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Nucleotide sequence of beet cryptic virus 3 dsRNA2 which encodes a putative RNA-dependent RNA polymerase

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The nucleotide sequence of a DNA copy of beet cryptic virus 3 double-stranded RNA2 was determined, and one strand was found to contain a single long open reading frame of 1431 nucleotides which encoded a putative polypeptide containing 478 amino acid residues with an M_r of 54·9K. This polypeptide contained conserved amino acid sequence motifs found in the genes that encode putative RNA-dependent RNA polymerases of other RNA viruses.

The viruses in the plant cryptic virus group are small isometric particles of 30 to 38 nm that are transmitted only through seed, cause no apparent symptoms and contain two or three molecules of dsRNA (Boccardo *et al.*, 1987; Milne, 1991). The beet cryptic virus (BCV) first identified in sugar beet has isometric particles about 30 nm in diameter and is widespread in different cultivars of *Beta vulgaris* (Pullen, 1968, 1969; Kassanis *et al.*, 1977). Further analysis showed that BCV is a mixture of two viruses, BCV1 and BCV2, each with a genome of two dsRNA components with sizes of 2060 and 1740 bp (BCV1), and 1420 and 1320 bp (BCV2) (Accotto & Boccardo, 1986). There is no significant sequence homology among these four dsRNA genome components (Antoniw *et al.*, 1986; Xie *et al.*, 1989). In *in vitro* translation experiments the two dsRNAs of BCV1 behave as monocistronic messengers; the smaller dsRNA codes for the coat protein (53K) and the larger dsRNA might code for a polymerase (67K) (Accotto *et al.*, 1987). Recently, a third beet cryptic virus (BCV3) was found in Japanese leaf beet *B. vulgaris* cv. Fudanso. BCV3 particles are of a similar size to those of BCV1 and BCV2, and contain two dsRNA genome components with sizes of about 1740 and 1600 bp (Xie, 1992). In this paper, we have determined the nucleotide sequence of a DNA copy of BCV3 dsRNA2 and discuss possible functions of the polypeptide encoded.

A cDNA library of BCV3 dsRNAs was made as described by Antoniw *et al.* (1986) but with the following modifications. Because it was not known whether the genome segments of BCV3 were polyadenylated, the dsRNAs were first polyadenylated using poly(A) polymerase (BRL) before cDNA synthesis using the Amersham cDNA synthesis kit with an oligo(dT) primer. The cDNA was ligated into the *Eco*RI site of pUC13 and used to transform *Escherichia coli* strain TB1 (BRL). Specific clones of dsRNA2 of BCV3 were identified by screening the cDNA library with random-primed ^{32}P-labelled cDNA of dsRNA2 electroeluted from a polyacrylamide gel. The identity of the cDNA clones was confirmed by using them as probes to hybridize with Northern blots of isolated dsRNAs using the methods described by Xie *et al.* (1989).

Three of the cDNA clones obtained which were specific to dsRNA2 of BCV3 contained inserts of approximately 1600 to 1650 bases, which was close to the full length of the original dsRNA2. These clones were used for the determination of the nucleotide sequence. Nested deletions were obtained by digesting the clone containing the largest insert with exonuclease III and mung bean nuclease (Stratagene). The cloned cDNAs were sequenced by the dideoxynucleotide chain termination method using Sequenase Version 2.0 (USB). The nucleotide sequence of cDNA of BCV3 dsRNA2 was determined in both orientations and the corresponding plus strand RNA sequence is shown in Fig. 1. As far as we know this is the first published nucleotide sequence of a cryptic virus. One long open reading frame (ORF) of 1431 nucleotides was found which encodes a predicted polypeptide of 478 amino acids with a calculated M_r of 54·9K. The sequence context of the initiation codon UACAUGG is not thought to be a strong initiation codon (Kozak, 1987). The conserved amino acid sequence motif GDD was found in the deduced polypeptide (Table 1), suggesting that it is probably an RNA-dependent RNA polymerase (Poch *et al.*, 1989). This was supported by the presence of three other conserved amino acid sequence motifs (Habili &

