inside the intact particle (Pereira *et al.*, 1994). PMTV MAb 70 also strongly reacted with denatured BSBV and BVQ coat proteins in Western blotting, but not with intact virus particles in the immunoelectron microscopical decoration test (Milne, 1984). None of the other PMTV-specific MAbs described by Pereira *et al.* (1994) was bound by BVQ or BSBV particles, or the denatured coat proteins (results not shown). The coat protein of BVQ, like that of BSBV, lacks the ten immunodominant N-terminal amino acids which are found in PMTV coat protein (Kashiwazaki *et al.*, 1995; Koenig *et al.*, 1997). This is apparently the reason why no serological relationships are found between the intact particles of PMTV and those of the two beet viruses.

The coat protein gene of BVQ, like those of PMTV and BSBV, is terminated by an amber (UAG) stop codon (position

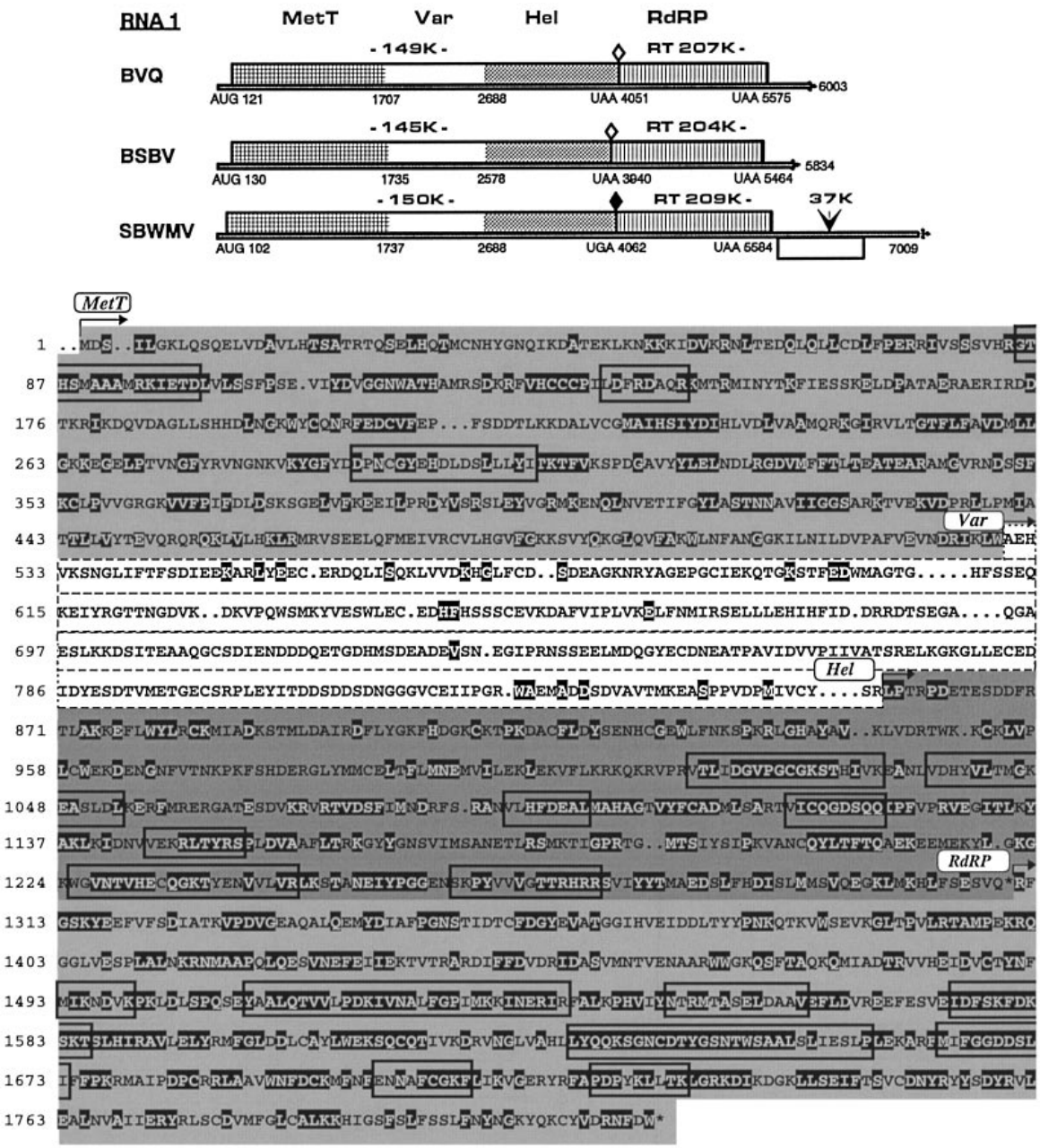

Fig. 2. Upper part: genetic organization of RNA 1 of BVQ, BSBV (Koenig & Loss, 1997) and SBWMV (Shirako & Wilson, 1993). Lower part: deduced amino acid sequence of the putative 207K readthrough protein of BVQ. White lettering on a black background denotes amino acid residues which are conserved in the BVQ, BSBV and SBWMV proteins. Additional amino acids not shown may be identical in the proteins of two of the three viruses. Dots in the sequence indicate that additional amino acids are present in the corresponding areas of the BSBV and/or SBWMV proteins. The regions containing the methyltransferase (MetT), helicase (Hel) and RNA-dependent RNA polymerase (RdRP) motifs (Koonin & Dolja, 1993) are highlighted by different shades of grey. The MetT motifs I to III, the Hel motifs I, Ia and II to VI and the RdRP motifs I to VIII in the respective regions are boxed. The variable region (Var) (Koenig & Loss, 1997), which contains few conserved residues, is shown on a white background.

823–825 of BVQ RNA 2). It is followed in-frame by a 972 nt ORF which reaches a UGA stop codon at position 1795. The two codons (CAA) and (UCA) immediately 3′ of the amber stop codon conform to the optimal leaky UAG context CAR–YYA (Skuzeski *et al.,* 1991). The leakiness of this stop codon has recently been proven for PMTV (Cowan *et al.,* 1997). Readthrough of the UAG stop codon on BVQ RNA 2 would result in a protein which has a molecular mass of 54 kDa, much smaller than the putative 104 kDa coat protein readthrough protein of BSBV. Two further ORFs with coding

