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Nucleotide Sequence of the Australian Bluetongue Virus Serotype 1 RNA Segment 10

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SUMMARY

The complete nucleotide sequence of the RNA segment 10 of Australian BTV serotype 1 has been deduced from a combination of sequencing cDNA inserts cloned into pBR322 and synthetic deoxynucleotide priming on purified double-stranded RNA molecules. The gene segment was 822 nucleotides in length and capable of coding for two proteins on either 229 or 216 amino acids, having net charges of either +4.5 or +5.5 respectively at neutral pH. Comparison with the RNA segment 10 from BTV serotype 10 revealed a high degree of amino acid sequence conservation as well as regions of nucleic acid sequence conservation.

Bluetongue virus (BTV) is the type member of the orbivirus genus, which is one of six genera in the family Reoviridae. A total of 24 serotypes of BTV have been identified, on the basis of serum neutralization tests, and at present eight of these have been identified in Australia (Gould, 1988). The genome of this arthropod-borne virus consists of 10 double-stranded RNA (dsRNA) molecules which are enclosed by a double-shelled capsid. The inner core of the virus is composed of two major (VP3 and VP7) and three minor (VP1, VP4 and VP6) proteins which in turn are surrounded by an outer coat of two proteins, VP2 and VP5 (Verwoerd et al., 1972). Apart from the seven structural viral proteins, three non-structural proteins are also synthesized from individually purified segments of the BTV genome. In vitro translation studies revealed that NS1, NS2 and NS3 were coded for by RNA segments 5, 8 and 10, respectively (Sangar & Mertens, 1983; Eaton & Gould, 1987) (Fig. 1). In particular, RNA segment 10 was capable of coding for two proteins (termed VP8 and VP8a or NS3 and NS3a) either in vitro or in vivo in approximately equimolar proportions. Tryptic peptide analyses (Sangar & Mertens, 1983; B. T. Eaton, unpublished observations) have shown NS3 and NS3a to be related. Comparative sequence analyses of genome segments from North American BTV, South African BTV and Australian BTV isolates have demonstrated that different BTV serotypes have variable nucleic acid sequences for each segment, although the proteins that they encode remain highly conserved. The exception to this appeared to be VP2, the protein responsible for the elicitation of virus-neutralizing antibody (Huismans & Erasmus, 1981; Inumaru & Roy, 1987). In VP2, only certain well defined regions were conserved while the other regions appeared to be variable (Gould, 1987b).

To date no comparative sequence data have been available for the non-structural proteins of BTV. Hybridization analyses (Huismans & Cloete, 1987) have suggested that the most highly conserved RNA segment for BTV serotypes from all geographical regions was that coding for NS1. Hybridization responses for RNA segment 5, which codes for NS1, appeared to be similar to that for VP3; the latter is one of the major core antigens and recombinant DNA probes from its gene were capable of differentiating BTV on a geographical basis (Gould, 1987a, 1988), as it also preferentially hybridized to BTV isolates from the same geographical region. Kowalik & Li (1987) showed that NS1, NS2 and NS3 were all highly conserved within the North American BTV isolates. However no data were available (either by hybridization or sequence analysis) for the relatedness of nucleotide sequences of BTV NS3 (RNA segment 10) across geographical...
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Fig. 1. In vitro translation products of total BTV genome dsRNA or individually purified RNA segments. Lane 1, total genomic dsRNA; lane 2, RNA segment 8; lane 3, RNA segment 9; lane 4, RNA segment 10 from BTV serotype 1 (CSIRO 156). Translations were carried out using rabbit reticulocyte lysate as described in Eaton & Gould (1987) and electrophoresed on a 10% SDS-polyacrylamide gel.

Fig. 1 illustrates that purified RNA segment 10 from BTV1 coded for two proteins of approximate Mr 2.5 x 10^4 and 2.4 x 10^4 (termed NS3 and NS3a, respectively). This was almost double the expected coding capacity for that RNA species. Fig. 2 shows the complete nucleotide sequence of the sense strand of RNA segment 10 and, for comparative purposes, the nucleotide sequence of the North American BTV10 RNA segment 10 as well as any deduced amino acid differences are also given. The complete sequence of RNA segment 10 was 822 nucleotides long and contained one open reading frame which began at the presumed initiator codon (nucleotides...
Fig. 2. The complete nucleotide sequence of RNA segment 10 from the Australian BTV serotype 1 and its deduced amino acid sequence. Nucleotide position numbers and the differences with respect to the BTV10 RNA segment 10 are given above the nucleotide sequence while amino acid changes reflected by sequence differences are given below the amino acid sequence.

20 to 22) and terminated at a TGA codon (nucleotides 707 to 709). This enabled a protein of 229 amino acids or Mr 25 504 to be translated. The presence of a second AUG codon at nucleotide positions 59 to 61, previously reported by Lee & Roy (1986), was also conserved and raised the question as to which methionine was actually used to initiate synthesis of NS3. Unlike the other BTV gene segments previously sequenced (Purdy et al., 1985, 1986; Ghiasi et al., 1985; Gould, 1987a, b; Gould & Pritchard, 1987), the RNA segment coding for NS3 was the only one in which the presumed initiator codon did not conform to the Kozak consensus sequence (Kozak, 1981, 1984). NS3 is poorly translated in vivo, which may be a consequence of the pyrimidine present at position +4. In vitro, two proteins of approximate Mr 2.5 x 10^4 and 2.4 x 10^4 were translated from RNA segment 10 (Fig. 1, lane 4). This may have arisen from inefficient recognition of the initial AUG codon by ribosomes scanning the 5' terminus of the sense strand and initiation of synthesis at the second in-phase AUG codon, which has a purine at position +4 (Fig. 2; Kozak, 1981). This explanation appears to be reasonable as the apparent difference in Mr between NS3 and NS3a (Fig. 1, lanes 1 and 4) approximated to the calculated difference of 1.55 x 10^3 deduced from their sequence differences (Fig. 2).
higher than that for VP5 (68%; Gould & Pritchard, 1987) or VP2 (52%; Gould, 1987b). The 3' terminus of RNA 10 also differed from that of other RNA segments in that it was unusually long, having 113 nucleotides, as well as being G + C-rich (nucleotides 727 to 757 and 790 to 822 were 63% and 67% G + C, respectively). Other BTV gene segments examined so far have been slightly A + T-rich (56% to 58%). It is not clear why a 3' terminus that was three to four times longer than any previously sequenced BTV RNA segment should be conserved. Presumably some sequences were conserved as either RNA replication signals and/or for encapsidation, whilst other areas may have been involved in the formation of 'head to tail' concatemers of this RNA segment (Eaton & Gould, 1987). If this were the case then it would be expected that RNA segment 9, which can also form concatemers, should show similar features at its 3' terminus.

The deduced amino acid sequences for BTV1 RNA segment 10 codes for an NS3 protein very similar in composition to that of BTV10 from North America (Lee & Roy, 1986). Of the 14 deduced amino acid differences, exactly half were conservative changes and thus the distribution of cysteine residues, charged amino acids and the hydropathy profiles were almost identical to those of BTV10 NS3 (Lee & Roy, 1986). To date no function has been assigned to NS3. However, as this protein is so highly conserved, it may well play an essential role in either BTV replication or morphogenesis.

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REFERENCES


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