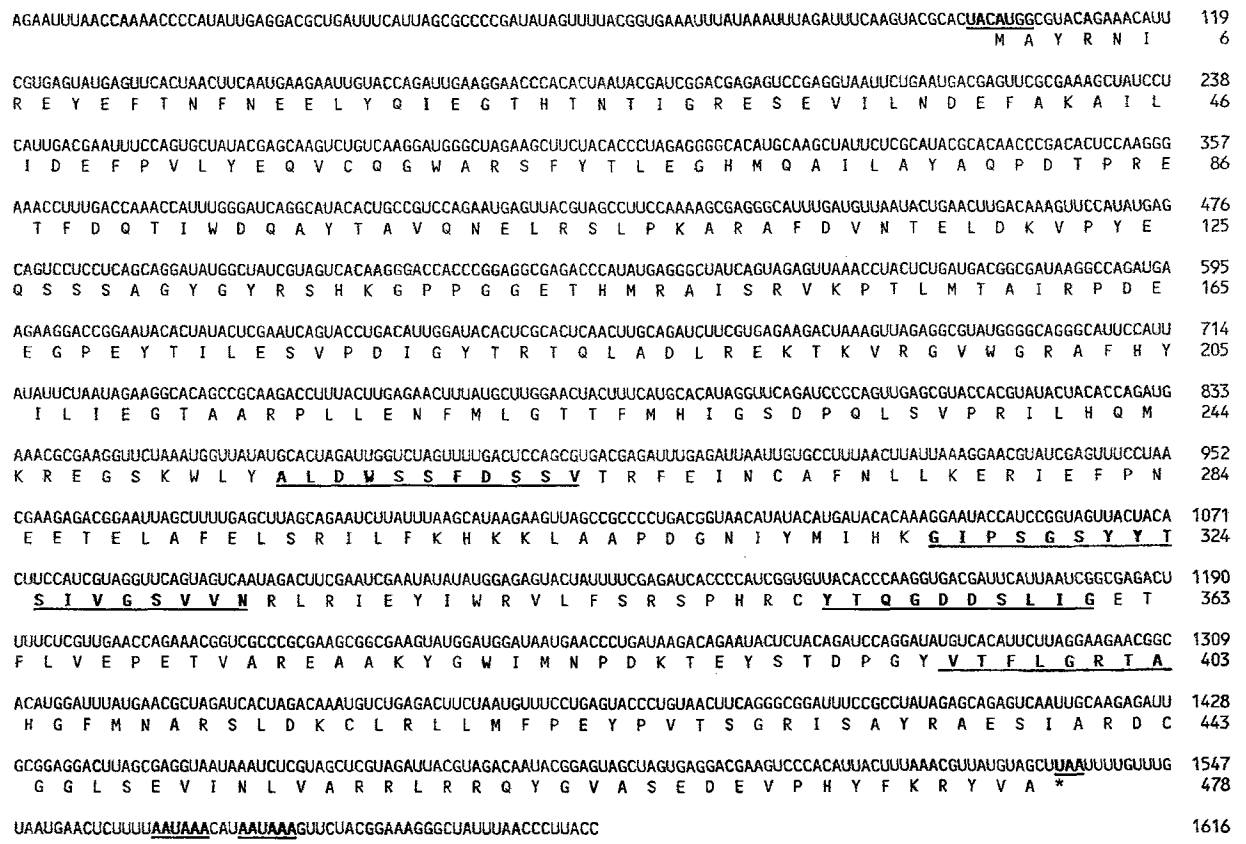

Fig. 1. The nucleotide sequence of the plus-strand dsRNA2 of BCV3 and amino acid sequences of the coding regions are shown. The termination codon is indicated by an asterisk. The sequence context of the initiation codon, possible polyadenylation signals and the conserved amino acid sequence motifs are highlighted.