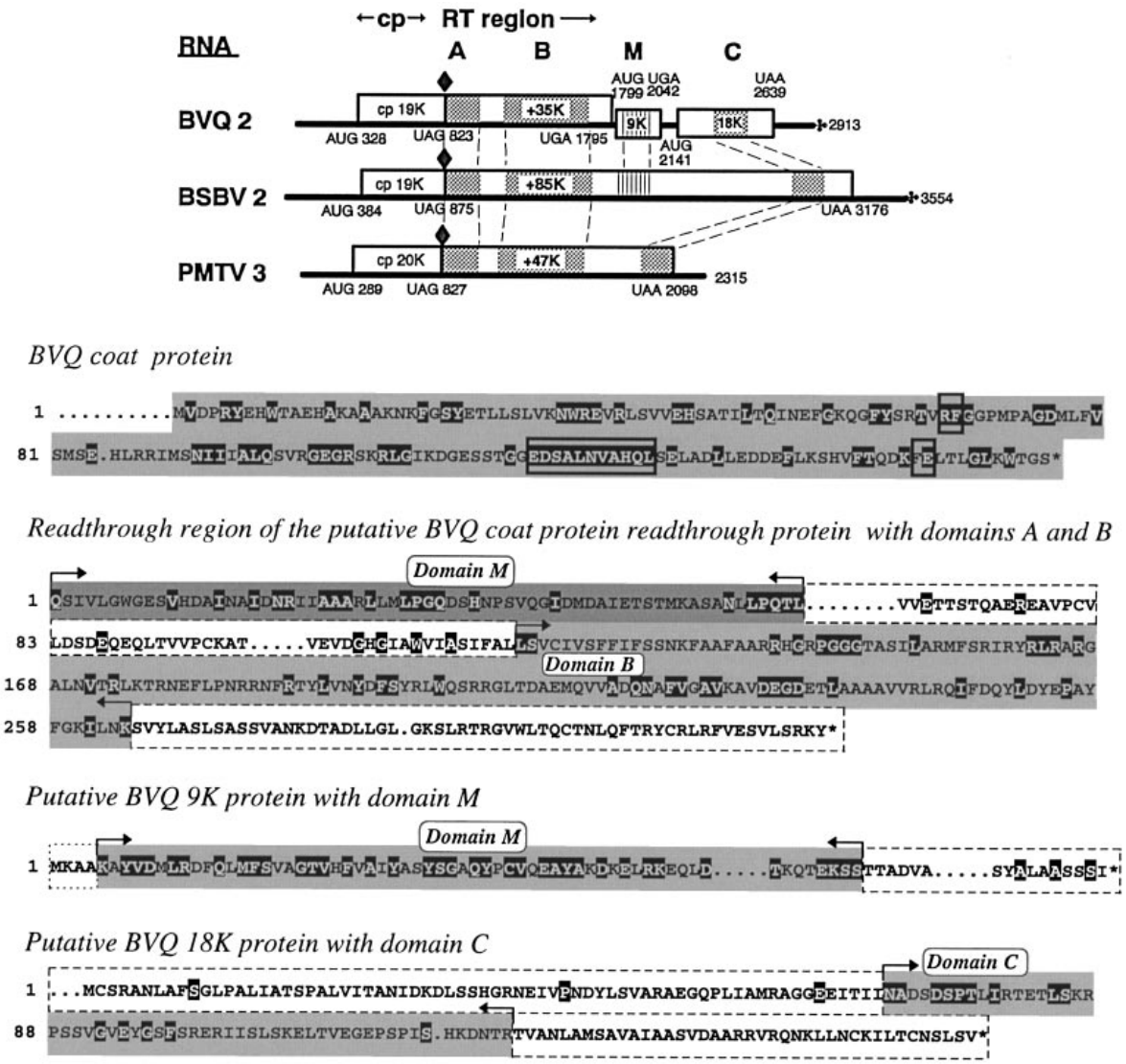

Fig. 3. Upper part: genetic organization of RNA 2 of BVQ and BSBV (Koenig *et al*., 1997) and RNA 3 of PMTV (Kashiwazaki *et al*., 1995). Lower parts: deduced amino acid sequences of the putative coat protein, coat protein readthrough protein and two further RNA 2-encoded proteins of BVQ. Dots in the sequences indicate that additional amino acids are present in the corresponding areas of the BSBV and/or PMTV proteins. White lettering on a black background denotes amino acid residues which are conserved in the proteins of all three viruses. However, the area of the 9K protein is found only in the BSBV readthrough protein but not in that of PMTV, and thus comparison in this area is only between the BVQ and BSBV proteins. The A, B and C domains which were previously identified in the BSBV and PMTV readthrough regions (Koenig *et al*., 1997), and the additional domain M which is found only in the BVQ and BSBV proteins, are highlighted by a grey background. Regions outside these domains are shown on a white background. The B domain may extend for a further 15–20 amino acids towards the C terminus.

capacities for 9 kDa and 18 kDa proteins start at AUG 1799 and 2141 and terminate with UGA and UAA stop codons at positions 2042 and 2639, respectively (Fig. 3, upper part).

The deduced amino acid sequence for the readthrough part of the BVQ coat protein readthrough protein contains the A and B domains which have been identified previously in the coat protein readthrough proteins of PMTV and BSBV (Koenig *et al.*, 1997). The C domain is present in the putative BVQ 18K protein. The 9K protein shows amino acid identity with a central region of the putative BSBV coat protein readthrough protein (Fig. 3, domain M). Alignments of the deduced amino acid sequences of the putative 9K and 18K proteins with that of the putative BSBV coat protein readthrough protein suggest that the two 3′-proximal ORFs of BVQ RNA 2 may have resulted from deletion events which have mutilated an originally longer coat protein readthrough ORF (Fig. 3). Deletions have frequently been observed in the coat protein readthrough domains of several rod-shaped viruses (e.g. Tamada & Kusume, 1991; Chen *et al.*, 1994; Koenig *et al.*, 1997).

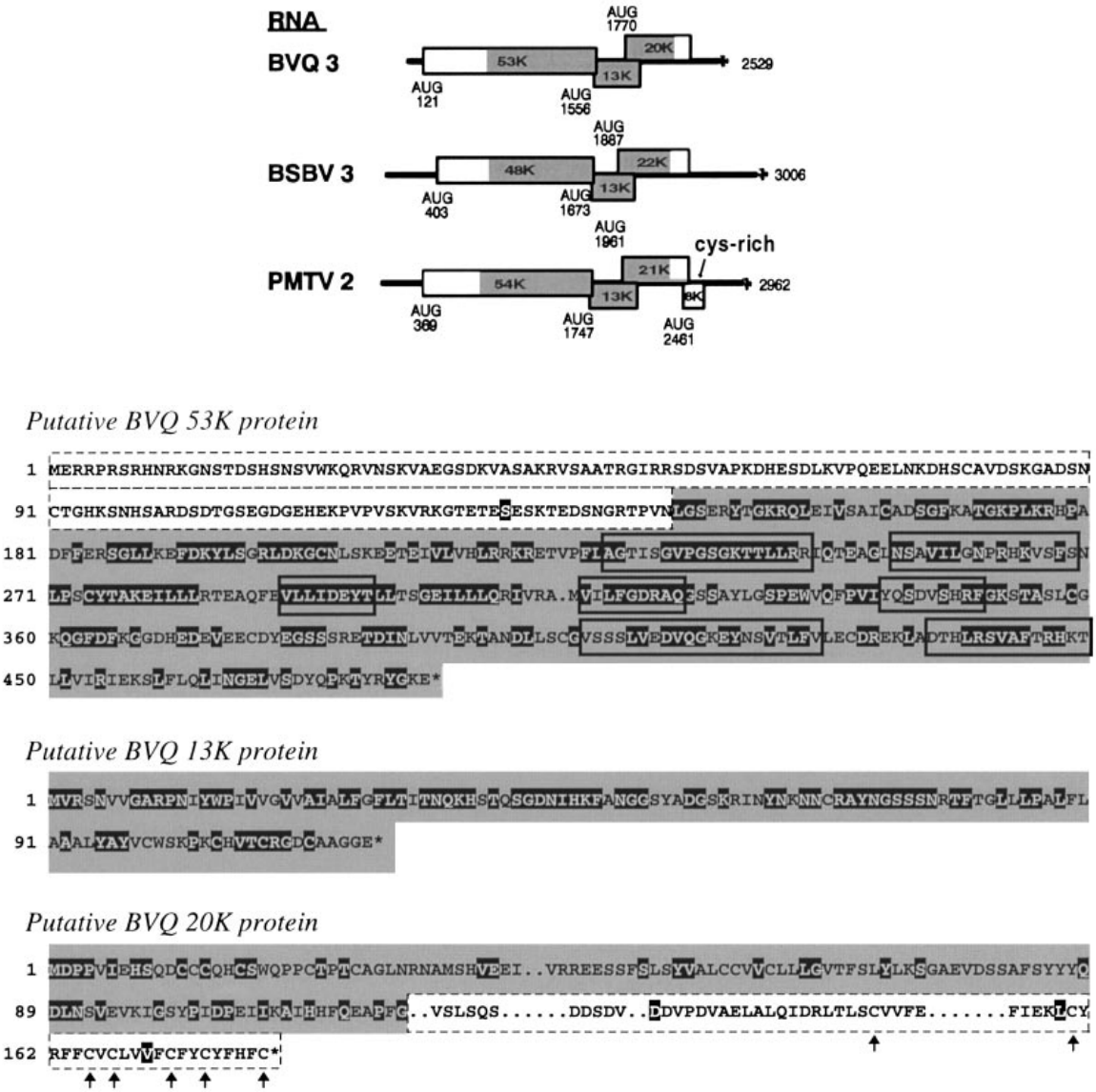

Fig. 4. Upper part: genetic organization of RNA 3 of BVQ and BSBV (Koenig *et al*., 1996) and RNA 2 of PMTV (Scott *et al*., 1994). Lower parts: deduced amino acid sequences of the putative translation products of BVQ RNA 3. Dots in the sequences indicate that additional amino acids are present in the corresponding areas of the BSBV and/or PMTV proteins. White lettering on a black background denotes amino acid residues which are conserved in the proteins of all three viruses. Additional amino acids not shown may be identical in the proteins of two of the three viruses, especially in areas highlighted by a grey background. Regions which are highly specific for each virus are shown on a white background. The helicase motifs I, IA and II to VI (Koonin & Dolja, 1993) in the putative 53K protein are boxed. The accumulation of C residues in the C-terminal part of the 20K protein sequence is marked by arrows.

BVQ RNA 3

BVQ RNA 3 contains a typical triple gene block (TGB) similar to those found on BSBV RNA 3 and PMTV RNA 2 (Fig. 4). However, with only 2529 nt, BVQ RNA 3 is considerably shorter than BSBV RNA 3 (3006 nt) (Koenig *et al*., 1996) and PMTV RNA 2 (2962 nt) (Scott *et al*., 1994). This is mainly due to the much shorter length of its 5′ UTR (120 nt versus 402 in BSBV RNA 3) and 3′ UTR (220 nt versus 547 in BSBV RNA 3) (Fig. 4, upper part). The three partially overlapping ORFs extend from nt 121–1566, 1556–1909 and 1770–2309, and have coding capacities for proteins with molecular masses of 53, 13 and 20 kDa, respectively. These proteins are presumably involved in virus movement, as has been shown for the TGB-encoded proteins of BSMV (Petty *et al*., 1990), white clover mosaic potexvirus (Beck *et al*., 1991) and BNYVV (Gilmer *et al*., 1992). The putative 53K protein carries the typical helicase motifs I, IA and II–VI (Koonin & Dolja, 1993) in its central and C-terminal part, which is strongly conserved in the corresponding proteins of BSBV and