Symons, 1989; Morozov, 1989) also found in genes encoding RNA-dependent RNA polymerases from other characterized ss and dsRNA viruses (Table 1). BCV3 therefore appears to be different from the other cryptic viruses, alfalfa cryptic virus (Accotto *et al.*, 1990), BCV1 (Accotto *et al.*, 1987) and white clover cryptic virus 1 (Boccardo *et al.*, 1989, 1990), in which the smaller genome segment seems to encode the coat protein as shown by *in vitro* translation and immunoprecipitation with whole virus antiserum. The amino acid sequence of the polypeptide derived from ryegrass cryptic virus (RCV) dsRNA5 (Xie, 1992) showed strong homologies within that of BCV3 dsRNA2 and contained three of the four amino acid sequence motifs, suggesting that it may also be an RNA-dependent RNA polymerase.

In addition to the long ORF, BCV3 dsRNA2 contained a 5′ leader sequence of 101 nucleotides and a 3′ untranslated region of 69 nucleotides (Fig. 1). Two possible eukaryotic polyadenylation signals of sequence AAUAAA, which are located 32 to 46 nucleotides upstream of the 3′ end of the plus-strand RNA, may suggest the presence of a poly(A) tail at the 3′ end. This is consistent with the observation that the 3′ end of the coding strand of white clover cryptic virus 1 dsRNAs is polyadenylated (Boccardo *et al.*, 1989, 1990). A possible second smaller ORF was also found in the complementary strand of dsRNA2. It extends for 321 nucleotides (starting from nucleotide 571) and contains 107 amino acid residues with a calculated M_r of 11·7K. However, whether this smaller ORF is actually translated into protein *in vivo* has yet to be determined.

The sequence of the cDNA clone of BCV3 dsRNA2 suggests that the plus-strand RNA has terminal sequences of 5′ AGAAUUU–UUACC 3′. These terminal sequences are as yet tentative, since they were not determined directly. Also, because poly(A) tails were added before the cDNA synthesis and cloning, it is possible that one or more uracil nucleotides may be present at the 5′ end of the plus strand and one or more adenine nucleotides may be present at its 3′ end. The 5′-terminal sequence of a cDNA clone of BCV3 dsRNA1 also contained the same nucleotide sequence 5′ AGAATTT-, suggesting that, in cryptic viruses, the same terminal sequences may be shared by both dsRNA genome components of the same virus. In reoviruses the different genome components also have identical terminal sequences and these are thought to play an important role during the replication process. Sequence

Table 1. *Conserved amino acid sequences of putative RNA polymerases encoded by RNAs of plus-strand RNA viruses, cryptic viruses and dsRNA viruses*