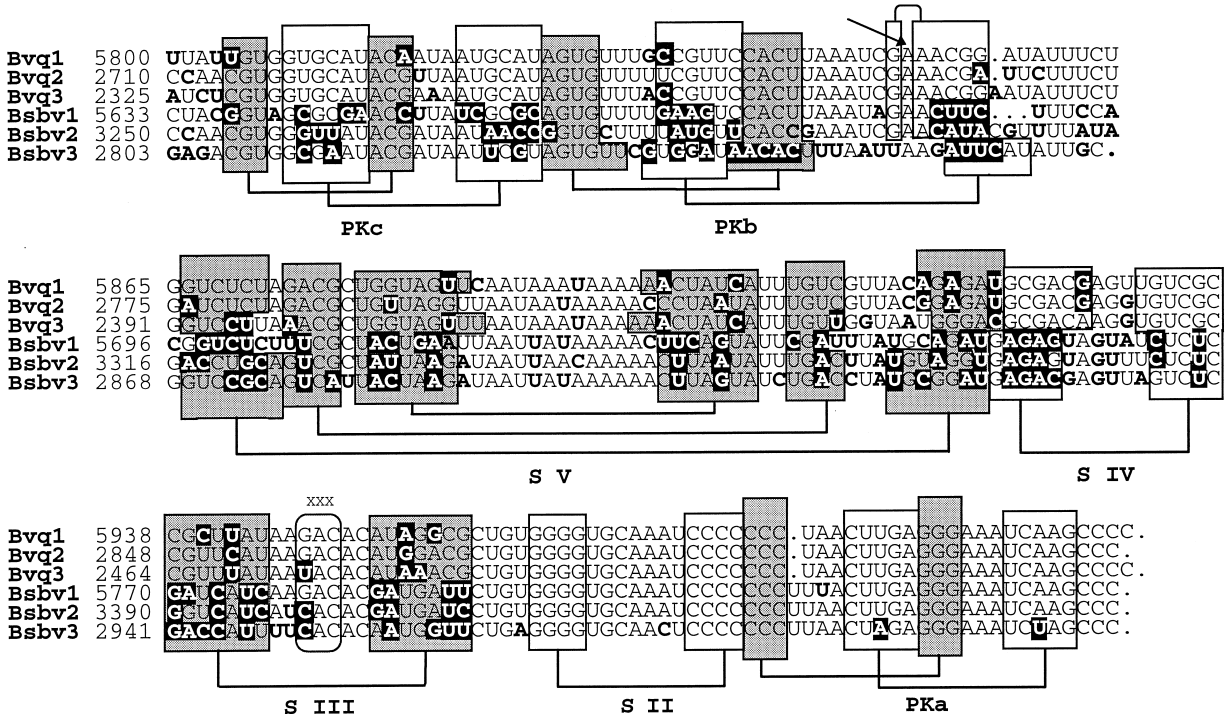

Fig. 5. Alignment of the 3′ ends of the three BVQ and BSBV RNA species showing the tRNA-like structures consisting of pseudoknot a (PKa) and stem–loops (S) II–IV, an upstream hairpin SV and an upstream pseudoknot domain consisting of PKb and PKc. White letters on a black background indicate covariations in the stems and also, in stem–loop SIII, variations in the anticodon for valine. The arrow points to the bulged A residue in PKb.

PMTV also. The N-terminal third of these proteins, however, is unique for each of the three viruses (Fig. 4). The amino acid sequences of the second TGB-encoded protein are highly conserved among the three viruses, and to a somewhat lesser extent also those of the N-terminal and central parts of the third protein, whereas the C-terminal parts of the third protein are specific for each virus (Fig. 4). The TGB-carrying RNA 2 of PMTV has an additional gene for a cysteine-rich protein (Scott *et al.*, 1994) which is not found on RNA 3 (Fig. 4) or any other genome parts of BVQ or BSBV. The C-terminal part of the third TGB-encoded protein of BVQ, however, contains numerous cysteine residues which are not found on the corresponding proteins of BSBV or PMTV. Nevertheless, no stretches of more than two identical amino acids were found between the C-terminal portion of the third TGB protein of BVQ and the 8K protein of PMTV.

The 5′ and 3′ UTRs of BVQ and BSBV RNAs

The 5′ ends of most furo-, pomo- and pecluviral RNAs consist of the sequences $GUA(U)_n$**..** (Shirako & Wilson, 1993; Scott *et al.*, 1994; Kashiwazaki *et al.*, 1995; Manohar *et al.*, 1993; Herzog *et al.*, 1994) or $GU(A)_2(U)_n$**..** (BSBV RNAs 1–3, Koenig *et al.*, 1996, 1997; Koenig & Loss, 1997). BVQ RNA 3 starts with $GUA(U)_5$**..** BVQ RNAs 2 and 1 deviate somewhat in starting with $GU(A)_4(U)_2$**..** and $AU(A)_2(U)_5$**..**, respectively. No stretches of more than four or five identical nucleotides were found between the 5′ UTRs of the three BVQ RNAs. The 5′ ends of BVQ and BSBV RNAs, like those of other furoviruses (Shirako & Wilson, 1993; Manohar *et al.*, 1993; Herzog *et al.*, 1994; Scott *et al.*, 1994; Kashiwazaki *et al.*, 1995) have the potential to fold into a series of long hairpins. In many of them, internal loops were detected with C–A and C–C mismatches, reported so far only for tymoviral RNAs (Hellendoorn *et al.*, 1996) where they have been shown to be important for replication and symptom production (Hellendoorn *et al.*, 1997). The first hairpin extends from nt 8–39, 12–36 and 9–43 in BVQ RNAs 1, 2 and 3, and from nt 6–38, 10–40 and 7–44 in BSBV RNAs 1, 2 and 3, respectively. In the first hairpins, C–A mismatches occur between nt 15–32 and 17–30 of BVQ RNA 1, between nt 17–31 of BVQ RNA 2 and between nt 20–36 of BVQ RNA 3, respectively. The loop of the second hairpin of the coat protein gene-carrying RNA 2 of BVQ and BSBV consists of seven nucleotides, sequence ACAGGNN (nt 73–79 and 83–89 of BVQ and BSBV RNA 2, respectively). With PMTV such a loop is found on the third hairpin of its coat protein gene-carrying RNA 3 (nt 145–151).