		Motif							
	Virus	I		II		III		IV	
A†	Consensus	* * ** *		** * *		* * *		* * *	
	SNBV‡	ETDIASFDKSQ	47§	MMKSGMFLTLFVNTVLN	18	AFIGDDNIIH	31	PYFCGGFI	
	AlMV	EIDFSKFDKSQ	47	QRRTGDALTYLGNTIVT	18	VASGDDSLIG	31	PFICSKFL	
	BMV	EADLSKFDKSQ	47	QRRTGDAFTYFGNTLVT	16	IFSGDDSLII	27	PYVCSKFL	
	BSMV	EIDFSKFDKSK	47	QQKSGNCDTYGSNTWSA	16	VFGGDDSLIL	29	PAFCGKFL	
	TRV	EIDMSKFDKSA	47	QQKSGDADTYNANSDRT	16	TYGGDDSLIA	29	PMFCGKFL	
	TMV	ELDISKYDKSQ	47	QRKSGDVTTFIGNTVII	16	AFCGDDSLLY	29	GYFCGRYV	
	BNYVV	VIDAAACDSGQ	44	VKTSGEPGTLLGNTILM	16	AMKGDDGFKR	30	ITFCGYAL	
	TYMV	ANDYTAFDQSQ	41	MRLTGEPGTYDDNTDYN	15	MVSGDDSLID	27	PLFCGYYV	
	PVX	ANDYTAFDQSQ	41	MRLTGEGPTFDANTECN	16	VYAGDDSALD	32	PEFCGWLI	
B	BCV3	ALDWSSFDSSV	51	GIPSGSYYTSIVGSVVN	19	YTQGDDSLIG	34	VTFLGRTA	
	RCV	AVDWSGFDASV				IVQGDDSLSA	34	VTFLGRSS	
C	Consensus	* * ** *		** * *		* * *		* * *	
	ROT	YTDVSQWDSSQ	59	AVASGEKQTKAANSIAN	22	RVDGDDNYAV	49	KIFFRAGI	
	REO	NIDISACDASI	85	TFPSGSTATSTEHTANN	34	VCQGDDGLMI	47	IFGCRIPN	
	BTV	AIDYSEYDTHL	115	THLSGENSTLIANSMHN	24	QYVGDDTLFY	48	KQGCYVPQ	
	RDV	LADCSSWDQTF	72	YMWSGRLDTFFMNSVQN	23	QVAGDDAIMV	52	MHFRDPSI	
	ScV	LDGASSFCFDY	60	TLLSGWRLTTFMNTVLN	18	VHNGDDVMIS	48	AQYLSRSC	
	HAV	IADATAYDSNC	179	GGGTGQSATSWDNTATF	25	YNTSDDTVWW	34	VEYLSKLP	
	IBDV	SIDLEKGEANC	56	GQGSGNAATFINNHLLS	33	ERSIDDIRGK	51	RLFCSAAY	
	Φ6	ATDVSDHDTFW	57	GLSSGQGATDLMGTLLM	42	ISKSDDAILG	35	GAFLGDIL	

* Highly conserved residues.

† A, plus-sense RNA viruses (Morozov, 1989); B, cryptic viruses; C, dsRNA viruses.

‡ SNBV, Sindbis virus; AlMV, alfalfa mosaic virus; BMV, brome mosaic virus; BMSV, barley stripe mosaic virus; TRV, tobacco rattle virus; TMV, tobacco mosaic virus; BNYVV, beet necrotic yellow vein virus; TYMV, turnip yellow mosaic virus; PVX, potato virus X (Morozov, 1989); ROT, bovine rotavirus (L1) (Cohen *et al.*, 1989); REO reovirus serotype 3 (L1) (Wiener & Joklik, 1989); BTV, bluetongue virus serotype 10 (L1) (Roy *et al.*, 1988); RDV, rice dwarf virus (S1) (Suzuki *et al.*, 1992); ScV, (Diamond *et al.*, 1989); HAV, hypovirulence-associated virus (Koonin *et al.*, 1991; Shapira *et al.*, 1991); IBDV, infectious bursal disease virus (Morgan *et al.*, 1988); and Φ6, bacteriophage Φ6 (Mindich *et al.*, 1988; Bruenn, 1991).

§ Numbers indicate the number of amino acids contained between each motif.

data of cDNA clones of RCV showed that the plus strand of RCV dsRNA5 also has a 5′-terminal sequence of 5′ AGAAU-, similar to that of BCV3 dsRNA2 (Xie, 1992).

Other characterized dsRNA viruses, orthoreovirus (McCrae, 1981; Antczak *et al.*, 1982), orbivirus (Mertens & Sangar, 1985; Roy, 1989), rotavirus (Imai *et al.*, 1983; Both *et al.*, 1984), phytoreovirus, fijivirus (Kudo *et al.*, 1991), rice ragged stunt virus (Yan *et al.*, 1992), cystovirus (Mindich, 1988) and *Saccharomyces cerevisiae* virus L-A (ScV; Icho & Wickner, 1989) have terminal sequences that differ from those found in BCV3 dsRNA2. There are, however, some similarities with the terminal sequences of cypovirus, 5′ AGUAA–GUUAGCC 3′ (Kuchino *et al.*, 1982).

Although cryptic viruses are a unique and as yet incompletely characterized virus group, their genomes encode some of the same conserved amino acid sequence motifs as other well characterized viruses, suggesting that cryptic viruses have a common evolutionary origin with other plant viruses.

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