The 3′ ends of the three BVQ RNAs, like those of BSBV (Koenig & Loss, 1997) and several other furo- and pomoviral RNAs (e.g. Shirako & Wilson, 1993; Kashiwazaki *et al.*, 1995) have the capacity to fold into tRNA-like structures (Fig. 5), strongly resembling those of the valylatable 3′ ends of the

Table 1. Percentage identity of deduced amino acid sequences of various gene products of BVQ and other rod-shaped viruses with similar genome properties

Putative gene product	BVQ/BSBV	BVQ/PMTV	PMTV/BSBV	BVQ/most closely related other viruses
RNA 1				
ORF 1 protein (containing MetT- and hel-encoding domains)	54	–	–	45 (SBWMV) 39 (PCV)
Readthrough portion of ORF 2 protein (containing RdRP-encoding domain)	73	–	–	66 (SBWMV) 58 (PCV)
RNA 2				
Coat protein	53	51	52	33 (SBWMV) 26 (PCV)
RNA 3				
First TGB protein*	58	53	54	38 (PCV)
Second TGB protein*	75	69	68	51 (PCV)
Third TGB protein*	37	41	35	24 (PCV)

* Not present in SBWMV.
–, No data available for PMTV RNA 1.

turnip yellow mosaic tymovirus (TYMV) (Rietveld *et al*., 1982) and sunn-hemp mosaic tobamovirus (SHMV) RNAs (Rietveld *et al*., 1984). Stem–loop SII and pseudoknot PKa, forming the aminoacyl acceptor arm, are almost completely conserved among the six BSBV and BVQ RNAs. Moreover, the seven-membered hairpin loop of SII, equivalent to the T-loop of canonical tRNA, is identical to that of TYMV RNA in the case of BSBV RNA 3, and almost identical for the other five RNAs. The variability in loop 1 and the invariance in loop 2 (AAA) of PKa are characteristic features of many other pseudoknots, e.g. in the tymovirus tRNA-like structures (Mans *et al*., 1991).

The seven base-pair anticodon stem of hairpin III has a high number of covariations, thereby giving strong circumstantial evidence for its existence, and in all cases it has an anticodon for valine. The invariant ACAC sequence at the 3′ side is not only typical for the tymovirus tRNA-like structures, but is also conserved in canonical $tRNA^{val}$ (for a review see Mans *et al*., 1991). The U at the wobble position in the case of BVQ RNA 3, which was confirmed with four independent clones, however, is so far unique among valylatable tRNA-like structures. SIV is characterized by six (BVQ) or five (BSBV) base-pairs and a more variable loop, as also found for tymovirus tRNA-like structures (Mans *et al*., 1991).

Upstream of the tRNA-like structure, a long and stable hairpin is predicted, harbouring many covariations, again leaving little doubt about its existence. This hairpin is characterized by a long hairpin loop with a stretch of three to six A residues at the 3′ side. It is striking that for the RNAs of other pomo- (PMTV), peclu- (PCV) and furoviruses (SBWMV) a similar long hairpin can be predicted immediately upstream of SIV of the tRNA-like structure, although for SBWMV a pseudoknot (pseudoknot 1) was proposed previously in this region (Shirako & Wilson, 1993). This hairpin is strongly reminiscent of the one found in SHMV RNA, located between its valine-specific tRNA-like structure and the upstream pseudoknot domain (UPD). Even in the hordeivirus BSMV, a long hairpin was predicted between its tyrosine-specific tRNA-like structure and the UPD region (Pleij *et al*., 1987).

The pseudoknots PKb and PKc are strongly conserved in all BVQ RNAs and in BSBV RNAs 1 and 2. They show strong similarities to those described earlier in SBWMV (Shirako & Wilson, 1993), PMTV (Kashiwazaki *et al*., 1995), tobamoviruses (van Belkum *et al*., 1985), hordeiviruses (Pleij *et al*., 1987) and even one species of tymovirus (Hellendoorn *et al*., 1996). In most cases the 3′ proximal pseudoknot (PKb) has a bulged A residue at a specific position in the 3′ strand of stem 2, as also found for all the BVQ RNAs and for BSBV RNA 1 and 2 (Fig. 5, marked by an arrow), thereby ensuring linkage and coaxial stacking of the two stem regions of this pseudoknot. Loop 2, in the case of the three BVQ RNAs (UAAAUC), is identical to that of the tobamovirus pseudoknot which was shown to be a very important region for its function as a translational enhancer in tobacco mosaic virus RNA (Leathers *et al*., 1993). However, PKb of BSBV RNA 3 deviates even more in this respect.

The pseudoknot structure PKc has all the features of the so-called 'classic' pseudoknot: three and six base-pairs, respectively, for stems S1 and S2; one single nucleotide spanning the deep groove (mostly G); and the AU-rich three nucleotide loop 2 as in PKc-like pseudoknots at identical positions in the

3′ UTRs of other unrelated viral RNAs (Pleij *et al.*, 1987). Especially in the BSBV RNAs, PKc is highly supported by covariations. Interestingly, a partial repeat of this UPD motif is found just upstream of PKb and PKc in some of the BVQ and BSBV RNAs (results not shown).

Classification and evolutionary relationships of BVQ

In its genetic organization, BVQ resembles BSBV and differs from all other rod-shaped viruses for which complete genome analyses have been reported. Amino acid sequence alignments for the putative gene products of RNAs 2 and 3 reveal relationships especially to BSBV and PMTV. The calculated amino acid sequence identities range from slightly above 50% for the coat proteins, to slightly above 70% for the RNA-dependent RNA polymerase domains of the putative RNA 1-encoded readthrough proteins and the second TGB proteins (Table 1). With potyviruses, amino acid sequence identities in the coat proteins have been found to be above 90% for strains of the same virus, but below 73% for different virus species (Shukla & Ward, 1989). Similar percentages should also be useful for differentiation between viruses and strains in other genera of plant viruses (van Regenmortel *et al.*, 1997). The data listed in Table 1 suggest that BVQ represents a new virus species, rather than a strain of BSBV. BVQ and BSBV are about as distantly related as BSBV and PMTV, or BVQ and PMTV (Table 1). With their tripartite genomes, in which the movement functions are encoded in a TGB, these three viruses show the typical properties of the recently proposed genus *Pomovirus* (Torrance & Mayo, 1997).

The RNA 1-encoded proteins, which are presumably involved in replication, reveal relatively close relationships of BVQ and BSBV to SBWMV, a furovirus in the restricted sense of Torrance & Mayo (1997), and PCV, a member of the recently proposed genus *Pecluvirus* (Torrance & Mayo, 1997) (Fig. 2; Table 1), and even to hordei- and tobraviruses (Koenig & Loss, 1997). Affinities to tymoviruses are revealed by the 5′ and 3′ UTRs of the BVQ and BSBV RNAs (this paper) and to potex- and carlaviruses, as well as to hordeiviruses by the TGB-encoded proteins (Koenig *et al.*, 1996). The 3′ UTRs of the BVQ and BSBV RNAs, in addition, reveal affinities to hordei- and tobamoviruses.

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